

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: My main criticism of the manuscript is that data about the abundance of denitrifiers are excluded from the manuscript, even though we are told they exist. Why are the nirS and nirK data separated from this manuscript? Are those data really so abundant as to warrant their own manuscript? Being able to add quantitative information about the entire denitrifying community would make the present manuscript exceptional.

Our response: We indeed measured nirS gene qPCR abundance. As mentioned in our manuscript, however, these data will be presented in another paper entitled "Di-

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versity and abundance of nitrite reductase (nirS) genes in the subsurface biosphere at hydrothermal vents of the Juan de Fuca Ridge" that will soon be submitted to the journal *Geobiology*. Since these nirS qPCR data are the first of their kind, we think they should be discussed in greater detail than the incorporation in the present article would allow. That is why we chose a separate manuscript to present these data, along with an in-depth analysis of the nirS biodiversity. Including all these data in the same paper would significantly add length and distract from the main scope of the present manuscript, which was to provide the first rate measurements of N-elimination processes in hydrothermal vent systems. We think that just referring to these data in the discussion (giving a range for the %nirS genes (relative to total bacteria) measured by qPCR) already helps the discussion, without extending the length of the manuscript too much. Also, please note that we were not able to successfully amplify nirK genes in our samples.

Specific comments:

Reviewer's comment: Abstract: The authors write: 'Little is known about nitrogen transformations in general . . .' I think a lot is known about nitrogen transformations in general.

Our response: We now write: "Little is known about fixed nitrogen (N) transformation and elimination at diffuse vents where anoxic hydrothermal fluids are mixed with oxygenated crustal seawater prior to discharge."

Reviewer's comment: p.4184/section 2.1 Would be good to indicate how much time passed between sample collection and the initiation of the rate measurements.

Our response: We clarified in section 2.1 that samples were stored "for typically less than 1 hour" in a cold room after each dive until further processing. In section 2.3, we mention that only samples collected during the June 2008 were pre-incubated during 12 hours before the start of the incubations.

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Reviewer's comment: p. 4184, line 21: How is DI equilibrated with ship air a 'blank' for N₂O? Also, it would be good to note which/how many standards were used.

Our response: We clarified this section. We used DI water as blank, since N₂O concentrations should be negligible in DI water, and treated the blanks the same way as our samples. The average value for the blanks measured in this study was about 4 times lower than our most diluted standard. With respect to which/how many standards were used, we added the following sentence, section 2.2, in the manuscript: "Calibration curves were constructed from five serial two-fold dilutions of a concentrated N₂O standard (~1250 nmol N₂O L⁻¹) from a reference gas tank of ultra-pure N₂O".

Reviewer's comment: p.4185: Were the rate measurements replicated in any way? It is difficult to tell if there were replicates for each type of rate measurement. From Fig 2 it looks like maybe not?

Our response: We clarified in section 2.3 and in the Figure 2 caption that all the 2009 ¹⁵N-labeled incubations were performed in duplicate (in Figure 2, we report the average values for duplicate measurements). Furthermore, as mentioned in section 3.2, we also performed parallel incubations with 2009 samples, with amendments of either ¹⁵N-labeled NO₃⁻ or NO₂⁻. These incubations generally yielded similar denitrification and DNRA rates, usually with less than 30% variation between the two series of incubations.

Reviewer's comment: p. 4192, line 16: What is the lower limit of detection for the rate measurements? i.e. is 0.5 nM d⁻¹ significantly different than zero? I think this is an important thing to calculate and discuss as some of the 'rates' are quite low.

Our response: The theoretical detection limit based on analytical accuracy and gas/water volume would be >50 pM d⁻¹ (p= picomoles). However, the actual detection limit was dependent on other factors related to sample handling and age etc, which is why we report relevant statistics based on a linear increase. The lowest denitrification rate of 0.5 nM d⁻¹ was measured at Easter Island in August 2008. $\delta^{29}\text{N}_2$ and $\delta^{30}\text{N}_2$

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linearly increased by 1.3‰ and 9.0‰ respectively, during the incubation period, which is well within the range of average precision of the measurements (i.e. 0.05‰ for $\delta^{29}\text{N}_2$ and 2‰ for $\delta^{30}\text{N}_2$, as reported in section 2.3, and see Fig. 2 (a, and b)). Furthermore, the error propagation of the standard error of the slope of the linear relationship was 0.2 nM d⁻¹ (see Fig. 3). We added a sentence to better explain these points in section 3.2.

Reviewer's comment: Section 3.3 title terminology is correct (16S rRNA gene) but the text of this section uses the not-quite correct term rDNA

Our response: We adopted a consistent terminology and changed all "16S rDNA" to "16S rRNA gene".

Reviewer's comment: p.4193 line 10: in order of abundance, not importance

Our response: Changed!

Reviewer's comment: Why are rates reported as N per L in the Results, but as N per kg in the Discussion?

Our response: We now report all rates in N L⁻¹ day⁻¹ in the Results and Discussion, to avoid confusion. We also removed this part of the sentence in the discussion: "and were up to 22 nmol N L⁻¹ day⁻¹ in the Black Sea (Fuchsman et al., 2008; Jensen et al., 2008)" (line 22, p.4195). In their paper, Jensen et al. (2008) are referring to anammox rate only, not denitrification rate.

Reviewer's comment: p.4199, line 2: would it be more correct to refer to SUP05 as a clade of bacteria?

Our response: Yes! We have changed the text accordingly.

Reviewer's comment: Section 4.3: There were no data presented here on the abundance of denitrifiers, only abundance of SUP05, so how can this section be about 'environmental controls on denitrifier abundance?'

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Our response: We changed the title of this section to: Environmental controls on denitrifier (and anammox) activity and abundance. True, only SUP05 abundances are presented, but the term "denitrifiers" is inclusive, and we decided not to go with a more constrained subtitle.

Reviewer's comment: Fig. 6: It would be a lot more useful to have the actual gene abundance on this figure, rather than a percent of total community.

Our response: We disagree. It is more quantitatively accurate to normalize the SUP05 abundance with respect to the total bacterial abundance (from 16S rRNA gene abundance), which, in part, depends on the absolute amount of DNA extracted at each site. Furthermore, since bacterial abundances (in copies/mL seawater) were highly variable among vent sites, it is more informative to express SUP05 abundances as a percent of total bacterial community, allowing us to directly compare the different vent sites. Other microbiological studies have also used this approach to compare their qPCR results (e.g. Chon et al. (2009), and Huber et al. (2010), listed in our reference list). We will however include a table with actual gene abundances (in copies/mL seawater) for total bacteria and SUP05 bacteria in the Supplementary Materials (Table S1) in the final version of the manuscript.

Reviewer's comment: Fig.7: reporting r2 and p values out to four decimal places is not really appropriate. Also, non-parametric rank correlations would probably be more appropriate for this data set, especially panel b.

Our response: We reduced the number of decimals. We also calculated Spearman's rank correlations for non-parametric data instead of Pearson's correlation for all our data and changed the text accordingly. Indeed, overall significance was reduced. Since the positive linear relationships observed between the DNRA/denitrification ratio and nitrate deficit were no longer significant, we removed this consideration from the Results and Discussion sections. The weak linear relationship between N₂O and SUP05 bacteria relative abundance was also no longer observed when using Spearman's rank

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correlation, so we also removed this statistic from the Results.

Technical comments: p.4180, line 22: bacteria and archaea p.4187 line 8: μM should be μm p.4193, line 16: missing a 'the', should be 'the 16S rRNA . . .' p.4195: should be 'productive' not 'production' p.4197, line 9: a few too many 'generally' p.4199, line 1: should be rRNA gene p.4199, line 2: proteobacterium p.4201: a small style point: three sentences on this page alone start with 'However, . . .' and it is also used very frequently throughout the manuscript. Besides being repetitive, it is not grammatically correct p.4216, table 2 caption: should be rRNA gene

Our response: We corrected all these technical points in the new version of the manuscript.

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