

Diversity and dynamics of protist communities in subtidal North Sea sediments in relation to metal pollution and algal bloom deposition

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The overall objective of this study was to obtain a better insight in the interactions between microbial communities, metal contaminants and algae-derived organic matter in subtidal, metal-contaminated sediments in the Belgian Coastal Zone (BCZ). The BCZ is characterized by extensive, annual spring phytoplankton blooms, and it has been estimated that 24% of the phytoplankton production is deposited onto the sediments. Remineralisation of this phytodetritus has been shown to lead to effluxes and hence bioavailability of trace metals (TM). The activity of benthic microbial organisms (pro- and eukaryotes) has been pointed out as one potential factor acting upon the release of TM into the water column. It remains unclear however, how microbial communities, phytodetritus deposition and TM behaviour interact. In order to predict/understand the response of marine ecosystems to metal contamination and algal bloom deposition, good baseline knowledge of the inhabitants of this environment is required. During this study, we focussed on the protist (~unicellular eukaryotes) communities, as these are largely understudied compared to bacterial, meio- and macrobenthic communities, despite their important role in the benthic food web, the functioning of the microbial loop and benthic-planktonic coupling of cycles of matter and energy.

During two field campaigns (in 2007-2008), we studied the diversity and spatio-temporal variation in diversity and community structure in the subtidal sediments of the BCZ in relation to a suite of physical, chemical (incl. TM) and microbiological parameters. The *in situ* studies were complemented with laboratory experiments in which sediment cores were incubated under various environmental conditions, viz. with and without phytodetritus deposition, and with different arsenic (As) concentrations. This not only allowed giving a more detailed picture about the effect of phytodetritus deposition on protist community structure and trace metal mobilization, and their interaction, but also to assess the impact of different degrees of As contamination on protist community structure in these marine sediments.

First (Chapter 2 of the thesis) we described microeukaryotic community composition in 9 BCZ stations with different granulometries and TM loads, before and after the spring phytoplankton bloom and compared the upper sediment layer (0-1) and deeper (9-10cm) layers. Variation in community structure was studied using 18S rDNA-based DGGE fingerprint analyses of microeukaryotic communities, complemented with the construction of group-specific (ciliates and cercozoans) clone libraries. Our results showed that microeukaryotic diversity was dominated by Stramenopila (mainly diatoms), Metazoa and Fungi. Protozoan diversity was underestimated based on DGGE analyses with general eukaryotic primers, but the clone libraries using group-specific primers showed that especially ciliates were much more diverse. Our data revealed that sediment type was the prime variable determining microeukaryotic community structure, and its overriding influence probably masked temporal and depth-related variability. Metals were strongly correlated to the sediment characteristics (grain size, CHL α , silt content); the effect of the trace metals on community composition could not be distinguished from the differences in sediment type.

Then (Chapter 3 of the thesis) we performed a more detailed, monthly analysis of protist community variation with depth (0-10 cm) in one silty, metal-contaminated station, for the period February to July 2008 (including periods of intense algae blooms). Microeukaryote and protozoan community composition changed with depth (~ redox, sulphides and pH) and with time, from February to July (~phytoplankton bloom deposition), with especially May and July being distinct. Increased microbial mineralization caused pronounced changes in the redox environment and the bioavailable metal concentrations in the sediment, which correlated with the observed seasonal and vertical variation patterns in community structure. Eh and pH were the dominant factors structuring the communities, but trace metals as well had a significant, independent impact (14-19 %) on the variation in microbial community structure.

The effects of phytoplankton bloom deposition and TMs on the protist communities were analyzed in more detail in two microcosm experiments.

In the first experiment (Chapters 4 and 5 of the thesis), the deposition of a phytoplankton bloom was simulated in metal-contaminated sediment cores, and sampling was performed immediately (2h), 2 days and 7 days after the start of the experiment. We focussed on the effect of bacterial mineralization of the phytoplankton-derived detritus on the release of metals and also studied the effect of the phytodetritus deposition on the structure of the total and active bacterial and microeukaryotic communities (with focus on Protozoa), and assessed how this related to metal mobilization. This allowed giving a more detailed picture about the short-time response of benthic microbial communities to organic matter enrichment and metal contamination, as well as the potential role of microbial communities in metal mobilization.

We observed that the onset of mineralization (as revealed by CLPP analysis) was very fast and started within 2h after phytodetritus deposition. Increased bacterial production was observed after two days, while bacterial biomass was stable and probably controlled by heterotrophic nanoflagellates (HNF). A clear link was established between heterotrophic microbial activity and effluxes of the TMs As, Co and Mn. Furthermore, DNA- and RNA-based fingerprints of bacteria and Protozoa showed distinct changes in the total and active community structure as a consequence of the algal enrichment, and with time. The enrichment effect was most pronounced after 2 and 7 days for bacteria and Protozoa respectively. Our results suggest that phytodetritus deposition activates and stimulates the microbial loop, via changes in bacterial activity, biomass and community composition, together with subsequent changes in numbers and relative abundance of heterotrophic nanoflagellates and ciliates, and composition and activity of protozoan communities. No effect of the metal fluxes was observed for bacterial and microeukaryotic diversity (~ species richness). Calculations based on our results suggest that during phytoplankton blooms the microbial activity alone may induce the release of substantial amounts of dissolved As in areas of the BCZ covered by muddy sediments.

In the second microcosm experiment (Chapter 6 of the thesis) we studied the effect of As, a notoriously toxic TM, on active protist communities (based on 18S rRNA-DGGE analyses). Sediments from a metal-contaminated silty station and an unpolluted sandy sediment were spiked with a range of As concentrations ($0\text{--}960\mu\text{g L}^{-1}$) and incubated in the dark for 2 days. Diversity (~number of DGGE bands) significantly decreased at contamination levels $\geq 480\mu\text{g As L}^{-1}$ in both sediment types, but the decrease was more pronounced in the sandy (43%) than in the silty sediment (32%), suggesting higher tolerance to As contamination in the silty sediment. In addition, a significant shift in community composition occurred at contamination levels $\geq 120\mu\text{g As L}^{-1}$ and again at $\geq 480\mu\text{g As L}^{-1}$ in silty sediment. Surprisingly, the effect of As on protist community composition was not significant in the sandy sediment. Fungi responded most sensitively to high As concentrations, while only some ciliates increased in relative abundance with higher As levels. These included representatives from various classes such as the *Phyllopharyngea*, *Spirotrichea*, *Litostomatea* and *Colpodea*. The observed responses contributed to the interpretation of OTU-specific positive and negative correlations with several metals that had been found in chapter 3 and 5, as in natural environments, it was not possible to isolate the effect of the metals from that of the environmental variability. Indeed, trace metals often strongly co-vary with many physico and- biogeochemical parameters.

Finally (In Chapter 7 of the thesis), the results of the field campaigns and the microcosm experiments were integrated and discussed. An overview was given of the overall composition of the microeukaryotic communities in the subtidal sediments of the BCZ. Generally, Stramenopila, Alveolata, Fungi, Rhizaria, and Amoebozoa were the dominant groups, ecologically important as primary producers (mainly diatoms), grazers (Rhizaria, Alveolata, Amoebozoa, heterotrophic stramenopiles), saprobes (Fungi) or pathogen-parasites (e.g. Marine Alveolates Group I, several heterotrophic Stramenopila, e.g. *Pirsonia* sp.) in these sediments. The importance of spatial and seasonal variation in various environmental factors was demonstrated for shaping microeukaryote community structure and diversity, with a pronounced impact of sediment parameters and phytoplankton bloom deposition on the activity and composition of the communities. Finally, we can conclude that microeukaryotic communities in the sediments are affected by trace metals, as evidenced by both the field and experimental data.