

Discriminative biogeochemical signatures of methanotrophs inhabiting different chemosynthetic fields in an active mud volcano of the Canadian Beaufort Sea

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In this study, we investigated vertical variations in methanotrophic communities in association with varying methane fluxes within a mud volcano (420 m water depth, MV420) of the Canadian Beaufort Sea, by analysing geochemical properties, microbial lipids, and nucleic acid signatures. Three push cores were collected from visually discriminative chemosynthetic fields that were (i) bare of organisms (BO) to the naked eye, (ii) covered with bacterial mats (BM), or (iii) siboglinid tubeworms (ST), by using a remotely operated vehicle (ROV). The methane fluxes were highest at the sites of BO ($0.06 \text{ mmol cm}^{-2} \text{ y}^{-1}$) and BM ($0.04 \text{ mmol cm}^{-2} \text{ y}^{-1}$) and substantially lower at the ST site ($0.01 \text{ mmol cm}^{-2} \text{ y}^{-1}$). All MV420 sites showed the presence of aerobic methane oxidation (MOx)- and anaerobic methane oxidation (AOM)-related lipid biomarkers (4-methyl sterols and *sn*-2-hydroxyarchaeol, respectively), which were distinctly different in comparison to the reference site where these compounds could not be detected. Our lipid biomarker results are furthermore in good agreement with 16S rRNA analyses, which revealed the presence of MOx-related bacteria (*Methylococcales*) and AOM-related archaea (ANME-2 and ANME-3 groups) at the MV420 sites. The 4-methyl sterols derived from *Methylococcales* were predominant in the surface layer at the BM site with a moderate methane flux, while their occurrence was limited at the BO and ST sites. Notably, the abundances of AOM-related lipid biomarkers were minor at the BO site where the methane flux was highest. However, ANME-3 and sulfate reducing bacteria (*Desulfobacteraceae* and *Desulfobulbaceae*) were abundant in the downcore sediments at the BM site. On the other hand, the abundances of the ^{13}C -depleted *sn*-2-hydroxyarchaeol potentially derived from ANME-2 and/or ANME-3 groups was predominant at the ST site, indicating their co-occurrence under a lower methane flux. Accordingly, our results suggest that a niche diversification within this MV system has shaped distinct methanotrophic communities due to the availability of electron acceptors in association with varying degree of methane fluxes and bioirrigation activity.

Keywords: Arctic, Mud volcano, chemosynthetic organism, Anaerobic methane oxidation, Aerobic methane oxidation, Methanotroph