Biogeosciences
Discussions

Interactive comment on "Contribution of dinitrogen fixation to bacterial and primary productivity in the Gulf of Aqaba (Red Sea)" *by* E. Rahav et al.

Anonymous Referee #2

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In this study, Rahav et al. report results from field campaigns in the Gulf of Aqaba where they measured nitrogen fixation (Nfix), primary production (PP), bacterial production (BP), and did enrichment experiments to test for P and C limitation of Nfix. Results are compared for three different cruises, two of which happened in 2010 (March, 'winter', mixed water column, and September, 'summer', stratified water column) and one other cruise which was carried out 2 years later in July 2012 ('summer', stratified water column). In Sept. 10, the investigators additionally sampled for metatranscriptomics from the stratified water column- cDNA samples from 3 depths were pooled, sequenced, and analyzed for the presence of nif genes, none of which were cyanobacterial, but all matching either Euryarchaeota or bacterial diazotrophs (d-Proteobacteria

or Chlorobia).

The main conclusions from these data are 1) that summer/winter differ regarding the magnitude of Nfix (higher during stratification) 2) that non-cyanobacterial diazotrophs are mainly responsible for Nfix during stratification (based on positive correlation between BP and Nfix, P stimulation of Nfix but not PP in summer, and the analysis of the nif cDNA in Sept 10), and 3) that Nfix by these diazotrophs is P and C limited. While these conclusions, especially 2), sound interesting and definitely worth of publishing, I fear that the data do not support them, and therefore I can hardly recommend this study to be published in Biogeosciences. These are my main reasons:

1) The conclusions on the seasonal patterns of nitrogen fixation and responsible organisms are problematic. What is a representative station, representative for what? 2010 was by no means a normal year climatologically, with a strong 2009-2010 El Nino, thus a comparison to 2012 is problematic- how do we know that 2010 samplings are typical seasonal situations? The stratification appears weak as well.

2) More importantly, the proof and possible triggers for a community shift between winter and summer are not well explained and hardly justify the conclusions. The correlations between bacterial production measurements and other rate measurements appear weak; for example, have all rate measurements been initiated at the same time? This information is not given in the methods section, but they could influence whether or not rate measurements correlate. BP incubations lasted 4-8h, (details? which ones 4, which ones 8?) which is very different to incubations lasting 24h (a lot can happen in 24 h... DON, NH4 release, re-uptake by phytoplankton, bacteria...). Also, it is difficult to confirm that nitrogen-fixing bacteria are be responsible for Nfix, based on looking at a correlation with bacterial production- such bacteria are usually orders of magnitude lower abundant than other pelagic bacteria, but BP incubations represent the uptake of a large part of the bacterial community. Therefore, I fear that correlative analyses are not justified.

3) The support drawn from the metatranscriptomic analyses is unfortunately weak as well. Have the authors checked the DNAse-treated RNA samples for DNA contamination? This could e.g. be done with a PCR. Some people do DNAse treatment twice because it can be very difficult to get rid of low-level DNA contamination. I don't even want to argue that the nif genes from this study are all contaminants, but while it can be expected that any nif expression makes up only a minor part of such a transcriptome, isn't it very suspicious that in the Arabian Sea, where cyanobacterial diazotrops including Trichodesmium are known to occur, none of the recovered sequences match cyanobacteria, in a situation that has been traditionally viewed as a prime habitat for Trichodesmium (stratification, warm waters, low N:P)? Are the described 6309 sequences unique ones representing many more sequences or are they indeed the only nif Sequences from 2.3 million reads * 100nt? Have the samples from the different depths been barcoded or was it indeed only a pooled sample? For what reason has the surface sample been left out- because there would have been mainly cyanobacteria in there? So the point is, even if these sequences are native oceanic, it would need much stronger data, and better presentation of the data, to make the point. Is it possible to run an assembly to see whether the reads build contigs that allow for a better sequence comparison vs. the databases?

End of review