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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.

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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 2 General comments: -The diversity and abundance of functional genes are not directly related to the functions of the ecosystems, which are performed by proteomes and are more related to gene expression and post-transcriptional processes. So any direct functional speculation based only on the diversity and abundance of functional genes should be avoid. For an instance, nir gene can NOT represent the denitrification rate (result 3.2). Check throughout the manuscript.

Response (R): According to the review comments, we revised some sentences to be more clearly (e.g., L31-34, L455-465). Denitrifying gene abundance was obviously related to the potential denitrification ability of microbes, which has been demonstrated by several studies (Wallenstein et al., 2005; Bulow et al., 2008; Dang et al., 2009). Gene numbers were more significant than gene expression when studying the relationship between bacterial community and environmental characteristics.

-The main molecular tool used in this manuscript is Q-PCR, but the quality control was not included. The detection limit should be reported. For the SYBR based QPCR, the specificity of the primers for all the functional genes should be tested and melting curves should be included either in the main paper or in a supplementary material.

R: These primers were tested by several researchers (Harms et al., 2003; Bowman et al., 2004; Chon et al., 2009; Ye et al., 2009), and also suitable for our studies. Melting curve analysis for SYBR Green assay was prepared after amplification to distinguish the targeted PCR product from the non-targeted PCR product, and results show unimodality. (L165-167)

Specific comments: [1] The language should be checked by a native English-speaking person, a lot of parts were quite redundant.

R: The language has been checked to eliminate grammar errors and redundancy.

[2] If the methods used in this study have been published in previous papers, no need to repeat all the detailed steps, check throughout.

R: The method and material section was condensed.

[3] Which sample was used for QPCR should be described (p,5256)

R: Each sediment core was sectioned into 5 cm slices (0-5, 5-10, 10-15, and 20-25 cm,). All samples were used for the QPCR experiments. (L124-129)

[4] DNA extraction efficiency should be considered and the quality of DNA (conc, 260/280) should be tested. (part 2.3)

R: We tested the quality of DNA with the value of OD 260/280, which was all around 1.8. The DNA was also quantified by image analysis on a 1

[5] Why only nirS was selected for clone library analysis? (nosZ gene is also a important gene related to N2O emission, part 2.5)

R: The reaction, in which NO2- is reduced to nitric oxide (NO), distinguishes denitrifier community from other nitrate-reducing bacteria. NirS genes characterized this key step and were significant in our study sites, therefore, were selected to build the clone library (Cole et al., 2004; Heylen et al., 2006). The nos genes are usually used for N2O and N2 emission research (Philppot, 2002), which is not the issue to be dealt with in this study.

[6] Page 5262, line 4-6, based on which threshold the sequences were clustered?

R: Clusters in the phylogenetic tree were divided by class and function. (L305-306)

[7] Page 5265, the lower abundance of nirK does not mean the nirK-containing group is not functionally important, more information about the gene expression and proteome should be considered before drawing any conclusion.

R: “In this study, both nirK and nirS were detected in the Pearl River sediment. However, nirK abundance was much lower than nirS abundance in the sediment because nirK only prevails in the conditionally oxygen-exposed environment (Desnues et al., 2007; Knapp et al., 2009). The enzymes encoded by nirS and nirK respectively require different substrates. Particularly environmental conditions may alter the proportion of denitrifiers with nirK or nirS. Moderate levels of NO3- in the river sediment increase nirS diversity (Yan et al., 2003). The nirS gene is found to be more widespread in the bacterial communities in various sediments (Priemé et al., 2002; Liu et al., 2003; Throbäck et al., 2004; Tiquia et al., 2006; Oakley et al., 2007; Dang et al., 2009). Therefore, we used the nirS gene to characterize the diversity of denitrifier communities in the Pearl River sediment.” (L400-410)

[8] Coverage was calculated to show the quality of the clone libraries, on which criterion the number of clone in a library was decided? Was it by coverage or rarefraction analysis? If by the latter, the rarefraction curves should also be included in the paper.

R: The number of clone in a library was decided by coverage and rarefaction analysis. The coverage and clone coverage were all more than 70

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.