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Biogeosciences Discussions

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

S. Falk et al.

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We acknowledge the referees' comments. Several issues were addressed by all three referees and will be commented first. Comments on specific issues mentioned by the individual referee are addressed as specific comments at the end of the text.

General comments

The aim of this study was to comparatively evaluate denitrifier community composition from two brakish water habitats, water column and sediment. Several studies have explored marine *nirS*-type denitrifier communities in either of these two habitats (Braker *et al*., 2000; Jayakumar *et al*., 2004; Liu *et al*., 2003; Castro-Gonzalés *et al*., 2005; Hannig *et al*., 2006). Phylogenetic analysis of *nirS* sequences in all these studies revealed that both habitats were largely dominated by novel *nirS* genotypes from as yet unknown denitrifiers. In addition these genes were mostly grouped into subclusters

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according to the habitat from which they were derived (Braker *et al*., 2000; Jayakumar *et al*., 2004; Castro-Gonzalés *et al*., 2005). Thus the structure of the respective denitrifier communities seems to be determined at least in part by habitat specific parameters. Whether there are intrinsic factors of water column or sediments that lead to the development of distinct microbial communities is unknown. Differences in water column versus sedimentary denitrification regime or separation of microbial communities by large geographic distances were speculated to drive the development of *nirS*-type denitrifier communities (Jayakumar *et al*., 2004). However, which of these drivers has a major impact could not be resolved from the datasets available to date.

Sampling locations in previous studies that focused on either water column or sediment denitrifier communities were separated by large geographic distances of thousands of kilometres, e.g. from the Pacific Northwest (Braker *et al*., 2000), the eastern tropical North Pacific (Liu *et al*., 2003), the eastern South Pacific (Castro-Gonzalés *et al*., 2005) or even by continents as for the Arabian Sea (Jayakumar *et al*., 2004) and the Baltic Sea (Hannig *et al*., 2006). In this study sampling sites were included which were located only 550 km apart from each other. We agree that this is a large distance from microbial scales. We are also aware that environmental conditions such as nutrient status but not salinity (Bodden sediment, 7 to 9 psu; Gotland deep water column, 7 to 13) were different between the two sites we sampled. In contrast to the Baltic proper, coastal areas are affected by e.g. high riverine nitrogen inputs which lead to high denitrification N-losses (Voss *et al*., 2005), but summertime denitrification rates were low for the coastal area included in our study (Dahlke, 1990). However, we still consider our experimental sites to be suitable for this type of study since comparable chemical gradients within water column and sediment can never be found at the same site. In addition, a distance of this extent is rather small compared to those between locations of the sampling sites of other studies and second, the Baltic Sea is a semi-closed system with limited exchange with the North Sea water masses. This suggests a rather strong geographic separation of communities by the geographic realities over extended periods of time. However, within the central Baltic water masses are exchanged hor-

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izontally between coastal areas and the central Baltic and vertically between water column and sediment. Despite the fact that the Baltic proper may represent a closed circulation cell (Voss *et al*., 2005) approximately once in a decade an inflow of North Sea water occurs such as those in 2003. Feistel *et al*., 2004 described the hydrographic situation after the inflow of water masses into the central Baltic from the Darss Sill (in proximity to our sampling site at the Rassower Strom) area via the Bornholm Deep to the Gotland Basin in summer 2003. Their results indicate successive changes in bottom water temperature and salinity along this transect. Together with local currents this suggests that not only water masses are exchanged but also microbial communities thus allowing a comparison of the denitrifier communities at these locations. This assumption is also supported by the occurrence of *nirS* subclusters which comprised clones from both locations of the Baltic Sea (Fig. 5).

We focused on *nirS*-type denitrifiers on purpose since comparably few data sets were available on *nirK*-type denitrifiers, i.e. from sediments of Puget Sound and the tropical eastern North Pacific off Mexico. No water column *nirK* sequences were generated in any other study thus making a comparison between the respective communities from distinct geographic locations impossible. Whether the lack of *nirK* sequence information from the water column is due to researchers focussing exclusively on *nirS*-type denitrifier communities or is a result of failure to detect *nirK*-type denitrifiers by the molecular methods used currently is unknown. Failure to amplify *nirK* from sediments was indeed described for samples obtained from Puget Sound (Braker *et al*., 2000) and no transcripts of *nirK* genes could be detected in sediment samples of an estuary (Nogales *et al*., 2002). We also restricted ourselves to exploring the genetic potential for denitrification of water column and sediment communities since our attempts to use mRNA based analyses to eventually link structure and function of these communities failed. From our experience denitrification genes under natural conditions are most likely not continuously expressed but only if the respective organisms are sufficiently supplied with nitrogen and carbon sources as electron acceptors and donors, respectively. Since the genetic potential of only part of the community, the *nirS*-type

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denitrifiers, was addressed in our study denitrification activity or potential denitrification could not be linked directly to community structure and was therefore not measured.

Specific comments

Referee 1:

Replicate DNA extractions, PCR amplifications and T-RFLP analysis were performed from replicate samples from the same sediment cores resulting in highly repetitive T-RFLP patterns. Additional sediment samples taken in June 2003 were analysed showing also rather similar *nirS* T-RFLP patterns which indicates rather stable communities over time.

Similarly, the communities from the Gotland Deep water column in August and October 2003 are comparable to the communities in August 2004. The main T-RFs (36 bp, 47 bp, 111 bp) were found in both years and also the characteristic T-RF of the sulphidic zone (296 bp in 2003 and 295 bp in 2004). The main difference between both years is the reduced number of T-RFs in oxygenated water layers (up to 17 in 2003 and up to 8 in 2004) in 2004.

The lowest level of identity (45%) of any clone from marine habitats was found for the pairwise comparison of 166 amino acids of clone wA20 to *nirS* from isolate B9-12.

The conclusion that in 'sediments with comparable environmental conditions similar *nirS*-type denitrifier communities can develop despite large geographic distances' was not drawn from the T-RFLP patterns as interpreted by the referee but from the analysis of sequences from Baltic Sea and Puget Sound sediment. Most of these sequences cluster as sister groups within Cluster I. We therefore think that this conclusion is valid.

Referee 2:

Capitalizing *nirS* was only done at the beginning of a sentence which is in agreement with conventions of at least some other journals. Apart from these positions we do not find any disagreement with the conventions.

Bootstrapping is comparably easy to perform but its value is still discussed (Soltis and

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Soltis, 2003). Therefore we chose to calculate *nirS* trees using distance matrix based neighbour joining, parsimony methods and maximum likelihood procedures. Whenever, the grouping of a given group of sequences was supported by all three algorithms these sequences were considered to be consistently grouped together and were designated as clusters (Cluster I to VII) and subclusters which were indicated by polygons. Phylogenetic analysis of *nirS* genes as shown in Fig. 4 indeed shows overlap of water column and sediment sequences within clusters but they were not grouped together in common subclusters except for sequences from both habitats in some but not all subclusters within the Baltic Sea in Cluster I. This shared Cluster I may be a result of the geographic proximity of our sampling sites. In addition, one subcluster within Cluster I was found to contain sequences from the Arabian Sea water column, from Baltic Sea sediment as well as from the Pacific Northwest.

We agree that T-RFLP results should be considered as fingerprints of communities and may oversimplify our view of community structure. Therefore we cloned *nirS* genes from samples from different depths from both habitats for more specific insights. Our results indicate that at least for the 111-bp T-RF there some overlap of clones from both habitats in Cluster I, but we agree that the 36-bp T-RF was not found in clusters common to water column and sediment of the Baltic Sea. T-RFs of the clones could be added to a revised version of the tree.

Concerning suboxic conditions in the Baltic Sea, there is no formal or consistent definition of suboxic conditions as stated in Adams *et al*., 2005 (http://geoinfo.nmt.edu/staff/mclemore/documents/adams_sme.pdf). However, Murray *et al*., 1995 defined the suboxic zone in the Black Sea as the biochemical transition zone between the oxic surface layers and the the sulfidic deep waters where oxygen and hydrogen sulfide do not overlap. Adams *et al*., 2005 specified suboxic conditions between >10 to <30 μM of dissolved oxygen which is in the range we found in the zone of the water column that was defined as suboxic for the Baltic Sea.

Referee 3:

We did not include sequences published for the study by Santoro *et al*., 2006 since

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they were derived from a coastal aquifer, which is a habitat subjected to conditions clearly distinct to those found in marine water column and sediments. However, we agree that we could have included sequences from the River Colne estuary sediments. This could be done in a revised version of the manuscript.

We have added the now published sequence of *Thiomicrospira denitrificans* to our alignment which was not available when the manuscript was prepared to our *nirS* tree. However, according to our alignment it does not cluster closely to any other known sequence and consequently also not to one of our clones. Generally it is difficult if not impossible to infer phylogenetic relationships of denitrifiers from a *nirS* sequence.

Interactive comment on Biogeosciences Discuss., 3, 697, 2006.