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Quarterly Research Performance

Progress Report (Period ending 03/30/2016)

Assessing the response of methane hydrates to environmental change at the Svalbard continental margin

Project Period (11/1/2013 to 10/31/2016)

Submitted by:
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EXECUTIVE SUMMARY

In November 2013, Oregon State University initiated the project entitled: **Assessing the response of methane hydrates to environmental change at the Svalbard continental margin**. In this project, we will take advantage of a unique opportunity to collect samples from the Svalbard continental margin. The overall objective of this research is to constrain the biogeochemical response of the gas hydrate system on the Svalbard margin to environmental change. Because of a delay in the planned expedition, we reconfigured the program based on discussions with NETL program managers and submitted a revised SOPO. In the new plan, we will collect samples in three expeditions, the first of which happened Oct 7-21, 2014. We were able to also join an expedition to the area onboard the RV Helmer Hanssen during May15-29, 2015 and another one onboard the RV Heincke August-September 2015. We are currently discussing a modification of the SOPO to include participation in an upcoming MeBo drilling expedition in this region, scheduled for August-September, 2016. We continue with analyses of the samples collected during these expeditions as well as completed a computational model for methane hydrate formation under conditions of variable salinity.

PROGRESS, RESULTS, AND DISCUSSION

1. Expedition(s) update: We participated in a cruise onboard the RV Heinke in August-September 2015, which was focused on collecting water column samples along the Svalbard and Barents Sea margins. Cruise reports have been submitted.
2. Water column results. We conducted extensive sampling campaign of the water column along the entire Barents Sea-Svalbard margin in August-September 2015, including the shelf regions, to document the significance of methane release at the upper limit of gas hydrate stability relative to additional sources on the shelf. Water and air samples collected during two RV Heinke expeditions indicate that the methane released in the slope does not contribute to the atmospheric input, however the shelf regions do. In addition extensive hydroacoustic surveys along the entire edge of has hydrate stability do not show any methane discharge expect for the well-studied region offshore Prins Karl Foreland. A summary of these results is being written for publication. Preliminary results were presented at the 2016 Gordon Research Conference on Natural Gas Hydrate (Galveston, TX, March 2016) and will also be presented at the upcoming EGU General Assembly (Vienna, Austria, 17–22 April 2016). Abstract is attached
3. Geochemistry: Preliminary data from a series of cores recovered at on the fan of Storfjordrenna, west Barents Sea from a location where gas hydrate was recovered as shallow as 0.82 mbsf indicate that the increase in methane flux inferred sulfate profile, may be linked to an enhanced gas hydrate dissociation in this area. Ongoing studies are aimed at testing this postulate, with the aim to bridge the gap between hydroacoustic flare detection in the water column and the mapping of hydrate reservoir at depth, and provide additional clues to unravel the complex interactions among ice, ocean, microbiology and climate and their sensitivity to both natural and anthropogenic change in Arctic regions. We presented these results at the Gordon Conference on Natural Gas Hydrates March, 2016. We expect to submit a manuscript on these observations by June 2016. Additional analysis of those fluids is underway. We now completed analyses of major and minor ions as

well as some selected Sr isotope data. Plots of these new data are attached. Preliminary evaluation of these data suggests a complex system with various fluid sources and advective flow regimes. More analyses are currently being conducted

4. Microbiology. Marine sediment samples obtained from gas seep sites offshore Svalbard (Barents Sea) were incubated in sterile anoxic seawater media at in situ temperatures and pressures at two different methane partial pressures (0.2 MPa; and 3.8 MPa, at methane saturation). Sediments were sampled after 7, 25, and 129 days. Sulfate reduction rates, calculated from the accumulation of sulfide in the media, totaled ~50 nm/cm3/day at both methane partial pressures after 129 days of incubation. Incubations enriched for sulfate-reducing Deltaproteobacteria of the families Desulfobulbaceae and Desulfobacteraceae that are known to partner with ANME in consortia that anaerobically oxidize methane (Figure 1). ANME-2 abundance may have also increased slightly in these incubations. Communities incubated for 129 days are currently being analyzed. Quantification of genes and gene transcripts involved in AOM (mcrA, dsrAB) will be used to infer whether AOM community response times vary with methane concentration. These new data were presented at the 2016 Gordon Research Conference on Natural Gas Hydrate (Galveston, TX, March 2016).
5. Modeling- We are currently working on revisions for the paper: "Methane hydrate formation in Ulleung Basin under conditions of variable salinity: reduce model and experiments", which was accepted pending revisions on March 5, 2016. This paper is the first of two in which we present an approximate or reduced model of methane hydrate evolution in subsea sediments under conditions of variable salinity. We describe a two-phase three-component physical model. The model is rich enough to allow the study complex dynamics of hydrate formation under the conditions of variable salinity such, and yet is robust and very efficient compared to the published comprehensive fully implicit approaches. In particular, we discuss in detail how the thermodynamics constraints are incorporated in the model and calibrated using experimental data.

Problems or delays

No new problems since the set-back last year when the planned expedition in the R/V M.S. Merian got cancelled due to massive engine failure of the vessel. We completed our expedition plan to the area as documented in the revised project plan and added an additional cruise on the RV Hanssen in May 2015.

Products

- Abstract of poster presentation to the EGU General Assembly (Vienna, Austria, 17–22 April 2016).
- New data results from analyses of pore water samples from the Svalbard margin
- Posters presented at the Gordon Conference on Gas Hydrate in Galveston, TX, March 2016.

Methane seepage along the continental margin off Svalbard - from Bjørnøya to Kongsfjorden

S. Mau[1], M. Römer[1], M. Torres[2], T. Pape[1], T. Gentz[3], E. Damm[3], P. Wintersteller[1], J. C.-W. Hsu[1], M. Loher[1], S. Buchheister[1], M. Lange[1], A.-C. Melcher[1], N. Brückner[1], S. Gaide[1], G. Bohrmann[1] and shipboard scientific party HE449 and HE450

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Widespread methane seepage was observed along the continental margin from northern Norway along the edge of the Barents Sea towards Spitsbergen up to Kongsfjorden in August/September 2015. 68 hydrocasts deployed along this section indicated elevated concentrations of dissolved methane up to 878 nM and thus above the atmospheric methane equilibrium concentrations of 2.6-3.5 nM. Methane concentrations were decreasing towards the sea-surface or showed numerous peaks, which suggest gas seeps in the vicinity of the stations. Flare imaging revealed seep clusters from west off Bjørnøya to the known seep-fields off Prins Karls Forland. Most of the hydrocasts were taken within 30 km distance to a flare, therefore, most of the methane concentrations appear to originate from seepage. Preliminary interpretation of δ^{13}C-methane values cannot distinguish the source(s). The values between -50.7 to -33.6 ‰ suggest rather a thermogenic source, but a mixture of biogenic and thermogenic methane or biogenic methane altered by microbial oxidation cannot be excluded. Flare locations are situated in the area of the NNW-SSE striking Hornsund Fracture Zone suggesting that methane might ascend from depth along this pathway. Our data reveal that methane seepage off Svalbard is not focused off Prins Karls Forland, but is more widespread than previously thought.

Appendix

Additional geochemical data from Pingo-Like features found south of Spitzbergen, Barents Sea

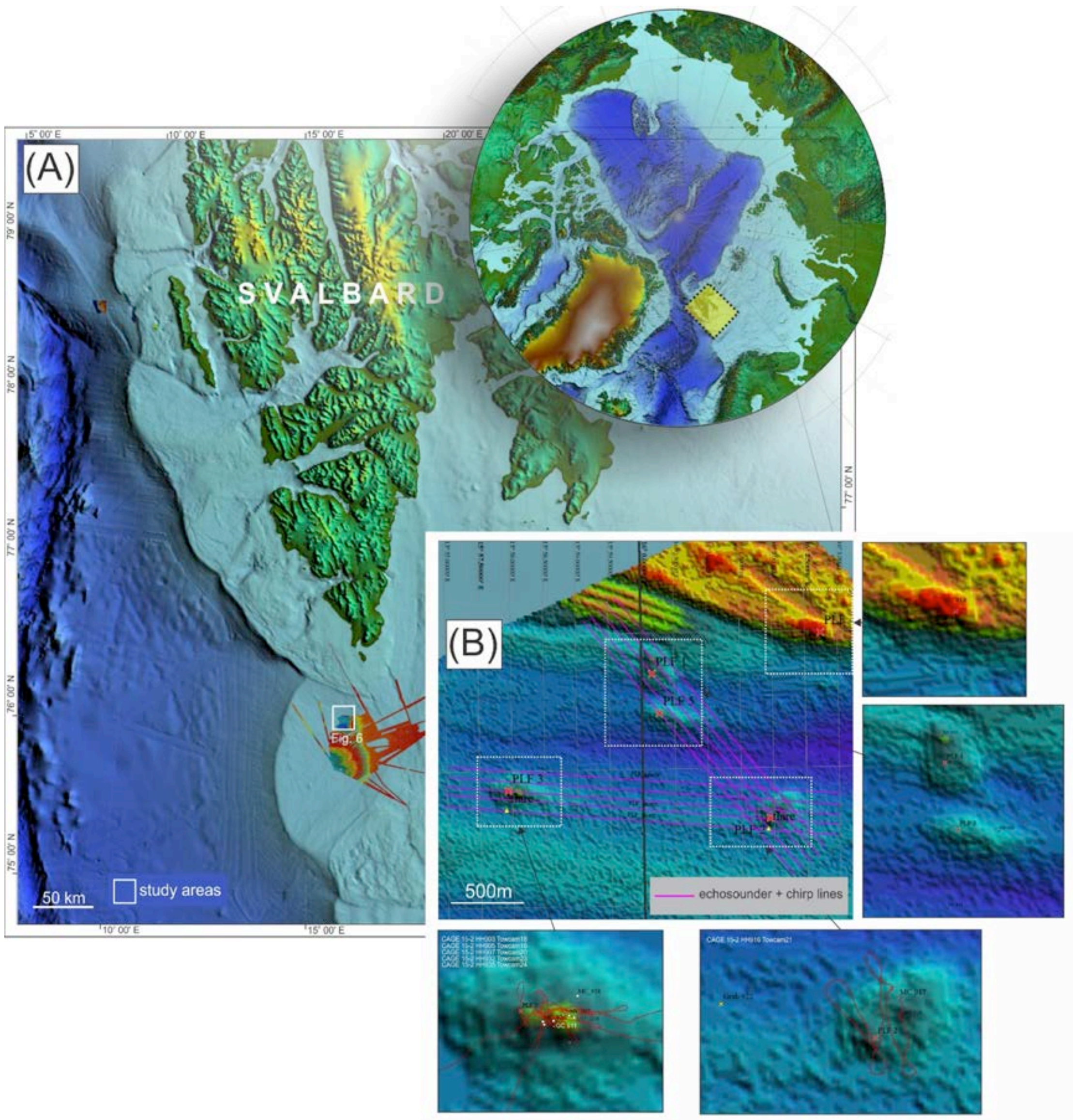
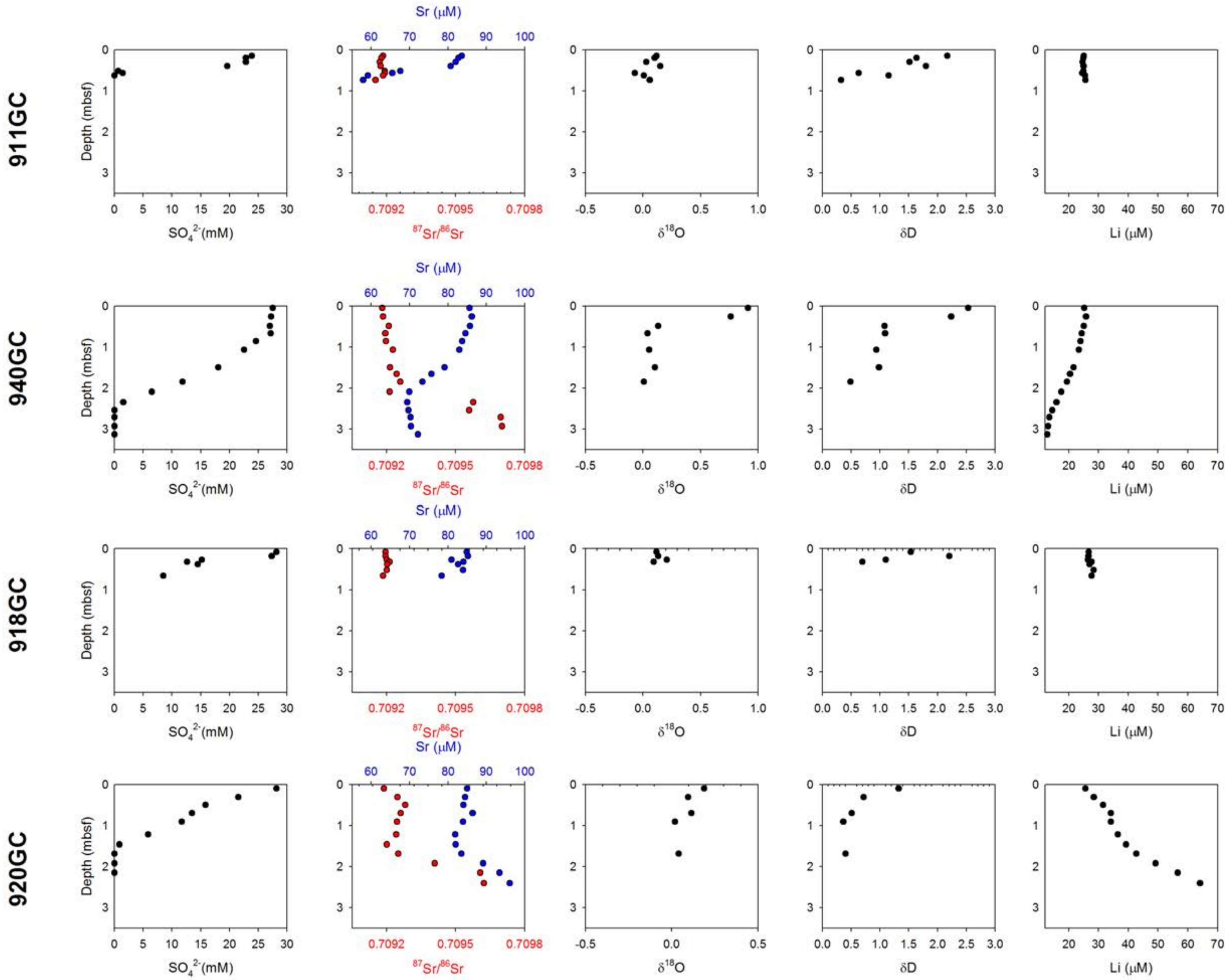
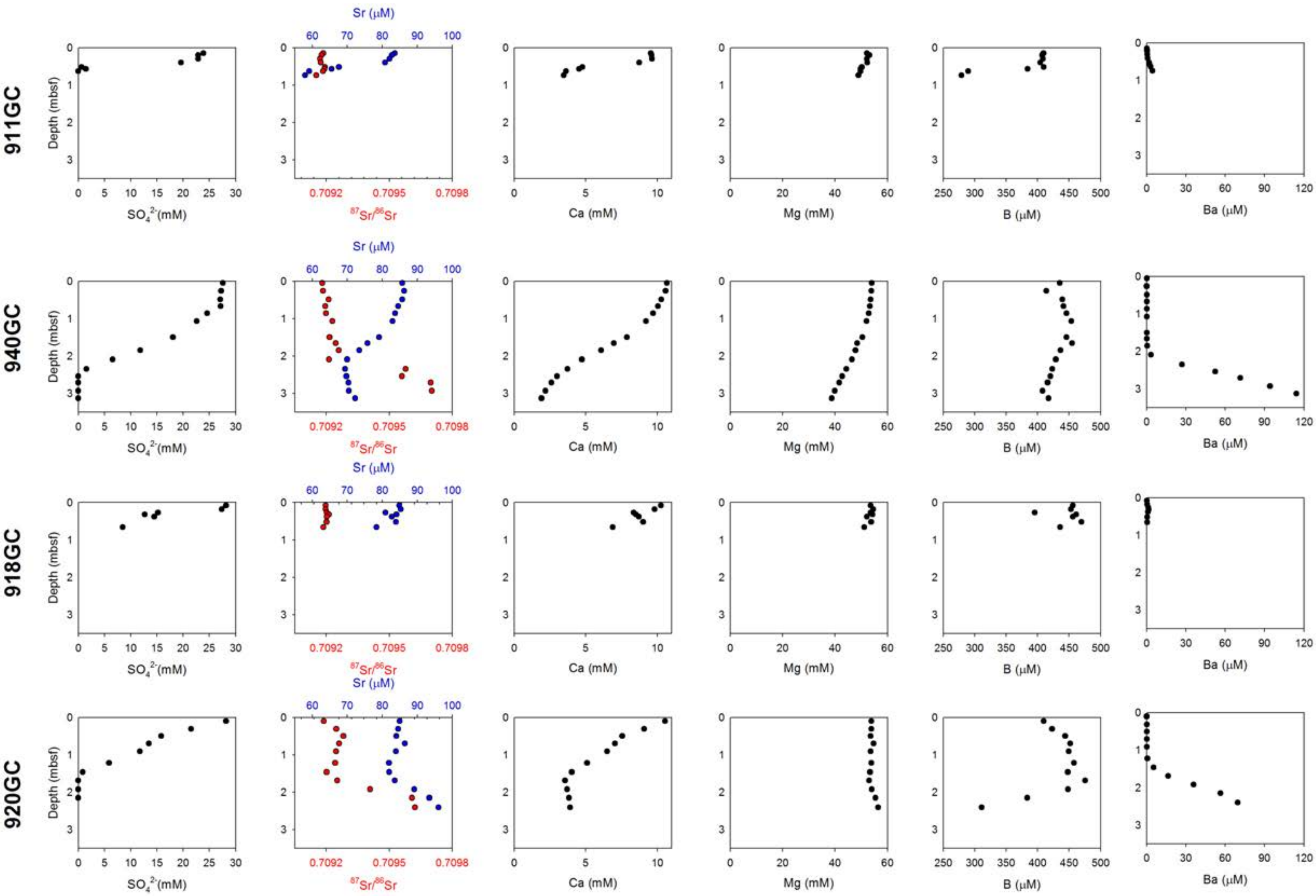


Figure 1- Map showing locations of gas-hydrate bearing structures



911GC
940GC
918GC
920GC
Depth (mbsf)
SO4 2- (mM)
Sr (μM)
87Sr/86Sr
δ18O
δD
Li (μM)


911GC
940GC
918GC
920GC
Depth (mbsf)
SO4 2- (mM)
Sr (μM)
87Sr/86Sr
0.7092
0.7095
0.7098
Ca (mM)
Mg (mM)
B (μM)
Ba (μM)

Temporal dynamics of communities that anaerobically oxidize methane in response to short-term changes in methane partial pressures

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3 College of Earth, Ocean, and Atmospheric Sciences, Oregon State University, Corvallis, Oregon, USA

Question

How do microbial communities that anaerobically oxidize methane in marine sediments change when stimulated by different concentrations of methane?

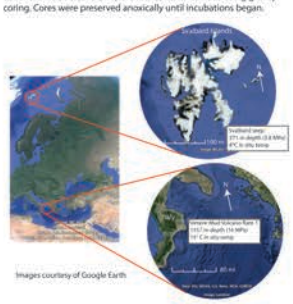
Background

- Methane, a potent greenhouse gas, is the most abundant atmospheric hydrocarbon, responsible for about 20% of anthropogenic warming[1].
- Marine sediments produce tens to hundreds of teragrams of methane annually, but up to 90% is consumed before reaching the hydrosphere, through microbial anaerobic oxidation of methane (AOM)[2,3,4].
- Remaining methane is released at thousands of seabed cold seeps distributed globally along continental margins[5,6,7].
- Consortia of anaerobic methanotrophic archaea (ANME) and sulfate-reducing bacteria (SRB) carry out AOM in marine sediments, coupling methane oxidation to bicarbonate with the reduction of sulfate to sulfide[8,9,10] (equation below).
- Cold seeps exhibit temporal variation in gas flux intensity, on scales of hours to years[11,12], but regional-scale seepage may persist for thousands of years[13].
- Incubations may lend insight into successional patterns in cold seep environments and will enable more accurate prediction of methane release from the seafloor, especially under changing methane flux regimes.

$$CH_4 + SO_4^{2-} \longrightarrow HS^- + HCO_3^- + H_2O$$

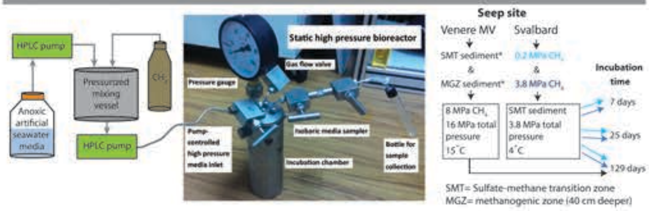
Sample location and collection

Samples were collected from methane seeps offshore Svalbard and at Venere Mud Volcano (MV) in the Mediterranean Sea using gravity coring. Cores were preserved anoxically until incubations began.



Images courtesy of Google Earth

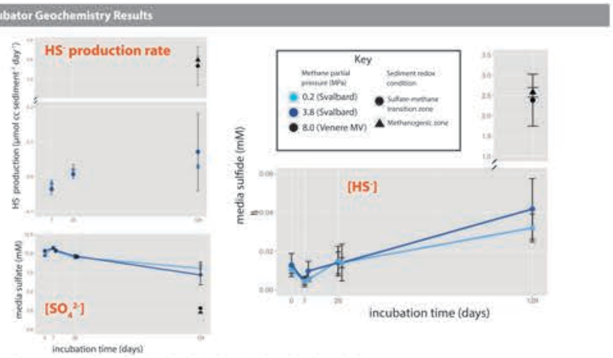
Experimental setup



Methods

- Sediments from two methane seeps were incubated up to 4.3 months in static high-pressure bioreactors simulating *in situ* conditions at different methane partial pressures (above).
- Incubators were subsampled for sediment, which was immediately frozen at -80˚C for nucleic acid extraction, and repressurized under original conditions.
- Media sulfide and sulfate were measured at each sampling and repressurization.
- DNA was extracted using an SDS- and heat-based approach[14]. The V4 region of the 16s rDNA gene was amplified and 250 bp paired-end reads were sequenced on an Illumina Miseq. Sequence data were processed and chimeras removed in Mothur (v. 1.32.1)[15].

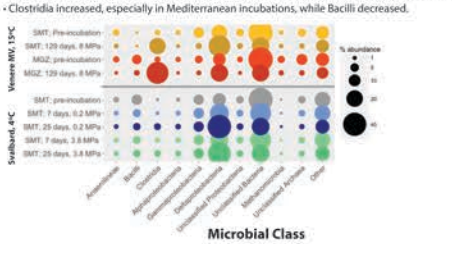
Incubator Geochemistry Results

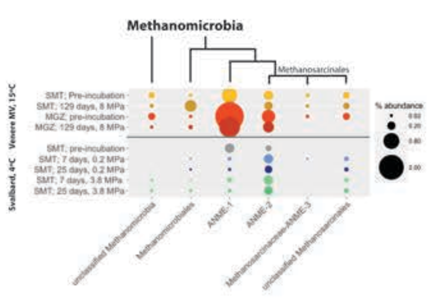


- Sulfate decreased and sulfide accumulated in all incubations beyond 25 days (below).
- Sulfide production in Venere MV sediments approximated 400 nM cc^{-1} d^{-1} and did not vary by sediment redox type (SMT or MGZ). These rates were an order of magnitude higher than measured in Svalbard cold seep SMT sediments when incubated for the same duration (28–71 nM cc^{-1} d^{-1}).
- Both 0.2 and 3.8 MPa CH_4 treatments showed comparable sulfide production rates in Svalbard SMT sediments.
- Media methane concentrations from Svalbard incubations after 4.3 months were all below 1 mM but were significantly higher in the 3.8 MPa methane treatment.

Microbial Community Results

- Deltaproteobacteria, particularly of the family Desulfobulbaceae, increase in all incubations. Desulfobulbaceae are SRB known to partner with ANME in AOM consortia.
- Methanomicrobia did not increase. ANME-2 was slightly stimulated in Svalbard incubations, but ANME-1 and -3 were not. Communities were insensitive to methane concentrations tested.
- Clostridia increased, especially in Mediterranean incubations, while Bacilli decreased.





Conclusions

- Sulfate reduction rates ~3-100x higher than sulfide production rates suggests sulfur is mineralized (as iron sulfide) and/or assimilated into biomass.
- Higher sulfide production rates in Venere MV sediments may reflect higher temperature and methane pressures than used in Svalbard incubations[16]. Venere MV sulfide production was lower than from a shorter incubation of mud volcano sediment with 8 MPa methane[17], though sulfide production likely decreased with accumulation over the 129 day incubation.
- Assuming equimolar methane oxidation with sulfide production, incubated Svalbard SR-AOM rates were comparable to *in situ* AOM measurements from seeps at several continental margins and mud volcanoes, but lower than in prior incubations (table below). Higher methane pressures did not appear to stimulate increased sulfate reduction-in contrast to previous findings[17]-or any other discernible community response.

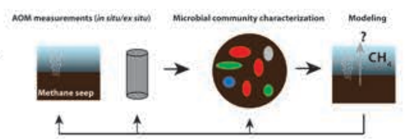
Location	Habitat	SR-AOM rate (nmol cc-1 day-1)	Reference
Svalbard margin	reduced sediment	20-70	this study
Capt. Aryutinov MV, Gulf of Cadiz	reduced sediment	69-3500	17
Hakon Mosby MV, Barents Sea	reduced sediment	4	18,19
Hydrate Ridge, Cascadia Margin	bacterial mat	5-99	20,21,22

■ = incubation ■ = *in situ*

- The increase in Deltaproteobacteria, particularly Desulfobulbus, suggests that SRB are initially stimulated on the order of weeks. SRB use sulfate to oxidize organic material in sediments, and/or partner with ANME to oxidize methane.
- Low abundances of ANME (2% or less) in pre-incubated samples suggests neither of these seeps were likely active for a long time.
- Longer incubations are required to stimulate ANME growth. Percent abundance decreases in ANME (below) likely reflect increased Deltaproteobacteria and Clostridia in incubated samples.
- The bloom in Clostridia is suggestive of organic matter fermentation. Clostridia may be responsible for a significant portion of the sulfate reduction during incubation, and therefore could compete with SRB that support AOM.
- Community dynamics should be considered when attempting to connect ANME/SRB abundance and activity with AOM rates.

Future directions

- 16s rRNA amplicon sequencing will be performed to analyze Svalbard SMT communities incubated for 4 months at 0.2 and 3.8 MPa CH_4.
- Functional genes methyl coenzyme reductase (*mcrA*) and dissimilatory sulfate reductase (*dsrAB*), from ANME and SRB respectively, will be quantified by ddPCR.
- Fluorescent microscopy of ANME/SRB and total cell counts are anticipated to reveal increases in cell numbers throughout enrichment-- particularly SRB in AOM consortia.
- A follow-up study with adequate controls (no CH_4, non-seep communities) and CH_4 measurements is recommended. We are in the process of developing a high-pressure incubation system to rerun several enrichments.
- Metagenomics will be considered to explore functional diversity and activity of incubated communities in higher detail.



Acknowledgments

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- Sequencing was performed by OSU's Center for Genome Research and Biocomputing (CGRB).
- Incubations were conducted in the laboratory of Dr. Xiang Xiao at Shanghai Jiao Tong University. Chiara Cassarini, Yan Wen Kai, and Ding Jian provided crucial support in the operation of high pressure equipment.

References

Methane discharge from Arctic gas hydrates: warming or natural ventilation?

Wei-Li Hong[1], Marta E. Torres[2], JoLynn Carroll[1], Antoine Cremiere[3], Giuliana Panieri[1], and Pavel Serov[1]

[1]CAGE - Centre for Arctic Gas Hydrate, Environment and Climate, Department of Geology, UiT The Arctic University of Norway, Tromsø, Norway
[2]College of Earth, Ocean, and Atmospheric Sciences
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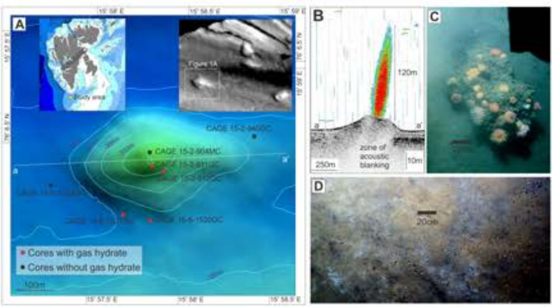
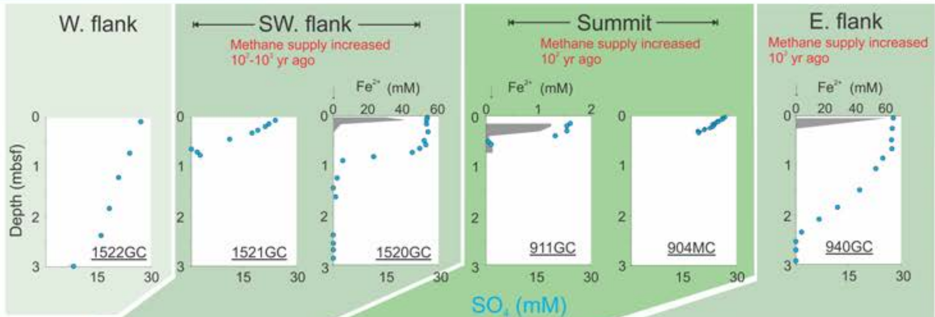
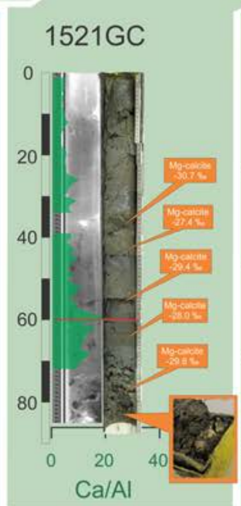
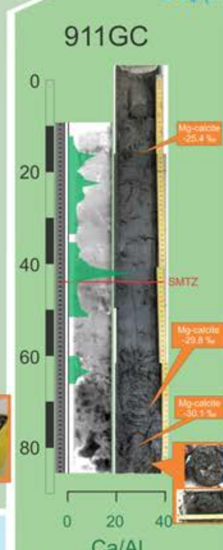
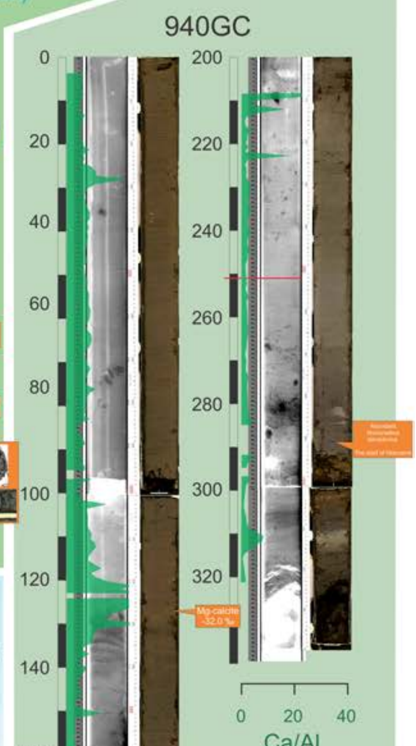


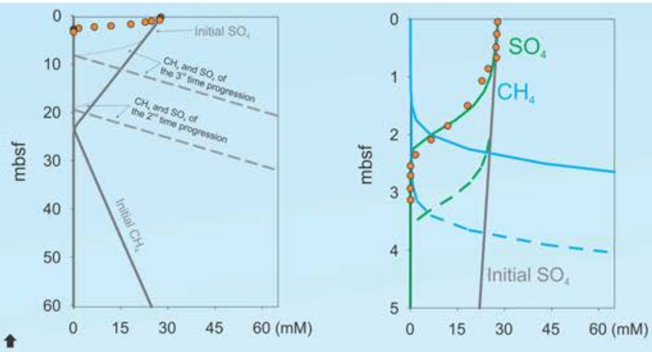








Litho-stratigraphy columns and porewater profiles of several selected cores. Three of the sulfate profiles exhibit a non-steady state trend which we attributed to a recent increase in methane supply. The abundant reduced iron (shown as the grey area) in the porewater rejects the interpretation of enhanced bottom seawater mixing as the low iron in bottom seawater (the arrows) will dilute the porewater iron rapidly. Layers of carbonate in the sediments suggest several episodic events of methane seepages. Gas hydrates were recovered from four of the cores.



⬆

Time-elapsing transport-reaction model to interpret the nen-steady state porewater sulfate profiles.The initial condition is derived from the upper part of the profile. Rapid anaerobic oxidation of methane (AOM) is induced by a strong pulse of methane from the bottom of the model frame (60 mbsf). Replenish of sulfate from seafloor is significantly slower than the sulfate consumption by AOM at sulfate-methane-transition-zone and there results in the kinked sulfate profile. From this model, we can estimate the time when methane supplies strated to increase at each site.

Results of heat propagation model for a modern scenario with cold bottom water (blue bar) and a stadial scenario with warm bottom water (red bar). (A) The average temperature in this area is 1.8 °C with an annual fluctuation of ~6 °C. (B) Seasonal bottom water fluctuation can only disturb the sub-bottom temperature above ~15 mbsf. Only gas hydrate shallower than ~5 mbsf will surfer potential melting during summer seasons. (C) Gas hydrates from the entire 60-meter sediment column are likely to melt after 10 years of increase in bottom water temperature.

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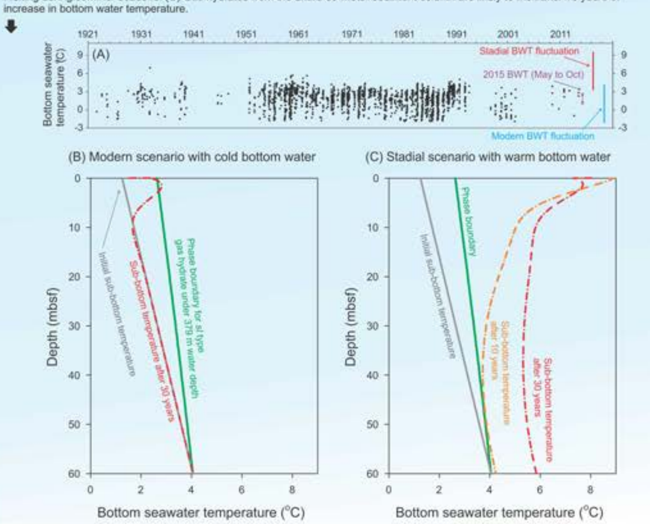

Evolution model of Storfjordrenna gas hydrate pingos. We identified more than two past emission episodes which correlate to the authigenic carbonate layers we observed. The fine hemipelagic sediments between the carbonate layers represent quiescent periods with weak methane supply.

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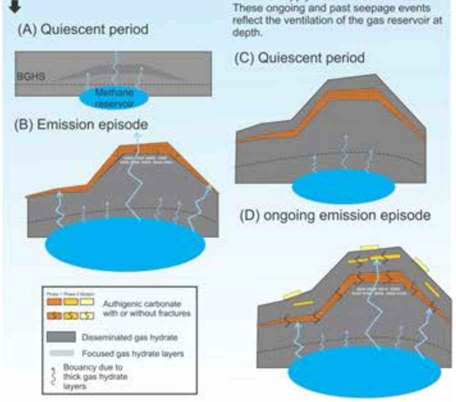
The non-steady state sulfate profiles predict the end of the most recent quiescent period and the increase of a methane supply within the sediments. These ongoing and past seepage events reflect the ventilation of the gas reservoir at depth.



Methane Seepage along the Continental Margin off Svalbard - from Bjørnøya to Kongsfjorden

S. Mau[1], M. Römer[1], M. Torres[2], T. Pape[1], T. Gentz[3], E. Damm[3], P. Wintersteller[1], J. C.-W. Hsu[1], M. Loher[1], S. Buchheister[1], M. Lange[1], A.-C. Melcher[1], N. Brückner[1], S. Gaide[1], G. Bohrmann[1] and shipboard scientific party HE449 and HE450

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to be submitted SOON

INTRODUCTION:

Emissions of methane are well known phenomena at numerous places in the oceans. However, the publication by Skarke et al. (2014) who found >500 new gas plumes along the northern US Atlantic margin indicates that there might be many more seepage sites undiscovered worldwide. Up to date, three seepage areas are well known and studied along the western margin of the Barents Sea: the Hammerfest Basin in the SW Barents Sea, the Håkon Mosby Mud Volcano, and sites offshore Prins Karls Forland (**Fig. 1**). However, methane supersaturation in the water column along the western Svalbard coast hints to further seepage. We investigated the western Barents Sea margin to determine the extent of seepage.

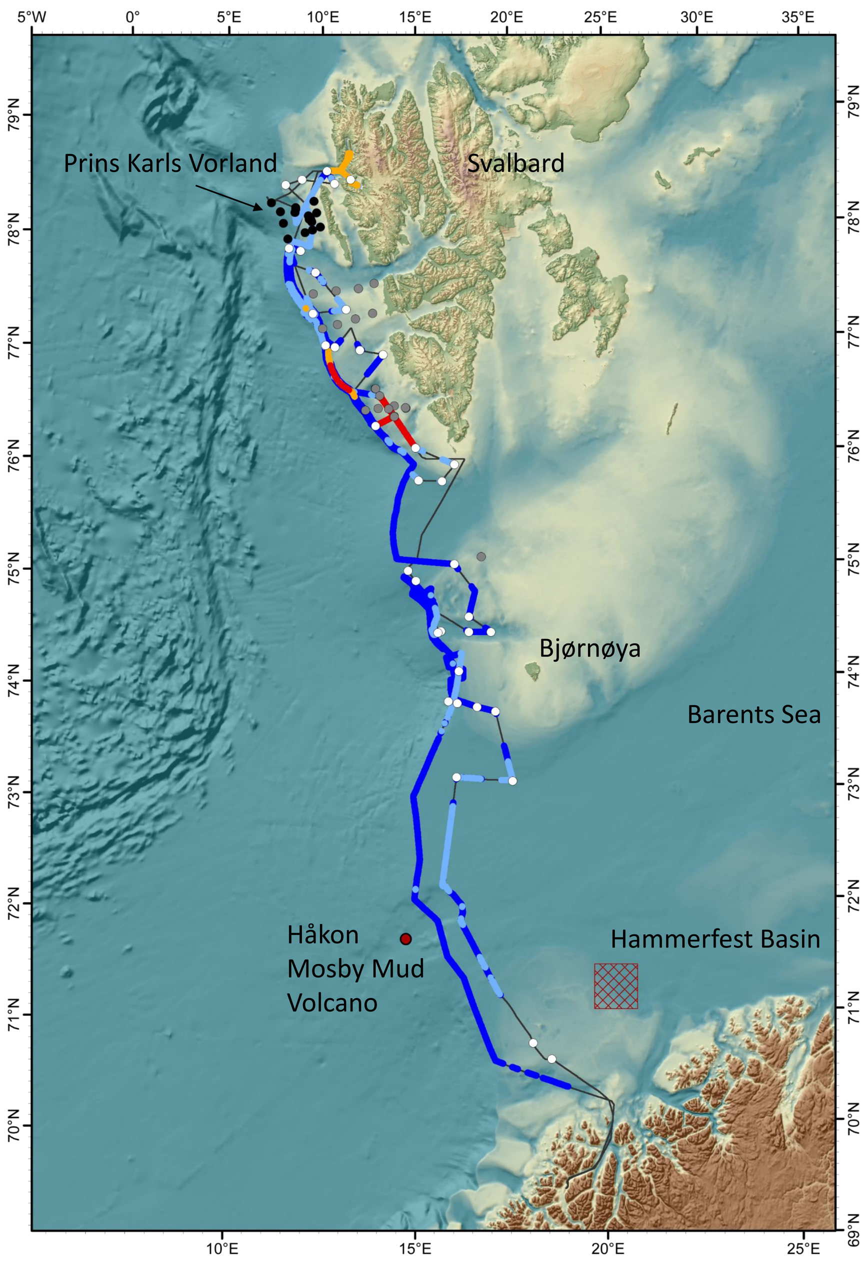


Fig. 1: CTD stations of cruises HE 387 (black), HE449 (gray), and HE450 (white). The line marks the cruise track of HE450, along which atmospheric methane concentrations were measured by ICOS (Los Gatos Research). The color code is: dark blue <1.76 ppm, blue 1.76-1.77 ppm, orange 1.77-1.78 ppm, and red >1.78 ppm.

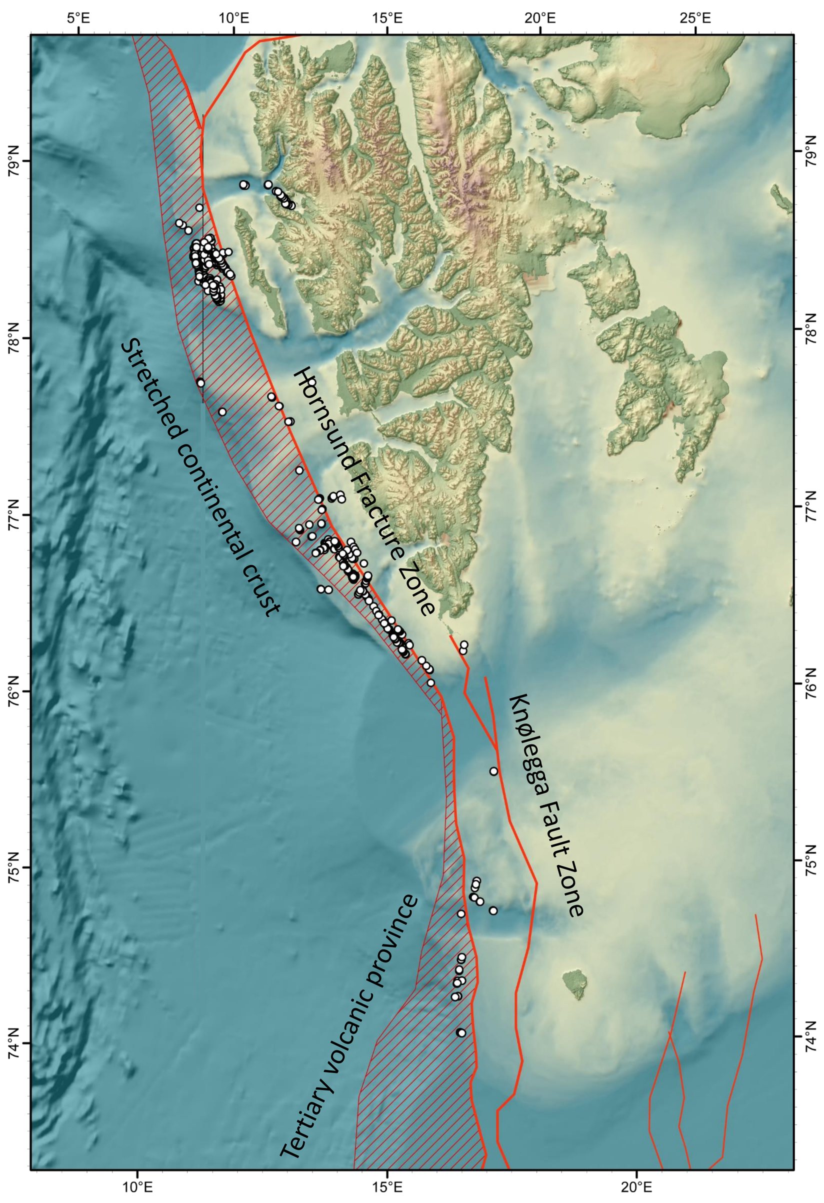


Fig. 3: Flare locations observed during HE449 and HE450 as well as flares already described by Sahling et al. (2014) illustrated as white dots. Map shows also the main structural features in red according to Faleide et al. (1991).

FLARES:

Flare clusters were found west off Bjørnøya, at Sørkappbanken, and at Hornsundbanken (**Fig. 3**). Additional seeps were also discovered in-between these clusters and farther north. The flares are all located in the vicinity of the Hornsund Fracture Zone. In addition, most gas emissions occur on bathymetric highs (**Fig. 4**), only a few single flares were found in troughs. We speculate that glaciomarine to marine sediments cover the structural pathways in the troughs. Our data reveal that methane seepage off Svalbard is not focused off Prins Karls Forland, but is more widespread than previously thought.

METHANE CONCENTRATIONS:

At almost all CTD-stations (**Fig. 1**) we found elevated dissolved methane concentrations. Methane concentrations were increasing towards the seafloor or showed numerous peaks, which suggest gas seeps in the vicinity of the stations (**Fig. 2**). Most of the hydrocasts were taken within 30 km distance to a flare, therefore, most of the methane concentrations appear to originate from seepage.

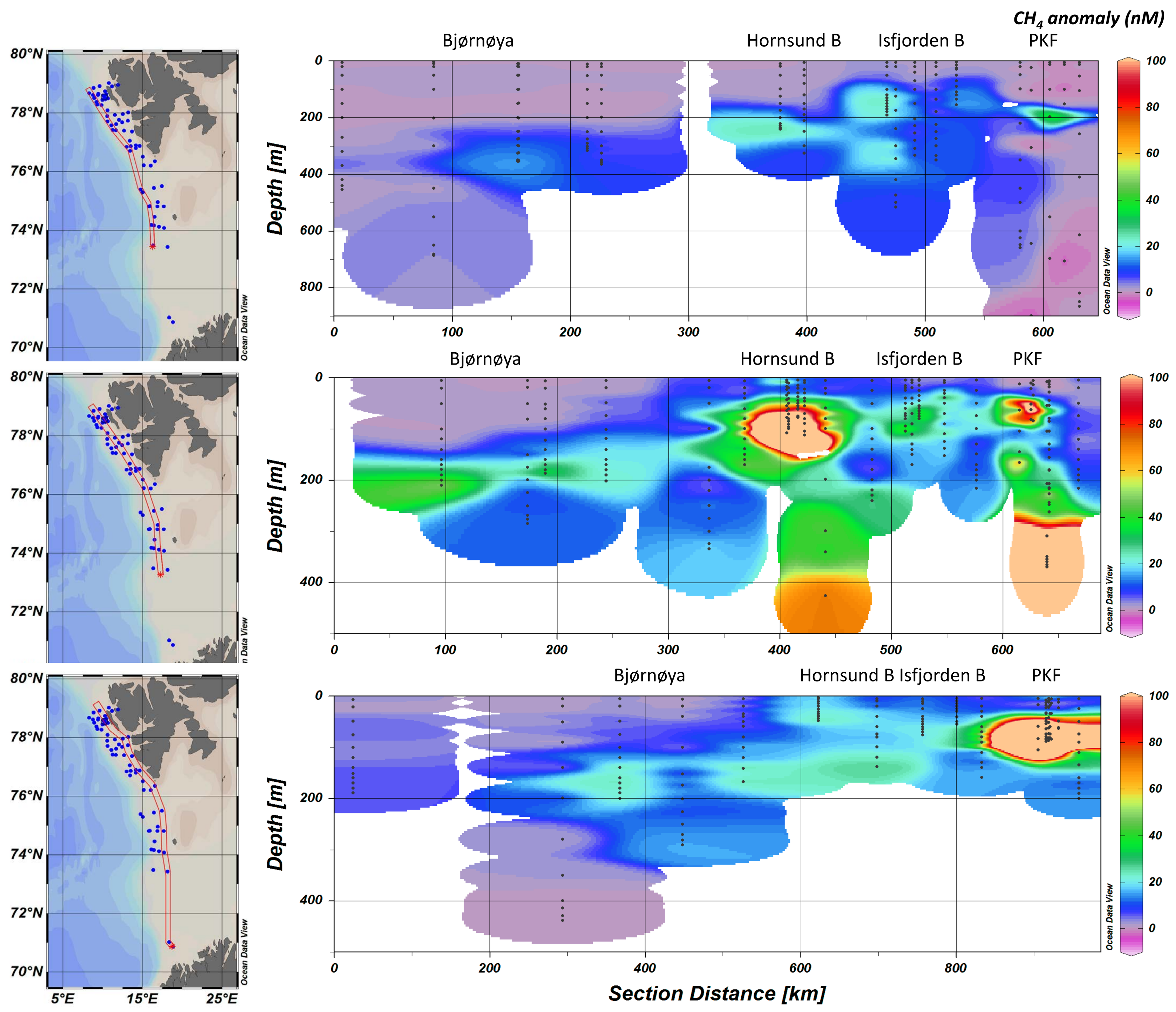
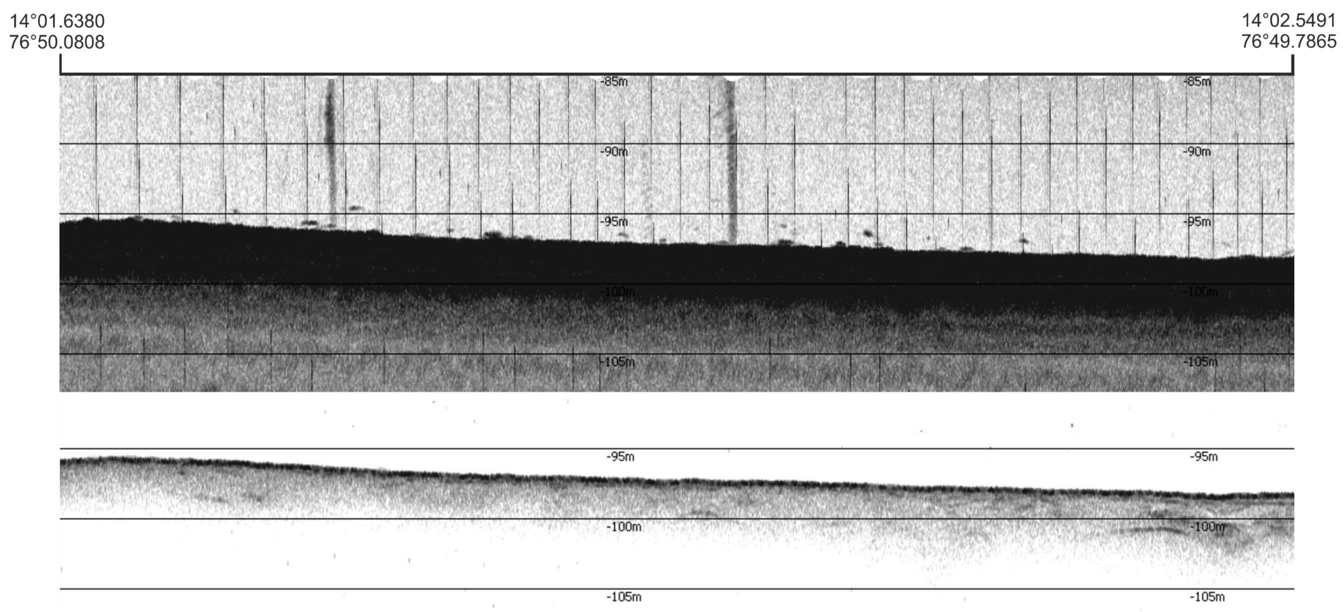


Fig. 2: South – North transects of dissolved methane concentrations. The upper section shows the transect farthest from the coast, the middle section the transect over the shelf, and the lower one the transect closest to land. Above each contour plot, the approximate location along the transect is indicated. The abbreviations stand for Hornsund B – Hornsund Banken, Isfjorden B – Isfjorden Banken, and PKF – Prins Karls Forland. Methane anomaly was derived by subtracting the atmospheric methane equilibrium according to the temperature and salinity of the water sample (2.6-3.5 nM).

Fig. 4: Example of a sub-bottom profile showing flares on top of hard ground. Most of the flares occurred on such reflectors. Sub-seafloor structures were visualized with the sediment echosounder *Innomar* SES-2000 medium.



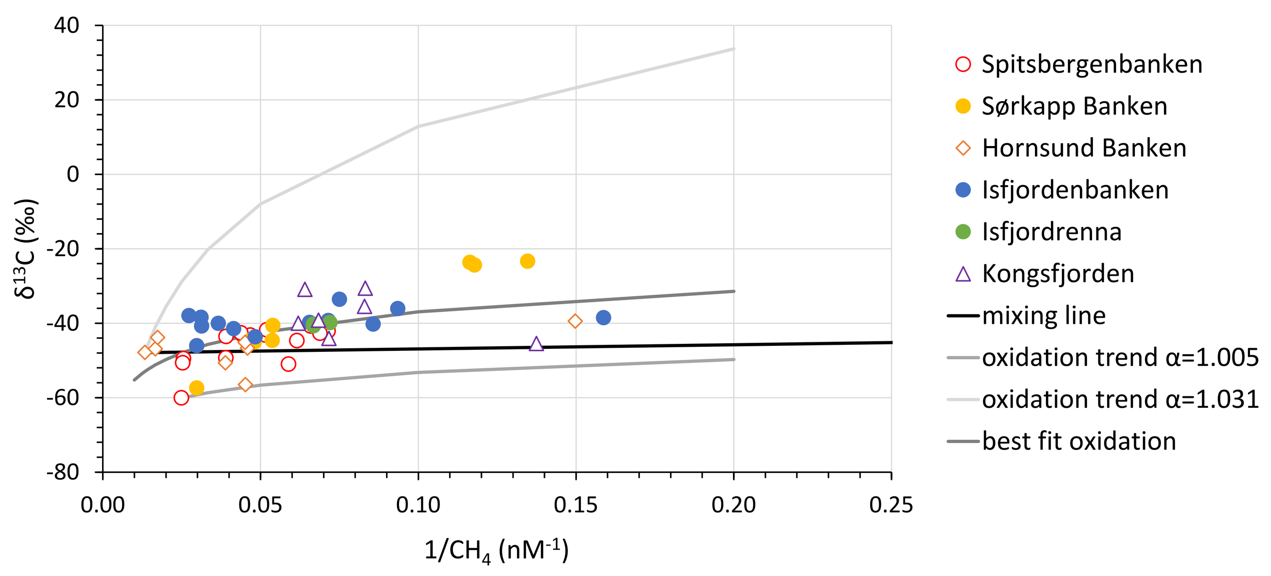


Fig.5: $\delta^{13}C$ vs. $1/CH_4$ concentration for water samples. Samples are grouped according to their location from south to north. The black line marks a mixing line between the sample with the highest methane concentration and background methane values of the ocean. The data do not fit to the mixing line. The gray lines are microbial oxidation trends calculated according to Coleman et al. (1981). The best fit had an R^2 of 0.36 with a $\delta^{13}C$-value of the source methane of -64 ‰. Apparently part of the sampled methane was already microbially oxidized, which leads to a shift of the $\delta^{13}C$-values to higher values.

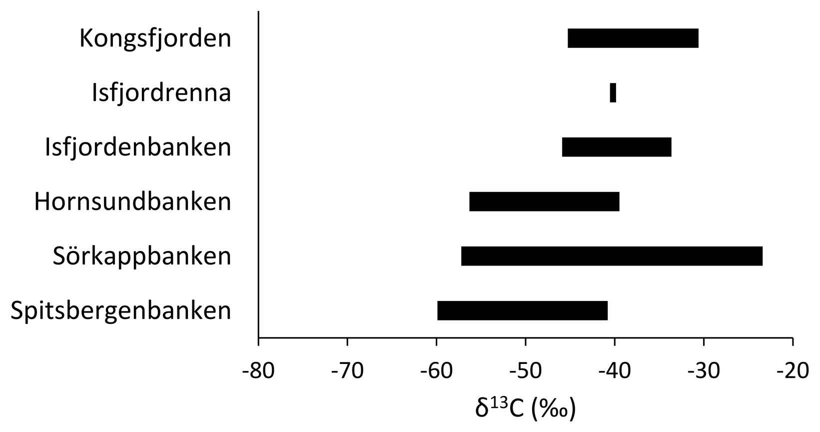


Fig. 6: Ranges of $\delta^{13}C$-methane measured at different areas along the western Barents Sea margin. Our data indicates a stronger microbial methane component in the southern regions than in the northern areas.

SOURCE:

We measured the stable carbon isotopic composition of methane derived from water samples, which were collected 2-10 m above the seafloor. Mixing with background methane in the water column appears to be negligible. However, part of the methane seems to have been consumed by methane oxidizing microorganisms in surface sediments or the bottom water (**Fig. 5**). The oxidation and the lack of higher hydrocarbons suggest a biogenic source of a fraction of the methane while the remainder might be of thermogenic origin (**Fig. 6**).

EMISSION TO THE ATMOSPHERE:

Continuous methane concentration measurements of the air revealed a possible link to shallow seep sites. We observed elevated methane concentrations while working in the area of Hornsundbanken and in Kongsfjorden (**Fig. 1** & **Fig. 7**). Flares were observed at both areas, which gas emissions might have contributed to the elevated gas content in the atmosphere.

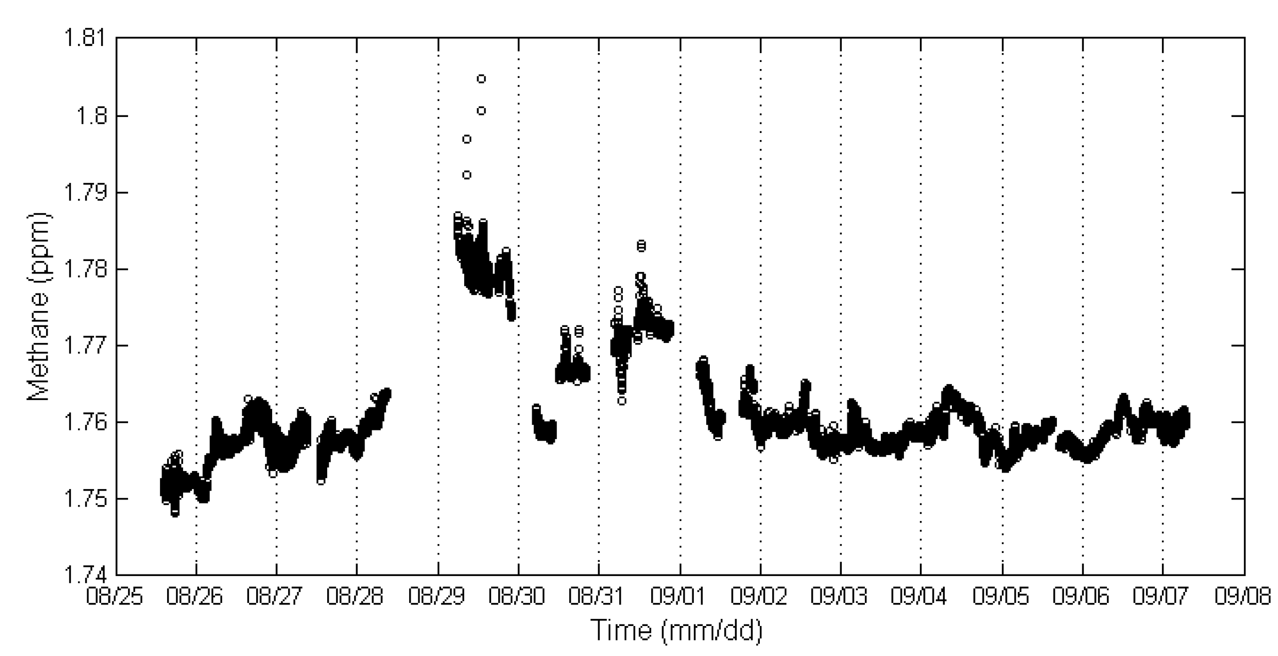


Fig.7: Methane concentrations of air measured during cruise HE450 using ICOS (Los Gatos Research) when the instrument was not used for other applications. A tubing attached to ICOS was adjusted to a porthole (~10 m above sea-surface) and air was continuously pumped to the ICOS. Elevated concentrations were measured crossing Hornsundbanken (08/29) and near Kongsfjorden (08/31).

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