

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 #2

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The biological mechanism of denitrification is of great ecological significance since this process is the most dominant source of N₂O emission, which is proved recently to be the single most important ozone-depleting substance. Huang's paper entitled 'Distribution of typical denitrifying functional genes and diversity of the nirS-encoding bacterial community related to environmental characteristics of river sediments' investigated the functional groups of the denitrification by analyzing the abundance of functional genes and found the spatial distribution of the denitrifiers is correlated with the dissolved inorganic N. These data are of ecological significance for understanding the mechanism of

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denitrification in the point of view of microbial ecology and molecular ecology.

main points:

Basically the most important part of this manuscript is to investigate the functional gene diversity and distribution. 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 avoided. For an instance, nir gene can NOT represent the denitrification rate (result 3.2). Check throughout the manuscript.

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.

There are additional points requiring attention by the authors:

1. The language should be checked by a native English-speaking person, a lot of parts were quite redundant.
2. If the methods used in this study have been published in previous papers, no need to repeat all the detailed steps, check throughout.
3. Which sample was used for QPCR should be described (p,5256)
4. DNA extraction efficiency should be considered and the quality of DNA (conc, 260/280) should be tested. (part 2.3)
5. part 2.5, why only nirS was selected for clone library analysis? (nosZ gene is also an important gene related to N₂O emission)
6. Page 5262, line 4-6, based on which threshold the sequences were clustered?

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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.

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 rarefaction analysis? If by the latter, the rarefaction curves should also be included in the paper.

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