



# The archaeal community in tidal flat sediments of the German Wadden Sea and its contribution to anaerobic oxidation of methane



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## Introduction

- Discovery of anaerobic oxidation of methane (AOM) in the 1970s
- Recent assignment of three archaeal phylogenetic groups to anaerobic methanotrophs (ANME-1 to ANME-3)
- Consortia of ANME and sulfate reducing bacteria (SRB) mediate AOM at most sites
- Rare findings of ANME-2/SRB aggregates in partially methane-saturated surface sediments of the German Wadden Sea [1]

## Objectives

To identify → key populations and → „hot-spots“ of AOM in a tidal flat by → CARD-FISH and → comparative sequence analysis of archaeal 16S rDNA clones from surface and subsurface sediments (0-30, 200 and 490 cm depth)

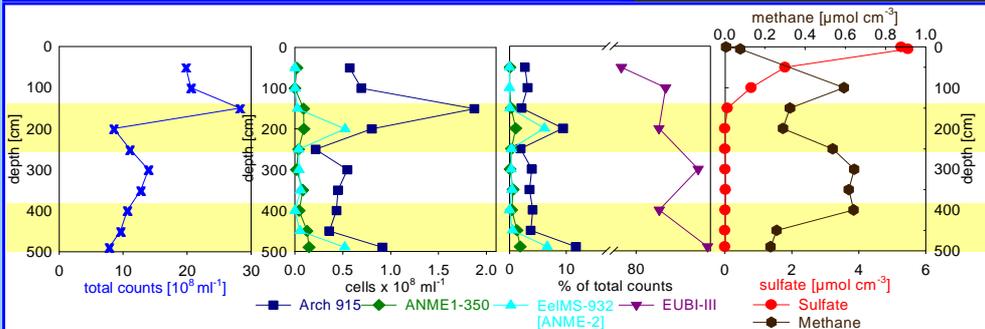
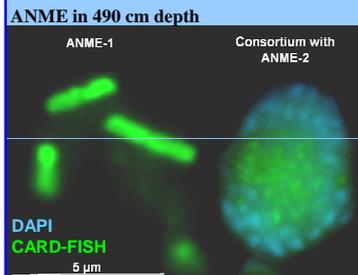
## Results

### I Surface sediments (0-30 cm depth)

- Total cell count:  $2 \times 10^9$  cells ml<sup>-1</sup>
- << 1% of all cells affiliated to the archaea
- No ANME detected by both methods

### II Shallow subsurface sediments (50 – 490 cm depth)

- Total cell counts decrease from  $2 \times 10^9$  cells ml<sup>-1</sup> in 50 cm to  $8 \times 10^8$  cells ml<sup>-1</sup> in 490 cm depth
- Detection of ANME-1 and ANME-2 (mostly small consortia with a diameter of  $\pm 2 \mu\text{m}$ ) by both methods
- Maximal 9 and 12% of all cells affiliated to the archaea in 200 and 490 cm depth
- ANME-2 account for about 65 and 57% of archaea in those layers (ANME-1: 12 and 16%)
- Archaeal clones affiliated to ANME-3 were only retrieved from 200 cm depth, but not detected by CARD-FISH
- No strong indications on the involvement of detected *Desulfosarcina* related species in AOM by CARD-FISH



Higher percentage and abundance of ANME-2, ANME-1, and archaea coincides with methane consumption in two distinct layers of the vertical profile of 5 m

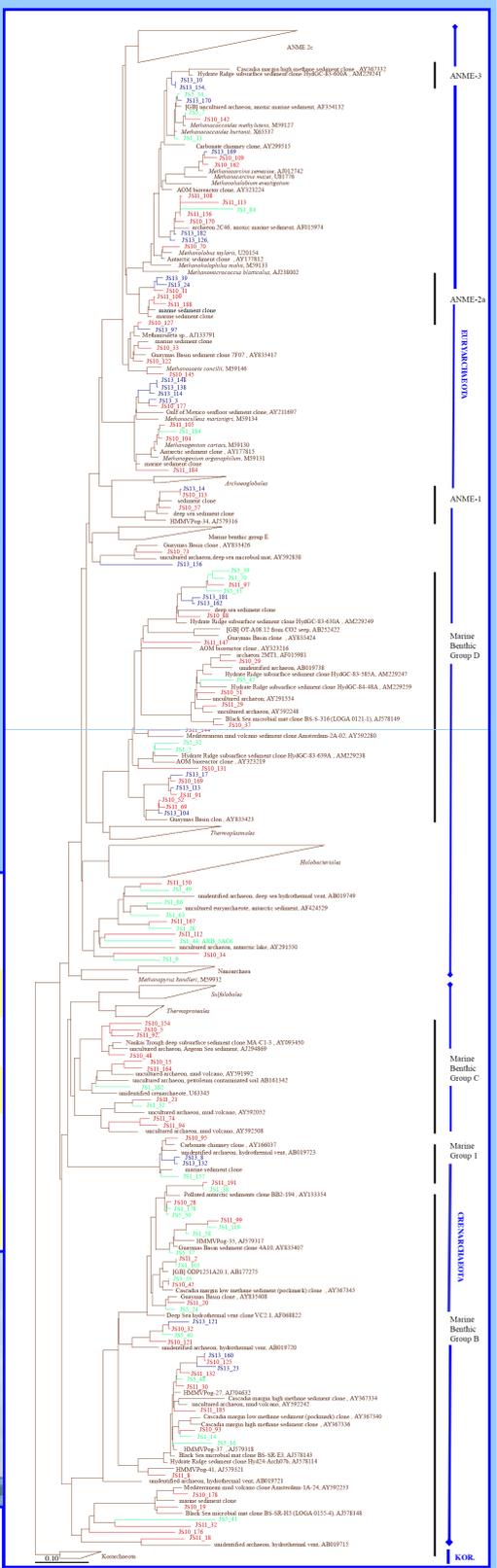
## Conclusions

- Probably globally widespread occurrence of ANME
- High archaeal abundance especially of ANME-2 in sediment layers low in methane (200 and 490 cm) even at sulfate depletion (490 cm)
- High archaeal diversity
- Potentially important role of ANME-1, ANME-2 and other archaea in methane cycling in the tidal flat



## Outlook

- Probe design for novel archaeal groups and identification of their in-situ abundance by CARD-FISH
- Analysis of possible functions of archaea by metagenomics → further screening of a metagenomic library of about 23000 fosmid clones from Janssand sediment of 490 cm depth - an insert containing an MCR-gene is currently sequenced



Phylogenetic relationship of selected archaeal 16S rRNA gene sequences retrieved from sediment (0-30 cm, 200 cm and 490 cm sediment depth) from the Janssand. This preliminary tree was constructed by parsimony analysis. The bar represents 10% sequence divergence.



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[1] Ishii et al., 2004, FEMS Microbial Ecology 50, 203-212.