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Biogeosciences Discussions

Interactive comment on "Distribution of typical denitrifying functional genes and diversity of the *nirS*-encoding bacterial community related to environmental characteristics of river sediments" *by* S. Huang et al.

S. Huang et al.

huangsh3@mail2.sysu.edu.cn

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Dear Editor:

We are submitting the revision of the manuscript, titled "Distribution of typical denitrifying functional genes and diversity of the nirS-encoding bacterial community related to environmental characteristics of river sediments" by Shan Huang, Chen Chen, Xunan Yang, Qunhe Wu, and Renduo Zhang. We greatly appreciate the reviewers' comments

and suggestions about the manuscript, which indeed assist us to improve the quality of the manuscript significantly. Based on the comments and suggestions, we have revised the manuscript. The response to the reviewer's comments was summarized as follows.

Referee 1: General comments: -Throughout the manuscript, the presence of genes potentially involved in denitrification is interpreted as a measure of denitrification activity (e. g., p. 5252, l. 7-8; p. 5267, l. 13-17), however, denitrification rates or transcriptional activity of these genes were not investigated in this study. Here, the authors need to be more careful in their assumptions and conclusions.

Response (R): According to the reviewer comments, we revised some sentences to make them more accurate (e.g., L31-34, L455-465). Denitrifying gene abundance was clearly related to potential denitrification ability of microbes, which has been shown by several studies (Wallenstein et al., 2005; Bulow et al., 2008; Dang et al., 2009). In this study, we determined gene abundance but not its transcriptional activity for two reasons. First, long term environmental effects mostly control distribution and diversity of DNA; otherwise mRNA may mirror some short term environmental changes, such as substrate addition. As a result, gene numbers are more appropriate when studying the relationship between bacterial community and environmental characteristics. Secondly, it is difficult to extract and amplify RNA in the sediment, therefore, RNA's coverage and measurement accuracy are much lower than those of DNA (Bulow et al., 2008).

-Their data clearly demonstrate the genetic potential for denitrification in the different sediment layers or at the different sites; however, this does not necessarily mean that the process takes places, especially since a lot of these organisms are facultative denitrifiers. Here, passages in the introduction and in the discussion part that deal with the relationship between presence of functional genes and assumptions about activities should be rephrased to point out more clearly that the intensity of biogeochemical processes cannot directly be inferred from the molecular data. Here, more investigations

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would be needed to find out if all of these potential denitrifiers are actually playing a role in the process in situ, and to gain insight into the relevance and intensity of the process itself.

R: The issue was addressed in several paragraphs (L387-399, L455-465). We agreed with the reviewer that denitrifying gene appearances may not necessarily mean the processes take places. However, it is reasonable to believe that the environment, in which denitrifiers settle down and have great abundance, should promote the related biogeochemical processes. Currently, we are performing additional studies to find out if these denitrifiers actually participate in the process.

-Canonical Correspondence Analysis certainly is a nice tool to bring data of community composition and environmental parameters together, however, it was originally designed to analyze plant communities where data elevation differs from the clone library data used here in this study. When the authors are using the frequency of certain sequences in their libraries as analogues to the fraction of a certain species in a community, they should keep in mind that the coverage of their clone libraries ranged from only 65 to 86

R: The number of clone in a library was decided by the coverage and rarefaction analysis. Thus, the data should well represent the real composition of the in situ communities. In fact, the coverage values were around 75

Specific comments: [1] Sequence similarities to genes obtained in certain environments may point to a similar origin of the sequences found in this study, however 78

R: In this study, sequences with identities higher than 90

[2] This paragraph should be shifted to the methods section. (p. 5260, l. 13-24)

R: Done. (L169-181)

[3] These two sentences provide redundant information. (p. 5261, l. 7-9)

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R: Done. (L278-279)

[4] The authors provide some decent references for their statement; nevertheless I think that conclusions regarding denitrification activity should be handled more carefully. (p. 5261, l. 13-15)

R: This passage has been revised. (L283-286)

[5] This has not directly been tested. So far, the authors have only shown some statistical correlation between gene abundances and environmental parameters. (p. 5263, l. 25-27)

R: The statistical correlation should be a way to reflect the relationship between the distribution of gene and environmental factors. (L354-356) More information about the gene transcriptional activity and response to substrate addition was under investigation.

[6] The authors should provide more explanations for the independent variation of the different functional genes involved in denitrification. Organisms capable of complete denitrification should harbor all these genes. How can the strong differences in gene abundances and in the correlation of individual genes with environmental parameters be explained? Have the authors taken into account that genome copy numbers of the different functional genes may differ among genes and organisms? (p. 5263, l. 25-27 and p. 5264, l. 1-2 and other places in the manuscript)

R: Organisms may not capable harbor all these genes, each bacterial species may participate in only one step of the denitrification process (Burgin and Humilton, 2007). These reductase genes are generally used as makers of the denitrification steps because an approach involving 16S rRNA is not suitable to investigate diverse communities of denitrifying bacteria (Zumft, 1992; Braker et al., 2000). Gene numbers is a different index from abundance of organism. Therefore, in this study, we focused on denitrifying gene abundance. (L47-68)

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[7] What does this sentence mean? (p. 5264, l. 13-14)

R: The sentence was changed to: “Various denitrifier communities were found among the different locations and sediment depths”. (L371-372)

[8] Please be more precise. (p. 5264, l. 19-20)

R: Done (L 378-380)

[9] In this passage, the authors mix information from literature, their own results, and some aspects of the outlook. It is a bit difficult to follow their ideas here. (p. 5265, l. 1-14)

R: This passage was carefully revised. (L387-399)

[10] It is not the genes that have different substrate requirements but the enzymes. (p. 5265, l. 18)

R: The sentence was changed to: “The enzymes encoded by nirS and nirK require different substrates.” (L403-404)

[11] This conclusion is too generalized. (p. 5266, l. 1-3)

R: This sentence has been deleted.

[12] How do the authors know about the physiologies of the different organisms, only functional gene sequences are available? Are these assumptions based on sequences that were closely related to cultured organisms? (p. 5266, l. 11-17; l. 27-29)

R: Physiologies of the different organisms were based on comparison of our data with the gene bank and also some isolation studies in the literatures (Jung et al., 2007; Bulow et al., 2008; Martins et al., 2010).

[13] Here the authors should point out more clearly that these are assumptions based on the results of other studies. Oxygen concentrations were not measured in this study, so it is difficult to say in which depth there are optimum conditions for the different

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processes or nitrate reduction pathways. (p. 5267, l. 13-22)

R: In our previous study, the concentration of dissolve oxygen in the same site was 0.30 mg L-1 in the water-sediment interface and decreased rapidly to 0.01 mg L-1 within 10 cm sediment (Huang et al., 2011). (L120-121)

[14] This is likely to be the case but has not been proven. Here, the authors should be a bit more careful. (p. 5267, l. 25-26)

R: The sentence was changed to: “Organic carbon was the primary electron donor for the respiratory denitrifying bacteria (Burgin and Hamilton, 2007)”. (L468-469)

[15] Has this been proven experimentally? (p. 5267, l. 29; p. 5268, l. 1)

R: The relationship between distribution of denitrifier clusters and NO2- concentrations were analyzed statistically.

[16] The language needs revision in some places.

R: The manuscript has been revised to eliminate all grammar errors and to enhance writing style.

We hope now that that manuscript is publishable in Biogeosciences. Thank you for your consideration on our manuscript. Best regards.

Sincerely yours,

Shan Huang, Ph.D.

CC: Chen Chen, Xunan Yang, Qunhe Wu, Renduo Zhang

Interactive comment on Biogeosciences Discuss., 8, 5251, 2011.