

We would like to thank both referees for their critical and constructive comments to our manuscript. Their comments helped to significantly improve the quality and clarity of the manuscript. We hope that our answers and revisions are sufficient to accept this work for publication in Biogeosciences. Please find our responses to each of the individual comments below.

Referee # 2 Dr. Ionescu

Received and published: 11 November 2015

The paper by Gier et al discusses N fixation in oxygen minimum zones in marine sediments (specifically off the coast of Peru). The study suggests a link between sulfate reduction and N fixation in these environments and supports this previously mentioned hypothesis by rates measurements and phylogenetic data. This paper adds to our understanding regarding diazotrophy in sediments as well as highlights our gap in knowledge on the matter by showing that not all patterns can be explained by the presented data. The paper is generally well written with some exceptions where the English can be improved and the wording can be phrased in a more accurate manner.

[The manuscript was cross-checked by an English speaker.](#)

I tried to highlight these places in the comments below. Additionally as stated below the figures are not suited to the page size used by the journal and hence are often not readable.

[We tried to improve the readability and clarity of the figures.](#)

Page 14408 line 4 – The definition of formalin is an aqueous solution of 37% (m:v) formaldehyde. Hence 37 % formalin would mean 13 % formaldehyde. I guess this is not what the authors meant. To avoid misunderstandings, I suggest using 37% formaldehyde solution.

[We agree with the referee and changed the information according to his suggestion.](#)

Page 14408 line 5 – The acetylene reduction assay should not be used for longer than 48 h. Some consider this to be too long as well. The reason is that the saturation of the enzyme with acetylene leads to a lack of fixed N and reduction in cell viability and accordingly N-fixation (See for examples Seitzinger and Garber, 1987 MEPS 37 and references therein).

[We agree with the referee and we are aware that incubation with acetylene can lead to a potential lack of fixed N, however to the best of our knowledge this is the standard method used for the determination of N₂ fixation in sediments \(15N rate determinations are not feasible in sediments as incubation times would need to be several weeks to months to achieve a signal above the natural 15N sediment background\). We have added in a recent citation \(Bertics et al., 2013\) that describes the method in further detail and we point towards this limitation of the method in the manuscript.](#)

Page 14408 line 14. If you have converted the NA from C₂H₄ reduction to N fixation, why do the graphs in Fig 3 still discuss C₂H₄. While the value of 3 is not fixed for all environments it is indeed widely used. If you used it you can now refer to N₂ rather than C₂H₄.

[As both referees pointed out that it is confusing to have nitrogenase activity \(NA\) and N₂ fixation in the manuscript, values were recalculated for N₂ fixation and all figures, tables and text were changed accordingly and we now only refer to N₂ fixation.](#)

Page 14409 line 27: 1 µl of BSA is not very informative as we don't know the concentration of the stock solution nor the reaction volume.

[The information has been added.](#)

Page 14410 line 25: No need for "The" in "The St. 9".

[Changed.](#)

Page 14411 line 3: “The deepest St. 10” means that there are several stations named St. 10 and this is the deepest of them. I suggest “The deepest station (10; 1025 m)...”
Or “St. 10 (the deepest; 1-25 m) ...”
[Changed.](#)

Page 14411 line 11: Erase “The” in “The St. 4 and 6”.
[Corrected.](#)

Page 14411 line 16: The shallowest St 1 – see my previous comment about the deepest St 10.
[Corrected.](#)

Page 14412 line 2: “Sediment depth profiles of N₂ fixation activity are expressed in nitrogenase activity (NA), i.e. without the conversion factor of 3 C₂H₄: 1 N₂” – Why convert in some cases (integrated rates) and not everywhere. Either you trust the conversion factor or you don’t – no need to confuse the reader. Providing N₂ fixation rates also allows for direct comparison with other studies. Please change this.

[As both referees pointed out that it is confusing to have nitrogenase activity \(NA\) and N₂ fixation in the manuscript, values were recalculated for N₂ fixation and all figures, tables and text were changed accordingly and we now only refer to N₂ fixation.](#)

Page 14412 line 9: In all cases so far you used the abbreviation St. even when several stations were mentioned why here the full word stations.
[Corrected.](#)

Page 14412 line 8-10: The choice of sentence structure is not clear – Simply state: NA and SR rates were high (or highest) at the shallow St.... and lowest at deep St...
[Changed.](#)

Page 14412 line 11 – page 14413 line 13: This section is messy and hard to follow. For example, St 1 has its own paragraph while the other stations are mentioned in a single paragraph. I also find this section too detailed. I believe you should only highlight the important things from the figures and not literally describe the graphs.

[The paragraph has been shortened and only highlights from the graphs are specified. We hope this improves the clarity of this section.](#)

Page 14413 line 15: The rate conversion was done from C₂H₄ to N₂ and not to N (same in Fig. 4). Also the units (mmol) is missing.
[Corrected.](#)

Page 14413 line 25, 27, 28: mmol N₂
[Corrected.](#)

Page 14414 line 7: Instead of “three novel clades and seven novel clades...” write “three and seven novel clades were detected, respectively”.
[Changed.](#)

Page 14414 line 15: For the sake of correctness add: for a “known” Vibrio species...
[Corrected.](#)

Page 14416 line 21: The term heterotrophic N₂ fixation is a bit obscure as autotrophy refers to carbon. If the authors refer to N₂ fixation by heterotrophs this should be stated in such a manner.
[The term heterotrophic has been clarified.](#)

Page 14416 line 23: The integrated N₂ fixation rate and the Corg concentration clearly showed similar trends. Nevertheless, the use of the word “correlated” requires a statistical measure

which I believe was not provided. Either provide such data (which should be straight forward) or rephrase the sentence to address the similarity in trends.

We agree with the referee and have rephrased the sentences accordingly.

Page 14417 line 22. Fig 5 should be Fig 4.

Corrected.

Figures:

Fig 2 – The figure is probably designed to cover an entire page (A4 or Letter). However, this is not the format used by this journal. Hence the printed figure is not readable. Online viewing requires as well magnification to 250 % for clear reading. Consider splitting into two panels spanning two pages.

The final format of Biogeosciences is letter format, hence the Fig. will be printed on a full page.

Fig. 3 – A similar problem as above with the addition of long text as the axis title. This cannot be read at 100% magnification on a screen or print.

The figure, as well as the axis title has been changed and the fonts were increased.

Fig. 4. As stated before I believe the correct unit is mmol N₂ and not mmol N. Fonts need to be increased.

We agree with the referee and changed the unit. Also the fonts were increased.

Fig. 5. The same comment as above. Additionally, the yellow line and text are hardly visible.

The whole figure and all fonts have been increased, the yellow line has been darkened and the unit was changed accordingly.

Fig. 6. Needless to say that this is useless in print or at standard screen viewing. The fonts need to be larger. Sequences from this study should be bold. The shaded frames should be positioned in the background of the tree and not above it as they hide the text. Consider cutting the tree into two sections on two pages.

We agree with the referee and tried our best to increase the quality of the whole figure. The sequences from this study have been increased and were made bold. The shaded frames were changed to a transparent design for a better visibility. We considered cutting the tree into two sections, however this would make a direct comparison and association of the sequences more difficult for the reader and therefore we decided to show the tree on one page.