Molecular ecological analysis of methanogenic communities in terrestrial and submarine permafrost deposits of Siberian Laptev Sea area

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What we know is
a drop
What we don’t know is
an ocean

Isaac Newton

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PREFACE

This work was part of the joint research project “Process studies of permafrost dynamics in the Laptev Sea” financed by the German Federal Ministry of Education and Research (BMBF) and of the Russian-German Cooperation “System Laptev Sea”. The main focus of the Laptev Sea System Project was the formation of the submarine permafrost within the near-shore zone of the shallow shelf. Studies of permafrost evolution in the coastal zone allow us to understand the on-shore/off-shore permafrost system evolution more precisely (Overduin et al., 2007).
The coastal drilling transect campaign was conducted in spring 2005 in the Cape Mamontov Klyk area (Siberian Laptev Sea Shelf area, Russia). Cores were drilled along an 11.5 km transect from terrestrial to submarine permafrost. Field research and sampling were carried out during the expedition COAST I 2005. This study is presented in English as a cumulative Ph.D. thesis at the University of Potsdam, Institute of Biochemistry and Biology.
This thesis includes an introduction of the particular research field including the scientific background and descriptions of the study area. The main part of this thesis contains three publications as first author. The first was published (under my maiden name *Koch)*. The second has been submitted and the third is in preparation for submission (both under my married name *Feige)*. The final part of my thesis comprises a publication as co-author and a synthesis representing the conclusions and future perspectives of the work.

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work and the always friendly and humorous atmosphere. Special thanks goes to Lars Ganzert alias Larsen, Juliane Griess alias Jule, Esther Rickert, Hugues Lantuit, and Georg Schwamborn for the deep, useful, and upbeat discussions and exchanges we have had. Both at and outside of work, I had a great time with you. The same goes for Dr. Daria Morozova and Dr. Susanne Liebner, who are no longer at our institute. Dear Susi and Daria, I thank you for your fond encouragement and for listening. Thank you to all my colleagues in the periglacial department!

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And last but not least my thanks go to my most humorous husband, Henrik. I treasure your humour, your love, your encouragement, and our wonderful conversations.

ABBREVIATIONS

bp	base pairs
Cy3	fluorescent dye
DAPI	4`,6-diamidino-2-phenylindole
DGGE	denaturing gradient gel electrophoresis
DNA	deoxyribonucleic acid
DOC	dissolved organic carbon
FISH	fluorescence *in situ* hybridization
IPCC	Intergovernmental Panel on Climate Change
OUT	operational taxonomic unit
PCR	polymerase chain reaction
ppm	parts per million
RNA	ribonucleic acid
rRNA	ribosomal RNA
RT PCR	real time PCR
TOC	total organic carbon
TCC	total cell count

Abbreviations are explained in the publications in which they appear. No explanation, mathematic symbols, or physical units is otherwise offered.

SUMMARY

Despite general concern that the massive deposits of methane stored under permafrost underground and undersea could be released into the atmosphere due to rising temperatures attributed to global climate change, little is known about the methanogenic microorganisms in permafrost sediments, their role in methane emissions, and their phylogeny.

The aim of this thesis was to increase knowledge of uncultivated methanogenic microorganisms in submarine and terrestrial permafrost deposits, their community composition, the role they play with regard to methane emissions, and their phylogeny. It is assumed that methanogenic communities in warmer submarine permafrost may serve as a model to anticipate the response of methanogenic communities in colder terrestrial permafrost to rising temperatures.

The compositions of methanogenic communities were examined in terrestrial and submarine permafrost sediment samples. The submarine permafrost studied in this research was 10°C warmer than the terrestrial permafrost. By polymerase chain reaction (PCR), DNA was extracted from each of the samples and analyzed by molecular microbiological methods such as PCR-DGGE, RT-PCR, and cloning. Furthermore, these samples were used for in vitro experiment and FISH.

The submarine permafrost analysis of the isotope composition of CH_4 suggested a relationship between methane content and in situ active methanogenesis. Furthermore, active methanogenesis was proven using ^{13}C-isotope measurements of methane in submarine permafrost sediment with a high TOC value and a high methane concentration.

In the molecular-microbiological studies uncultivated lines of *Methanosarcina*, *Methanomicrobiales, Methanobacteriacea* and the Groups 1.3 and Marine Benthic from *Crenarchaeota* were found in all submarine and terrestrial permafrost samples. Methanosarcina was the dominant group of the Archaea in all submarine and terrestrial permafrost samples. The archaeal community composition, in particular, the methanogenic community composition showed diversity with changes in temperatures. Furthermore, cell count of methanogens in submarine permafrost was 10 times higher than in terrestrial permafrost.

In vitro experiments showed that methanogens adapt quickly and well to higher temperatures. If temperatures rise due to climate change, an increase in methanogenic activity can be expected as long as organic material is sufficiently available and qualitatively adequate.

ZUSAMMENFASSUNG

Trotz allgemeiner Bedenken, dass auf Grund des Temperaturanstieges im Zusammenhang mit der globalen Klimaerwärmung große Mengen des in terrestrischen und submarinen Permafrostsedimenten gespeicherten Methans freigesetzt werden könnte, ist bisher wenig über die in diesen Böden lebenden methanogenen Mikroorganismen, ihre Phylogenese und sowie ihre Bedeutung hinsichtlich der Methanemissionen bekannt.

Das Ziel dieser Doktorarbeit war die Erweiterung der bisherigen Kenntnisse über unkultivierte methanogene Mikroorganismen in submarinen und terrestrischen Sedimentablagerungen, die Zusammensetzung ihrer Lebensgemeinschaft, ihrer Phylogenese und ihrer Bedeutung bei der Emission von Methan. Es wird vermutet, dass methanogene Gemeinschaften submarines Permafrostes zur Erstellung von Modellen genutzt werden können, um Aussagen bezüglich potenzieller Reaktionen methanogener Gemeinschaften des kälteren terrestrischen Permafrostes auf steigende Temperaturen, zu ermöglichen.

Die Zusammensetzung der methanogenen Gemeinschaft wurde in terrestrischen und submarinen Permafrostproben untersucht. Der im Rahmen dieser Forschungsarbeit untersuchte submarine Permafrost wies eine im Vergleich zum terrestrischen Permafrost um circa 10°C höhere Temperatur auf. Mittels Polymerasenkettenreaktion (PCR) wurde von jeder der Proben DNA extrahiert und mittels weiterer molekular-mikrobiologischen Methoden wie DGGE, RT-PCR und Klonierung analysiert. Des Weiteren wurden die Proben für *in vitro* Experimente und Zellzählungen (DAPI und FISH) verwendet.

Die Analyse der Isotopenzusammensetzung von CH_4 in submarinen Permafrostsedimenten ließ einen Zusammenhang zwischen Methangehalt und aktiver in situ Methanogenese vermuten. Überdies konnte aktive Methanogenese, mittels ^{13}C-Isotopenmessungen von Methan in submarinem Permafrostsediment mit hohem TOC-Wert und hoher Methankonzentration, bewiesen werden.

Im Rahmen der molekular-mikrobiologischen Untersuchungen wurden in allen submarinen und terrestrischen Permafrostproben unkultivierte Linien von *Methanosarcina, Methanomicrobiales, Methanobacteriacea* und den Gruppen 1.3 und Marine Benthic von *Crenarchaeota* gefunden. *Methanosarcina* war in allen submarinen und terrestrischen Permafrostproben die dominierende Gruppe der Archaeen. Die Zusammensetzung der archaealen Gemeinschaft, insbesondere die Zusammensetzung der methanogenen Gemeinschaft, variierte zwischen den submarinen und terrestrischen Proben. Des Weiteren fand sich bei der Zellzählung der Methanogenen im submarinen Permafrost eine 10-fach höhere Zellzahl als im terrestrischen Permafrost.

Die *in vitro* Experimente zeigten, dass Methanogene sich schnell und gut an höhere Temperaturen anpassen können. Im Falle weiter steigender Temperaturen auf Grund

der Klimaveränderungen, kann – bei ausreichender Verfügbarkeit und Qualität organischen Materials – mit einer Zunahme der methanogenen Aktivität gerechnet werden.

1. INTRODUCTION

1.1 Scientific background

1.1.1 Arctic climate change and methane emission

The Earth's climate is changing with increasing global temperature and a change that is particularly amplified in the Arctic (Serreze et al., 2000, McGuire et al., 2007). Over the past few decades, the temperature in the Arctic has been rising at almost twice the rate as compared to the rest of the world (ACIA, 2004). Evidence of arctic warming includes widespread melting of sea ice and a shortening of the snow season (McBean et al., 2005).

Global warming has resulted from greenhouse gases (mostly carbon dioxide and methane), from natural and anthropogenic sources such as emissions from wetlands, transport, energy production, industry, agriculture, and landfills. Emissions from natural sources are largely determined by environmental variables such as temperature and precipitation. The IPCC 2001 documents global methane emissions from natural sources at approximately 190 Tg per year. Most natural sources of atmospheric methane are from wetlands, accounting for approximately 145 Tg of methane per year (IPCC, 2001). In wetlands, methane production is the dominant terminal process in microbial degradation of organic matter (Hedderich & Whitman, 2005).

Due to increases of greenhouse emissions, the temperature in the Arctic has increased by 3°C to 4°C and could further increase by about 4°C to 7°C by 2100 (ACIA, 2004). Methane is an effective greenhouse gas since it absorbs infrared radiation from earth more effectively than carbon dioxide. According to diverse bibliographical references, the greenhouse effect, the heat-trapping potency of methane is estimated to be 20 to 36 times greater than carbon dioxide (ACIA, 2004). Measurements of the atmospheric methane concentration over the last decades are shown in Figure 1 (NOAA, 2006 & 2008). Arctic warming leads to an increase of these emissions creating a feedback cycle whereby more warming causes additional methane emission, which in turn causes more warming, and so on (ACIA, 2004).

1.1.2 Permafrost – a sensitive indicator for global climate change

Permafrost is ground (soil or rock) that remains at or below 0°C for at least two consecutive years. Figure 2 (a.) shows the distribution of terrestrial and submarine permafrost in the Northern Hemisphere, where permafrost zones cover approximately 12 to 17 x 10^6 km^2 which is about 25% of the exposed land area (Zhang et al., 2000). Very cold permafrost (at -10°C and lower) occurs mainly in the northern Arctic and is usually 500-600 m thick (Hubberten & Romanovskii, 2003). Warm permafrost (at -2 to -1°C, close to its melting point) occurs typically further south and is generally several meters thick (ACIA, 2004). Surface temperatures over

most permafrost areas are increasing. The temperature measurements from permafrost boreholes in Canada, Alaska, Norway, and Russia show that the surface temperature has increased by up to 2.8°C since the early 1980s (Couture et al., 2003, Isaken et al., 2001, Osterkamp, 2003, Romanovsky et al., 2002). Smaller temperature increases (1°C or less) have been observed in north-western Siberia (Chudinova et al., 2003). Due to these facts, the results from the five ACIA-designated models suggest that the total permafrost in the Arctic will decrease by approximately 23 % by 2080 (ACIA, 2004).

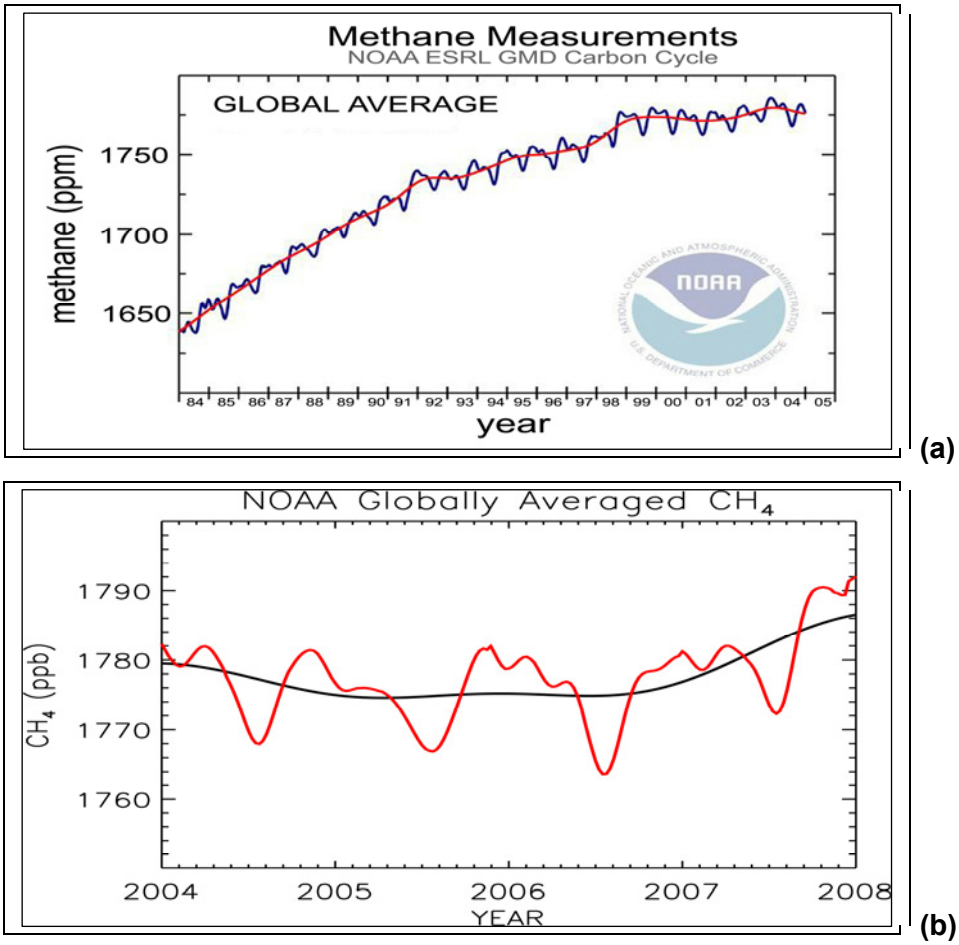

Figure 1. (a) Global average atmospheric methane mixing rations (blue line) determined using measurements from the GMD cooperative air sampling network. The red line represents the long-term trend (National Oceanic and Atmospheric Administration, NOAA, 2006) (b) Actual global methane concentrations; the red line shows the trend together with seasonal variations. The black line indicates the trend that emerges when the seasonal cycle has been removed (NOAA, 2008)

Thawing permafrost may act as a positive feedback mechanism to climate change through increasing emissions of greenhouse gases (i.e. carbon dioxide and methane; Anisimov & Nelson, 1997). Arctic wetland (permafrost soils), boreal, and tundra vegetation contain a significant portion (5.7×10^{12} kg) of the global organic carbon (102×10^{12} kg terrestrial C; Jonasson et al., 2001). This is roughly 5.6 % of the

Earth's soil carbon pool. Natural wetlands are the largest natural methane sources, contributing to approximately 24 % of the total emissions. Several studies of methane fluxes from Arctic wetlands have been conducted (Christensen 1993; Christensen et al., 1996; Tamura et al., 1995; Nakano et al., 2000; Wagner et al., 2003; Bohn et al., 2007). Current methane emissions from northern wetlands and tundra are estimated to be approximately 35 Tg per year (Reeburgh et al., 1993), corresponding to nearly 6 % of the total global methane source (Ehhalt et al., 2001).

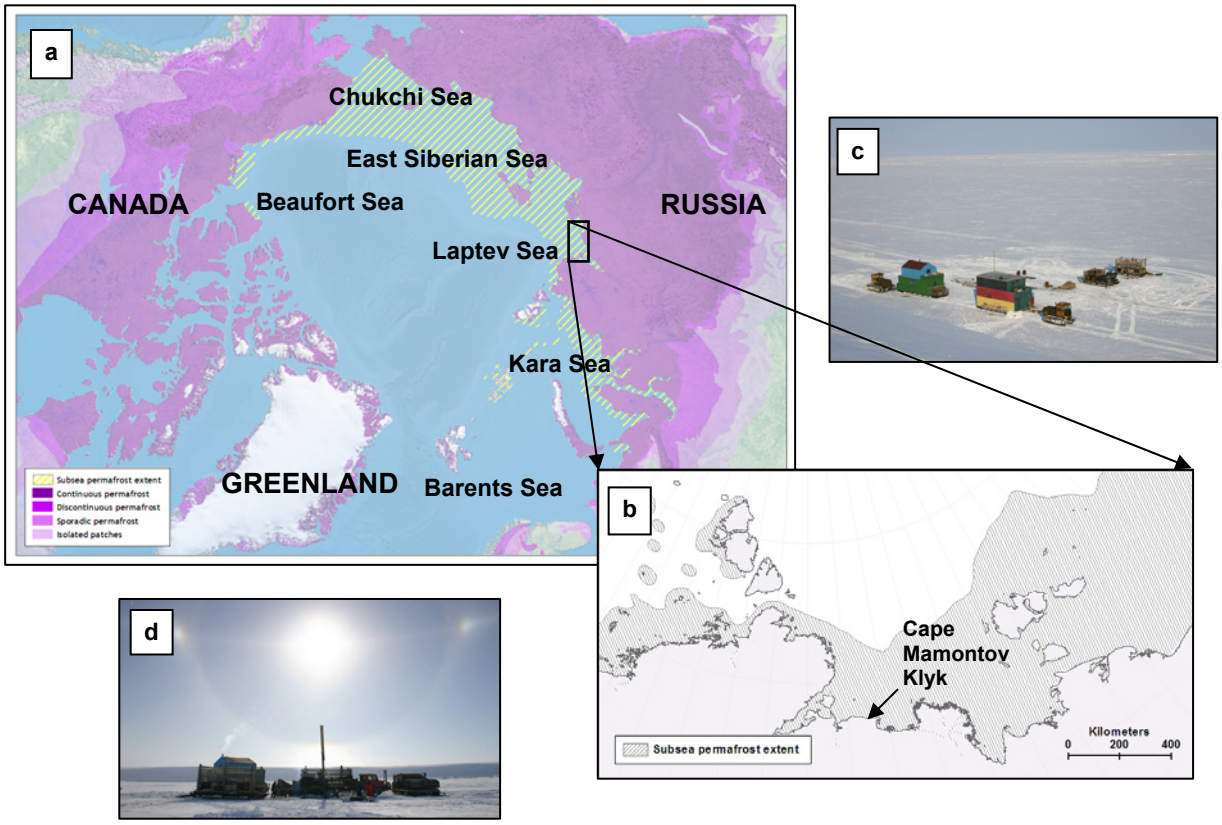

Figure 2. (a) Distribution of the terrestrial (violet) and submarine (striped) permafrost on the northern hemisphere (b) Map showing the location of Cape Mamontov Klyk (c) and (d) The images of the Russian drilling team and of drilling rig on Laptev Sea ice during spring 2005 (photo by V. Rachold, IASC)

1.1.3 Terrestrial permafrost – stressed with several processes

The expansion and temperature of terrestrial permafrost, located near coastal areas, is influenced by the proximity to the sea. The top of the terrestrial permafrost soil is called active layer, which thaws during summer and refreezes during autumn. The thickness of the active layer is strongly dependent on the air temperature (Anisimov & Nelson, 1997) and can be up to 5 m. According to global climate models, by 2099 the mean annual ground temperatures could rise by 2 to 6°C and the active layer

thickness could increase by 0.5 to 2 m everywhere within the East Siberian transect (Sazonova et al., 2004).
Conditions of terrestrial permafrost near the coast are influenced by oceanographic and meteorological processes (Walsh et al., 2005). Geomorphologically permafrost is affected by marine processes such as seawater temperature, sea ice distribution, storm, wave and tidal activity. These factors trigger the erosion of cliffs and bluffs. The rate of destabilization of permafrost is also dependent on the ground ice content and sea ice action. Studies of air temperature and active layer thickness in the Arctic are well documented. Rising sea levels may further destabilize terrestrial permafrost near the coast. In fact, the global average sea level has risen by 0.2 m during the 20th century (IPCC, 2001) through the warming of Atlantic and Pacific Ocean waters.

1.1.4 Submarine permafrost – a natural laboratory for studying global change

Submarine permafrost was formed by exposure of the sea bottom to negative mean annual temperatures or as a result of flooding of terrestrial permafrost (see Figure 3). Not all submarine permafrost is frozen, since the thawing point of sediments may be below 0°C by the presence of saline pore water (Overduin et al., 2008). The capillary effects in fine-grained material also play a role (Walsh et al., 2005).
Figure 3 shows the development of submarine permafrost of Siberian Laptev Sea Shelf area. Formerly terrestrial permafrost exists today as submarine permafrost in the Laptev Sea. Global warming with increasing seawater temperatures may accelerate the diminishment of this permafrost, since it is warmed from above by shelf seawater and from below by geothermal heat. The degradation of permafrost may lead to the escape of organic carbon, methane, or gas hydrates in or below the submarine permafrost. These substances would be released into the water column, and subsequently into the atmosphere. Thus, the degradation of submarine permafrost is thought to be a significant factor in global climate change system on a middle or long-term timescale. However, the rate of degradation of submarine permafrost in the Laptev Sea Shelf area is still largely unclear.
Drilling of onshore (terrestrial) and offshore (submarine) permafrost in the Laptev Sea region (see section 1.3) took place within the framework of the Russian-German Cooperation “Laptev Sea System”. It was discovered that the temperature of the submarine permafrost was 10°C higher than terrestrial permafrost. The comparison of sediments from onshore and offshore sites provides a good opportunity to study the effects of higher temperatures on processes affecting carbon stocks and microbial communities in permafrost environments.

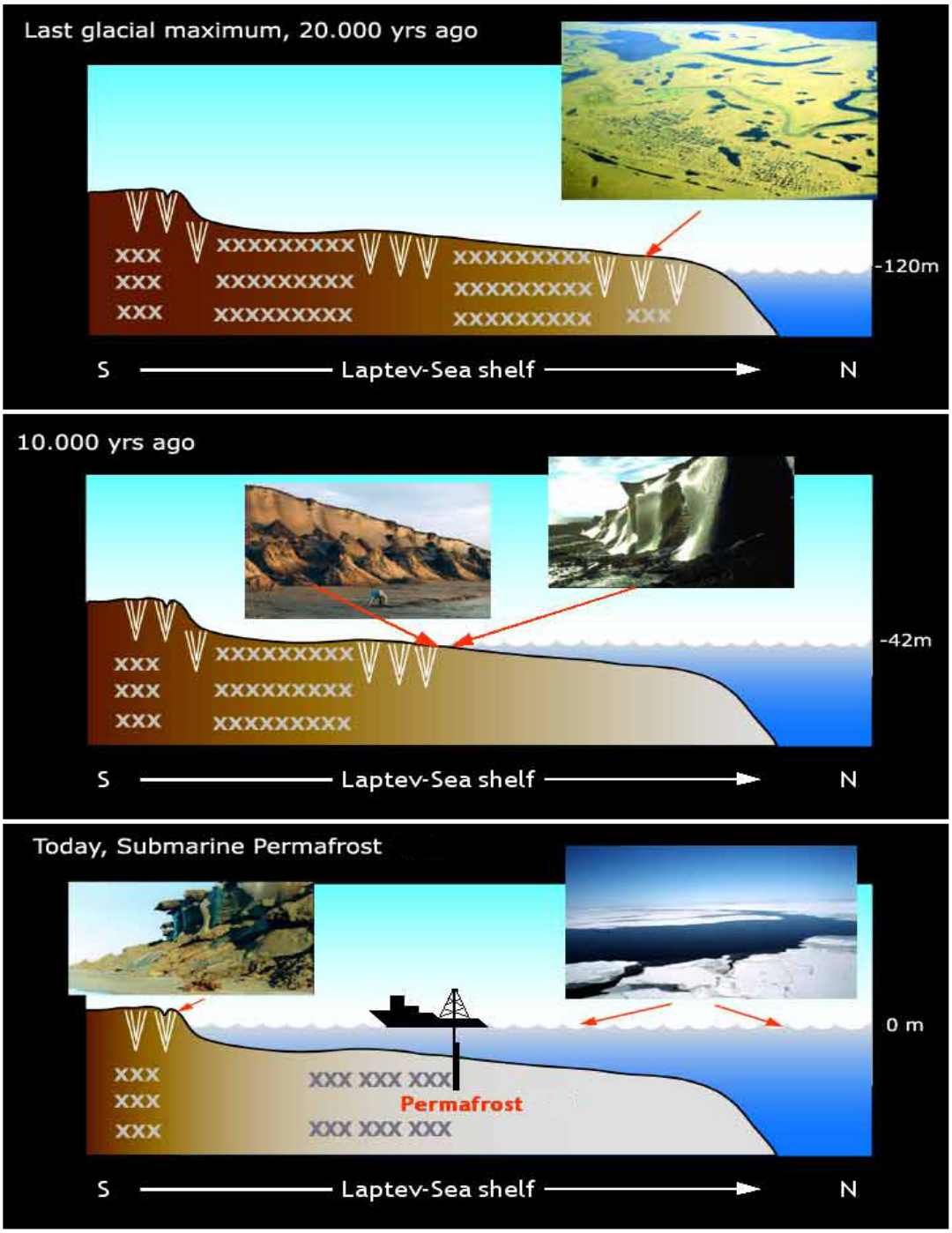

Figure 3. The Siberian Laptev Sea Shelf contains submarine permafrost, which was formed by flooding of terrestrial permafrost with ocean water during the Holocene sea level rise. (source: http://www.ifm-geomar.de)

1.1.5 Methanogens in permafrost – robust microorganisms

Methanogenic archaea are widespread in nature and in extreme environments, tolerating low and high temperatures, high salinity, and low and high pH-values (Wagner & Liebner, 2009). They are characteristic members of microbial communities of anoxic habitats where they are responsible for biogenic methane formation (methanogenesis). Methanogenesis (see Tab. 1) is the final step of

anaerobic degradation of organic matter within the anaerobic food chain. It is catalyzed by methanogenic archaea using substrates provided by other microorganisms. Most methanogens use carbon dioxide as electron acceptor and reduce it with hydrogen to methane. Some methanogens are able to use acetate for methane production.

The first step of carbon degradation within this food chain is conducted by extracellular enzymes of fermenting bacteria which decompose polymers to monomers. Subsequently the monomers are catabolised by other primary fermenters, producing acetate, propionate, butyrate, succiante, alcohols, hydrogen, and carbon dioxide. Propionate, butyrate, succiante, and alcohols can be degraded by themophile syntrophic bacteria to hydrogen, formate, or acetate. The involvement of thermophile syntrophic bacteria, however, is improbable because of low temperature (Hattori et al., 2000; Schink & Stams, 2001). Fatty acids could accumulate in cold environments, though it is unclear, how these fatty acids would be degraded. However, using injections with ^{14}C-labeled acetate, Rivkina and colleagues demonstrated (Rivkina et al., 2000) that permafrost bacteria were still metabolically active at temperatures down to -20°C. A different way of decomposition monomers at low temperature is also conducted by acetogenic bacteria. Acetogenic bacteria convert H_2 and CO_2 into acetic acid at low temperatures, when hydrogen concentrations are high, and pH-values exceed 7. Reversely, at high temperatures, when hydrogen concentrations are low, and pH-values are below 7, acetogenic bacteria convert acetic acid into H_2 and CO_2 (Thauer et. al, 2008). The substrates used for the methanogenic catabolic pathway can be assigned to three major groups (Table 1.1): methylotrophic, acetoclastic and CO_2-reductive methanogenesis.

Table 1. Methanogenic reactions (source: http://www.agen.ufl.edu)

CO_2-reductive methanogenesis:			
Hydrogen:	$4H_2 + CO_2$	>	$CH_4 + 2H_2O$
Formate:	$4HCOOH$	>	$CH_4 + 3CO_2 + 2H_2O$
Carbon Monoxide:	$4CO + 2H_2O$	>	$CH_4 + 3H_2CO_3$
Acetoclastic methanogenesis:			
Acetate:	CH_3COOH	>	$CH_4 + CO_2$
Methyltrophic methanogenesis:			
Methanol:	$4CH_3OH$	>	$3CH_4 + CO_2 + 2H_2O$
Trimethylamine:	$4(CH_3)_3N + 6H_2O$	>	$9\ CH_4 + 3CO_2 + 4NH_3$
Dimethylamine:	$2(CH_3)_2NH + 2H_2O$	>	$3\ CH_4 + CO_2 + 2NH_3$
Monomethylamine:	$(CH_3)NH_2 + 2H_2O$	>	$3\ CH_4 + CO_2 + 4NH_3$
Methyl Mercaptans:	$2(CH_3)_3S + 3H_2O$	>	$3\ CH_4 + CO_2 + H_3S$

In terrestrial as well as submarine permafrost areas geochemical processes are profoundly affected by microbial processes, such as methanogenesis. According to studies of Wagner et al. (2003), Høj et al. (2005), Metje & Frenzel (2007) the methane production in the active layers at low in situ temperatures reached 39 nmol

CH_4 h^{-1} g^{-1} soil. Methane production in cold habitats has been found to depend more on the quality of organic matter necessary for anaerobic degradation than on temperature (Wagner et al., 2005, Ganzert et al., 2007). Another important factor affecting methanogenic communities in permafrost habitats is the water regime (Høj et al., 2006). Christensen et al. (2004) found that variations of methane emissions depended on peat water availability. Low water content leads to a drastic reduction in methane emissions as such soil hydrology acts as a kind of “on-off switch” regulating large-scale methane emissions.

Methanogenic archaea are a phylogenetically diverse group of strictly anaerobic *Euryachaeota*. The members *Methanobacteriales*, *Methanococcales*, *Methanomicrobiales* and *Methanopyrales* belong to the group hydrogenotrophic methanogens and are substrate specialists, characterized by the absence of cytochromes and methanophenazine. They produce methane from hydrogen and carbon dioxide, while the genus *Methanosaeta* belongs to the group of acteoclastic methanogens which only grows on acetate. The species of *Methanosarcinales* are by comparison generalists. They show a larger physiological diversity and the use of a broad substrate spectrum (methanol, hydrogen, carbon dioxide, acetate, methylamines, methylthiols). All members of the *Methanosarcinales* (with the exception of *M. stadtmanae)* have cytochromes and methanophenazine (Thauer et al., 2008).

Molecular studies of methanogenic populations in Arctic permafrost, soils and sediments are reported in Bergman et al. (2000), Ganzert et al. (2007), Høj et al. (2006, 2008). These habitats are characterized by subzero temperatures, low water activity, and low nutrient availability. Further studies on methanogenic Archaea from Siberian permafrost have shown, that methanogens from these environments are more stress tolerant than methanogens from nonpermafrost habitats (Morozova & Wagner, 2007). Low numbers of cultivatable methanogenic Archaea have also been found in cold environments (Franzmann et al., 1992, Simonovka et al., 2003; Nozhevnikova et al., 2001; Rivina et al. 1998), despite the difficulty in isolating them in cold terrestrial environments due to their slow growth rates, high sensitivity to oxygen, and their close association to syntrophic partner microorganisms (Kotsyurbenko, 2005; Wagner, 2008).

Very little is known about the ecology and diversity of methanogens in such habitats; these are the first molecular research of archaeal communities in submarine permafrost sediments.

1.2 Objectives

The primary objective of this thesis was to increase the knowledge about archaeal communities in colder terrestrial and warmer submarine permafrost sediments from the Siberian Laptev Sea area, with special emphasis on methanogenic Archaea. A further aim was to obtain findings on the response of microbial communities to changing environmental conditions, which may provide insight into forthcoming changes of these communities due to the global warming.
In particular, methanogenic composition, abundance, and diversity in submarine and terrestrial permafrost were to be investigated and *in vitro* research conducted. For this purpose, several samples from different permafrost sites were obtained and analysed by molecular-biological methods such as PCR-DGGE, cloning, RT-PCR and FISH. Since the temperature of submarine permafrost is higher than the temperature of terrestrial permafrost, it offers the opportunity to study the abundance of microbial, especially methanogenic communities at higher temperatures as a kind of natural laboratory. Furthermore, several samples were used for *in vitro* experiments to investigate the response to diverse conditions.
To increase understanding of the methanogenic ecology in permafrost habitats and possible responses due to global warming, the following questions were posed and were the main focus of this study:

- How is the vertical distribution of methanogenic Archaea in submarine permafrost (at nearly 0°C) and how does distribution correlate with TOC value, methane concentration and methane isotope ratio?
- How does the archaeal community composition, and in particular, the methanogenic community composition vary in abundance and diversity in the terrestrial and submarine permafrost according to temperature, water content, and TOC value?
- What are dominant groups within the permafrost archaeal communities and which are driving carbon cycling processes?
- Which methanogens best adapt and assert themselves under *in vitro* conditions with regard to temperature and substrate availability within a certain time period?

1.3 Study site

The Laptev Sea is a semi-enclosed marginal sea of the Arctic Ocean. The Laptev Sea Shelf covers an area of approximately 450,000 km^2 north of the Anabar and Olenek rivers of northern Siberia. The Anabar, Olenek, Khatanga, Lena, and Yana rivers flow into the shelf sea (Naidina and Bauch, 2001). The Lena River, with its massive delta, is the largest river flowing into the Laptev Sea. The Lena River accounts for 70% of the total water and suspended matter entering the Laptev Sea (Kassens et al., 1998). During winter, the Laptev Sea is affected by a large area of constantly open water (polynya) separating the fast ice from the pack ice (Razina et al., 2007). Large parts of the Laptev Sea Shelf are fairly shallow, averaging less than 50 m water depth, whereas the mean depth of 75% of the Laptev Sea area is between 15 and 25 m, near 100 m water depth the continental slope breaks steeply (Razina et al., 2007, Kosobokova et al., 1998). The climate is cold, with low temperatures in February at -30°C and below and highs in July between 0 to 8°C (Razina et al., 2007). The annual precipitation is less than 300 mm (Grosse, 2005). Today the landscape directly adjacent to the Laptev Sea is treeless tundra (Naidina and Bauch, 2001).

During the COAST I expedition in spring 2005 permafrost samples were collected from five boreholes along a drilling transect that ran approximately 12 km northward from the coastal bluff at Cape Mamontov Klyk (73°42´N 117°10´E) (Fig. 2 and Fig. 4). The COAST team used a drilling rig (URB-2A-2) with a hydraulic rotary-pressure mechanism. All samples were transported in a frozen state to AWI Potsdam. The microbial investigations of this thesis are based on results of sediment samples from the terrestrial permafrost (Core C1) and submarine permafrost (Core C2).

C1 (terrestrial permafrost) was drilled on the mainland at a distance of approximately 100 m from the coastline and obtained a depth of approximately 60 m. The sediments consist of completely frozen and terrestrial material. It contains loam (soil containing sand, silt, and clay) and layers of dark gray autochthonous peat at depths between 0.3 m - 0.7 m, ice wedges down to 20.7 m, and fine sand (mostly dark gray) with varying organic content at depths between 20.7 – 60.8 m (Overduin et al., 2007). After drilling, the borehole temperature was measured using calibrated thermistors (MMT-4). The borehole temperature ranged between -18°C and -12.4°C (Junker et al., 2008).

At 11.5 km from the coast core C2 (submarine permafrost) was drilled from the sea ice (1.35 m thick) in water of 6 m depth through submarine and terrestrial deposits and reached a depth of approximately 77 m. At a depth of approximately 40 m below sea level frozen material was encountered, at a depth of approximately 65 m unfrozen material was encountered. Evidence suggests that the underlying unfrozen material was originally marine sediment (Rachold et al., 2007). At sediment depths between 6 m and 35.5 m the submarine permafrost consisted mostly of dark gray

fine sand (unfrozen); at sediment depths between 35.5 m and 40.3 m the submarine permafrost consisted of brownish gray fine sand (ice-bonded and unfrozen); at sediment depths between 40.3 m and 64.7 m the submarine permafrost consisted of brownish gray fine sand (frozen) with varying organic material contents; and at sediment depths between 64.7 m and 77 m the submarine permafrost consisted of mostly dark gray fine sand. Borehole temperatures measured after drilling were between -2°C and -1.1°C (Junker et al. 2008).

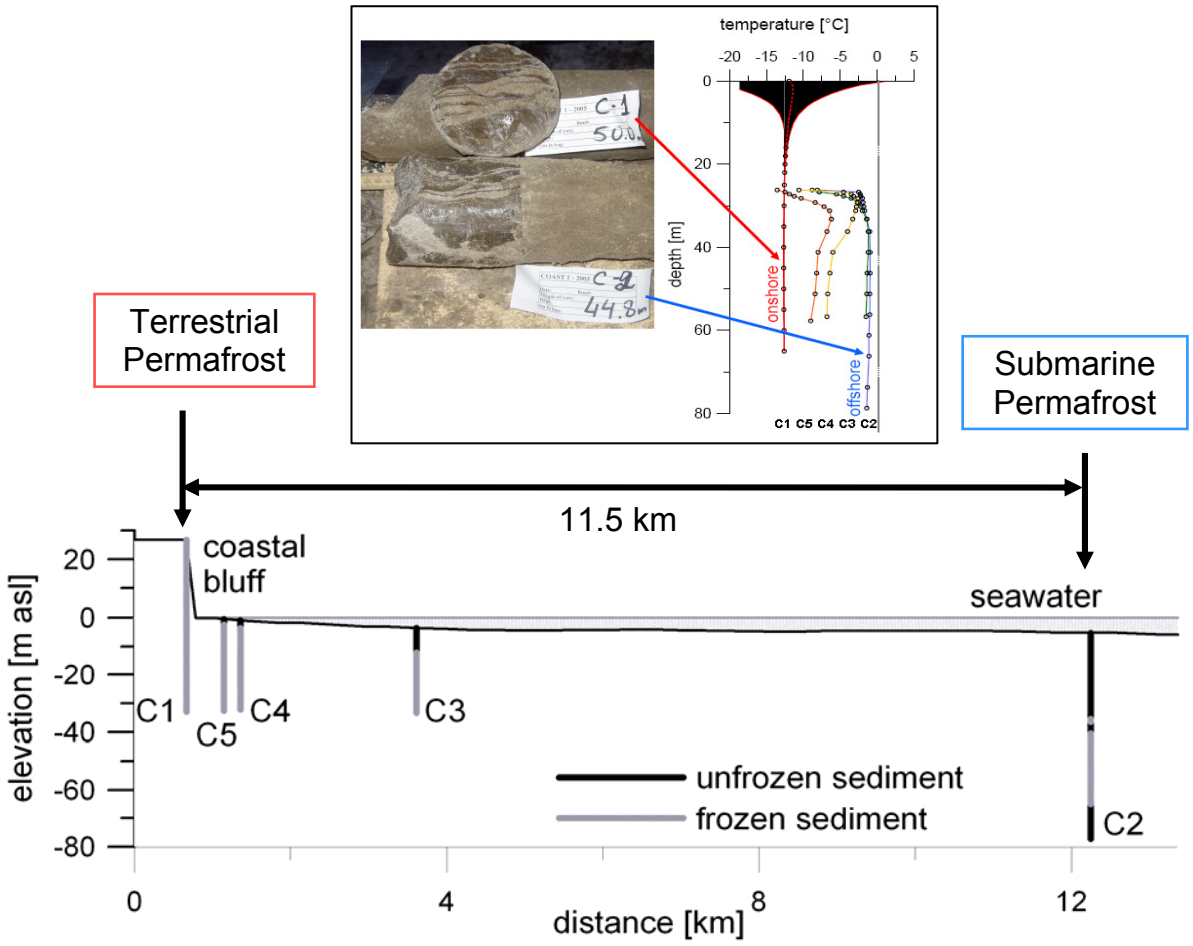

Figure 4. (above) Drilling cores and their depth; core C1 is terrestrial permafrost and core C2 is submarine permafrost. The drilling sites are 11.5 km apart; the other cores C3, C4, and C5 were not used in this dissertation (Overduin et al., 2008, modified); in the photograph similarities in deposits in the permafrost and terrestrial permafrost can be seen (the temperature difference between cores is approximately 10°C (Rachold et al., 2007)

1.4 Overview of publications

In section 3 the results of this thesis are presented in the form of four publications. The first and fourth (the fourth as co-author) have been published, the second has been submitted and the third is in preparation for submission.

1st Publication (published in 2009 in *Environmental Microbiology 11, 657-668*):

Methanogenic community composition and anaerobic carbon turnover in submarine permafrost sediments of the Siberian Laptev Sea

Authors: Katharina Koch[1], Christian Knoblauch[2], and Dirk Wagner[1]

Topic: Permafrost is a significant factor in controlling hydrological processes in the submarine permafrost environment. The submarine permafrost of the Laptev Sea Shelf is influenced by rising temperatures of seawater and plays an important role in climate change. This paper describes methanogenic communities along depth profiles of submarine permafrost by DNA-based analysis and by comparing methane isotope ratio, TOC value, and methane concentration. At a depth of 58 m, high intensities of DGGE bands correlated with lowest methane isotope ratio, highest TOC value, and highest methane concentration proving that methanogens are microbial active under *in situ* conditions in the frozen ground.

This paper was written, compiled, and illustrated by Feige (née Koch) with supervision by Wagner. Feige conducted the molecular analyse using DGGE (100%). Wagner carried out the sampling and measurements of methane content along the core (100%). Knoblauch carried out the measurements of isotope ratio and TOC (100%). Wagner and Knoblauch contributed also with helpful discussion to the interpretation of the results and improved the structure of the manuscript (30%).

2nd Publication (Final draft for *ISME Journal*):

Comparison of microbial communities in terrestrial and submarine permafrost sediments from the Siberian Laptev Sea area

Authors: Katharina Feige[1], Paul Overduin[1], and Dirk Wagner[1]

Topic: Submarine permafrost from the Laptev Sea Shelf was used as model to understand the potential effect of on microbial communities in permafrost habitats. The submarine permafrost (-1.5°C) is warmer than the terrestrial permafrost (-12°C) obtained from the Laptev Sea region. The primary goal of this paper is to identify microbial communities from permafrost-affected soil, deep terrestrial permafrost, submarine permafrost, and unfrozen marine sediments using Real Time PCR, FISH, and cloning. Furthermore, the results were interpreted with regard to chemical und

physical parameters. The diverse permafrost environments showed distinct differences in their archaeal communities. High copy numbers and cell count of methanogens were found in submarine permafrost at a depth of 58 m where the highest methane concentrations were found.

This paper was mainly written and compiled as well as illustrated by Feige under supervision of Wagner. Furthermore, Feige conducted all molecular analyses (100%). Furthermore, Overduin provided the physical and chemical parameters of terrestrial and submarine permafrost sediments (100%). Wagner and Overduin contributed by supportive discussion to the interpretation of the results and reworked parts of the manuscript (30%).

3rd Publication (in preparation):

Methanogenic communities analysed by *in vitro* experiments using denaturing gradient gel electrophoresis on terrestrial and submarine permafrost samples of the Siberian Laptev Sea area

Authors: Katharina Feige[1] and Dirk Wagner[1]

Topic: Each sample of submarine and terrestrial permafrost was used for *in vitro* enrichment experiments of methanogenic Archaea. The experiments were intended to stimulate methanogenic communities by application of diverse substrates, more precisely, LB medium for organic-rich nutrient conditions and artificial pore water for quasi *in situ* conditions. The substrates were applied at three different temperatures (0°C, 4°C, and 10°C). After this *in vitro* experiment DNA was extracted from stimulated as well as non-stimulated sediment samples for comparison of the community composition using PCR-DGGE. Samples with different experiment conditions showed significant differences in their methanogenic community profiles. Sequences obtained from DGGE gels were closely related to *Methanosarcinales, Methanomicrobiales, Methanobacteriaceae,* and uncultured *Euryarchaeota*. However, *Methanobacteriaceae* and *Methanosaeta* were first detected *in vitro* experiment at higher temperature, but had not been initially detected in their deep permafrost environments.

This paper was written, compiled, and illustrated by Feige under supervision of Wagner. Feige conducted in vitro *experiment (100%).*

4th Publication (as co-author, published in 2008 in *Ninth International Conference on Permafrost* edited by D.L. Kane and K. M. Hinkel):

Methane cycle in terrestrial and submarine permafrost deposits of the Laptev Sea region

Authors: Dirk Wagner[1], Katharina Koch[1], Andreas Gattinger[3], and André Lipski[4]

Topic: Carbon-rich permafrost sediments at high latitudes are an important source of methane. Methane, a greenhouse gas, contributes to about 20% of global warming (IPCC, 2001). In order to better understand the methane cycle in terrestrial and submarine permafrost, methanogenic activity and biomass, geochemical and molecular ecological studies on methanogenic diversity have been carried out which specifically examine the role of methanogens. For this study frozen sediments from cores in the Laptev Sea region in northeastern Siberia which have been dated as Holocene and Late Pleistocene were used. The results show that the methane in permafrost deposits is of recent origin and formed by cold-adapted methanogens. Methane production by methanogenesis in permafrost deposits is an important factor for future climate development.

My personal contribution to this publication consists of the methanogenic community analyses in submarine permafrost (100%).

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2. PUBLICATIONS

2.1 First Publication

Environmental Microbiology
Volume 11, Issue 3, March 2009, Pages. 657 – 668

Environmental Microbiology (2009) **11**(3), 657–668 doi:10.1111/j.1462-2920.2008.01836.x

Methanogenic community composition and anaerobic carbon turnover in submarine permafrost sediments of the Siberian Laptev Sea

Katharina Koch,[1]* Christian Knoblauch[2] and Dirk Wagner[1]
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Summary

The Siberian Laptev Sea shelf contains submarine permafrost, which was formed by flooding of terrestrial permafrost with ocean water during the Holocene sea level rise. This flooding resulted in a warming of the permafrost to temperatures close below 0°C. The impact of these environmental changes on methanogenic communities and carbon dynamics in the permafrost was studied in a submarine permafrost core of the Siberian Laptev Sea shelf. Total organic carbon (TOC) content varied between 0.03% and 8.7% with highest values between 53 and 62 m depth below sea floor. In the same depth, maximum methane concentrations (284 nmol CH_4 g^{-1}) and lowest carbon isotope values of methane (–72.2‰ VPDB) were measured, latter indicating microbial formation of methane under *in situ* conditions. The archaeal community structure was assessed by a nested polymerase chain reaction (PCR) amplification for DGGE, followed by sequencing of reamplified bands. Submarine permafrost samples showed a different archaeal community than the nearby terrestrial permafrost. Samples with high methane concentrations were dominated by sequences affiliated rather to the methylotrophic genera *Methanosarcina* and *Methanococcoides* as well as to uncultured archaea. The presented results give the first insights into the archaeal community in submarine permafrost and the first evidence for their activity at *in situ* conditions.

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Introduction

Arctic permafrost environments play an important role within the global methane cycle. Estimates of the methane emissions from these environments range between 10 and 39 Tg a^{-1} (Terragramm per anno), which represents up to 8% of the global methane emissions (Bartlett and Harriss, 1993; Cao *et al.*, 1998). Methane contributes to about 18% of the actual increases in global warming via the so-called greenhouse effect (Forster *et al.*, 2007), which may lead to long-term global climate change. Most warming occurs in high northern latitudes (Mann *et al.*, 1999; Oldfield and Alverson, 2003). These regions are characterized by permafrost that underlay about 25% of the land surface (Zhang *et al.*, 1999) and significant parts of the coastal sea shelves. Enormous quantities of carbon are stored in permafrost, which are by far larger than previously assumed (Anisimov and Reneva, 2006; Zimov *et al.*, 2006). Currently most strongly discussed with reference to permafrost is therefore the question: 'What will happen to the carbon accumulated in permafrost, in the event of a climate change?' Recently published data show that an increase of the permafrost temperature in Siberian permafrost deposits would lead to substantial rise in microbially produced methane and carbon dioxide (Wagner *et al.*, 2007). This is in accordance with updated model calculations predicting an increase of methane release for northern wetlands of up to 38% (Zhuang *et al.*, 2004; Anisimov, 2007; Khvorostyanov *et al.*, 2008).

Several studies demonstrate the presence of viable and cultivable microorganisms, including anaerobes, in terrestrial permafrost sediments up to 3 million years in age (Vainshtein *et al.*, 1995; Shi *et al.*, 1997; Rivkina *et al.*, 1998; Gilichinsky *et al.*, 2005). Although these environments are characterized by freezing temperatures, it was recently shown that the abundance and composition of the methanogenic population is similar to that of communities of similar temperate soil ecosystems (Wagner *et al.*, 2005). Direct bacterial counts in the order of 10^7-10^8 were reported for the active layer and the perennially frozen ground in north-east Siberia (Rivkina *et al.*, 1998; Kobabe *et al.*, 2004). Other studies reported only low numbers of

methanogens for the Mallik gas hydrate production research well (Colwell *et al.*, 2005; Mangelsdorf *et al.*, 2005). Nevertheless, methanogenic archaea in permafrost environments are well adapted to the extreme environmental condition of their habitat (Morozova and Wagner, 2007). These microorganisms do not only survive under permafrost conditions, but also can sustain active metabolism under *in situ* conditions (Rivkina *et al.*, 2004; Wagner *et al.*, 2007).

A particular variety of Arctic permafrost is the submarine permafrost, which underlay the shallow shelves of the Arctic coastal areas such as the Laptev Sea in north-east Siberia. This permafrost was formed by flooding of terrestrial permafrost with ocean water during the Holocene sea level rise (Rachold *et al.*, 2007). Flooding of the cold terrestrial permafrost (–5 to –15°C) with relatively warm (–0.5 to –2°C) saline sea water changed the system profoundly and resulted in a warming of the frozen sediments (Overduin, 2007), which might enable and enhance microbial carbon turnover. As the submarine permafrost originates from frozen terrestrial Holocene and Pleistocene deposits, the autochthonous microbial community is expected to be of terrestrial origin.

Due to its poor accessibility, studies on the microbial diversity and activity in submarine permafrost neither have been conducted by cultivation dependent methods nor by cultivation independent molecular approaches. Therefore, the microbial abundance and diversity in the carbon-rich permafrost as well as the significance of microbial activity and response to rising temperatures is completely unknown. The purpose of this investigation was a basic characterization of methanogenic communities in submarine permafrost sediments from the Laptev Sea and its potential for carbon mineralization in the frozen sediments. A cultivation-independent approach was used based on denaturing gradient gel electrophoresis (DGGE) of sediment DNA followed by sequencing of reamplified DNA bands. Geochemical and stable carbon isotope data were collected from the submarine permafrost core to trace evidence for microbial activity in the permanently frozen terrestrial sediments under the sea floor.

Results

Characteristics of the submarine permafrost sediments

The total organic carbon (TOC) content, methane concentrations and stable carbon isotope ratios of methane and TOC were measured in samples from the frozen part of the retrieved sediment core between 40.5 and 63.4 mbsf (metres below sea floor) (Fig. 1), representing submarine permafrost deposits. Generally, analysed parameters showed minor variations in the upper part of the core

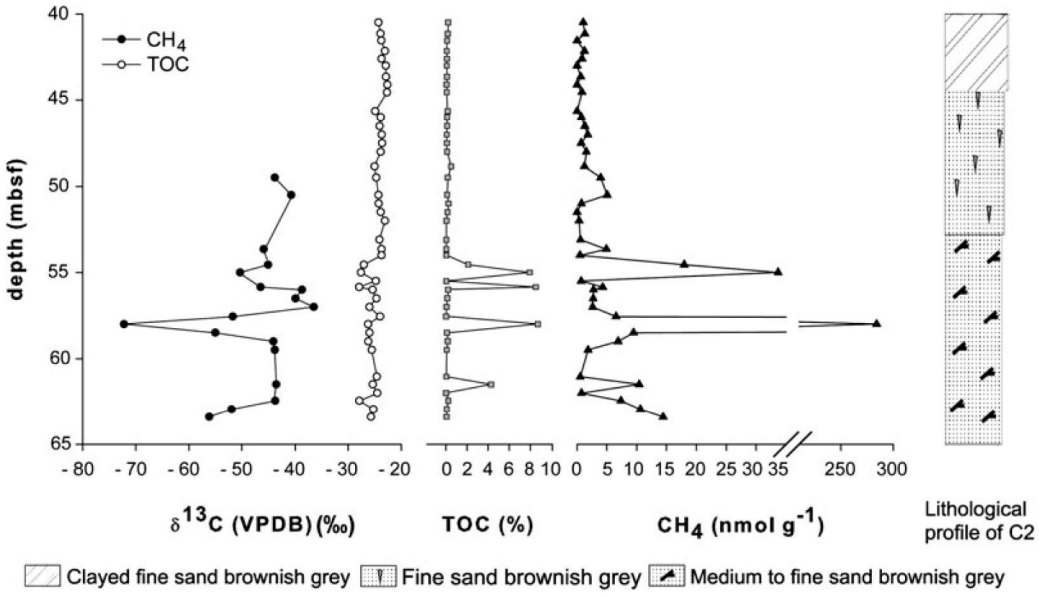

Fig. 1. Vertical profiles of $\delta^{13}C$ values of methane and soil organic carbon as well as concentrations of total organic carbon (TOC), methane and cross-section of the submarine permafrost sediments according to Overduin (2007, modified)

(40–53 mbsf) but differed stronger in the deeper part (53–64 mbsf). The TOC content varied between 0.03% and 8.7% in depths between 40.5 and 63.4 mbsf with the highest concentration at 58 mbsf. Elevated TOC concentrations were also found at depths of 55, 55.9 and 61.5 mbsf. Methane concentrations varied strongly (0–284 nmol g^{-1}) with two distinct peaks at 55 and 58 mbsf. The $\delta^{13}C$ values of TOC ranged between –23.1‰ and –27.9‰ VPDB with generally lower values in the deeper part of the core where also elevated TOC and methane concentrations were measured. Due to the small sample size $\delta^{13}C$ values of methane could only be determined if dissolved CH_4 concentrations were above 2 nmol g^{-1}, which was the case in core depths below 49 mbsf. Methane $\delta^{13}C$ values ranged between –36.5‰ and –72.2‰ VPDB with the lowest values in the zone of highest methane and TOC concentrations.

DGGE analysis of submarine permafrost sediments

Community analyses with specific primers for methanogenic *Archaea* were accomplished to study a possible link between these microorganisms and the elevated methane concentration in the submarine permafrost sediments. The DGGE profile (Fig. 2) showed the presence of up to 34 bands along the submarine permafrost profile between 35 and 66.7 mbsf. The dissimilarity of these bands showed the occurrence of different archaea in every depth. Generally, the number of bands varied with depth. Only in the upper two layers between 35 and 43.1 mbsf the number of bands was constant with about five bands. At least two well-defined bands were present in every depth apart from the depth of 50.6–50.7 mbsf (one well-defined band). Between 57.7 and 58.2 mbsf up to seven different bands were detected showing a higher variability of methanogenic archaea in the sediments at this depth. In the depth between 57.7 and 58.2, where TOC and methane concentrations were highest and $\delta^{13}CH_4$ values lowest, DGGE bands were considerably stronger than in the rest of the submarine permafrost core. At the depth from 52.6 to 52.7 mbsf up to nine different bands were detected, but the intensities of all bands in this sediment layer were too weak to be exploited. Twenty-six sequences of the 16S rRNA gene fragments from submarine permafrost environments could be reamplified from the DGGE gel and were tested by the Ribosome Database Project II. Sequences could be assigned to the genera *Methanosarcina* (9 sequences), *Methanococcoides* (9 sequences), *Methanoculleus* (1 sequence) and *Methanogenium* (1 sequence). The similarity of all sequences to its closest relative varied between 98% and 99%. Six sequences were affiliated with uncultured archaea (92–99% similarity; Table 1).

Phylogenetic analysis of methanogenic sequences from submarine permafrost

A total of 34 DGGE bands were sequenced. Eight sequences were excluded from the further analysis because of their short length (< 230 nucleotides). The remaining 26 sequences were used for the construction of a phylogenetic tree (Fig. 3). Every sequence (> 230 nucleotides) of a phylogenetic group was based on adding the sequences to archaeal phylogenetic trees of nearly full-length 16S rRNA gene sequences. Eighteen sequences fell in the family *Methanosarcinaceae*, forming three new *Submarine Permafrost Clusters*. Nine sequences could be classified as members of the genus *Methanosarcina* and another nine as members of the genus *Methanococcoides*. One sequence from the depth of 58 mbsf showing highest methane concentrations and the lowest methane $\delta^{13}C$ values affiliated to the Permafrost Cluster I described by Ganzert and colleagues (2007). Furthermore, nine sequences, including two submarine permafrost clusters (II and III), were nearly identical to *Methanococcoides*

Table 1. Archaeal species detected by PCR-DGGE from different study sites in north-east Siberia.

Taxonomic units	Genus	Submarine permafrost sediments, Laptev Sea shelf[a]	Permafrost-affected soils, Laptev Sea region[b]	Late Pleistocene permafrost sediments, Lena Delta[c]
Methanosarcinaceae	*Methanosarcina*	9	11	2
	Methanococcoides	9	–	–
Methanomicrobiaceae	*Methanogenium*	1	–	–
	Methanoculleus	1	–	–
	Others	–	12	10
Methanosaetaceae	*Methanosaeta*	–	2	2
Crenarchaeota		n.d.	n.d.	10
Uncultured *Archaea*		6	3	–
Sequences (total)		26	28	24

a. This study.
b. Ganzert and colleagues (2007).
c. G. Jurgens and D. Wagner, unpubl. data.
n.d., not determined.

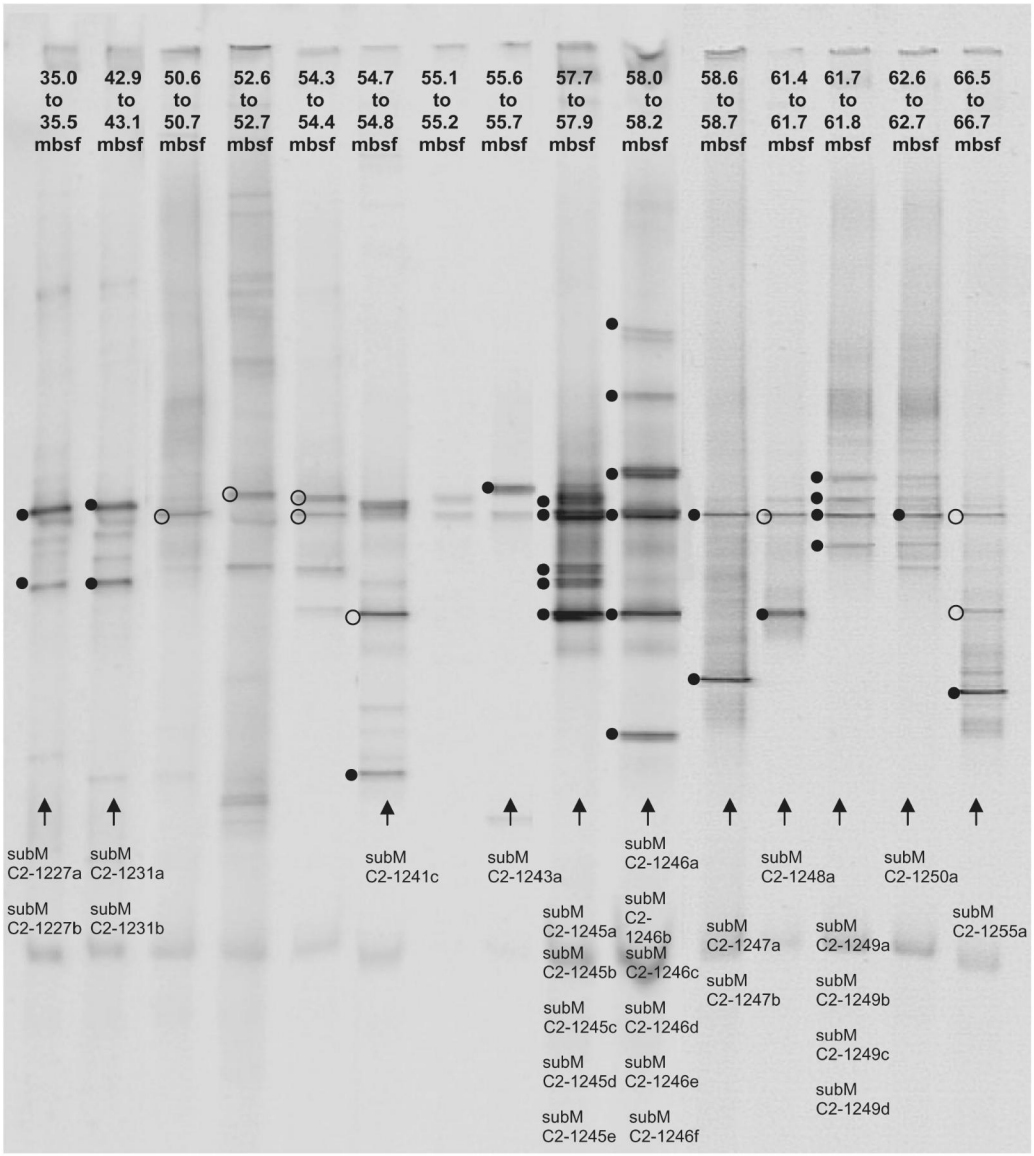

Fig. 2. DGGE profiles of 16S rRNA genes amplified from submarine permafrost community DNA obtained from various depths of the sediments between 35 and 66.7 mbsf. Marked bands were selected for sequencing (black circles ≥ 230 bp; open circle ≤ 230 bp). Only sequences longer than 230 bp were used for phylogenetic analysis.

burtonii from Ace Lake, Antarctica. Two more sequences (subM_C2_1247b and subM_C2_1255a) belonged to the order *Methanomicrobiales* and the remaining six to unidentified *Archaea*. Four of them constitute the fourth new 'Submarine Permafrost Cluster'. Sequences assigned to *Methanococcoides* were found in deeper submarine permafrost sediments (55.6–62.7 m), whereas sequences associated with *Methanosarcina* were mainly observed in

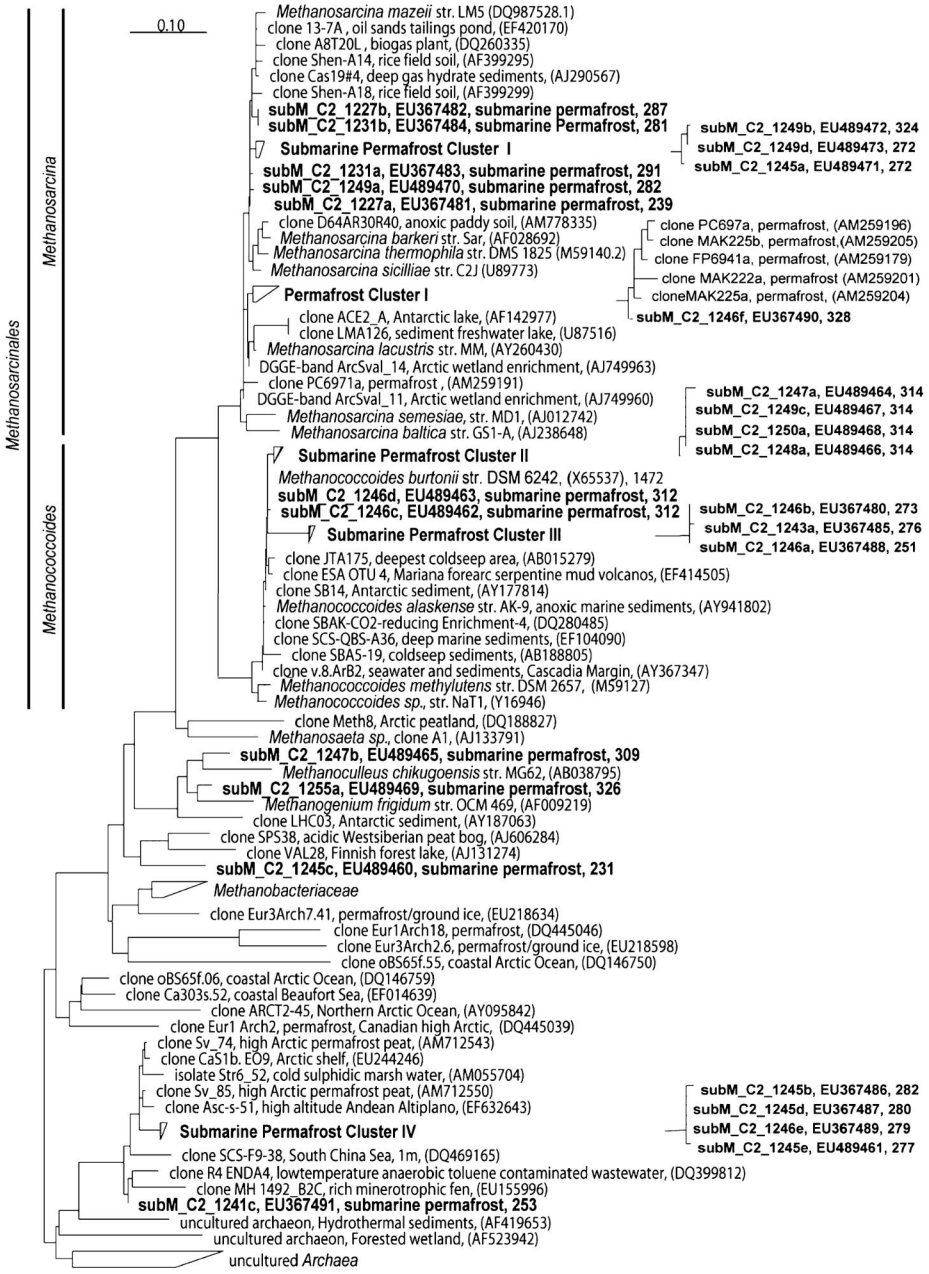

Fig. 3. Phylogenetic analysis (maximum likelihood) illustrating the affiliation of methanogenic 16S rRNA gene sequences reamplified from DGGE bands. The sequences recovered from submarine permafrost belong to *Methanosarcina*, *Methanococcoides*, *Methanomicrobiales* and uncultured archaea. The scale bar represents 0.10 changes per nucleotide. Identification of the bands is shown in Fig. 2. Environment, clone name, accession number and length of each sequence are indicated.

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the top and bottom of the submarine permafrost sediments. Most of the sequences related to uncultured archaea were found in the zone with maximum methane concentration.

Discussion

Permafrost ecosystems of the Siberian Arctic are characterized by low *in situ* temperatures and high water contents, which leads to the accumulation of organic carbon. The fate of this carbon reservoir is of particular interest, considering possible feedback mechanisms that might result in increasing methane fluxes in a warming Arctic. Submarine permafrost, which was warming to near 0°C after being flooded by marine waters, is a natural laboratory for studying the impact of environmental changes on microbial decomposition processes. This study presents the first results on the methanogenic community composition and evidence for methanogenic activity in submarine permafrost sediments.

Our results show a distinct methane profile along the Late Pleistocene deposits with trace amounts in the upper part of the frozen sediments, maximum values in two distinct peaks between about 53 and 63 mbsf and again low methane concentrations at the bottom of the profile. The two methane peaks between 53 and 63 mbsf cannot be explained by an upward diffusion of methane from the unfrozen sediment layers as latter explanation would require high methane concentrations in the bottom of the sediment that, however, were not present. Water saturated and frozen sediment layers are an effective diffusion barrier preventing gas diffusion between the sediment layers. Rivkina and Gilichinsky (1996) and Rivkina and colleagues (2007) measured methane concentration profiles in different aged permafrost deposits of north-east Siberia. At several sites, they found elevated methane concentrations in deep (20–50 m) permafrost material but almost no methane in overlaying permafrost. They concluded from these results that methane cannot diffuse through frozen sediments. Furthermore, Cramer and Franke (2005) measured hydrocarbon concentrations and stable carbon isotope signatures in sea water and surface sediments of the NE Laptev Sea and found thermally generated methane seeping through the sediments only in the northern part of the Shelf. They concluded that the submarine permafrost layer in the central Laptev Sea is preventing hydrocarbons from being released into the sea water. As the methane profile in the investigated core gives no indication for vertical methane diffusion from below the frozen sediment and methane diffusion is impeded inside the frozen permafrost, we are confident that the high methane concentrations in the permafrost core at depths of 53 and 59 mbsf are due to *in situ* methanogenesis. The potential for methane production in Holocene and Pleistocene permafrost deposits at subzero temperatures has already been shown in previous investigations (Rivkina *et al.*, 2007; Wagner *et al.*, 2007).

Further evidence that methane was indeed produced *in situ* is given by the profile of TOC. Maximum methane concentrations and highest TOC values of up to 8.7% were measured in the same sediment layers. As methane is the final end-product of anoxic organic matter degradation the elevated methane concentrations most likely originate from the high amount of organic matter in the same sediment layer. However, besides the amount of organic matter, also its availability for microbial degradation (Hogg, 1993; Wagner *et al.*, 2007), as well as the distribution of methanogens along the sediment profile (Fig. 2), may explain the large variability of methane concentrations in the core profile (Fig. 1).

The stable carbon isotope signatures of methane in the studied core indicates conclusively that elevated methane concentrations in the deeper part of the core (Fig. 1) are of microbial origin. Methane produced during methanogenesis is strongly depleted in ^{13}C resulting in $\delta^{13}CH_4$ values in the range of –55 to –90‰ VPDB (Rice, 1992; Thomsen *et al.*, 2001, Webster, 2005). The low $\delta^{13}CH_4$ value of –72.2‰ VPDB and the high methane concentration in a sediment depth of about 58 mbsf indicate that methanogenic archaea actively form methane at *in situ* temperatures of –1.5°C. Flooding of the terrestrial permafrost resulted in a significant temperature rise from –12°C in the terrestrial permafrost to about –1.5°C in the submarine permafrost (Rachold *et al.*, 2007). The microbial degradation of the available organic matter in the submarine permafrost was most likely enhanced as a result of this post flooding temperature rise. This interpretation is in accordance with results from terrestrial Holocene permafrost deposits from the Lena Delta, indicating modern methanogenesis in the frozen ground by cold-adapted methanogens at temperatures as low as –6°C (Wagner *et al.*, 2007).

The DGGE profile of 16S rRNA genes was used to study the distribution of methanogenic archaea along the submarine permafrost sediments and to get a deeper insight into the phylogenetic relationship of the methanogenic community in comparison to terrestrial permafrost deposits. The DGGE fingerprints showed significant differences within the depth profile of submarine permafrost. Particularly, the analysis revealed a more divers methanogenic population in the frozen sediments in a depth of around 58 mbsf. In this layer, up to 9 DGGE bands were found with a noticeable density in comparison to the other depths. This increased diversity is comparable with the diversity of methanogens within the active layer of permafrost soils from the same study region (Ganzert *et al.*, 2007).

Sequences obtained from the DGGE bands affiliated with *Methanosarcinaceae*, *Methanomicrobiaceae* and hitherto uncultured archaea, while species of the families *Methanobacteriaceae* and *Methanosaetaceae*, as reported in other studies on archaeal diversity in Arctic permafrost environments (Høj *et al.*, 2005; Ganzert *et al.*, 2007), were not detected. A total of 18 sequences out of 26 belonged to *Methanosarcinaceae* and only 2 sequences belonged to *Methanomicrobiaceae*. Further 6 sequences were affiliated with uncultured archaea. This result suggested that the methanogenic community in submarine permafrost prefer among other substrates C1 compounds such as methanol and methylated amines.

The phylogenetic analysis showed that nine of the *Methanosarcinaceae*-like sequences were related to the genus *Methanosarcina* and further nine sequences were affiliated with the genus *Methanococcoides* (Table 1). Previous studies of permafrost-affected soils from the Laptev Sea coast reported that about 50% of the detected sequences were related with the order *Methanomicrobiales*, which is characterized by hydrogenotrophic methanogens, while the remaining sequences mainly affiliated to *Methanosarcina*-like methanogens and uncultured archaea (Ganzert *et al.*, 2007). However, on the other hand latter study did not find any sequences related to *Methanococcoides* (Table 1). Also data from Late Pleistocene permafrost sediments from the Lena Delta showed a dominance of *Methanomicrobiales*-related sequences, while sequences related to *Methanococcoides* were not detected (G. Jurgens and D. Wagner, unpubl. results, Table 1).

The observed differences between the microbial community composition in submarine permafrost (particularly in the zone of high methane concentration) and its terrestrial counterpart are particularly noteworthy, because submarine permafrost sediments developed by the flooding of terrestrial permafrost due to sea level rise or coastal erosion (Rachold *et al.*, 2007). We therefore expected a similar composition of the microbial community in the submarine as in the terrestrial permafrost but found distinct differences. The difference in the methanogenic communities in both permafrost types indicate that the environmental changes affecting submarine permafrost as flooding of the coastal areas, possibly caused the observed variation in the microbial community structure.

The *Methanococcoides*-related sequences were found mainly in the submarine permafrost layer with the highest methane concentration. Moreover, in this zone sequences are accumulated, which are closely related to uncultured archaea. However, so far the genus *Methanococcoides* comprises three species; two of them were isolated from polar regions. *Methanococcoides burtonii* was isolated from the anoxic hypolimnion of the Ace Lake, Antarctica (Franzmann *et al.*, 1992), and *Methanococcoides alaskense* was obtained from marine sediments from Skan Bay, Alaska (Singh *et al.*, 2005). Both organisms are cold-adapted with a minimum temperature for growth of 1.7 and –2.3 respectively. This shows that at least *Methanococcoides* relatives can be active and grow under *in situ* temperature conditions of the submarine permafrost sediments.

Another reason for the dominance of *Methanococcoides* relatives might be the elevated sulfate concentration in the zone between 57.7 and 58.2 mbsf, which was 10 times higher (between 1.5 and 2 mmol l^{-1} sulfate; P. Overduin, pers. comm., 2008) in comparison to the sediment layers above and below the zone of highest methane concentration. If sulfate is present, sulfate reduction is the major catabolic process due to the higher affinity of sulfate reducers for hydrogen and acetate and a higher energy yield of sulfate reduction in comparison with methanogenesis (Winfrey and Zeikus, 1977; Schönheit *et al.*, 1982; King, 1984). Under these conditions methanogenesis is very low (Franzmann *et al.*, 1991; Senior *et al.*, 1982) and only favoured if using competitive substrates like methanol or methylated amines (Oremland and Polcin, 1982; Ferdelman *et al.*, 1997), as it is the case for *Methanococcoides* in this zone.

Six of the *Methanosarcina*-like sequences (subM_C2_1227a, subM_C2_1227b, subM_C2_1231a, subM_C2_1231b, subM_C2_1246f, subM_C2_1249a) clustered with cultured methanogens (e.g. *Methanosarcina barkeri*, *Ms. thermophila*) and several environmental sequences with the closest relatives from rice field soils (Ramakrishnan *et al.*, 2001), freshwater-lake sediments (MacGregor *et al.*, 1997) and gas hydrate sediments (Marchesi *et al.*, 2001). One of these sequences (subM_C2_1246f) was most closely related to the Permafrost Cluster I isolated from the terrestrial permafrost of the Laptev Sea hinterland (Ganzert *et al.*, 2007). The remaining three sequences (subM_C2_1245a, subM_C2_1249b, subM_C2_1249d) form the *Submarine Permafrost Cluster I*, which are most closely related to clone Shen-A18 (Ramakrishnan *et al.*, 2001) and clone D64AR30R40 (Peng *et al.*, 2008) from rice field soils. One of the sequences in this cluster was recovered from the sediment layer with the highest methane concentration, while the remaining two sequences were obtained from the depth between 61.4 and 61.7 mbsf characterized by low concentration of organic carbon and methane.

The closest relative of the *Methanococcoides*-like sequences is *Methanococcoides burtonii* (Franzmann *et al.*, 1992), a cold-adapted species isolated from the Ace Lake, Antarctica. Four sequences (subM_C2_1247a, subM_C2_1248a, subM_C2_1249c, subM_C2_1250a) form the *Submarine Permafrost Cluster II*, which were obtained from three different sediment layers (58.65, 61.70, 62.60 mbsf) below the zone of maximum methane

concentration. Further three sequences (subM_C2_1243a, subM_C2_1246a, subM_C2_1246b) form the *Submarine Permafrost Cluster III*. Two of them originated from the zone of highest methane concentration and one from the layer above this zone. Additional two sequences (subM_C2_1246c, subM_C2_1246d) recovered also from the zone of maximum methane concentration belonging to the group of *Methanococcoides*.

Only two sequences (subM_C2_1255a) were affiliated with *Methanomicrobiaceae*. The closest relative to the first sequence subM_C2_1247b, obtained from the bottom of the submarine permafrost above the unfrozen sediments, is *Methanoculleus chikugoensis* isolated from paddy field soil in Japan (Dianou *et al.*, 2001). The second sequence subM_C2_1255a, recovered from the sediment layer above the zone of highest methane concentration, is closest related to *Methanogenium frigidum* isolated from the Ace Lake, Antarctica (Franzmann *et al.*, 1997).

The final six sequences, which were mostly recovered from the zone of highest methane concentration, were closest related to uncultured archaeal sequences (similarity between 91% and 99%). Sequence subM_C2_1245c was closest related to a sequence obtained from an acidic peat bog from Siberia (Jurgens *et al.*, 2000). Sequence subM_C2_1241c was closest related to a sequence from a minerotrophic fen peatland in central New York State (Cadillo-Quiroz *et al.*, 2008). The final four sequences (subM_C2_1245b, subM_C2_1245d, subM_C2_1245e, subM_C2_1246e) form *Submarine Permafrost Cluster IV*.

The sequences that were assigned to the submarine permafrost clusters might include methanogenic archaea characterized by specific adaptation mechanisms maintaining their survival and metabolic activity under the harsh environmental conditions of the permafrost environment. Previous studies on the response of methanogenic archaea, isolated from Siberian permafrost-affected soils, against different environmental stress conditions, showed a remarkable resistance of these organisms against extreme low temperature (down to −78°C), high salinity (up to 6 M NaCl), starvation (up to 3 months) and desiccation (up to 25 days; Morozova and Wagner, 2007). Obviously, permafrost environments force the adaptation of microbial communities to low temperature conditions with species, which have been untraced in temperate ecosystems so far.

In conclusion, this study has provided the first knowledge of the methanogenic community composition in submarine permafrost sediments from the Laptev Sea coast. The results indicated a dominance of methylotrophic methanogenic archaea, particularly in the zone with maximum methane concentration, low $\delta^{13}CH_4$ values, and elevated sulfate concentrations (up to 2 mmol l^{-1}), which is contrary to detected archaeal groups in previous studies on Siberian permafrost environments. The observed differences in the methanogenic community structure in submarine permafrost sediments in comparison to its terrestrial counterpart may result from the up to-10°C higher *in situ* temperature of submarine permafrost. To understand the carbon dynamics in permafrost environments under changing environmental conditions, it is of particular interest to know how the changes in the microbial community structure affect the decomposition of organic carbon under anoxic conditions. Therefore, submarine permafrost is considered as an ideal natural laboratory to study the impact of changing environmental conditions, particularly increasing permafrost temperatures, on the structure and function of microbial communities in climate-sensitive permafrost habitats.

Experimental procedure

Investigation area

The studied core was retrieved from the Laptev Sea shelf near Cape Mamontovy Klyk, north-east Siberia (73°42′N, 117°10′E, Fig. 4). The investigation area is characterized by an Arctic climate, with low mean annual precipitation (less than 300 mm), short summers and, long and very cold winters (Grosse, 2005). This climate leads to the development of the typical landscape in the terrestrial hinterland known as the Arctic tundra. The entire area is located within the zone of continuous permafrost (Rachold *et al.*, 1996). In contrast to the temperature minimum measured in terrestrial permafrost of this region (−13°C, Wagner *et al.*, 2007), the average temperature in the submarine permafrost amounted to −1.5°C (Rachold *et al.*, 2007).

Drilling program and sample communication

During the COAST expedition in spring 2005 five sediment cores were drilled in a transect from the Laptev Sea coast at Mamontovy Klyk to the shallow Laptev Sea shelf (73°42′N, 117°10′E). The Laptev Sea shelf was selected as suitable area for the coastal drilling transect, because the region between Olenyek and Anabar River Delta was not influenced by fluvial waters and deltaic depositions (Overduin, 2007). The goals of the COAST expedition were to analyse geochemical processes related to the interactions between sea water and permafrost as well as the characterization of microbial processes and communities. Analyses were conducted to trace the transformation of unchanged terrestrial permafrost via offshore permafrost to submarine permafrost. Detailed data on drilling are presented elsewhere (Overduin, 2007; Rachold *et al.*, 2007).

Five cores of up to 77 m length were drilled on the Laptev Sea shelf and the coast. Drilling was carried out using a drilling rig (URB-2A-2) with a hydraulic rotary pressure mechanism. Drilling was conducted dehumidified and without flushing so that the natural structure of the drill cores remained intact. The core segments were transported in frozen condition to the Alfred Wegener Institute in Bremer-

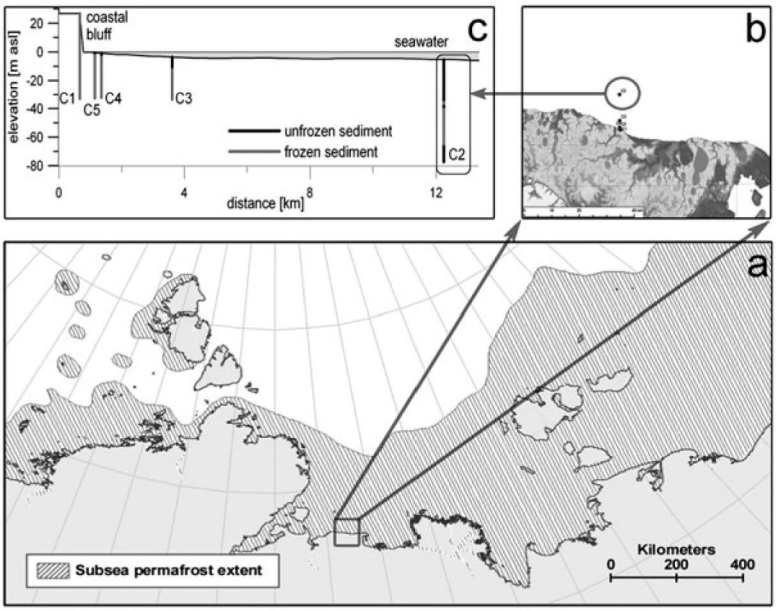

Fig. 4. (A) Investigation area of the Laptev Sea in north-east Siberia. (B) The right-handed small figure (with courtesy of G. Grosse, UAF) shows the exact position of submarine permafrost core 2 and (C) the left-handed figure shows its depth profile (Overduin *et al.*, 2008, modified).

haven, Germany and archived at –22°C. Core segments from drill holes were split along their vertical axis into two halves under aseptic conditions with a diamond saw in an ice laboratory at –15°C. One half of each core was photographed and stored in a climatic room at –22°C at the Alfred Wegener Institute for future reference. Afterward, the other half of the core was cleaned with a sterile knife for lithological and geocryologically descriptions (Overduin, 2007). Subsamples (small cylindrical pieces of approximately 10 g each) of each segment were extracted for analysis of the TOC and methane concentrations as well as stable carbon isotope signatures in the frozen sediments. The remaining material of each subsample was thawed at 4°C and homogenized under anoxic and sterile conditions for analysis of the microbial communities. Subsamples for the different analyses were filled into sterile plastic Nalgene boxes. Samples were used directly for the analyses or refrozen for later investigations at –22°C.

Soil properties

The core C2 (Fig. 4) was chosen for the current study. The drill site was most distant (11.5 km) from the coast. A channel temperature sensor was installed after drilling and the measured temperatures varied between –1°C and –1.7°C. The depth of water was approximately 6.0 m with a sea ice thickness of 1.4 m and a bottom water salinity of 29.2‰. The drilling reached frozen sediment at a depth of 35.5 mbsf. As from approximately 36–40.3 m the sediment is again unfrozen and as well at depth 64.7 unfrozen sediments were discovered (P. Overduin, pers. comm.), the definitive upper permafrost boundary is still open to interpretation.

A precise description of the lithological profile of core C2 is given by Overduin (2007). Briefly, the sediments in all depths contain brownish and dark grey, fine sand. The sediments between 51.5 and 64.7 mbsf have found embedded deposits of dark plant and wood remains. The typical cryogenic structures of submarine permafrost deposits are visibly. The core age was determined for two selected samples with 86 ka (44.5 m) and 111 ka (77 m) by IR-OSL at the Institute of Geology, Tallinn University of Technology, Research Laboratory for Quaternary Geochronology (A. Molodkov).

Vertical profiles of sediment CH_4 concentrations were obtained from each segment by extracting CH_4 from sediment porewater after thawing frozen subsamples (approximately 10 g). The samples were placed in glass bottles (*c.* 50 ml) containing 20 ml of a saturated NaCl solution and sealed gas tight with black rubber stoppers. The thawed samples were shaken and the CH_4 headspace concentration was analysed with gas chromatography. Subsequently, the stable carbon isotope composition of methane was determined.

For determining TOC and its stable carbon isotope signature subsamples were treated with H_3PO_4 overnight to remove inorganic carbon. Samples were subsequently washed repeatedly with distilled water to remove the remaining H_3PO_4. Samples were sieved (< 2 mm), milled in a planetary ball mill, dried at 105°C, and TOC was finally measured with an elemental analyser (Elementar VarioMAX).

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Methane analysis

Gas analysis was performed with an Agilent 6890 gas chromatograph equipped with a Carbonplot capillary column (Ø 0.52 mm, 30 m) and a flame ionization detector. Helium was used as the carrier gas. The injector, oven and detector temperatures were set at 45°C, 45°C and 250°C respectively. All gas sample analyses in the various experiments were done after calibration of the gas chromatograph with standard gases. After calibration the analytical reproducibility is > 98.5%. Details of methane analysis were described previously in Wagner and colleagues (2003).

Isotope measurement

The $\delta^{13}C$ values of particulate organic carbon were measured with an isotope ratio mass spectrometer (Finnigan Delta Plus) equipped with an elemental analyser (CE 2500) and a conflow II interface. Samples were calibrated against external standards (NBS 19, 1.95‰ VPDB; IAEA C6, 10.8‰ VPDB; IVA soil standard –27.46‰ VPDB). The $\delta^{13}C$ values were expressed relative to the international VPDB standard.

Stable carbon isotope ratios of methane were determined at the same isotopic ratio mass spectrometer (Finnigan Delta Plus) equipped with a PreCon (Brand, 1995). Samples (0.1–2 ml) were injected with a gas tight syringe (Hamilton) through a sample port into the sample container that was flushed with a constant flow (20 ml min^{-1}) of He (> 99.999%). The PreCon was used to remove CO_2 from the samples, subsequently combust methane to CO_2 at 1000°C, and to concentrate the derived CO_2 in two serial cryotraps at –196°C. The derived CO_2 was transferred via a GC (Agilent 6890, Pora Plot Q column) and a GC/C III interface (Thermo, Bremen, Germany) to the mass spectrometer. ^{13}C-isotope measurements of methane could only be conducted if concentrations were higher than about 2 nmol g^{-1}. The reproducibility of the measurement was generally better than ±0.5‰. Isotope values are given as $\delta^{13}C$ (‰) calculated by the formula: $\delta^{13}C = [(R_{sample}/R_{standard}) - 1]$, with R representing the ratio between ^{13}C and ^{12}C in the sample and the standard (VPDB $R = 0.0112372$).

Molecular analyses

DNA was extracted from the submarine permafrost sediments using UltraClean™ Soil DNA Isolation Kit (Mo Bio Laboratories, Carlsbad, USA). Methanogenic 16S rRNA was amplified in a nested PCR protocol and analysed by DGGE. In the first amplification the primers ArUn4F (5′-TCY ggT TgA TCC TgC CRg-3′) and Ar958R (5′-YCC ggC gTT gAV TCC AAT T-3′) were used. In the second amplification the primer combination GC-357F and 0691R specific for methanogens (Watanabe *et al.*, 2004) was applied to the identical DNA fragments. The reaction mixture of 50 µl contained 1× PCR buffer, 0.25 mM of each dNTP, 2 mM $MgCl_2$, 0.4 µM of each primer, 2.5 Unit HotStarTaq DNA Polymerase (Qiagen) and 1–2 µl of template. The PCR cycle for the first amplification was 95°C in 10 min followed by 40 cycles of 94°C for 1 min, 57°C for 1 min and 72°C for 2 min, followed by a final extension at 72°C for 10 min. The following PCR cycle was used for methanogenic amplifications: 95°C in 10 min followed by 30–35 cycles of 94°C for 1 min, 53°C for 1 min and 72°C for 2 min, followed by a final extension at 72°C for 10 min. PCR was made using an iCycler Thermal Cycler (Bio-Rad). Electrophoresis was performed with 2% agarose gel in 0.5× TAE. Agarose gel was stained with SYBR Gold (1:10 000 dilution, Molecular Probes). Amplified fragments were separated on 8% polyacrylamide gels in 1.0× TAE with a linear gradient of 30–55% [100% denaturant was 7 M urea and 40% (v/v) formamide]. DGGE was performed at 60°C and 100 V for 840 min. The gels were stained with SYBR Gold (1:10 000 dilution, Molecular Probes) for 30 min, rinsed with distilled water and photographed under UV illumination. DGGE bands were cut from the gel using a sterile scapel, reamplified and PCR-purified as previously described (Ganzert *et al.*, 2007). Sequencing was done by MWG Biotech AG (Martinsried, Germany).

Phylogenetic analysis

Sequences for methanogens were manually edited to get sequences (Sequencer 4.7, Gene Codes, USA), and contigs were assembled with the same program. Sequences for methanogens were tested using Ribosomal Database Project II (RDP, Taxonomic Outline of the *Bacteria* and *Archaea*, release 7.8, July 2007) and analysed using the BLAST tool at the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/blast/). Sequence and descriptive data for development of the ARB tree were downloaded from RDP (http://rdp.cme.msu.edu), EMBL (http://www.ebi.ac.uk), European rRNA Database (http://bioinformatics.psb.ugent.be/webtools/rRNA/) and DSMZ (https://www.dsmz.de). The selected reference sequences were downloaded from NCBI/BLAST (http://www.ncbi.nlm.nih.gov/blast/Blast.cgi). Sequences for methanogens were added to a prealigned database using the aligning tools in the ARB program package (Technical University of Munich, Munich, Germany). The phylogenetic tree (maximum likelihood) was construced with filters termini and pos_var_arc_1450_aug04 and the Xfig software for tree view. All sequences determined in this study are edited with the software Sequin and deposited in the GenBank databases under accession number EU367469–EU367491 and EU489460–EU489473.

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2.2 Second publication

Final draft for ISME Journal

Comparison of microbial communities in terrestrial and submarine permafrost sediments from the Siberian Laptev Sea area

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Abstract

Global warming accelerates the degradation of organic material in permafrost and permafrost-affected soils in Arctic polar regions. The methane emissions from thawing carbon-rich permafrost are a positive feedback to the global carbon cycle. In order to understand the effects of increasing temperature on carbon dynamics, the microbial community, and methanogenic diversity in the climate-sensitive Arctic permafrost environments, submarine permafrost of -2°C was compared to i terrestrial permafrost of -12°C. This temperature difference provides an opportunity to study the effects of warming on permafrost and understand including changes in pore water space, and chemistry. Methane concentrations varied in the submarine permafrost sediments between 0 nmol CH_4 $g^{-1}$and 284 nmol CH_4 g^{-1}, and in the terrestrial permafrost deposits between 0.4 nmol CH_4 $g^{-1}$and 40 nmol CH_4 g^{-1}. 608 archaeal 16S rRNA sequences were analysed from samples taken from submarine and terrestrial permafrost at three depths, respectively. Sequencing and phylogenetic analyses of representative clones showed that most of the rRNAs were closely related to *Methanosarcinales*. Quantitative analysis of bacteria and methanogenic Archaea using TaqMan gene expression assay for Real Time PCR indicated that the rRNA copy densities of bacteria and methanogenic Archaea were approximately 10 times higher in the submarine than in the terrestrial permafrost. A similar trend was found using FISH.

Running title: Microbial communities in permafrost sediments

Keywords: Clone, FISH, Methanogens, RT PCR, Permafrost

Introduction

Perennially frozen ground (permafrost) in the northern hemisphere covers between 13 and 18 % of the exposed land surface area (Zhang et al., 2000). In addition, relic terrestrial permafrost from the continental shelf areas exposed during last ice age (Rachold et al., 2007) now lies undersea as submarine permafrost following sea level rise that followed the last glacial maximum. Estimates of it's the extent of submarine permafrost vary. The relatively warm arctic saline seawater (about -1.8°C; Wegner et al., 2005) separates the submarine permafrost from the cold atmosphere and warms the sediment. During the past thirty years changing climate has affected the northern Arctic (ACIA, 2004). For example, the pack-ice cover has been dramatically reduced (Stroeve et al., 2008), winter air temperatures have increased (ACIA, 2004), and Atlantic water mass inflow into the Laptev Sea has increased (Taldenkova et al., 2008). These changes will probably lead to increasing Arctic water temperatures and may lead to increased rates of degradation of submarine permafrost.

In eastern Siberia, the temperature at the top of the permafrost has increased by approximately 1.3°C since 1960 (Romanovsky, 2001). Anisimov & Nelson (1997) estimate a 20-35 % reduction in permafrost distribution in the northern hemisphere by 2080. Permafrost stores significant amounts of organic carbon and gas hydrates within and beneath the permafrost. Permafrost warming has the potential to release large amounts of greenhouse gases, such as methane, into the atmosphere. This positive feedback to further climate change has been considered one of a dozen globally significant tipping points in the Earth's climate system (Lenton et al., 2008).

Koch et al. (2009) demonstrated methanogenic activity in submarine permafrost at a depth of approximately 58 m and temperatures between -2°C and -1°C. Methanogenesis can increase with increasing temperature. The rate depends to a greater degree on the quality than on the quantity of the organic matter, subject to the availability of qualitatively good microbial substrates (Wagner et al., 2007). As permafrost degrades and becomes discontinuous, methanogenic activity will also expand spatially (Christensen et al., 2004). A similar effect may affect microbial processes in submarine permafrost that slowly thaws by geothermal heat from below and by warmer saline sea water from above. High concentrations of methane have been observed in submarine permafrost sediments (Koch et al. 2009).

We propose to use submarine permafrost as a natural laboratory in which to observe effects of environmental changes on microbial activity in permafrost habitats. Submarine permafrost as a natural laboratory is essential for understanding the long-term effects on microbially produced gas hydrates to climate change. We investigate how the temperature adaptations of the dominant archaeal populations are affected by warming, because temperature is a fundamental environmental regulator for microbiological activity. Temperature can also affect species composition and microbial community structure. Long-term temperature increases due to climate

change will vary the composition of archaeal communities - these changes may further enhance the positive feedback to climate change.
The goal of this paper is to describe the prevailing communities of methane producing archaea in cold terrestrial (12°C) and warm submarine permafrost (-2°C to 1°C). We ask: how does temperature affect the community composition in terrestrial permafrost sediment? We use the archaeal community structure in submarine permafrost sediment with mean temperatures between -2°C to 0°C as a model for the archaeal community in terrestrial permafrost which will warm due to global climate change.

Materials and Methods

We quantify the bacterial and methanogenic DNA (using Real Time PCR) and measure the physiological condition of the bacteria and archaea (using fluorescence *in situ* hybridization) in terrestrial and submarine permafrost samples. In both cases, we characterize the permafrost temperature, pore space volume and pore water geochemistry of the samples and describe how these change with warming. Furthermore, clone library analyses were carried out to get a more detailed might the archaeal community structure in both permafrost environment.

Study site and sampling

The Laptev Sea Shelf area near Mamontov Klyk is located at latitude 73°42`N and longitude 117°10`E in northeastern Siberia. In 2005 five borehole cores from onshore to offshore was drilled along a transect. The campaign and the core material are described in Rachold et al. (2007). For this study, samples were selected from a terrestrial permafrost core (C1) located about 100 m inland from the coastline and from a submarine permafrost core (C2) located on the Laptev Sea shelf about 11.5 km from the coast. The core was cut longitudinally into quarters, one of them was used for molecular-microbiological investigations, and one for sedimentological and hydrochemical investigations. Three terrestrial permafrost samples one from the active layer (0.50 m– 0.51 m) and two from the ice-bonded sediments (43.0 m– 43.02 m and 59.50 m – 59.52 m) as well as three submarine permafrost samples from two ice-bonded sediments (56.0 m – 56.12 m and 58.0 m – 58.24 m) and one from the unfrozen sediments (70 m) were taken for the molecular microbiological investigation.
To understand the microbial environment, sediment characteristics and pore water composition were investigated. The core sections tended to be longer for these measurements in order to provide enough pore water for analysis. Sampling in C1 occurred over the intervals 0.42 m - 0.55 m for the active layer and 42.90m - 43.05 m, and 59.52 m - 59.66 m for the deep permafrost. Sedimentological hydrochemical

samples from submarine permafrost were taken from the depths 55.91 m - 56.05 m, 57.88 m - 58.04 m and 70 m (Winterfeld, 2009).

Sediment Sampling and Characteristics

Coring procedures are described in Overduin et al. (2007). Borehole temperatures were measured at the coring site using a thermistor string and an infrared device. Both devices, uncertainties and the resulting data are described in Overduin et al. (2008) and Junker et al. (2008). Core material remained frozen until the cores were described and sectioned at the Alfred Wegener Institute in Potsdam, Germany. Before thawing, core section volume and weight were measured. After thawing, 0.2 µm pore-size Rhizon™ suction lysimeters were used to sample sediment pore water, and core sections were freeze-dried and weighed. Gravimetric and volumetric water contents were calculated from section weights and volume.

Sediment pH was determined following German industrial standardized methods (DIN 19684-1), for which 2.5g air-dried sediment and 6.125ml 0.01M calcium chloride were mixed and pH determined with a bench top pH meter. Total organic carbon (TOC) content of the sediment samples was measured on an Elementar Vario EL III carbon, nitrogen and sulphur (CNS) device. Prior to analysis, samples were ground and homogenized. Samples from which carbonates had been removed by heating for three hours with 4% hydrogen chloride were combusted catalytically with oxygen. Total organic carbon of the resulting gas phase was measured using thermal conductivity in at least duplicate samples. Pore water electrical conductivity was measured and adjusted to a reference temperature of 25°C. Sulphate and nitrate concentrations were measured using a Dionex ion chromatograph. Total dissolved iron and manganese concentrations were measured on samples acidified to below pH 2 with Suprapure HNO_3^- using inductively coupled plasma optical emission spectroscopy (ICP-OES).

DAPI staining and fluorescent in situ *hybridization (FISH)*

Permafrost samples (0.5 g) for microscopy were fixed in 1.5 ml 3.7 % formaldehyde (Formaldehyde Solution for molecular biology, 36.5 %, Sigma-Aldrich Chemie, Steinheim, Germany) diluted with 1 x phosphate-buffered saline (PBS) at 4°C overnight, washed thrice with 1 x PBS, transferred to an equal volume of PBS/absolute ethanol and stored at -20°C. Fixed samples were treated to optimize the dispersion with mixed 10% SDS/0.5 mM EDTA/0.1 % SPP (25 ml/ 250 µl/ 5ml) and shacked at 150 rpm for 30 min at 35°C. The dispersed soil samples were pipetted on slides (10 well, 6.7 mm, W/Adhesion, Menzel, Braunschweig, Germany) with 10 wells. Replicated of 10 µl of fixed and dispersed permafrost soil sample were dropped onto each well. Slides were dried at 45°C for 15 min and dehydrated in 50 % for 1 min, 80 % for 1 min and 96 % for 3 min.

FISH analysis with cyanine dye CY3-labeled oligonucleotide probes (final concentration, 50 ng μl^{-1}, TIB MOLBIOL, Berlin, Germany) and DAPI (4`,6-diamidino-2-phenylindole, Merck, Darmstadt, Germany) staining were performed. For FISH, a 1 µl oligonucleotide probe, 9 µl hybridization buffers (0.9 M NaCl, 20 mM Tris-HCl, pH 8.0, 0.02% SDS, and formamide in concentration according to Table (1) were mixed and dropped onto each well. The slides were transferred to an equilibrated 50 ml polypropylene top tupe and incubated at 46°C overnight. Slides were then washed at 48°C for 20 min in washing buffer (20mM Tris-HCl; pH 8.0, 5 mM EDTA, 0.01% SDS w/v and 225 mM NaCl according to a formamide concentration (see Table 1) in the hybridization buffer). Afterwards they were rinsed with milliQ water three times and dried in an air stream. Subsequently, DAPI staining and mounting media were carried out according to Liebner *et al.* (2007).

Table 1. rRNA-targeted oligonucleotide probes used for FISH

Probe	Target group	Target site	FA (%)	References
EUB338 I	Domain *Bacteria*	16S rRNA (338)	35	Amann *et al.* 1990
EUB338 II	Domain *Bacteria*	16S rRNA (338)	35	Daims *et al.* 1999
EUB338 III	Domain *Bacteria*	16S rRNA (338)	35	Daims *et al.* 1999
NON338	Control probe (complement. to EUB338)	16S rRNA	n.d.	Wallner *et al.* 1993
ARC915	Domain *Archaea*	16S rRNA (915)	20	Stahl and Amann 1991
MER 1	Methanogenic *Archaea*	16S rRNA (MER 1)	10-20	Hales *et al.* 1996

Cell count determinations

Cell numbers were calculated by DAPI and by FISH using the oligonucleotid probe for Bacteria (mixed of EUB I, II and III Cy3), for Archaea (ARC915 Cy3), and for methanogens (MER Cy3). Microscopy was constructed with a Zeiss Axioskop 2 equipped with filters 02 (DAPI), 10 (FLUOS) and 20 (Cy3), mercury-arc lamp and an AxioCam digital camera. For each hybridization and sample at least 1000 DAPI-stained cells were counted on 20 randomly chosen counting squares (Böckelmann *et al.,* 2003). Microscopic performance was carried out using 100 x 100 magnifications giving an area of 0.014884 mm^2 per counting square. Independent checks for non-EUB338 hybridization by FISH procedure were negative. Total cell counts and FISH cell counts were calculated for 0.5 g wet permafrost sediments.

Total DNA extraction

From each sample was weighted 500 mg for the DNA extraction. Nucleic acids were extracted from terrestrial permafrost sediment core (C1) at three depths and from submarine permafrost sediment core (C2) at three depths using Power Soil™ DNA Isolation Kit (Mo Bio Laboratories Inc., Carlsbad, USA). The extracted DNAs were used for molecular cloning and RT PCR using a TaqMan probe and primer set.

Real-time PCR analysis

Pseudomonas fluorescens for the measuring of bacterial 16S rRNA copy number and *Methanosarcina* spec. stain SMA-21 (Morozova & Wagner, 2007) for the measuring of methanogenic 16S rRNA copy number were selected as standard DNAs for real-time PCR and extracted from our pure culture using UltraClean™ Soil DNA Isolation Kit (Mo Bio Laboratories Inc., Carlsbad, USA). Those DNAs were diluted with pure water and measured DNA concentration in ng/µl using NanoPhotometer™ (UV/Vis spectrophotometer, Implen, Munich, Germany).

The amplifying primer set for Bacteria (Bact-0348 forwards and Bact-0786 reverse and the double-dye probe of Bact-0515) and methanogenic Archaea (MArch-0348 forwards and MArch-0786 reverse and the double-dye probe of MArch-0515) were checked with primer match by ARB and were used for measuring of bacterial and methanogenic 16S rRNA copy number. The double-dye probe was labelled with 6FAM at the 5´end and BBQ at the 3´end.

RT PCR was constructed with a Rotor-Gene™ 6000 real-time rotary analyser (Corbett Research Pty Ltd, Mortlake, NSW, Australia) and TaqMan Universal PCR Master Mix (Applied Biosystems, Foster City, CA, USA). Reaction mixtures for the TaqMan PCR were prepared in which the concentrations of each primer and the TaqMan probe, respectively, were optimized. Each sample was serially diluted and tested to determined PCR efficiency to see if there are any PCR inhibitors present (Zhang *et al.,* 2006). The quantitative measurement by RT PCR was constructed in triplicate.

The RT PCR amplification for bacteria followed a three-step (40 cycles) with HOLD-program (50°C for 2 min, then 95°C for 10 min), 20 s denaturation (95°C), 20 s annealing (50°C) and, 30 s elongation (72°C). The regression range of the standard curves with six dilutions (10^1 - 10^6) obtained by real-time PCR measurements was 0.998 - 0.999.

A nested RT PCR based Halliday et al. (2005) and Takahashi & Nakayama (2006) was designed and modified for detection of methanogenic archaea. Oligonucleotide primer pairs (ArUn4 forward and Ar958 reverse) were used in the first-round PCR amplification. The reaction mixture of 25 µl contained 1 x PCR buffer, 0.25 mM of each dNTP, 2 mM $MgCl_2$, 0.4 µM of each primer, 2.5 Unit Hot StarTaq DNA Polymerase (Qiagen, Hilden, Germany), and 1 µl of DNA from permafrost sample and of SMA 21. DNA amplification was performed by incubation at 95°C for 10 min, followed by 35 amplification cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 1 min, and a final extension at 72°C for 10 min. After this first amplification followed a three-step (40 cycles) with HOLD- program (50°C for 2 min, then 95°C for 10 min), 20 s denaturation (95°C), 20 s annealing (55°C) and, 30 s elongation (72°C). The regression range of the standard curves with dilutions (10^1 - 10^7) obtained by second-round real-time PCR measurements was 0.993 – 0.996.

In order to calculate the initial copy number of methanogenic archaea in samples before passing them through the extraction and the two PCR amplification steps, the equation on the basis of Takahashi & Nakayama (2006) was modified and formulated for this study as follows.

(1) a : b = c : d ; a = b x c/d

In equation (1), a represents the initial copy number of methanogenic DNA in the 500 mg of permafrost sample; c represents the initial copy number of the control (SMA 21, 10^3 copies of d) calculated after measurement with the NanoPhotometer™ (Implen), and b and d represents the copy numbers of methanogenic DNA and the control (SMA 21), respectively, after second-round RT PCR.

All RT PCR amplifications were analysed with automatic setting of the baseline and threshold values, using the standard curve method (the log of initial DNA concentration in standard templates).

Table 2. Combinations of Clone-PCR primer, Real-time primer and probe set

Target	Primer name and Sequence* (5`-3`)	References
Methanogenic archaea (RT-PCR)	S-P-March-348-S-a-17 (GYGCAGCAGGCGCGAAA) S-D-Arch-0786-A-a-20 (GGACTACVSGGGTATCTAAT) S-P-March-515-S-a-25 (TGCCAGCMGCCGCGGTAAYACCGGC)	Takai and Horikoshi (2000) Sawayama *et al.* (2004) Sawayama *et al.* (2006)
Bacteria (RT-PCR)	S-D-Bact-0348-S-a-17 (AGGCAGCAGTDRGGAAT) S-D-Bact-0786-A-a-20 (GGACTACYVGGGTATCTAAT) S-D-Bact-0515-S-a-25 (TGCCAGCAGCCGCGGTAATACRDAG)	Takai and Horikoshi (2000) Sawayama *et al.* (2006)
Archaea (PCR for cloning and 1. amplification for RT PCR)	ArUn4f (TCYGGTTGATCCTGCCRG)	Hershberger *et al.* (1996)
	Arc8f (TCCGGTTGATCCTGCC)	Banning *et al.* (2005)
	Arc109f (ACKGCTCAGTAACACGT)	Grosskopf *et al.* (1998)
	March348f (GYGCAGCAGGCGCGAAA)	Sawayama *et al.* (2006)
	Arc958r (YCCGGCGTTGAVTCCAATT)	DeLong (1992)
	Arc1492r (GGCTACCTTGTTACGACTT)	Lane (1991)

*** D = G, A or T; H =A, T or C; K =G or T; M= A or C; R = A or G; S =G or C; W=A or T; Y = C or T.**

PCR amplification

For DNA samples at depths 56 m, 58 m, 70 m (submarine permafrost) and 0.5 m (permafrost-affected soil), amplification used Archaea specific primers 8F and 1492R. PCR incorporated a hot start of 95°C for 10 min, followed by 50-55 cycles depending DNA samples (94°C for 1 min, 61°C for 1 min, and 72°C for 2 min) with a final extension at 72°C for 30 min. Two times PCR per DNA sample from 43 m and 59.5 m (both deep terrestrial permafrost) were necessary to get sufficient PCR product for building gene libraries, because the DNA templates of primary amplification were very weak. Archaeal 16S rRNA was amplified in a PCR with shorter bp products. In the amplification, the primers 109f and 958r were used with a start of 95°C for 10 min, followed by 42 cycles depending DNA samples (94°C for 1 min, 61°C for 1 min, and 72°C for 2 min) with a final extension at 72°C for 30 min. Clone yield from the submarine permafrost sample at 58 m was weak. Therefore, the amplification with the primer pairs 348 and 958 was performed by 38 cycles depending DNA samples (94°C for 30 sec, 53°C for 30 sec, and 72°C for 45 sec) with a final extension at 72°C for 10 min.

For a 50 µl reaction contained 25 µl of MangoMIX PCR buffer including deoxynucleoside triphosphate mix (Bioline, Luckenwalde, Germany), 0.5 µl of 50mM $MgCl_2$, 1 µl of forward primer (20 µM), 1 µl of reverse primer (20 µM), 2µl of template DNA and PCR water (Bioline) to the 50 µl volume.

Construction of clone libraries and sequencing

The PCR products for each sample were ligated into pGEM®-T Easy vector (Promega GmbH, Mannheim, Germany) and transformed into JM 109 High Efficiency Competent cells (Promega) on LB medium containing 20 mg/ml of ampicillin (D[-]-α-Aminobenzylpenicillin, Sigma-Aldrich Chemie). Before the Ligation, 100 µl of 100 mM IPTG (SERVA Electrophoresis, Heidelberg Germany) and 20 µl of 50 mg/ml X-Gal (Eppendorf, dissolved in N,N´-dimetyl-formamide) were spread over the surface of a LB plate with ampicillin and allowed to absorb for 30 minutes at 37°C. The resulting clones were screened for ribosomal DNA. Recombinants each from the terrestrial permafrost sediment and submarine permafrost sediment libraries were inoculated onto fresh fluid LB medium including ampicillin and then incubated overnight at 37°C. Just-visible smears of each colony were transferred to the bottoms of individual wells in a 96-well microtiter plate by using autoclaved toothpicks. A master PCR mix was made up containing, for each reaction of 30 µl, 1 µl cloning culture (diluted in 1:10 bidest. water), 15 µl of MangoMix PCR buffer including deoxynucleoside triphosphates (Bioline), 0,1 µl of primer M13F (100µM, 5`-GTAAAACGACGGCCAG-`3), 0,1 µl of primer M13R (100µM, 5´-CAGGAAACAGCTATGAC-´3), and 10.80 µl of sterile water. Aliquots (30µl) of the master mix were added to each well, and PCR was carried out under the conditions: hot start 94°C for 3 min, followed by 30 cycles (94°C for 1 min, 55°C for 1 min, and 72°C for 3 min) with a final extension at 72°C for

10 min. Subsamples (25 µl) of each PCR mixture were purified using Sepha dex™ G-50Superfine (GE Healtcare, Biosciences AB, Uppsala, Sweden).
The sequencing unique clones were carried out by MWG and GATC (MWG, Martinried Germany, and GATC Biotech AG, Konstanz, Germany). An aliquot of the PCR product from 660 randomly chosen positive clones (336 from the three different submarine permafrost sediment samples and 326 from the three different terrestrial permafrost samples) was sequenced by using the universal sequencing primer M13F and M13R, respectively.

Sequence analysis
Sequences for Archaea were edited following Koch et al. (2009) and were checked for chimeras with the Chimera-Check of the Ribosomal Database Project (RDP, Michigan State University). The resulting sequences, each comprising > 592 bp (depends using of primer combinations), were aligned into the ARB 16S rRNA database and against reference archaeal sequences. Following alignment, the sequences were analyzed by using ARB programs. All sequences were compared to > 97 % similarities as clone sequences and showed in four phylogenetic trees. These sequence data have been submitted to the GenBank database under accession No. FJ982666 – FJ982776.
Estimation of diversity indices (Shannon diversity index `H`, Chao1 and the ACE richness estimators, and the Simpson evenness index) were performed with DOTUR (Schloss and Handelsmann, 2005). Library coverage was calculated described by Høj et al. (2008).

Results

Environmental characteristics

Cores from terrestrial and submarine permafrost have been shown elsewhere to contain similar terrestrial permafrost deposits (Rachold et al. 2007). Since its inundation as a consequence of tectonics, sea level rise and/or coastal erosion, however, submarine permafrost has been submerged for about the last 2500 years, based on current shoreline retreat rates. Its temperature is about 10 °C higher than that of terrestrial permafrost, which has consequences for the volume of pore space inhabitable by microbes and for the hydrochemistry of the water in that pore space.
Temperature and gravimetric water content for both cores are shown in Figures 1 and 2. The terrestrial permafrost is cold, at between -12 and -13°C below the depth of diurnal to seasonal fluctuations (about 20 m). The profile shown here represents conditions in April of 2005, so that the trend to colder temperatures at the ground surface is due to end of winter conditions. The permafrost at submarine permafrost is 11 °C warmer, between -1 and -2°C, and shows little variation with depth, which is

typical for profiles in which phase change, in this case from ice to water, is taking place (Overduin et al. 2008).

In the terrestrial permafrost samples (42.9-43.05 m and 59.52-59.66 m), the in situ temperature was -12.6 °C, and water contents for both samples were 35% (vol.) and 20% (grav.), respectively, reflecting the low salinity of the pore water (0 and 1.0 ‰; 100 and 1840 µS/cm, respectively). The upper submarine permafrost samples (56.0-56.12 m, 58.0-58.24 m) were at about -1.45°C in situ, while the deepest one (70 m) was at -0.9 °C. Total volumetric water contents (ice + water) for the upper samples were 35 and 38 %, respectively, by volume, translating to gravimetric water contents of 25 and 39% respectively. Both samples were probably close to the melting point in situ, but frozen (i.e. most of the pore water was present as ice). The ice was present as pore ice (massive ice), so that the sediment was ice-bonded, with individual small ice lenses. At 70 m depth in the submarine core, the in situ temperature of -0.9 °C, it is unlikely that any ice remained (Overduin et al., 2008). The sediment was plastic (i.e. deformable) when recovered from the drill casing. Based on the sediment (a silty fine sand, dark grey in colour), the volumetric water content was probably 25-30%.

Variations in salinity over depth were observed in both cores, but were much greater in the submarine sediments due to diffusion of sea water into the sediment from above and to deposition of marine sediments at the lower end of the core. No sediments in the terrestrial core showed direct marine influence although salinities were high (up to 2000 µS/m) in the lower portion of the core. Overduin et al. (2008) describes the influence of brines on the liquid water and ice contents of the sediment, and shows that a third of the pore space can remain unfrozen even at temperatures as low as -10 °C at the salinities described here. Of the samples analysed here, only the pore water from submarine permafrost at 70 m was saline, with a conductivity of 24000 µS/cm and a pH of 7.8. In the submarine permafrost, the thawed and extracted pore water had electrical conductivities of 640 (0.3 ‰) and 600 µS/cm (0.2 ‰) and pHs of 7.5 and 7.0, respectively, while the terrestrial permafrost had salinities of 172 (0 ‰) and 1940 µS/cm (1.1 ‰) and pH values of 6.4 and 7.6.

Pore water chemistry was dominated by Ca, Na, Cl and SO_4. Sulphate concentrations in the freshwater sediment were usually less than 20 mg/l, but a peak of up to 200 mg/l occur around 58 m in the submarine sediment. Iron concentrations lay below detection limits at 56 m, but at 36 µg/l at 58 m. Numerous shallower samples (54-55 m) contain higher (detectable) $[Fe]_{aq}$ (up to 600 µg/l).

In the terrestrial permafrost sediments (Fig. 1 (d)), from 0-0.7 m and from 22.16-60.69 m (between 0.7 m and 22.16 the core contained almost purely ice), the methane concentration lay between 0.5 and 39.8 nmol CH_4 g^{-1} sediment. The methane concentrations in the submarine permafrost sediments (Fig. 2 (d)) varied with depth from 40.5 m to 63.4 m and lay between 0 and 284 nmol CH_4 g^{-1} sediment.

Table 3. Summary of physical and chemical descriptions for sediment samples investigated in this study; submarine permafrost at 70 m is unfrozen due to the high salt concentration

Sediment Samples	pH-value*	Total Nitrogen [%]	Total Organic Carbon [%]	Dissolved organic Carbon [µMol/L]	Fe (µmol/l)	Mn (µmol/l)	Sulfat (µmol/l)	Na (µmol/l)	Phosphat (µmol/l)
TERRESTRIAL PERMAFROST									
PERMAFROST-AFFECTED SOIL									
00.42 - 00.55 m (C1)	4.85	0.41	5.95	34580	637	10.4	4	400	6.
DEEPER PERMAFROST									
42.90 - 43.05 m (C1)	6.04	< 0.05	0.35	4235	6.7	9.3	44	970	< 1
59.52 - 59.66 m (C1)	7.87	< 0.05	0.17	2611	< 0.4	1.6	314	9570	< 1
SUBMARINE PERMAFROST									
FROZEN									
55.91 - 56.05 m (C2)	7.46	< 0.05	0.25	3447	< 0.4	3.9	124	2400	35
57.88 - 58.04 m (C2)	7.05	0.05	8.71	917	0.7	9.0	2012	1320	10
UNFROZEN									
70.00 m (C2)	7.77	0.07	0.59	1510	n.a.	n.a.	195	178030	n.a.

***pH-value using by DIN (dried)**

Abundance of methanogenic and bacterial microorganisms

The results of the real-time PCR analysis (Fig. 1 and 2, both (f)) indicated that the number of bacterial rRNA gene copies is approximately 100 times higher than the number of the methanogenic rRNA gene copies. The number rRNA gene copies of bacteria was 1000 times more than the total cells counts determined by DAPI (Fig. 1 and 2, both (e)). The numbers of methanogenic 16S rRNA gene copies were 1.90 x 10^8 and 1.42 x 10^9 g^{-1} in the submarine permafrost sediments, and 4.48 x 10^8 and 3.38 x 10^{10} g^{-1}, in the terrestrial permafrost sediments, using real time PCR analysis. PCR analysis and the total cell counts showed that the highest relative abundance of methanogens in the permafrost-affected soils is at 0.5 m, in the submarine permafrost sediments at 58 m. The latter corresponds with the location of highest methane concentrations. Generally, the numbers of rRNA copies of bacteria and methanogenic archaea in the submarine permafrost sediments were approximately 10 times higher than in the deep terrestrial permafrost sediments.

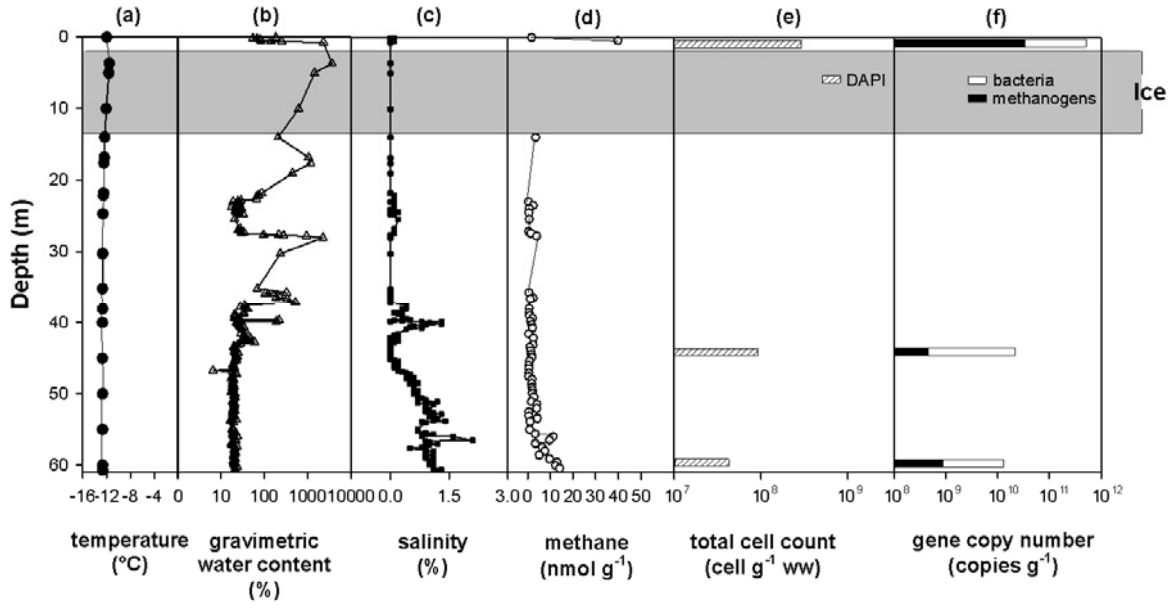

Figure 1. Depth profiles of geochemical and microbiological markers along the terrestrial permafrost core (C1)

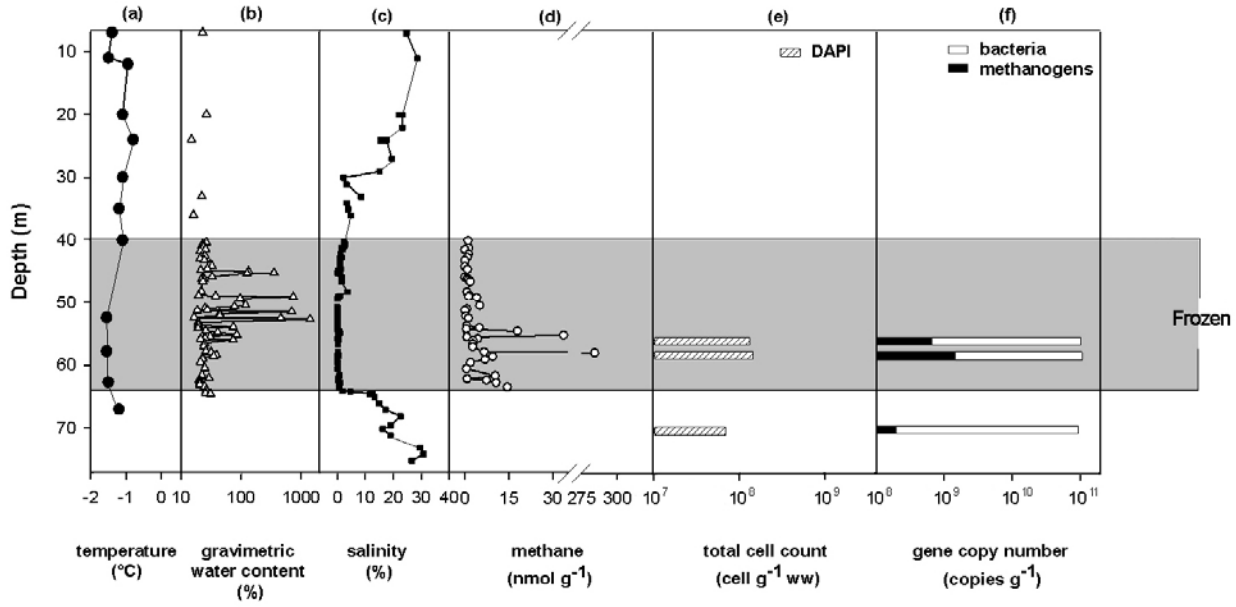

Figure 2. Depth profiles of geochemical and microbiological markers along the submarine permafrost core (C2)

The FISH archaeal, methanogenic and bacterial domain-specific probes (Table 4) were performed on each of the three selected terrestrial and submarine permafrost sediments (Tab. 4). High cells counts of methanogens (not less than $2x10^7$ cells g^{-1}) and bacteria (not less than $1x10^8$ cells g^{-1}) were found in the permafrost-affected soil and in the submarine permafrost sediment at 58 m. The latter contained: archaea 22.4% ±5.5, methanogens 15.5% ±2.9, and bacteria 71.7% ±15.9. The proportion of methanogens of all archaea is 69%, which is the highest of all examined samples. Low cells counts of methanogens and bacteria were found in the deep terrestrial permafrost and unfrozen marine sediments. Moreover, at 43 m of the deep terrestrial permafrost sediment and at 56 m of the submarine permafrost sediment, proportions of methanogens of all archaea were at their lowest, which corresponded to lowest methane concentrations.

Table 4. Community structure of bacteria, archaea and methanogenic group

Sediment Samples	Total Count (cells/g ww)	Cell Count (cells/g ww)			% of Total Count			% of ARCH 915
	DAPI	EUB338	ARCH915	MER	EUB	ARC	MER	MER
TERRESTRIAL PERMAFROST								
PERMAFROST-AFFECTED SOIL								
00.50 - 00.51 m (C1)	**2.89 x 10^8** (± 3.4 x 10^7)	**1.31 x 10^8** (± 7.5 x 10^6)	**4.29 x 10^7** (± 6.5 x 10^6)	**2.77 x 10^7** (± 3.6 x 10^6)	45.4 (± 2.6)	14.8 (± 2.3)	9.6 (± 1.3)	64.6 (± 8.5)
DEEPER PERMAFROST								
43.00 - 43.02 m (C1)	**8.98 x 10^7** (± 1.5 x 10^7)	**5.29 x 10^7** (± 6.7 x 10^6)	**6.13 x 10^6** (± 1.9 x 10^6)	**1.78 x 10^6** (± 1.7 x 10^6)	58.9 (± 7.4)	6.8 (± 2.1)	2 (± 1.9)	29.1 (± 27.3)
59.50 - 59.52 m (C1)	**4.22 x 10^7** (± 9.2 x 10^6)	**1.88 x 10^7** (± 8.9 x 10^6)	**3.41 x 10^6** (± 1.8 x 10^6)	**1.94 x10^6** (± 6.6 x 10^5)	44.7 (± 21)	8.1 (± 4.2)	4.6 (± 1.6)	56.8 (± 19.3)
SUBMARINE PERMAFROST								
FROZEN								
56.00 - 56.12 m (C2)	**1.21 x 10^8** (± 3.2 x 10^7)	**6.93 x 10^7** (± 1.9 x 10^7)	**2.01 x 10^7** (± 1.1 x 10^7)	**4.58 x 10^6** (± 3.1 x 10^6)	57.1 (±15.3)	16.5 (± 8.9)	3.8 (± 2.6)	22.8 (± 15.5)
58.00 - 58.24 m (C2)	**1.32 x 10^8** (± 2.4 x 10^7)	**9.45 x 10^7** (± 2.1 x 10^6)	**2.96 x 10^7** (± 7.3 x 10^6)	**2.04 x 10^7** (± 3.8 x 10^6)	71.7 (±15.9)	22.4 (± 5.5)	15.5 (± 2.9)	69 (± 12.9)
NOT FROZEN								
70.00 m (C2)	**6.37 x 10^7** (± 7.1 x 10^6)	**2.54 x 10^7** (± 4.4 x 10^6)	**9.93 x 10^6** (± 4.5 x 10^6)	**5.74 x 10^6** (± 4.8 x 10^6)	39.8 (±6.8)	15.6 (± 7)	9 (± 7.6)	57.8 (± 48.7)

Phylogenetic analysis of archaeal communities

A clone library analysis with 119 operational taxonomic units (OTUs, identified with > 97% similarity, 608 sequences) was conducted, of which 70 OTUs (279 sequences) were from terrestrial permafrost sediments, and 49 OTUs (329 sequences) were from the submarine permafrost sediments.

Overall, four archaeal 16S rRNA gene libraries were constructed: Methanosarcinales (Fig. 4a), *Methanomicrobiales* (Fig. 4b), *Methanobacteriales* (Fig. 4b) and *Crenarchaeota* (Fig. 4 b). In particular, 88 OTUs belonged to *Methanosarcinales*, 1 OTU to *Methanobacteriales*, 10 OTUs to *Methanomicrobiales* and 20 OTUs to *Crenarchaeota* (s. Figures 4 (a), (b), (c) and (d)). In five of the six different samples the dominating group of OTUs was *Methanosarcina*. Only at the depth of 56 of the submarine permafrost sediments Group 1.1b and Marine Benthic Group C of *Crenarchaeota* was prevailing.

Four new clusters (SMPF 58m (2), DTP 59.5 m, PAS 0.5 m and DTP 43 m) were formed in the library of *Methanosarcinales*. The clusters PAS and DTP 43 m were closely related to *Methanosarcina* stain MS (Simanova et al., 2003) and clone ARG4 (Stein et al., 2001) from metal-rich sediments from Green Bay.

Most of the crenarchaeal OTUs have similarities to their next relatives. Sequences isolated from cold habitats such as recently deglacialed soil, cold sulphidic marsh water and deep cold-seep area.

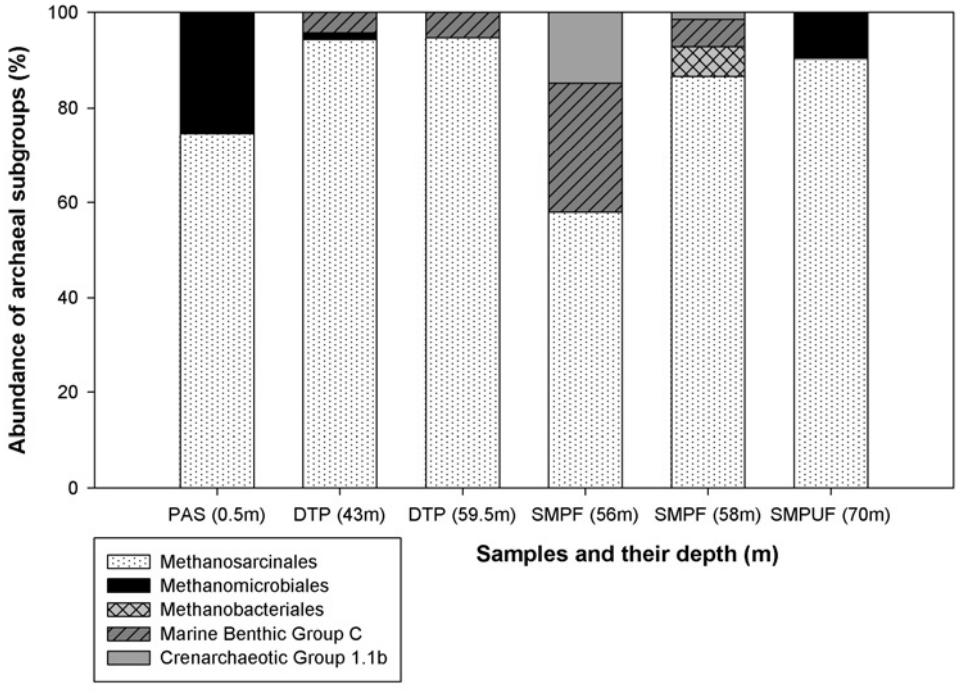

Figure 3. Differences in the abundance of archaeal subgroups founded in permafrost sediment samples

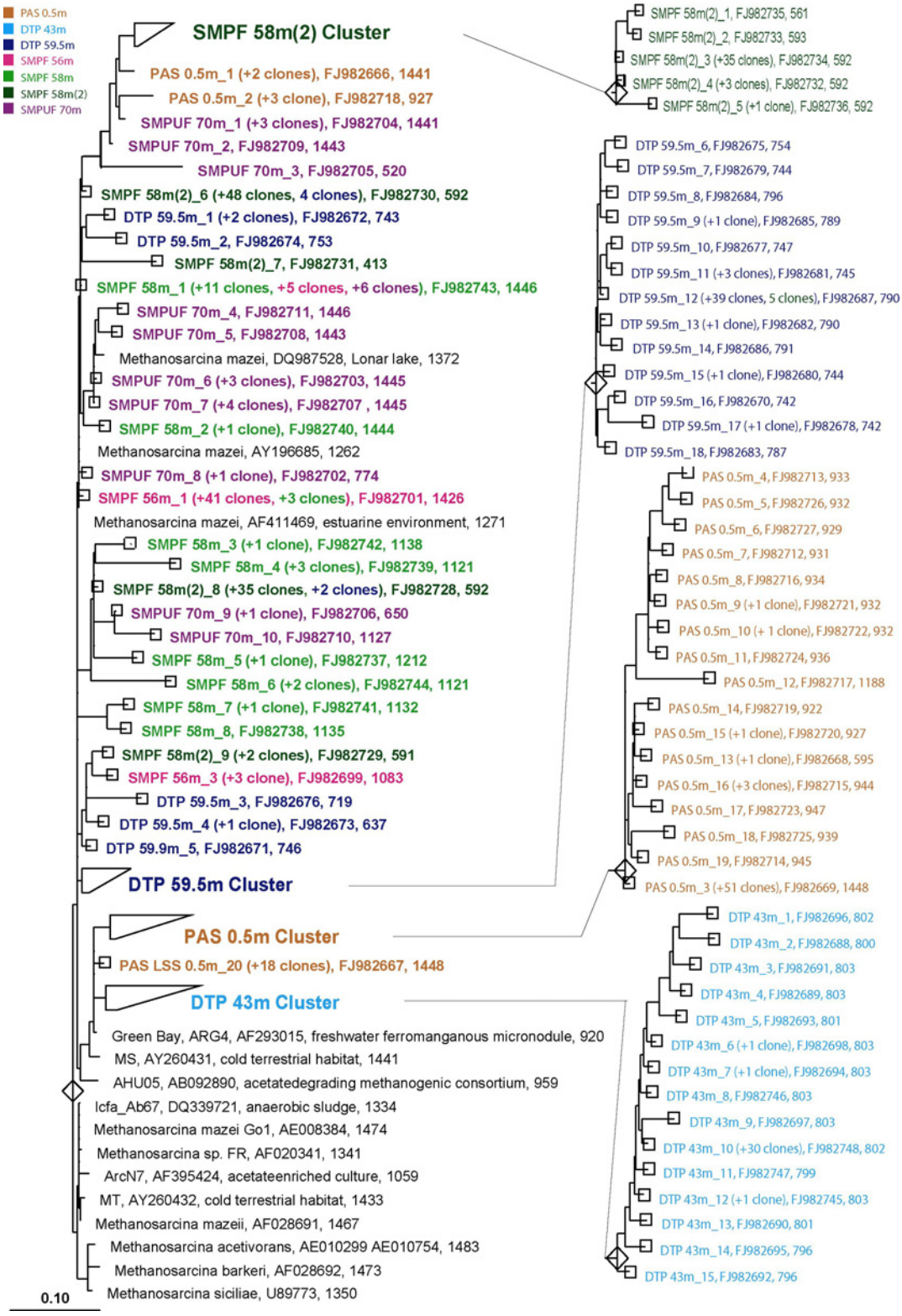

Figure 4. Maximum likelihood phylogenetic tree of *Methanosarcinales* showing the position of archaeal 16S rRNA phylotypes retrieved from terrestrial and submarine permafrost sediments of Laptev Sea region. Sequences are color-marked according to location and depth (PAS = Permafrost-affected soil, DTP = Deep terrestrial permafrost, SMPF = Submarine permafrost frozen, SMPUF = Submarine permafrost unfrozen). One representative of sequence groups >97% identical is shown; additional number of clones represent by a sequence.

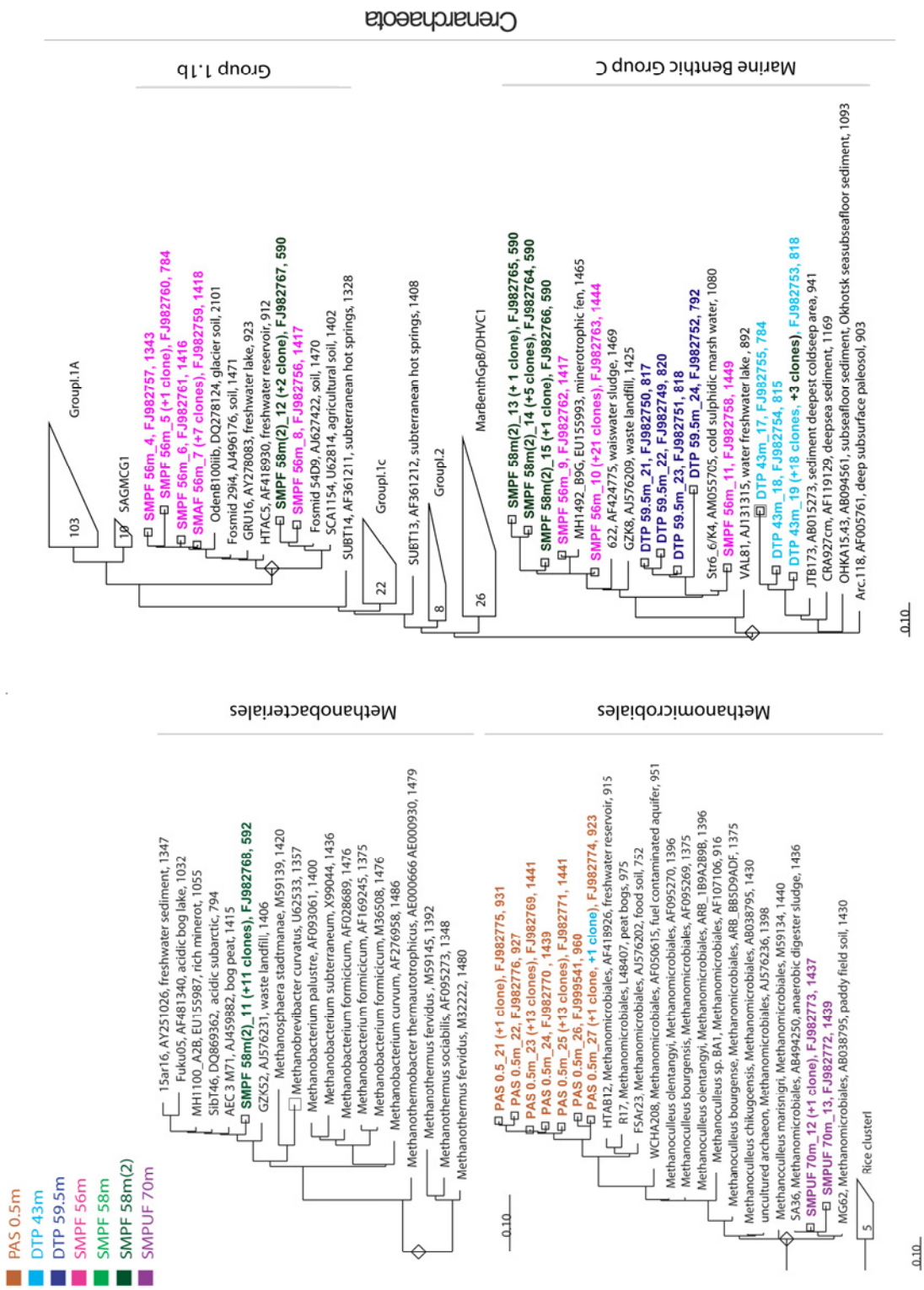

Figure 5. Maximum likelihood phylogenetic trees of *Methanomicrobiales*, *Methanobacteriales*, and *Crenarchaeaota* showing the position of archaeal 16S rRNA phylotypes retrieved from terrestrial and submarine permafrost sediments of Laptev Sea region. Sequences are color-marked according to location and depth (PAS = Permafrost-affected soil, DTP = Deep terrestrial permafrost, SMPF = Submarine permafrost frozen, SMPUF = Submarine permafrost unfrozen). One representative of sequence groups >97% identical is shown; additional number of clones represent by a sequence.

Table 5. Statistical diversities for clone libraries of terrestrial and submarine sediments

	PAS [1)]	DTP [2)] 43 m	DTP 59.5 m	SMPF [3)] 56 m	SMPF 58 m	SMPF 58 m (2)	SMPUF [4)] 70 m
Archaea							
max seq. length	1400	800	800	1400	1400	592	1400
seq. no	134	70	75	89	33	176	31
OTU´s	**27**	**19**	**24**	**11**	**9**	**16**	**13**
Shannon (min, max)	**2.24** (2.00; 2.48)	**1.54** (1.14; 1.94)	**2.18** (1.84; 2.51)	**1.42** (1.17; 1.66)	**1.70** (1.33; 2.07)	**1.52** (1.33; 1.72)	**2.27** (1.97; 2.58)
Chao1 (min, max)	**61** (37; 141)	**139** (65; 330)	**110** (49; 317)	**19** (12; 54)	**10** (9; 20)	**18** (16; 28)	**20** (14; 50)
ACE (min, max)	**70** (41; 161)	**118** (42; 451)	**107** (47; 327)	**29** (15; 99)	**11** (10; 14)	**21** (17; 39)	**23** (15; 55)
Simpson	**0.19**	**0.45**	**0.2**	**0.35**	**0.25**	**0.35**	**0.1**
Library coverage	**79.9**	**72.9**	**68**	**87.6**	**72.7**	**90.9**	**58.1**
Methanogenic Archaea							
seq. no	134	67	71	51	33	160	31
OTU´s	**27**	**16**	**20**	**3**	**9**	**11**	**13**
Shannon (min, max)	**2.24** (2.00; 2.48)	**1.37** (0.98; 1.76)	**2.00** (1.67; 2.33)	**0.32** (0.09; 0.55)	**1.70** (1.33; 2.07)	**1.20** (1.021.37)	**2.27** (1.97; 2.58)
Chao1 (min, max)	**61** (37; 141)	**94** (43; 239)	**73** (34; 213)	**3** (3; 7)	**10** (9; 20)	**14** (12; 33)	**20** (14; 50)
ACE (min, max)	**70** (41; 161)	**82** (31; 317)	**72** (34; 222)	**4** (3; 18)	**11** (10; 14)	**17** (12; 45)	**23** (15; 55)
Simpson	**0.19**	**0.49**	**0.23**	**0.85**	**0.25**	**0.09**	**0.1**
Library coverage	**79.9**	**76.1**	**71.8**	**94.1**	**72.7**	**93.1**	**58.1**

[1)]Permafrost-affected soil; [2)]Deep terrestrial permafrost; [3)]Submarine permafrost (frozen); [4)]Submarine permafrost (unfrozen)

Discussion

As a result of global warming, increased permafrost degradation and release of significant quantities of the currently conserved organic matter is predicted for high latitudes (Anisimov et al., 1999). The future development of permafrost environments as source of methane depends primarily on the response of the methane-cycling microorganisms to a changing environment. This study focussed on methanogenic archaea in submarine permafrost, which has been warmed to near 0°C since it was flooded by marine waters since the last glaciation. Submarine permafrost is therefore a natural laboratory for studying the impact of rising temperature on the structure and function of microbial communities in permafrost environments.

Our study indicates changes both in the abundance and structure of bacterial and archaeal communities between submarine and terrestrial permafrost. Although the samples from both permafrost cores originated from different depths, they are comparable in their lithology and stratigraphy (Overduin et al., 2007, 2008). The results revealed significantly higher cell counts both for the total community as well as for bacteria and archaea in the submarine permafrost sediments at depths of 56 and 58 m than in the terrestrial permafrost at 43 and 59.5 m. The most striking differences between the two different permafrost habitats are the temperature as well as the liquid water content and methane concentration. The bulk porewater chemistry is similar in both habitats, apart from a higher TOC content at a depth of 58 m in the submarine sediment. In the submarine permafrost the temperature is about 10°C higher than in its terrestrial counterpart and showed the highest methane concentration of all drilled permafrost sediments. These findings correspond with former results indicating that the methane in the submarine permafrost is presently produced by methanogenic archaea (Koch et al., 2009). Wagner and colleagues (2007) reported in situ activity also for Holocene terrestrial permafrost deposits in the Siberian Lena Delta. They also showed that the activity of methanogens increases with rising in situ temperatures. This shows that the higher cell numbers found in this study can be the result of a higher metabolic activity in the warmer submarine permafrost sediments. A higher activity of methanogens in the submarine permafrost is also indicated by the results of fluorescence in situ hybridization, which revealed higher cell numbers for methanogens in the submarine permafrost. Although FISH signals can not be directly translated to activity status of all cells, a positive relationship between bacterial metabolism and the capacity to detect active cells has been demonstrated (Bouvier & del Giorgio, 2003).

The diversity of archaea varied with depths and permafrost habitats (Fig. 3 and Tab 5). Generally, the numbers of archaeal OTUs were low in all permafrost samples which is comparable with previous studies of high Arctic peat (Høj et al., 2008), continental margin sediments (Harrison et al., 2009), and methane-rich cold seep sediments (Reed et al., 2006).

Most of the sequences analysed were related to the group of *Methanosarcinales* and *Crenarchaeota*. The remaining sequences were affiliated with the group of *Methanobacteriales,* which were found only in submarine permafrost at 58 m depth, and *Methanomicrobiales*, solely found in deep terrestrial permafrost at 43 m depth. The archaeal communities in both permafrost environments were represented by all major methanogenic groups currently known, including new sequence clusters. Sequences from all permafrost samples were phylogenetically related to most environmental clones from cold habitats. Nonetheless, the affiliations of sequences in the phylogenetic trees are different.
Most notably, *Methanosarcina* is the dominant genus of the archaeal communities (Fig.3) in both permafrost habitats. This result indicates that the group of *Methanosarcina* in the permafrost environment is dominated by psychrophiles or psychrotolerant species. Good survival potential of methanogenic archaea in permafrost sediments have also been demonstrated at low water content, where – before thawing of permafrost – the onset of methanogenesis was limited by low substrate and water availability (Høj et al., 2006; Morozova & Wagner, 2007). In particular, two large *Methanosarcina*-like clusters from deep terrestrial permafrost (Fig. 4; DTP 43 m and 59.5 m) were inhabited by methanogens from a cold environment. The sequences from submarine permafrost did not form a large cluster, but rather showed a very high diversity. We conjecture that the carbon turnover is more versatile in these sites because of increasing temperature and water content, whereas the methanogenic community composition in the deep permafrost sediments is more stable due to unchanged permafrost temperature. Hence, permafrost temperature and water content are an important factor in regulating the metabolic activities of methanogens and their partner microorganisms. We suggest that the Methanosarcina in deep terrestrial permafrost become well adapted to increasing permafrost temperature.

For the other archaeal groups detected in the permafrost sediments, *Crenarchaeota* has, as far as we know, been detected in deep terrestrial (40 m and deeper) and submarine permafrost habitats for the first time. Jurgens & Wagner (unpublished) have found *Crenarchaeota* in Siberian terrestrial permafrost as well, but only to a depth of 25 m at Kurungnakh Island, northeastern Siberia, which was reasonable due to the decreasing carbon content and a material shift to fluvial sands. *Crenarchaeota* have also been found in low temperature environments (Steven et al., 2007, Kemnitz et al., 2004, Metje & Frenzel, 2007, Høj et al., 2006 and 2008). In submarine as well as in deep terrestrial permafrost Marine Benthic Group C was detected. It was found that the abundance of *Crenarchaeota* based on cloning was noticeably lower in deep terrestrial permafrost than in the warm submarine permafrost (Fig.3).Only the Group 1.1b of *Crenarchaeota* was found in submarine permafrost at 56 m. According to Nicol & Schleper (2006) group 1.1b of *Crenarchaeota* could be involved in nitrogen

cycles. Furthermore, it remains unclear what role *Crenarchaeota* plays in influencing microbial communities (Schleper, 2007).
However, the change of the archaeal community compositions due to higher temperatures in the submarine permafrost has shown that the population of methanogenic archaea is flexible enough to compensate drastically environmental changes by alteration of their species composition. Particularly the genus Methanosarcina has been identified as the dominant archaeal group under varying environmental conditions.

It is of interest to compare terrestrial permafrost-affected soils with deep terrestrial permafrost. Permafrost-affected soils are the upper seasonally thawing and freezing horizons where the warm summer temperature leads to high microbial turnover. Hence, TOC and DOC values are very high in permafrost-affected soil. Sazonova et al. (2004) showed that by 2099 the seasonal thaw depth in East Siberia transect could increase by 0.5 - 2 m. The changing properties of the deeper permafrost also play an important role in regulating the microbial community compositions and their ecosystem balance.
The highest total cell counts in terrestrial permafrost samples were found in the permafrost-affected soil, which is in accordance with other studies based on fluorescence microscopy (Kobabe et. al., 2004; Liebner & Wagner, 2007). Cell counts decreased with increasing terrestrial permafrost depths. The archaeal community compositions differed between permafrost-affected soil and deep terrestrial permafrost due to different temperatures and physiochemical conditions. Generally, microbial communities in permafrost-affected soil are more exposed to seasonal changes than communities in the deep permafrost, where physiochemical conditions are more stable.
Permafrost-affected soil might be a source as well as a sink for both atmospheric CH_4 and produced CH_4. CH_4 produced in permafrost soil and sediments diffuses to the surface via the water column, gas bubbles, or plants. In northern Siberia the mean methane emissions range between 4.3 and 53.2 CH_4 mg m^{-2} d^{-1} during late spring and beginning autumn, depending on the permafrost soil's temporal and spatial variability, vegetation, and soil structure (Wagner et al., 2003, Kutzbach et al., 2004, Wille et al., 2008). Furthermore, Wagner and co-workers (2007) have shown that methane production under frozen conditions increased with increasing temperature. The microbial communities' compositions and the methanogenic activities were mainly influenced by the micro-relief, formed by cryogenic processes, which changed the micro environments (Wagner et al., 2005).
It has to be assumed that, through alteration of the structure of terrestrial permafrost below the permafrost-affected soil due to the influence of global warming, transformations of microbial communities will occur and, hence, lead to higher productions of biomass.

Mostly *Methanomicrobiales* were found in permafrost-affected soil. The result suggests that the detected members of *Methanomicrobiales,* being strictly carbon dioxide reducing methanogens, were relatively better competitors for substrates under such conditions (Høj et al., 2006).
Phylogenetic analyses of methanogens did not agree with methanogenic community studies reported elsewhere (Høj et al., 2006, Ganzert et al., 2007, Metje & Frenzel, 2007). Families such as *Methanobacteriaceae*, and *Methanosaetaceae* were not found in the permafrost-affected soil in this study. *Methanosaeta* and *Methanobacteria* could be not detected by the large archaeal primer set used for cloning. However, the application of primer pairs which lead to amplification of longer DNA fragments is less efficient than the amplification of shorter DNA fragments, since the polymerase shows lower efficiency for amplification of longer DNA fragments (Suzuki & Giovanni, 1996). Apart from these possible insufficiencies, the results suggested that *Methanobacteriaceae*, and *Methanosaetaceae* are nutrition specialists and hence limited by substrate availability, whereas *Methanosarcina* have a broad nutrition spectrum.
We assume that the depth-related variations in the archaeal community compositions are determined by different TOC and DOC values, and high salinity and sodium concentrations.

The comparison of frozen submarine permafrost with the underlying unfrozen submarine sediments is of importance. These underlying sediments were probably deposited in a marine environment during the last interglacial cycle, when sea levels were similar to modern levels (Rachold et al., 2007). The sediment at 70 m has a temperature of less than 0 °C, but contains little or no ice due to the lowering of the freezing point due to the saline porewater. In frozen submarine permafrost, the abundance of total and active cells counts and gene copy numbers detected for bacteria and archaea were similar at both depths (56 m and 58 m). However, the highest methanogenic cell counts at depth of 58 m were similar to methanogens cell counts from northeastern Siberian surface permafrost soils (Morozova et al., 2006; Rikina et al. 1998), where high methane concentrations indicated active methanogenesis. This coincided with the highest observed TOC value and a low δ^{13} CH_4 value (Koch et al., 2009) as well as the low DOC value, indicating that the bacteria involved in methanogenic degradation of organic matter are psychrophilic and/or psychrotolerant. In the unfrozen submarine sediments at 70 m the total cell counts and FISH signals were two times lower than in the submarine permafrost sediments. The effect of high salinity on the abundance of archaea and bacteria can be explained by their limited activity to the unfrozen submarine permafrost at 70 m. Further, the archaeal population from frozen submarine permafrost was different from populations from the unfrozen submarine permafrost. In contrast to frozen submarine permafrost, Crenarchaeota were not detected in unfrozen submarine permafrost, but

the *Methanomicrobiales* were found. This result suggested that these different diversities of the microorganisms correlated with some physiochemical factors such as frozen/unfrozen condition and salinity. Finally, the archaeal community compositions in unfrozen and frozen submarine permafrost were restricted on the genus-level and displayed a dominance of *Methanosarcina* closely related to known psychrotolerant strains. *Methanosarcina* can grow on H_2 + CO_2, methanol, methylamines and acetate. Further, it can adapt to high salinity (Morozova & Wagner, 2007; Spanheimer & Müller, 2008).

This study shows that the methanogenic archaea and specially *Methanosarcina* were found, have higher survival potential in permafrost sediment, and are also microbial active. The latter is more the case when physiochemical conditions such as temperature and water content change. The methanogenic population increase at rising permafrost temperature. Through global warming the thawing of permafrost could lead to instability of the methane hydrate, which produced from methanogenic archaea. Hence, the methane as greenhouse gas could be released into atmosphere and influence the global climate. Independent of the influences of human activity on climate, the global warming will activate and promote the methanogenic archaea.

Conclusion

Submarine permafrost is considered as an ideal natural laboratory to study the impact of changing environmental conditions, particularly increasing permafrost temperatures, on the structure and function of microbial communities in climate-sensitive permafrost habitats. Previously it was not clarified whether microbial communities of methanogenic archaea can adapt to increasing temperatures in the Arctic permafrost soils and sediments. The microbiological results have shown that permafrost microorganisms, especially the methanogenic communities, increase with higher *in situ* temperatures and water contents. The abundance of bacteria and methanogenic archaea in submarine permafrost sediments were approximately 10 times higher than in the deep terrestrial permafrost deposits. The clone library analyses shows that the community of methanogenic archaea is flexible enough to compensate drastically environmental changes by alteration of their species composition. Particularly the genus *Methanosarcina* has been identified as the dominant archaeal group under varying environmental conditions. We conclude that the abundance and activity of the methanogenic community in permafrost environments will not negatively effected by global warming.

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analyser. We are grateful to Katharina Knobel for support to interpretation of RT PCR results.

2.3 Third publication

In preparation

Methanogenic communities analysed by in vitro using denaturing gradient gel electrophoresis on terrestrial and submarine permafrost samples of the Siberian Laptev Sea Shelf area

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Abstract

To better understand the effects of temperature, pore water, and organic matter on methanogenic communities in terrestrial and submarine permafrost, samples from Laptev Sea Shelf area were studied using denaturing gradient gel electrophoresis (DGGE) of amplified 16S rRNA genes. Permafrost samples with different *in vitro* conditions showed significant differences in their methanogenic community compositions. Differences in methanogenic community compositions were observed at different temperatures and were compared to the methanogenic community compositions from unaltered environmental samples. The number of DGGE bands from unaltered environmental samples was higher than in the *in vitro* samples. This result suggests that the methanogenic communities in the *in vitro* samples were still in a stationary phase. It was shown that the methanogens can survive under various *in vitro* conditions. 16S rRNA genes from excised DGGE gels were belonged to *Methanosarcinales*, *Methanomicrobiales*, *Methanobacteriaceae,* and uncultured *Euryarcheota*. *Methanosaeta* and *Methanobacteria* were detected only under *in vitro* conditions at higher temperatures, but not in the unaltered environmental samples.

Keywords: *in vitro*, DGGE, methanogenic community

Introduction

Methanogenesis in permafrost soils and sediments play an important role in the carbon cycle. Most of the methane trapped in permafrost is of biogenic origin and is a significant source of natural gas. Methanogens produce methane as their main catabolic product (Valentine et al., 2000). Arctic tundra soils release 17 – 42 Giga ton methane per year (Cao et al., 1996; Christensen et al. 1996), which accounts for 25% of total atmospheric methane intake from natural sources (Fung et al., 1991). An

increase in Arctic air temperature of almost 2°C over five decades is a serious consequence of climate change (ACIA, 2004) and will lead to an increase in permafrost temperature. The thawing of permafrost affects hydrological systems, ecological systems and carbon release of degraded carbon from permafrost. The release of frozen methane trapped in permafrost would contribute to global warming. In particular, methane hydrates (frozen methane in combination with water molecules) occur in and under permafrost and evolve where organic matter accumulates. These methane hydrates could destabilize as permafrost temperature rise. The total amount of methane hydrates in and under permafrost is unknown, but 7.5 to 400 Giga ton carbon has been estimated (Gornitz and Fung, 1994).

More than 80% of the biosphere is permanently cold (Simankova et. al, 2003). Permafrost temperature is a factor which influences the activity of methanogenic Archaea, although the understanding of cold adaptation in this domain of life is limited (Cavicchioli et al., 2000). In a study on northeastern Siberian permafrost habitats, active methanogenesis was demonstrated in -1.5°C submarine permafrost sediments by detection of isotope ratio in correlation with DGGE method (Koch et al., 2009). Phylogenetic analysis of DNA extracted from these permafrost habitats has revealed the presence diverse Archaea including dominant members of the order Methanosarcinales (Koch et al., 2009; Feige et al. 2009, final draft). Very few analyses of thermal adaptation or dependence in frozen and arctic soils have been conducted (Morozova &Wagner, 2007; Simankova et al. 2003). The growth rate of methanogens depends on various environmental factors of which the most important are temperature, bioavailability, and water regime (Ganzert et al., 2007; Høj et al., 2006, 2008; Wagner et al. 2005). Nonetheless, much remains unknown about interactions with other microorganisms and the process of emergence of a dominant species. The partner microorganisms, which provide substrates for methanogens also play an important role.

Investigation is essential to determine whether permafrost contains active microbial ecosystems that have important implications for global nutrient cycling and biochemical processes. Development of methodologies to detect methanogenic communities in permafrost could help explain in vitro biochemical processes at different temperatures.

In this study, we investigated the response of methanogens to different temperatures and lack of organic nutrients in submarine and terrestrial permafrost sediment samples from different depths. Supply of organic matter and artificial pore water to these permafrost sediment samples was used for in vitro experiments under anaerobic conditions at three different temperatures to analyse methanogenic communities. Before and after the in vitro experiments, we detected the methanogenic Archaea using PCR-DGGE.

Materials and Methods

In situ *terrestrial and submarine permafrost sampling*

Permafrost samples were extracted from the Laptev Sea Shelf area near Cape Mamontovy Klyk, in northeastern Siberia (Koch et al., 2009) using a drilling rig (URB-2A-2) (Overduin et al., 2007, 2008).

Six samples were selected for in vitro studies (described in Feige et al., 2009 final draft). Three of the samples were terrestrial permafrost sediment taken from 0.5 m, 43 m and 59.5 m depth and three were submarine permafrost taken from 56 m, 58 m and 70 m depth.

The chemical parameter of pore water (Table 1) and proportions of pore water (Table 2) are shown below. One litre of artificial pore water was made for *in vitro* experiments shown in Table 3. For *in vitro* organic availability, 1/10 LB medium (Luria - Bertani medium) in 100 ml artificial pore water was made with 0.1 g NaCl (MERCK, Darmstadt, Germany), 0.1 g Tryptone Peptone (BBL™ Trypticase™ Peptone, BD, Le Pont de Claix, France), and 0.05 g Yeast Extract (Bacto™ Yeast Extract, Technical, BD).

Table 1. Chemical parameter of environmental pore water (Overduin et al., 2008; Winterfeld, 2009)

Sample	Depth [m]	Al [mg/l]	Ba [mg/l]	Ca [mg/L]	Fe [mg/l]	K [mg/L]	Mg [mg/L]	Mn [mg/l]	Cl [mg/L]
TERRESTRIAL PERMAFROST									
Active layer									
C1 00.42 - 00.55	0,50	0,432	0,04	29,40	35,55	0,38	11,70	0,57	27,02
deep									
C1 42.90 - 43.05	43,00	< 0,02	< 0,02	15,50	0,37	4,06	13,00	0,51	12,50
C1 59.52 - 59.66	59,60	< 0,02	0,11	60,40	< 0,02	11,60	66,30	0,09	530,30
SUBMARINE PERMAFROST									
frozen									
C2 55.91 - 56.05	56,00	< 0,02	0,10	78,10	< 0,02	5,21	19,00	0,22	29,51
C2 57.88 - 58.04	58,00	< 0,02	0,16	104,00	0,04	6,01	17,10	0,49	32,16
unfrozen									
C2 70.00 FM	70,00	n.a.	n.a.	741,71	< 0,02	102,18	477,79	n.a.	9299,00

Table 2. Proportions of selected chemical parameter of pore water in *in vitro* experiments

TERRESTRIAL PERMAFROST	
Active layer	
C1 00.42 - 00.55	Fe > Ca > Mg > Mn > Al > K > Ba
Deep	
C1 42.90 - 43.05	Ca > Mg > K > Mn > Fe
C1 59.52 - 59.66	Mg > Ca > K > Ba > Mn
SUBMARINE PERMAFROST	
Frozen	
C2 55.91 - 56.05	Ca > Mg > K > Mn > Ba
C2 57.88 - 58.04	Ca > Mg > K > Mn > Ba > Fe
Unfrozen	
C2 70.00 FM	Ca > Mg > K

Table 3. Chemicals (MERCK, Darmstadt, Germany) for simulated pore water [g] for 1 liter (milli pore water) used in *in vitro* experiments

Sample	$AlCl_3$ *$6H_2O$ M=241.45 g/mol	$BaCl_2$ *$2H_2O$ M=244.28 g/mol	$CaCl_2$ *$2H_2O$ M=147.02 g/mol	$FeCl_3$ *$6H_2O$ M=270.3 g/mol	KCl M=74.55 g/mol	$MgCl_2$ *$6H_2O$ M=203.30 g/mol	$MnCl_2$ *$4H_2O$ M=197.91 g/mol
TERRESTRIAL PERMAFROST							
active layer							
C1 00.42 – 00.55	0.043	0.004	2.940	3.555	0.038	1.170	0.065
deep							
C1 42.90 – 43.05	0	0	1.005	0.008	0.288	0.770	0.041
C1 59.52 – 59.66	0	0.011	6.040	0	1.160	6.630	0.010
SUBMARINE PERMAFROST							
frozen							
C2 55.91 – 56.05	0	0.011	7.810	0	0.523	1.906	0.025
C2 57.88 – 58.04	0	0.018	10.400	0.004	0.601	1.710	0.049
unfrozen							
C2 70.00 FM	0	0	74.170	0	10.220	47.76	0

Each frozen sediment sample was placed clean side up in an anaerobic glove box and scrape off with a sterile scalpel. Each scraped sample was mixed and separated into ten parts of approximately 2.6 g each. One 2.6 g part of every sample did not undergo *in vitro* testing and was stored at 22°C. The nine other 2.6 g parts of every

sample were placed in each nine autoclaved 250 ml four squared glass (Schott flask). Then 5 ml of milli Q water, artificial pore water, and LB medium were added to three Schott flasks, respectively. Then all flasks were sealed with gum septum and capped. All flasks were incubated at 0°C (in ice water), 4°C and 10°C, respectively (Fig. 1) for twelve weeks. The system was kept under anaerobic conditions by 76 % nitrogen, 19 % carbon dioxide and 5 % methane. Microbial analyses were then conducted.

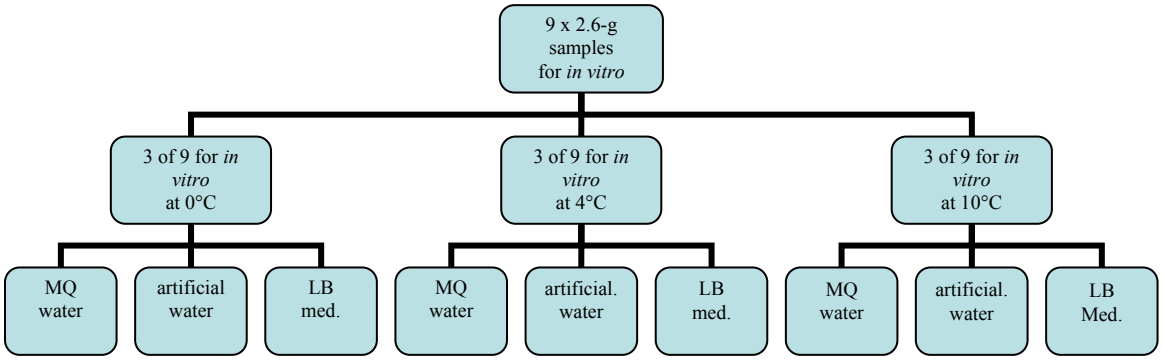

Figure 1. Total samples from one environmental permafrost habitat incubated for 12 weeks (LB = LB medium; MQ water = Millipore water)

Molecular analysis of origin and in situ samples

DNA was extracted from samples by using a Power Soil™ DNA Isolation Kit (Mo Bio Laboratories Inc., Carlsbad, USA). Methanogenic 16S rRNA was unsuccessfully amplified by a direct polymerase chain reaction (PCR) using the primer set GC20_MARCH (5´-gCggCCCgCCgCCCCCgCCggYgCAgCAggCgCgAAA) (Sawayama et al. 2006, modified for this study) and S-D-Arch-0786-A-a-20 (5´-ggACTACVSgggTATCTAAT) (Sawayama et al. (2006). Some samples show a DNA product weakly. Because of this, a nested PCR was performed on all samples. In the first amplification the primers ArUn4F (5`-TCYggTTgATCCTgCCRg) (Hershberger et al., 1996) and Ar958R (5`-YCCggCgTTgAVTCCAATT) (DeLong, 1992) were used according to the following program: an initial denaturation at 95°C for 10 min; 30 cycles of denaturation (30 sec at 94°C), annealing (30 sec at 57°C) and extension (1 min at 72°C), and a final extension at 72°C for 10 min before storing at 4°C. The primer set of GC20_MARCH and S-D-Arch-0786-A-a-20 was used for a second PCR amplification using the following program for denaturing gradient gel electrophoresis (DGGE): an initial denaturation at 95°C for 10 min; 17 - 40 cycles (depending of samples) of denaturation (30 sec at 94°C), annealing (30 sec at 53°C) and extension

(1 min at 72°C); and a final extension at 72°C for 10 min before storing at 4°C. All PCR amplifications were conducted in a 25 µl reaction mixture containing 12.5 µl of SAHARA MIX (including deoxynucleotide triphosphates and buffer) (Bioline, Luckenwalde, Germany), 7.5 µl PCR water (Bioline), 5 mM $MgCl_2$ (Bioline), 0.8 µM each primer, and 2 µl template.
DGGE was performed with a DCode Universal Mutation Detection System (?, Bio-Rad, Germany). A denaturant gradient maker was used to make 8% (w/v) polyacrylamide gels with denaturing gradient from 40% to 60% [100% denaturant corresponds to 7M urea and 40% (v/v) deionised formamide], using acrylamide-bisacrylamide gel stock solution (37.5:1). Electrophoresis was conducted in a 1 x TAE buffer for 14 h at 100 V and 60°C. After loading, the gel was stained with SYBR Gold (1:10000 dilution, Molecular Probes) for 1 h, rinsed with distilled water, and photographed under UV illumination.

Re-amplification, sequencing and phylogenetic analysis

DGGE bands were cut from the gel using a sterile scalpel, diluted in 30 µl milli Q water, overnight at 4°C, and amplified using following PCR program with the primer combination of S-P-MARCH-348-S-a-17 (5`-gYgCAgCAggCgCgAAA) and S-D-Arch-0786-A-a-20 (5´-ggACTACVSgggTATCTAAT): an initial denaturation at 95°C for 10 min; 35 - 40 cycles of denaturation (30 sec at 94°C), annealing (30 sec at 53°C) and extension (1 min at 72°C); and a final extension at 72°C for 10 min before storing at 4°C. All PCR amplifications were conducted in a 28 µl reaction mixture containing 12.5 µl of SAHARA MIX (including deoxynucleotide triphosphates and buffer) (Bioline, Luckenwalde, Germany), 7.5 µl PCR water (Bioline), 4 mM $MgCl_2$ (Bioline), 0.7 µM each primer, and 5 µl template. Each PCR-amplified DGGE band was purified and sequenced with forward primer MARCH 348 by GATC Biotech AG (Konstanz, Germany). Every sequence was compared to reference microorganisms available in the GenBank (NCBI BLAST). The closest 16S rRNA sequences were retrieved from GenBank, and aligned using the ARB program package (Technical University of Munich, Munich, Germany). A phylogenetic tree was then created with filters termini and pos_var_arc_1450_aug04.

Results

In vitro *experiments*

Methanogenic archaea were incubated for 12 weeks on six selected sediment samples of terrestrial and submarine permafrost. The objective was to discover which methanogens would persist under experimental conditions. The methanogens of each sample were exposed to 1/10 LB-medium, artificial pore water (AP), and sterile water (SW) (Millipore water) at three different temperatures (0°C, 4°C and 10°C) under anaerobic conditions.

The addition of 1/10 LB medium (LB) was used to discover at what temperatures microbial communities depend on organic matter. It is assumed that organic matter is biologically degraded by partner microorganisms which provide important substrates for methanogenic Archaea. The addition of artificial pore water (AP) should reveal at what temperature microbial communities depend on artificial pore water (AP) when organic availability is absent. Sterile water (SW) was used to discover how methanogenic Archaea respond to the absence of organic matter and how their development varies at different temperatures. The samples in the *in vitro* experiments were then compared to samples of unaltered environmental sediments and conclusions drawn.

PCR-based DGGE analysis of permafrost samples

Denaturing gradient-gel electrophoresis (DGGE) was used to investigate 16S rRNA gene diversity in six different samples before and after the *in vitro* experiments. These six permafrost samples from the Laptev Sea Shelf area were compared with regard to variation of methanogenic community composition. The DGGE profiles show that the methanogenic community compositions changed during the *in vitro* experiments (Fig. 1 and 2). Furthermore, DGGE images of the *in vitro* experiments samples showed up to four well-defined bands. However, every DGGE image of the PCR-amplified DNA from the unaltered environmental samples showed more detectable bands than any sample after *in vitro* experimentation which indicated that methanogenic diversity was highest in the unaltered environmental samples. The DGGE bands for methanogenic DNA in the six permafrost samples showed significant differences in distribution. In general, the DGGE profiles of the PCR-amplified DNA from permafrost samples showed that the number of DGGE bands increased with higher temperature. The number of DGGE bands from permafrost-affected soil increased by addition of LB medium and artificial pore water and rising temperature, whereas the number of bands decreased by addition of sterile water. The other DGGE profiles of the PCR-amplified DNA from the deep terrestrial and submarine permafrost samples showed that the DGGE bands varied with temperature, availability of organic, and availability of inorganic matter. The differences between all DGGE profiles suggest a significant shift in methanogenic community composition through addition of 1/10 LB medium, artificial pore water and sterile water. Most methanogenic DGGE bands indicated that the best growth temperature was 10°C.

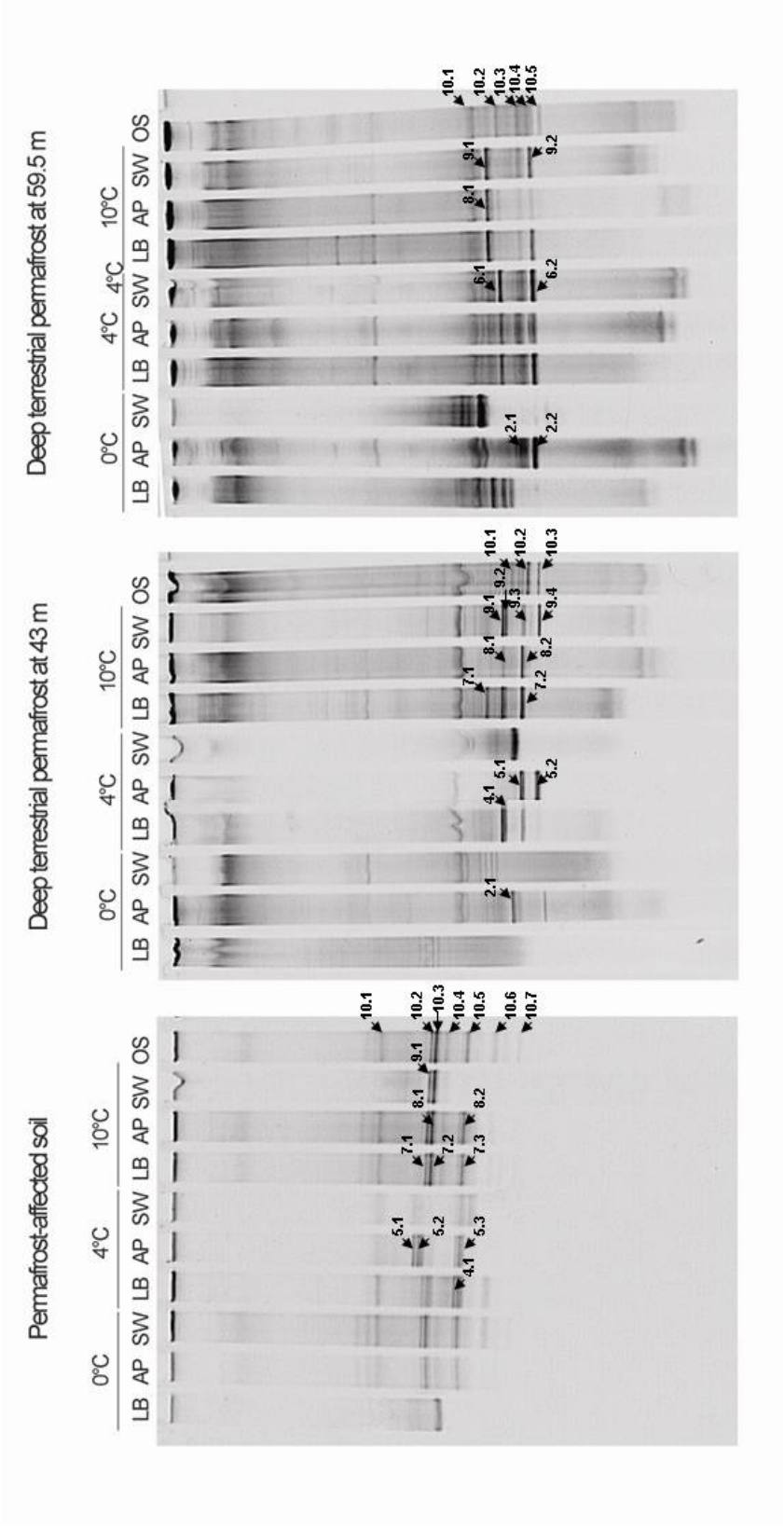

Figure 1. DGGE profile of one unaltered environmental (OS=original state) and nine *in vitro* samples from permafrost-affected soil and deep permafrost sediments at 43 m and 59.5 m; the abbreviations LB = LB medium; AP = artificial pore water, SW = sterile water, OS = unaltered environmental samples; the marked excised bands were used for phylogenetic tree.

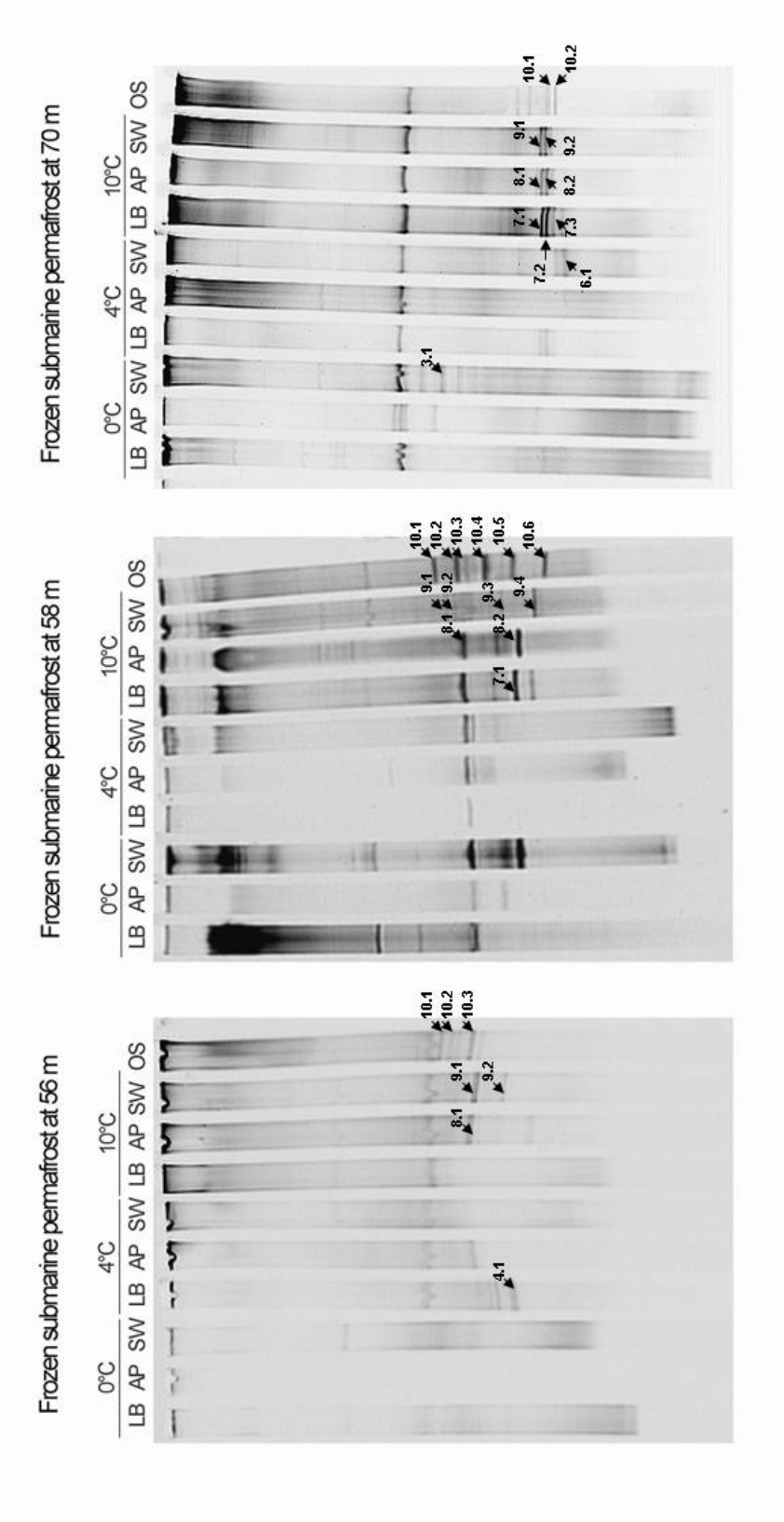

Figure 2. DGGE profile of one unaltered environmental (OS=original state) and nine *in vitro* samples from frozen submarine permafrost sediments at 56 m and 58 m and unfrozen permafrost sediment at 70 m; the abbreviations LB = LB medium; AP = artificial pore water, SW = sterile water, OS = unaltered environmental samples; the marked excised bands were used for phylogenetic tree.

Phylogenetic analysis of permafrost samples

All DGGE bands were excised from DGGE gels. Some of the DGGE bands could not be re-amplified. After discarding the short sequences, a total of 73 sequences (> 270 bp) from excised DGGE were analysed and obtained for gene libraries of 16 rRNA. These sequences could be distinguished by genus and order.

These sequences could be classified into *Methanosarcinales, Methanomicrobiales, Methanobacteriaceae* and uncultured *Euryarchaeota*. 26 sequences belonged to the genus *Methanosarcina* of the order *Methanosarcinales. Methanosarcina* was the most detected genus of methanogenic *Archaea* (Fig. 3 and 4). 29 sequences belonged to the *Euryarchaeota* forming two new clusters: “Deep Terrestrial Permafrost” Cluster and “Peat” Cluster. “Peat“ Cluster contains reference sequences from peatland (Fig. 4). The remaining 18 sequences belonged to the genera *Methanogenium*, *Methanobacterium*, *Methanosaeta*, uncultured *Methanomicrobiales*, and uncultured *Methanosarcinales*. In contrast to the unaltered samples, *Methanosaeta* and *Methanobacterium* were only detected *in vitro* at high temperature after the addition of artificial pore water and sterile water (Tab. 5).

The affiliations of the detectable bands are shown below (Tab. 4). Some bands attributed to *Methanosarcina,* which most of them were found in submarine permafrost at 58 m depth. Some detected bands in deep terrestrial permafrost at 43 m and 59.5 m depth belonged to unidentified *Euryarchaeota* and belonged to the new “Deep Terrestrial Permafrost” Cluster.

Table 4. The number of amplified 16S rRNA gene sequences excised from all DGGE gels including unaltered environmental and *in vitro* samples

Highest similarity	DGGE bands PAS* 0.5 m	DTP* 43 m	DTP* 59.5 m	FSMP* 56 m	FSMP* 58 m	UNSMP* 70 m
Methanosarcinales						
Methanosarcina	6	8		1	11	
Methanosaeta		1			1	1
Uncultured	2	3	2			
Methanomicrobiales						
Methanogenium						3
Uncultured				4		
Methanobacterium	1					
Euryarchaeota	8	3	8	2	1	7
Total	17	15	10	7	13	11

*PAS = Permafrost-affected soil; DTP = Deep terrestrial permafrost; FSMP = Frozen submarine permafrost; UNSMP = Unfrozen submarine permafrost

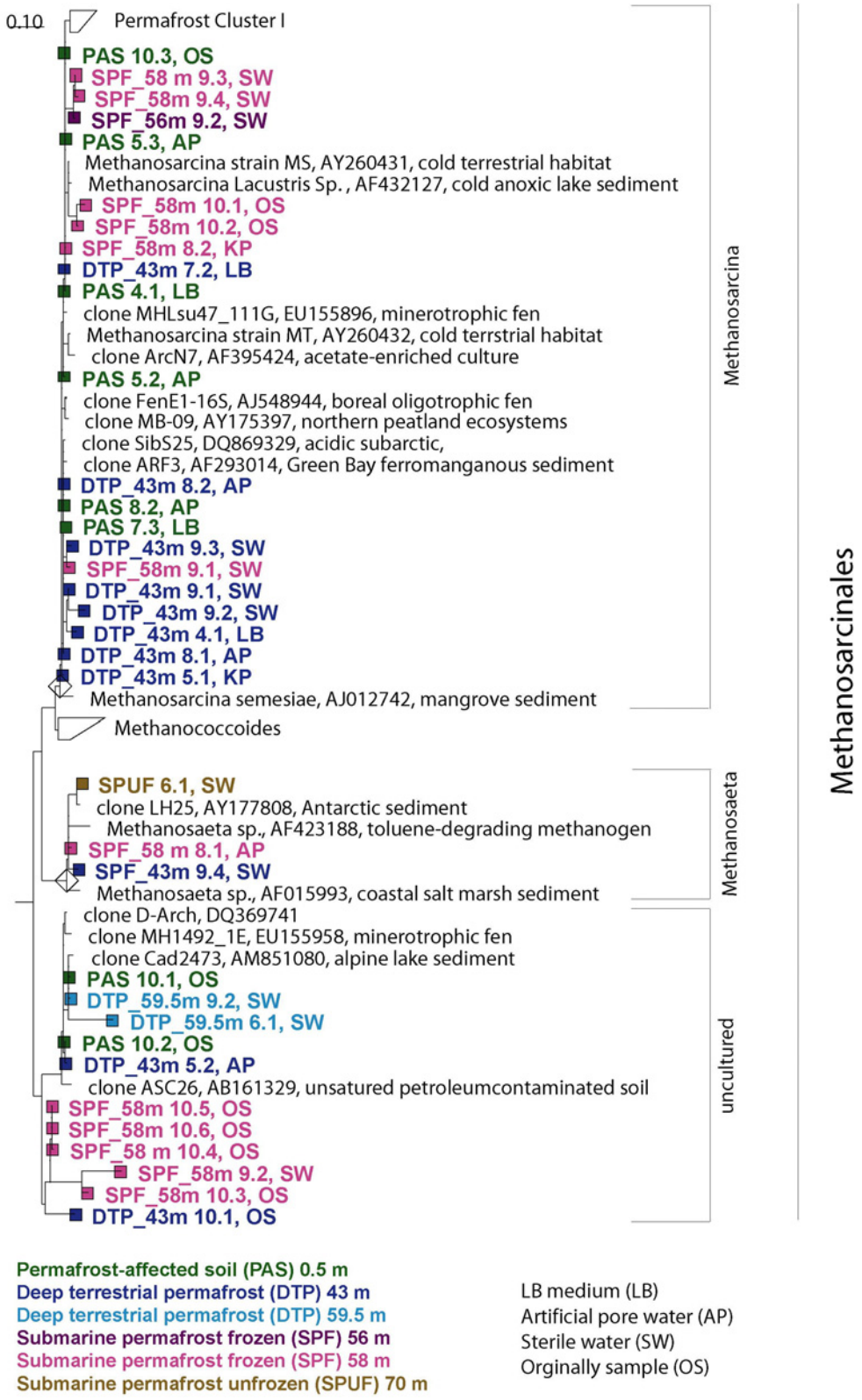

Figure 3. Maximum likelihood phylogenetic tree of *Methanosarcinales* showing the position of archaeal 16S rRNA phylotypes retrieved from terrestrial and submarine permafrost sediments. Sequences are color-marked according to location and depth (PAS = Permafrost-affected soil, DTP = Deep terrestrial permafrost, SMPF = Submarine permafrost frozen, SMPUF = Submarine permafrost unfrozen). Further, the abbreviations: LB = LB medium; AP = artificial pore water; SW = sterile water, and OS = originally sample

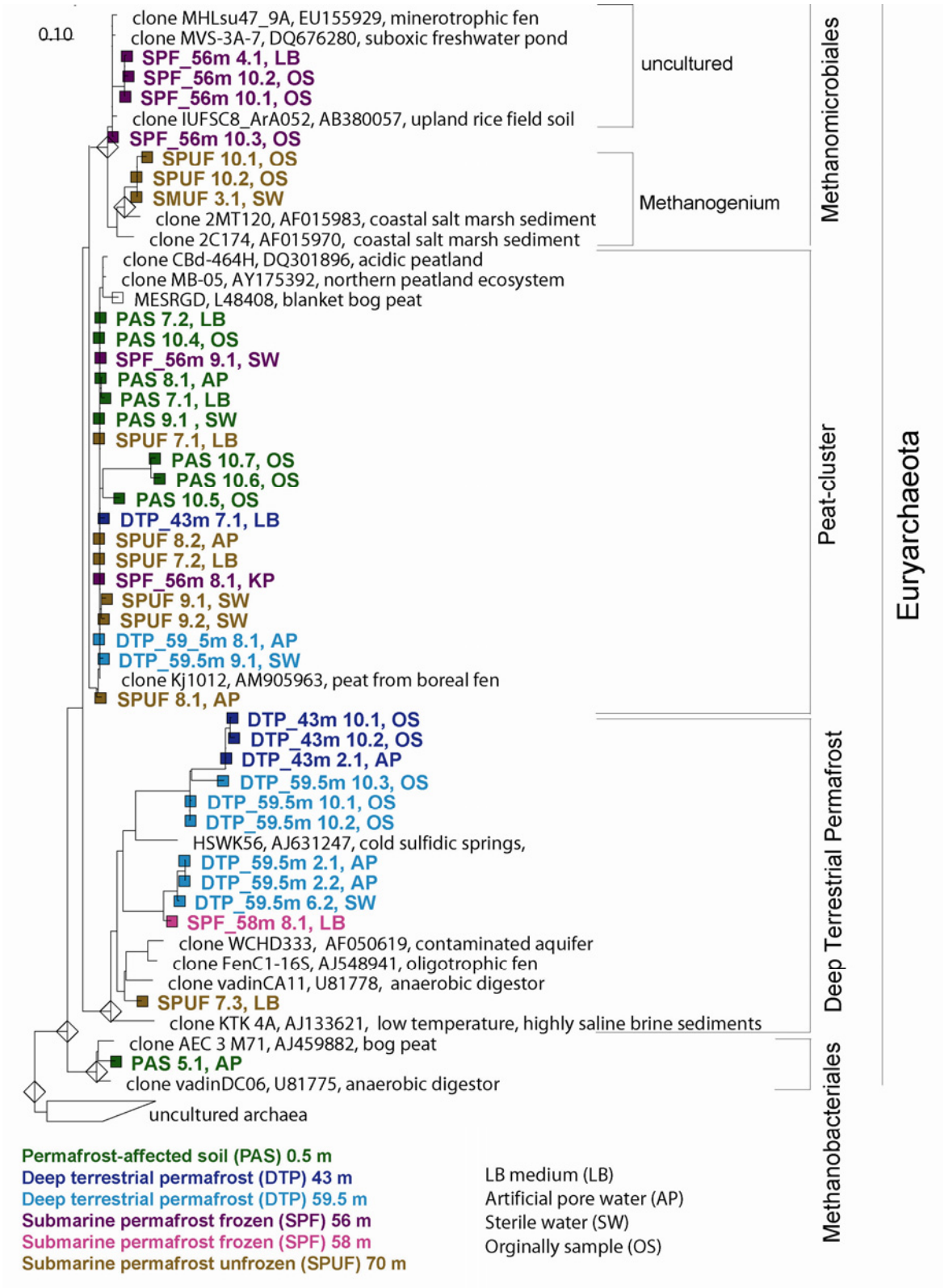

Figure 4. Maximum likelihood phylogenetic tree of *Euryarchaeota* showing the position of archaeal 16S rRNA phylotypes retrieved from terrestrial and submarine permafrost sediments. Sequences are color-marked according to location and depth (PAS = Permafrost-affected soil, DTP = Deep terrestrial permafrost, SMPF = Submarine permafrost frozen, SMPUF = Submarine permafrost unfrozen). Further, the abbreviations: LB = LB medium; AP = artificial pore water; SW = sterile water, and OS = originally sample

Table 5. Detection of all amplified 16S rRNA gene sequences excised from all DGGE gels for all unaltered environmental and *in vitro* samples

	Unaltered samples	LB medium	Artificial pore water	Sterile water
Methanosarcinales				
Methanosarcina	✓	✓	✓	✓
Methanosaeta			✓	✓
uncultured	✓		✓	✓
Methanomicrobiales				
Methanogenium	✓		✓	
uncultured	✓	✓		
Methanobacterium			✓	
Euryarchaeota	✓	✓	✓	✓

Discussion

Permafrost change affects ecosystems. An increase of permafrost temperature alters characteristics of soil and sediment systems thus affecting microbial communities. One consequence is that microorganisms produce higher amounts of carbon dioxide and methane, since they profit in particular by increasing water levels, temperature, and bioavailability. Among the many microorganisms found in these habitats are methanogens who are capable of methanogenesis, even at low temperatures.

Before discussing the results, it should be noted that conditions of *in vitro* experiments differed from environmental conditions particularly with regard to quality and quantity of organic matter, pore water supply, and temperature.

The observations under *in vitro* conditions showed a reduction of methanogenic diversity compared to the unaltered environmental samples. It should be assumed that other microorganisms were also affected by the experimental conditions. Since methanogens exist in symbiotic relationships with specific protozoan species, changes in protozoan community composition would likely affect associated methanogens. However, the protozoan species was not examined in this study.

The detection of methanogens by DGGE was generally higher in the unaltered environmental samples than in the *in vitro* samples. A possible explanation could be that the microorganisms were in a log phase under the *in vitro* conditions, which is rare in natural habitats (Morita, 1997). In natural habitats due to homogenous environmental conditions, balance is maintained between log and stationary phases such that populations of microorganisms remain constant, albeit still active. Under laboratory conditions such as *in vitro* experimentation the microbial population remained in the log phase after incubation at different temperatures.

Another difficulty was that thawing of the permafrost sediments at the beginning of the *in vitro* condition destroyed the physical structure of permafrost (Rivkina et al*.,* 2000). Hence, besides differences in bioavailability as compared to natural habitats,

the *in vitro* experiments reflect methanogenic activity and physiological growth under altered geophysical conditions.

According to studies on microbial community compositions at higher permafrost temperatures (Feige at al., 2009, final draft), changes in composition might cause differences in detection of methanogens at different temperatures. Due to greater methanogenic detection in permafrost samples at 10°C in contrast to less methanogenic detection at lower temperatures, it is evident that the growth rate of methanogens is related to temperature. In another study, the doubling time of microbial growth rate increased as the temperature decreased using ^{14}C-labeled acetate (Rivkina et al., 2000).

In this study, the DGGE bands of methanogens in unaltered environmental samples and *in vitro* samples showed considerable differences which could be due to the application of organic and inorganic matter *in vitro*. The phylogenetic analysis partly confirmed this assumption.

A high number of sequences belonged to the order *Methanosarcinales*, which was the dominant methanogenic order. This is probably explained by the fact that *Methanosarcinales* are generalists and utilize a broad substrate spectrum as compared to other methanogenic groups. Another relatively high number of sequences belonged to uncultured *Euryarchaeota*, forming two new clusters: “Peat” Cluster and “Deep Terrestrial Permafrost” Cluster.

Most sequences (clones PAS) belonging to the “Peat Cluster” which was formed on reference sequences from peatland, were found in the upper peat active layer (permafrost-affected soil). Other sequences from deep permafrost habitats belonging to “Peat Cluster” originated from sites where herbal remains where found. Thin layers (< 1 cm) of organic matter were found at these depths, which were sandy sediments probably redeposited from a river bank or beach environment (Winterfeld, 2009). It is not likely that these were *in situ* peat, suggesting that the microbes were redeposited with the organic matter, or that they originated in a non-peat layer. However, no methanogenic Archaea could be found in the unaltered environment samples. Only *in vitro* with the addition of organic matter, artificial pore water, and sterile water at higher temperatures, was methanogenic Archaea belonging to the “Peat Cluster” detected. This shows that these methanogens could survive in the deep permafrost should temperatures rise.

Other sequences belonged to “Deep Terrestrial Permafrost” Cluster of uncultured *Euryarchaeota.* Most of these sequences were in the unaltered environmental deep terrestrial permafrost at of 43 m and 59.5 m depth. The phylogenetic characterizations of these methanogens are unknown. Some sequences from the same samples were again found under *in vitro* conditions. This result indicates that these methanogens could also adapt to higher temperatures, should the permafrost temperature continue to increase due to the global change.

Another three sequences belonging to genus *Methanosaeta*, were detected only at >4°C from *in vitro* samples of submarine permafrost (58 m), deep terrestrial permafrost (43 m), and unfrozen marine sediment. This observed detection of *Methanosaeta* may reflect that these slow-growing microorganisms need acetate provided by partner microorganisms (which could require higher temperatures) to become established. Another study found *Methanosaeta* only in Arctic soil layers toward the end of the summer season (Høj et al., 2005). In previous studies on the same terrestrial and submarine permafrost samples the genus *Methanosaeta* was not found in subzero environments (Koch et al., 2009; Feige et al., 2009, final draft).
Low detection of methanogenic Archaea in submarine permafrost samples at 56 m and unfrozen marine permafrost samples under *in vitro* conditions could be due to low spreading in this specific permafrost environment. Highest detection of methanogens in unfrozen marine sediment was noted at 10°C. It is of interest to discover why the methanogenic detection in submarine permafrost at 56 m is significantly lower as compared to relatively high methanogenic detection in other permafrost samples. A possible reason could be that there was a high contribution of group 1.3b of *Crenarchaeota*, (described in Feige et al., 2009, final draft). Several studies have indicated that Crenarchaeota are relatively prevalent in low temperature environments (Kemnitz et al., 2004; Metje & Frenzel, 2007; Høj et al., 2008). It is thought that *Crenarchaeota* contribute to important degradation pathways. This assumption should be examined in further studies.
The interaction between temperature and microbial communities is relevant to estimate to what impact forthcoming climate change will have on methane emissions in permafrost environments. An increase in permafrost temperatures might exponentially increase methanogenesis in deep permafrost habitats. Methanogenic populations might also increase as a result of their production and consumption of the substrate pool on which methanogenesis depends. Further studies should pay special attention to the effect of greater substrate availability and rising temperatures on microorganisms which interact with methanogenic Archaea. Such studies can provide a better understanding of the permafrost environment and the effects of rising temperatures due to the global warming on such environments.

Conclusion

Based on *in vitro* experiments using a PCR-DGGE approach, the two most predominant groups of methanogens in this study closely belonged to *Methanosarcina* and new uncultured *Euryarchaeota*. The cell count of methanogenic *Archaea* increased with rising temperature. Possibly, they are in the log phase. Rising temperatures in permafrost environments due to climate change can lead to changes in methanogenic community composition.
Further studies of the effect of temperature on the methanogenic activity should be estimated by FISH results in conjunction with measured methane production rates.

However, it is possible not all methanogens which can be detected by DGGE are detectable by FISH probes.

3. SYNTHESIS AND CONCLUSION

3.1 Synthesis

3.1.1 Introduction

In this doctoral thesis, diverse studies have been carried out and discussed in detail in the first three publications. The objective of this synthesis is to provide a deeper understanding of archaeal communities and, in particular, methanogenic communities in the warm submarine and cold terrestrial permafrost environment in the Siberian Laptev Sea Shelf area. Methanogenic archaea are involved in methane production in permafrost environments and are one source of methane emissions in thawing permafrost deposits.

The studies were based on samples from submarine permafrost and from terrestrial permafrost of the Laptev Sea Shelf area near Mamontov Klyk. Both permafrost sites have been investigated by Rachold et al. (2007) who observed similarity in deposits in the submarine permafrost and terrestrial permafrost (Fig. 4). The average terrestrial permafrost temperature lies between -12 and -13°C at a depth of about 20 m where there is no influence by diurnal or seasonal fluctuations. The submarine permafrost is approximately 10°C warmer at a varying temperature between -1°C and -2°C. Higher temperature in submarine permafrost occurred following the inundation of seawater after the last glaciation which raised the temperature to nearly 0°C. Thus seen terrestrial and submarine permafrost habitats are of interest to study the characteristics of archaeal communities, in particular, methanogenic communities under different permafrost conditions. Moreover, the submarine permafrost serves as natural laboratory for studying the impact of increasing temperatures on microbial community compositions in permafrost environments.

3.1.2 Archaeal community abundance and structure

Understanding archaeal community abundance and structure in diverse permafrost environments, particularly with regard to temperature is necessary for an ecological interpretation of the results and help anticipate the response of microbial communities to future climate change. The main parameters of analysis were temperature adaptability, water content adaptability, spatial variability, and phylogeny of the archaeal communities, in different permafrost environments in the Laptev Sea Shelf area.

In an initial study 15 sediment samples were obtained from a submarine permafrost core extending from 35 m to 65 m depth which were used to investigate the vertical variability of methanogenic Archaea. Using PCR-DGGE (see first publication) a total of 21 different sequences were obtained from these samples all of while belong to methanogenic Archaea.

In a second study, the influence of temperature and water content on archaeal community compositions was investigated in six diverse sediment samples: one permafrost-affected soil, two deep terrestrial permafrost sediments, two submarine permafrost sediments and one unfrozen submarine sediment. After cloning a total of 547 sequences were obtained (second publication). It was found that both core samples of terrestrial and submarine permafrost sediments of Laptev Sea Shelf area contained representatives of *Methanosarcinales*, *Methanomicrobiales* and *Methanobacteriales*, but no *Methanosaetaceae,* whereas other recent molecular studies of methanogenic Archaea in the Arctic have detected *Methanosarcinales*, *Methanomicrobiales* and *Methanobacteriales* as well as *Methanosaetaceae* (Ganzert et al., 2007; Høj et al., 2006, 2008; Metje & Frenzel 2007). Most of these sequences were 97-100% related to sequences in the GenBank. Some sequences formed new clusters. These new clusters of methanogenic Archaea were shown to be a recently cold-adapted species, since psychrophilic and psychrotolerant methanogens have 16S rRNA gene sequences nearly identical to their representative sequences from cold environments. 50 sequences (see second publication) were related to sequences of the 1.3 Group and Marine Benthic Group of *Crenarchaeota.* These were presented in the recent review by Schleper and colleagues (2005). The physiology of these sequences of *Crenarchaeota* is presently unknown. Sequences affiliated to *Methanococcoides burtonii,* which were detected by the DGGE method (see first publication), could not be obtained by cloning possibly because the large primer pairs used for cloning do not cover all species or because the polymerase amplifies longer DNA fragments less efficiently than shorter ones (Suzuki & Giovanni, 1996).

Furthermore, the cloning study of terrestrial and submarine permafrost (see second paper) has shown low heterogeneity of archaeal communities, possibly due to methodological limitation such as biases in the PCR-amplification and complex melting behaviour of diverse fragments in permafrost samples.

Depth-dependent variability in the archaeal community composition was observed between upper permafrost-affected soil and deep terrestrial permafrost sediments as well as between frozen submarine permafrost sediments and unfrozen marine sediments (see second publication). Generally, microbial communities in upper permafrost-affected soil are more affected by seasonal changes (see second publication) than more stabile communities in deep permafrost sediments where living conditions are more homogenous. Due to vegetation growing on upper permafrost-affected soils, the carbon availability for microbial populations in these sites is greater. Moreover, the stronger presence of *Methanomicrobiales* in permafrost-affected soil could be explained by tolerance of soil aeration to a certain extent making them better competitors for substrates in such environments (Høj et al., 2006). Methanomicrobiales were detected in unfrozen permafrost but have not been detected in frozen permafrost.

Archaeal community composition varied between permafrost environments with a temperature difference of approximately 10°C. The studies of deep permafrost sites demonstrated that the population of methanogens increased by up to 10 times as temperatures and water content rise (see second publication). This has been shown by direct cell counts using FISH and by gene copy numbers using TaqMan method (RT-PCR). The highest number of methanogenic cells (acquired by methanogenic cell counts, see second publication) was found in submarine permafrost at 58 m depth. This correlates with the highest number of DGGE bands, high methane concentration, highest observed TOC value and lowest methane isotope ratio also found at 58 m depth which indicates active methanogenesis (see first and fourth publications). Due to the dominance of *Methanosarcina* (detected by cloning) in all samples, the potential for methanogenesis could be relatively high. However, several depths have shown no correlation between TOC and δ^{13} CH_4 values. This might be due to low carbon quality available to microbial communities (Wagner et al., 2007).
In general, the investigated archaeal communities, in particular, methanogenic communities in both permafrost sites of the Laptev Sea Shelf area showed their specialization for cold environments. The observed variability in the archaeal community compositions suggests that the extend of carbon degradation and the volume of methane formation are dependent on temperature of the environment and physiochemical conditions such as water and salinity content. According to the process-related results (see third publication), the DGGE-profiles lead to the conclusion that the dynamics of methanogenic communities were primarily dependent on the temperature and substrate availability. Moreover, the results have shown that these methanogens are able to adapt to changes in temperature and water content. Their adaptability to rising permafrost temperatures might explain why they remain the dominant group of Archaea even where *Crenarchaeota* populations increase. Which environmental factors influence interactions between methanogenic populations and *Crenarchaeota* in deep permafrost sediments at increasing temperatures is yet unknown.

3.1.3 In vitro experiment with methanogenic communities

The objective of this study was to determine under which conditions methanogenic archaeal communities could – within a certain time frame – best adapt to an increase of their environment temperature of origin. This is the first study of how quickly microbial communities of methanogenic microorganisms from deep permafrost environments are able to adapt to higher temperatures. This *in vitro* experiment furthermore examined which methanogens best prevail. The *in vitro* experiment was conducted with a LB medium as organic substrate, artificial pore water as inorganic substrate, and $N_2/CO_2/CH_4$ as a gas mixture. Several samples were incubated at three different temperatures (0°C, 4°C, and 10°C) for 12 weeks. Thereafter, DGGE profiles of all samples were taken, obtaining a total of 75 sequences in this *in vitro*

experiment on varied temperatures and substrates. Some of these sequences were attributed to *Methanosarcinales*, *Methanosarcina* being the dominant genus. The other sequences obtained from the *in vitro* experiment belonged to uncultured and unknown *Euryarchaeota*. It was hypothesized that these *Euryarchaeota* have a specific physiological ability to adapt under particular temperature and physiochemical conditions. The remaining sequences belonged to *Methanomicrobiales*, *Methanobacteriales* and *Methanosaetaceae. Methanosaetaceae* have not been found in submarine and deep terrestrial permafrost before (see first and second papers). Two sequences belonged to *Methanosaeta* from deep terrestrial permafrost at 43 m and submarine permafrost at 58 m, both of which were only detected after incubation at 10°C, which is a remarkably higher temperature compared with actual low permafrost temperatures. In contrast to the faster-growing *Methanosarcina*, which are capable of utilizing a broad substrate spectrum, *Methanosaeta* is a slow-growing specialist that grows only on acetate.

The results of the *in vitro* experiment have shown that methanogenic Archaea have good survival and adaptation skills under *in vitro* condition. Nevertheless, detection of methanogenic Archaea is generally low in the *in vitro* experiment at low temperature which primarily can be explained by the fact that the methanogenic populations used for the experiment were in a log phase which in natural environments is rare (Morita*,* 1997), since in natural habitats under stable environmental conditions microbiological populations are well balanced, and, hence, are active, but do not grow. However, the *in vitro* results demonstrated that temperature change lead methanogens to change from a stationary phase to an adaptation and/or growth phase.

It is not known how the varying of temperature and physiochemical parameters affect the interaction of methanogenic and other microbial communities and how partner organisms influence the composition of methanogenic communities. These questions should be studied in future research.

In summary, the in vitro experiments showed that methanogenic Archaea are capable of rapid adaptation to rising temperatures far beyond the current temperature values in their natural habitats. Such temperatures are expected as a consequence of climate change.

3.2 Conclusions

Inundation of terrestrial permafrost with relatively warm seawater resulted in submarine permafrost approximately 10°C warmer than terrestrial permafrost. Submarine permafrost offers as a natural laboratory to study the impact of warming on microbial abundance and community structure and as a model for the effects of global warming on microbial communities in permafrost environments.
The main conclusions of this thesis are:

- The composition of methanogenic Archaea detected in submarine permafrost sediments, using PCR-DGGE, varied with depth with regard to TOC value, methane concentration, and methane isotope ratio.
  - ⇒ The highest number of detected well-defined DGGE bands for methanogens correlate with the highest TOC value, highest methane concentration and lowest methane isotope ratio which indicated active methanogenesis in frozen sediment.
  - ⇒ The obtained sequences were affiliated to *Methanosarcina*, *Methanococcoides,* Permafrost Cluster I, four new clusters (Submarine Permafrost Cluster I - IV), and so far uncultured Archaea.
- The Archaea, especially methanogenic Archaea, detected in permafrost-affected soil (active layer), deep terrestrial permafrost sediments, frozen submarine permafrost sediment unfrozen submarine permafrost samples showed diversity with changes in temperatures and changes of physiochemical characteristics of the permafrost sediments.
  - ⇒ The sequences obtained by cloning belonged to *Methanomicrobiales*, *Methanobacteriales*, *Methanosarcina*, and several new clusters of methanogens as well as Group 1.3b and Marine Benthic group of *Crenarchaeota*. *Methanosarcina* is the dominate genus of Archaea in all permafrost samples, indicating a high tolerance of these methanogens to extreme variation in their habitat.
  - ⇒ FISH and RT-PCR analysis with six different samples from terrestrial and submarine permafrost revealed a ten times higher cell count and gene copy numbers of bacteria and methanogenic Archaea in submarine permafrost sediment in comparison to the cold deep permafrost sediments.
- *In vitro* incubation experiments were carried out with one sample from the active layer, two samples from deep terrestrial permafrost sediments, two samples from frozen submarine permafrost sediments, and one sample from unfrozen submarine permafrost sediments to analyze the changes in the methanogenic community structure under different nutrient conditions by using PCR-DGGE.
  - ⇒ DGGE-profiles showed that most groups of methanogenic Archaea can grow at all tested temperatures.

- ⇒ Sequences obtained by cloning belong to *Methanomicrobiales*, *Methanobacteriales*, *Methanosarcina, Methanosaeta* and uncultured *Euryarchaeota.*
- ⇒ The methanogenic communities varied in different permafrost samples. Such differences can be related to differences in the degradation pathways and/or the archaeal community structure.
- ⇒ Sequences belonging to *Methanosaeta* were only found at higher temperatures, originally not detected in the unaltered environmental samples.

The results of this study demonstrate that methanogenic Archaea are well adapted to their low temperature environment and able to adapt to increases in permafrost temperature due to climate change. Higher temperatures in permafrost sediments alter the microbial community structure of methanogenic Archaea and lead to an increased rate of methane production with so far unknown consequences for the global atmospheric methane budget.

3.3 Future Research

Future research of methanogenic Archaea and bacteria in microbial communities in submarine permafrost sediments and in coastal terrestrial permafrost will lead to a better understanding of their different roles and interactions in such communities. Bacteria should studied using in cloning, FISH with specifically labelled probes, and DGGE since such methods may reveal which bacterial populations are responsible for the degradation of organic matter to monomers, H_2, CO_2, acetate, fatty acid, or alcohol and clarify the specific role they play with regard to methanogenic Archaea.

Understanding the dynamics and activity of methanogenic populations may enhance our understanding of microbial processes in general and clarify how methanogenic communities interact with others microorganisms and inhibitors. Stabile isotope using ^{13}C labelling should be conducted for identification of active methanogens as well as bacteria, Archaea and other microorganisms. Microbial communities can then be phylogenetically analyzed using DGGE. Chemical and/or physical inhibitors in conjunction with gas chromatography can facilitate detection of methane production.

Isolates should be extracted from submarine permafrost sediments which may lead to new isolates as well as indentification of new specific methanogens. New specific isolates may prove more robust than methanogens from non-permafrost environments. They could also be employed in further studies under Martian conditions in analogy to studies of Morozova & Wagner (2007) using *Methanosarcina* spec. (stain SMA 21). Furthermore, new isolates with their specific abilities could be a more effective means for organic degradation in bioreactors in countries such as Alaska, Canada, northern Europe, and Russia which have cooler climates. The methanogens commonly used in bioreactors have a higher temperature optimum than psychrophilic methanogens.

The existence of anaerobic methanotrophs in submarine permafrost is unknown. This question should be pursued using PCR and DGGE with specific primer pairs for methanotrophic bacteria in analogy to the studies of Liebner and colleagues (2009).

Studying the three other cores (C3, C4, C5) which were obtained at the same time and were located on the transect between as the terrestrial and submarine permafrost cores used in this study could explain how methanogenic communities change and vary along the transect between the two cores which contain permafrost sediments at temperatures between –12°C (C1) and –1°C (C2) The results of such a study could then be compared to the findings of this doctoral thesis.

APPENDIX

FOURTH PUBLICATION

Ninth International Conference on Permafrost, edited by Douglas L. Kane and Kenneth M. Hinkel, Volume 2, 2008, Pages: 1875 – 1880

Methane Cycle in Terrestrial and Submarine Permafrost Deposits of the Laptev Sea Region

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Abstract

Permafrost environments within the Siberian Arctic are natural sources of the climate-relevant trace gas methane. In order to improve our understanding of present and future carbon dynamics in high latitudes, we studied the activity and biomass of the methanogenic communities in terrestrial and submarine permafrost deposits. For these investigations, permafrost cores of Holocene and Late Pleistocene age were drilled in the Laptev Sea region. A high CH_4 concentration was found in the upper 4 m of the Holocene deposits, which correlates well with the methanogenic activity and biomass. Even the incubation of core material at -3°C and -6°C showed a significant CH_4 production (range: 0.04–0.78 nmol CH_4 h^{-1} g^{-1}). The results indicated that the methane in permafrost deposits originated from modern methanogenesis by cold-adapted methanogenic archaea. Microbial-generated methane in permafrost sediments is, so far, an underestimated factor for future climate development.

Keywords: Laptev Sea; methane; methanogenesis; permafrost deposits; phospholipid biomarker; psychrophiles.

Introduction

The Arctic plays a key role in Earth's climate system, as global warming is predicted to be most pronounced at high latitudes and because one third of the global carbon pool is stored in ecosystems of the northern latitudes. Global warming will have important implications for the functional diversity of microbial communities in these systems. It is likely that temperature increases at high latitudes will stimulate microbial activity and carbon decomposition in Arctic environments, and accelerate climate change by increasing trace gas release (Melillo et al. 2002, Zimov et al. 2006). Currently, the functioning of microbial communities and their impact on changing environmental conditions are not adequately understood, and the potential methane release from frozen sediments is not adequately quantified.

Methane is chemically very reactive and more efficient in absorbing infrared radiation than carbon dioxide. Estimates of methane emissions from arctic and sub-arctic wetlands range between 10 and 39 Tg a^{-1}, or between 2.2 and 8.6% of global methane emissions (Bartlett & Harriss 1993, Cao et al. 1998). Methane, as a powerful greenhouse gas, contributes to about 20% of global warming (IPCC 2001).

In general, temperature is one of the most important variables regulating the activity of microorganisms. The growth potential, as well as the molecular, physiological and ecological aspects of microbial life at low temperatures, has been investigated in many studies (e.g., Gounot 1999, Wagner 2008). Certain key processes of the methane cycle are carried out exclusively by highly specialised microorganisms such as methanogenic archaea and methane oxidising bacteria. The microbial methane production (methanogenesis) in the active layer of permafrost is the terminal step during the anaerobic decomposition of organic matter, while the methane oxidation is the primary sink for methane in Arctic wetlands (Wagner et al. 2005).

However, there are only a few studies investigating the geochemistry and microbiology of permafrost deposits, which were mainly done in Siberia and Canada. Direct bacterial counts in the order of 10^7 to 10^8 were reported for permafrost deposits from Northeast Siberia (Rivkina et al. 1998). Shi and colleagues (1997) found viable bacteria in permafrost sediments up to 3 million years in age in the Kolyma-Indigirka lowlands. Most of the isolated bacteria showed mesophilic growth characteristics. In contrast, the minimum temperature for growth of permafrost bacteria was recently calculated to be -20°C (Rivkina et al. 2000). Furthermore, molecular life markers and low numbers of methanogens were found in the Mallik gas hydrate production research well (Colwell et al. 2005, Mangelsdorf et al. 2005). However, methanogenic activity could not be detected in the permafrost sediments using radiolabelled ^{14}C-substrates.

For the understanding and assessment of recent and future carbon dynamics in high latitudes, we have to answer the question: "What will happen to the carbon stored in permafrost, in the event of a climate change?" From this view point, we studied the methane concentration, the quantity and quality of organic matter, and the activity, biomass and diversity of methanogenic communities in permafrost deposits of the Laptev Sea region.

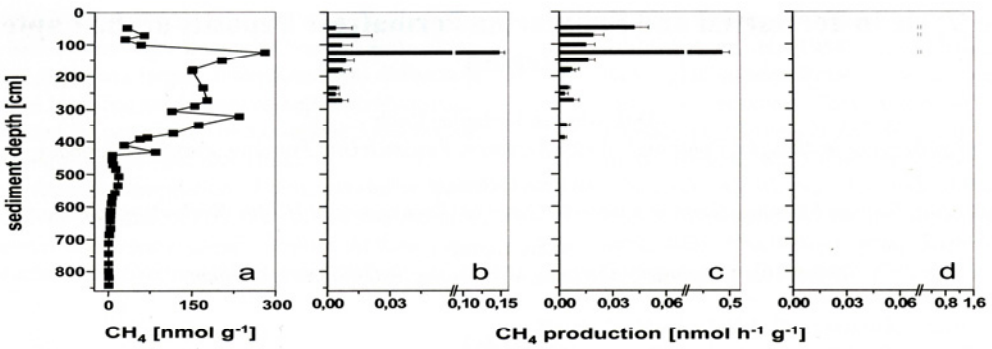

Figure 1. Vertical profiles of methane concentration (a), and methane production rates determined at 5°C without any additional substrate (b), with acetate (c) with hydrogen (d) as methanogenic substrates.

Study Sites

Within the scope of long-term studies on carbon dynamics in the Siberian Arctic, several expeditions were carried out by the Alfred Wegener Institute for Polar and Marine Research.

The Holocene permafrost core was drilled during the LENA 2001 expedition on the main study site Samoylov Island (72°22′N, 126°28′E, Pfeiffer & Grigoriev 2002). Samoylov, with the Russian-German Research Station, is located in the active part of the Lena Delta (Hubberten et al. 2006). The Lena Delta lies at the Laptev Sea coast between the Taimyr Peninsula and the New Siberian Islands. Continuous permafrost, which occurs throughout the investigation area, extends to depths of about 100–300 m (Yershov 1998), with active layer thicknesses between 30 and 60 cm depth.

The submarine permafrost cores of Late Pleistocene age were recovered in the framework of the COAST expedition from the western Laptev Sea along a transect running perpendicular to the coastline (Rachold et al. 2007). The Laptev Sea region is characterised by an arctic continental climate with low mean annual air temperature of about -15°C and low summer precipitation of <198 mm. Further details of the study sites were described previously in Wagner et al. (2003) and Rachold et al. (2007).

Drilling of Permafrost Deposits

The drilling of an 850 cm long core was carried out with a portable gasoline powered permafrost corer without using drilling fluid to avoid microbiological contamination of the permafrost samples. A mixing of the permafrost sediments was not observed due to the frozen state of the core material. The individual core segments, which were up to 50 cm in length, were placed immediately after removal from the corer into plastic bags and stored at about -8°C in the permafrost cellar of the Research Station Samoylov. After drilling of the core, the borehole temperature was monitored with a string of 9 thermistors. The cores were transported in a frozen state to Potsdam, Germany. During transport, the temperatures in the containers were monitored by micro data loggers. The storage temperature in the Potsdam laboratory was -22°C.

Core segments were split along their long axis into two halves under aseptic conditions with a diamond saw in an ice laboratory at -22°C. Afterwards, one half of the core was cleaned with a sterile knife for lithological and geocryological descriptions. Subsequently, one half was cut into segments of about 10–30 cm length according to the lithology and the geocryology. Small pieces (approx. 10 g) of each sub-sample were taken for analysing the methane concentration in the frozen sediments. The remaining material of each sub-sample was thawed at 4°C and homogenized under anoxic and sterile conditions for analysis of the sediment properties and the microbial activities and biomarkers. Sub-samples for the different analyses were placed into sterile plastic Nalgene boxes. Separated samples were used directly for the experiments (methane concentration, methane production rates, and biomarker analysis) or were freeze-dried for the organic carbon analyses. The second half of the core is kept as an archive in the ice core storage at the Alfred Wegener Institute.

Methanogenesis in Terrestrial Permafrost

Our results show significant amounts of methane in the first four meters of frozen sediments (up to 282 nmol CH_4 g^{-1} sediment, Late Holocene, 5000 yr BP until today) and only trace amounts of methane in the bottom section of the core (0.4–19 nmol CH_4 g^{-1} sediment; Middle Holocene, 9000–5000 yr BP; and Early Holocene, 11500–9000 yr BP; Fig.1a). Different amounts of methane in different aged permafrost deposits from northeastern Eurasia were reported by Rivkina & Gilichinsky (1996). They detected methane in modern (Holocene) and old permafrost deposits (Middle and Early Pleistocene, 1.8–0.78 mill. yr BP), but not in Late Pleistocene ice complexes (ice rich permafrost, 130000–11500 yr BP). They concluded from their findings that methane cannot diffuse through permafrost sections. If methane is unable to diffuse through permafrost from deeper deposits, it must either be entrapped during the deposition of the sediments or originate from recent methane production by methanogenic archaea (methanogenesis) in the frozen ground.

The analyses of methane production in selected sediment samples at 5°C, revealed activity only in permafrost layers with significant concentrations of methane (upper 4 m of the sediments; Fig. 1b). An important finding from the activity analyses is that no methane production was detectable in the bottom part of the permafrost section (>4 m) characterized only by traces of methane. This was also the case after addition of acetate or H_2/CO_2 as energy and carbon source (Fig. 1c, d). This indicates that the absence of methanogenesis does not depend on deficiency of methanogenic substrates in the Middle and Early Holocene deposits. Methane was only found in permafrost sediments with verifiable methane production activity.

The investigation of phospholipids as molecular biomarkers for *Bacteria* (PLFA) and *Archaea* (PLEL) shows a vertical profile with the same trend as the methane concentration. Specifically, significant amounts of phospholipids were determined in the upper Late Holocene deposits (<4 m sediment depth), which correlates (r = 0.632, P = 0.05) with the highest amount of methane (Fig. 2). In contrast, the biomarker concentrations in the Middle and Early Holocene permafrost sediments (>4 m sediment depth) drastically decreased to values below 10 nmol g^{-1} sediment, which corresponds with the detected traces of methane. Phospholipids are compounds of cell membranes that rapidly degraded after cell death (Harvey et al. 1986, White et al. 1979). They are regarded as appropriate biomarkers for viable microorganisms (e.g., Ringelberg et al. 1997, Zelles 1999). Therefore, the positive correlation of methane concentration with viable bacteria and archaea gives us the first strong evidence of recent methanogenesis under in situ conditions in permafrost deposits.

Although only a few psychrophilic strains of methanogenic archaea have been isolated, there are some indications of methanogenic activity in cold permafrost environments (Kotsyurbenko et al. 1993, Ganzert et al. 2006). However, the incubation of permafrost samples from 45–63 cm depth at sub-zero temperatures with acetate and hydrogen as methanogenic substrates, indicated a relatively high methane production rate under permafrost temperature conditions. At a temperature of -3°C, a significant increase in methane production was found, which rose linearly to headspace concentrations of about 1000 ppm (with acetate) and 2500 ppm (with hydrogen) during 300 h after the initiation of the experiment. At a temperature of -6°C, methanogenesis was lower; however, after a lag phase of about 300 h, a significant increase to 200 ppm (with acetate), and 500 ppm (with hydrogen) within 200 h, was observed. The calculated activity of methanogenic archaea with hydrogen reached values of 0.78 ± 0.31 nmol CH_4 h^{-1} g^{-1} and 0.14 nmol CH_4 h^{-1} g^{-1} at incubation temperatures of -3°C and -6°C, respectively. This was 2.5 and 3.5 times higher compared to the activity with acetate (0.31 ± 0.04 nmol CH_4 h^{-1} g^{-1} and 0.04 ± 0.01 nmol CH_4 h^{-1} g^{-1}) at the corresponding temperatures.

The quality of organic carbon is a limiting factor in the microbial metabolism process. Our results reveal a high organic carbon content (on average 2.4%) for the Holocene

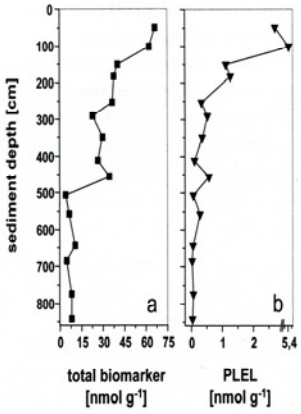

Figure 2. Vertical profiles of total lipid biomarkers (a) and phospholipid ether lipids (PLEL, b) within the Holocene permafrost core.

permafrost deposits (Table 1). However, the quantity of organic matter in permafrost ecosystems provides no information on the quality, which determines the availability of organic compounds as energy and carbon sources for microorganisms (Hogg 1993, Bergman et al. 2000). For this purpose, the humification index (HIX), which is a qualitative parameter, can give suitable information with regard to microbial metabolism. Wagner and colleagues (2005) demonstrated that the availability of organic carbon in permafrost soils decreased with increasing HIX. This is in agreement with the present study. It was shown for the permafrost sequence that the HIX increased continuously with depth. This indicates that the organic carbon is less available for microorganisms with depth because of the higher degree of humification. Consequently, at this point, we can summarize that the zone with significant concentrations of methane and activity of methanogenic microorganisms is characterized by the highest concentration of high quality organic carbon.

In contrast to the results of the soil-ecological variables (methane production activity, PLEL biomarker concentration, TOC, HIX), we do not achieve any hint for a possible entrapment process of methane during sedimentation, which was deduced from data of paleoclimate research carried out in the same study area (Andreev et al. 2004, Andreev & Klimanov 2005).

More than 20 percent of the terrestrial Arctic is characterized by ice rich permafrost (Zhang et al. 1999). Large areas, mainly dominated by continuous permafrost, exist in Siberia with thicknesses up to 900 m (Yershov 1998). The present study revealed that considerable parts of these cold habitats are recent sites of methane production, probably catalyzed by specific cold-adapted methanogenic archaea. This increasing reservoir of climate-relevant trace gases becomes of major importance against the background of global warming which could result from a thawing of

permafrost area up to 25% until 2100 (Anisimov et al. 1999) and subsequent disposal of the methane reservoirs into the atmosphere. Additionally, the results show that an increase of the permafrost temperature would lead to substantial rise in microbiologically-produced methane in the frozen ground. This would further strengthen the contribution of permafrost to the atmospheric methane budget.

Table 1. Borehole temperature, total organic carbon (TOC), and humification index (HIX, dimensionless) of the Holocene permafrost deposits from Samoylov Island.

Depth [cm]	T [°C]	TOC [%]	HIX
49	-1.9	4.82	3.71
72		2.50	5.74
84		3.64	5.33
102	-4	4.47	6.39
126		4.91	5.47
151		4.01	3.80
179		2.63	5.62
183	-7.4	3.42	6.64
235		n.d.	6.88
254		2.54	5.69
273		1.65	8.13
291		3.11	6.95
307	-9.4	0.87	0.68
323		1.88	6.08
350		2.11	6.83
375		2.49	8.01
389		n.d.	8.07
393	-12.5	n.d.	7.65
412		1.19	6.42
433		1.57	7.06
442		2.46	8.34
456		2.90	n.d.
471		3.00	7.65
485		2.54	8.10
507	-12.8	1.85	n.d.
534		2.27	8.25
557		2.49	8.70
570		2.65	7.80
590	-12.7	2.52	6.65
613		2.39	9.20
626		1.92	8.42
644		1.51	9.10
667		1.85	9.08
686		0.96	9.58
712		0.61	9.23
743		1.25	11.29
774		1.04	8.38
798	-11.5	1.69	9.11
819		1.97	9.46
843		2.56	8.42

n.d. = not detected

Methanogenesis in Submarine Permafrost

Coastal erosion and sea level rise created the shallow shelf of the Laptev Sea whose bottom is formed by the formerly terrestrial permafrost (Rachold et al. 2007). Flooding of the cold (-5 to -15°C) terrestrial permafrost with relatively warm (-0.5 to -2°C) saline sea water changed the system profoundly and resulted in a warming of the permafrost. Therefore, we consider submarine permafrost as a natural laboratory for studying the impact of environmental changes on permafrost habitats.

First results obtained from submarine permafrost deposits of the Laptev Sea shelf revealed methane concentrations of up to 284 nmol CH_4 g^{-1} sediment (Fig. 3a). Highest methane values were found in the layers with the highest amount of organic carbon (up to 9%). Extremely low $\delta^{13}CH_4$ values of -75 ‰ indicated active methanogenesis in this zone (Knoblauch, pers. com.). According to the studies of Rivkina & Gilichinsky (1996), who did not find any significant amounts of methane in Late Pleistocene permafrost sediments, it can be concluded that our findings in submarine permafrost are also a result of recent methanogenesis. This interpretation is supported by first data of DNA-based analyses of methanogenic communities in the sediments, which revealed a higher diversity and abundance of methanogens within the core segment with the highest amount of methane (Fig. 3b).

Conclusions

This work shows, for the first time, that methanogenic archaea do not only survive in permafrost habitats, but also can be metabolicly active under in situ conditions. Due to the sub-0°C experiments and the in situ temperatures of permafrost sediments, we can conclude that the methanogenic community is dominated by psychrotolerant or even psychrophilic microorganisms. Despite this adaptation to cold environments, we show that a slight increase of the temperature can lead to a substantial increase of methanogenic activity. In the event of degradation of terrestrial or submarine permafrost sediments, this would lead to an extensive expansion of the methane deposits with subsequent impacts on total methane emissions. A future in-depth characterization of the metabolism of these cold-adapted methanogens will reveal biotic and abiotic factors which influence the methane production activity of these organisms.

Methane of microbial origin in perennially frozen deposits probably represents an unconsidered source for the global methane budget. Methane release to the atmosphere from frozen ground is mediated by ongoing permafrost degradation through enhanced thermokarst formation and accelerated coastal erosion in the Arctic. Although the change in permafrost conditions by global warming is examined in the framework of several international projects (e.g., ACD: Arctic Coastal Dynamics, CALM: Circumpolar Active Layer Monitoring), these investigations should be linked more closely with microbiological process studies and biodiversity research. Microbial parameters important

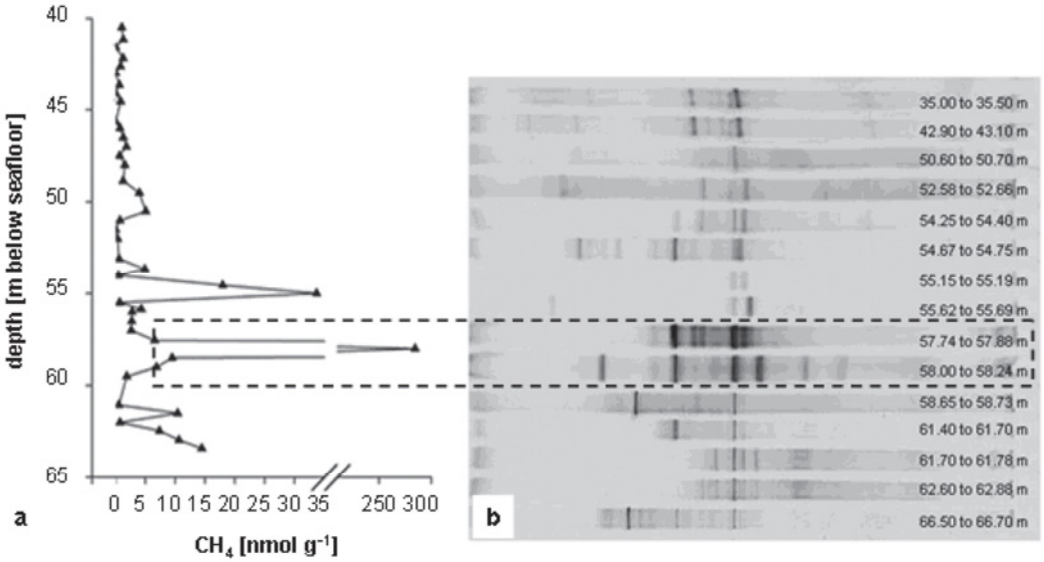

Figure 3. Vertical profiles of methane concentration (a), and DGGE fingerprinting of 16S rRNA genes (b) amplified from the submarine permafrost sediments (between 35.0 and 66.7 m depth).

for the assessment of the carbon turnover (e.g., cell numbers, activities, biodiversity and stability of microbial communities) should be analysed at observation areas in the Arctic, where long-term ongoing monitoring programs are undertaken. The evaluation of microbiological data and their correlation with climatic and geochemical results represents the basis for the understanding of the role of permafrost in the global system, in particular feedback mechanisms related to material fluxes and greenhouse gas emissions in the scope of a warming Earth.

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Erklärung

Ich versichere, dass ich meine Dissertation selbstständig, ohne unerlaubte Hilfe angefertigt und mich dabei keiner anderen als der von mir ausdrücklichen bezeichneten Quellen und Hilfen bedient habe. Die Dissertation wurde in der jetzigen Form noch bei keiner anderen Hochschule eingereicht und hat noch keinen sonstigen Prüfungszwecken gedient.

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