Biogeosciences Discuss., 12, C1456–C1461, 2015
www.biogeosciences-discuss.net/12/C1456/2015/

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.

T. Shiozaki et al.

shiozaki@aori.u-tokyo.ac.jp

Received and published: 20 April 2015

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 -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 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.

// Although we set the transect from inside bay to open ocean to examine the gradient of environmental variables and diazotrophy, there were no significant difference between inside and outside the bays (Fig. S2 and S5) except at the OT line during the KK-13-6_Sep when nitrate, ammonium, and phosphate concentration increased inside the bay. This similarity could be because intense exchange of surface waters often occurred between inside and outside the bays (Furuya et al., 1993). According to the TS diagram (Fig. 3), surface waters collected during the same cruise in a particular season generally belonged to the same water system that was prevalent in the investigated

region at the time of our sampling. Therefore, for simplicity, the average value of each variable was shown in each cruise. We have added these statements in L191-201. The clone library analysis showed that UCYN-A, Trichodesmium, and γ-24774A11 were likely important diazotrophs from early summer to fall when nitrogen fixation occurred. Therefore, the present study quantified these nifH phylotypes by a qPCR analysis to examine their relative importance in these seasons. In addition, UCYN-B which is considered a major diazotrophs in the tropical and subtropical oligotrophic ocean (Moisander et al., 2010) has been quantified. The qPCR analysis demonstrated that the target groups were quantified even at stations where these clones were not recovered from the clone library analysis, suggesting that the number of clones was not sufficient to capture the diazotroph community structure on each cruise. Despite this limitation, the sequences more frequently recovered in the clone library generally corresponded to the most abundant group revealed by the qPCR analysis. For example, UCYN-A was frequently recovered in the library during the KT-12-20_Aug, KK-13-1_Jun, and KK-13-6_Sep cruises; for these samples, the qPCR results showed that UCYN-A was the most abundant group among the four examined. Similarly, qPCR data indicated that Trichodesmium was the most abundant group during fall, when this group was frequently recovered in the library (during the KT-12-27_Oct cruise). This consistency in the general results obtained by the clone library and qPCR suggests that both of these approaches captured a similar seasonal trend in community composition changes for at least the major diazotroph groups. We are now clearly stated in L390-402. Abundance of Richelia is generally lower than that of Trichodesmium and it inhabits tropical and subtropical regions as with Trichodesmium (Shiozaki et al., 2010). The UCYN-B and UCYN-C are also observed in tropical and subtropical regions (Moisander et al., 2010; Taniuchi et al., 2012), and not in the temperate regions (Needoba et al., 2007; Rees et al., 2009; Mulholland et al., 2012). Our results thus were consistent with the previous ones. We have added number of recovered sequences at each station in every cruise in Table 1. //

P. 2, l. 11 The nifH sequences were only recovered, not quantified. It is hard to say as

'played key role' in this study.

// In the revised manuscript, we have quantified the four diazotroph group as mentioned above. According to the results of the qPCR analysis. We have revised the description as follows. "Quantitative PCR analysis revealed that UCYN-A was relatively abundant from early to late summer compared with Trichodesmium and γ-24774A11, whereas Trichodesmium abundance was the highest among the three groups during fall." (L25-27). //

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

// We have moved the related sentences to the Discussion (L376-381). //

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

// We have shown these horizontal distributions in Figure S2. //

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

// The obtained clone number has been shown at each station in every cruise in Table 1. //

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 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).

// Although the geostrophic current fields showed that Stn. ON7 during the KT-12-27_Oct cruise was located in anticyclonic eddy, the nitrogen fixation rate was similar with that at the other stations. Furthermore, according the TS diagram, water belonged to the Kuroshio water system did not exist (Fig. 3). //

P. 10, l. 25-P. 11 l. 4 The approach is interesting. But the authors should compere

the community structure to Blais et al (2012) and discuss the different usage of organic materials of bacteria.

// Blais et al (2012) suggested this hypothesis on the basis of their observation that gradient of nitrogen fixation rate occurred from the river mouth to the open ocean, and did not mention the specific diazotroph groups. //

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

// High DIN concentration are generally regarded to inhibit nitrogen fixation (Falkowski, 1983). We wrote this in L53-54. //

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.

// Nitrogen fixation rate in the OT line has been shown in Fig. S2. //

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.

// We agree. Since we do not have data before the tsunami, we have deleted these sentences. //

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

// We have added this information in Table 1 and Fig. S5. //

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 be include the bottom layer and sediments.

// We have deleted the sentences related with the damage of tsunami from the Discussion. //

Fig. 4 (b) The line of N2 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.

// In the revised figure, we have used color to distinguish each parameter. Further, we have added information of season in each cruise. //

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

// We have deleted this figure from the revised manuscript. //

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