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1 15N-labeling to Trace Slow Growing Anaerobically Methane Oxidising Communities

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The anaerobic oxidation of methane (AOM) is one of the most important sinks for the greenhouse gas methane in marine ecosystems. AOM is known to be mediated by at least two phylogenetically different groups of Archaea, ANME-I and ANME-II, most closely related to the *Methanosarcinales*. These Archaea are often found in close association with sulphate reducing bacteria of the *Desulfosarcina* group. So far, no pure cultures of these microorganisms have been obtained. Consequently, the mechanism and regulation of this process remained unknown for a long time. Recently, using naturally enriched environmental samples from Hydrate Ridge, dominated by ANME-II, and the Black Sea and Gulf of Mexico, both dominated by ANME-I, we established in-vitro experimental systems to investigate the physiology of AOM. Increasing rates of methane dependent sulfate reduction in subsequent incubation periods indicated growth of responsible microorganisms. This effect was 5-10 fold more pronounced under elevated methane partial pressures (1.35 MPa). The increase in AOM-dependent biomass was investigated by stable isotope labeling and further confirmed by cell counts. Using 15N-labeled ammonium as sole N-source, a significant and methane-dependent increase of 15N in the biomass was detected. This was accompanied by an increase in N- and C-contents compared to controls without methane. Further experiments were carried out to attribute this isotopic enrichment to an increase in biomass

and more specifically to ANME-populations. Cell counts demonstrated for different samples an 5-10 fold increase of ANME-microorganisms over time, thus confirming the substantial enrichment of AOM-dependent biomass. Changes in microbial community composition during the enrichments were studied by 16S rDNA and functional gene-based DGGE analysis. However, no changes were detected for neither Archaea nor sulfate reducing bacteria over an incubation period of up to six months. This indicates a stable community adapted to methane as main carbon and energy source.