Diverse active microbial communities in a tidal flat sediment as deciphered by a multidisciplinary approach

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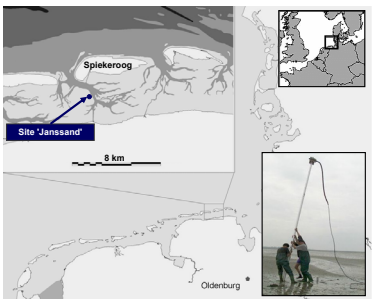
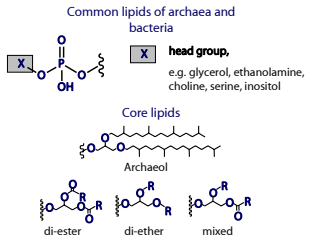
Introduction



Location of the sampling site in the backbarrier tidal flats of Spiekeroog island in the Northwest German Wadden Sea and sampling of a 6 meter long sediment core.

Most of the global prokaryotic biomass is presumably harbored in the marine subsurface. So far, the investigation of marine sediments mainly focused on the 'deep biosphere' at open-ocean and continental-margin settings but less attention was paid to the deeper layers of coastal sediments. In recent environmental studies, however, the community composition of subsurface tidal-flat sediments was analyzed using a cultivation-based approach (Köpke et al., 2005) or the phylogenetic characterization of bacterial communities was targeted with molecular biological tools (Wilms et al., 2006).

Here, we present a multidisciplinary approach to analyze the vertical distribution of active microbial populations in the upper meters of a tidal flat sediment. A combination of complementary geochemical, molecular biological and microbiological methods was applied to investigate the activity and composition of microbial communities. One of our analytical approaches presented here is the analysis of **intact polar lipids (IPLs)** using HPLC-ESI-MS.

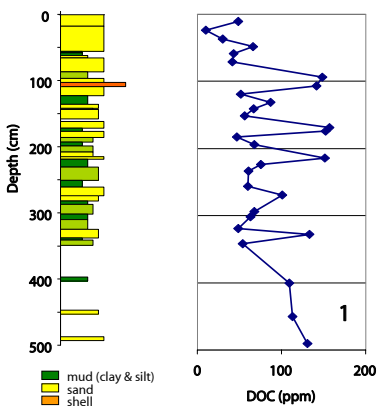


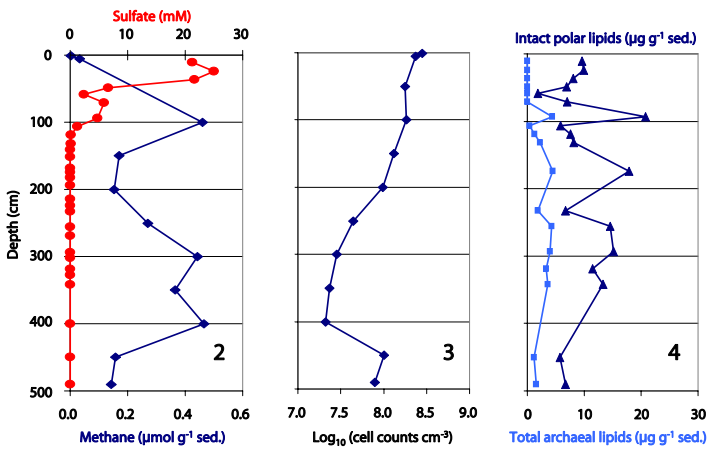
General chemical structure of phospholipids. These biomarkers can be analysed using HPLC-MS or indirectly *via* their fatty acid side chains (**PLFA**) by GC or GC-MS.

Results

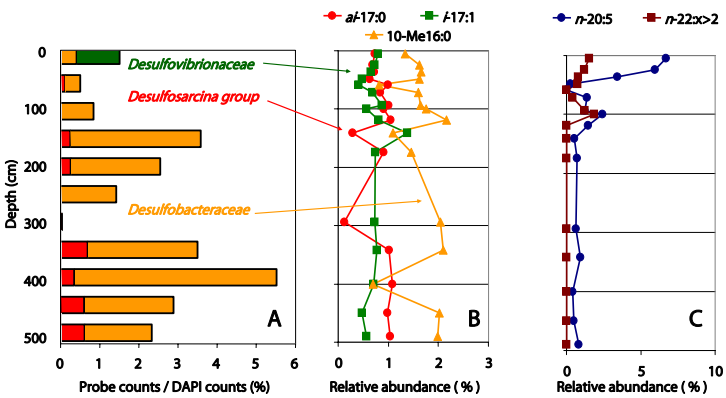
Geochemical profiles

- The upper 120 cm were almost sandy whereas deeper layers contained a higher fraction of mud.
- DOC increased slightly with depth and showed a few peaks of higher values in sandy layers indicating higher permeabilities (Fig. 1).





Polar lipid fatty acids & *in situ* abundance of cultured bacteria



- Detection of IPLs with mixed alkyl-acyl side chains indicate a substantial proportion of sulfate-reducing bacteria (SRBs) even in deeper sediment layers because these lipids were detected in mesophilic SRBs (Rütters et al., 2001).
- Molecular nucleic acid probe counts and typical PLFA patterns confirm substantial proportion of SRBs in all sediment layers, with members of the *Desulfobacteraceae* being the most abundant ones (Fig. A and B).
- Polyunsaturated PLFA at the sediment surface represent meio- and microfauna but also algal material (Fig. C).
- Detection of a polyunsaturated $C_{20:5}$ PLFA points toward a bacterial origin in deeper sediment layers.

Intact polar lipids of bacterial and archaeal origin

- The diminished methane concentrations at app. 200 cm and 490 cm indicate anaerobic oxidation of methane (AOM; Fig. 2).
- The total cell numbers obtained by SYBR Green staining and the quantities of the detected total IPLs decreased only slightly with depth showing elevated numbers at 100 cm depth (Fig. 3 and 4).
- Up to a depth of 45 cm phospholipid-type diethers of archaeal origin were absent and even in deeper layers bacterial IPLs were more abundant.
- Higher percentage of archaeol-containing lipids at layers with lower methane concentrations point to active ANME-2 methanotrophs.

Conclusions

- Intact polar lipids of bacterial origin form a major fraction of the microbial lipids even in deeper layers of a Wadden Sea sediment.
- Different intact polar lipid patterns in intertidal subsurface sediments reflect the diversity of their microbial communities, *e.g.* bacterial *vs.* archaeal.
- The relatively high abundance of archaea and sulfate-reducing bacteria throughout the sediment column was shown with both microbiological and geochemical methods.

References
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