

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

Anonymous Referee #2

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This manuscript examines and compares denitrifying microbial communities across environmental gradients within the water column (Gotland Deep) and coastal sediments (Rassower Strom) of the Baltic Sea. The cultivation-independent approach employed relies on the use of the *nirS* nitrite reductase gene as a molecular marker for denitrifiers – an approach that has been used extensively in many previous studies. Unfortunately, no *in situ* denitrification rate or even ‘denitrification potential’ measurements were performed as part of this study. Furthermore, only DNA (and not RNA) samples were analyzed, so the link between *nirS*-based denitrifier diversity and the actual process of denitrification is fairly weak.

What is particularly curious about this study is why the authors chose to compare the denitrifying communities in water column and sediment samples collected 550 km away

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(and over 1 month apart)? By the way, since when is 550 km considered geographically close? It is stated that these environments share ‘comparable physico-chemical gradients’, but expecting to see major similarities in community structure between these disparate environments seems questionable at best. Not surprisingly, shifts in denitrifier community composition along these ‘gradients’ were observed ONLY in the water column, while the communities were fairly uniform in the upper 3 cm of sediment.

The recent study by Hannig et al. (2006) examined *nirS*-based denitrifier community structure in the water column of two stations in the vicinity of the Gotland Deep station examined in the present study. The primary difference between these two studies is that the Hannig et al. paper (which has 3 overlapping authors with this study) focused solely on water column communities and included more rigorous statistical analysis attempting to link community structure to biogeochemical parameters. With that information in mind, I would have expected to see this additional Baltic Sea water column study take it to the next level and actually employ quantitative (e.g., qPCR) or mRNA-based (e.g., RT-PCR) approaches to gain further insights into these denitrifying communities. Instead, this study just adds the dimension of a sediment/water column comparison, which is much less informative.

I am a bit concerned by the finding that ‘two dominant T-RFs (36 and 111-bp) were found in all layers of the sediment and were identical to T-RFs dominating the oxygenated and the suboxic zones of the water column’. While this is by no means impossible, the Hannig et al. (2006) paper indicated that these T-RFs actually correspond to multiple sequence types distributed widely throughout the *nirS* phylogenetic tree. Thus, unless a clone library is generated and exhaustively sequenced for every sample for which T-RFLP is applied, it is not possible to definitively rule out that these T-RFs may correspond to more than one distinct *nirS*-containing denitrifier. Thus, T-RFLP may severely oversimplify our view of denitrifier community structure in the environment.

I did not realize that oxygen concentrations of 10-50 μM were considered ‘suboxic’? I thought the working definition of ‘suboxic’ was $<10 \mu\text{M}$ or $<5 \mu\text{M}$ in such environments.

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One unfortunate shortcoming of the present study is that no attempt was made to examine the ‘functionally equivalent’ *nirK* gene in these samples. Because previous studies using inappropriate *nirK* primers were met with little success, many studies (like this one) now choose to focus solely on *nirS*. As a result, there is now an extremely large database of *nirS* sequences from marine water column and sedimentary environments (which is great), but a severe lack of *nirK* sequences from such systems (which is bad). It would be quite interesting and worthwhile to apply some of the recently described *nirK* primers to these Baltic Sea samples.

Much to my surprise, there are a number of *nirS* database sequences previously reported from marine/estuarine sedimentary systems that were NOT included in the comparative phylogenetic analysis in this paper. In particular, the *nirS* mRNA clones obtained by RT-PCR from sediments of two sites within the River Colne Estuary (Nogales et al., 2002) are particularly pertinent to the ‘brackish’ sediment sample in this study. In addition, there are a number of relevant sequences from a recent study of a coastal beach aquifer (Santoro et al., 2006), which examined denitrifier communities across environmental gradients (this paper was even cited in this paper). Considering that this Baltic Sea study is purposely ‘*nirS*-only’, a more thorough comparison with additional relevant environmental sequences (especially from sediments) seems warranted. Finally, although referred to repeatedly as a ‘marine’ environment, the salinities of the two Baltic Sea sampling sites are 7-9 psu and 7-13 psu, which is brackish at best. Thus, comparisons with *nirS* sequences from marine/estuarine systems spanning a wider range of salinities is recommended.

The dates are incorrect for several of the citations (Page 710, lines 9-12) of previous *nirS* diversity papers.

Why have no attempts been made to obtain *nirS* sequences from *Thiomicrospira denitrificans* isolates. This seems highly relevant, especially considering the extensive speculation that these types of autotrophic denitrifiers are so important at the suboxic-sulfidic interface in the Baltic Sea.

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