Referee #1

The paper has been substantially improved by revision. Especially, I appreciated the addition by the authors of the qPCR analysis. However, on one point we continue to disagree. As all of the reviewers mentioned, the number of the recovered nifH sequence was not enough. The authors did not change this point. It is still difficult to discuss about the dominance or seasonal variation from the clone library analysis, even if the results was similar to those of qPCR.

I suggest that the authors discuss about the abundance and variation of four groups based on the results of qPCR. The result of clone library experiments shows only the existence of the other diazotroph (Leptolyngbya, δ-Proteobacteria, and Cluster III) if the number of the sequences will not be changed.

Line 315-317: I suggest adding the cruise KK-13-6 and referring to figure S5.
Line 399-402: The result of clone library analysis cannot say “seasonal trend”.
Line 452-453: The result of clone library analysis cannot say “dominant”.
Line 455-456: The number of the recovered sequence is not enough to say “major diazotroph”.
Line 472-474: It is difficult to say that the organism was inactivated by the flushed out from the coastal region. There is no data which shows the organism condition (active or inactive) before the flushed out.
Fig. 7 the number of copies should be shown with log scale.

Referee #2

Nitrogen fixation data is rare in the temperate marine ecosystems, and the authors clarified the accuracy of their rate measurement data. Therefore, this paper can provide significant contribution to our understanding of nitrogen fixation in the ocean. After the revision, the authors added quantitative PCR (qPCR) data of the three major phylotypes, which provided more useful molecular information and compensated the limitation of their sequencing results. Hence, they can explain (discuss) the seasonal and spatial variations of nitrogen fixation in a more convincing way. However, there are still some problems and questions needed to be considered, especially the influences of environmental factors to nitrogen fixation.

Specific comments:
P.4, L.72: the methods of Pearson’s correlation matrix were missing. At least, the authors should explain what the “surface water” meant (Table 2). Did they average the surface data of different stations for each cruise or input each data point during calculation? It seems that the error bars of the averaged nitrogen fixation rate and nutrient data were very large. Therefore, it is not appropriated if they used averaged data when they were calculating the correlation matrix. The author can simply display the data used for calculation in a table in supplementary information. Also, why didn’t the author use the data of other water layers during calculation? It seems that nitrogen fixation was also significant in other water layers.

P.13, L.222: the unit of nitrogen fixation rate should be uniform throughout the paper.

The author used “L” or “l” in different places of the paper.

P.14, L.252: according to Table 2, correlation between nitrate, phosphate and temperature was strong, and all this factors had significant correlation with nitrogen fixation rate. However, it is not necessary that all these factors influenced nitrogen fixation directly. For examples, the negative correlation of nitrate (phosphate) with nitrogen fixation rate can be due to their negative correlations with temperature. Therefore, this issue is needed to be considered during discussion. Especially, both DIN concentration (ammonium should only contribute very small portion) and N:P ratio did not show significant negative correlation with nitrogen fixation rate.

P.18, L.308: It is suggested not to use one page to discuss the two rate measurement methods in the beginning of discussion, as this study was not focusing on this issue.
Moreover, if gas dissolution method is better in this study, further discussion of these two methods is not helpful to the objective of this study.

P.22, L388: Correlations of the qPCR result and nitrogen fixation rate and environmental factor are suggested to be analyzed. The authors tried to discuss numbers of different phylotypes with nitrogen fixation rate and environmental factors. Conducting environmental correlation analysis will support their points and provide clear picture. In order to estimate gene copies of different nifH phylotypes accurately, the authors can use qPCR of 16S rRNA to normalize the data of nifH.

P.24, L.415: “disappearing during in spring” seems grammatically incorrect to me.

p.26, L.452: as the author did not do qPCR of cluster III, it is inappropriate to imply that cluster III was increased in abundance with “suspension of sediment”. The abundance of cluster III might not be changing a lot throughout different seasons, and their higher relative abundance in cold condition may be simply due to repression of the cyanobacterial diazotrophs. Therefore, without qPCR data of cluster III, the author should not strengthen the importance of cluster III in cold conditions. Also, the nitrogen fixation of marine cluster III is still not well confirmed and this study was based on DNA works, so, it is not helpful and convince to mention too much about cluster III in discussion and conclusion. Besides that, relatively abundant cluster III was also reported in Arctic (Farnelid et al 2001).

S3,Phylogenetic tree: the Trichodesmium should not be clustering with cluster I proteobacteria. The tree was not stable, and the reason may be due to insufficient sequences of cyanobacteria. The author can consider adding more reference sequences of cyanobacteria, which should make the tree more stable.