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Comment

***Interactive comment on* “Significance of N₂ fixation in dissolved fractions of organic nitrogen” by U. Konno et al.**

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Thank you very much for your comments on our manuscript. We would like to revise the manuscript in accordance with your suggestions.

Responses to your comments are as follows:

1. Definitions of PON and DON: Because there has been not clear definition on the boundary between ‘particulate fraction’ and ‘dissolved fraction’ (Hansell and Carlson, 2002), in this manuscript (MS), we define the terms ‘retentate’ and ‘filtrate’ for the ‘impassable fraction’ and ‘passable fraction’ respectively, obtained by using a GF/F filter. The primary aim of this study is to estimate the total N₂ fixation rate by adding the fixation rates of the retentate and filtrate fractions and we focused on the contribution of

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N₂ fixation rates in filtrate fractions, which had been ignored in past many studies. If the purpose is to estimate the total N₂ fixation rate, there is no problem with size of filter. Moreover, the GF/F filter (mesh size: $\sim 0.7\mu\text{m}$) is traditionally used for the determination of N₂ fixation rates in the field because that has a low blank for the determination of nitrogen-isotope compositions. Thus, we would like to clarify the abovementioned definitions (page 767, line 10 in original MS).

2. Title: The main objective of this study is the estimation of the total N₂ fixation rate. We focused on the N₂ fixation rates in filtrate fractions, which have been ignored in previous studies. The title that you have suggested appears to indicate that the main aim of the study is to determine the N release rate. Therefore, we would like to revise the title of the MS to ‘Significance of N₂ fixation in filtrate fraction’.

3. Fixation in DON fraction: The boundary between retentate ($>0.7\mu\text{m}$) and filtrate ($<0.7\mu\text{m}$) has been defined as above; hence, picoplanktonic-sized diazotrophs may fix N₂ in the filtrate fractions.

4. Global fixed N cycle: Because the global oceanic fixed N is considered to be in balance, it is assumed that the efflux and influx of N would be equal. Therefore, several studies have reported on the expected increase in N₂ fixation as described in MS.

5. Glibert & Bronk (1994): The word ‘culture’ in the original MS is misleading. In fact, Glibert & Bronk (1994) incubated *Trichodesmium* that had been collected and picked in the field. Therefore, we would like to correct the whole sentence to “incubation experiments” (page 767, line 14; page 773, line 21; and page 775, line 26 in the original MS).

6. Total N₂ fixation rate: When N₂ fixation occurs during ¹⁵N₂ tracer incubation experiments, ¹⁵N₂ must be moved into either the retentate fraction or the filtrate fraction. The term ‘total N₂ fixation rate’ refers to the sum of the N₂ fixation rates in the retentate and filtrate fractions. Hence, we would like to add sentences to define the term ‘total N₂ fixation rate’ (page 767, line 15 in the original MS).

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7. In response to comment No. 11, we would like to add a sentence to clarify the objectives of this study (page 768, line 15 in the original MS).

8. Response to comment No. 12 is as follows: The aim of this study is to estimate the total N₂ fixation rates and the contribution of the N₂ fixation rates in the filtrate fractions; previous studies have been limited to the determination of the retentate fractions. If there are microorganism activities in incubation bottle, we can detect all ¹⁵N cycle in the bottle in both retentate and filtrate as N₂ fixation. Thus, there are no problems in the determination of the total N₂ fixation rate. Clarification of the mechanisms and aspect of $\delta^{15}\text{N}$ are future challenge.

9. In response to comment No. 13, to discuss the concentrations in detail, we would like to add a few sentences (page 770, line 10 in the original MS).

10. In response to comment No. 14, to improve the paper flow, we would like to divide Chapter 2 into the sub-chapters and added a sentence into chapter 1 (page 768, line 15 in the original MS).

11. Response to comment No. 15 is as follows: While the N₂ fixation incubation in Capone et al., (1994) or Glibert and Bronk (1994) were done for *Trichodesmium* collected by using a plankton net, our study was carried out using natural seawater samples. This is the essential difference between those and ours. In view of the recent findings on N₂ fixation by *Richelia* and nanoplanktonic and picoplanktonic diazotrophs, it is considered that the studies of Capone et al. and Glibert and Bronk could estimate only partial oceanic N₂ fixation. While, our study follows past studies and additionally estimate N₂ fixation rates including other diazotrophs. We believe that our study promote step forward on oceanic N₂ fixation.

12. Response to comment No. 17 is as follows: Because the concentrations of filtrate N were larger than those of retentate N by an order of magunitude, the amount of change of delta values of filtrate N should be smaller than those of retentate N. Therefore the delta values of the combined retentate + filtrate should change smaller than those of

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the retentate alone. Figure 1 shows such results. Because error bars are enough small to include those symbols, we added the figure caption.

13. We would like to correct Figure 2, Table 1, and few other words and sentences in our MS based on the comments of the reviewer (comment Nos. 3, 4, 8, 9, 16, and 18).

We trust that the revisions made in response to your comments are satisfactory. Please find the pdf files of our revised manuscript attached. Thank you for your consideration.

Please also note the supplement to this comment:

<http://www.biogeosciences-discuss.net/7/C907/2010/bgd-7-C907-2010-supplement.pdf>

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