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

Anonymous Referee #2

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The aim goal of this paper is to quantify 1) nitrogen fixation b organisms collected by GF/F filters and 2) the fraction of N₂ recently fixed that can be transferred to the DON pool. I do not feel comfortable with this paper. I am sympathetic to the point being addressed, and I think this work would be done to improve the knowledge of the fate of nitrogen fixation in oceanic systems. The topic is timely and important. That said there are some major issues with the study that greatly affect the conclusions and interpretation, and necessarily prevent suitability for publication The main problems come from the estimation of the DON release:

1) Filtrate obtained by using GF/F filter can not considered as containing only dissolved DON. 2) Calculation of DON release rates has not been explained 3) Time zero experiments are lacking

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1) The lower efficiency of GF/F filters for PON retention compared to 0.2 μm membrane is now well-documented (Altabet 1990; Sondergaard and Middelboe 1993; Libby and Wheeler 1994; Lee et al. 1995; Slawyk and Raimbault 1995). Altabet (1990) found some 15N accumulation in the <GF/F fraction retained on 0.2 μm filters. In short, the use of GF/F filters is not adapted to determine 15N accumulation in the DON pool. Then all the paper, from the title to the conclusion, is based on an wrong statement.

2) Measurement of DON release, i.e. the transfer of nitrogen from intracellular organic nitrogen to extracellular DON, is based on the assumption that soluble intracellular organic nitrogen is the source pool. Then, the 15N enrichment of the intracellular pool is required to quantify DON release rates. In fact, as long as the species and the 15N enrichment of intracellular source pool for DON release are not exactly known, it is impossible to measure total DON release by phytoplankton with the 15N-labeled DIN pool. The 15N enrichment of this internal source pool has not been measured here. Then, I think authors have used initial 15N-N₂ enrichment to calculate nitrogen fluxes in the <GF/F filtrate. But in this case, the rate of DON transfer, then named as DIN loss by Slawyk et al. (1998), may only represent rough estimates of the total amount of DON release. Because of dilution of the 15N tracer by intracellular nitrogen during uptake, 15N excess-enrichment of internal DON lost by phytoplankton cells is lower than the 15N excess-enrichment of the DI15N entering these cells. Then, these authors have argued that extremely high DON Loss rates (>50%) of gross uptake would produced negative changes in PON concentrations of cells taking DIN and are very unlikely.

Only DON loss is actually available and high percentages reported in this work (66 to 84%) seem unrealistic.

In conclusion high 15N enrichment found in the <GF/F filtrate was certainly not due to DON release.

3) More, using information available in this paper (surface concentration of DON, integrated rates of DON release) and assuming initial 15N-N₂ enrichment around 10 %

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(e.g., 29222 ‰, we can estimate the excess enrichment for samples collected in summer 08. The calculated values, ranging from 0.21 to 2.63 ‰, are lower than the detection limit given in the materials and methods section (2‰, page 8) and do not seem significant. However, this crucial information can be only obtained correctly from “time zero” experiments using the ^{15}N tracer and running the experiments under standard experimental conditions.

Some results need to be clarified: Figure 1: $\delta^{15}\text{N}$ on PON and DON are measured separately. Then, how can the authors calculate PON + DON enrichment?

Figure 2; Vertical profiles of N_2 fixation rate in this figure show that the highest rates are found at station 7 and lowest at station 1. But integrated values given in table 2 do not follow this pattern.

In conclusion, deep modification and important additional information are needed. Until this situation can be cleared up, the manuscript is not as credible or newsworthy as the title suggests.

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