

Discriminative signatures of methanotrophic communities according to the chemosynthetic habitats at the active mud volcano of the Canadian Beaufort Sea

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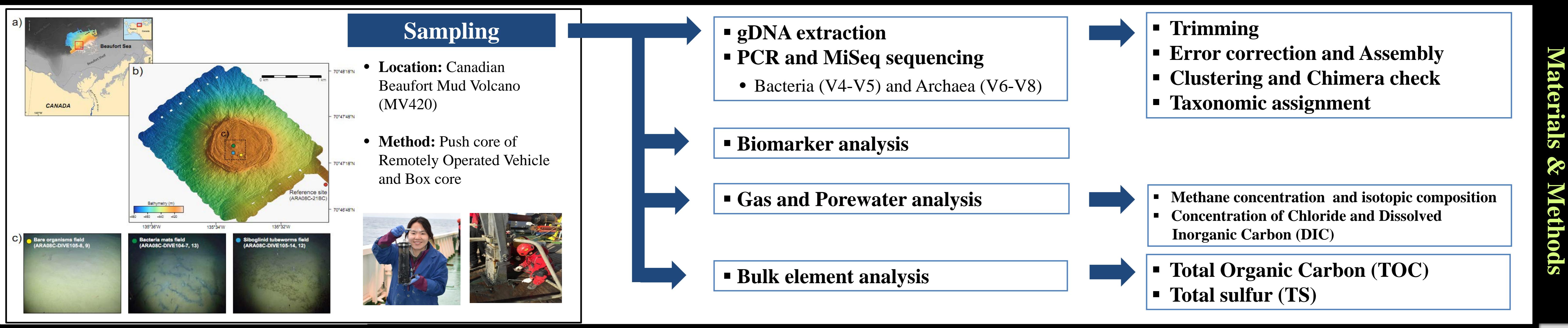
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To obtain knowledge on the spatial variations of methanotrophic guilds along the methane flux regimes, we performed geochemical and microbiological analyses within a mud volcano (MV420) of the Canadian Beaufort Sea. Sediments were collected from visually discriminative chemosynthetic fields, bare of organisms (BO) with the naked eye, covered with bacterial mats (BM), or siboglinid tubeworms (ST) of MV420 and reference site. The methane flux decreased in the order of the BO, BM and ST sites. With the down core stratification of biogeochemical and microbial profiles within each site of MV420, lipid biomarker and 16S rRNA analyses revealed the spatial difference of aerobic methane oxidation (MOx)- and anaerobic methane oxidation (AOM)-related groups along the sites of MV420, which were distinctly different with the reference site. MOx-related bacteria, *Methylococcales* dominated the surface layer of BO site whilst AOM-related archaea, mostly ANME-3 and sulfate reducing bacteria of *Desulfobacterace* and *Desulfobulbaceae* were predominant in the sediments of BO and ST sites. 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.

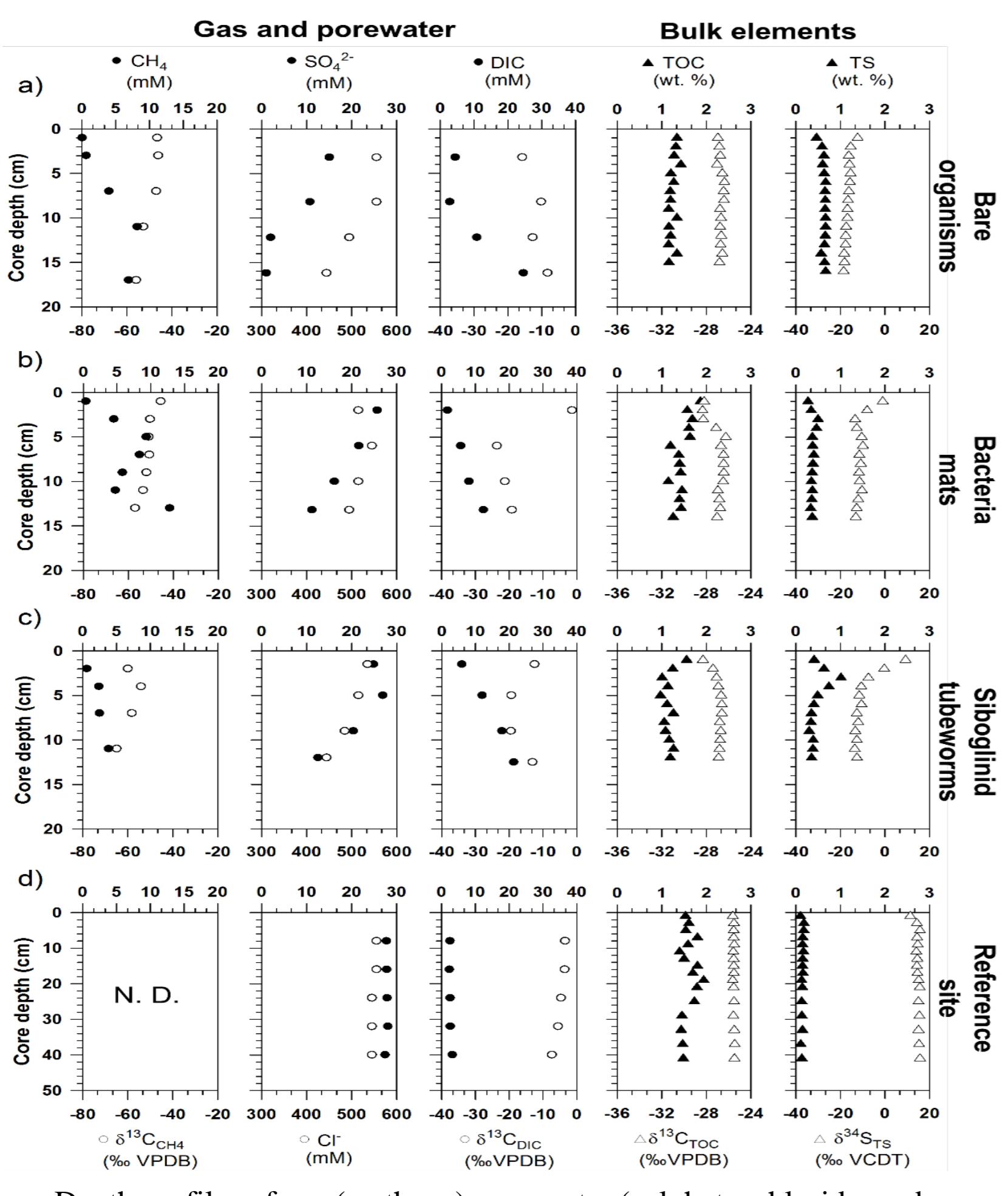
Abstract



Materials & Methods

Results

1. Profiling of environmental factors



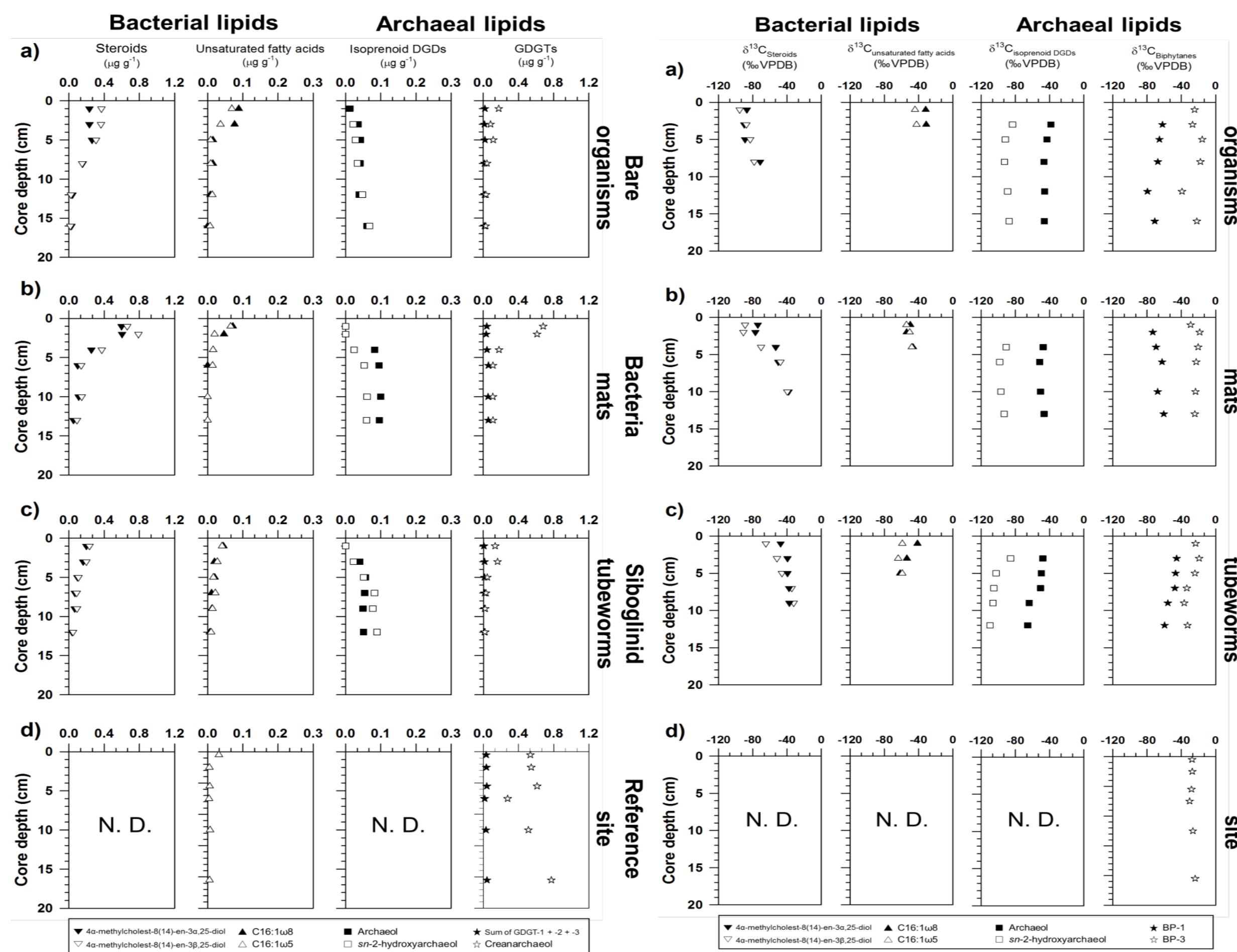
Depth profiles of gas (methane), porewater (sulphate, chloride, and dissolved inorganic carbon), and bulk element (total organic carbon and total sulphur)
N.D. denotes 'not detected'.

	BO	BM	ST	Reference site
Temperature (°C) (surface seafloor)	0.36	0.37	0.37	3.9
Methane flux (mmol cm ⁻² y ⁻¹)	-0.06	-0.04	-0.01	N. D.
Sulfate flux (mmol cm ⁻² y ⁻¹)	0.07	0.04	0.02	N. D.
Penetration sulfate depth (ca. cm)	1.5	4	7	>40

Methane and sulphate flux calculated from their concentration profiles and the penetration depths (cm) of sulphate. '-' denotes 'not determined'.

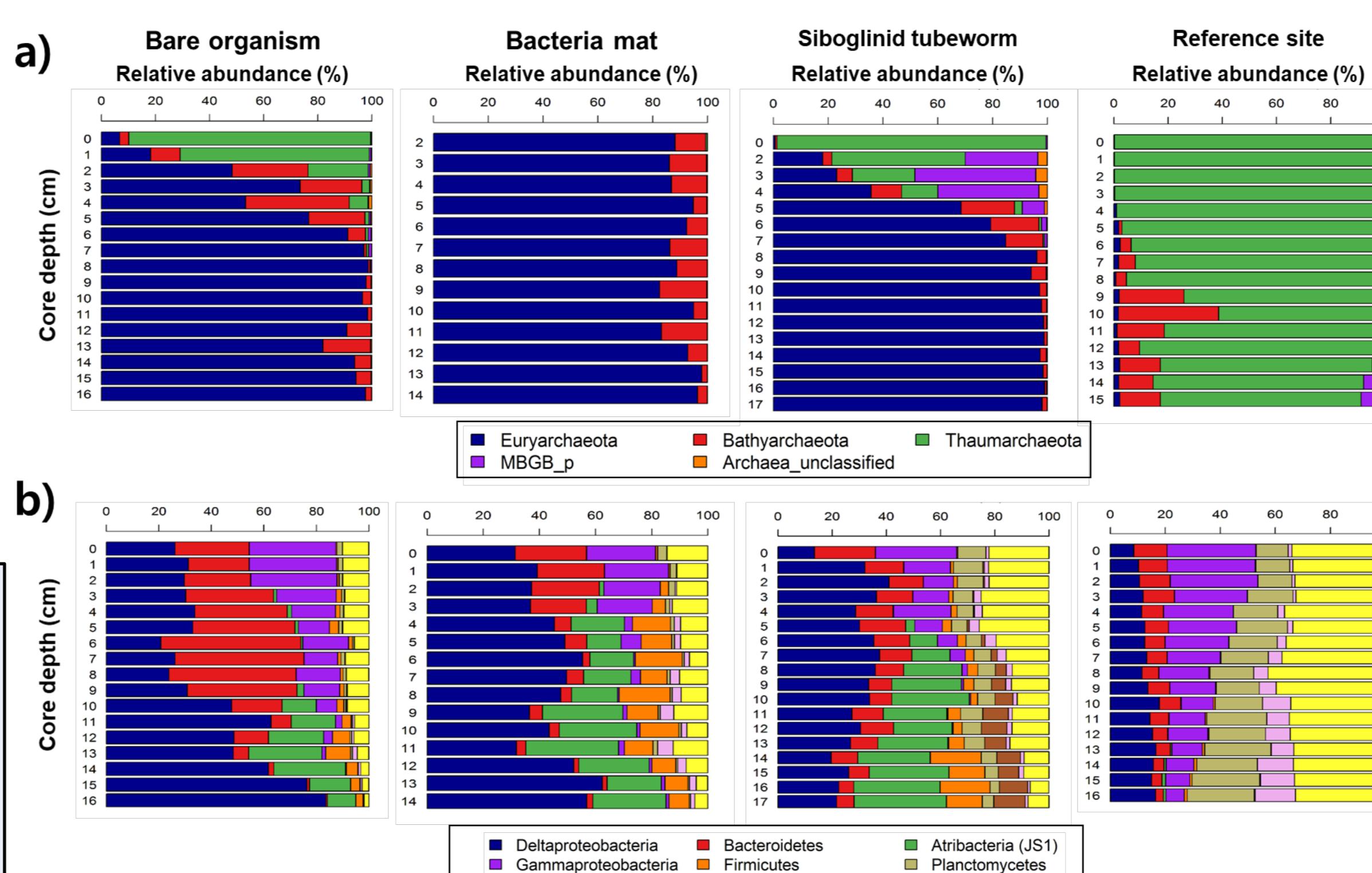
- Methane and sulfate fluxes calculated by their concentration gradients varied among three sites of MV420 with a high heterogeneity.
- The surface of the BO site which were devoid of even mobile taxa showed the highest methane and sulfate flux. On the other hand, in accordance with decreasing fluid fluxes, the free-living bacteria communities and the siboglinid tubeworm patches were differently developed on the surface of MV420.

2. Lipid composition



Vertical profiles of selected bacterial and archaeal lipid biomarker concentrations and their isotopic compositions. N.D. denotes 'not detected'.

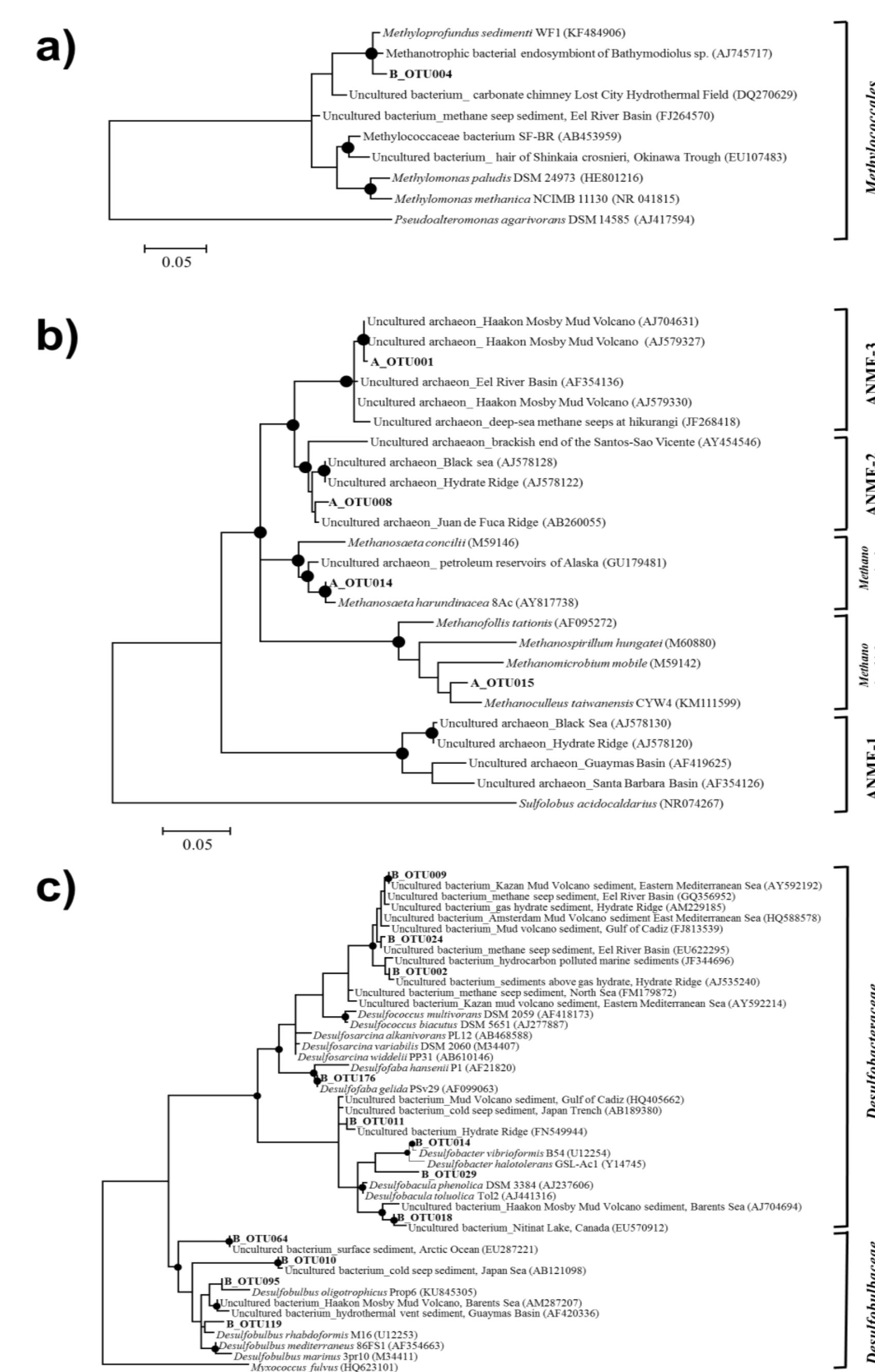
3. Microbial composition



Relative abundance of archaeal (at class level) (a) and bacterial taxa (at phylum level and class level of Proteobacteria) (b) along the core depths.

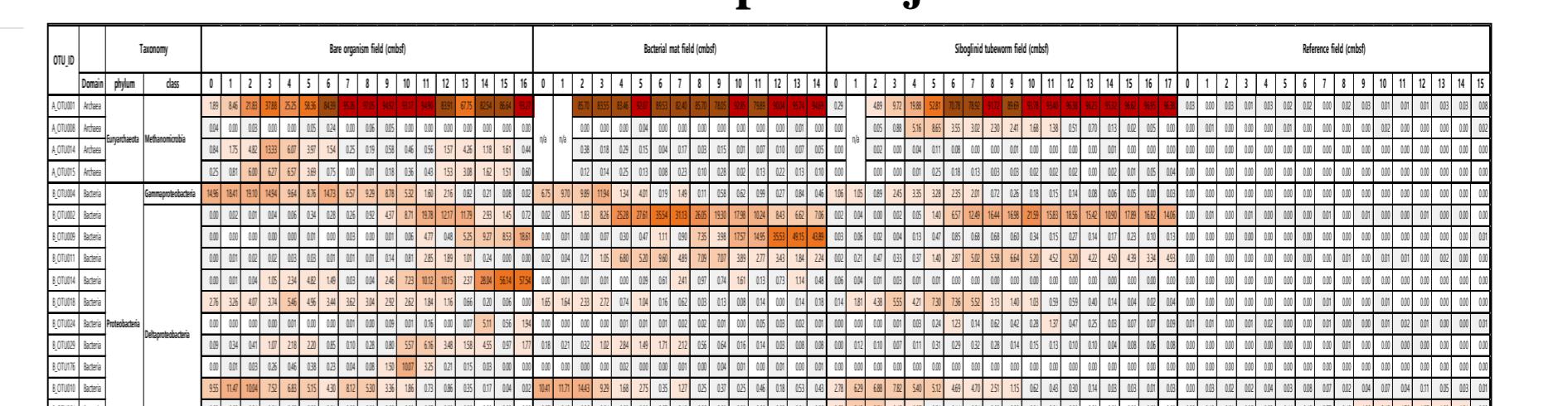
MOx and AOM-related methanotrophic communities assessed based on the lipid biomarkers and 16S rRNA gene sequences showed a clear down-core shift within the site and variance among the sites of MV420.

4. Phylogenetic analysis of major OTUs and their abundance



Phylogenetic tree of major OTUs of (a) *Methylococcales*, (b) *Methanomicrobia*, and (c) *Deltaproteobacteria*

Heat map of major OTUs



Major OTUs (>5%) of *Methanomicrobia*, *Deltaproteobacteria*, and *Methylococcales* were selected.

'-' denotes that the samples were not included due to the low number of sequences.

- Discriminative biogeochemical signatures of methanotrophs were shown along the chemosynthetic communities at MV420 of the Canadian Beaufort Sea.
- Niche diversification of MOx- and AOM-related communities (*Methylococcales*, and ANME-2 and -3 groups, respectively) within MV system seems to be shaped by the availability of electron acceptors in association with varying degree of methane fluxes and bioirrigation activity.

Conclusion