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Biogeosciences Discussions

Interactive comment on "Activity and abundance of denitrifying bacteria in the subsurface biosphere of diffuse hydrothermal vents of the Juan de Fuca Ridge" *by* A. Bourbonnais et al.

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Reviewer's comment: The paper is overall well written, my only argument is for the bacterial community analysis and q-PCR. I am afraid that the 16S rRNA gene library analysis performed in this study is weak. Only very limited clones were sequenced from the five bacterial 16S rDNA clone libraries, for example, only 27 clones from library of Phang were sequenced, thus the coverages of the libraries were low, mostly around 50-60%. Therefore, the compositional analysis of different bacterial groups in the environments were not convincing at all. I understand that one of the main focuses of this paper is to determine the relative contributions of different Nitrogen elimination

processes (i.e. denitrification, annamox, and DNRA) in the diffuse vents, thus the authors didn't make enough efforts for community analysis. Then I would recommend the authors just to make a brief description of the bacterial community analysis, in my opinion, it's almost worthless to calculate the percentage of different groups using so limited data, Fig.4 could be eliminated from the text.

Our response: In our study, we preferred obtaining high quality, near full-length 16S rRNA gene sequences by sequencing the 16S rRNA gene using 4 different primers, i.e. B8F, B1492R, B515F and B907R, instead of just two primers (generally M13 primers), as is often the case in other microbiological studies. This additional effort puts some constraints on the overall quantity of sequence analyses performed. Due to the high cost associated with Sanger sequencing, we were not able to sequence more clones. Other studies have previously described hydrothermal vent bacterial communities using a comparably modest number of 16S rDNA clones. For example, Huber et al., (2002) (in FEMS Microbiology Ecology) constructed 16S rRNA gene clone libraries using $\sim$40 clones from 6 diffuse flow hydrothermal habitats, with coverage ranging from 19% to 77.5%. Furthermore, increasing the number of clones sequenced would not necessarily increase the coverage. For example, Forget et al. (2010) (cited in our reference list) sequenced up to 176 16S rRNA gene clones from hydrothermal sediments at a South Tonga Arc submarine volcano, but the coverage remained below 45%. Is it also well known that quantitative bias can occur during PCR amplification (generally favoring the most abundant groups), and that the % of each group cannot be accurately determined from 16S rRNA gene clone libraries only, even if the coverage would be close to 100%. While we agree that sequencing more clones would have increased the confidence in the reported 16S rRNA gene community composition, we believe that our current sequencing effort still allows us to speculate about potential denitrifiers in vent fluids, and significantly add value to the discussion of the paper. We also maintain that a figure is more informative than simply describing the data in the text, with regard to communicating the presence of potential denitrifiers in these systems. For this reason, and unless the Associated Editor has a strong and opposing opinion, we intend

to keep Fig. 4 as part of the manuscript. We added this sentence in the Fig. 4 legend: "Note that only a limited number of clones were sequenced at each site".

Reviewer's comment: And actually I couldn't understand why the authors only did qPCR for SUP05, based on the fact that SUP05 is actually not the main players in the samples, why not do quantitative analysis of the epsilonproteobacteria?

Our response: SUP05 bacteria were abundant, if not dominant, denitrifiers at some sites (e.g. Hulk, 2008, Cloud, 2007). As for other vents, ε-proteobacteria seem to be the most abundant group in some 16S rRNA gene clone librairies (e.g. Phang, 2009), though these data are not quantitative. However, since most ε-proteobacteria bacteria have been shown to be quite versatile and can use different electron donors and acceptors (as further discussed in section 4.2 of the manuscript), measuring the total abundance of ε-proteobacteria using qPCR would have not been useful to indicate the actual abundance of denitrifiers. Therefore, we decided to just give an example of how abundant a known clade of denitrifiers (SUP05) could be in vent fluids, and we only discuss in the text (using our 16S rRNA gene clone libraries), the likely presence of other possible denitrifying bacteria.

Other comments:

1. The rates presented in this study were measured at ambient pressure, possible influences by high pressure on the activities were ignored. The authors already discussed and assumed that the depths encountered in this study (1500- 2200m) may not change bacterial metabolisms from those at atmospheric pressures. I make an argument here that pressure of âĹij 20 MPa may have significant influences on bacterial metabolic rates and growth rates, therefore, in-situ measurements were needed to prove or disapprove the assumptions.

Our response: We agree that performing in-situ rates would have been the best way to prove this assumption. In situ incubations at these depths are not trivial and require technology that was not available. It was challenging enough to collect enough fluid to

perform our 15N-labeled incubations. Furthermore, as mentioned in section 4.1, there is no conclusive evidence that bacterial metabolic and growth rates really are affected by elevated pressures at these depths ($\sim$1500-2000 m depth). To better address the reviewer's concern, we added the following sentence at the end of the first paragraph, section 4.1: "Future studies should thus better investigate the effect of pressure on microbial metabolism at these depths."

2. Fig.2 is difficult to read, need some modifications. And I could not find numbers in brackets.

Our response: We will improve Fig. 2 in the new version of the manuscript.

3. Fig. 5 could be used as a supplementary Figure.

Our response: We could include Fig. 5 in the supplementary material (along with the ε-phylogenetic tree (Fig. S1)), at the discretion of the Editor. Please let us know.

4. Introduction, second paragraph, need to add refs

Our response: We added a reference to this paragraph, i.e. Jannash and Mottl., 1985, which is a pioneering study in this field.

Interactive comment on Biogeosciences Discuss., 9, 4177, 2012.