

Review of manuscript bgd-11-17151-2014

General comments

This work recounts diazotrophic activity in the iron-rich, Kerguelen area, measured during the Keops2 cruise. Authors evoke the co-occurrence of nitrogen fixation activity and environmental factors, such as light intensity and iron availability, as well as their statistical correlation, either observed in the present study (but the statistical analysis and its results are missing) or reported from the literature.

Although original, this work is presented and discussed with some lack of specificity and precision (a few suggestions are listed in the detailed comments). In particular, the description of N₂ fixation strategies and diazotrophs diversity is wrong, due to imprecision in the text. Figures are also incomplete and loose, making sections 3 and 4 quite tough to follow.

I believe the discussion requires clarification and reformulation. It presently considers stations one after the other, which generates redundancy and confusion, making it difficult to capture the thought process that authors wished to communicate. It also seems that the discussion largely refers to the literature as a support to the present assumptions, while some results from the present study may remain under-exploited. In particular, this work first brings forward the fact that N₂ fixation was assessed in an environment offering a gradient in Fe availability. Iron limitation is one hypothesis to explain why HNLC regions present such low biomass standing stocks. As announced in the introduction, I was expecting authors to develop this point in the discussion. There is a section dealing with Fe, but surprisingly, hypotheses are fed with reports from the literature, more than with author's results. I would suggest reorganizing the text to first synthesize the information provided by all figures (coming from all stations) into a more global picture, discussed in a structured discussion resolving around questions (and not around sampling stations).

Detailed comments

Nota: The comments below follow the reading order and refer to slides numbers as read on the printed version.

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?

- Slide 17154, Line 20. As is, this statement is erroneous: replace "diazotrophic organisms" with filamentous strains.

- Slide 17154, Line 24. Likewise, this statement is lacking precision. Temporal separation of N₂ 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.
- Slide 17155 The sentence lines 3-4 requires a citation.
- Slide 17155, Lines 15-16 “The Kerguelen area in particular has a deep reservoir of Fe coming from sediment contribution”. Did authors mean that the Kerguelen sediments constitute a deep Fe reservoir (remove “contribution”)? A statement on the process allowing for the upward transfer of iron from deeper layers would be relevant here.
- Slide 17155, Lines 18-19 “than artificial Fe-fertilization with respect to atmospheric CO₂ sequestration below 200m”. This sentence could be detailed some more.
- Slide 17155, Lines 22-23. “as it is the occurrence of N₂ fixation in environments with micro molar concentrations of DIN and DIP”. I did not understand this part of the sentence. Is a verb missing?
- Slide 17155, Line 24 “its regulation”: it is very unclear what “its” refers to.

Material and Methods

- Slide 3, title of paragraph 2.1. Replace “Sampling” with “Sampling stations”
- 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 onto Fig 1.

Section 2.2.

- Slide 17156, Line 18 Replace “Physical-chemical” with “Physico-chemical”
- Slide 17157, Line 8 "Water samples (prefiltered by 25 μm) 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? This part of the Methods is incomplete.
- 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?
- Slide 17157, Lines 14-15 “Additionally, incubations were done for 2 size fractions (total and < 5 μm) 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?

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?

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?

Results

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

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.

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

- 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.

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

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

- Slide 17160, Line 13 “Ze” is not defined.

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? .

- 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)

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

- Slide 17161, Lines 27-28 "The highest fluorescence values ($>4\mu\text{g/L}^{-1}$)". Why is fluorescence expressed in $\mu\text{g/L}$ (same comment below about Fig 3)? Do authors mean Chlorophyll concentration instead of fluorescence?

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

- Slide 17162, Line 20 "rates at R-2 station increased (1.05 to $3.13 \text{ nmol N L}^{-1} \text{ d}^{-1}$) under 1% light ". This sentence is unclear.

- 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 N₂ fixation rates?
- Slide 17163, Lines 5 to 12: same remark.
- 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.
- Slide 17164, Line 2 "PC1" is not defined.
- Slide 17164, Lines 3-4 "Therefore N₂ fixation seems overall correlated with Chlorophyll a, dFe, NH₄⁺ and temperature". Isn't this a discussion already?
- Slide 17164, last sentence is a discussion of results.

Section 4 Discussion

- Slide 17165, Line 15 "This study provides the first evidence of high N₂ 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.
- 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.
- 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".
- 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.

- Slide 17166, Line 1 “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. 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? Please add this information in the legend of table 1 and detail how calculations were made.
- Did nitrogen fixation mostly occur during the light period or the dark period? The failed amplifications make it impossible to related 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.
- 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)?
- Slide 17166, Lines 4-7. Why “indeed”? Did authors mean “already”? I do not understand how this sentence relates to the previous one.
- 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.
- Slide 17166, Lines 17-19. What would a graph showing depth profiles of N₂ fixation as a function of iron look like? (with X= Fe concentration, Y = depth and N₂ fixation rates as a color gradient). Such presentation of results might reveal the correlation or non-correlation between iron and N₂ fixation across the study area.
- 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 N₂ fixation could be regulated by primary productivity.
- 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 N₂ 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 N₂ 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)?
- Slide 17169, Lines 4-5 “Considering an average NO₃ uptake rate of 0.94 μmol/L”. I did not understand where this rate comes from. Please explain.
- Slide 17169, Lines 6-7 “N₂ fixation could add an additional 0.5%”. Please explain how this value was calculated
- 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?
- 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.

- 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.
- Figure 2: TWE seems to cross Station E-2, which is not mentioned in the Material and Methods. Where is this station E-2?
- 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 ($\mu\text{g/L}$) doesn't seem correct. If graph b depicts N^* , why is the color bar scaled between 100 and 900?
- Figure 4: replace “Mix Layer Depth” with “Mixed Layer Depth”
- Table 1. “PZ” is not defined. How were the assimilation of NH_4^+ and NO_3^- estimated (to be detailed in the methods section)?