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Biogeosciences Discussions

Interactive comment on "Significance of N_2 fixation in dissolved fractions of organic nitrogen" *by* U. Konno et al.

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This manuscript reports on the impact that the release of dissolved organic nitrogen during 15N2 incubations has on estimates of nitrogen fixation rates. The paper has the potential to be an important addition to the literature and is clearly within the scope of Biogeosciences. It is addressing an important issue using state of the art measurements. Unfortunately, the authors made a poor choice when it came to filtration that renders the paper, in its current form, unpublishable for the reasons outlined below (comment #12). As a result I recommend rejection at this time. All is not lost, however. I offer a number of suggestions below for how the data could be reevaluated and a new paper constructed. They report valuable data on an important issue. I hope they will take the time to present it correctly.

Specific comments on the manuscript:

1. Title – I don't think the title is that representative of the significance of the work. I suggest something like – Significance of nitrogen release on measured nitrogen fixation rates.

2. Page 766, line 4 - The authors repeatedly using the term ". . ..fixation in the dissolved organic nitrogen (DON) fraction." This is awkward and not technically correct. It sounds as if the DON was somehow fixing the nitrogen. A clearer alternative would be to say "the fixation signal in the DON fraction."

3. Page 766, line 7 – The lack of clarity holds true for the statement, ". . ..those estimated from the DON fraction. . ."

4. Page 766, line 24-26 - There are lots of sentences in the paper that are open to multiple interpretations or are just unclear. This is a prime example.

5. Page 767, line 6-8 - In this time of apparent rapid global change, I don't think that the assumption that nitrogen fixation input estimates should increase just because denitrification loss estimates have, is a robust one.

6. Page 767, line 14 and elsewhere – The authors refer to the studies published in Glibert and Bronk (1994) as culture studies. This is incorrect. The Glibert and Bronk (1994) paper presents data from field studies in the Caribbean. The list of papers that directly measured these rates is very small. It is disconcerting that the authors of the submitted manuscript do not appear to have read this one closely.

7. Page 767, line 16-20 - The authors use the term "total nitrogen fixation rate" but never explicitly define what that is or how they calculate it.

8. Page 767, line 28 – Accuracy is a poor word choice in this sentence.

9. Page 768, line 8 – Clarify what "on a daily basis" means in the context of this sentence.

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10. Page 768, line 10-11 –Doug Capone will be very happy to learn he has done the impossible when he published nitrogen fixation rates using the C2H2 method! The problem in the sentence is the use of the term total again – it needs to be defined.

11. Page 768 – Before launching into the sampling and methods, it would be useful for the reader to know the objectives of the study. I suggest adding a sentence or two to make that clear.

12. Page 769, line15 – The authors only filtered their "DON" samples through a GF/F filter. Unfortunately this is a serious flaw as the paper is currently written – I'd even call it fatal. We know that GF/F filters only retain some fraction of the bacteria (Lee and Fuhrman 1987; Glibert et al. 1995; Lee et al. 1995; Gasol and Morán 1999; Berg et al. 2001). We know that the fraction that is retained is highly variable and depends on where the study was done and the volume of water filtered. We also know that Trichodesmium produces ammonium in addition to DON (Mulholland et al. 2004; Mulholland et al. 2006). Finally, we know that bacteria use ammonium and DON (reviewed in Kirchman 2000). The authors are aware of these issues because they discuss them later in the paper (top page 775). The presence of bacteria in their "DON" signal could affect their interpretation in a number of ways (see Bronk and Glibert 1994). For example, is the similarity between the isotopic ratios of the PON and "DON" fractions observed at some sites (Table 2) due to the isotopic signal of the bacteria, which are likely present in both pools?

The inclusion of bacteria in what the authors are calling DON pool lumps a number of processes together. For them to report actual DON δ15N values they will need to correct for a bacteria signal that is no doubt in their "DON" sample. If they have data on the retention of bacteria on GF/F filters and the δ15N value for those bacteria, they can do this. If not, they need to revise how they are presenting their data.

Unfortunately I cannot recommend publication of the manuscript it its present form because of this issue. I recommend that the authors take another look at their data for

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what it really is – δ15N in PON and δ15N in DON+some fraction of the bacteria. Then they need to redo the manuscript, starting by explicitly defining all terms they use and how they calculate everything they report.

13. Page 770, line 10 – This paper is relatively short, why not discuss the concentration data here?

14. Page 770, line 25 – The DNA extraction seemed to come out of the blue. It was not mentioned in the abstract. Having a short statement at the end of the introduction describing the objectives of the study would really help in this regard. In the methods section, a sentence outlining the premise behind the molecular work would also make the paper flow better.

15. Page 776 – As written I do not see where there has been any real step forward here. Capone et al. (1994) estimated that approximately 50% of recently fixed nitrogen by Trichodesmium was released as DON in the form of amino acids. Glibert and Bronk (1994) measured that approximately 50% of recently fixed nitrogen by Trichodesmium was released as DON. Here the present study takes another average and comes up with approximately 50% of recently fixed nitrogen by Trichodesmium was released as DON. With the geographic coverage they have, I'd love to see a more detailed estimate of how inclusion of the DON+bacteria δ15N data would change estimates of nitrogen fixation in the region. This would be a much more useful contribution to the literature than another grand average.

16. Table 1 – I assume the nutrient columns are μmol N or P /L. These need to be added. As written it would be μmol/L of the compound. This is a common point of confusion, which is why I'm still a big fan of the μg-at N L-1 unit – regardless what my most esteemed colleagues Peter LeB. Williams or George Luther say!

17. Figure 1 – Something seems wrong in this figure. As written it notes that the PON and DON signals are added. If this was a direct addition, the summed value would have to be higher than the PON alone, but that's not what the graph says. I assume

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is has to do with a weighted average. This needs to be clarified. Also, the addition of error bars is a must. If they are included and are too small to see, this should be noted.

18. Figure 2 – I suggest making the triangles solid to make the figure easier on the reader.

The science behind this paper is a really nice piece of work and I'm sorry the review was not more positive. I truly hope the authors take the time to reevaluate their data and its presentation. If the authors are more specific about what they actually measure it will be much easier to compare the results to future and past work. If any of my comments are unclear or off the mark, I'd be happy to have them contact me directly.

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