

# **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.**

### **Response to Anonymous Referee #1**

We acknowledge the comments of referee #1 and value the suggestions he made. They largely improved the manuscript. We improved our methodological section adding a section describing our PCA analysis and mass spectrometry calibration. We revised our figures and extensively worked on the text. Responses to all specific comments are reported as supplementary comments.

Introduction - Slide 17154, Line 14. Check expression. “the diversity of diazotrophs is increasingly important”. The statement of an actual increase in diversity should be supported by appropriate references. Didn’t the authors actually mean that the awareness (or discovery) of diazotrophs diversity is increasing or moving forward?  
A: The text was modified according to the referee’s suggestion.

- Slide 17154, Line 20. As is, this statement is erroneous: replace “diazotrophicorganisms”with filamentous strains.

A: The text was modified according to the referee’s suggestion.

- Slide17154, Line 24. Likewise, this statement is lacking precision. Temporal separation of N<sub>2</sub> fixation and photosynthesis evolved in unicellular strains. Consider reformulating the paragraph dealing with strategies (lines 17-27) to attribute each adaptation strategy to the appropriate type of diazotroph.  
A: The text was modified according to the referee’s suggestion.

-Slide 17155 The sentence lines 3-4 requires a citation.

A: A citation was added to the text

- Slide 17155, Lines 15-16“The Kerguelen area in particular has a deep reservoir of Fe coming from sedimentcontribution”. Did authors mean that the Kerguelen sediments constitute a deep Fereservoir (remove “contribution”)? A statement on the process allowing for the upwardtransfer of iron from deeper layers would be relevant here.  
A: The paragraph was rephrased.

- Slide 17155, Lines 18-19 “than artificial Fe-fertilization with respect to atmospheric CO<sub>2</sub> sequestration bellow200m”. This sentence could be detailed some more.  
A: The paragraph was rephrased.

- Slide 17155, Lines 22-23. “as it is the occurrence of N<sub>2</sub> fixation in environments with micro molar concentrations of DIN and DIP”. I did not understand this part of the sentence. Is a verb missing?  
A: The sentence was rephrased.

- Slide17155, Line 24 “its regulation”: it is very unclear what “its” refers to.  
A: The text was modified according to the referee’s suggestion.

## Material and Methods

- Slide 3, title of paragraph 2.1. Replace “Sampling” with “Sampling stations”

A: The text was modified

- The description of the two transects is missing; does TNS strictly follow one longitude, and does TWE strictly follow one latitude? Please add the transect stations on to Fig 1.

A: Transects were defined and represented in Fig 1.

### Section 2.2.

- Slide 17156, Line 18 Replace “Physical-chemical” with “Physicochemical”

A: Replaced

- Slide 17157, Line 8 "Water samples (prefiltered by 25 \_m) were taken in 1 L Nalgene bottles". How many water samples were taken, and at which depth(s) at each station? Were the sampling depths the same from a station to the next? Or, since all incubations were performed on deck with light filters, were the sampling depths chosen so that the local light extinction at the depth of sampling would match that of the filters?

A: Details were added to this paragraph and the entire mat and methods section was reorganized to improve clarity.

- Slide 17157, Lines 11-12. At the time of the experiment, to which depths and temperatures (at each depth) did these attenuations correspond? Because all incubations were performed at surface temperature, some experimental biases in the fixation rates might be expected in bottles representative of deep samples. Also, were these depths within or below the mixed layer?

A: All depth were within the euphotic zone and were all within the MLD as well.

- Slide 17157, Lines 14-15 “Additionally, incubations were done for 2 size fractions (total and < 5 \_m) with an intermediate sampling time after 12 h of incubation.” This sentence is unclear. Were new incubations started (and in new bottles) 12h after the beginning of the first series of incubations? How did these times compare with the light:dark cycle?

A: Comparisons were not always possible since the time was not the same at the beginning and end of the incubations. Details were included in the text.

Section 2.3 Were nutrient concentration measured along vertical profiles and how many depths were sampled? Since  $N^*$  is plotted on Fig2, the way this parameter was estimated should be precisely described here. In particular, which N and P values were used to calculate the N:P ratio used in the estimation of  $N^*$ ?  $N^*$  also uses a constant: how was it calibrated in the present study?

A: Nutrient samples were analyzed at all stations and depths. Details are described in a companion paper in this special issue[*Blain et al., 2014*]. We added details to the text in the Mat and Met section.

Section 2.4 - Slide 17158, Lines 5-7 “In spite of an extended sampling effort (1 L water filtered at each process station and depth), amplification was only effective at station E-1 using large volume sampling filters.” Does this statement mean that amplification failed at all stations but E-1? Is there any putative explanation for this?

A: We filtered large volumes of water at 3 stations (F-L, E-1 and R-2) in order to improve our chances of amplifying *nifH*. However amplification was only successful at E-1. The DNA extract from F-L was lost during shipment for sequencing. The extract from R-2 could not amplify for *nifH*. In all other stations we filtered 1L of seawater and was clearly not enough for successful amplification. Another explanation for our unsuccessful amplification of *nifH* is the specificity of primers that might not be adapted for southern ocean diazotrophs. We tried to include these aspects in the revised text.

## Results

The result section contains some elements of discussion that could be removed, for a more concise and clearer data reporting.

A: We made an effort eliminate unnecessary discussion elements in the result section.

Section 3.1 - Slide 17159, 1st paragraph of section 3.1. The geographical location of the polar front should be provided (reference could be made to a published work, e.g. the recent Park et al., 2014 in JGR Oceans).

Please draw the front on Fig.1.

A: The polar front was drawn on Figure 1. We also added a citation.

- Slide 17160 Line 2, about stations located South of the polar front: replace "lower salinity (> 34)" with "higher salinity (> 34)".

A: Replaced

- Slide 17160, Lines 23 and 26-27. Stations TNS1, 2, TEW1, 2, 7, 8, and TNS3-10, TEW3-6 should be documented in the method section. A representation of these transects on Fig1 is needed.

A: Stations are now represented in Fig 1.

- Slide 17160, Line 10 "Photosynthetically Active Radiation (PAR) reaches 1 %": add "of the incident irradiance at the surface".

A: The text was added.

- Slide 17160, Line 13 is unclear. "On the contrary, Ze values": on the contrary to what? (i.e. what does this sentence oppose to?)

A: The sentence was rephrased.

- Slide 17160, Line 13 "Ze" is not defined.

A: Ze was defined in the text.

Section 3.2 - Slide 17160, Lines 16-17. The following first discusses N\* values, giving no information on the nutrient level to the reader, before presenting the absolute nutrient concentrations on Fig 4. Wouldn't it be more logical to discuss absolute concentrations and their vertical distribution first, and then infer on N\* distributions?

A: The text was reorganized.

- Slide 17160, Line 18. Although authors refer to two published papers when first using the term N\*, it would in addition be necessary to recall, in the methods section, how precisely this parameter was estimated in the present study (see comment above on section 2.3)

A: Details were included in the Mat and Method section.

- Slide 17160, Line 19. Why did authors chose to express N\* in \_mol/L instead of \_mol/kg (as done in the cited literature)? Could the former introduce biases when comparing N\* values in waters with different densities?

A: Our choice was based on the limited scale of our study as we mostly worked on the MLD (which could exceed 200m depth).

- Slide 17161, Lines27-28 "The highest fluorescence values (>4\_gL<sup>-1</sup>)". Why is fluorescence expressed in g/L (same comment below about Fig 3)? Do authors mean Chlorophyll concentration instead of fluorescence?

A: Fluorescence values reported is given by the CTD. These values were corrected for chlorophyll (already calibrated). We chose not to express values in mg/m<sup>3</sup>.

- Slide 17162, Line 6. Replace "picoeukariotesandnanoeukariotes" with "picoeukaryotes and nanoeukaryotes" (and check throughout the text).

A: Text was corrected.

- Slide 17162, Line 20 "rates at R-2 station increased (1.05 to 3.13 nmol N L<sup>-1</sup> d<sup>-1</sup>) under 1% light ". This sentence is unclear.

A: The sentence was rephrased

- Slide 17162, Lines 21 to 25 ("Bacterial abundance and fluorescence for R-2 [...] subsurface peak (Fig. 4b and f). "). This sentence is about bacterial abundance and nutrients; why does it appear in the results section related to N<sub>2</sub> fixation rates?

A: The entire section was rewritten for clarity.

- Slide 17163, Lines 5 to 12: same remark.

A: The entire section was rewritten for clarity.

- Slide 17163, Line 23. Imprecise statement. "A multivariate Principal Components Analysis(PCA) indicated..." What PCA, and on which variables was it performed? Was it run in the present study or are authors referring to a published analysis in the literature? If this statistical analysis was carried out on the present data, then it should be detailed in the manuscript.

A: The PCA was run in the present study (see figure S1 below, not included in the text). Details were included in the Mat and Methods section and the result section.

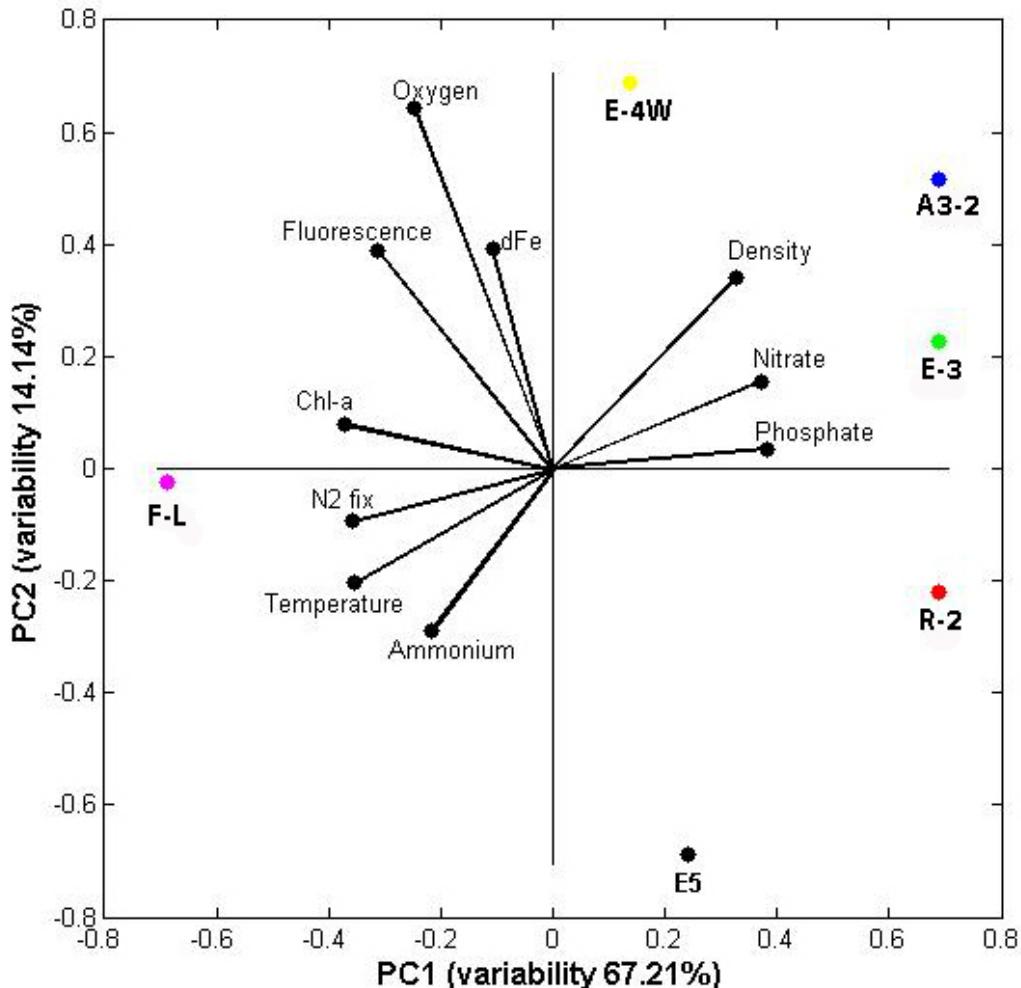


Figure S1: PCA performed with data obtained during the KEOPS2 study.

- Slide 17164, Line 2 "PC1" is not defined.  
 A: PC1 was defined in the text. We also added an additional section on our materials and methods to explain the PCA analysis.

- Slide 17164, Lines 3-4 "Therefore N<sub>2</sub> fixation seems overall correlated with Chlorophyll a, dFe, NH<sub>4</sub><sup>+</sup> and temperature".  
 Isn't this a discussion already?

A: We moved the sentence to the discussion section

- Slide 17164, last sentence is a discussion of results.  
 A: We moved the sentence to the discussion section.

Section 4 Discussion - Slide 17165, Line 15 "This study provides the first evidence of high N<sub>2</sub> fixation rates for the Southern Ocean". I am missing a discussion of this statement. Are these rates high compared to other oceans, or because they sustain an important fraction of primary production? If primary production was assessed during the cruise, what fraction of it was supported by nitrogen fixation? I was expecting here an argued comparison of the rates measured in this study with what is currently reported in the literature for other oceanic regions.

A: The discussion was rewritten.

- Slide 17165, Lines 21-22. Asis, this statement does not seem correct to me. It is already known that N2 fixation is not excluded from cold environments. See for instance Hiltbrunner et al 2014 (Oe-section 2.3)

A: The paragraph was rephrased.

- Slide 17162, Line 6. Replace "picoeukariotes and nanoeukariotes" with "picoeukaryotes and nanoeukaryotes" (and check throughout the text).

A: We corrected the text throughout the text

- Slide 17165, Lines 21-22. As is, this statement does not seem correct to me. It is already known that N2 fixation is not excluded from cold environments. See for instance Hiltbrunner et al 2014 (Oecologia), Lett and Anders 2014 (Plant and soil). Authors may want to discuss instead that given the fact that N2 fixation had already been observed in cold terrestrial environments, one could expect to also find active nitrogen fixers in cold oceanic waters – with the present results as a proof.

A: A statement was added to the text including a citation for terrestrial N2 fixation in the arctic.

- Slide 17165, Line 26 and Slide 17166, Line1, "The N2 fixation rates detected in the Southern Ocean, in general, were higher than in other HNLC and oligotrophic areas, (up to 5 times higher; Table 2)". If comparison is made here between the present measurements and reports from the literature for other areas, consider replacing the sentence with something like "The N2 fixation rates observed in the present study were higher than those already reported for other HNLC and oligotrophic areas".

A: The sentence was rephrased

- Slide 17166, Lines1-2. The reference to Table 2 and comparison of N2 fixation rates between different oceanic regions are found here. This is the core information in the manuscript but I found it hard to grasp. Table 2 presents integrated rates of N2 fixation but does not specify the depth over which these rates were assessed: are they all comparable? Whether these measurements were taken over the euphotic zone, the mixed layer or over a surface layer, differences in estimations

could arise that are not due to a difference in activity but a difference in the way calculations were performed, in particular if the depth ratios between the euphotic zone and mixing zone vary significantly between the considered regions. It seems important to include another column in table 2 to specify the integration depth and, if possible, the correspondence with the depth of the euphotic and mixed layers.

A: We included the depth of integration of all references.

- Slide 17166, Line1

"up to 5 times higher considering integrated values". This factor does not appear when comparing tables 1 and 2. Table 2 reports integrated activities while table 1 provides activities per unit of volume. Why aren't the integrated values presented in table 1? This statement is thus unclear to me; I am missing a more developed comparison and discussion of these results. In particular, the Methods section indicates that N2 fixation was assessed using 8 light levels (plus dark), while table 1 only provides 1 estimated rate per station.

A: The section was rephrased to improve clarity. Table 2 specifies the depth of integration for each study including our own. Table 1 provides average values over the MLD. The legend of Table 1 was also improved.

I thus assume the rates provided on table 1 are the average values deduced from the 8 light incubations from each profile? Or was the dark incubation used also in the average estimate?

A: The dark incubation was not included in the average value.

Did nitrogen fixation mostly occur during the light period or the dark period? The failed amplifications make it impossible to relate the observed activities to any phylotype of diazotroph. But the known physiology of unicellular diazotrophs would already be helpful to bracket a few potential groups.

A: We agree with the referee. Unfortunately there was not a clear trend in day and night time N<sub>2</sub> fixation.

- Slide 17166, Line 2. How was the “average integrated rate [: :] for the study area” calculated? Does this number include all stations, whether South or North of the polar front (and if not, then why so?)?

A: All stations were included. What is reported is the average of all integrated rates. We rephrased the sentence.

- Slide 17166, Lines 4-7. Why “indeed”? Did authors mean “already”? I do not understand how this sentence relates to the previous one. -

A: The sentence was rephrased

Slide 17166, Line 7. Vague sentence. What field experiments? This sentence either requires references to the literature or an argued discussion supported by data from the present study.

A: The sentence was deleted as it did not provide valuable arguments for our discussion.

- Slide 17166, Lines 17-19. What would a graph showing depth profiles of N<sub>2</sub> fixation as a function of iron look like? (with X= Fe concentration, Y = depth and N<sub>2</sub> fixation rates as a color gradient). Such presentation of results might reveal the correlation or non-correlation between iron and N<sub>2</sub> fixation across the study area.

A: We agree. Unfortunately dFe data is not available for all depths and stations where our N<sub>2</sub> fixation experiments were carried out. Such representation of results is not possible in this case. A companion paper in the KEOPS special issue reports dFe data. We cited that work.

- Slide 17166, Lines 20-21. The present statement is not supported by data. I am missing a description of the way this statistical analysis was performed (in the methods section) and a presentation of results. I also did not understand how N<sub>2</sub> fixation could be regulated by primary productivity.

A: We modified our statement in order to improve clarity.

- Slide 17166, Line 28 to Slide 17167, Line 1. Determination of N\* is particularly appropriate to infer whether the considered area is rather a source or sink of nitrogen, i.e. if N<sub>2</sub> fixation is prevalent or not relatively to denitrification. It seems to me that it would be relevant to compare the N\* values deduced in the present study to other oceanic regions to support the discussion of the potential role of N<sub>2</sub> fixation in this area of the Southern Ocean: how do the highest N\* measured in the present study compare with e.g. those reported by Gruber and Sarmiento (1997)?

A: A comment was included in the text.

- Slide 17169, Lines 4-5 “Considering an average NO<sub>3</sub> uptake rate

of 0.94 \_mol/L". I did not understand where this rate comes from. Please explain. –  
A: This values was obtained from  $^{15}\text{NO}_3$  uptake experiments performed during the KEOPS2 cruise and reported in a companion paper in the KEOPS2 special volume. This was detailed in the revised manuscript.

Slide 17169, Lines 6-7 "N2 fixation could add an additional 0.5%". Please explain how this value was calculated

A: This was calculated using the estimates of new production reported in Cavagna et al 2014.

- Slide 17169, Lines 7-8 "Moreover, N2 fixation is likely to co-occur with nitrification" Please argue, this statement needs some supporting information. Couldn't there be a lag time between N2 fixation and remineralization?

A: Data from the KEOPS cruise suggest high rates of nitrification in the MLD. Moreover, nitrification has been proven to account for about half of bioavailable nitrate in the euphotic zone of open ocean waters (Yool et al., 2007).

- Slide 17169, Lines 18-20. I don't think the second part of this sentence is a proof for the statement presented at its beginning.

A: The sentence was rephrased.

- Figure 1: A description of the background color is missing in the legend, as well as a color bar on the figure. If there is only one black dot, please remove the "s" in "Black dots". A representation of transects, whose data are shown in Fig 2, would be really needed on Figure 1. Given its complex shape, the Polar front should also be located on Fig. 1, for a better comprehension of the local, prevalent conditions at each station.  
A: Figure 1 was redrawn and currently includes the polar front and transect stations

- Figure 2: TWE seems to cross Station E-2, which is not mentioned in the Material and Methods. Where is this station E-2?

A: E-2 was sampled as the second visit to lagrangian station E. It appears in figure 1.

- Figure 3. If this figure shows different parameters observed at "E" stations, why aren't the stations located at the top of the graph, instead of (or in addition to) the TNS stations? Graphs titles do not agree with the legend. Also, the fluorescence unit (\_g/L) doesn't seem correct. If graph b depicts N\*, why is the color bar scaled between 100 and 900?  
A: Figure 3 describes only stations located in Transects. It does not describe parameters at station E. This was a mistake and was corrected in the revised version.

- Figure 4: replace "Mix Layer Depth" with "Mixed Layer Depth"

A: Replaced

- Table 1. "PZ" is not defined. How were the assimilation of NH4+ and NO3- estimated (to be detailed in the methods section)?

A: PZ was replaced by Ze and defined in the table legend.

NH4 and NO3 assimilation data is reported in a companion paper in the KEOPS2 special issue.

## References

Blain, S., J. Capparos, A. Guéneugues, I. Obernosterer, and L. Oriol (2014), Distributions and stoichiometry of dissolved nitrogen and phosphorus in the iron fertilized region near Kerguelen (Southern Ocean), *Biogeosciences Discussions*, 11(6), 9949-9977.