Biogeosciences Discuss., 11, C9139–C9144, 2015
www.biogeosciences-discuss.net/11/C9139/2015/

Biogeosciences Discussions
Open Access

Interactive comment on "Nitrogen fixation in the Southern Ocean: a case of study of the Fe-fertilized Kerguelen region (KEOPS II cruise)" *by* M. L. González et al.

Anonymous Referee #2

Received and published: 15 March 2015

General comments In this study, the authors report for the first time N2 fixation activity as well as diazotrophic diversity along a natural Fe gradient in the Southern Ocean during the KEOPS II cruise and from this point of view, this study could be of interest for the scientific community. They compared their measured rates and the community composition to environmental conditions and try to depict the functioning of this under-studied ecosystem. Unfortunately I have a lot of concerns about the veracity of the results for both activity and diversity and I think these issues need to be addressed before a possible publication in BG (see my specific comments below). In general, this manuscript needs to be clarified (misspellings, station names and position, methods. . .), the result section is very difficult to follow, the conclusions should be supported by the results

Full Screen / Esc
Printer-friendly Version
Interactive Discussion
Discussion Paper

and not so much on speculations based on other studies and the figures are often too small and unclear.

Abstract - slide 17153, line 8. For the calculation of the N*, why didn't you use a constant? (+2.9, Gruber and Sarmiento, 1997, GBC) - slide 17153, lines 12 and 15. Redundancy: "Molecular analysis showed a diazotrophic community dominated by heterotrophic bacterioplankton." and "diazotrophic community dominated by heterotrophic bacterioplankton" - slide 17153, lines 17 to 20. The last sentence is speculative and based on unrelated studies. In the present state of the study, this sentence should be removed. However, if you can provide statistical analysis showing the possible role of DFe for N2 fixation and regenerated primary production, it could support the first statement (lines 17-19) and, if they are available, dissolved organic matter concentrations (DOC, DON or DOP) and bacterial production should also be included in your statistical analysis to support your second statement (lines 19-20). - Keywords: replace "dissolve" with "dissolved".

Introduction - slide 17154, lines 1-4. "the N cycle in the current ocean seems to be out of balance." You forgot to say that most of the budgets and models have used stable isotopes incubations, which are for N losses "potential rates" (so, often overestimated rates) and for N2 fixation, underestimated rates as most of the incubations for the last decades have been done with "the bubble method", which can underestimate the rates from 2- to 6-fold (Mohr et al., 2010, PlosONE; Grosskopf et al., 2012, Nature; Wilson et al., 2012, Applied and Environmental Microbiology). - slide 17154, line 14. Rephrase "the diversity of diazotrophs is increasingly important and it currently includes groups within alpha and gamma-proteobacteria and archaea", I would suggest "the diversity of diazotrophs is important and includes different groups within Cyanobacteria, alpha and gamma-proteobacteria and archaea, and the discovery of new diazotrophs is still increasing". - slide 17154, line 21. Replace "Cianobacteria" with "Cyanobacteria". - slide 17154, line 25. After "oxygenic photosynthesis during the day", add "such as Crocosphaera watsonii" or "UCYN-B and UCYN-C" and spell what UCYN means. -

slide 17155, line 4. Add references. - slide 17155, lines 19-22. I don't understand this sentence, please rephrase.

Materials and Methods Section 2.1, - slide 17156. Here all the stations/transects mentioned later in the manuscript are not described and I would suggest to add all of them on the map (Figure 1) to make it clearer. I would also suggest to precisely say in the legend of this figure where the plateau is and add a line to represent the polar front. Section 2.2 - slide 17156, line 16. "Experiments were done at all process stations and at the pseudo-Lagrangian stations "E"." is repeated again on slide 17157, line 6. - slide 17157, line 8. "Water samples (prefiltered by 25 μm)". Why did you pre-filter your seawater on 25 μm? Especially you knew that diatoms are very abundant in this area (you mentioned a bloom in your Results section, slide 17160, line 22), and if there were present, you have possibly missed part of the activity of the Diazotroph-Diatom associations, which can be very important. Could you please comment about it. - slide 17157, line 9. In addition to the pre-filtration, you used the bubble method to do your measurements, which can again underestimate your rates. Your cruise took place 1.5 years after the Mohr's paper, why did not you use the 15N2 dissolved method? Did you at least check your 15N2 enrichment at the different time points of your incubations? You don't even mention the possible underestimation of your rates until your conclusion and I think you should mention it before. Finally, you don't say anything about the company which have produced your 15N2. As you might know, some batches of 15N2 are not "clean" and are contaminated with 15N-NH4+ and 15N-NO3- (Dabundo et al., 2014, PlosONE) and could explain (1) why you have such high rates and (2) why you did detect "N2 fixation" (which could actually be NH4+ or NO3- uptakes) at every depths/stations. Could you please provide the name of the company and the batch number of your 15N2 bottles, and if they are part of the possible contaminated batches, could you please check the contamination, because at the moment, I am very suspicious about your results. - slide 17157, line 16. "precombusted glass fiber filters (GF/F) of 0.7 μm pore (450°C for 12 h)" already said line 4. - slide 17157, line 18. "filters were kept at 60°C until laboratory analysis". Usually filters are kept dry but

BGD

11, C9139–C9144, 2015

not at 60°C for so long. How did you ship/send them back to Europe? Do you have any references for this protocol? Section 2.3 - slide 17157, line 27. "150 μL of 1 % gluteraldehyde". Usually 1% is the final concentration for the fixation, do you have any references for the fixation you used at $\sim$0.1% gluteraldehyde? Section 2.4 I have again issues with your sampling strategy and the process of the data for the molecular analysis. - slide 17158. First, if your rates are real, you should have been able to amplify DNA at more stations, even with only 1L. In oligotrophic area, where the organisms are also rare and often less active, 1-2L are enough. Do you have any ideas of why it failed? It is usual to detect diazotrophs even when there is no activity, but the opposite is very surprising for me. Why the size fractionation is not the same as for the rates? At least you would have something comparable. From which size fraction your positive results are coming? The paper you cited line 12 (Beier et al., 2014) is now published in Environmental Microbiology Reports (2015). It is not very easy to access and the details about the DNA extraction are in the Supplementary informations, I would suggest to add few sentences about the protocol in the present manuscript. - slide 17159, line 8. What kind of technique have been used for the sequencing? (454, Illumina, other ?), add a sentence. - slide 17159, line 10. How did the software determine low quality sequences? Please add a threshold value. If you only have one positive sample where you have been able to detect diazotrophs (at one depth of one station) and knowing that this region is highly variable, how can you state that this unique sample can be representative of the whole area?

Results Section 3.1 - slides 17159 and 17160. Some stations are new and not mentioned before. Please add them on the map (Figure 1) and describe then in the materials and methods section. - slide 17160. Define Ze as the depth of the euphotic zone line 9. Section 3.2 - slide 17160, line 19. TNS is not define and add this transect on the map. - slide 17160, line 24. Define precisely N*, the values you used and what it means in the materials and methods section. - slide 17161, lines 21-22."In general, a phytoplankton bloom with fluctuating biomass in time and space was developed at all stations". This sentence is very vague, please rephrase more specifically. - slide

BGD
11, C9139–C9144, 2015

17161, line 24. “surface fluorescence values >2 μg L-1”. Do the authors mean fluorescence of chlorophyll a ? If yes, then the fluorescence is a proxy of the biomass and it should be mentioned in the manuscript and precisions should be done on the title of this section. Furthermore the bacterioplanktion should not be in a section called “nutrients and fluorescence distribution”. - slide 17162, line 6. Replace “picoeukariotes and nanoeukariotes” by picoeukaryotes and nanoeukaryotes. Section 3.3 Why do the author talk again about bacterial abundances and nutrients in this section about N2 fixation? Please rewrite this section. As mentioned before, the statistical tools should be described and the authors should add the results here. I would rather suggest a RDA instead of a PCA. It would help you to determine the possible relative “contribution” of each variables (DFe, DOM, primary production, bacterial production) on your N2 fixation rates and support the hypothesis in your discussion. -slide 17164, line 8. “total community” is not true if you have pre-filtered on 25 μm. -slide 17165, line 8. “heterotrophic bacteria”. Are they all heterotrophs or do you mean non-cyanobacterials?

Discussion As mentioned before I have difficulties to trust the results as the rates might not be true and as the molecular data are weak to draw any conclusions. After validation of the rates, I would suggest to discuss more the geographical repartition of the results in regard to Fe and other nutrients availability as it is one of the main focus for the KEOPS II project (statistical analysis should be shown and better than a PCA, I would rather suggest a RDA “redundancy analysis”), it would be interesting to try to do a budget of N (and compared it to the results of the Table 2) in this area and compared N2 fixation to any other sources of external N in this area. Finally I would be curious to know how much of this N2 fixation is performed during the day compared to during the night, and how much is performed in the euphotic zone compared to the aphotic zone. The proposed discussion could be sustained by the results available in this study, but at the moment a lot of uncertainties (rates, diversity) and speculations (DOM driven N2 fixation), and the utilization of other studies are too present. - slide 17168, line 16. “Moreover the Fe limitation for diazotrophy is known to control diazotrophic cyanobacteria such as Trichodesmium (Bonnet et al., 2009), which were not detected in this

BGD
11, C9139–C9144, 2015

study". I would remove this sentence, which is not appropriate here for this discussion. - slide 17169, line 10. "Ammonium by diazotrophy could support 20 % of ammonium oxidation". Did you measure the NH4+ production from N2 fixation? These results would be interested to show.

Tables and figures -Table 1. Defined MLD and PZ in the legend. Ze have been used along the manuscript instead of PZ, please check and be consistent. It is not clear what those rates mean. Are they areal rates? Then the units are wrong. Or are they averages? Then it needs to be mentioned in the legend, with the number of depths, the deepest depth and the number of measurements per depth.

-Table 2. Please add your integrative areal rates here.

-Figure 1. Please add dots for all the stations mentioned in the manuscript, lines for the transects and a line for the Polar Front.

-Figure 2 and 3. The subfigures are very small and difficult to read. You should add over a) and c) TNS and TWE respectively. For Figure 3, check fluorescence and its unit, the titles on the graphs are not the same as in the legend.

-Figure 4. Too small again and check fluorescence.

-Figure 6. Add explanation about the day and the night time points.

-Figure 7. Your sequences in the tree are not clear. Change OUT to OTU and check misspellings.

Interactive comment on Biogeosciences Discuss., 11, 17151, 2014.