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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.

Anonymous Referee #1

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In this study the authors investigated the denitrifying bacterial community in river sediments using the functional genes narG, nirK, nirS, nrfA, and nosZ involved in denitrification or DNRA as molecular markers. The depth-dependent distribution of all of these genes over a depth profile of 25 cm was investigated using quantitative PCR while the analysis of denitrifier diversity focused on nirS only. The manuscript addresses the important topic of nitrogen cycling in river sediments, which is especially relevant where river ecosystems are threatened by eutrophication as a result of human impact and where mechanisms of nitrate removal are of special interest. So the overall topic is

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certainly of interest to the readership of this journal. The introduction covers all the relevant aspects and also gives a good overview of the molecular markers used in this study. Moreover, the manuscript provides nice data about the distribution of the selected marker genes in river sediments indicative of the presence of potentially denitrifying microorganisms, which also correlated with some key environmental parameters such as nitrate or organic carbon content as one would expect.

However, I have some concerns regarding the interpretation of the data. 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. 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 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.

Another issue that the authors should pay attention to is the statistical analysis of the data. 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

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only 65 to 86 % (p. 5261, l. 24-25), and that the frequency of a certain sequence in a library may be affected by PCR or cloning bias. So there are some uncertainties about how representative the data are of the real composition of the in situ communities. Here, the authors should at least comment on the potential errors or uncertainties associated with the statistical analysis of clone library data as performed in this study.

Moreover, some parts of the discussion should be reorganized to avoid redundancies.

Specific comments:

p. 5252, l. 11-14: Sequence similarities to genes obtained in certain environments may point to a similar origin of the sequences found in this study, however 78 % sequence identity as the lower range given is not very high. Sequences might just as well share a certain degree of identity without necessarily implying restriction to certain habitats.

p. 5260, l. 13-24: This paragraph should be shifted to the methods section.

p. 5261, l. 7-9: These two sentences provide redundant information.

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

p. 5263, l. 25-27: 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 and p. 5264, l. 1-2 and other places in the manuscript: 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?

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p. 5264, l. 13-14: What does this sentence mean?

p. 5264, l. 19-20: Please be more precise.

p. 5265, l. 1-14: 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. 18: It is not the genes that have different substrate requirements but the enzymes.

p. 5266, l. 1-3: This conclusion is too generalized.

p. 5266, l. 11-17; l. 27-29: 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. 5267, l. 13-22: 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 processes or nitrate reduction pathways.

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

p. 5267, l. 29; p. 5268, l. 1: Has this been proven experimentally?

The language needs revision in some places.

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