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Comment

## ***Interactive comment on “NirS-containing denitrifier communities in the water column and sediment of the Baltic Sea” by S. Falk et al.***

**Anonymous Referee #3**

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This study targets the diversity of denitrifying bacteria by analyzing one of the two nitrite reductase genes involved in dissimilatory nitrite reduction. The objectives are not clearly laid out and the authors make sweeping statements without much evidence. The major objectives appear to be, to find the difference between the denitrifying communities in the sediment and water column. They come to the conclusion that distinct marine nirS-type denitrifier communities occupy different ecological niches which are defined by their habitat, water column or sediment, shaped by the prevalent environmental conditions, and can be isolated by large geographic distances.

Contrary to their conclusion, their results show that there are few TRFs exclusively found in the water column and few exclusively in the sediment and the rest overlap between the two habitats. Again, with their phylogenetic analysis of nirS gene from the

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Baltic Sea and sequences from marine habitats from all over the world, they report that it indicated distinct denitrifier communities that grouped mostly according to their habitat. By habitat they mean sediment or water column. Closer look at the phylogenetic relationship in Fig.4 does not show any such groupings. Of the seven clusters defined in Fig.4 all the seven clusters have sequences from both sediment and water column. Cluster VII alone has sequences predominantly from water column, but this cluster also has sequences from the Pacific NorthWest sediment. Again, there appears to be no clustering in Fig.4 by geographical location. So, in fact, neither their TRFLP analysis nor the phylogenetic analysis shows any distinct separations between sediment and water nor geographical distance. Hence their main conclusion is not justified.

The method used in this study is partly sequencing and partly TRFLP analysis. Sequence data is also available from the water column from this region from earlier studies. However, there is no attempt to assign the TRFs to the possible nirS sequences from the water column as well as the sediment, which would have made the study more relevant. The authors fail to follow the conventions in naming the genes, where the gene names should always start with a lower case letter; very often the authors capitalize the n in nirS. Phylogenetic trees do not give any confidence, unless one knows what the bootstrap values are for each branching, the authors have failed to do this, the trees presented are without any Boot Strap values. The manuscript is poorly written and is often not clear what the authors mean to state. The authors have made no attempt to amplify nirK gene and report that few studies reported failure in amplifying this gene. Occasional failure by previous workers to amplify nirK genes from the environment is no excuse for not attempting to amplify this gene from a different environment. nirS gene alone do not complete the denitrifier community, and failure to amplify a gene does not make the organisms that possess that gene irrelevant in the environment.

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