

## ***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 #3**

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General comments: This manuscript provides the seasonal variability of nitrogen fixation and diazotrophs in the coastal region of the western North Pacific Ocean where was seriously affected by the 2011 Tohoku earthquake-induced tsunami. The authors showed that the high nitrogen fixation rate was observed in summer and fall, and the *nifH* sequence of UCYN-A and  $\gamma$ -proteobacteria was detected at the same time. They concluded that the origin of these diazotroph is Tsugaru warm current and the findings will be re-evaluate the nitrogen fixation in temperate ocean. They also recovered *nifH* sequences assigned with benthic strains or terrestrial strains in this region and discussed the influence of the 2011 Tohoku earthquake-induced tsunami. The observation field is relatively interesting. The authors have made a good attempt at the

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understanding of nitrogen fixation in the temperate region. However, I feel that this study is not adequately describe.

Specific comments: 1. The meaning of the transect sampling is not clear. There are some hydrographic difference between coastal and offshore stations, due to Tsugaru water current, Kuroshio and Oyashio. The authors sometimes mentioned terrestrial effects. However authors showed the average of each line. I suggest the analysis region should be divide with each stations or coastal/offshore stations. It may be better if the authors would like to discuss about the influence of the 2011 Tohoku earthquake-induced tsunami. The influence of the damage of benthic environment may be clear in coastal stations than those of offshore stations. 2. To discuss the seasonal variation of diazotrophic community, the number of recovered *nifH* sequences is not enough. Much more sequences should be analyzed. In addition, I wonder why the *Richelia*, UCYN-B and UCYN-C were not recovered. Were the sequence number and the volume of sampling water appropriate? Furthermore, if the authors describe the benthic strains or terrestrial strains, the data also should be shown as each stations. I think that the quantitative PCR also useful method for this study, such as Shiozaki et al (2014).

I encourage the authors to reanalyzing the nitrogen fixation rate, environmental factors and *nifH* sequences at each station.

P. 2, l. 11 The *nifH* sequences were only recovered, not quantified. It is hard to say as ‘played key role’ in this study.

P. 7, l. 25 This sentence belongs to the ‘discussion’.

P. 8, l. 10-11 The horizontal distributions of nitrogen fixation and nutrients should be shown.

P. 9, l. 5-22 It would be shown the stations where the *nifH* sequences were recovered.

P. 10, l. 4-24 It would be added the discussion about the influence of eddy which seems bring the water mass from Kuroshio. The sea surface temperature showed

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some currents from south to north around offshore stations in KT-12-27 and KK-13-6 (Fig. S1). And the nitrogen fixation rate of offshore station OT7 was higher than the other stations (Fig.2).

P. 10, l. 25-P. 11 l. 4 The approach is interesting. But the authors should compare the community structure to Blais et al (2012) and discuss the different usage of organic materials of bacteria.

P. 11, l. 5-23 This sentence should be improved to clarify why the low nitrogen fixation rates were observed.

P. 11, l. 20 Add the nitrogen fixation rate along the OT transect line to the Fig. S3. It would be clear the influence of Typhoon Man-yi.

P. 12, l. 7-11 This part is not convincing. There is no evidence that the cluster III was not exist in this region before the tsunami. It is not clear that the cluster III recovered in this study is the same as those living in sediment or not.

P. 12, l. 11-15 It would be better to show the horizontal distribution of diazotroph.

P. 12, l. 24 The experiments were conducted to surface layer, and did not consider any bottom structure or re-suspending. If the authors would like to evaluate the damage of tsunami, the study should include the bottom layer and sediments.

Fig. 4 (b) The line of N<sub>2</sub> fixation is hard to see in KK13-6 and KS14-2. To help the reader, seasons should be added with the cruise name, such as Fig.3.

Fig. 6 The data should not be summarized with different stations.

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