Response to Referee #1

We thank Reviewer #1 for his constructive comments. Below are copied the comments in regular font with our point by point responses in blue. Changes in the manuscript appear in 'track change' mode.

Bonnet and coauthors measured nitrogen fixation rates and diazotroph abundance along a west-east transect in the western tropical South Pacific Ocean. They report some astonishingly high rates along this transect and offer explanations for the driving factors. This is a solid piece of work with some important and interesting findings. Most of my comments below are minor although there are a couple of major typographical errors that need to be fixed. But this manuscript can be improved to make it something more than a data report by providing a good oceanographic context to the observations reported. It seems very likely that the researchers encountered different water masses along the transect that account for some of the variances in nitrogen fixation rates reported and providing that context would be useful. I suggest not using acronyms when not necessary – the difference between GY and gyre is two letters and it just makes it easier to read. I also suggest some minor modifications to the figures to make them more useful.

I do have a couple of pet peeves to express and hope the authors will pay attention to at least the second and change the manuscript accordingly.
1) While I realize that this was a major oceanographic expedition with many groups, all working at different pace and thus necessarily, some results are available earlier and already published while others more recently processed, it is still frustrating to read a manuscript where critical bits of information are presented elsewhere, either already published, in review or in preparation. It is unfortunate that success in the modern scientific enterprise is measured by numbers of papers and careers of especially young scientists are determined by first authorships, resulting in piece meal papers. I don't expect the authors can do much about this but do wish to raise this issue because it is especially important for major interdisciplinary field expeditions such as OUTPACE.
I totally understand this comment and share this view in some ways. The OUTPACE special issue is divided in 27 papers, each dealing with a specific part of the 'story'. Since the submission of this manuscript, several of the cited papers are now available online on the special issue webpage https://www.biogeosciences.net/special_issue894.html, and some are accepted, which should improve accessibility. I acknowledge that a synthesis paper would be necessary to give a broad and multidisciplinary view of the ecosystem functioning in this region.

2) The word "hotspot" is starting to get overused. It would seem that each investigator's favorite geography is a "hotspot" and I am having a difficult time with the concept of claiming a quarter or even one eighth of the largest ocean (western tropical South Pacific) as a hotspot. As the authors themselves say, "WTSP is a vast oceanic region" (page 2, line 21). The data to support the idea that the entire WTSP is a hotspot is still sparse and much too variable - 631286 in Melanesian archipelago waters - is a range of almost 45% in this cruise alone. The findings in this manuscript are significant even without that claim. In addition, there is one, potentially two real hotspots within this transect that are important in my view and that get lost when the claim is made to the whole area – I am both supportive and excited by the idea that there is a "sweet spot" (to use a different term) for diazotrophy at the interface where there is a supply of iron and phosphorus (around station LDB). This zonal gradient is similar to the meridional gradient in Fe and P that Moore et al characterized in the Equatorial Atlantic. But the South Pacific is more complex and thus interesting in that there is clearly some sort

of island effect with higher rates closer to the islands as well as Fe supply from the seafloor.
We agree with this comment and made a more reasonable use of the term hot spot throughout the new version of the manuscript. In addition, we now discuss the possible origin of the high N2 fixation rates at LDB, in relation with physical parameters and nutrient inputs page 15 line 10-28.

Specific comments and suggestions:
Page 2, line 5 – is it ammonia or amino acids?
It is first transformed into ammonia (see reaction below) then in amino acids
$N2 + 8\ H+ + 8\ e- + 16ATP \rightarrow 2\ NH3 + H2 + 16\ ADP + 16\ Pi$

Page 7, line 4 – should be per cell, not par cell
Yes, sorry, this has been fixed

Page 7, line 18 (and elsewhere) – it would be good to discuss what is special about station LDB. This station is clearly a hotspot. Why? Was there any eddy activity here? Why is the water warmer here? Why is the chl higher all along the water column? Are we at the edge of water masses? Actually for that matter, what is going on at LDA where warmer waters are mixed down to almost 150 m.
We added a section regarding the bloom at LDB page 15, line 23-28 and also refer to a video showing the evolution of the origin of the bloom: 'However, the huge surface bloom observed at LDB (Figure 1) and extensively studied by (de Verneil et al., 2017) was mainly sustained by N_2 fixation (secondary fueling picoplankton and diatoms) (Caffin et al., in review, 2018)), rather than deep nutrient inputs (de Verneil et al., 2017). This bloom had been drifting eastwards for several months and initially originate from Fiji and Tonga archipelagoes (https://outpace.mio.univ-amu.fr/spip.php?article160), which may have provided sufficient Fe to alleviate limitation and triggered this exceptional diazotroph bloom'.

Page 8, line 2 – what is DL? If it is detection limit, what is it? Why is the range 0-4 in line 4 – i.e. why is this not from detection limit?
We acknowledge that DL was not defined in the submitted version. We have added in the Methods section the following sentence: 'The minimum quantifiable rate (quantification limit, QL) calculated using standard propagation of errors via the observed variability between replicate samples measured according to Gradoville et al. (2017) was 0.035 nmol $N\ L^{-1}\ d^{-1}$.' We now refer to QL instead of DL in the Results section. Line 4 has been fixed as well.

Page 9, lines 34-38. This is a very important and interesting finding. While pressure could be one reason, clearly temperature would also play a role (although that would be in the opposite direction?) – i.e. there is a temperature gradient of 6-8 C between surface and 150m.
We acknowledge that there is temperature gradient with depth, but if temperature would have played a role on dissolution, we would have had higher dissolution at depth (colder temperatures) compared to the surface, which was not observed. However, we thought that it was important to be mentioned and we have added a sentence as follows in the revised version: 'Despite the A_{N2} value was different according to the incubation mode, it did not change with the depth of incubation on the mooring line, indicating that a slightly higher pressure than atmospheric pressure (1.5 bar at 5 m depth) is enough to promote the $^{15}N_2$ dissolution. It also indicates that the slightly lower seawater temperature (22-24°C) recorded at ~100-180 m where the deepest samples were incubated likely did not affect the solubilization of the $^{15}N_2$ gas'.

We partly agree with this comment. For samples collected above 50 m, the seawater temperature in the deck incubators and in situ was identical, so it seems that only pressure played a role in the higher dissolution. However, we acknowledge that seawater temperature is lower below 50 m and this may have enhanced the gas dissolution. Therefore, we have modified the text as follows: 'The seawater temperature checked regularly in the on-deck incubators was equivalent to ambient SST and likely did not explain the differences observed for samples collected above 50 m. However, we cannot exclude that the colder temperatures measured below 50 m-depth (~23-26°C instead of 29-30°C in surface) may have, in addition to pressure, slightly enhanced the $^{15}N_2$ gas dissolution, despite the AN_2 value did not change with depth'.

Page 10, line 11 – suggest using "differing" or some other word such as changing rather than differed?
We changed by 'Despite the A_{N2} value was different according to the incubation mode'

Page 10, line 15 – what does under in-situ-simulated mean? Why not just on-deck incubations that simulated appropriate light levels?
We agree and changed the text accordingly

Page 10, line 21 – it would appear that there is quite a bit of variability even in the archipelago waters. I am concerned that the contouring for figure 2 makes it appear as though it is a lot more uniform than it really is. While I understand the attraction of presenting the data along a linear transect this way, I do worry that real numbers are getting lost in this presentation and that the rates are actually a whole bunch more variable.
We now present all vertical profiles as supplementary information, clearly showing that rates are higher at some stations, which is also discussed in the new version of the manuscript.

Page 11, lines 3-22 – why only discussion of DIP – what about DOP? Trichodesmium can use DOP and it would have been interesting to see what was going on with that.
We have added a section regarding DOP in section 4.2: 'During the OUTPACE cruise, the DIP turn-over time was variable but close or below two days in MA waters (Moutin et al., in review, 2018), indicating a potential limitation by DIP at some stations. *Trichodesmium*, the most abundant and major contributor to N_2 fixation during the cruise, is known to synthesize hydrolytic enzymes in order to acquire P from the dissolved organic phosphorus pool (DOP) (Sohm and Capone, 2006). Moreover, *Trichodesmium* spp. differs from the other major diazotroph UCYN-B enumerated on the cruise in the forms of organic P it can synthesize. It is thus likely that DOP species that favored *Trichodesmium* over UCYN-B played a role in maintaining high *Trichodesmium* biomass in MA waters'.

Page 11, lines 26-27 – what is the range for the DFe concentrations?
The range of DFe for MA waters was 0.2-66.2 nM and 0.2-0.6nM for GY waters. This has been added in the revised version page 14 lines 13-14.

Page 12, lines 15-16 – Is it not the other way around – PAR explains depth?
We changed the text as follows 'Besides DFe, N_2 fixation rates were significantly negatively correlated with depth and logically positively correlated with PAR and seawater temperature, those two parameters being depth dependent (the thermocline was roughly located around 50 m)'.

Page 12, line 35 – dominated not dominating
This has been fixed

Page 13, line 28 – suggest saying “more than” rather than above
I do not understand this comment

Page 14, line 18-19 – the sentence construction suggests that rates have gone up rather than our understand of rates have changed
Yes, we totally agree and have changed the sentence as follows: ‘The number of N_2 fixation estimates have increased dramatically at the global scale over the past three decades (Luo et al., 2012)’.

Page 24, Table 3. It would seem that the table header for the second row is wrong. Spent a lot of time trying to figure out why the numbers were different till I figured out that it is actually for UCYN B rather than Trichodesmium
Thanks a lot, it was a mistake, it is fixed in the new version.

Page 25, Table 4. Why are the numbers for cDNA gene copies different from that reported in the text?
I am not sure to get this comment. The cDNA numbers are not reported in the text. However, the nifH copies per liter from the qPCR assay are given in Table 3 and in the text to give the context of the nanoSIMS studies. Those numbers are different of course but it is well specified in the text page 10 lines 30-31 that we are not talking about cDNA in this section but about quantification of nifH.

Page 27 Figure 1: Suggest improving 1a and show the ocean currents better – the superimposition of a big arrow does not do much. I am not clear how 1b was done – am just surprised that there are no clouds in the image. This is not critical expect to understand if the high chl patches seen are temporally relevant.
The currents have been redrawn on Figure 1a.
Figure 1b is actually a quasi-Lagrangian weighted mean Chl map in which the satellite data are weighted in time by each pixel’s distance from the ship’s average daily position for the entire cruise. As there were a large number of images due to the 45 days duration of the cruise, the resolution is nice despite there were clouds.

Page 28, Figure 2: Suggest adding the parameters to the various subfigures.
The parameters have been added

Response to Referee #2

We thank Carolin Löscher for the time devoted to this review and for her constructive comments. Below are copied the comments in regular font with our point by point responses in blue. Changes in the manuscript appear in 'track change' mode.

Bonnet and colleagues fill again one of the white spots on the maps of nitrogen fixation by presenting data from the OUTPACE cruise in 2015 to the western and central tropical South Pacific. N2 fixation rates from the photic are resented and complemented with a metadata analysis and a mining for the major diazotrophic clades Trichodesmium and UCYN-B. N2 fixation can be massive in that region, which is shown to be split in two different biogeochemical parts, and can reach up to 631±286µmol N/m/d. Those rates are extremely high and thus not only confirm the predicted importance of that region for N2 fixation but actually show that the N supply via N2 fixation is much higher than predicted. Moreover, they challenged the statistical approach of Luo and colleagues suggesting SST as a key regulating factor of N2 fixation in that region. This study now provides some realistic evidence for a limitation by either DIP or Fe. A discussion on the contribution of N2 fixation to primary and export production again emphasizes the importance of this region, which is from my point of view the best part of the manuscript. As in previous studies, I am missing direct C fixation rates, this would be easy to do in future studies and make your point of N2 fixation to primary production much stronger. Altogether, this is another piece of high-quality work of this group on this critical topic. The presentation is clear, the figures and tables are well chosen. I don't quite get, why there is no abstract, maybe it would be good to directly summarize your key findings, there. I only have minor comments and recommend publication in Biogeosciences.

Specific comments:

p. 2, l. 3 the biological carbon pump: the sentenced is phrase in a way that it sounds strange, here, also it deserves the Azam reference. Given your discussion on the contribution of N2 fixation to the carbon pump, this needs more than a sentence, please explain the connection between N2 fixation, C fixation and export production in some sentences.

The first paragraph of the introduction has been modified according to the suggestions as follows: 'In the ocean, nitrogen (N) availability in surface waters controls primary production and the export of organic matter (Dugdale and Goering, 1967; Eppley and Peterson, 1979; Moore et al., 2013). The major external source of new N to the surface ocean is biological N_2 fixation (100-150 Tg N yr^{-1}, (Gruber, 2008)), the reduction of atmospheric di-nitrogen gas (N_2) dissolved in seawater into ammonia (NH_3^+). The process of N_2 fixation is mediated by diazotrophic organisms that possess the nitrogenase enzyme, which is encoded by a suite of *nif* genes. These organisms provide new N to the surface ocean and act as "natural fertilizers", contributing to sustaining ocean productivity and eventually carbon (C) sequestration through the N_2-primed prokaryotic C pump (Karl et al., 2003; Karl et al., 2012). This N source is continuously counteracted by N losses, mainly driven by denitrification and anammox, which convert reduced forms of N (nitrate, NO_3^-, nitrite NO_2^-, NH_4^+) into N_2. Despite the critical importance of the N inventory in regulating primary production and export, the spatial distribution of N gains and losses in the ocean is still poorly resolved'.

p. 2, l. 6. nifH is only one gene, and it codes for one subunit of the nitrogenase reductase

We agree and the sentence has been changed as follows: 'The process of N_2 fixation is mediated by diazotrophic organisms that possess the nitrogenase enzyme, which is encoded by a suite of *nif* genes'.

p.4, l. 3 spell out HgCl2

It has been spelled: 'fixed with mercuric chloride ($HgCl_2$, final concentration 20 mg L^{-1})'

p.4, l. 8 and is throughout the text: submitted is capitalized, please change

We updated the references throughout the manuscript. Since the submission of this manuscript, most of the submitted ones are now under review or accepted. Anyways, when appropriate, we indicated 'submitted' without the capital S.

p.4, l. 10 As the paper is in review a brief description of the method would be helpful

The following paragraph has been added in section 2.1: 'Samples for determining dissolved Fe concentrations were collected and analyzed as described in Guieu et al. (Under review). Briefly, samples were collected using a Titane Rosette mounted with 24 teflon coated 12 L GoFlos deployed with a Kevlar cable. Dissolved Fe concentrations were measured by flow injection with online preconcentration and chemiluminescence detection according to Bonnet and Guieu (2006). The reliability of the method was monitored by analyzing the D1 SAFe seawater standard (Johnson et al., 2007), and an internal acidified seawater standard was measured daily to monitor the stability of the analysis'.

p.4, l. 13 ff and discussion: I see and understand why you chose for the bubble method here and I respect that you give me an argument to not start this discussion, again. I assume my opinion on the method issue is anyway clear by now, and seeing your discussion shows how reflective and aware of the importance of that topic you are. For the sake of having one day a truly comparative method, let me add a very pragmatic argument: I used both methods excessively, as a practical conclusion I can conclude (and I am sure you know what I am talking about) shaking the bottles with the gas bubbles in them for 100 times each is much more exhausting then pre-dissolving the gas in a bag with water. This limits the number of bottles you can handle, and with that the spatial resolution you will get. In addition, if you try shaking on a culture of Trichodesmium, you kill parts of the culture as they seem to dislike the shaking even more than I do. This would lead to a possible underestimation in addition to the one caused by the method. I appreciate the text you added to the discussion, but I feel it is a bit of an overkill. I would recommend to shorten it, but leave the justification with the DOM and Fe contamination potential in, so that everyone can understand why you chose for it. In addition, Grosskopf et al showed, and this is actually what plays into your cards, that the bubble method is mostly problematic for low rates- you have massive rates, so you don't really run into that problem.

Yes I know what you are talking about ☺ I will not give more details here as I can see that we understand each other. Ideally, we should have done proper comparisons onboard but as long as we have MIMS measurement, we can correct our data. Anyways, we shortened the discussion as suggested to keep only the 'contamination' argument, the rest is dedicated to the MIMS measurements during the in situ versus on-deck incubations. We also now make reference to a new study by Wannicke et al., (2018) and have added the following sentence: 'Moreover, a recent extensive meta-analysis (13 studies, 368 observations) between bubble and enriched amendment experiments to measure $^{15}N_2$ rates reported that underestimation of N_2 fixation is negligible in experiments that last 12-24 h (e.g. error is -0.2 %); hence our 24 h based experiments should be within a small amount of error (Wannicke et al., 2018)'.

p. 5, l.5: Could you add a sentence on what a trapezoidal integration is?

We have added the following sentence in the new version of the manuscript: 'Discrete N_2 fixation rate measurements were depth integrated over the photic layer using trapezoidal integration procedures. Briefly, the N2 fixation at each pair of depths is averaged, then multiplied by the difference between the two depths to get a total N2 fixation in that depth interval. These depth interval values are then summed over the entire depth range to get the integrated N2 fixation rate. The rate nearest the surface is assumed to be constant up to 0 m (JGOFS, 1988)'.

p. 6, l.2 If You follow your classical screening approach, here, and there is nothing bad about it. Still, I would be extremely curious seeing a nifH sequencing doe on those samples- how else can we know who is doing the N2 fixation, who could do it if conditions change? In the results it seems, your single cell rates add up to the bulk at station SD2 (I am referring to table 3), not at the others- so there must be something else. In this context, I appreciate the gene expression assay and indeed it could be that the het groups fill the gap. But obviously, the microbes you don't look at will not show up. I see, you are referring to Stenegren et al., 2017, but it would be beneficial for future studies to have a proper deep sequencing included.

I totally agree with this comment. Indeed we cannot totally reconcile the single-cell rates of dominant groups with bulk rates, which mean that there are other players. Of course DDAs quantified by Stenegren et al. are part of the story. We also observed some *Katagnymene spiralis* during the cruise, which despite scarce are really big and may contribute to N2 fixation, together with the heterotrophs mentioned in Benavides et al. (same issue) and probably fungi etc.. so yes, deep sequencing is really needed to fill this gap…

Response to Referee #3

We thank reviewer 3 for the time devoted to this review and for his/her constructive comments. Below are copied the comments in regular font with our point by point responses in blue. Changes in the manuscript appear in 'track change' mode.

The manuscript presented by Bonnet et al. reports N2 fixation rate measurements and diazotroph abundances from the Western tropical South Pacific. Complementary single-cell measurements of the two most abundant diazotrophs reveal the biogeochemical importance of each of these organisms in this region. Since this manuscript reports a subset of data collected on the OUTPACE cruise, these measurements are analyzed in correlation to a comprehensive set of nutrients, including dissolved iron, and other biogeochemical parameters. The manuscript is well written and the conclusions of this manuscript are appropriately based on the presented data. This new data is a nice addition of N2 fixation rate measurements in relation to biogeochemical parameters and a contribution of individual organisms (not solely based on abundances) hat will ultimately help confine the extent and magnitude of N input by N2 fixation into the global ocean. I only have a few comments (please see below).

Abstract: Maybe the authors could add a little more discussion/conclusions to the abstract as it currently reads almost like results only.

The abstract has been extended in the new version of the manuscript and now includes more discussion/conclusions.

p 3, l 5: I would use either ammonia (NH3) or ammonium (NH4+)

Totally right, this has been fixed

p 3, l 6: Isn't this 'nif genes' rather than 'nifH genes'?

Yes, the sentence has been changed as follows: 'The process of N_2 fixation is mediated by diazotrophic organisms that possess the nitrogenase enzyme, which is encoded by a suite of *nif* genes'.

p 3, l 7: anammox uses nitrite and ammonium as substrates, maybe those could be added to the fixed N?

I had added 'anammox' just before submission thanks to a suggestion of a co-author, so yes of course, I have added these substrates in the new version of the manuscript.

Section 2.2: As far as I understand, the authors used the time-zero samples rather than incubated controls as the natural abundance value in the N2 fixation rate calculation. In many cases, this is OK; however, I have also seen quite large changes in the natural abundance over time in incubated samples/bottles without the addition of stable isotope. These are usually the result of fractionation during the incubation time, e.g. due to the uptake of residual nitrate or remineralization of organic material. The fractionation effects can lead to higher or lower d15N values of the natural abundances. In the absence of incubated controls, the detection limits of N2 fixation might be a bit worse than if those values are available. I would therefore recommend that the authors add their detection limits to the manuscripts, such as the minimum change in d15N that was used as a cutoff for a significant 15N enrichments or reporting the actual d15N values measured in their

incubations and the time-zero values. This would also be coherent with general criticisms brought up in the recent paper by Gradoville et al. (2017; DOI: 10.1002/lno.10542).

We acknowledge that isotopic fractionation may occur during the incubation period and that it would be generally more correct to use the natural abundance value after incubation. In the present case, the rates were so high so the impact of fractionation is probably negligible. However it may impact the quantification limit, and we have added a sentence regarding the minimum quantifiable rates in section 2.2: 'The minimum quantifiable rates (quantification limit, QL) calculated using standard propagation of errors via the observed variability between replicate samples measured according to Gradoville et al. (2017) were 0.035 nmol N L^{-1} d^{-1}.

Section 2.4: Was the at% 15N in the N2 pool measured here as well?

It was measured in triplicates at every station but only in the bottles dedicated to bulk N2 fixation measurements and this value was used for the group-specific rate calculations as the same methodology was used (same bottles, same amount of 15N2 added) for both types of measurements.

Section 3.4.: Was primary production measured on this cruise? Based on the description in this section, it sounds to me as if N2 fixation somewhat scales with productivity and/or turnover of organic material.

Yes PP was measured using the 14C labeling method (not 13C). It appears in the correlation table but not in text. We have thus added PP and bacterial production in this section (which are both correlated with N2 fixation). The sentence is now: 'Regarding the main biogeochemical stocks and fluxes measured during the cruise, N_2 fixation rates were significantly positively correlated with dissolved Fe, dissolved organic N (DON), phosphorus (DOP) and carbon (DOC), particulate organic N (PON), particulate organic carbon (POC), biogenic silica (BSi), Chl *a* concentrations, primary and bacterial production ($p<0.05$), and significantly negatively correlated with NO_3^-, NH_4^+, DIP and silicate concentrations ($p<0.05$)'.

p 15, l 5: I assume that the "%" dropped from the 9.7? If not, does that mean that more organic matter is exported than produced at a given point in time?

Yes, this is 9.7 %, I have added the % in the new version of the manuscript.

p 15, l 14: With respect to the structure of the sections, I would almost move the entire section 4.2 here, as the rest of the discussion nicely scales from a more detailed and organism-centric discussion to a more system-oriented discussion. This would also have the side effect that the end of your discussion is not quite as focused on so many references that are related to other OUTPACE data which are not actually presented here.

I tried to do that and the structure of the manuscript did not seem coherent anymore to me. As the 4.2 section discusses the N2 fixation results, which are the first presented, it was not consistent for me to present detailed group-specific data before presenting the big picture. However, I acknowledge that section 4.4 Ecological relevance of N2 fixation in the WTSP contains many references of the OUTPACE SI not really related to the present study. Therefore, I propose to merge this section and the conclusion section to set our study in the general context of the OUTPACE study.

Table 1: Any idea on why the d15N value is so low for stn 11 (i.e. -7.05 ‰? Do you have any depth distribution of the d15N values for PN?

We measured d15N of PN at only 2 depths (the surface, 5 m and the deep chlorophyll maximum) so it's not a very good resolution. I was also wondering why such low values at SD 11. I know that this is the area where we have seen some high Fe inputs likely coming from underwater volcanoes. This may alter the isotopic composition of plankton…

Response to Referee #4

Below are copied the comments in regular font with our point by point responses in blue. Changes in the manuscript appear in 'track change' mode.

Review of the manuscript: In depth characterization of diazotrophs activity across the Western Tropical South Pacific hot spot of N2 fixation by S. Bonnet et al. I can't recommend this manuscript for publication in its current form, which is a shame, because it partially presents a very interesting data set about a large, but relatively understudied part of the world ocean. I have made this decision primarily because the authors do not present sufficient data or a convincing analysis to make their case. I think they need to show the reader a lot more information, a more expansive and rigorous analysis and be more up front about how they arrive at their conclusions. At present, I am unconvinced. I don't think that the problem can be simply rectified by rejigging the existing manuscript, adding some additional figures/tables or doing more statistics as in a normal 'major' revision. The authors need to make a fresh start, think carefully about where they want to go and the strengths/limitations of their data, and then re-write carefully. If they do that properly, I think this work has the potential to make a very significant contribution to the global N-fixation literature.

We are quite surprised that the reviewer opinion is that this paper do not present sufficient data. However, we believe that our story is compelling because:
-we provide accurate bulk N2 fixation over a 4000 km transect and the whole photic layer, which represent the first data for this region
-we tested several methods of incubations and provide a critic opinion of each method in the context of the methodological issues associated with N2 fixation measurements in the N2 fixation community
-we further investigate the contribution of the main diazotroph groups using single-cell isotope approaches (nanoSIMS)
-we provide extensive environmental data (dissolved iron, DIP turnover times…) to discuss the spatial variability of the N2 fixation activity observed. This part has been improved following the suggestions (please see below)

Some general comments: This reviewer recognizes that English may not be the lead author's prime language. However, in places, some of the word choices and syntax are ambiguous so that different readers are likely to take different things away from the manuscript. Specific examples are noted below. The authors should take advantage of the native English speakers in the author list to ensure that the wording is done with more precision to ensure that the intended meaning is communicated.

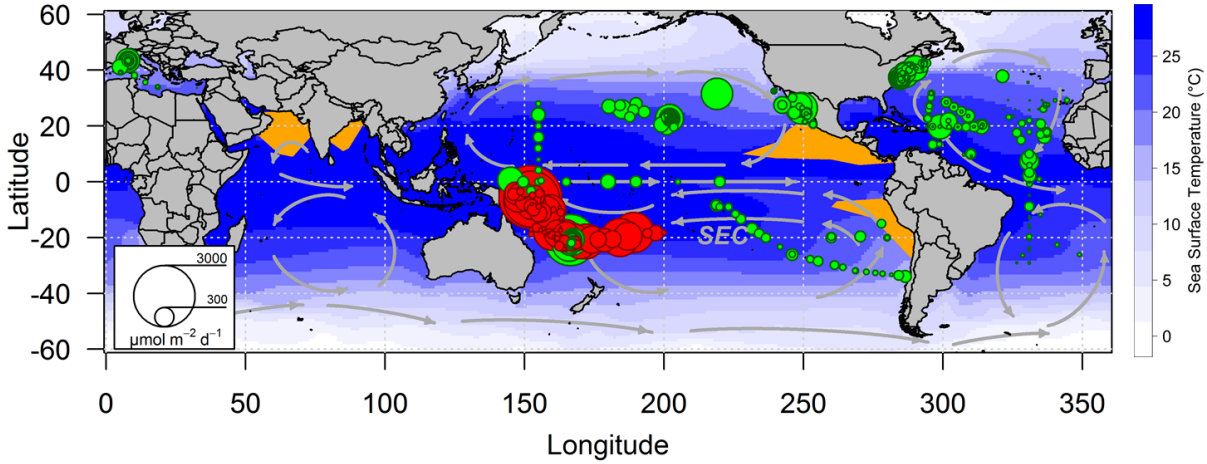
We acknowledge that English was not perfect as it is not the native language of the first author. The revised version of the manuscript has been checked by the native English speaker of the author list.

Repeated references are made throughout to works which are 'submitted' or 'in review'. While this indicates the present manuscript is quite timely, the reader can make no critical or objective use of these references as they haven't seen the light of day, might be rejected or heavily altered before they are eventually published. It's hard to take these citations at face value. Where possible, I would include actual data from closely related studies in your paper to genuinely demonstrate your point, noting by citation that a fuller description will be published in other work. If not possible, stick to your own dataset.

We totally understand this comment. The OUTPACE special issue is divided in 27 papers, each treating a specific part of the 'story'. Since the submission of this manuscript, several of the cited papers are now available online on the special issue webpage https://www.biogeosciences.net/special_issue894.html, and some are accepted (this has been updated in the revised version), which should improve the accessibility.

The authors need to be a lot more precise about your geography. The 'regions' used herein are quite large, loosely defined and contain a number of somewhat similar, but different oceanographic regimes. For example, 'Western Tropical South Pacific' would also include the Warm Pool region north of PNG – it's western, south of the equator and certainly tropical – but a different setting altogether. Likewise, 'Eastern Tropical South Pacific' also includes extensive areas of Ekman driven upwelling where it would be difficult to extrapolate your measurements. The longitude scale on Fig. 1 (bottom) is seriously wrong.
We agree that the WTSP is a vast region that includes the warm pool region north of PNG, but also the Solomon Sea, the Coral Sea etc… these regions were previously documented for N2 fixation, and we clearly state in the introduction section that our goal is to specifically study the central and eastern parts of the WTSP, that are critically undersampled + the border of the south pacific gyre. This was the goal of the OUTPACE project, so we only report results from this cruise. To avoid any misunderstanding, we have added 'OUTPACE cruise' in the title of the manuscript to specify that this article is about the results of this specific cruise and not the results about the entire WTSP.
The longitude scale on Figure here is the conventional one decided for all outpace papers of the Special issue. However we fixed that point in the revised version and provided a new version of Figure 1 to provide a general context of the study area.

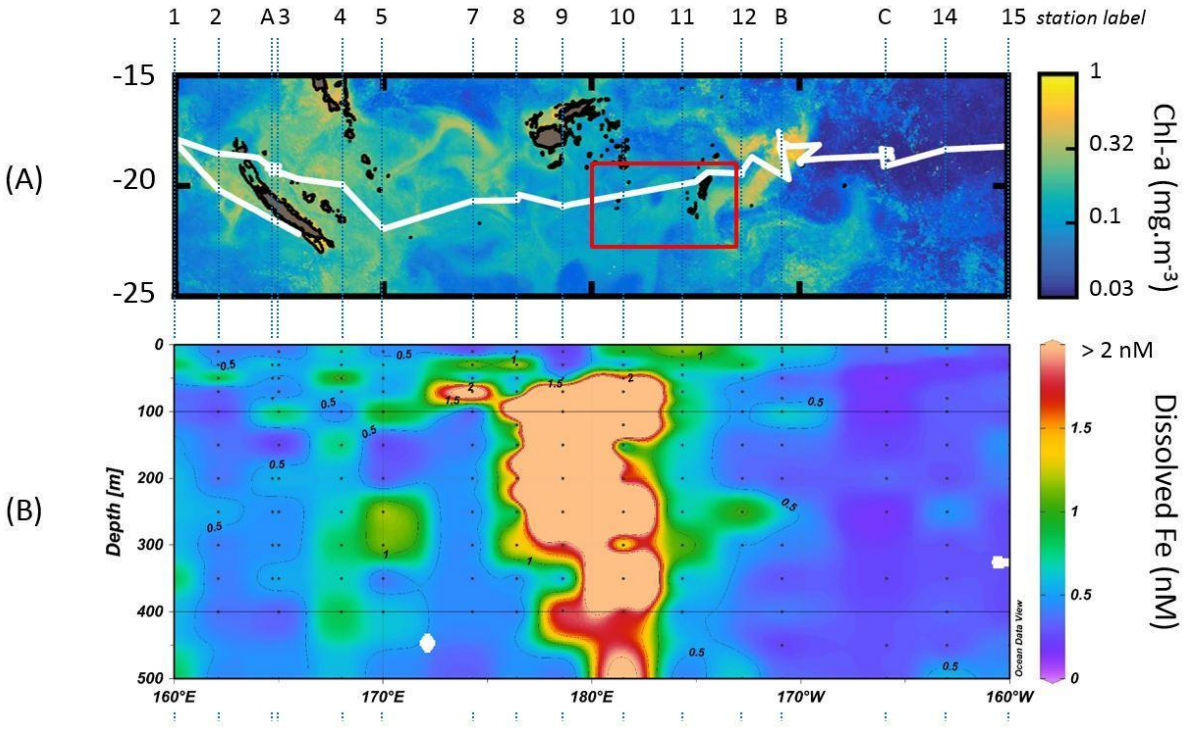
For starters, what exactly do you mean by a N-fixation 'hot spot'? What's the cut-off? A summary table at least summarizing ranges of measured or reliably estimated N-fixation in other 'hot-spot' parts of the world ocean (e.g. Arabian Sea, Caribbean, Arafura Sea, etc., etc.) would be very useful to set the scene and would tie your work into the wider literature of global N-fixation. As a reader, I'm thinking a lot about how is this paper compares with the much larger body of published work done by Capone, Carpenter and their collaborators/students, etc. There seems to be little quantitative integration (show me the numbers) with even the many more recent measurements of N-fixation in the SW Pacific. These need to be tied together, or shown why not.
To the best of our knowledge, there is no 'official' cut-off to design a hot spot but we clearly mentioned in the manuscript that the rates reported here are in the upper range of the higher caregory (100-1000 µmol N m^{-2} d^{-1}) of rates reported in the N_2 fixation MAREDAT database for the global ocean (Luo et al., 2012). This was our argument to say that it is hot spot. However, as suggested by Reviewer 1, we tone down the hot spot story throughout the revised version. We also recently published the map below, which gathers N2 fixation rates from the Luo et al. global database (in green) compared to the rates measured in the WTSP (in red, this cruise and others) by our team. It speaks for itself and reveals the WTSP as a high N2 fixation area in the global ocean, at least from our current knowledge. We acknowledge that there are also other high N2 fixation areas such as the Caribbean Sea and probably other parts which are to date particularly undersampled. This map was published in a very short letter in PNAS to argue for a spatial decoupling between N2 fixation in the western Pacific and N losses in the eastern Pacific, and only present depth-integrated rates, with no details. The present paper aims at describing vertical and horizontal N2 fixation rates during the OUTPACE cruise in relation with environmental parameters, identify the main players etc…



From Bonnet, S., Caffin, M., Berthelot, H., and Moutin, T.: Hot spot of N2 fixation in the western tropical South Pacific pleads for a spatial decoupling between N2 fixation and denitrification, Proceedings of the National Academy of Sciences of the United States of America, 114, E2800-E2801, 10.1073/pnas.1619514114, 2017.

Figures and tables – Need more of them!, and more quantitative! ODV color contour plots are nice, but awfully hard to interpret quantitatively, and quite impossible if looking at a B/W version of your paper. Show some convincing/representative profiles, hard contour lines and East-West quantitative values. The discussion, by and large, is mostly hand-waving and speculation. While quite a few papers are cited (several of which haven't been published), there is a lack of quantitative information and data presented from these studies with which to compare the authors' results, assess their veracity and draw comparisons. The bit about regional differences being due to iron (etc.) inputs from submarine volcanos is wholly speculative on the information provided. Not a shred of quantitative information is presented to back this assertion up. Might the regional difference in fixation be due to regional differences in wind stress and water masses which affects the depth of the mixed layer and vertical mixing through the thermocline? I'd like to see a more focused discussion.

We now provide the vertical profiles of N2 fixation in the supplementary information. The vertical and longitudinal variability of N2 fixation are now better described in the results section and discussed in the section 4. The depth of the mixed layer has been also added to the revised manuscript and is homogeneous across the zonal transect and may not explain the differences observed (all this is now presented and discussed). The full set of dissolved iron data (see figure below) cannot be provided in the revised version as they are used in another paper under (minor) revision in Scientific reports. However, the discussion on the role of iron has been updated page 14 lines 11-36 to give more quantitative data.



Surface Chlorophyll-a concentration (mg m^{-3}) during the 45-day transect of the OUTPACE cruise (A) (The ocean color satellite products are produced by CLS. Figure courtesy of A. De Verneil). (B) Cross-section of dissolved Fe nM (0-500 m). From Guieu et al., (Under review, minor revisions)

Why no comparison with the extensive work done on N-fixation, fluxes and driving processes done at station ALOHA? An opportunity is missed.
We performed an extensive comparison between the OUTPACE results and the ALOHA station data in our other OUTPACE paper by Caffin et al., (2018) regarding the functioning of the ecosystem, the role of N2 fixation on export etc…

Some specific comments:
Page Line(s) Comment 1 2 What exactly do you mean by 'hot spot' – see above 3
Please see comment above

10-11 By 'highest rates of N2 fixation' do you mean on an area-specific basis (I doubt it) or aggregate fixation on a regional basis primarily because of the very large area involved? The oxygen deficient zones of the eastern Pacific are due to higher regional productivity arising from Ekman-driven upwelling at the basin scale, not N-fixation. Indeed, fixation tends to be lower in upwelling regions. Simple N:P ratios are a poor predictor in this case.
Yes, I mean that Deutsch et al., (2007) predicted the highest rates of N2 fixation in the global ocean in the ETSP. I totally agree that this is a high productivity area mainly due to the upwelling, so it was counter intuitive to find N2 fixation there, but their argument was that the decrease of P* in this region would be the result of intense N2 fixation. This Deutsch et al study motivated many cruises in the ETSP to quantify N2 fixation there. Most of them found low rates (average range ~0-60 µmol N m^{-2} d^{-1}, (Dekaezemacker et al., 2013; Fernandez et al., 2011; Fernandez et al., 2015; Knapp et al., 2016; Loescher et al., 2014)).

10-20 This paragraph seems to do a logical U-turn 21 etc. References to regional N-fixation ignore large database of published historical estimates by Capone, Carpenter, etc., etc., etc.

I am not sure to get this comment. In this paragraph we review the existing literature in the ETSP and WTSP and all along the manuscript we compare our results with the global MAREDAT database, which includes historical data from Capone, Carpenter... There are very few studies in this South Pacific. I wish I could cite more papers including Capone, Carpenter... I may have missed something, so if you know more papers published for this area, please provide the references. Some of them include Capone results especially the Knapp et al. 2016 and Dekaezemacker et al. for which D.G Capone is co-author (he was also the chief scientist onboard the cruise from which these papers originate). The Montoya et al., 2004 paper in the Arafura Sea also include D.G Capone data…

4 11 Change "equalled to" to "reliably extrapolated to estimates of"
This has been changed

18 Change "previously undocumented" to "new"
This has been changed

22 What is the basis of 'selection' – suggest leave out
nanoSIMS analyses are very expensive and time consuming. It was this not possible to perform such analyses at all stations, and we thus selected three stations to perform those analyses based on the diazotroph community composition assessed microscopically onboard. We remove the terms 'selected stations' in the introduction and specify in the Methods section that those analyses were performed at only 3 stations

22 What do you mean by 'potential ecological impact' of N-fixation? Poor wording.
We changed by 'biogeochemical impact'

29 Suggest changing 'contrasted' to something about a gradient of conditions. What's the essential difference between 'oligotrophic' and 'ultra-oligotrophic'? Strictly speaking that's like saying something is 'more better'.
The depth of the deep chlorophyll maximum was the main criteria. We changed the sentence as follows: 'It covered a trophic gradient from oligotrophy (deep chlorophyll maximum (DCM) located at ~80-100 m) in MA waters around New Caledonia, Vanuatu, Fiji up to Tonga, to ultra-oligotrophy (DCM located at 115-150 m) in GY waters located at the western boundary of the South Pacific Gyre (see Introduction article Moutin et al., 2017 for details on the cruise implementation)'.

33 (etc.) By 'fluorescence', I presume you mean 'chlorophyll fluorescence' – say it because lots of other things fluoresce if you measure it right.
It has been changed

5 4 Use 'stored' instead of 'preserved. More importantly for low-level nutrient analyses – how long were the samples stored before analysis (hours, days, months)?
This has been changed. The nutrient samples were stored for ~3 months before analysis (the time for the 4°C container to be back from French Polynesia (Tahiti) to Marseille (France). Previous experience from our team have confirmed that this way of conservation ($HgCl_2$, 4°C) is a valid methodology.

7-10 Are there any actually published papers that describe these methods? Preferable. In the case of dissolved iron, the more widely used and less confusing notation would be: Fediss.
The manuscripts mentioned in the submitted version are now all available online on the webpage of the special issue https://www.biogeosciences.net/special_issue894.html and most of them have been accepted, except the Fe paper (presently in minor revision) , so the method for dissolved Fe concentration determination is now given in this section.

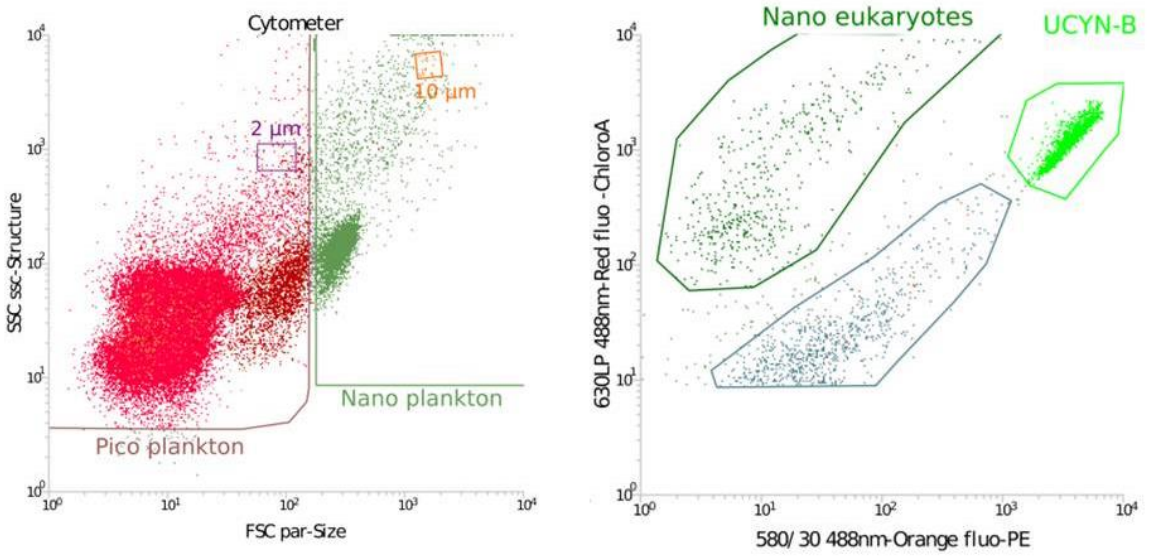
14 In using open-ocean communities, it is almost universally observed that metal and organic contamination results in under-estimation of rates due to toxicity of these materials to finicky oceanic bugs. Why do you think they are over-estimated?
The most likely metal contamination onboard a metallic ship is iron, which would likely fertilize the studied water mass and consequently would over estimate rates (together with DOM contaminations) (see previous work from Bonnet et al., 2009). We acknowledge that other metals such as Hg or Cu at high concentrations would have the opposite effect due to toxicity, but this is unlikely to occur with the protocols we use as they are scarce compared to iron.

20-21 Presumably you mean sub-samples taken from the Niskins. Explicit reading suggests you collected the water in situ in the poly-carb bottles.
This was stated in the first paragraph of the method section (common to all other sub-sections of the methods) 'Seawater samples were collected by 12-L Niskin bottles mounted on the CTD rosette'. Wee re-specified that seawater was collected from niskin bottles here.

33 How were these filters stored and for how long? Text suggests they were analysed almost right-away (good if you can do it correctly!).
We stored the dried filters in a desiccator for 3-4 months before analysis. As long as there is no water anymore on the filters, they can be stored for months.

6 20 What's the "them"?
The polycarbonate bottles mentioned in the previous sentence. We changed as follows for more clarity: 'eight additional polycarbonate (2.3 L) bottles were collected from the surface (50 % light irradiance) to determine *Trichodesmium* and UCYN-B specific N_2 fixation rates by nanoSIMS and quantify their contribution to bulk N_2 fixation. Two of these bottles were amended with $^{15}N_2$ as described above for further…'

26-28 What are the flow cytometric characteristics you sorted and counted the UCYN cells with? A lot of those don't have much, if any photosynthetic pigment, and if they did, the near-surface ones would likely be bleached a bit? It would be nice to see a cytogram.
Pico- and nano- phytoplankton were clustered on FSC vs. SSC cytograms (left panel on the figure below) using 2 µm beads. UCYNs were associated to the nano- phytoplankton (right panel on the figure) and the UCYN associated cluster was established using orange fluorescence vs. red fluorescence cytograms. The cluster corresponding to UCYNs is shown in light green on the cytogram of the right panel on the figure, and could be clearly separated from other clusters.



8 2 Strictly speaking, you're 'estimating' this, not determining it.
Yes, this has been changed

16 (etc) By 0-50 m, I presume you mean the 'surface mixed layer'. This is a key matter herein as the surface mixed layer thickness changes along your transect. Strictly speaking, surface is surface (say 0-5 m). Even small and ephemeral density gradients in the near-surface layer and surface mixed layer can have profound influences on vertical mixing rates and hence the light histories of cells embedded in the surface layer. Be very specific!
We changed the text to be more specific. 'The mixed layer depth (MLD) calculated according to the de Boyer-Montegut et al., (2004) method was located around 20-40 m throughout the zonal transect: Maximum temperatures were measured in the surface mixed layer (~0-20/40 m) and remained almost constant along the longitudinal transect with 29.1 ± 0.3°C in MA waters and 29.5 ± 0.4°C in GY waters'.

.
23 By DCM, I presume you mean 'deep chlorophyll maximum'?
Yes, it is now defined in the methods section.

29-34 It's not clear what, if anything, this paragraph contributes to the paper.
This paragraph is about a methodological aspect regarding the comparison of the solubilization of the 15N2 tracer according to the mode of incubation of samples used. This is the first time that such comparisons are done, and these results will be helpful for people who measure N2 fixation in on-deck incubators (a method widely used) and will hopefully convince them to perform MIMS measurements on their samples.

37-38 This seems very wrong. I'm presuming you actually mean the per-mil deviation of the particulate matter (δ15N) from the normal natural abundance of 15N (0.367%). Normally, N-fixation has a δ15N value close to 0‰. Larger deviations would suggest other fractionation processes such as denitrification. Clarify and fix up.
The term 15N/14N ratio was probably misleading. We changed the sentence by 'The natural N isotopic signature of suspended particles measured over the photic layer was on average -0.41‰ in MA waters and 8.06‰ in GY waters (Table 1)'.

9 1-8 This is all the 'new' N-fixation data in the results text. Pretty thin. As an interested party, I'd like to see a lot more. Graphics and tables too. 10-17 Correlations – So what?

Tables of correlation coefficients fill space, but are instantly forgettable. What's the point of the correlations other than that you can do them?
The presentation of N2 fixation rate results have been extended, we have merged sections 3.3 (N2 fixation results) and 3.4 (correlations) in the new version on the manuscript, and have added correlations between N2 fixation and primary and bacterial production as requested by another reviewer. Correlations are used in the discussion section and help the interpretation of the results.

18-23 Show 'em or ditch this.
Not sure to get this comment

10 1-5 etc Decimals on figures. It's easy to calculate lots of decimal places on figures, but they clutter up the text. Given the analytical and natural variability of these processes and the analytical processes, how many decimal places are really justified and meaningful?
For N2 fixation rates, given our quantification limit, one decimal only is justified and realistic. This is what is done in the manuscript.

17-18 Realistically, one never really 'measures' a process. Given all of the factors at work, the best we can do is 'estimate' its magnitude. Best to be frank about that.
We agree and replaced 'measurements' by 'estimates'

10-11 16-9 You probably overdid this bit of text. The 'bubble' problem is well known. Best to just say that you used the Montoya method to minimize contamination, but corrected for incomplete dissolution by measuring the 15N/14N ratio. It is interesting that you get higher dissolution in the samples incubated in situ and that needs to be explicitly corrected for
We shortened the text as suggested and removed the following part 'Two methods are routinely used by the scientific community to perform direct N_2 fixation measurements in marine systems: 1) the method developed by (Montoya et al., 1996), which consists of the addition of the $^{15}N_2$ tracer as a bubble in the incubation bottles (hereafter referred to as the 'bubble addition method') and the measurement of the $^{15}N/^{14}N$ ratio of PN before (time zero) and after incubation, 2) the method consisting of adding the $^{15}N_2$ as dissolved in a subset of seawater previously N_2 degassed (Mohr et al., 2010) (hereafter referred to as the '$^{15}N_2$-enriched seawater method'). The second method was developed because the first had been observed to potentially underestimate N_2 fixation rates (Großkopf et al., 2012; Mohr et al., 2010; Wilson et al., 2012) due to the incomplete (and gradually increasing during the incubation period) equilibration of the $^{15}N_2$ in the incubation bottles when injected as a bubble. This results in a lower $^{15}N/^{14}N$ ratio of the N_2 pool available for N_2 fixation (the term A_{N2} used in the Montoya et al. (1996) equation) as compared to the theoretical value calculated based on gas constants, and therefore potentially leads to underestimated rates in some studies (see references above), whereas other studies do not see any significant differences between both methods (Bonnet et al., 2016c; Shiozaki et al., 2015)'.

28 Fig. 1 Bottom: longitude scale is very wrong.
The longitude scale provided here is the conventional one decided for all outpace papers. However we fixed that point.

Would like to see some comparative profiles of measured variables in different regions
The present paper is about the OUTPACE cruise results. We have added N2 fixation ranges from other part of the world for comparison, but this paper is not designed as a review of all the existing literature. Therefore we decided to stay focus on our data.

29 Fig. 2 ODV scales need to be properly annotated.

The x axis label has been added

Fig. 3 Potentially useful, but ... Bottom: should have x-axis scale 0-10 with vertical dotted lines clearly showing natural abundance of 15N (0.367%) and the theoretical 15N/14N ratio if all 15N2 in bubble dissolved. Is the (very) slight mid-water increase in 15N excess statistically valid?
This has been fixed

In depth characterization of diazotroph activity across the western tropical South Pacific hot spot of N_2 fixation (OUTPACE cruise)

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Abstract

Here we report ~~quantification of~~ N_2 fixation rates ~~over a~~from a ~4000 km transect in the western and central tropical South Pacific, a ~~region~~ particularly under-sampled region in the World's Ocean. Water samples were collected in the euphotic layer along a west to east transect from 160°E to 160°W that, ~~-~~covered~~ing~~ contrasting trophic regimes, from oligotrophy in the Melanesian archipelagoes (MA) waters to ultra-oligotrophy in the South Pacific Gyre (GY) waters. N_2 fixation was detected at all 17 sampled stations with an average depth integrated rate of 631 ± 286 µmol N m^{-2} d^{-1} (range 196-1153 µmol N m^{-2} d^{-1}) in MA waters and of 85 ± 79 µmol N m^{-2} d^{-1} (range 18-172 µmol N m^{-2} d^{-1}) in GY waters. T~~he~~wo cyanobacteria, the larger colonial filamentous *Trichodesmium* and the smaller UCYN-B, ~~cyanobacteria~~ dominated the enumerated diazotroph community (>80 %) and gene expression of ~~nitrogenase genes~~the *nifH* gene (cDNA >10^5 *nifH* copies L^{-1}) in MA waters. ~~, and s~~Single-cell isotopic analyses performed by nanoscale secondary ion mass spectrometry (nanoSIMS) at select ~~ed~~ stations ~~reveal~~ identified that *Trichodesmium* was always the major contributor to N_2 fixation in MA waters, accounting for 47.1 to 83.8 % of bulk N_2 fixation. The most plausible environmental factors explaining such exceptionally high rates of N_2 fixation in MA waters are discussed in detail emphasizing the role of macro- and micronutrients (e.g. iron) availability, seawater temperature and currents.

1 Introduction

In the ocean, nitrogen (N) availability in surface waters controls primary production and the export of organic matter (Dugdale and Goering, 1967; Eppley and Peterson, 1979; Moore et al., 2013)~~, a process commonly referred to as 'the biological carbon pump'~~. The major external source of new N to the surface ocean is biological N_2 fixation (100-150 Tg N yr^{-1}, (Gruber, 2008)), the ~~conversion~~ reduction of atmospheric ~~N_2~~ di-nitrogen gas (N_2) dissolved in seawater into ammoni~~uma~~a (~~NH_4~~NH_3^+). The process of N_2 fixation is mediated ~~. This process is performed~~ by diazotrophic organisms that possess~~ing~~ the ~~enzyme nitrogenase~~ nitrogenase enzyme, which is encoded ~~encoded~~ by ~~the~~ a suite of *nif*~~H~~ genes. These organisms provide new N to the surface ocean and act as "natural fertilizers", contributing to sustaining ocean productivity and eventually ~~CO_2~~carbon (C) sequestration through the N_2-primed prokaryotic ~~carbon (C)~~C pump (Karl et al., 2003; Karl et al., 2012). This N source is continuously counteracted by N losses, mainly driven by denitrification and anammox, which convert ~~fixed~~ reduced forms of N (nitrate, NO_3^-, nitrite NO_2^-, NH_4^+) into N_2. Despite the critical importance of the N inventory in regulating primary production and export, the spatial distribution of N gains and losses in the ocean is still poorly resolved.

A global scale modeling study predicted that the highest rates of N_2 fixation are located in the South Pacific Ocean (Deutsch et al., 2007; Gruber, 2016). These authors also concluded that processes leading to N gains and losses are spatially coupled to oxygen deficient zones such as in the eastern tropical South Pacific (ETSP), which harbors NO_3^--poor but phosphate-rich surface waters, i.e. potentially ideal niches for N_2 fixation (Monteiro et al., 2011). However, recent field studies based on several cruises and independent approaches, including biological $^{15}N_2$ incubations-based measurements and geochemical $\delta^{15}N$ budgets, ~~revealed~~ have consistently measured low N_2 fixation rates (average range ~0-60 µmol N m^{-2} d^{-1}) in the surface ETSP waters (Dekaezemacker et al., 2013; Fernandez et al., 2011; Fernandez et al., 2015; Knapp et al., 2016; Loescher et al., 2014). Low activity in the ETSP has been largely attributed to ~~, presumably due to~~ iron (Fe) limitation (Bonnet et al., 2017; Dekaezemacker et al., 2013), as Fe is a major component of the nitrogenase enzyme complex required for N_2 fixation (Raven, 1988). ~~On the opposite,~~However, the western tropical South Pacific (WTSP) ~~has~~ was recently ~~been~~ identified as a of high N_2 fixation activity (Bonnet et al., 2017) and together, these studies plead for a basin-wide spatial decoupling between N_2 fixation and denitrification in the South Pacific Ocean.

The WTSP is a vast oceanic region extending from Australia in the west to the western boundary of the South Pacific Gyre in the east (hereafter referred to as GY waters) (Figure 1). It has been chronically under-sampled (Luo et al., 2012) as compared to the tropical North Atlantic (Benavides and Voss, 2015) and the North Pacific Oceans (e.g. (Böttjer et al., 2017)) ~~oceans~~, however ~~, However, , but~~ recent oceanographic surveys performed in the western part of the WTSP, in the Solomon, Bismarck (Berthelot et al., 2017; Bonnet et al., 2009; Bonnet et al., 2015) and Arafura (Messer et al., 2016; Montoya et al., 2004) Seas report extremely high N_2 fixation rates (>600 µmol N m^{-2} d^{-1}, i.e. an order of magnitude higher than in the ETSP) throughout the year. In these regions, high N_2 fixation~~, that~~ have been attributed to sea surface temperature >25 °C and continuous nutrient inputs of terrigenous and volcanic origin (Labatut et al., 2014; Radic et al., 2011). The central and eastern parts of the WTSP, a vast oceanic region bordering Melanesian archipelagoes (New Caledonia, Vanuatu, Fiji) up to the Tonga trench (hereafter referred to as MA waters) have been far less investigated. One study (Shiozaki et al., 2014) reported high surface N_2-fixation rates close to Melanesian islands in relation with nutrient supplied by land runoff. However, the lack of direct N_2 fixation measurements over

the full photic layer ~~prevents the implementation of~~ impedes accurate N budget estimates in this region. In addition, the reasons for such an ecological success of diazotrophs in the WTSP are still under debate (Bonnet et al., 2017) as the horizontal and vertical distribution of environmental parameters potentially controlling N_2 fixation, in particular measured Fe concentrations, are still scarce in this region.

Recurrent blooms of the filamentous cyanobacterium *Trichodesmium*, one of the most abundant diazotrophs in our oceans (Luo et al., 2012), have been consistently reported in the WTSP since the James Cook (Cook 1842) and Charles Darwin's expeditions and later confirmed by satellite observations (Dupouy et al., 2011; Dupouy et al., 2000) and microscopic enumerations (Shiozaki et al., 2014; Gradoville et al., 2017). However, molecular studies based on the *nifH* ~~diversity also revealed the presence~~gene abundances have shown high densities of unicellular diazotrophic cyanobacteria (UCYN) in the WTSP (Moisander et al., 2010). Three main groups of UCYN (A, B and C) can be distinguished based on *nifH* gene sequences. In the warm (>25 °C) waters of the Solomon Sea, UCYN from group B (UCYN-B) co-occur with *Trichodesmium* at the surface, and together dominate the diazotrophic community (Bonnet et al., 2015), while UCYN-C are also occasionally abundant (Berthelot et al., 2017). Further south in the Coral and Tasman Seas, UCYN-A dominate the diazotroph community (Bonnet et al., 2015; Moisander et al., 2010). Both studies reported a transition zone from UCYN-B-dominated communities in warm (>25 °C) surface waters to UCYN-A-dominated communities in colder (<25° C) waters of the western part of the WTSP. Further east in the MA waters, *Trichodesmium* and UCYN-B co-occur and account for the majority of total *nifH* genes detected (Stenegren et al., 2018). Although molecular methods greatly enhanced our understanding of the biogeographical distribution of diazotrophs in the WTSP, DNA-based *nifH* counts ~~cannot be~~do not ~~equaled reliably extrapolated to estimates of to~~equate~~d~~ to metabolic activity. Thus, the contribution of each dominant group to bulk N_2 fixation is still lacking ~~in this globally important high N_2 fixation area~~the WTSP~~'hot spot' of N_2 fixation~~. Previous studies showed that different diazotrophs have different fates in the ocean: some are directly exported, others release and transfer part of the recently fixed N to the planktonic food web and ~~fuel~~ indirectly fuel export of organic matter (Berthelot et al., 2016; Bonnet et al., 2016a; Karl et al., 2012). Consequently assessing the relative contribution of each dominating group of diazotrophs to overall N_2 fixation is critical to assess the biogeochemical impact of N_2 fixation in the WTSP.

In the present study, we report ~~previously undocumented~~new bulk and group-specific N_2 fixation ~~quantification~~ rate measurements from ~~over a~~a ~4000 km transect in the western and central tropical South Pacific. The goals of the study were i) to quantify ~~to~~ both horizontal and vertical distribution of N_2 fixation rates in the photic layer in relation with ~~hydrological and biological~~environmental parameters, ii) to quantify the relative contribution of the dominant diazotrophs (*Trichodesmium* and UCYN-B) to N_2 fixation ~~at selected stations~~ based on cell-specific measurements, and iii) to assess the potential ~~ecological~~ biogeochemical impact of N_2 fixation in this region.

2 Methods

Samples were collected during the 45-day OUTPACE (Oligotrophic to UlTra oligotrophic PACific Experiment) cruise (DOI: http://dx.doi.org/10.17600/15000900) onboard the R/V *L'Atalante* in February-March 2015 (austral summer). The west to east zonal transect along ~19 °S started in Noumea (New Caledonia) and ended in Papeete (French Polynesia) (Figure 1). It~~,~~ covered~~ing~~ a trophic gradient from ~~contrasted trophic regimes (~~oligotrophy (deep chlorophyll

maximum (DCM) located at ~100 m) in MA waters ~~y to ultra-oligotrophy) (see Moutin et al., 2017 for details), crossing MA waters~~ around New Caledonia, Vanuatu, Fiji up to Tonga, to ultra-oligotrophy (DCM located at 115-150 m) in ~~and~~ GY waters located at the western boundary of the South Pacific Gyre (see Introduction article Moutin et al., 2017 for details on this~~e~~ cruise ~~implementation~~). Data were collected at 17 stations including 14 ~~short~~ short-duration (SD; 8 h) stations (SD1 to SD15, note that SD13 was not sampled) and three ~~long~~ long-duration (LD; 7 days) stations (LDA, LDB and LDC). Vertical (0-200 m) profiles of temperature, salinity, and chlorophyll fluorescence were obtained at all 17 stations using a Seabird 911 plus CTD equipped with a Wetlabs ECO-AFL/FL fluorometer. Seawater samples were collected by 12-L Niskin bottles mounted on the CTD rosette.

2.1 Macro-nutrient and dissolved Fe concentrations analyses

Samples for quantifying nitrate (NO_3^-) and dissolved inorganic phosphorus (DIP) concentrations were collected at 12 depths between 0 and 200 m in acid-washed polyethylene bottles, ~~fixed~~ amended with mercuric chloride ($HgCl_2$, ~~(~~ final concentration 20 mg L^{-1}) and ~~preserved~~ stored at 4 °C until analysis. Concentrations were determined using standard colorimetric techniques (Aminot and Kerouel, 2007) on a Bran Luebbe AA3 autoanalyzer. Detection limits for the procedures were 0.05 µmol L^{-1} for NO_3^- and DIP.

Samples for ~~determing~~determining dissolved Fe ~~(DFe)~~ concentrations ~~determination~~ were collected and analyzed as described in Guieu et al. (Under review). Briefly, samples were collected using a Titane Rosette mounted with 24 teflon coated 12 L GoFlos ~~and operated along a~~deployed with a Kevlar cable. Dissolved Fe concentrations were measured by flow injection with online preconcentration and chemiluminescence detection according to Bonnet and Guieu (2006). The reliability of the method was monitored by analyzing the D1 SAFe seawater standard (Johnson et al., 2007), and an internal acidified seawater standard was measured ~~every day in order~~daily to monitor the stability of the analysis.

The sampling and analytical methods used to analyze the parameters reported in the correlation table (Table 2) are described in detail~~s~~ in the methods sections for related papers in this issue (Bock et al., in review, 2018; Fumenia et al., in review, 2018; Stenegren et al., 2018; Van Wambeke et al., Accepted). ~~Samples for dissolved Fe (DFe) concentrations determination were collected and analyzed as described in Guieu et al. (Under review).~~

2.2 Bulk N_2 fixation rate measurements

Whole water (bulk) N_2 fixation rates were measured in triplicate at all 17 stations using the $^{15}N_2$ isotopic tracer technique (adapted from Montoya et al. (1996)). The $^{15}N_2$ bubble technique was intentionally chosen due to the time limitation to make enriched $^{15}N_2$ seawater inoculates (e.g. 6-9 depths = 6-9 inoculates) and the larger sample bottles required for making proper estimates of activity in oligotrophic environments. In addition, we aimed to avoid any potential overestimation due trace metal and dissolved organic matter (DOM) contaminations often associated with the preparation of the $^{15}N_2$-enriched seawater (Klawonn et al., 2015; Wilson et al., 2012) in our incubation bottles as Fe and DOM have been found to control N_2 fixation or *nifH* gene expression in this region (Benavides et al., 2017; Moisander et al., 2011). However, the $^{15}N/^{14}N$ ratio of the N_2 pool available for N_2 fixation (the term A_{N2} used in the Montoya et al. (1996) equation) was measured in all incubation bottles by ~~M~~membrane ~~I~~inlet ~~M~~mass ~~S~~spectrometry (MIMS) to ensure accurate rate calculations (see below).

Seawater samples were collected from ~~niskin~~Niskin bottles into 10 % HCl-washed, sample-rinsed (3 times) light-transparent polycarbonate (2.3 L) bottles from 6 depths (75 %, 50 %, 20 %, 10 %, 1 %, and 0.1% surface irradiance levels) at all short-duration stations SD1 to SD15 and 9 depths (75 %, 50 %, 35 %, 20 %, 10 %, 3 %, 1 %, 0.3 % and 0.1 % surface irradiance levels) at LDA, LDB and LDC, corresponding to the sub-surface (5 m) down to 80 to 180 m depending on the station. Bottles were sealed with caps fitted with silicon septa and amended with 2 mL of 98.9 atom% $^{15}N_2$ (Cambridge isotopes). The purity of the $^{15}N_2$ Cambridge isotopes stocks was previously checked by Dabundo et al. (2014) and more recently by (Benavides et al., 2015) and (Bonnet et al., 2016a). They were found to be lower than 2 x 10^{-8} mol:mol of $^{15}N_2$, leading to a potential N_2 fixation rates overestimation of <1 %. Each bottle was ~~agitated~~ shaken 20 times ~~for 10 minutes~~ to break the $^{15}N_2$ bubble and facilitate its dissolution and incubated for 24 h. At SD stations, bottles were incubated in on-deck incubators connected to surface circulating seawater at the specified irradiances using blue screening as the duration of the station (8 h) was too short to deploy in situ mooring lines. At LD stations (7 days), one profile was incubated following the same methodology in on-deck incubators and another replicate profile was incubated in situ for comparison on a drifting mooring line located at the same depth from which the samples were collected. Incubations were stopped by filtering the entire ~~sample~~ incubation bottle onto pre-combusted (450 °C, 4 h) 25-mm diameter glass fiber filters (GF/F, Whatman, 0.7 µm nominal pore size). Filters were subsequently ~~, that were~~ dried at 60 °C for 24 h before analysis of ~~being analyzed onshore for~~ $^{15}N/^{14}N$ ratios and particulate N (PN) ~~concentrations~~ determinations using an elemental analyzer coupled to a mass spectrometer (EA-IRMS, Integra CN, SerCon Ltd) as described in (Bonnet et al., 2011).

To ensure accurate rate calculations, the $^{15}N/^{14}N$ ratio of the N_2 pool in the incubation bottles was measured on each profile from triplicate surface incubation bottles from SD1 to SD14 and at all depths at SD15 and LD stations. Briefly, 12 mL were subsampled after incubation into Exetainers® fixed with $HgCl_2$ (final concentration 20 mg L^{-1}) that were preserved upside down in the dark at 4 °C until analyzed using a ~~membrane inlet mass spectrometer (~~MIMS~~)~~ according to (Kana et al., 1994). Lastly, we collected time zero samples at each station to determine the natural N isotopic signature of ambient particulate nitrogen (PN). The minimum quantifiable rates (quantification limit, QL) calculated using standard propagation of errors via the observed variability between replicate samples measured according to (Gradoville et al., 2017) were 0.035 nmol N L^{-1} d^{-1}.

Discrete N_2 fixation rate measurements were depth integrated over the photic layer using trapezoidal integration procedures. Briefly, the N_2 fixation at each pair of depths is averaged, then multiplied by the difference between the two depths to get a total N_2 fixation in that depth interval. These depth interval values are then summed over the entire depth range to get the integrated N_2 fixation rate. The rate nearest the surface is assumed to be constant up to 0 m (JGOFS, 1988)

~~assuming that surface N_2 fixation rates were identical to those in subsurface (5 m) and considering that rates below the deepest sampled depth were zero (JGOFS, 1988).~~

2.3 Statistical analyses

Spearman's rank correlation was used to examine the potential relationships between N_2 fixation rates, hydrological, biogeochemical, and biological parameters across the longitudinal transect (n=102, α=0.05). A non-parametric Mann-

Whitney test (α=0.05) was used to compare the MIMS data obtained following on-deck versus in situ incubations, and to compare nutrient and Chl *a* distributions between the western part and the eastern part of the transect.

2.4 Group-specific N_2 fixation rate measurements ~~at selected some?select stations~~

2.4.1 Experimental procedures

At three ~~selected~~ stations along the transect (SD2, SD6, LDB), where *Trichodesmium* and UCYN-B accounted for >90 % of the total diazotrophic community (see below and Stenegren et al. (2018)), eight additional polycarbonate (2.3 L) bottles were collected from the surface (50 % light irradiance) to determine *Trichodesmium* and UCYN-B specific N_2 fixation rates by nanoSIMS and quantify their contribution to bulk N_2 fixation. Two of these bottles~~m~~ were amended with $^{15}N_2$ as described above for further nanoSIMS analyses on individual cells (the 6 remaining bottles were used for DNA and RNA analyses, see below) and were incubated for 24 h with the incubation bottles dedicated to bulk N_2 fixation measurements in on-deck incubators as described above. To recover large-size diazotrophs (*Trichodesmium*) after incubation, 1.5 L were filtered on 10 µm pore size 25 mm diameter polycarbonate filters. The cells were fixed with paraformaldehyde (PFA) (2 % final concentration) for 1 h at ambient temperature (~25 °C) and the filters were then stored at -20 °C until nanoSIMS analyses. To recover small size diazotrophs (UCYN-B), samples were collected for further cell sorting by flow cytometry prior to nanoSIMS. 1 L of the remaining $^{15}N_2$ labelled bottle were filtered onto 0.2 µm pore size 47 mm polycarbonate filters. Filters were quickly placed in a 5 mL cryotube® filled with 0.2 µm filtered seawater with PFA (2 % final concentration) for 1 h at room temperature in the dark. The cryovials were vortexed for 10 s to detach the cells from the filter (Thompson et al., 2012) and stored at -80 °C until cell sorting. Cell sorting of UCYN-B was performed on a Becton Dickinson Influx™ Mariner (BD Biosciences, Franklin Lakes, NJ) high speed cell sorter of the Regional Flow Cytometry Platform for Microbiology (PRECYM), hosted by the Mediterranean Institute of Oceanography, as described in Bonnet et al. (2016a) and (Berthelot et al., 2016). After sorting, the cells were dropped onto a 0.2 µm pore size polycarbonate 13 mm diameter polycarbonate filter connected to low pressure vacuum pump, then stored at -80 °C until nanoSIMS analyses. Special care was taken to drop the cells on a surface as small as possible (~5 mm in diameter) to ensure the highest cell density possible to facilitate subsequent nanoSIMS analyses.

2.4.2 Abundance of diazotrophs by microscopy and qPCR methods. ~~and *nifH* gene expression~~

The abundance of *Trichodesmium* filaments and the average number of cells/filament were~~as~~ determined microscopically: 1 to 2.2 L were filtered on 2 µm polycarbonate filters. The cells were fixed with PFA prepared with filtered seawater (2 % final concentration) for 1 h at 4 °C and stored at -20 °C until counting using an epifluorescence microscope (Zeiss Axioplan, Jana, Germany) fitted with a green (510–560 nm) excitation filter. The whole filter was counted and the number of cell per trichome was counted on at least 10 filaments per station.

Four other diazotrophic phylotypes were quantified using quantitative PCR (qPCR) as they were ~~too scarce or not enumerabl~~not easily quantifiable by standard epifluorescence microscopy: UCYN-A1, UCYN-B and two heterocystous symbionts of diatom-diazotroph associations (DDAs): *Richelia intracellularis* associated with *Rhizosolenia* spp. (het-1) and ~~*Richelia* *R.*~~ *intracellularis* associated with *Hemiaulus* spp. (het-2). Triplicate 2.3 L-bottles were filtered onto 25 mm diameter 0.2 µm Supor filters with a 0.2 µm pore size at each station using a peristaltic

pump. The DNA extraction and TaqMAN qPCR assays are fully described in (Stenegren et al., 2018). To ~~evaluate~~ quantify~~the~~ *nifH* gene expression, additional triplicate 2.3 L bottles were filtered as described above. The filters were placed into pre-sterilized bead-beater tubes (Biospec Products Inc., Bartlesville, OK USA) containing 250 µL RLT buffer (Qiagen RNeasy) amended with 1 % ß-mercaptoethanol and 30 µL of 0.1 mm glass beads (Biospec Products Inc.). The time of filtering for RNA varied between stations (17-21:00). Filters were flash frozen in liquid nitrogen and stored at -80 °C until RNA extraction. The RNA extraction and Reverse Transcription (RT) were performed as previously described using a Super-Script III first-strand cDNA synthesis kit (Invitrogen Corp., Carlsbad, CA, USA) including the appropriate negative controls (water ~~,~~ and No ~~-~~RT) (Foster et al., 2010). The *nifH* gene expression for het-1, het-2, *Trichodesmium*, UCYN-A1, and UCYN-B was as described previously (Foster et al., 2010).

2.4.3 nanoSIMS analyses, data processing and group-specific rate calculations

NanoSIMS analyses were performed using a nanoSIMS ~~N~~50L instrument (Cameca, Gennevilliers France) at the French National Ion MicroProbe Facility according to (Bonnet et al., 2016a; Bonnet et al., 2016b) and (Berthelot et al., 2016). Briefly, a ~ 1.3 pA Cesium (16 KeV) primary beam focused onto ~100 nm spot diameter was scanned across a 256 x 256 or 512 x 512 pixel raster (depending on the image size) with a counting time of 1 ms per pixel. Samples were pre-sputtered prior to analyses with a current of ~10 pA for at least 2 min to achieve sputtering equilibrium and ensure a consistent implantation and analysis of the cell interior by removing cell surface. Negative secondary ions ($^{12}C^{14}N^-$, $^{12}C^{15}N^-$) were collected by electron multiplier detectors, and secondary electrons were also imaged simultaneously. A total of 10-50 serial quantitative secondary ion images were generated, that were combined to create the final image. Mass resolving power was ~8000 in order to resolve isobaric interferences. 20 to 100 planes were generated for each cells analyzed. NanoSIMS runs are time intensive and not designed for routine analysis, but a minimum of 250 cells of UCYN-B per station and 30 *Trichodesmium* filament portions were analyzed to take into account the variability of activity among the population.

Data were processed using the LIMAGE software. Briefly, all scans were corrected for any drift of the beam and sample stage during acquisition. Isotope ratio images were created by adding the secondary ion counts for each recorded secondary ion for each pixel over all recorded planes and dividing the total counts by the total counts of a selected reference mass. Individual *Trichodesmium* filaments and UCYN-B cells were easily identified on nanoSIMS images that were used to define regions of interest (ROIs). For each ROI, the $^{15}N/^{14}N$ ratio was calculated.

Trichodesmium and UCYN-B cellular biovolume was calculated from cell-diameter measurements performed on 50 ~cells or trichomes per station ~~on an~~using an epifluorescence microscope (Zeiss Axioplan, Jana, Germany) fitted with a green (510–560 nm) excitation filter. UCYN-B had a spherical shape and *Trichodesmium* cells were assumed to have a cylindrical shape. The carbon content per cell was ~~determined~~ estimated from the biovolume according to Verity et al. (1992) and the N content was calculated based on a C:N ratios of 6 for *Trichodesmium* (Carpenter et al., 2004) and 5 for UCYN-B (Dekaezemacker and Bonnet, 2011; Knapp et al., 2012). ^{15}N assimilation rates were expressed 'pe~~a~~r cell' and calculated as follows (Foster et al., 2011; Foster et al., 2013): assimilation (mol N cell^{-1} d^{-1}) = (^{15}Nex x N_{con})/N_{sr}, where ^{15}Nex is the excess ^{15}N enrichment of the individual cells measured by nanoSIMS after 24 h of incubation relative to the time zero value, N_{con} is the N content of each cell determined as described above, and N_{sr} is the excess ^{15}N enrichment of the source pool (N_2) in the experimental bottles determined by MIMS (see above).

Standard deviations were calculated using the variability of N isotopic signature measured by nanoSIMS on replicate cells. The relative contribution of *Trichodesmium* and UCYN-B to bulk N_2 fixation was calculated by multiplying cell-specific N assimilation by the cell abundance of each group, relative to bulk N_2 fixation determined at the same time.

3 Results

3.1 Environmental conditions

Seawater temperature ranged from 21.4 to 30.0 °C in the sampled photic layer (0 to ~80-180 m) over the cruise transect (Figure 2a). ~~Maximum temperatures were measured a~~at ~~the~~t the surface ~~depth~~in the upper 30m ~~(0-50 m, 28.7 °C on average) (~0-30 m) and remained constant along the longitudinal transect, the average measured~~temperature was , ~~with one exception when slightly higher sea surface temperatures (SST) were observed at LDB (29.9°C) compared to the transect average SST (28.7°C).~~ The mixed layer depth (MLD) calculated according to the (de Boyer Montégut et al., 2004) method was located around 20-40 m throughout the zonal transect: Maximum temperatures were measured in the surface mixed layer (~0-20/40 m) and remained almost constant along the longitudinal transect with 29.1 ± 0.3°C in MA waters and 29.5 ± 0.4°C in GY waters.

Based on other hydrographic measurements (dissolved nutrients~~For nutrients~~, dissolved ~~D~~Fe and Chl *a* concentrations), the longitudinal transect ~~was divided~~divided into two ~~main characteristic~~ sub-regions: 1) the MA region from station SD1 (160_°E) to LDB (165_°W), and 2) the GY sub-region from station LDB (165_°W) to SD15 (160_°W) located in the South Pacific Gyre. Chl *a* concentration~~s~~ in the upper 50 m ~~were~~ was significantly ($p<0.05$) higher in MA waters (0.17 µg L^{-1} on average ~~over 0-50 m~~) than in GY waters (0.06 µg L^{-1} on average ~~over 0-50 m~~) (Figure 1, Figure 2b). The DCM was located around 80-100 m in MA waters and deepened ~~at~~ to ~150 m in GY waters indicating higher oligotrophy in the GY region. Surface NO_3^- concentrations (Figure 2c) ~~were~~ were consistently close or below the detection limit (0.05 µmol L^{-1}) ~~all over the surface~~in the upper water column (0-50 m) ~~waters~~ throughout the transect~~, but~~ and the depth of the nitracline gradually deepened from ~75~~80~~-100 m in MA waters ~~down~~ to ~115~~0~~ m in GY waters. DIP concentrations were slightly higher or close to detection limits (0.05 µmol L^{-1}) in MA surface waters (0-50 m) ~~waters~~, ~~where~~and ~~–~~ the phosphacline was more shallow~~er~~ (20-45 m) than the nitracline and DIP concentrations increased significantly ($p<0.05$) in GY waters ~~to reach~~and ranged 0.13-0.17 µmol L^{-1} (Figure 2d).

3.2 N isotopic signature of the N_2 pool after incubation ~~(MIMS results)~~

The ^{15}N enrichment of the N_2 pool after 24 h of incubation with the $^{15}N_2$ tracer was on average 6.145 ± 0.798 atom% (n=54) in bottles incubated in on-deck incubators and significantly higher ($p<0.05$) in bottles incubated on the *in situ* mooring line (7.548 ± 0.557 atom% (n=44), Figure 3a). However, the depth of incubation on the *in situ* mooring line (between 5 and 180 m) did not have any significant effect ($p>0.05$) on the isotopic signature of the N_2 pool at LDB and LDC, which remained constant over the water column (Figure 3b).

3.3 Natural isotopic signature of suspended particles and N_2 fixation rates

The natural N isotopic signature ~~$^{15}N/^{14}N$ ratio~~ of suspended particles measured over the photic layer was on average -0.41_‰ in MA waters and 8.06_‰ in GY waters (Table 1). Those isotopic values~~numbers~~ were used as time zero samples to calculate N_2 fixation rates.

N_2 fixation was detected at all 17 sampled stations and the average measured N_2 fixation rates ~~in the surface waters (X – X m)~~ in the two~~2~~ previously defined sub-regions were 1) ~~the transect could also be divided into the two main characteristic sub-areas: 1) the MA waters exhibiting average N_2 fixation rates of~~ 8.9 ± 10 nmol N L^{-1} d^{-1} (range: QD~~D~~L-48 nmol N L^{-1} d^{-1}) over the photic layer in MA waters, and 2) ~~the GY waters exhibiting average N_2 fixation rates of~~ 0.5 ± 0.6 nmol N L^{-1} d^{-1} (range: QL~~0~~-4.0 nmol N L^{-1} d^{-1}) in GY waters (Figure 2e). In MA waters, N_2 fixation was ~~mostly~~ largely restricted to the ~~surface~~ mixed layer (~0-30~~25~~ m), where average rates were 15 nmol N L^{-1} d^{-1}, with local maxima (> 20 nmol N L^{-1} d^{-1}) at stations SD1, SD6 and LDB and local minima (<5 nmol N L^{-1} d^{-1}) at SD8 and SD10. ~~rates commonly peaked at 30 to 48 nmol N L^{-1} d^{-1}~~. In GY waters, maximum rates reached 1-2 nmol N L^{-1} d^{-1} and were located deeper in the water column (~50 m). When integrated over the photic layer, N_2 fixation represented an average net N addition of 631 ± 286 µmol N m^{-2} d^{-1} (range 196-1153 µmol N m^{-2} d^{-1}) in MA waters and of 85 ± 79 µmol N m^{-2} d^{-1} (range 18-172 µmol N m^{-2} d^{-1}) in GY waters.

~~3.4 Correlations between N_2 fixation and hydrological, biogeochemical and biological parameters~~

N_2 fixation rates were significantly positively correlated with seawater temperature and photosynthetically active radiation (PAR) ($p<0.05$) and significantly negatively correlated with depth and salinity ($p<0.05$) and not significantly correlated with dissolved oxygen concentrations ($p>0.05$) (Table 2). ~~Regarding the main biogeochemical stocks and fluxes measured during the cruise,~~ N_2 fixation rates were significantly positively correlated with dissolved Fe, dissolved organic N (DON), phosphorus (DOP), ~~dissolved iron~~ and carbon (DOC), particulate organic N (PON), particulate organic carbon (POC), biogenic silica (BSi), ~~and~~ Chl *a* concentrations ~~($p<0.05$)~~, primary and bacterial production ($p<0.05$), and significantly negatively correlated with concentration of dissolved nutrients: NO_3^-, NH_4^+, DIP and silicate ~~concentrations~~ ($p<0.05$).

~~In terms of diazotrophic groups based on the quantification of *nifH* genes by qPCR,~~ N_2 fixation rates were significantly positively correlated with *nifH* abundances for *Trichodesmium* spp., UCYN-B and the three symbionts of DDAs (het-1, het-2 ~~and het-3~~) ~~abundances~~ ($p<0.05$) and not significantly correlated with UCYN-A1 and UCYN-A2 abundances ($p>0.05$) (Table 2). Regarding non-diazotrophic plankton ~~determined by flow cytometry~~, N_2 fixation rates were significantly positively correlated with *Prochlorococcus* spp., *Synechococcus* spp., heterotrophic bacteria and protist~~s~~ abundances ($p<0.05$) and significantly negatively correlated with picoeukaryotes ($p<0.05$).

3.5 Contribution of *Trichodesmium* and UCYN-B to N_2 fixation and nitrogenase gene expression

At the three stations where cell specific N_2 fixation rates were estimated by nanoSIMS (SD2, SD6 and LDB), the most abundant diazotroph phylotype was *Trichodesmium* with 1.3 x 10^5, 3.3 x 10^5 and 1.2 x 10^5 cells L^{-1} respectively, followed by UCYN-B, which abundances were 2.0 x 10^4, 1.5 x10^5 and 3.8 x 10^2 *nifH* copies L^{-1} respectively. Het-1 and het-2 combined were one to two orders of magnitude lower, ranging from 1.0 to 9.9 x 10^3 *nifH* copies L^{-1} and UCYN-A1 were below detection at the three stations. In summary, *Trichodesmium* and UCYN-B accounted for 98.2 %, 99.8 % and 92.1 % of the total diazotroph community (based on the phylotypes targeted here) at SD2, SD6 and LDB, respectively (Table 3).

The $^{15}N/^{14}N$ ratio of individual cells/trichomes of UCYN-B and *Trichodesmium* were measured via nanoSIMS analyses and used to estimate single cell N_2 fixation rates. A summary of the enrichment values and cell-specific N_2

fixation is provided in Table 3. Individual trichomes exhibited significant ^{15}N enrichments (0.610 ± 0.269, 0.637 ± 0.355 and 0.981 ± 0.466 atom% at stations SD2, SD6 and LDB, respectively) compared with time zero samples (0.369 ± 0.002 atom%). UCYN-B were also significantly ^{15}N-enriched with 1.163 ± 0.531 atom% and 0.517 ± 0.237 atom% at SD2 and SD6, respectively (note that no UCYN-B could be sorted and analyzed by nanoSIMS at LDB as they accounted for only 0.3 % of the diazotroph community). Cell-specific N_2 fixation rates of *Trichodesmium* were 38.9 ± 8.1, 29.3 ± 5.4 and 123.8 ± 24.8 fmol N cell d^{-1} at SD2, SD6 and LDB. Cell-specific N_2 fixation of UCYN-B were 30.0 ± 6.4 and 6.1 ± 1.2 fmol N cell d^{-1} at SD1 and SD6. The contribution of *Trichodesmium* to bulk N_2 fixation was 83.8 %, 47.1 % and 52.9 % at stations SD2, SD6 and LDB, respectively. The contribution of UCYN-B was 10.1 %, 6.1 % at SD2 and SD6, respectively (Table 3).

The *in situ nifH* expression for all diazotroph groups targeted by qPCR was estimated using a TaqMAN quantitative reverse transcription PCR (RT-QPCR) (Table 4). The sampling and filtering time (17:00-21:00 h) was not optimal for quantifying the *nifH* gene expression for all diazotrophs, however it ~~is useful evaluation for~~provides useful information about which diazotrophs were potentially active during the experiment and compliments the nanoSIMS analysis which measures the *in situ* activity. Both *Trichodesmium* and UCYN-B dominated the biomass (Stenegren et al., 2018)–, as did their *nifH* gene expression at all three stations, especially SD2 and SD6. Of the two het-groups, het-1 had a higher *nifH* gene expression, which was consistent with its higher *nifH* abundance by ~~standard~~ DNA qPCR (Stenegren et al., 2018). UCYN-A1 was consistently below detection for the *nifH* gene expression and was also the least detected diazotroph by *nifH* qPCR (Stenegren et al., 2018).

4 Discussion

4.1 Methodological considerations: the importance of measuring the $^{15}N/^{14}N$ ratio of the N_2 pool

Our understanding of the marine N cycle relies on accurate ~~measurements~~ estimates of N fluxes to and from the ocean. ~~Two methods are routinely used by the scientific community to perform direct N_2 fixation measurements in marine systems: 1) the method developed by (Montoya et al., 1996), which consists of the addition of the $^{15}N_2$ tracer as a bubble in the incubation bottles (hereafter referred to as the 'bubble addition method') and the measurement of the $^{15}N/^{14}N$ ratio of PN before (time zero) and after incubation, 2) the method consisting of adding the $^{15}N_2$ as dissolved in a subset of seawater previously N_2 degassed (Mohr et al., 2010) (hereafter referred to as the '$^{15}N_2$ enriched seawater method'). The second method was developed because the first had been observed to potentially underestimate N_2 fixation rates (Großkopf et al., 2012; Mohr et al., 2010; Wilson et al., 2012) due to the incomplete (and gradually increasing during the incubation period) equilibration of the $^{15}N_2$ in the incubation bottles when injected as a bubble. This results in a lower $^{15}N/^{14}N$ ratio of the N_2 pool available for N_2 fixation (the term A_{N2} used in the Montoya et al. (1996) equation) as compared to the theoretical value calculated based on gas constants, and therefore potentially leads to underestimated rates in some studies (see references above), whereas other studies do not see any significant differences between both methods (Bonnet et al., 2016c; Shiozaki et al., 2015).~~ Here we decided to use the 'bubble addition method' to minimize potential trace metal and organic matter contaminations, which may have resulted in overestimating rates (Klawonn et al., 2015). Moreover, a recent extensive meta-analysis (13 studies, 368 observations) between bubble and enriched amendment experiments to measure $^{15}N_2$ rates reported that underestimation of N_2 fixation is negligible in experiments that last 12-24 h (e.g. error is ~0.2 %); hence our 24 h~~r~~ based experiments should

be within a small amount of error (Wannicke et al., 2018). However, we paid careful attention to accurately measure the term A_{N2} to avoid any potential underestimation and reveal that the way bottles are incubated (on-deck *versus* in situ) has a great influence of the A_{N2} value, and thus on N_2 fixation rates.

Our MIMS results ~~reveal~~ measured a significantly ($p<0.05$) lower ^{15}N enrichment of the N_2 pool (6.145 ± 0.798 atom%) when bottles were incubated in on-deck incubators compared to when bottles were incubated on the *in situ* mooring line (7.548 ± 0.557 atom%). This suggests that the $^{15}N_2$ dissolution is much more efficient when bottles are incubated in situ, probably due to the higher pressure in seawater at the depth of incubation (1.5 to 19 bars between 5 and 180 m) compared to the pressure in the on-deck incubators (1 bar). The seawater temperature checked regularly in the on-deck incubators was equivalent to ambient surface temperature~~SST~~ and likely did not explain the differences observed~~.~~. This result highlights the need to perform ~~systematic~~ routine MIMS measurements to use the most accurate A_{N2} value for rate calculations, independently of the $^{15}N_2$ approach used (gas or dissolved). In our study, the theoretical A_{N2} value based on gas constants calculations (Weiss, 1970) was ~8.2 atom%, so the deviation from this value is more important when bottles are incubated in on-deck incubators as compared to when they are incubated in situ. This suggests that the use of the bubble addition method without MIMS measurement potentially leads to higher underestimations when bottles are incubated in on-deck incubators, which is the case in the great majority of marine N_2 fixation studies published so far (Luo et al., 2012). 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.

Despite the A_{N2} value was differen~~t~~d according to the incubation mode, it did not change with the depth of incubation on the mooring line, indicating that a slightly higher pressure than atmospheric pressure (1.5 bar at 5 m depth) is enough to promote the $^{15}N_2$ dissolution. It also indicates that the slightly lower seawater temperature (22-24 °C) recorded at ~100-180 m where the deepest samples were incubated likely did not affect the solubilization of the $^{15}N_2$ gas. In our study, the vertical profiles performed at LD stations and incubated either on-deck in triplicate or in situ in triplicates reveal identical ($p>0.05$) N_2 fixation rates regardless of the incubation method used (Van Wambeke et al., Accepted). This indicates that in situ incubations and on-deck incubations that simulate~~s~~ appropriate light levels ~~under in situ simulated conditions (on-deck incubators)~~ are~~is~~ a valid methodology for $^{15}N_2$ fixation rate measurements on cruises during which in situ mooring lines cannot be deployed, as long as ~~systematic~~ routine measurements of the isotopic ratio of the N_2 pool is performed in incubation bottles.

4.2 Drivers of ~~What causes such high~~a ~~hot spot of~~ N_2 fixation rates in the WTSP?

N_2 fixation rates measured in MA waters (average 631 ± 286 µmol N m^{-2} d^{-1}) are three to four times higher than model predictions for this area (150-200 µmol N m^{-2} d^{-1}, Gruber (2016)). They are in the upper range of the ~~upper~~ higher category (100-1000 µmol N m^{-2} d^{-1}) of rates defined by Luo et al. (2012) in the ~~global~~ N_2 fixation MAREDAT database for the global ocean and thus ~~reveal~~ identify the WTSP as an area for high ~~'hot spot' of~~ N_2 fixation in the global ocean. Recent studies performed in the western part of the WTSP, i.e. in the Solomon, Bismarck (Berthelot et al., 2017; Bonnet et al., 2009; Bonnet et al., 2015) and Arafura (Messer et al., 2015; Montoya et al., 2004) Seas also reveal extremely high rates (>600 µmol N m^{-2} d^{-1}), indicating that this high N_2 fixation activity area ~~'hot spot' of N_2~~

~~fixation~~ extends geographically west-east from Australia to Tonga and north-south from the equator to 25-30 °S, covering a vast ocean area of ~13 x 10^6 km^2 , (i.e. ~20 % of the South Pacific Ocean area). However, the ~~reasons for such an ecological success of diazotrophs~~driver(s) for diazotrophy in this region ~~are~~ is still poorly ~~understood~~ resolved and raises the question of 'which factors influence the distribution and activity of N_2 fixation in the ocean?' In a global scale study conducted by ~~in 2014 at global scale,~~ Luo et al. (2014), which investigated the ~~statistical links~~ correlations between ~~the spatial variation of~~ N_2 fixation and ~~that of~~a variety of environmental parameters commonly accepted to control this process, they ~~: surface NO_3^- and DIP concentrations, the tracer P*, atmospheric deposition, sea surface temperature (SST), mixed layer depth, solar radiation in the mixing layer, wind speed and minimum oxygen concentration in the 0-500 m layer. They~~ concluded ~~that the best predictor to explain the spatial distribution of N_2 fixation in the ocean is~~ that SST (or surface solar radiation) was the best predictor to explain the spatial distribution of N_2 fixation in the surface ocean. Below we highlight the most plausible factors explaining such ~~this 'hot spot' of~~ high N_2 fixation rates in our study area~~: WTSP~~.

Seawater temperature~~ST~~. Seawater temperature~~SST~~ was unlikely the factor explaining the differences in N_2 fixation rates observed between MA and GY waters, as ~~SST~~ it was consistently high (≥28~~.7~~ °C in the surface mixed layer~~on average over the 0-50 m layer~~) and optimal for the growth and nitrogenase activity of most diazotrophs (Breitbarth et al., 2007; Fu et al., 2014) all along the cruise transect. This indicates that other factors such as nutrient availability may explain the distribution of N_2 fixation.

DIP availability. The ~4000 km transect was ~~clearly~~ divided into two main sub-regions: 1) the MA waters, harboring typical oligotrophic conditions with surface ~~(0-50 m)~~ NO_3^- and DIP concentrations close to detection limits (0.05 µmol L^{-1}), a nitracline located at 75-100 m, moderate surface ~~(0-50 m)~~ Chl *a* concentrations (~0.17 µg L^{-1}), a DCM located at ~80-100 m and very high N_2 fixation rates (631 ± 286 µmol N m^{-2} d^{-1} on average), and 2) the GY waters harboring ultra-oligotrophic conditions with undetectable NO_3^-, a deeper nitracline (115 m), and comparatively high DIP concentrations (0.15 µmol L^{-1}), very low Chl *a* concentrations (0.06 µg L^{-1}, DCM ~150 m) and low N_2 fixation rates (85 ± 79 µmol N m^{-2} d^{-1}).

In the NO_3^--depleted MA waters, ~~the~~ low DIP concentrations ~~close to the detection limit~~ are indicative of the consumption of DIP by the planktonic community, including diazotrophs. This is consistent with the negative correlation found between N_2 fixation and DIP turn-over time (T_{DIP}, the ratio between DIP concentrations and DIP uptake rates) ~~during the OUTPACE cruise~~ (Table 2~~), indicative~~) and suggests a ~~of~~ higher DIP limitation when N_2 fixation ~~increases~~ is high and consumes DIP. ~~On the opposite~~The high , DIP concentrations ~~are high~~ (>0.1 µmol L^{-1}) in GY surface waters compared to MA waters is , consistent with former studies that consider~~ing~~ the South Pacific Gyre as a High Phosphate, Low Chlorophyll ecosystem (Moutin et al., 2008), in which DIP accumulates in the absence of NO_3^- and low N_2 fixation activity. In the high Phosphate, Low Chlorophyll scenario, the community is , ~~which is suspected to be~~ limited by temperature and/or Fe availability (Bonnet et al., 2008; Moutin et al., 2008). ~~Moutin et al., (2005) have shown that seasonal variations in DIP availability control the growth and decline of *Trichodesmium* blooms in New Caledonian waters.~~ During the OUTPACE cruise, the DIP turn-over time T_{DIP} was~~ere~~ variable but close or below~~for~~ two days ~~variable~~ in MA waters (Moutin et al., in review, 2018), indicating a potential limitation by DIP at some stations. *Trichodesmium*, the most abundant and ~~one of the~~ major contributor to N_2 fixation during the cruise, is known to synthesize hydrolytic enzymes in order to ~~access~~acquire P from the ~~the~~ dissolved organic

phosphorus pool (DOP) (Sohm and Capone, 2006). Moreover, *Trichodesmium* spp. differs from the other major diazotroph UCYN-B enumerated on the cruise in the forms of organic P it can synthesize. It is thus likely that DOP species that favored *Trichodesmium* over UCYN-B played a role in maintaining high *Trichodesmium* biomass in MA waters. ~~For example during the occupation at station LDB, decreased to below detection and *Trichodesmium* dominated~~ It has to be noted that average DIP turnover times~~concentrations~~ in MA waters were ~~but~~ always much higher than those typically measured in severely DIP-limited environments such as the Mediterranean and the Sargasso Seas (e.g. (Moutin et al., 2008)), suggesting that DIP concentrations are generally favorable for the development of certain diazotrophs in the WTSP, and do not alone explain why N_2 fixation is high in MA waters and low in GY waters. However, it is likely that the depletion of DIP stocks at the end of the austral summer season forces the decline of diazotrophic blooms in the WTSP (Moutin et al., 2005), concomitantly with the decline of~~in~~ SST.

Fe availability. Before OUTPACE, our knowledge on Fe sources and concentrations in the WTSP was ~~patchy~~limited, especially in MA waters. During OUTPACE, Guieu et al. (Under review) reported high dissolved ~~D~~Fe concentrations in MA waters (range 0.2-66.2 nmol L^{-1}, 1.7 nmol L^{-1} on average over the photic layer), i.e significantly ($p<0.05$) higher than those reported in GY waters (range 0.2-0.6 nmol L^{-1}, 0.3 nM on average over the photic layer). The low dissolved~~D~~ Fe concentrations measured in the GY waters ~~are in accordance~~agree well with previous reports for the same region (Blain et al., 2008; Fitzsimmons et al., 2014). However, the high dissolved~~D~~ Fe concentrations measured in MA waters were previously undocumented, and reveal several maxima (> 50 nmol L^{-1}) between stations SD7 to SD11, ~~suggesting~~ indicative of intense fertilization processes taking place in this region. Guieu et al. (Under review) found that atmospheric deposition measured during the cruise in this region was too low to explain the observed dissolved ~~D~~Fe concentrations in the surface water column. ~~, and that the Fe inputs up to the euphotic layer is from a shallow (~500 m) hydrothermal source.~~

The seafloor of the WTSP hosts the Tonga-Kermadec subduction zone which stretches 2500 km from New Zealand to the Tonga archipelago. It has among the highest density of submarine volcanoes associated with hydrothermal vents recorded in the ocean (2.6 vents/100 km; Massoth et al. (2007)), which discharge large quantities of material into the water column, including biogeochemically relevant elements such as Fe and, manganese. ~~, etc. By use of modeling simulations,~~ Guieu et al., (Under review) used hydrological data recorded by Argo float in situ measurements, atlas data and simulations from a general ocean circulation model to argue that the high dissolved Fe concentrations may be sustained by a submarine source characterized by freshwater inputs. They ~~-~~hypothesize that such ~~shallow~~ Fe inputs~~sources~~ could spread throughout the WTSP through mesoscale activity and mainly predominant westward currents such as the South Equatorial Current, SEC (Figure 1) and thus may explain the high dissolved~~D~~ Fe concentrations in MA waters compared to the GY ones. In our study, dissolved ~~D~~Fe concentrations were significantly positively correlated with N_2 fixation~~, likely contributing~~ and help to ~~to~~ explain~~ing~~ the distribution of ~~contrasted~~ N_2 fixation rates ~~observed~~ measured across the OUTPACE transect. In addition, our measurements~~, observations and model predictions~~ are also ~~This is~~ in accordance with recent model simulations performed at the basin wide Pacific scale, which show that deep Fe sources controls the spatial distribution and the abundance of *Trichodesmium* in the WTSP (Dutheil et al., in review, 2018).

In summary, ~~Our~~ our hypothesis to explain the spatial distribution of N_2 fixation in MA waters ~~this region~~ is the following: when high DIP ~~rich~~ waters flow westward from the ETSP through the SEC and cross the South Pacific

Gyre, N_2-fixing organisms do not develop despite optimal SST (>25 °C), likely because GY waters are Fe-depleted (Bonnet et al., 2008; Moutin et al., 2008). When the high~~se~~ DIP, low DIN (dissolved inorganic N)~~-rich~~ waters from the gyre are advected west of ~~pass~~ the Tonga trench in Fe-rich and warm (>25°C) waters, all ~~the~~ environmental conditions are fulfilled ~~high DFe concentrations associated with SST >25°C altogether would provide ideal conditions~~ for diazotrophs to bloom extensively ~~and likely explain the 'hot spot' of N_2 fixation in the region~~. According to (Moutin et al., in review, 2018), the strong depth difference between the nitracline and the phosphacline in MA waters associated with winter mixing allows a seasonal replenishment of DIP, which creates an excess of P relative to N and thus also favors N_2 fixation in this region. Further investigations are required to better quantify Fe input from shallow volcanoes and associated hydrothermal activity along the Tonga volcanic arc for the upper mixed layer, study the fate of hydrothermal plumes in the water column at the local and regional scales, and investigate the potential impact of such hydrothermal inputs on diazotrophic communities at the scale of the whole WTSP.

~~Besides DFe,~~ N_2 fixation rates were significantly negatively correlated with NO_3^- concentrations, consistent with the high energetic cost of N_2 fixation compared to NO_3^- assimilation (Bock et al., in review, 2018). They were also negatively correlated with depth and logically positively correlated with ~~, which likely explains the significantly positive correlations between N_2 fixation and~~ PAR and ~~N_2 fixation and~~ seawater temperature, ~~those~~ two parameters which are ~~being also~~ depth dependent ~~(the thermocline was roughly located around 50 m)~~. Most N_2 fixation took place in the surface mixed layer and rates were ~15 nmol N L^{-1} d^{-1} in MA waters with local maxima at stations SD1, SD6 and LDB and local minima at SD8 and SD10. *Trichodesmium*, the most abundant diazotroph enumerated ~~organism in~~ at those stations.~~waters~~ ~~*Trichodesmium* spp.~~ (Stenegren et al., 2018). ~~This organism is~~are buoyant~~, often form surface slicks as was observed in the MA waters (Stenegren et al. 2018)~~, and horizontal advection is well known to result in ~~heterogeneous~~patchy distributions of *Trichodesmium* in the surface ocean, with huge surface accumulations named slicks, as observed during OUTPACE (Stenegren et al., 2018), surrounded by areas with lower accumulations (Dandonneau et al., 2003). These physical processes may explain the differences between stations rather than local enrichments of nutrients due to islands as those three stations where the highest rates were measured are not located close to islands. However, the huge surface bloom observed at LDB (Figure 1) and extensively studied by (de Verneil et al., 2017) was mainly sustained by N_2 fixation (secondary fueling picoplankton and diatoms~~),~~ (Caffin et al., in review, 2018)~~)~~, rather than deep nutrient inputs (de Verneil et al., 2017). This bloom had been drifting eastwards for several months and initially originate from Fiji and Tonga archipelagoes (https://outpace.mio.univ-amu.fr/spip.php?article160), which may have provided sufficient Fe to alleviate limitation and triggered this exceptional diazotroph bloom as ~~previsouly~~previously proposed by Shiozaki et al. (2014).

~~N_2 fixation rates were the highest were NO_3^- concentrations were the lowest, and both were significantly negatively correlated, consistent with the high energetic cost of N_2 fixation compared to NO_3^- assimilation (Falkowski, 1983).~~

4.3 *Trichodesmium*: the major contributor to N_2 fixation in the WTSP

In ~~this 'hot spot' of N_2 fixation (~~MA waters~~)~~, the dominant diazotroph phylotypes quantified using *nifH* quantitative PCR assays were *Trichodesmium* spp. and UCYN-B (Stenegren et al., 2018), which commonly peaked at >10^6 *nifH*

copies L^{-1} in surface (0-50 m) waters. DDAs (mainly het-1, but het-2 and het-3 were also detected) were the next most abundant diazotrophs (Stenegren et al., 2018). This result is consistent with the fact that abundances of those ~~four~~ phylotypes co-varied and were significantly positively correlated with N_2 fixation rates (Table 2). The two UCYN-A lineages (UCYN-A1 and UCYN-A2) were less abundant (<1.0-1.5 % of total *nifH* copies, Stenegren et al., 2017) and not significantly correlated with N_2 fixation rates (Table 2).

The relative contribution of different diazotroph phylotypes to bulk N_2 fixation has been largely investigated through bulk and size fractionation measurements (usually comparing > and <10 µm size fraction N_2 fixation rates), which may be misleading since some small-size diazotrophs are attached to large-size particles (Benavides et al., 2016; Bonnet et al., 2009) or form colonies or symbioses with diatoms (e.g. UCYN-B, (Foster et al., 2011; Foster et al., 2013)) and some diazotrophic-derived N released by diazotrophs is assimilated by small and large non-diazotrophic plankton (e.g. Bonnet et al. (2016a)). Here we directly measured the *in situ* cell-specific N_2 fixation activity of the two dominating diazotrophs groups ~~dominating the community~~ in MA waters: *Trichodesmium* and UCYN-B.

At all three studied stations, *Trichodesmium* ~~was~~ dominated~~ing~~, accounting for 68.0-91.8 % of the diazotroph community, followed by UCYN-B, accounting for 0.3 to 31.7 %. ~~Likewise~~ In addition, *Trichodesmium* and UCYN-B had the highest measured gene expression (10^2-10^5 cDNA *nifH* copies L^{-1}). It was not surprising that UCYN-B had high gene expression given that the sampling time occurred later in the day (17-21:00), however both *Trichodesmium* and het-1 (which typically reduce N_2 and express *nifH* highest during the day; ~~(~~Church et al. 2005), had detectable and often equally high expression as UCYN-B. Cell-specific N_2 fixation rates reported here are in the same order of magnitude as those reported for field populations of *Trichodesmium* (Berthelot et al., 2016; Stenegren et al., 2018) and UCYN-B (Foster et al., 2013). *Trichodesmium* was always the major contributor to N_2 fixation, accounting for 47.1 to 83.8 % of bulk N_2 fixation, while UCYN-B never exceeded 6.1-10.1 %, despite accounting for >30 % of the diazotroph community at SD6. This may be linked with the lower ^{15}N enrichment at SD6 (0.517 ± 0.237 atom%), which is due to a high proportion of inactive cells (atom% close to natural abundance) compared to SD2, where the majority of cells were active and highly ^{15}N-enriched (1.163 ± 0.531 atom%). Such heterogeneity in N_2 fixation rates among UCYN-B like cells has already been reported by Foster et al., (2013). Overall, these results show that the most abundant phylotype (*Trichodesmium*) accounts for the majority of N_2 fixation, but not in the same proportion~~, highlighting~~, further indicating that the abundance of micro-organisms in seawater cannot be equated to activity, which has already been reported for other functional groups such as bacteria (de Boyer Montégut et al., 2004). In the North Pacific Gyre (Station ALOHA), Foster et al. (2013) report a higher contribution of UCYN-B to daily bulk N_2 fixation (24-63 %) during the summer season, indicating that this group likely contributes more to the N budget at station ALOHA than in the WTSP, where *Trichodesmium* seems to be the major player.

4.4 Ecological relevance of N_2 fixation in the WTSP and conclusions

N_2 fixation was significantly positively correlated with Chl *a*, PON, POC and BSi concentrations, as well as with primary production, suggesting a tight coupling between N_2 fixation, primary production and biomass accumulation in the water column. Based on our measured C:N ratios at each depth, the computation of the N demand derived from primary production measured during OUTPACE (Johnson et al., 2007) indicates that N_2 fixation fueled on average 8.2 ± 1.9 % (range 5.9 to 11.5 %) of total primary production in the WTSP. This contribution is higher than in other

oligotrophic regions such as the Northwestern Pacific (Shiozaki et al., 2013), ETSP (Raimbault and Garcia, 2008), Northeast Atlantic (Benavides et al., 2013b) or the Mediterranean Sea (Bonnet et al., 2011; Ridame et al., 2013), where it is generally <5 %. However, it is comparable to results found further North in the Solomon Sea (N_2 fixation fueled 9.4 % of primary production, Berthelot et al. (2017)), which is part of the WTSP 'hot spot' ~~of~~ for N_2 fixation (Bonnet et al., 2017). Van Wambeke et al. (Accepted) show that N_2 fixation represents the major source (>90 %) of new N to the upper photic (productive) layer during the OUTPACE cruise, before atmospheric inputs and nitrate diffusion across the thermocline, indicating that N_2 fixation supported nearly all new production in this region during austral summer conditions.

The large amount of N provided by N_2 fixation likely stimulated the growth of non-diazotrophic plankton as suggested by significant positive correlations between N_2 fixation rates and the abundance of *Prochlorococcus* spp., *Synechococcus* spp., heterotrophic bacteria and protists. $^{15}N_2$ based transfer experiments coupled with nanoSIMS experiments designed to trace the ~~passage~~ transfer of ^{15}N in the planktonic food web demonstrated that ~10 % of diazotroph-derived N is rapidly (24-48 h) transferred to non-diazotrophic phytoplankton (mainly diatoms and bacteria) in coastal waters of the WTSP (Bonnet et al., 2016a,b; Berthelot et al., 2016). The same experiments performed in offshore waters during the present cruise confirm that ~10 % of recently-fixed N_2 are also transferred to picophytoplankton and bacteria after 48 h (Massoth et al., 2007). This is in accordance with (Van Wambeke et al., Accepted) who report that N_2 fixation fuels 430 to 70 % of the bacteria N demand in MA waters. This further demonstrates that N_2 fixation acts as an efficient natural N fertilization in the WTSP, potentially fueling subsequent export of organic material below the photic layer. Van Wambeke et al. (Accepted) estimated that the *e*-ratio, which quantifies the efficiency of a system to export particulate carbon relative to primary production (*e*-ratio = POC export/PP), was three times higher ($p<0.05$) in MA waters compared to GY waters. Moreover, *e*-ratio values were as high as 9.7 % in MA waters, i.e. higher than the *e*-ratios in most studied oligotrophic regions (Karl et al., 2012; Raimbault and Garcia, 2008), where it rarely exceed 1 %, indicating that production sustained by N_2 fixation is efficiently exported in the WTSP. Diazotrophs were recovered in sediment traps during the cruise (Van Wambeke et al., Accepted), but their biomass only accounted for ~5 % (locally 30% at LDA) of the N biomass in the traps, indicating that most of the export was indirect, i.e. after transfer of diazotroph-derived N to the surrounding planktonic communities that were subsequently exported. A $\delta^{15}N$-budget performed during the OUTPACE cruise reveals that N_2 fixation supports exceptionally high (>50 % and locally >80 %) of export production in MA waters (Knapp et al., Accepted). Together these results suggest that N_2 fixation plays a critical role in export in this globally important ~~'hot spot' of~~ region for elevated N_2 fixation.

~~5 Conclusions~~

The magnitude and geographic ~~extent~~ distribution of N_2 fixation control the rate of primary productivity and vertical export of carbon in the oligotrophic ocean, thus accurate estimates of N_2 fixation are of primary importance for oceanographers to constrain and predict the evolution of marine biogeochemical carbon and N cycles. The number of ~~Global~~ N_2 fixation estimates have increased dramatically at the global scale over the past three decades (Luo et al., 2012). The results ~~of this study~~ reported here show that some poorly ~~explored~~ sampled areas such as the WTSP provide unique conditions for diazotrophs to fix at high rates and ~~may contribute to revise upward the~~ contribute to the need to

update current N_2 fixation estimates for the Pacific Ocean. Further studies would be required ~~along the annual time scale~~ to assess the seasonal variability of N_2 fixation in this region and perform accurate N budgets. Nonetheless, such high N_2 fixation rates question whether or not these high N inputs can balance the N losses in the ETSP. A recent study based on the N* (the excess of N relative to P) at the whole South Pacific scale (Fumenia et al., in review, 2018) reveals a strong positive N* anomaly (indicative of N_2 fixation) in the surface and thermocline waters of the WTSP, which potentially influences the geochemical signature of the thermocline waters further east in the South Pacific through the regional circulation. However, the WTSP is chronically undersampled, and a better description of the mesoscale and general circulation would be necessary to assess how N sources and sinks are coupled at the South Pacific scale.

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Table 1. $^{15}N/^{14}N$ ratio of suspended particulate nitrogen (average over the photic layer) across the OUTPACE transect.

Station #	$^{15}N/^{14}N$ ratio-PN_{susp} (‰)
MA waters	
1	2.00
2	0.78
3	0.57
A	-
4	2.71
5	1.57
6	1.91
7	0.5
8	-2.45
9	-2.21
10	-2.7
11	-7.05
12	1.89
B	-2.88
Average MA waters	*-0.41*
GY waters	
C	7.91
14	8.72
15	7.55
Average GY waters	*8.06*

Table 2. Summary of relationships between measured N_2 fixation rates and various physical and biogeochemical parameters. Also shown are correlations between measured rates and the ~~and among N_2 fixation rates and~~ several diazotrophic or non-diazotrophic planktonic groups enumerated at the respective stations. The corresponding unit is given for each parameter, and ~~indicated by~~ Spearman's rank correlation (n=102, α=0.05)~~.~~ ~~The sig~~are provided; significant correlations ($p<0.05$) are indicated by an asterisk (*).

	Variable	Unit	N_2 fixation Spearman's correlation coefficient
Physical and biogeochemical parameters	Pressure	dbar	-0.705*
	Temperature	°C	0.658*
	Salinity	psu	-0.701*
	Oxygen	µmol Kg^{-1}	0,151
	PAR	µmol photons m^{-2} s^{-1}	0.319*
	NO_3^-	µmol L^{-1}	-0.544*
	NH_4^+	µmol L^{-1}	-0,024
	DIP	µmol L^{-1}	-0.770*
	$Si(OH)_4$	µmol L^{-1}	-0.724*
	Dissolved Fe	nmol L^{-1}	0.398*
	DON	µmol L^{-1}	0.517*
	DOP	µmol L^{-1}	0.418*
	DOC	µmol L^{-1}	0.573*
	PON	µmol L^{-1}	0.721*
	POC	µmol L^{-1}	0.723*
	Biogenic silica	µmol L^{-1}	0.274*
	Chl *a*	µg L^{-1}	0.266*
	Primary production	µg C L^{-1} d^{-1}	0.657*
	Bacterial production	µmol C L^{-1} h^{-1}	0.692*
	T_{DIP}	days	-0.721*
	Trichodesmium sp.	*nifH* copies L^{-1}	0.729*
	UCYN-A1	*nifH* copies L^{-2}	-0,051

	UCYN-A2	*nifH* copies L^{-3}	-0,147
	UCYN-B	*nifH* copies L^{-4}	0.511*
	het-1	*nifH* copies L^{-5}	0.538*
Planktonic groups	het-2	*nifH* copies L^{-6}	0.576*
	het-3	*nifH* copies L^{-7}	0.276*
	Prochlorococcus sp.	cells ml^{-1}	0.697*
	Synechococcus sp.	cells ml^{-1}	0.720*
	Pico-eukaryotes	cells ml^{-1}	-0.450*
	Bacteria	cells ml^{-1}	0.780*
	Protists	cells ml^{-1}	0.680*

Table 3. Summary of diazotroph abundances and nanoSIMS analyses at SD2, SD6 and LDB.

Station #	*Trichodesmium* abundance (cells L^{-1})	Contribution to diazotroph community (%)	Atom% ^{15}N (mean ± SD)	N_2 fixation rate (fmol $cell^{-1}$ d^{-1})	Contribution to bulk N_2 fixation (%)
SD2	1.3×10^5	84.9	0.610 ± 0.269	38.9 ± 8.1	83.8
SD6	3.3×10^5	68.0	0.637 ± 0.355	29.3 ± 5.4	47.1
LDB	1.2×10^5	91.8	0.981 ± 0.466	123.8 ± 24.7	52.9

Station #	~~*Trichodesmium*~~ UCYN-B abundance (cells L^{-1})	Contribution to diazotroph community (%)	Atom% ^{15}N (mean ± SD)	N_2 fixation rate (fmol $cell^{-1}$ d^{-1})	Contribution to bulk N_2 fixation (%)
SD2	2.0×10^4	13.2	1.163 ± 0.531	30.0 ± 6.4	10.1
SD6	1.5×10^5	31.7	0.517 ± 0.237	6.1 ± 1.2	6.1
LDB	3.8×10^2	0.3	n.d	n.d	n.d

Table 4. Summary of *nifH* gene expression data determined by qRT-PCR at selected stations (SD2, SD6, LDB), where the cell-specific N_2 fixation rates were measured.

Diazotroph	Station SD2 cDNA *nifH* (gene copies L^{-1})	Station SD6 cDNA *nifH* (gene copies L^{-1})	Station LDB cDNA *nifH* (gene copies L^{-1})
Trichodesmium spp.	1.1×10^5	5.1×10^5	5.78×10^4
UCYN-B	1.9×10^5	1.5×10^5	1.03×10^2
Het-1	6.83×10^2	1.56×10^3	2.04×10^2
Het-2	5.44×10^2	2.14×10^2	bd
UCYN-A1	bd	bd	bd

Figure captions

Figure 1. Upper panel: ~~general situation of the~~Map of the western and central Pacific and associated Seas. Lower panel: Sampling locations superimposed on a composite sea surface Chl *a* concentrations during the OUTPACE cruise (February 19th- April 3rd, quasi-Lagrangian weighted mean Chl *a*). Short- duration (X) and long (+) duration stations are indicated. The satellite data are weighted in time by each pixel's distance from the ship's average daily position for the entire cruise. The white line shows the vessel route (data from the hull-mounted ADCP positioning system).

Figure 2. Horizontal and vertical distributions of (a) seawater temperature (°C), (b) chlorophyll fluorescence (µg L^{-1}), (c) NO_3^- (µmol L^{-1}), (d) DIP (µmol L^{-1}) and (e) N_2 fixation rates (nmol N L^{-1} d^{-1}) across the OUTPACE transect. LD stations are noted ~~reported as well as the~~and the extent of the two defined sub-regions MA: Melanesian archipelago waters, GY: South Pacific Gyre waters. Y axis: pressure (dbar), X axis: longitude, black dots correspond to sampling depths at the various SD and LD stations.

Figure 3. (a) The average measured $^{15}N/^{14}N$ ratio of the N_2 pool in the incubation bottles incubated either in on-deck incubators (n=54) and *in situ* (mooring line) (n=44). The dashed line represent the theoretical value (~8.2 atom%) calculated assuming complete isotopic equilibration between the gas bubble and the seawater based on gas constants. Error bars represent the standard deviation (b) Depth profiles of $^{15}N/^{14}N$ ratio of the N_2 pool in the incubation bottles incubated either in on-deck incubators (filled symbols) or on an *in situ* mooring line (open symbols).

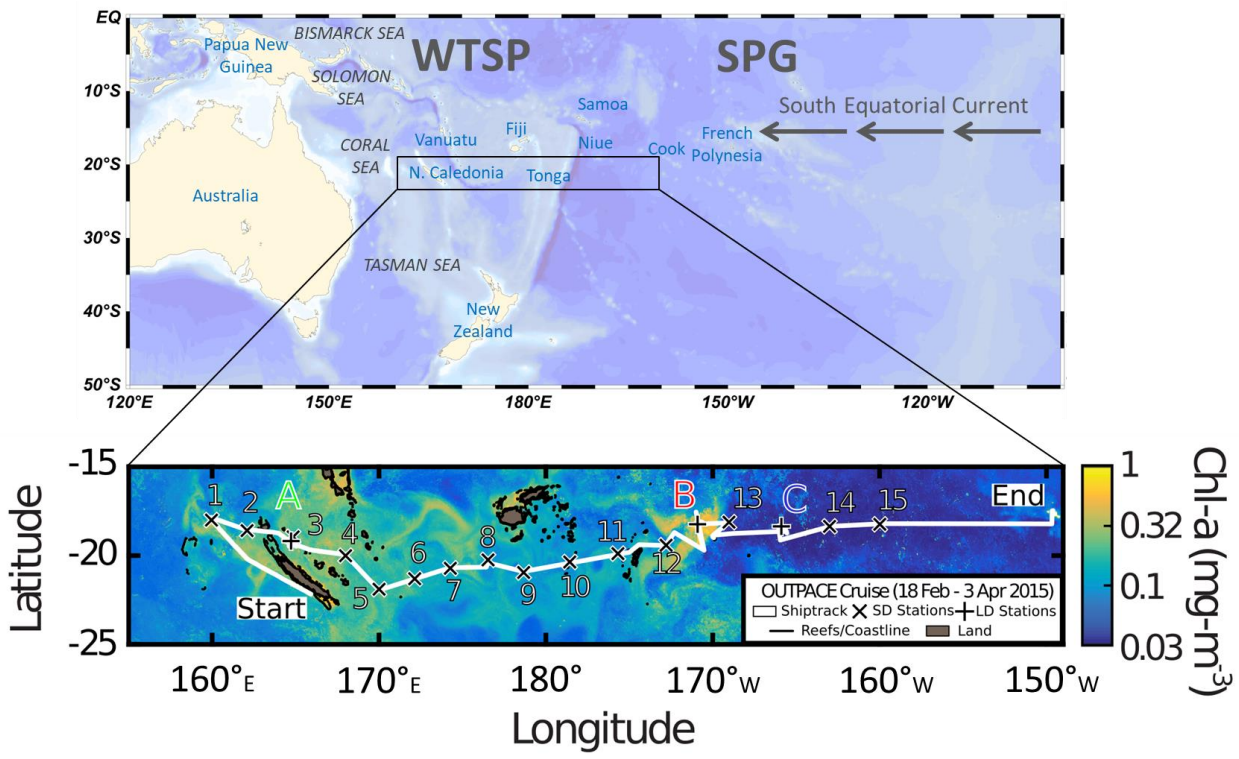

EQ
10°S
20°S
30°S
40°S
50°S
120°E
150°E
180°E
150°W
120°W
Papua New Guinea
BISMARCK SEA
SOLOMON SEA
CORAL SEA
Australia
TASMAN SEA
New Zealand
Vanuatu
Fiji
Samoa
Niue
Cook
French Polynesia
N. Caledonia
Tonga
WTSP
SPG
South Equatorial Current
-15
-20
-25
Latitude
160°E
170°E
180°
170°W
160°W
150°W
Longitude
Start
End
A
B
C
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
OUTPACE Cruise (18 Feb - 3 Apr 2015)
Shiptrack
SD Stations
LD Stations
Reefs/Coastline
Land
1
0.32
0.1
0.03
Chl-a (mg-m-3)

Figure 1.

1

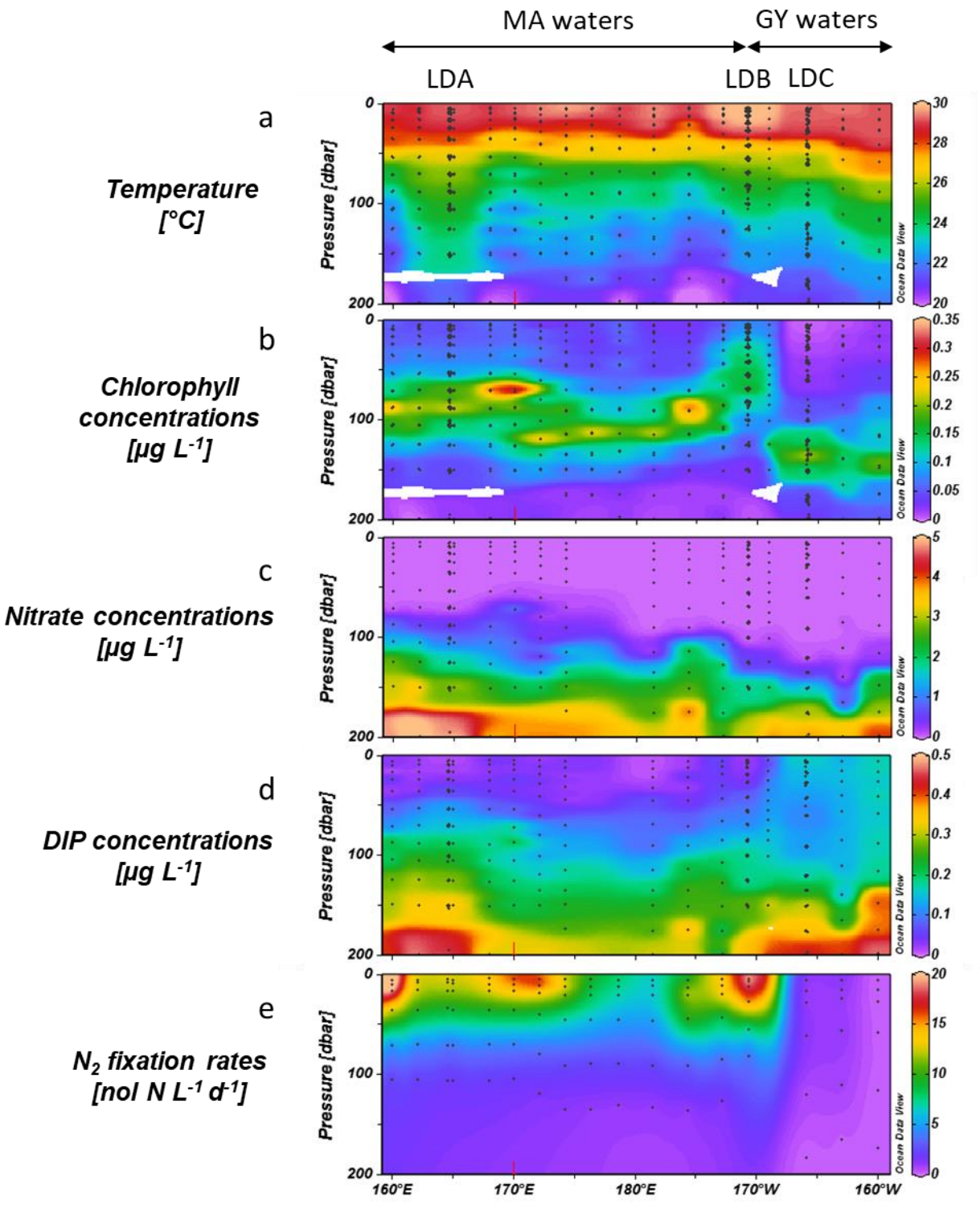
2

3

4

5

6


MA waters
GY waters
LDA
LDB
LDC
a
Temperature [°C]
b
Chlorophyll concentrations [µg L-1]
c
Nitrate concentrations [µg L-1]
d
DIP concentrations [µg L-1]
e
N2 fixation rates [nol N L-1 d-1]
Pressure [dbar]
0
100
200
Ocean Data View
30
28
26
24
22
20
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
5
4
3
2
1
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15
10
5
160°E
170°E
180°E
170°W
160°W

Figure 2.

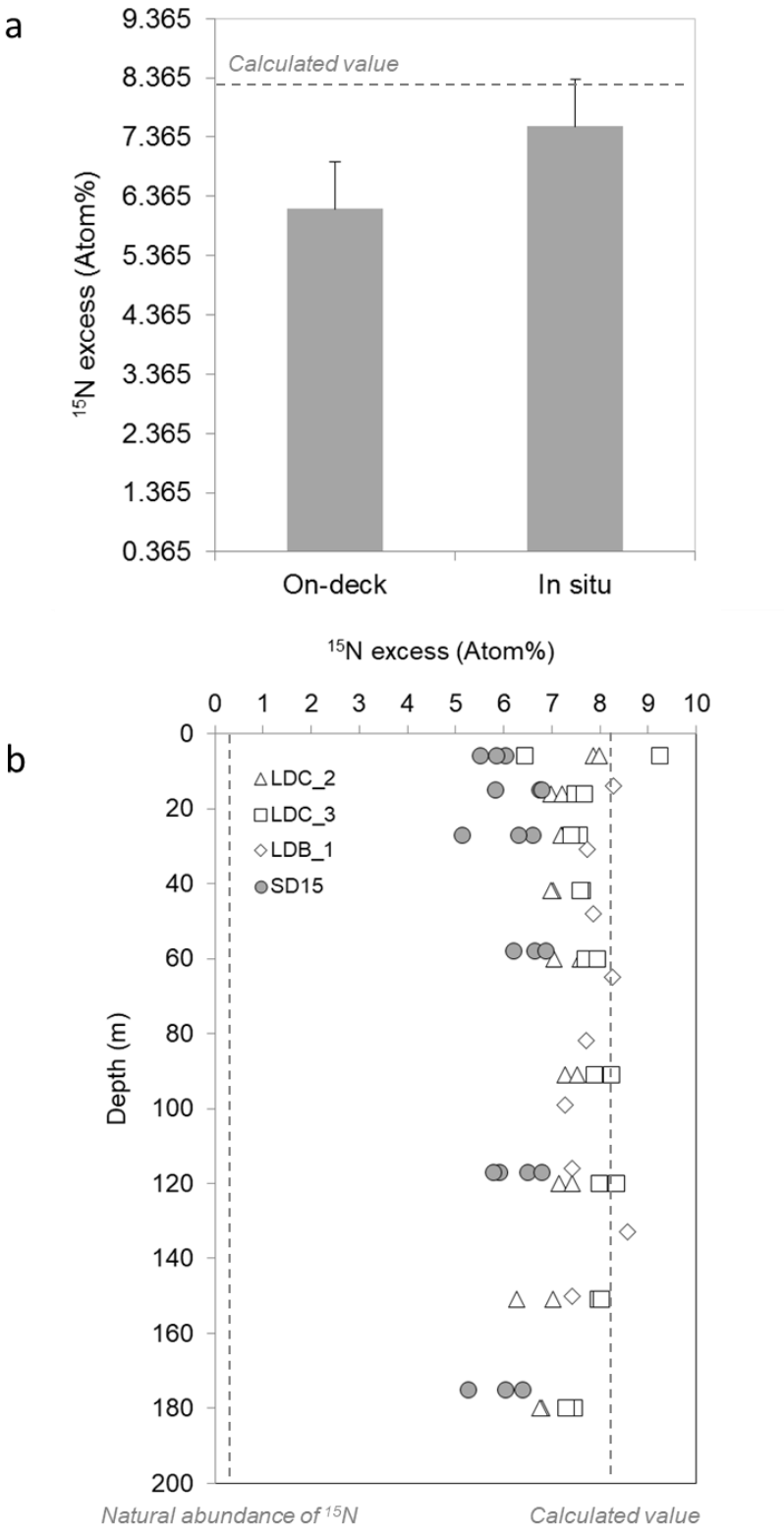
a
9.365
8.365
7.365
6.365
5.365
4.365
3.365
2.365
1.365
0.365
Calculated value
15N excess (Atom%)
On-deck
In situ
15N excess (Atom%)
0 1 2 3 4 5 6 7 8 9 10
b
0
20
40
60
80
100
120
140
160
180
200
Depth (m)
LDC_2
LDC_3
LDB_1
SD15
Natural abundance of 15N
Calculated value

Figure 3