Biogeosciences Discuss., 12, C366–C369, 2015
www.biogeosciences-discuss.net/12/C366/2015/

Biogeosciences
Discussions
Open Access

Interactive comment on "Seasonal dynamics of nitrogen fixation and the diazotroph community in the temperate coastal region of the northwestern North Pacific" *by* T. Shiozaki et al.

Anonymous Referee #2

Received and published: 28 February 2015

General comments:

Nitrogen fixation rate measurements and nifH gene based molecular studies in temperate regions of western North Pacific Ocean is relatively rare, comparing with the intensively studied Eastern Pacific Ocean and Atlantic Ocean. The authors reported nitrogen fixation rate and some nifH sequences of potential nitrogen fixers in the temperate coastal region of the western North Pacific Ocean, which can provide some missing knowledge in this field. In general, the patterns and explanations of nitrogen fixation rate presented in this study are good and making sense, while the part of nifH gene based molecular study is too weak to reveal the community structures in the studied regions. The authors only included less than 200 clones of nifH gene amplicons

from the six cruises and tried to discuss "diazotroph community structure", in which any statements made are not convince enough. Besides that, there are numbers of unclear issues, related to the methodologies of both rate measurement and molecular works, needed to be clarified or addressed.

The authors tried to link up the 2011 Tohoku-oki tsunami with the diazotroph community structure in discussion part and conclusion part. However, without comparing the diazotoph community structures before and after the tsunami, it is inappropriate to make any related conclusions.

Specific Comments:

A. nifH gene based molecular works:

The amount of nifH clones (200 clones) sequenced is really too few to reveal the community structure in 6 cruises. If the clones sequenced were evenly selected from the samples of 6 cruises, there should be approximately 33 clones sequenced per cruise and 8 sequences representing the diazotroph community in each sampling stations. In this case, the conclusions about distribution of diazotrophs will be very inaccurate. For examples, absence of Trichodesmium and other cyanobacterial diazotrophs in most of cruises may be due to the low coverage of sequencing and PCR primer induced bias(Langlois, Hümmer et al. 2008).

P.2, l.4: As mentioned by another referee, the authors should not use the term "diversity" here. The author can use "diazotroph community" or "identities of potential nitrogen fixers" to replace the term "diversity".

P.5 l.9-14: The authors mentioned that DNA was extracted from the samples collected in Stns. OT4, ON1, ON5, and ON7. However, it is unclear that how the data of relative abundances of diazotroph species in different cruises was generated. Did they mix the DNA samples or PCR products or sequence data of different stations in the same cruise? The authors should clarify this part.

P.5 l.26-27: The bootstrap values of most important branches (dividing the clusters of nifH) in the phylogenetic tree were lower than 50

P.9 l.6-22, fig.6 : the authors should describe the diazotroph community structure in different stations of the same cruise separately (if they would like to sequence more clones), rather than just presenting the total sequencing results of each cruise as one community. Inconsistent nutrient concentration and nitrogen fixation rate were detected in different stations during the same cruise (fig. S2), therefore, the diazotroph community in these stations may also be different.

B. Nitrogen fixation rate measurement:

P.6 l.7-8: Recently study reported contamination of commercial stock 15-N2 gas with 15N-labeled ammonia and nitrate, which could affect the results of nitrogen fixation rate measurement significantly (Dabundo, Lehmann et al. 2014). Therefore, the authors should ensure the purity of their 15-N2 gas.

C. Nitrogen Fixation and environmental data

P.2 l15-16: Previous study showed that ammonia is stronger inhibitor than nitrate (Ito and Watanabe 1983), and the inhibitory effect of nitrate to different diazotrophs is still not clear (Cejudo and Paneque 1986). However, the authors were just caring about nitrate in this paper. It seems that the data of ammonia was also included in the supplementary figures. Why did not the authors make use of the ammonia data?

P.9 l1-2: As exceptions were found in subsurface layer of OT4, statistical analysis (e.g. principal component analysis) is needed to find out the important and significant environmental variables. As mentioned before, I suggest the author to include ammonia as one of the environmental variables. Besides the concentrations of DIN, they can use N:P ratio as a better indicator of nitrogen limitation.

Cejudo, F. J. and A. Paneque (1986). "Short-term nitrate (nitrite) inhibition of nitrogen fixation in Azotobacter chroococcum." Journal of bacteriology 165(1): 240-243.

Dabundo, R., M. F. Lehmann, et al. (2014). "The Contamination of Commercial 15N2 Gas Stocks with 15N–Labeled Nitrate and Ammonium and Consequences for Nitrogen Fixation Measurements." PLoS One 9(10): e110335. Ito, O. and I. Watanabe (1983). "THE RELATIONSHIP BETWEEN COMBINED NITROGEN UPTAKES AND NITROGEN FIXATION IN AZOLLAâĂŘANABAENA SYMBIOSIS." New phytologist 95(4): 647-654. Langlois, R. J., D. Hümmer, et al. (2008). "Abundances and distributions of the dominant nifH phylotypes in the Northern Atlantic Ocean." Applied and environmental microbiology 74(6): 1922-1931.

Interactive comment on Biogeosciences Discuss., 12, 865, 2015.