nature portfolio

Peer Review File

Evidence of limited N2 fixation in the Southern Ocean

Corresponding Author: Professor Nicolas Cassar

This manuscript has been previously reviewed at another journal. This document only contains information relating to versions considered at Communications Earth & Environment.

This file contains all editorial decision letters in order by version, followed by all author rebuttals in order by version.

Version 0:

Decision Letter:

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Dear Professor Cassar,

Your manuscript titled "Evidence of limited N2 fixation in the Southern Ocean" has now been seen by our reviewers, whose comments appear below. In light of their advice we are delighted to say that we are happy, in principle, to publish a suitably revised version in Communications Earth & Environment under the open access CC BY license (Creative Commons Attribution v4.0 International License).

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We hope to hear from you within two weeks; please let us know if you need more time.

Best regards,

Alice Drinkwater,
Associate Editor
Communications Earth & Environment

REVIEWERS' COMMENTS:

Reviewer #1 (Remarks to the Author):

I have re-reviewed the Gu et al. manuscript for Communications in Earth and Environment and they have largely satisfied my comments from the first review round (e.g. dropping “Sediment” in the title.

They have certainly beefed up the manuscript overall and clarified many points in the current version.
I do note some minor items to bring to the author’s attention:
Line 71. “Conservative” is ambiguous. Do they mean stringent? A high threshold? Perhaps reword for clarity.
Pages 4-8. Several instances of unnumbered references in text (i.e. Just author(s) and year.

Regarding other reviewer comments about methodology to assess N2 fixation: 15N2 and C2H2 reduction measure different things- 15N2 is a direct measurement of uptake of N2 into biomass, and typically only into the particulate fraction (measuring the uptake into dissolved organic N is a specialty measurement). It probably provides an estimate of “Net” incorporation. C2H2 Reduction (here implemented thru FRACAS) is a measure of total nitrogenase enzyme activity as C2H2 freely diffuses to sites of activity. It probably more closely approximates “Gross” N2 fixation and as pointed out by the authors – requires conversion and assumptions to express it order to quantitative N2 fixed.

The use of these two approaches helps to independently corroborate the presence (or absence) of activity at those sites reported.

In terms of the significance of the observations, yes the rates are very low in the nearshore waters and below detection in the offshore component- but they do provide important information on the phylogenies of potential diazotrophs that are distinct from other previous studies in distinct Antarctic waters as well as in the Arctic. Thus, it provides a baseline which may have relevance as populations evolve in response to upper ocean warming, nutrient delivery and other correlated parameters. Finally, for those skeptical of the activity at low temperature and elevated nutrients, I point out the recent studies documenting N2 fixation in systems with elevated nutrients, as well as the long recognized studies at low temperature such as in the cyanobacterial mats along the Antarctic Dry Valley streams.
Doug Capone

Reviewer #2 (Remarks to the Author):

This study examined nitrogen fixation in the Southern Ocean using two different methods and further evaluated the occurrence of nitrogen fixation with several statistical methods, which are commendable. This study provided new evidence of nitrogen fixation in the western Antarctic region. This study and previous studies have shown that widespread nitrogen fixation can occur in the Southern Ocean. In this regard, this manuscript is worthy of publication.

On the other hand, the interpretation of nifH sequence data remains problematic.
As pointed out in the previous review, the possibility of sediment resuspension can be considered based on observed environmental changes. However, the authors did not obtain robust results on this issue. Therefore, the section “Potential for sedimentary diazotrophy” should be more modest. The discussion of L252-264 seems too speculative.

The source of the nitrogen fixation detected is difficult to conclude from their results. However, it is interesting to note that the obtained sequences differ from known sequences. Does this mean that most of the diazotrophs are endemic to the Southern Ocean? I don't understand well the description of L264-271. Please rewrite them a little more clearly.

Specific

L141: I do not understand how to normalize. Please clarify it in the Method section.

L370-374: Please specify the conditions and number of cycles for each PCR.

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We would like to thank the reviewers for the comments. Our responses to the reviewers' comments are addressed in detail below

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Changed "conservative" to "stringent" to clarify.

Pages 4-8. Several instances of unnumbered references in text (i.e. Just author(s) and year.

Added reference number in line 76 and moved the position of citation number in line 115.

Regarding other reviewer comments about methodology to assess N2 fixation:
15N2 and C2H2 reduction measure different things- 15N2 is a direct measurement of uptake of N2 into biomass, and typically only into the particulate fraction (measuring the uptake into dissolved organic N is a specialty measurement). It probably provides an estimate of "Net" incorporation.
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As demonstrated in the previous reply to the reviewers, we agree that it would be ideal to collect more samples to validate the sediment origins of the diazotrophs, but unfortunately, we haven’t had a chance. It is described as “potential” in the manuscript to clarify that the evidence we had were indirect and speculative.

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known sequences. Does this mean that most of the diazotrophs are endemic to the Southern Ocean? I don't understand well the description of L264-271. Please rewrite them a little more clearly.

It is possible that lots of the diazotrophs are endemic and new in the Southern Ocean, making them “unidentified”. In the meantime, the currently available nifH database may still be limited, as shown by the similarity between the unidentified diazotrophs and recently reconstructed heterotroph genomes.

Specific

L141: I do not understand how to normalize. Please clarify it in the Method section.

“Normalize” meant N2 fixation per unit biomass, calculated as “N2 fixation rate/PON concentration” here. We’ve added this description in the manuscript.

L370-374: Please specify the conditions and number of cycles for each PCR.

The thermal cycle conditions are added in the manuscript.