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Interactive comment on "In depth characterization of diazotroph activity across the Western Tropical South Pacific hot spot of N$_2$ fixation" *by* Sophie Bonnet et al.

Anonymous Referee #4

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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. 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. 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. 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. 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. 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. Why no comparison with the extensive work done on N-fixation, fluxes and driving processes done at station ALOHA? An opportunity is missed.

Some specific comments: Page Line(s) Comment 1 2 What exactly do you mean by 'hot spot' – see above 3 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. 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. 4 11 Change "equalled to" to "reliably extrapolated to estimates of" 18 Change "previously undocumented" to "new"

22 What is the basis of 'selection' – suggest leave out 22 What do you mean by 'potential ecological impact' of N-fixation? Poor wording. 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'. 33 (etc.) By 'fluorescence', I presume you mean 'chlorophyll fluorescence' – say it because lots of other things fluoresce if you measure it right. 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)? 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. 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? 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. 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!). 6 20 What's the "them"? 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. 8 2 Strictly speaking, you're 'estimating' this, not determining it. 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! 23 By DCM, I presume you mean 'deep chlorophyll maximum'? 29-34 It's not clear what, if anything, this paragraph contributes to the paper. 37-38 This seems very wrong. I'm presuming you actually mean the per-mil deviation of the particulate matter ($\delta15N$) 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. 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? 18-23 Show 'em or ditch this. 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? 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. 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

28 Fig. 1 Bottom: longitude scale is very wrong. Would like to see some comparative profiles of measured variables in different regions 29 Fig. 2 ODV scales need to be properly annotated. Some hard contours would be very useful 30 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?

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