Alfred Wegener Institut für Polar- und Meeresforschung,
Forschungsstelle Potsdam
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Microbial communities and their response to Pleistocene and Holocene climate variabilities in the Russian Arctic

Kumulative Dissertation
zur Erlangung des akademischen Grades
"doctor rerum naturalium"
(Dr. rer. nat.)

eingereicht an der
Mathematisch-Naturwissenschaftlichen Fakultät
der Universität Potsdam

von

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Potsdam, Mai 2013

Published online at the
Institutional Repository of the University of Potsdam:
URL http://opus.kobv.de/ubp/volltexte/2013/6889/
URN urn:nbn:de:kobv:517-opus-68895
http://nbn-resolving.de/urn:nbn:de:kobv:517-opus-68895

IT'S A MAGICAL WORLD, HOBBES OL' BUDDY …
LET'S GO EXPLORING.

Bill Watterson

This work is based on a joint project of the Alfred Wegener Institute for Polar and Marine Research, Research Unit Potsdam (AWI) and the Helmholtz Centre Potsdam, GFZ German Research Centre for Geosciences. The associated PhD thesis was supervised by Prof. Dr. Dirk Wagner (Geomicrobiology; AWI/GFZ) and Dr. Kai Mangelsdorf (Organic Geochemistry, GFZ) and laboratory work was conducted in both institutes.

This work was funded as a part of the Priority Program: International Continental Scientific Drilling Program (ICDP); SPP 1006 and grants of the Deutsche Forschungsgemeinschaft (DFG) were given to Kai Mangelsdorf and Dirk Wagner (MA 2470/2, WA 1554/10-1).

Travel grants for Juliane Bischoff were gratefully received from Potsdam University Graduate School (POGS), Deutsche Gesellschaft fuer Polarforschung (DGP), Vereinigung fuer allgemeine und angewandte Mikrobiologie (VAAM) and Helmholtz Graduate School for Polar and Marine Research (POLMAR), which enabled the attendance of various national and international conferences.

Abstract

The Arctic is considered as a focal region in the ongoing climate change debate. The currently observed and predicted climate warming is particularly pronounced in the high northern latitudes. Rising temperatures in the Arctic cause progressive deepening and duration of permafrost thawing during the arctic summer, creating an 'active layer' with high bioavailability of nutrients and labile carbon for microbial consumption. The microbial mineralization of permafrost carbon creates large amounts of greenhouse gases, including carbon dioxide and methane, which can be released to the atmosphere, creating a positive feedback to global warming. However, to date, the microbial communities that drive the overall carbon cycle and specifically methane production in the Arctic are poorly constrained. To assess how these microbial communities will respond to the predicted climate changes, such as an increase in atmospheric and soil temperatures causing increased bioavailability of organic carbon, it is necessary to investigate the current status of this environment, but also how these microbial communities reacted to climate changes in the past.

This PhD thesis investigated three records from two different study sites in the Russian Arctic, including permafrost, lake shore and lake deposits from Siberia and Chukotka. A combined stratigraphic approach of microbial and molecular organic geochemical techniques were used to identify and quantify characteristic microbial gene and lipid biomarkers. Based on this data it was possible to characterize and identify the climate response of microbial communities involved in past carbon cycling during the Middle Pleistocene and the Late Pleistocene to Holocene. It is shown that previous warmer periods were associated with an expansion of bacterial and archaeal communities throughout the Russian Arctic, similar to present day conditions. Different from this situation, past glacial and stadial periods experienced a substantial decrease in the abundance of *Bacteria* and *Archaea*. This trend can also be confirmed for the community of methanogenic archaea that were highly abundant and diverse during warm and particularly wet conditions. For the terrestrial permafrost, a direct effect of the temperature

on the microbial communities is likely. In contrast, it is suggested that the temperature rise in scope of the glacial-interglacial climate variations led to an increase of the primary production in the Arctic lake setting, as can be seen in the corresponding biogenic silica distribution. The availability of this algae-derived carbon is suggested to be a driver for the observed pattern in the microbial abundance.

This work demonstrates the effect of climate changes on the community composition of methanogenic archae. *Methanosarcina*-related species were abundant throughout the Russian Arctic and were able to adapt to changing environmental conditions. In contrast, members of *Methanocellales* and *Methanomicrobiales* were not able to adapt to past climate changes.

This PhD thesis provides first evidence that past climatic warming led to an increased abundance of microbial communities in the Arctic, closely linked to the cycling of carbon and methane production. With the predicted climate warming, it may, therefore, be anticipated that extensive amounts of microbial communities will develop. Increasing temperatures in the Arctic will affect the temperature sensitive parts of the current microbiological communities, possibly leading to a suppression of cold adapted species and the prevalence of methanogenic archaea that tolerate or adapt to increasing temperatures. These changes in the composition of methanogenic archaea will likely increase the methane production potential of high latitude terrestrial regions, changing the Arctic from a carbon sink to a source.

Zusammenfassung

Die Arktis ist in den gegenwärtigen Diskussionen zum Klimawandel von besonderem Interesse. Die derzeitig beobachtete globale Erwärmung ist in den hohen nördlichen Breiten besonders ausgeprägt. Dies führt dazu, dass ehemals gefrorene Böden zunehmend tiefer auftauen und daher im Boden enthaltene Kohlenstoffquellen für die mikrobielle Umsetzung und Mineralisierung zur Verfügung stehen. Aufgrund dieser Prozesse entstehen klimarelevant Gase, darunter Kohlendioxid und Methan, die aus den Böden und Sedimenten freigesetzt werden können. Wenn man bedenkt, dass in den nördlichen Permafrostgebieten die Hälfte des weltweit unter der Bodenoberfläche gelagerten Kohlenstoffs gelagert ist, wird die Bedeutung dieser Region für das Verständnis des globalen Kohlenstoffkreislaufes und der möglichen Treibhaus-gasemissionen sichtbar. Trotz dieser Relevanz, sind die am Kohlenstoffumsatz beteiligten Mikroorganismen in der Arktis wenig untersucht und ihre Anpassungsfähigkeit an die gegenwärtigen Klimaveränderungen unbekannt. Die vorliegende Arbeit untersucht daher, wie sich Klimaveränderungen in der Vergangenheit auf die Anzahl und Zusammensetzung der mikrobiellen Gemeinschaften ausgewirkt haben. Dabei liegt ein besonderer Fokus auf die methanbildenden Archaeen, um das Verständnis der mikrobiellen Methandynamik zu vertiefen.

Im Rahmen dieser Arbeit wurden drei Bohrkerne aus zwei verschiedenen Standorten in der russischen Arktis untersucht, darunter terrestrischer Permafrost und Seesedimente aus Sibirien und Chukotka, Russland. Mittels der Identifikation und Quantifizierung von mikrobiellen Genen und charakteristischen Bestandteilen der mikrobiellen Zellmembran war es möglich, fossile mikrobielle Gemeinschaften in Seesedimenten mit einem Alter von bis zu 480 000 Jahren und in Permafrostablagerungen mit einem Alter von bis zu 42 000 Jahren zu rekonstruieren. Es wurde gezeigt, dass es während vergangener warmen Perioden zu einem Wachstum von Bakterien und Archaeen in allen untersuchten Standorten gekommen ist. Dieser Trend konnte auch für die Gemeinschaft der methanogenen Archaeen gezeigt

werden, die während warmen und insbesondere feuchten Klimabedingungen in großer Anzahl und Diversität vorhanden waren, was wiederrum Rückschlüsse auf mögliche Methanemissionen erlaubt. In den terrestrischen Permafroststandorten wird der Temperaturanstieg als direkte Ursache für die gefundene Reaktion der mikrobiellen Gemeinschaft vermutet. Im Gegenzug dazu, führte der Temperaturanstieg im untersuchten arktischen See wahrscheinlich zu einer erhöhten Primärproduktion von organischem Kohlenstoff, die wiederum das Wachstum der Mikroorganismen antrieb. Weiterhin konnte im Rahmen dieser Arbeit gezeigt werden, dass *Methanosarcina*-verwandte Spezies in der Russischen Arktis weit verbreitet sind und sich an veränderte Umweltbedingungen gut anpassen können. Im Gegensatz dazu stehen Vertreter von *Methanocellales* und *Methano-microbiales,* die nicht in der Lage sich an veränderte Lebensbedingungen anzupassen.

Im Rahmen dieser Arbeit konnte erstmalig gezeigt werden, dass es in früheren Warmphasen zu einem vermehrten Wachstum der an der Umsetzung des organischen Kohlenstoffs beteiligten Mikroorganismen in der Russischen Arktis gekommen ist. Im Zusammenhang mit der zukünftigen Erwärmung der Arktis kann also von einer Veränderung der am Kohlenstoffkreislauf beteiligten Mikroorganismen ausgegangen werden kann. Mit den steigenden Temperaturen werden sich einige Vertreter der methanproduzierenden Mikroorganismen an die veränderten Bedingungen anpassen können, während Temperatur-empfindliche Vertreter aus dem Habitat verdrängt werden. Diese Veränderungen in der mikrobiellen Gemeinschaft können die Methanproduktion der hohen noerdlichen Breiten erhoehen und dazu beitragen, dass aus der Arktis als eine Kohlenstoffsenke eine Kohlenstoffquelle wird.

1. Introduction

1.1. Motivation

We only have the present and the past to look at, to predict the future…

The Arctic is changing... Recent studies suggest that the Arctic is particularly vulnerable to the currently observed climate change and warms at around twice the rate as the rest of the planet. This results in an advancing thawing and destabilization of previously frozen ground, having an impact on infrastructures, ecosystems and elemental cycles.

Previously frozen and thus protected reservoirs of soil stored carbon become available for microorganisms. Bearing in mind that the grounds of the Northern High latitude contain twice as much carbon as currently estimated for the atmosphere, the importance of the Arctic for the carbon cycling and global carbon budgets becomes obvious.

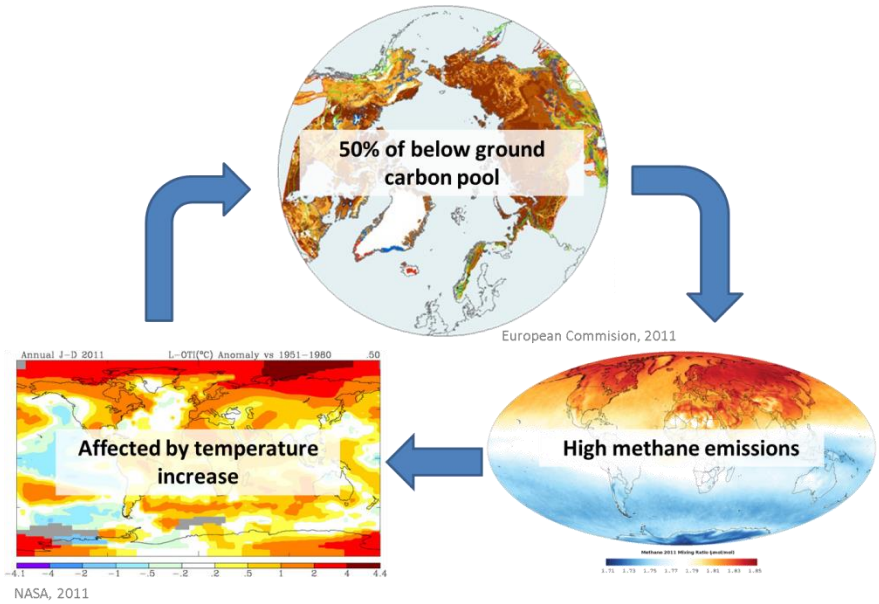

Figure 1: *Arctic environments are characterized by a high availability of carbon, high methane emissions and in particular affected by the on-going temperature changes.*

The microbial consumption and activity of these carbon pools lead to considerable amounts of climate relevant greenhouse gas emissions that can have a significant impact on the temperature regime of the Earth. However, challenges exist to predict how these environmental changes will impact the microorganisms and thus the production and emission of methane and carbon dioxide in Arctic environments. One opportunity to solve this question is to go back in time and investigate how the microbial communities that are involved in the cycling of carbon reacted to climate changes in the past. Even though the rate of the warming trend that is currently observed is exceptional, the Earth's climate was previously affected by distinct cold and warm periods, known as glacial-interglacial climate cycles.

Within this work, I investigated how carbon cycling and particularly, methane producing microorganisms reacted to environmental changes in the Arctic in the last 400 000 years by investigating traces of these communities in permafrost deposits and lake sediments in two different locations within the Russian Arctic.

1.2. **Scientific Background**

1.2.1. Permafrost in arctic environments

Permafrost is defined as ground (soil or rock and included ice and organic material) that remains below 0°C for at least two consecutive years (van Everdingen, 1998 revised May 2005). 24% of the exposed land surface in Northern Hemisphere (around 23 Mio km^2) is underlain by permafrost (Zhang, *et al.*, 2003), with permafrost mostly occurring between 60°N and 68°N, but also down to around 35°N in the Qinghai-Xizang (Tibet) Plateau (Zhang, *et al.*, 1999). Permafrost can be classified as isolated, sporadic, discontinuous and continuous according to its spatial distribution (e.g. Zhang, 2005). The study sites that were investigated in scope of this PhD thesis are classified as continuous permafrost, which means that over 90% of the area is frozen (Brown, *et al.*, 1997). Permafrost can reach thicknesses of several

hundred metres with the deepest permafrost layers reported for Siberia up to 1600 m (Romanovskii & Hubberten, 2001).

Figure 2: *Distribution of permafrost in the Northern Hemisphere*

The uppermost layers of permafrost are affected by changing temperatures throughout the yearly seasonal cycle, for example surface temperatures ranging from -30°C to + 15°C for a site in Siberia (Boike*, et al.*, 2012). This seasonally thawed and frozen layer, the so called 'active layer' can reach thicknesses of a few centimetres to metres (van Everdingen, 1998 revised May 2005). The underlying layers of permafrost remain continuously frozen, as indicated in Fig. 3. The temperature of the permafrost deposits differs throughout the Northern Hemisphere and ranges from -1 to -15°C, with the lowest temperatures only found in the Canadian Archipelago and northern Russia (Romanovsky*, et al.*, 2010). The temperature of the permafrost deposits is affected by the glaciation history, the large scale surface energy balance, the thermal properties of surface and subsurface and the water bodies (Yershov & Williams, 2004, Boike*, et al.*, 2012). Below the permafrost

base the temperatures rise to above 0°C due to the geothermal heat of the inner Earth (Yershov & Williams, 2004).

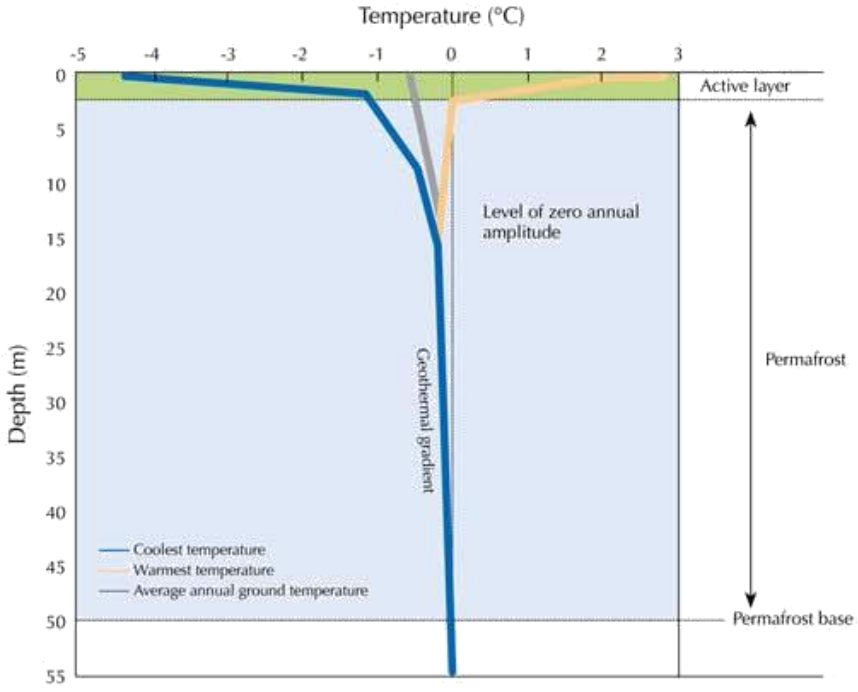

Figure 3: *Minimum, maximum and average temperature in permafrost with depth. (modified after Miguel Ángel de Pablo).*

Long-term measurements of permafrost temperatures (in scope of the International Polar Year (IPY) 2007-2009) of 575 boreholes in the circumpolar northern hemisphere demonstrated that mean annual ground temperatures are up to 2°C warmer than they were 20-30 years ago (Romanovsky, *et al.*, 2010). Furthermore an increase of the seasonally thawed layer throughout was observed. For Russia, a deepening of the active layer by about 20 cm in the period from 1956 to 1990 was reported (IPCC, 2007). These changes in the active layer depth are driven by several factors, including surface temperature, physical and thermal properties of the surface cover and substrate, vegetation, soil moisture and duration and thickness of snow cover (Brown, *et al.*, 2000, Frauenfeld, *et al.*, 2004, Zhang, 2005).

1.2.2. Carbon storage and emission in arctic environments

According to latest estimations, 1672 Pg (10^{15} g) carbon are stored in soil and deposits of northern permafrost regions, accounting to 50% of the globally belowground stored carbon (Tarnocai, et al., 2009). These large quantities of belowground stored organic carbon are explained by accumulation and reduced decomposition rates of organic matter due to cold temperatures and waterlogging (Davidson & Janssens, 2006).

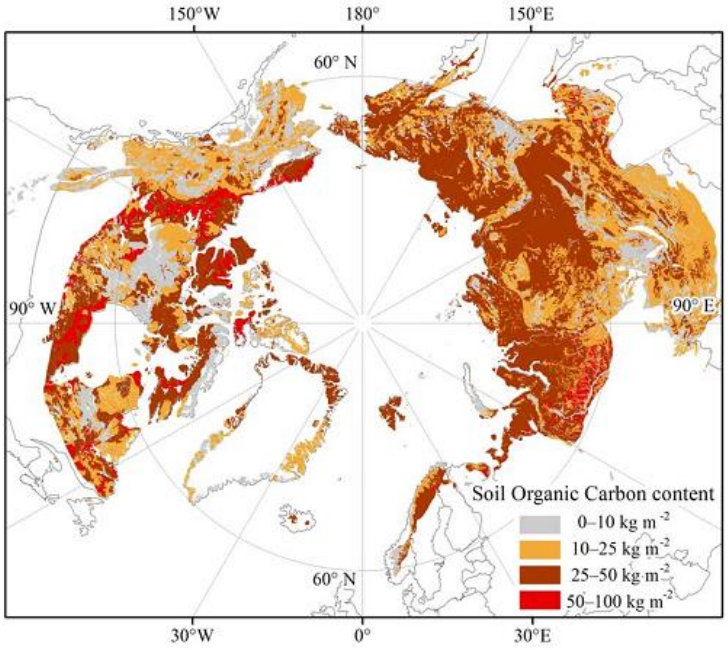

Figure 4: *Distribution of soil organic carbon in the circumpolar region in up to 1 m depth (map after Hugelius et al., 2012)*

Soil stored carbon in the high northern latitudes is particularly sensitive to changes in long-term climate (Schuur, *et al.*, 2008). Thawing of permafrost deposits is generally linked to a destabilization of stored carbon by top-down thawing at the active-layer/permafrost interface and by hydrologic changes (Striegl, *et al.*, 2005). Harden, *et al.* (2012) estimate that 61 to 399 Pg of permafrost stored carbon will thaw and is thus available for microbial consumption by the end of this century. Modelling further suggests that the Arctic permafrost will change from a carbon sink to a carbon source by 2020 (Schaefer, *et al.*, 2011) by emitting considerable amounts of the climate relevant gases, such as carbon dioxide and methane (Dutta, *et al.*, 2006).

Methane is a significant trace gas that contributes with ~20% to the total greenhouse gases effecting global warming (IPCC, 2007). Northern high latitude ecosystems are a major source for methane, accounting to 32 to 106 Tg methane per year (McGuire, *et al.*, 2009). The combination of methane production and consumption results in a net methane emission of 16 to 65 Tg methane per year for this environment (McGuire, *et al.*, 2009). Additionally, lakes were identified as sources for methane, contributing to 6-16% (8-48 Tg CH_4 a^{-1}) of the total methane emission (Bastviken, *et al.*, 2004). In particular, arctic thaw lakes emit massive amounts of methane and contribute significantly to the methane emission of arctic wetlands with 3.8 Tg CH_4 a^{-1} (Walter, *et al.*, 2006). However, more research in needed to constrain the sensitivity of the Arctic carbon cycle to climate changes (McGuire, *et al.*, 2009).

1.2.3. Methane cycling in arctic environments

Methane production is driven by methanogenic archaea, a strictly anaerobic group belonging to the kingdom *Euryarchaeota* (Hedderich, 2006). Methanogenesis is possible due to a syntrophic association between anaerobic bacteria, acetogenic bacteria and methanogenic archaea (Thauer, *et al.*, 2008). Methane is a major product of the anaerobic biomass degradation. Complex organic matter (biopolymers) is degraded to monomers and afterwards fermented to acetic acid, carbon dioxide and hydrogen. Methanogens can convert these products to methane using three different biochemical reaction pathways: CO_2-reductive, acetoclastic and methylotrophic methanogenesis.

Methane production in **terrestrial** permafrost deposits occurs mainly in the seasonally thawed active layer, which is characterized by many biogeochemical processes related to the carbon cycling such as microbial activity, but also the release of carbon in the gas, liquid and particulate form (Boike, *et al.*, 2012). The active layer of permafrost contains the highest cell counts (up to 3 x 10^8 cells g^{-1}) of methanogenic archaea (Kobabe, *et al.*, 2004) with a high diversity of species belonging to the families

Methanomicrobiaceae, *Methanosarcinaceae*, and *Methanosaetaceae* (Ganzert, *et al.*, 2007). Further studies show that the production of methane can occur also in permanently frozen deposits with temperatures of -6°C and lower (Rivkina, *et al.*, 2004, Wagner, *et al.*, 2007, Bischoff, *et al.*, 2013) as methanogenic archaea isolated from permafrost environments seem to be able to withstand these extreme environmental conditions (Morozova & Wagner, 2007, Wagner, *et al.*, 2013). Thus, methanogenic archaea are also expected to be viable and active in deeper, permanently frozen layers.

Table 1: *Methanogenic pathways, free reaction energy and representative methanogenic genera*

Pathway	DG°' [kJ/mol CH_4]		
CO_2 reductive pathway:			
$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O$	-	130.4	*Methanobacterium, Methanococcus*
$4HCOO^- \rightarrow CH_4 + 3CO_2 + 2H_2O$	-	119.5	*Methanobrevibacter, Methanospirillum*
Acetoclastic pathway:			
$CH_3COO^+ + H^+ \rightarrow CH_4 + CO_2$	-	36	*Methanosarcina, Methanosaeta*
Methylotrophic pathway:			
$4CH_3OH \rightarrow 3CH_4 + CO_2 + 2H_2O$	-	103	*Methanosarcina, Methanococcoides, Methanolobus, Methanosphaera*

The methane produced is released to the atmosphere by ebullition, plant-mediated transport via aerenchyma or diffusion through the active layer (Wagner et al. in Margesin, 2009). When the methane diffuses through near-surface aerobic layers, it can be oxidized by aerobic methanotrophic proteobacteria (Hanson & Hanson, 1996). The potential methane oxidation

rates in the northern hemisphere range from 0 – 15 Tg CH_4 per year (McGuire, *et al.*, 2009), showing the importance of aerobic methane oxidation as terrestrial sink for methane.

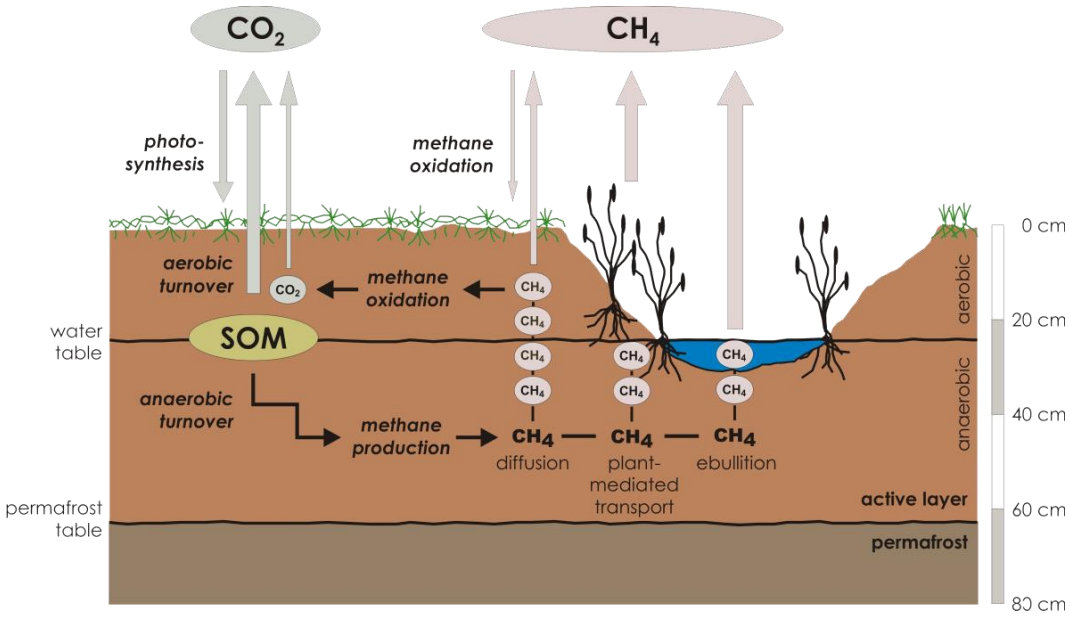

Figure 5: *Microbial driven methane-cycling in the Siberian tundra (modified after Wagner in Margesin (2009)).*

Methanogenesis also occurs in anoxic sediment layers of lakes and is considered as the most important terminal step in the degradation of OM in lake sediments (Glissmann, *et al.*, 2004). The methane produced can be released by ebullition, plant mediated emission and diffusion processes to the atmosphere. Methane oxidation can occur in the oxic water layers or along oxic sediment layers. Several studies characterised methanogenic archaea in lake sediments of temperate (Zepp Falz, *et al.*, 1999, Nusslein, *et al.*, 2001, Nusslein, *et al.*, 2003) and tropic (Conrad, *et al.*, 2010) environments. Studies on polar settings are limited to Antarctic sites, including Lake Fryxell (Karr, *et al.*, 2006) and Ace Lake (Coolen, *et al.*, 2004), where novel psychrophilic methanogenic species such as *Methanococcoides burtonii* and *Methanogenium frigidum* were isolated (Franzmann, *et al.*, 1992, Franzmann, *et al.*, 1997). However, arctic lakes are thought to be affected by the on-going climate changes as it was demonstrated that small increases in the water temperature have a high influence on the ice coverage and water

mixing (Vincent & Laybourn-Parry, 2008). This can have a further impact on several important environmental parameters such as bio productivity, water mass stratification and gas exchange of greenhouse gases and therefore, cause subsequent climate feedback processes on larger time and spatial scale ('Polar Amplification'; ACIA, 2005).

1.3. **Study Sites**

The studies conducted in scope of this thesis included two investigation sites in the Russian Arctic: Kurungnakh Island, Siberia and Lake El'gygytgyn, Chukotka (Fig. 6). Both sites are located in the zone of continuous permafrost; however, they are characterized by significant differences in water, carbon and nutrient availability, which will be explained in more detail in the respective chapters.

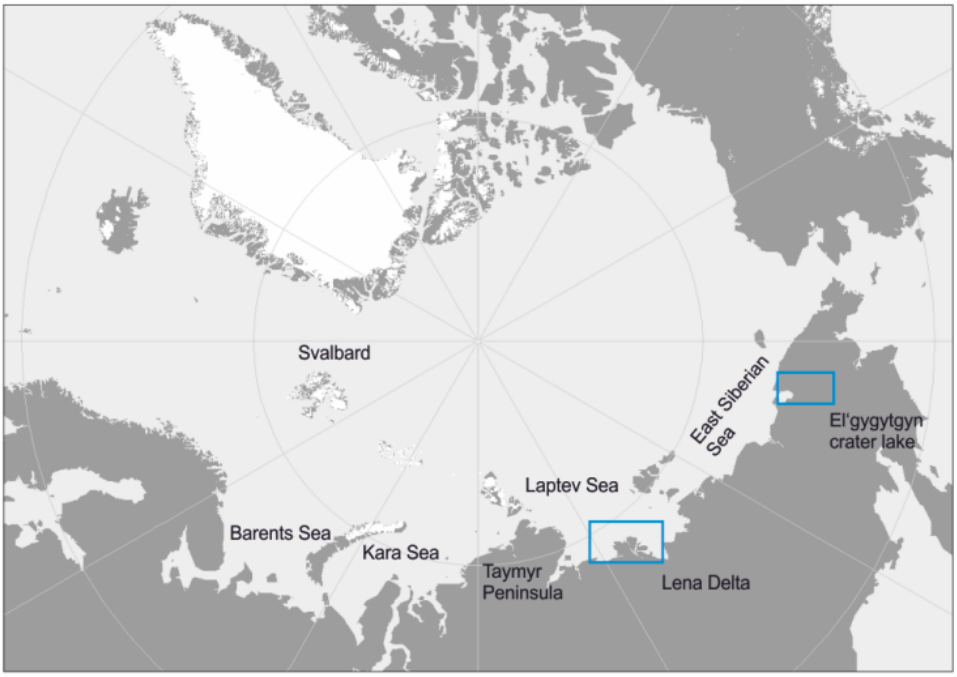

Figure 6: *Overview of study sites investigated in this thesis. The blue boxes indicate the study sites Kurungnakh Island, Lena Delta and Lake El'gygytgyn, Chukotka.*

1.3.1. Lena-Delta, Siberia

The Lena River Delta is the largest Arctic delta with an area of about 29 x 10^3 km^2 (Schneider, *et al.*, 2009). The Lena River, which feeds the delta is largest river in East Siberian region, with a discharge of 16,000 m^3/s (Dai and Trenberth, 2002). Kurungnakh Island is located in the southern part of the Lena River Delta close to the Olenyekskaya Channel and partly exposed up to 40 m above the river level.

Kurungnakh Island is composed of Late Quaternary sediments belonging to the oldest Lena River terrace and was formed during the Late Pleistocene. This so–called 'Third Terrace' is characterized by a lowest formation of sandy deposits and a huge 'Ice complex' formation about 20 m thickness with ice-rich, silty deposits and peat block inclusions of less decomposed material (Yedoma suite, Schwamborn, *et al.*, 2002). Holocene layers on top of the ice complex are about 2-3 m thick Ice wedges 3-5 m in width are widely distributed (Schirrmeister, *et al.*, 2003). This site is characterized by ice-rich and carbon rich permafrost deposits (Bischoff, *et al.*, 2013). During the Late Pleistocene and Holocene, Kurungnakh Island has undergone several climatic stages: cold and dry (Early Weichselian Stadial, Zyryan), warm and wet (Middle Weichselian Interstadial, Kargin), cold and dry (Late Weichselian Stadial, Sartan), warm and dry (Early Holocene) and warm and wet (Middle and Late Holocene), as reconstructed by Wetterich, *et al.* (2008) for this site.

The terrestrial permafrost deposits were obtained in the depression of a low-centered ice-wedge polygon during a Russian-German expedition (LENA 2002) in July 2002.

1.3.2. El'gygytgyn Crater Lake, Chukotka

The El'gygtgyn crater is located in central Chukotka, northeast Russia. A meteorite impact 3.6 million years ago (Layer, 2000) caused an impact crater of about 18 km in diameter that is partially filled with an off-centred lake of 12 km in diameter. The crater lake is one of the best conserved impact structures on Earth and represents a unique climate archive in the high

latitude due to its age, size and continuous sedimentation history (Glushkova, 2001, Melles*, et al.*, 2012).

The 'Lake El'gygytgyn Scientific Drilling Project' in 2008/2009 (Melles*, et al.*, 2011) was part of the International Continental Scientific Drilling Programme (ICDP). Two different sites were drilled:

(1) The drilling of the permafrost deposits from the western catchment of the lake was conducted between October and December 2008 (Melles*, et al.*, 2011, Schwamborn*, et al.*, 2012). A permafrost core (ICDP site 5011-3) of in total 141.5 m length was recovered. The work conducted in scope of this PhD thesis focuses on the uppermost 19 m of this core, representing deposits of marine isotope stages 5.5 to Holocene (Andreev*, et al.*, 2012).

(2) The drilling of lake sediments was conducted between January and May 2009 and sediments of Late Pliocene and Quaternary age were recovered (Melles*, et al.*, 2011, 2012). Three parallel, partly overlapping cores (ICDP site 5011-1A, B, C) of 143.7 m, 108.4 m and 517.3 m length were drilled, using the frozen lake ice cover as a surface for the drilling platform (Melles*, et al.*, 2011). The work conducted in scope of this thesis focusses on Middle to Late Pleistocene deposits of the marine isotope stages (MIS) 12 to 9, representing deposits of 480 to 300 ka BP (Melles*, et al.*, 2012).

1.4. **Objectives and approach**

The overall aim of this work is to advance our understanding of microbial carbon cycling and methane dynamics in the Russian Arctic during glacial-interglacial climate changes. Therefore, three records comprising Middle Pleistocene and Late Pleistocene to Holocene deposits from two different study sites in the Russian Arctic, including permafrost, lake shore and non-lake deposits from Siberia and Chukotka were investigated.

This PhD thesis aims to answer the following questions:

- Which microorganisms were/are involved methane cycling in past and present?
- How does the abundance and composition of microbial and in particular methanogenic communities differ in various permafrost environments?
- How did the microbial communities react to climate changes in the past?

In order to answer these questions, a combined stratigraphic approach of microbial and molecular organic geochemical techniques (Figure 7) was choosen. More detailed information on the respective studies and the applied methods are given in the respective manuscripts.

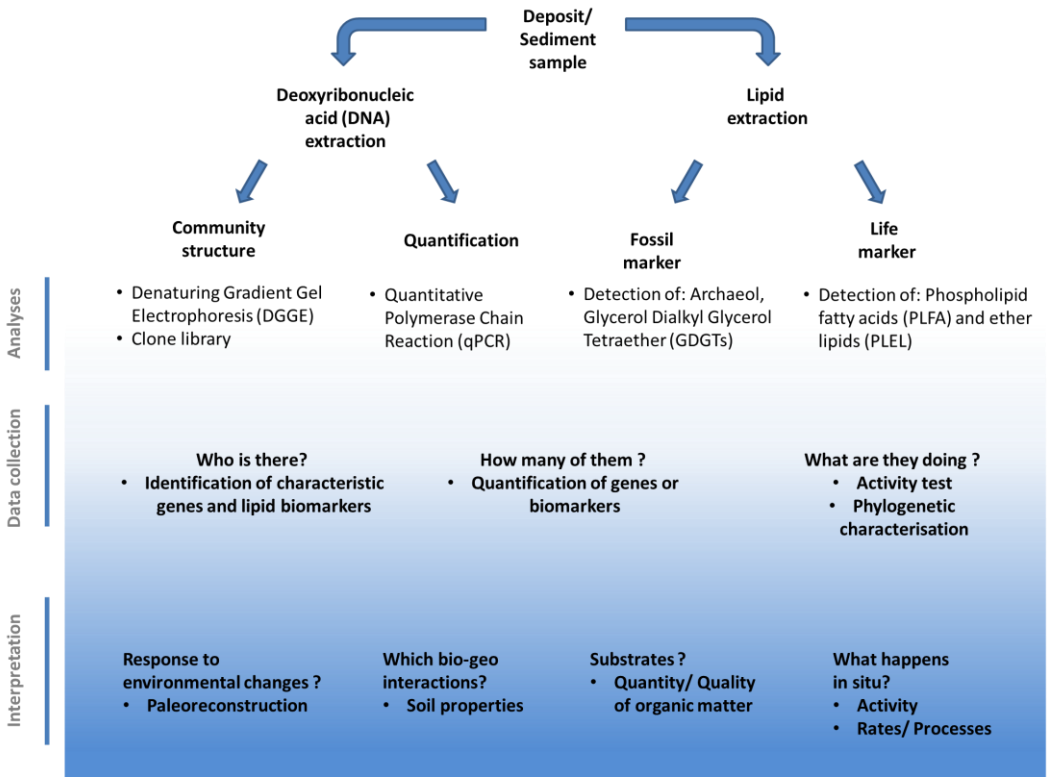

Figure 7 *Compact overview of the methodological approach used within this thesis for the characterization of microbial communities in permafrost deposits and lake sediments of the Russian Arctic.*

1.5. **Thesis organization**

This cumulative dissertations consist of an introductory chapter (*Chapter 1*), providing the scientific background, the approach and the aims and objectives of the overall thesis. The obtained results are presented in three manuscripts that are in the process to be published as original research articles in international peer-reviewed journals. Table 2 gives an overview of the respective publications and their current publication status.

Table 2: *Overview of the publications presented within this thesis. (Manuscript status refers to the date of submission of the overall thesis)*

Manuscript	Publication	Manuscript status
I	**Bischoff, J.**, Mangelsdorf, K., Gattinger, A., Schloter, M., Kurchatova, A. N., Herzschuh, U. and Wagner, D.: *Response of methanogenic archaea to Late Pleistocene and Holocene climate changes in the Siberian Arctic*	*Global Biogeochemical Cycles*, published online. doi: 10.1029/2011GB004238.
II	**Bischoff, J.**, Mangelsdorf, K., Schwamborn, G. and Wagner, D.: *Response of microbial communities to landscape and climatic changes in a terrestrial permafrost sequence of the El'gygytgyn crater, Far East Russian Arctic*	Final draft.
III	**Bischoff, J.**, Mangelsdorf, K., Goersch, J., Rosen, P., Lam, P., Wennrich V. and Wagner, D.: *Glacial-interglacial microbial community dynamics in Middle Pleistocene sediments in the Lake El'gygytgyn, Far East Russian Arctic*	*The ISME Journal*, under review.

1.6. Summary of the included manuscripts and contribution of the co-authors

1.6.1. Response of methanogenic archaea to Late Pleistocene and Holocene climate changes in the Siberian Arctic

***Juliane Bischoff**, Kai Mangelsdorf, Andreas Gattinger, Michael Schloter, Anna N. Kurchatova, Ulrike Herzschuh and Dirk Wagner*

In order to investigate the link between the methane dynamics in permafrost deposits and climate changes in the past, we studied the abundance, composition and methane production of methanogenic communities in Late Pleistocene and Holocene sediments of the Siberian Arctic. We detected intervals of increased methane concentrations in Late Pleistocene and Holocene deposits along a 42 ka old permafrost sequence from Kurungnakh Island in the Lena Delta (northeast Siberia). Increased amounts of archaeal life markers (intact phospholipid ethers) and a high variety in genetic fingerprints detected by 16S ribosomal ribonucleic acid (rRNA) gene analyses of methanogenic archaea suggest presently living and presumably active methanogenic archaea in distinct layers predominantly in Holocene deposits but also in deep frozen ground at 17 m depth. Potential methanogenic activity was confirmed by incubation experiments. By comparing methane concentrations, microbial incubation experiments, gene analysis of methanogens and microbial life markers (intact phospholipid esters and ethers) to already partly degraded membrane lipids such as archaeol and isoprenoid glycerol dialkyl glycerol tetraethers (GDGT), we demonstrated that archaeol likely represents a signal of past methanogenic archaea. The archaeol signal was used to reconstruct the response of methanogenic communities to past temperature changes in the Siberian Arctic and the data suggest higher methane emissions occurred during warm periods, particularly during an interval in the Late Pleistocene and the Holocene. This new data on present and past methanogenic communities in the Siberian terrestrial permafrost imply that these microorganisms will

respond to the predicted future temperature rise in the Arctic with increasing methane production as demonstrated in previous warmer periods.

Co-Authors' contribution: Dirk Wagner and Kai Mangelsdorf contributed with expertise in their respective fields and gave valuable input throughout the writing of the manuscripts. Dirk Wagner and Anna N. Kurchatova conducted the field work on Kurungnakh Island, Russia. Andreas Gattinger and Michael Schloter provided data on phospholipid fatty acid (PLFA) and ether lipid (PLEL) analyses. Ulrike Herzschuh contributed with her expertise on environmental and climate paleoreconstructions. Furthermore, remarks and comments of two anonymous reviewers were considered, improving the language and structure of the manuscript.

1.6.2. Response of microbial communities to landscape and climatic changes in a terrestrial permafrost sequence of the El'gygytgyn crater, Far East Russian Arctic

Juliane Bischoff*, Kai Mangelsdorf, Georg Schwamborn and Dirk Wagner*

Large areas of the Earth are affected by permanently or temporary low temperatures and furthermore affected by freeze-thaw cycles, low precipitation, low soil moisture and nutrient poor conditions. Extremophile microorganisms are able to withstand the harsh conditions and play an important role in the material- and energy cycles in these environments. However, the abundance and adaption of these extremophile microorganisms to environmental and climatic changes in terrestrial permafrost deposits in the Russian Arctic remain currently understudied. Here we present data on the distribution of bacterial and archaeal communities in deposits on the western shore of the Lake El'gygytgyn, Far East Russian Arctic. During a lake high stand, the investigation site was flooded and after the lake level dropped, ongoing from the Allrød, exposed to subaerial conditions. Using a combined approach of microbiological and lipid

biomarker analyses we can demonstrate a response of the microbial communities to these environmental changes. The composition of isoprenoid glyceroal dialkyl glycerol tetraether (GDGTs) and archaeol, as characteristic lipid markers for Archaea, reveal distinct patterns in the subaquatic when compared to subaerial deposits, indicating changes in the composition of the methanogenic communities. After the exposure of the investigation site to subaerial conditions, the abundance of Bacteria and Archaea increases strongly as was shown by the increasing 16S ribosomal ribonucleic acid (rRNA) gene copy numbers, GDGTs and phospholipid fatty acids (PLFAs). In particular the uppermost Holocene layers are characterised by comparably higher amounts of total organic carbon along with high microbial abundance. Our data shows the influence of the incorporation of organic material and beginning pedogenesis on the microbial communities, reinforcing the role of the uppermost 'active layer' as hotspots of carbon cycling in permafrost environments.

Co-Authors' contribution: Dirk Wagner and Kai Mangelsdorf contributed with expertise in their respective fields and gave valuable input throughout the writing of the manuscripts. Georg Schwamborn conducted the field work as a PI (Principle Investigator) of the terrestrial permafrost drilling in scope of the ICDP Project 'Scientific Drilling Lake El'gygytgyn'.

1.6.3. Glacial-interglacial microbial community dynamics in Middle Pleistocene sediments in the Lake El'gygytgyn, Far East Russian Arctic

***Juliane Bischoff**, Kai Mangelsdorf, Janine Goersch, Phyllis Lam, Peter Rosen, Volker Wennrich and Dirk Wagner*

Arctic lakes represent an important source for the greenhouse gas methane and are particularly sensitive to global warming. Microbial communities in arctic lakes, especially their changes in abundance and composition in

response to climate change are poorly understood. Here we show changing microbial abundances in response to glacial-interglacial variations in Middle Pleistocene sediments from Lake El'gygytgyn, NE Russian Arctic. A combined microbiological and organic geochemical approach revealed high abundances of bacterial and archaeal markers during interglacial intervals. This increase in microbial community size was most likely driven by a climatically induced increase in organic matter supply into the lake sediments. In particular, there was a substantial increase of algae-derived, autochthonous organic matter during interglacial periods. The increase in abundance of archaea is accompanied by a higher diversity of methanogens and concomitant with high methane production potentials, suggesting elevated methane production from the lake during the interglacials. Our findings demonstrate a previously unrecognized climate-driven impact on the microbial communities via changing organic matter supply and the significance of microorganisms for the carbon turnover in arctic lake sediments.

Co-Authors' contribution: Dirk Wagner and Kai Mangelsdorf contributed with expertise in their respective fields and gave valuable input throughout the writing of the manuscripts. Janine Görsch carried out the eco-molecular characterization of the methanogenic communities in the lake sediments as part of an associated Msc (Master of Sciences) project. Phyllis Lam contributed with her expertise on quantifying microbial genes using quantitative polymerase chain reaction (qPCR). Volker Wennrich supported the sampling of the lake sediment cores and Peter Rosen contributed with data on biogenic silica.

2. Response of methanogenic archaea to Late Pleistocene and Holocene climate changes in the Siberian Arctic

Juliane Bischoff, Kai Mangelsdorf, Andreas Gattinger, Michael Schloter, Anna N. Kurchatova, Ulrike Herzschuh and Dirk Wagner

Accepted for publication in *Global Biogeochemical Cycles,*
(doi: 10.1029/2011GB004238, published online 16 April 2013).

2.1. **Abstract**

In order to investigate the link between the methane dynamics in permafrost deposits and climate changes in the past, we studied the abundance, composition and methane production of methanogenic communities in Late Pleistocene and Holocene sediments of the Siberian Arctic. We detected intervals of increased methane concentrations in Late Pleistocene and Holocene deposits along a 42 ka old permafrost sequence from Kurungnakh Island in the Lena Delta (northeast Siberia). Increased amounts of archaeal life markers (intact phospholipid ethers) and a high variety in genetic fingerprints detected by 16S ribosomal ribonucleic acid (rRNA) gene

analyses of methanogenic archaea suggest presently living and presumably active methanogenic archaea in distinct layers predominantly in Holocene deposits but also in deep frozen ground at 17 m depth. Potential methanogenic activity was confirmed by incubation experiments. By comparing methane concentrations, microbial incubation experiments, gene analysis of methanogens and microbial life markers (intact phospholipid esters and ethers) to already partly degraded membrane lipids such as archaeol and isoprenoid glycerol dialkyl glycerol tetraethers (GDGT), we demonstrated that archaeol likely represents a signal of past methanogenic archaea. The archaeol signal was used to reconstruct the response of methanogenic communities to past temperature changes in the Siberian Arctic and the data suggest higher methane emissions occurred during warm periods, particularly during an interval in the Late Pleistocene and the Holocene. This new data on present and past methanogenic communities in the Siberian terrestrial permafrost imply that these microorganisms will respond to the predicted future temperature rise in the Arctic with increasing methane production as demonstrated in previous warmer periods.

2.2. **Introduction**

In recent years, the debate about climate change has increasingly focused on Arctic regions. The predicted temperature rise associated with climate change is especially pronounced for the polar regions of the Northern Hemisphere; both long-term measurements and future climate models indicate a significant increase in atmospheric and ground temperature for this particular environment. Permafrost, defined as ground remaining below 0 °C for two consecutive years (Washburn, 1980) and references therein, is widely distributed in the Arctic and covers about 25 % of the exposed land surface of the Northern Hemisphere (Zhang*, et al.*, 2008). Recently, a deepening of the seasonally thawed layer (active layer) and an increase in ground temperatures of the permafrost were reported for several sites in the Russian (Romanovsky*, et al.*, 2010) and North American Arctic (Smith*, et al.*,

2010). In general, increased permafrost thawing is assumed to be associated with enhanced carbon emission (Dutta*, et al.*, 2006) as carbon dioxide and methane, which are suspected to have a positive feedback on climate warming. Future modeling scenarios suggest that permafrost environments might change from a carbon sink to a carbon source (Oechel*, et al.*, 1993). Therefore, Arctic regions are of enormous importance for the global terrestrial carbon cycle (Zimov*, et al.*, 2006, Tarnocai*, et al.*, 2009).

Methane is a significant trace gas that contributes with ~20 % to the total greenhouse gases effecting on global warming (Denman, 2007). Presently, methane emission estimates from northern, high latitude, terrestrial environments range from 10 to 51 Tg a^{-1} (10^{12} g per year), which accounts for up to 8.6 % of the global, natural methane emissions (Bartlett & Harriss, 1993, Cao*, et al.*, 1998). With the thawing of permafrost, large amounts of carbon stored in permafrost successions might become available again for microbial degradation. Therefore, this carbon is a potential source of carbon dioxide and methane release to the atmosphere due to intensified microbial respiration of recent and older carbon pools.

Microbially mediated methane cycling includes processes of methane production (methanogenesis) and methane oxidation (methanotrophy); these processes are spatially separated. Methanogenesis is driven by methanogenic archaea (methanogens), a strictly anaerobic group belonging to the kingdom *Euryarchaeota* (Hedderich, 2006). Subsequently, methane is released to the atmosphere by ebullition, plant-mediated transport via aerenchyma, or diffusion through the active layer of the permafrost. When methane diffuses through near-surface aerobic layers, it can be oxidized by aerobic methanotrophic *Proteobacteria* (AMP) (Hanson & Hanson, 1996).

The first studies of Holocene permafrost deposits from the Lena Delta (Siberia) by Wagner*, et al.* (2007) revealed significant amounts of methane, which could be attributed to *in situ* activity of methanogenic archaea. The active layer of permafrost contains the highest cell counts (up to 3 x 10^8 cells g^{-1}) of methanogenic archaea (Kobabe*, et al.*, 2004) with a high diversity of species belonging to the families *Methanomicrobiaceae*, *Methanosarcinaceae,*

and *Methanosaetaceae* (Ganzert, *et al.*, 2007). A study on the Laptev Sea Shelf submarine permafrost (Koch, *et al.*, 2009) demonstrated that at depths where methane concentrations were at maximum the lowest carbon isotopic value for methane (up to −72.2 ‰ V-PDB) were measured. This indicated active methanogenesis in the frozen sediments. Samples with high methane concentrations were dominated by sequences affiliated with the methylotrophic genera *Methanosarcina* and *Methanococcoides*. Koch, *et al.* (2009) also demonstrated that intact deoxyribonucleic acid (DNA) was extractable from Late Pleistocene permafrost deposits up to 111 ka old. On Ellesmere Island, located in the Canadian Arctic Archipelago, Steven, *et al.* (2007) reported an archaeal community composed of 61% *Euryarchaeota* and 39% *Crenarchaeota*, displaying a diverse archaeal community in the perennially frozen sediments. Recently, several studies used lipid biomarkers to detect and quantify methanogenic archaea in an Antarctic lake (Coolen, *et al.*, 2004), permafrost soils (Wagner, *et al.*, 2005, Wagner, *et al.*, 2007), and peatlands (Weijers, *et al.*, 2004, Pancost, *et al.*, 2011). Microbial lipid markers indicating living microorganisms (phospholipids) and low numbers of methanogens were found at more than 1000 m depth in the Mallik gas hydrate production research well, drilled into the Canadian terrestrial permafrost at the northern edge of the Mackenzie River Delta (Colwell, 2005, Mangelsdorf, 2005). However, lipid biomarker studies in the Siberian Arctic on methanogenic communities in older permafrost deposits are currently missing.

Here we present a combined stratigraphic analysis using microbial lipid markers and rRNA genes on a permafrost sequence of Late Pleistocene and Holocene deposits from the Lena River Delta in Siberia, Russia. The aim was to analyze the abundance and composition of methanogenic communities in permafrost sediments deposited under different climatic conditions and to reconstruct the impact of past climate changes on the methane production.

2.3. **Materials and Methods**

2.3.1. Study site

The Lena River Delta (Fig. 1a) is the largest Arctic delta with an area of about 29 x 10^3 km^2 (Schneider, *et al.*, 2009). The entire Lena Delta is located in the zone of continuous permafrost, where the permafrost reaches a thickness of about 500-600 m (Romanovskii & Hubberten, 2001). The fieldwork for this

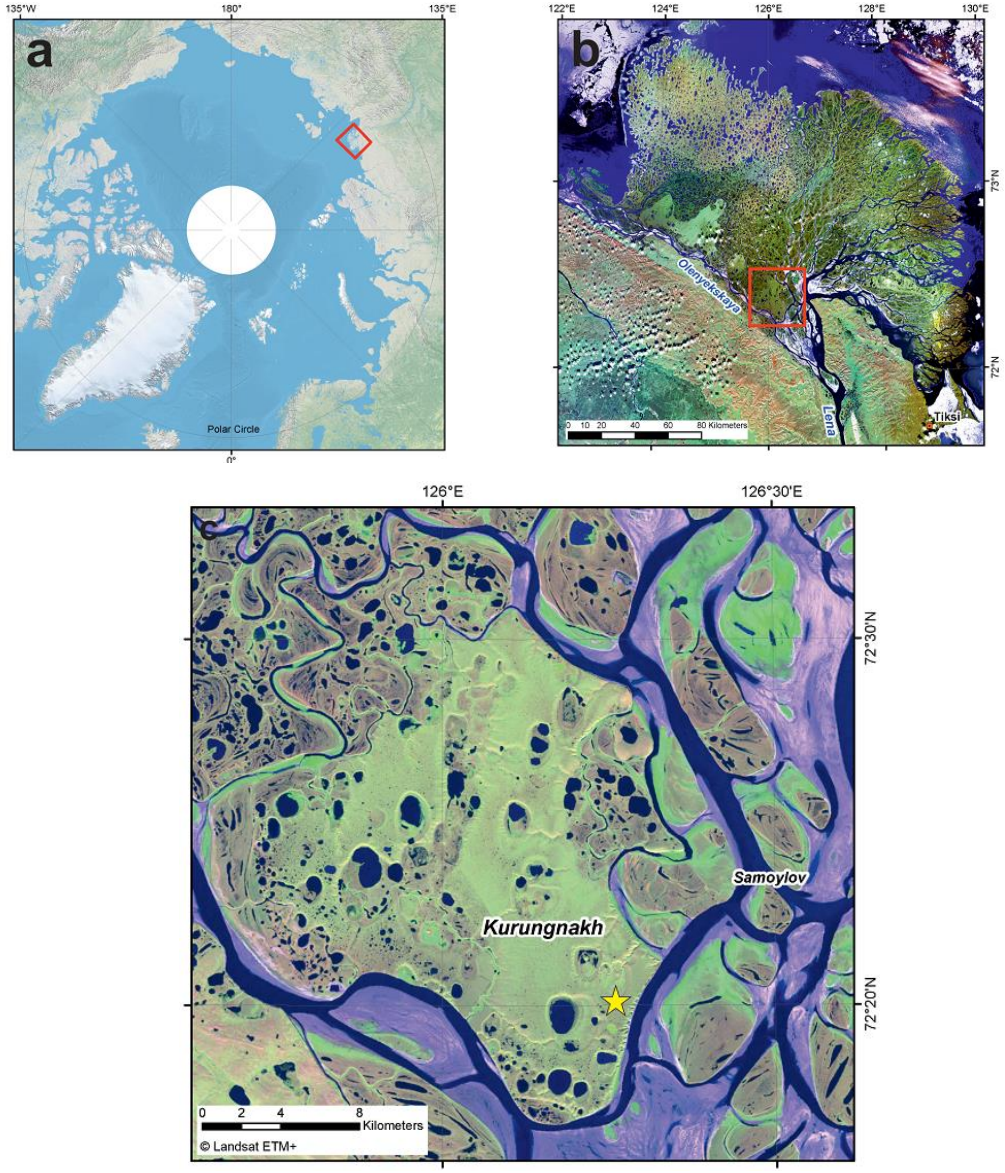

Figure 1: *Location of the Lena River Delta in the Russian Arctic (a). Kurungnakh Island study site in the Lena River delta (b), and location of drill site, marked with symbol (c). (ESRI data, Landsat ETM+).*

study was conducted on Kurungnakh Island, which is located in the southern part of the Lena River Delta close to the Olenyekskaya Channel (Fig. 1b).

Kurungnakh Island is composed of Late Quaternary sediments belonging to the oldest Lena River terrace, and is partly exposed up to 40 m above the river level. This so-called 'Third Terrace' was formed during the Late Pleistocene and is characterized by its lowest formation of sandy deposits covered by a huge characteristic 'Ice complex' (IC) formation about 20 m thick (Schwamborn*, et al.*, 2002). The IC is characterized by ice-rich, silty deposits with peat block inclusions of less decomposed material (Yedoma suite). Furthermore, on top of the IC, Holocene layers about 2-3 m thick and containing ice wedges 3-5 m wide are widely distributed (Schirrmeister*, et al.*, 2003). The depth of the active layer measured at the drill site in July 2002 was 32 cm.

During the Late Pleistocene and Holocene, Kurungnakh Island has undergone several climatic stages. This was indicated by the paleoclimatic and paleoenvironmental reconstruction presented in Wetterich*, et al.* (2008) for this site. This reconstruction was based on a stratigraphic analysis using a combination of several fossil bioindicators such as pollen, plant macro-fossils, ostracodes, insect remains and mammal bones as well as geochronology based on isochron uranium-thorium disequilibria technique and ^{14}C radiocarbon dating. Wetterich*, et al.* (2008) defined six climatic stages for the last 100 ka BP. Following this classification our Kurungnakh permafrost core was sectioned into five units (Fig. 2). Late Pleistocene deposits were divided into a cold and dry Unit II (2476 – 2400 cm; Early Weichselian Stadial, Zyryan), a warm and wet Unit III (2400 – 1400 cm; Middle Weichselian Interstadial, Kargin) and a cold and dry Unit IVa (1400 – 300 cm; Late Weichselian Stadial, Sartan). Holocene deposits were sectioned into a warm and dry Unit IVb (300 - 100 cm; Early Holocene) and a warm and wet Unit V (100 - 0 cm; Middle and Late Holocene).

2.3.2. Permafrost drilling and sample preparation

A 23 m long permafrost core was drilled in the depression of a low-centered ice-wedge polygon during the Russian-German LENA 2002 expedition in July 2002 on Kurungnakh Island (N 72°20, E 126°17; Fig. 1c) (Grigoriev, 2003). The drilling was carried out with a portable gasoline-powered permafrost corer. Drilling fluids were not used to avoid contaminating the permafrost samples with surface microorganisms as described previously (Wagner*, et al.*, 2007). The individual core segments, up to 50 cm long, were stored at about -8 °C in the permafrost cellar of the Samoylov Research Station, and were transported under frozen conditions to Potsdam, Germany (storage temperature in Potsdam -22 °C).

Core segments were split along their axes into two halves under aseptic conditions with a diamond saw in an ice laboratory at -10 °C. Afterwards, the surface of one half of the core was cleaned with a sterile knife for lithological and geocryological descriptions. Subsequently, this half was cut into segments about 10–30 cm long according to the lithology and the geocryology. Small pieces (approximately 5 g) of these subsamples were used for analyzing the methane concentration 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 sediment properties, microbial activity, and lipid biomarkers. The second half of the core is kept as an archive in the ice core repository at the Alfred Wegener Institute for Polar and Marine Research in Bremerhaven (Germany).

Overall, 73 samples were analyzed for their sediment properties (elemental analyses of carbon, grain size, methane concentration). According to the depth and the physico-chemical characterization (low/high total organic carbon (TOC), low/high methane concentration) we selected 31 samples for molecular and biomarker studies.

2.3.3. Sediment properties

The TOC and total carbon (TC) contents of the freeze-dried and homogenized permafrost samples were determined as described previously in Wagner, *et al.* (2007).

Pyrolysis of 10 selected freeze-dried homogenized samples was conducted by APT AS (Kjeller, Norway) using a Rock-Eval 6 instrument to obtain information on the characteristics of the organic matter in the permafrost deposits.

Accelerator mass spectrometry (AMS) ^{14}C-Radiocarbon dating of bulk organic matter was carried out for seven selected samples from the Kurungnakh permafrost sequence (Fig. 2) in the AMS facility at the Leibniz Laboratory for Radiometric Dating and Stable Isotope Research, University of Kiel, Germany as described previously in Wagner, *et al.* (2007).

Grain-size distribution was analyzed for bulk sediment samples every 30 cm. The sediments were oxidized using a 5% hydrogen peroxide (H_2O_2) solution to remove organic matter from each sample. The sand (0.063–2.0 mm) fraction was determined by wet sieving. The remaining silt (0.002–0.063 mm) and clay (<0.002 mm) materials were separated by sedimentation in ammonia solution (10 mL 25 % NH_3 in 100 L deionized water).

In order to obtain a vertical distribution of methane with depth, methane concentrations were analyzed by putting a slice (approx. 5 g) of frozen sediment in a glass bottle and immediately sealing the bottle gas-tight with a butyl-rubber stopper. After addition of 20 mL saturated saline (NaCl) solution, samples were shaken until thawed. Headspace gas samples were taken using a gas-tight syringe (Hamilton, Hamilton Bonaduz AG, Switzerland). Gas analysis was performed using a gas chromatograph (Agilent 6890, Agilent Technologies, USA) equipped with a bonded polystyrene-divinylbenzene-based column (HP-Plot-Q 15 m, 0.53 mm, 40 µm) and a flame ionization detector (FID) at operating conditions described previously (Koch, *et al.*, 2009).

2.3.4. Potential methane production rates

The potential methane production rates of permafrost sediments were analyzed for 25 selected samples both without any additional substrate and with hydrogen as a substrate for methanogenic archaea. Freshly thawed sediments (1 g) were weighed in 25 mL glass jars, closed gas-tight with a butyl-rubber stopper, and secured using aluminum crimp caps. The samples were evacuated and flushed with ultra-pure N_2/CO_2. Afterwards, the samples were supplemented with 0.1 mL sterile and anoxic tap water (tap water flushed with ultra-pure N_2/CO_2 and subsequently autoclaved) for analyzing methane production without substrate addition. In the case of potential hydrogen-driven methane production, sterile and anoxic tap water in combination with H_2/CO_2 (80:20 v/v, pressurized to 150 kPA) was added as substrate. Three replicates were used for each segment and substrate. The incubation temperature was 10 °C. Methane production was measured semi-weekly over a period of three months by sampling the headspace using a Hamilton gas-tight syringe and analyzed as described in section 2.3. Methane production rates were calculated from the linear increase in methane concentration.

2.3.5. Lipid biomarker analysis

Aliquots of the freeze-dried and ground samples were extracted using a flow blending system with a mixture of methanol/dichloromethane/ammonium acetate buffer (pH 7.6), 2:1:0.8 (v/v), modified after Bligh and Dyer (1959). For compound quantification, internal phospholipid standards (1-palmitoyl(D_{31})-2-hydroxy-glycero-3-phosphocholine and 1,2-distearoyl(D_{70})-glycero-3-phosphatidylcholine) were added. In a second step, the solvent extracts were collected in a separation funnel and for phase separation dichloromethane and water were added to achieve a ratio of methanol/dichloromethane/ammonium acetate buffer (pH 7.6) of 1:1:0.9 (v/v). Afterwards the organic phase was removed and the water phase was re-extracted two times with dichloromethane. For details on the method see (Zink & Mangelsdorf, 2004).

The obtained sediment extract was separated into fractions of different polarity (low polar lipids, free fatty acids, glycolipids, and phospholipids) using a pure silica column (1 g silica gel 63-200 µm) and a florisil column (1 g magnesium silica gel 150-250 µm) in sequence; for details see Zink and Mangelsdorf (2004). Blanks (pre-heated sea sand) were measured regularly and no phospholipids or GDGTs were detected.

2.3.6. Detection of archaeol and isoprenoid GDGTs

The low polar lipid fraction was dissolved in *n*-hexane to precipitate asphaltenes, which were removed from the soluble fraction by filtration over Na_2SO_4. The *n*-hexane-soluble fraction was separated by medium-pressure liquid chromatography (Radke*, et al.*, 1980) into fractions of aliphatic/alicyclic hydrocarbons, aromatic hydrocarbons, and nitrogen, sulphur, and oxygen (NSO) containing compounds.

The NSO fraction was investigated for tetraether lipids using an HPLC-APCI (high performance liquid chromatography-atmospheric pressure chemical ionization) mass spectrometry (MS) method modified after Hopmans*, et al.* (2000) and Schouten*, et al.* (2007). Chromatographic separation was achieved on an Econosphere Amino column (4.6 x 250 mm, 5 µm; Altech). The mobile phase consisted of *n*-hexane A and isopropanol B (5 min 99 % A, 1 % B) linear gradient to 1.4 % B within 22.5 min, in 1 min to 10 % B, holding 5 min to clean the column and back to initial conditions in 1 min, held for 6 min for equilibration. The flow rate was set to 200 µl/min and injection was performed via an autosampler with a 5 µl loop. APCI source conditions were as follows: corona current 5 µA giving a voltage of around 4 kV; vaporizer temperature 350 °C; capillary temperature 200 °C and voltage 7.5 V; nitrogen sheath gas at 60 psi; without auxiliary gas. Mass spectra were generated by selected (single) ion monitoring (SIM), in the positive ion mode. The multiplier voltage was 1500 V, and the scan rate was 1 s. For semi-quantitative determination of tetraether compounds an external synthetic archaeol standard (AvantiPolarLipids Inc., Al, USA) was regularly measured in parallel. Blanks did not contain any GDGTs.

2.3.7. Detection of PLFAs and PLELs

Lipids were extracted from the freshly homogenized material (ca. 30 g dw) from selected segments, according to the Bligh-Dyer method described in Zelles and Bai (1993). The resulting lipid material was fractionated into neutral lipids, glycolipids, and phospholipids on a silica-bonded phase column (SPE-SI; Bond Elute, Analytichem International, Harbor City, CA, USA) by elution with chloroform, acetone, and methanol, respectively. One half of the phospholipid fraction was used for PLFA analysis following procedures described in Zelles and Bai (1993) who also checked the composition of the polar lipid fraction and found no contamination with glycolipids under the elution conditions described. However, we are not sure if this holds true for all kind of environmental samples and sample matrices. After mild alkaline hydrolysis, the resulting fatty acid methylesters (FAMEs) were measured by gas chromatography/mass spectrometry (5973MSD GC/MS Agilent Technologies, Palo Alto, USA) at operating conditions described elsewhere (Zelles & Bai, 1993). The detected PLFAs comprises several saturated (*iso*-C_{14}, *n*-C_{14}, *iso*-C_{15}, *anteiso*-C_{15}, *n*-C_{15}, *iso*-C_{16}, branched-C_{17}, *n*-C_{17}, cyclo-C_{17}, *n*-C_{18}, branched-C_{19}, cyclo-C_{19}) , mono-unsaturated (*n*-$C_{15:1}$, *n*-$C_{15}\Delta^{9}$, *n*-$C_{16}\Delta^{7}$, *n*-$C_{16}\Delta^{9}$, cyclo-$_{16:1}\Delta^{7}$, *n*-$C_{16:1}\Delta^{11}$, *n*-$C_{17:1}\Delta^{9}$, *n*-$C_{17:1}\Delta^{11}$, *n*-$C_{18:1}\Delta^{9}$ *n*-$C_{18:1}\Delta^{11}$; *n*-$C_{18:1}$, *n*-$C_{20:1}\Delta^{11}$) and two poly unsaturated (*n*-$C_{18:2}\Delta^{9,12}$, 18:3) fatty acids. The sum of all detected phospholipid fatty acids resulted into a PLFA parameter.

The other half of the phospholipid extracts were subjected to PLEL analysis according to the method described by Gattinger, *et al.* (2003). After the formation of ether core lipids, etherlinked isoprenoids were released following cleavage of ether bonds with HI and reductive dehalogenation with Zn in glacial acetic acid. The resulting isoprenoid hydrocarbons were dissolved in 100 mL internal standard solution (nonadecanoic methyl ester) and subjected to GC/MS analysis at operating conditions described elsewhere (Gattinger, *et al.*, 2003). The detected ether cleavage products comprises phytane and biphytane and the sum of these compounds resulted into a PLEL parameter.

2.3.8. DNA extraction and polymerase chain reaction (PCR) amplification

Total genomic DNA was extracted using a Power Soil™ DNA Isolation Kit (Mo Bio Laboratories Inc., USA) according to the manufacturer's protocol. Amplifications were driven with a nested approach using a universal archaeal primer ArUn4F (Hershberger*, et al.*, 1996) plus Ar958R (DeLong, 1992), subsequently followed by amplification with the methanogenic archaea specific primer set 0357F and 0691R (Watanabe*, et al.*, 2004). DNA samples that were destined for denaturing gradient gel electrophoresis (DGGE) analysis were amplified using forward primer GC_0357F that was equal in sequence, but enlarged by a 20-mer GC clamp.

The PCR reaction mix contained 12.5 µl of premixed SAHARA-Mix™ including a PCR buffer, deoxyucleoside triphosphates (dNTPs), and polymerase (Bioline, Germany), 0.25 µl of each primer (20 mM), and 1 µl template, and was filled up to 25 µl with PCR-clean water (Mo Bio Laboratories Inc., USA). PCR was performed using an iCycler (Biorad, USA) and the following PCR cycle: 95 °C for 7 min, 30 cycles of 1 min at 95 °C, 1 min at 56 °C or 53 °C, 1 min at 72 °C, and a final elongation of 72 °C for 10 min. The amplified samples, positive and negative controls were checked by electrophoresis on a 1.5 % agarose gel in 0.5xTAE (diluted from 50xTAE, 5 Prime, Germany), that was prestained with GelRed™ (Biotium, USA). The PCR products were purified using a HiYield™PCR Clean-up / Gel Extraction Kit (Süd-Laborbedarf, Germany). Subsequently, DGGE analyses were conducted as described in Ganzert*, et al.* (2007) and sequence analyses were conducted by GATC Biotech AG (Konstanz, Germany).

2.3.9. Phylogenetic analysis

Sequence data obtained after DGGE analyses were checked for quality using the Sequencher® Software (Version 4.7., Gene Codes, USA). When indicated, sequences were cut or edited. Sequences were aligned with full length, quality controlled sequences provided by the 'Silva rRNA database project' (www.arb-silva.de; Pruesse*, et al.*, 2007). The phylogenetic analysis of partial

16S rRNA (ribosomal ribonucleic acid) gene sequences was performed using the ARB software package (www.arb-home.de; Ludwig, *et al.*, 2004). A phylogenetic tree (Maximum likelihood) was constructed using the pos_var_ssuref:archaea and termini filter provided by ARB. For a better overview only selected data are shown in the resulting tree. All sequences determined in this study were deposited in the GenBank database (JN635597 - JN635629).

2.4. **Results and Discussion**

2.4.1. Methane profile of the Kurungnakh permafrost sequence

Methane was detected at all depths from the Kurungnakh permafrost sequence, but the vertical profile shows a large variability from 4 to 3600 nmol methane g^{-1} sediment (Fig. 2c). Three intervals of increased methane concentration were identified in sediments at (i) 2300 – 2000 cm, (ii) around 1200 cm, and (iii) 500 – 100 cm depth. Overall, the highest methane concentrations were determined in Holocene deposits (300 – 100 cm depth).

High methane concentrations during the Holocene are comparable with a methane profile described for a permafrost sequence on Samoylov Island, which is located close to Kurungnakh Island (Fig. 1c). However, the methane concentrations reported for Samoylov Island were 10-fold lower than in the Kurungnakh sequence (Wagner, *et al.*, 2007). To our knowledge, enhanced methane concentrations in core material from terrestrial Late Pleistocene permafrost deposits have not been demonstrated before. Rivkina and Gilichinsky (1996) reported the occurrence of methane in Holocene deposits and in Early to Middle Pleistocene sediments (1.8 – 0.78 Ma BP), but were not able to detect methane in Late Pleistocene ice complex deposits (130 – 11.5 ka BP).

The observed methane profile in the Kurungnakh core corresponds to the distribution of the sedimentary organic matter (TOC; Fig. 2b). This implies that sedimentary organic carbon can be metabolized in a cascade of microbial

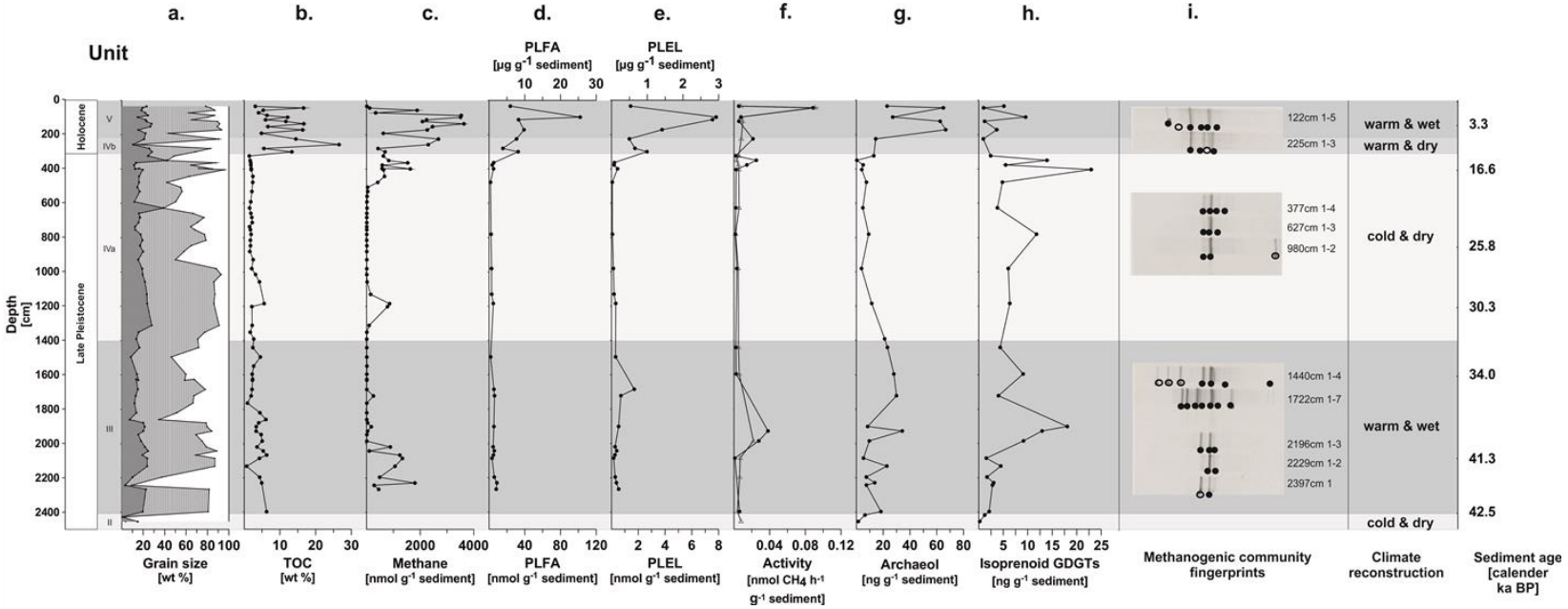

Figure 2: *Abiotic and biotic parameters of the permafrost deposits from Kurungnakh Island, Lena Delta (Siberian Arctic) presented with respect to sediment depth (left axis), sediment age in calendar years BP (right axis), and the underlining paleoclimate reconstruction provided by Wetterich et al. [2008]. The vertical profile shows: a. grain size distribution in wt% of clay (black), silt (grey), and sand (white), b. total organic carbon (TOC) content in wt%, c. methane content in nmol CH4 g-1 sediment, d. abundance of phospholipid fatty acids (PLFA) in nmol g-1 sediment dw and µg g-1 sediment dw, e. abundance of phospholipid etherlipids (PLEL) in nmol g-1 sediment dw and µg g-1 sediment dw, f. potential methanogenic activity without substrate (dots) and after addition of hydrogen (triangles), g. abundance of Archaeol in ng g-1 sediment dw, h. total isoprenoid glycerol dialkyl glycerol tetraethers (GDGTs) in ng g-1 sediment dw and i. denaturing gradient gel electrophoresis (DGGE) analysis profile with depth of 16S RNA genes of methanogenic archaea (sequenced bands marked with black dots were used for phylogentic analysis, bands marked with open circles were excluded due to sequence quality or length), respective sample ID of depth and consecutive band number.*

processes by a broad consortium of heterotrophic bacterial communities. This provides appropriate substrates (e.g. hydrogen, carbon dioxide, acetate, formate, and methanol) for methanogenic archaea and the final process of methanogenesis. The TOC content varied widely between 1.5 and 26.5 wt%. The deeper, older sediments of Unit III are characterized by relatively high TOC (up to 6 %) followed by a decrease to almost constant values of about 2 % in the younger sediments of Unit III and Unit IVa, with the exception of a small interval at ca. 1200 cm depth of higher TOC (around 4 %). With the transition from the Late Pleistocene to the Holocene (Unit IVa to Unit IVb) the TOC increases significantly, to an average value of 10 %. Generally, the intervals of higher methane concentrations correspond to higher TOC (Figs. 2b and 2c). Interestingly, the Late Pleistocene and Holocene organic matter show different compositions revealed by Rock-Eval pyrolysis (Fig. 3).

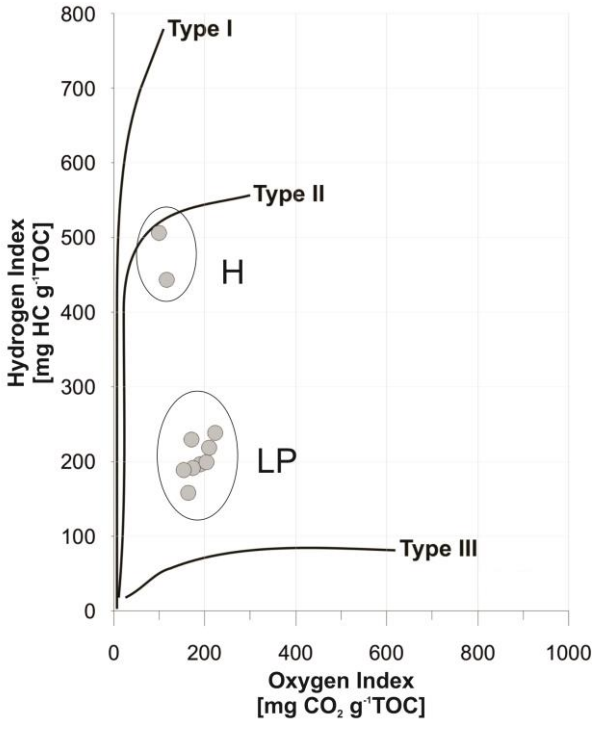

Figure 3: *Hydrogen index / oxygen index plot from Rock eval pyrolysis data characterizing the sediment organic matter of terrestrial permafrost deposits. The plot indicates differences in the quality of organic matter. Holocene samples (marked with 'H') cluster separately from Late Pleistocene (marked with 'LP') samples due to their higher Hydrogen index.*

The Holocene sediments have significantly higher Hydrogen index (HI) values. Higher HI values are often interpreted to represent a larger proportion of algal material in the organic matter. This algal material might

have been derived by occasional river flooding of Kurungnakh Island and as a consequence the formation of small algal-containing pools during the warmer Holocene period. Thus, in addition to higher and presumably less altered TOC, qualitative differences in the organic matter composition could explain the significantly higher methane contents in the Holocene deposits.

Overall, the corresponding TOC and methane concentration suggest that the TOC acted as a substrate source for the indigenous methanogenic communities. The fact that methane can be attached to an organic matrix might be another reason for the observed correlation (Chalmers & Bustin, 2007). However, in this context it has to be highlighted that the methane increase at the end of the Late Pleistocene is not reflected in the TOC data and that the higher TOC contents found in the middle part of Unit III did not result in higher methane values. This indicates that there are additional factors responsible for the observed methane profile.

One reason influencing the vertical distribution of methane is the lithological composition of the permafrost deposits. Kurungnakh permafrost deposits are heterogeneous, characterized by continuously changing proportions of the sand, silt, and clay fractions (Fig. 2a). Largest variations in the sediment compositions can be observed among the silt and sand fractions. In general silt is the dominant fraction, but there are distinct layers where sand significantly increases and becomes dominant. The intervals of low methane concentration found in Units III (1800-1400 cm) and IVa (1000-400 cm) contain generally higher proportions of sand. In contrast to soils made up of finer-grained material, which can trap gases more easily because of smaller pore sizes (Wang*, et al.*, 1993), the coarser grained sandy deposits are more permeable for gases, which might be an explanation for their low methane contents .

Carbon isotopic analyses of methane (Rivkina*, et al.*, 2007, Koch*, et al.*, 2009) as well as several microbiological studies on methanogenesis in arctic environments (Høj*, et al.*, 2005, Wagner*, et al.*, 2005, Metje & Frenzel, 2007) report that methane stored in shallow permafrost environments is of microbial origin. In this context, the questions to be discussed further are

whether the observed methane found in the terrestrial permafrost deposits on Kurungnakh Island (i) is recently produced by currently active methanogenic archaea within the permanently frozen ground, or (ii) was produced and trapped during the deposition of the Late Pleistocene and Holocene sediments? As a key to these questions traces of past and living microorganisms in the sediments were investigated.

2.4.2. Signals of living microbial communities in the Kurungnakh permafrost sequence

Several studies have reported that although permafrost environments represent an extreme habitat, microorganisms (bacteria and archaea) not only survive but can also be metabolically active (Rivkina*, et al.*, 2004, Wagner*, et al.*, 2005, Panikov & Sizova, 2007, Koch*, et al.*, 2009). Phospholipids (intact polar lipids) are considered as suitable life markers for microbial communities because the degradation of these abundant membrane compounds takes place rapidly after death of the source microorganisms (White*, et al.*, 1979, Harvey*, et al.*, 1986). Phospholipid fatty acids (PLFAs) are used as markers for viable *Bacteria* and *Eukarya* (Ringelberg*, et al.*, 1997, Zelles, 1999) and phospholipid ether lipids (PLELs) are characteristic markers for living archaeal communities in sediments (Zelles & Bai, 1993).

In the Late Pleistocene deposits the amounts of PLFA and PLEL are comparatively lower than in Holocene deposits (Figs. 2d and 2e), with the exception of a small interval at 1700 to 1500 cm depth in Unit III, where slightly higher amounts of PLELs (up to 2 nmol PLEL g^{-1} sediment dw) were detected. At the transition from the Late Pleistocene to the Holocene, the PLFA and PLEL concentrations significantly increase with the highest amounts occurring in the Holocene (Unit V). Comparable results on the vertical PLEL distribution in Holocene deposits were reported for permafrost sediments located close to our study site by Wagner*, et al.* (2005), but the abundance of total PLEL was considerably higher in the current study, indicating a greater number of living archaea in the Kurungnakh permafrost

sequence. For the intervals of increased PLEL concentrations (Holocene and in Unit III) genetic fingerprint analyses of the methanogenic communities (Fig. 2i) reveal a higher variability compared to other core sections. This indicates a diverse methanogenic community and implies the possibility of living methanogenic archaea.

Additionally, methanogenic activity incubation experiments with hydrogen and without any additional substrate (Fig. 2f) showed significant methane production in the Holocene deposits of up to 0.09 nmol CH_4 h^{-1} g^{-1} sediment. During the Late Pleistocene the potential methane production was usually low and could only be stimulated in two intervals representing the end of the Late Pleistocene (377-350 cm) and in the middle of Unit III (1985-1927 cm). Surprisingly, the addition of hydrogen as a substrate for methanogenic archaea did not lead to a significant increase or spatial shift in the potential methane production along the core as was observed by Wagner*, et al.* (2007).

Overall, in the Holocene sequence the PLFA and PLEL life marker profiles (Figs. 2d and 2e) and the methanogenic activity experiments (Fig. 2f) indicate the presence of living bacteria and archaea as well as potentially active methanogenic archaea. These results show that potential substrates for methanogenesis are present, either directly stored in the sediments or released due to alteration of the organic matter by psychrotolerant heterotrophic bacterial communities, whose presence is indicated by the PLFA profile (see Section 2.7). Furthermore, the data suggest that at least part of the methane detected in the Holocene sequence (Fig. 2c) might have been recently produced.

In the Late Pleistocene Unit III neither the enhanced PLEL concentrations at 1700 - 1500 cm depth nor the increased potential methanogenic activity at 1927-1985 cm depth correlate with the methane profile, suggesting living but inactive methanogenic communities at these depths (Figs. 2c, 2e, and 2f). In contrast, at the end of the Late Pleistocene higher potential methanogenic activity and at least a small increase of PLELs coincide with an increase of methane, indicating the possibility of active methanogens at the transition between the Late Pleistocene and the Holocene.

The higher methane concentrations detected in the older sediments of the Late Pleistocene Unit III (Fig. 2c) and at a depth of about 1200 cm in Unit IVa do not correspond with any life marker signal, indicating that this methane was produced and trapped in the past.

2.4.3. Reconstruction of past microbial communities in the Kurungnakh permafrost sequence

Biomarker analyses were conducted on the low polar lipid fraction. The abundance of archaeol was measured throughout the permafrost sequences as it was recently successfully used to reconstruct changes in the abundance of methanogenic archaea in a variety of ombotrophic peatland settings (Pancost*, et al.*, 2011). Additionally, isoprenoid GDGTs were detected and quantified as characteristic markers for archaea (De Rosa & Gambacorta, 1988, Koga & Morii, 2006). The GDGTs and archaeol are already partly degraded as indicated by the loss of their head groups. The remaining core lipid structures are less affected by degradation processes and are stable over geological time scales (Pease*, et al.*, 1998). Thus, in contrast to the life markers, archaeol and the GDGTs represent dead microbial biomass. Although it is very likely that these biomarkers largely represent past microbial communities, in particular in older sediments, the biomarkers per se provide no information about whether these remnants derived from past or presently living microbial communities. To get a better insight into the source of the biomarker signals, we compared the archaeol and GDGT distributions (Figs. 2g and 2h) with the previously discussed life marker profiles (Figs. 2d and 2e).

Archaeol was detected throughout the whole Kurungnakh permafrost sequence and varied between 0.3 and 66.7 ng g^{-1} sediment dw (Fig. 2g). The archaeol concentration increases at the transition from Late Pleistocene Unit II to Unit III. It remains relatively high in Unit III and decreases slowly to low values at the beginning of Unit IVa. The signal remains low until the end of the Late Pleistocene sequence. At the transition to the Holocene the archaeol signal increases again and shows the highest amounts of the whole

Kurungnakh permafrost core in the upper Holocene sequence (Unit V). The comparison with the life markers implies that higher archaeol concentrations in Late Pleistocene Unit III mainly represent increased microbial biomass of past archaeal communities. The archaeol signal during the Holocene is most likely the result of both past and presently living archaea. Thus, especially in the Late Pleistocene deposits, the archaeol profile seems to reflect the past distribution of the archaeal community in the Kurungnakh permafrost sequence.

Isoprenoid GDGTs (Fig. 2h) are comparatively low during the Holocene; their highest concentrations occur in Late Pleistocene Units III and IVa. A comparison with the PLEL life marker also suggests that the GDGTs accumulating during the Late Pleistocene mainly represent past microbial biomass.

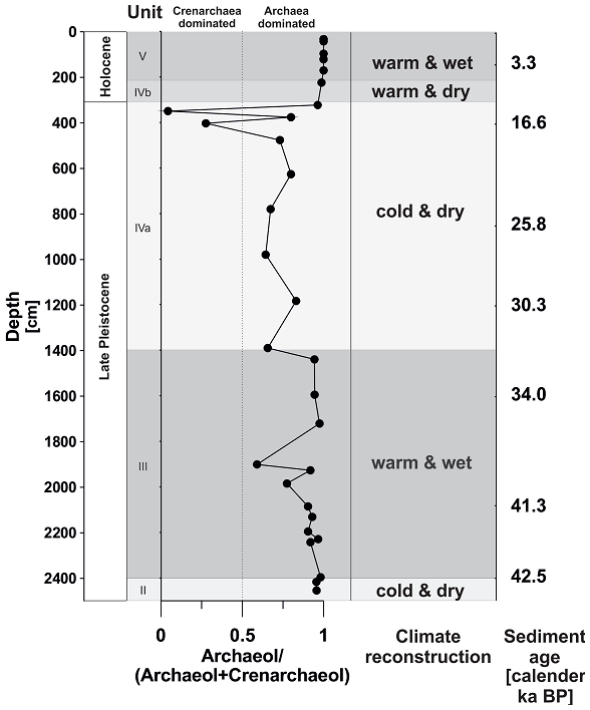

Figure 4: *Ratio of the relative abundances of archaeol and crenarchaeol along the Kurungnakh permafrost sequence.*

An interesting variability in the composition of the archaeal community is indicated by the archaeol/crenarchaeol ratio (Fig. 4). Archaeol is the dominating compound throughout the whole permafrost sequence. However, the archaeol/crenarchaeol ratio shows that enhanced amounts of crenarchaeol (Fig. 4) are detected in the Late Pleistocene deposits of Unit IVa.

This indicates a significant shift in the archaeal community during this time. Recently, crenarchaeol was considered to be characteristic for ammonium oxidizing archaea (AOA) (Pitcher*, et al.*, 2010). Ammonium oxidizing archaea have been identified in various soils and in arctic environments *(Leininger, et al., 2006, Yergeau, et al., 2010)*.

2.4.4. Climate impact on the distribution of microbial communities in the Kurungnakh permafrost sequence

As shown above (section 3.3) archaeol is an appropriate indicator to trace past variability of the archaeal likely the methanogenic community in the Kurungnakh permafrost sequence. Thus, in the following section the archaeol profile is compared to the Kurungnakh paleoclimate reconstruction by Wetterich*, et al.* (2008).

The Late Pleistocene Unit III (Middle Weichselian (Kargin) interstadial) was characterized by a change to relatively warm and wet summer conditions, by a distinct climate warming between 40 and 32 ka BP (Wetterich*, et al.*, 2008). This change in the climatic conditions was accompanied by a higher concentration of archaeol and a rise in the methane content (Figs. 2c and 2g) indicating an increase in the methanogenic community during this period. Thus, an increase in the paleo production of methane under warm and wet conditions is suggested. Part of this generated methane was surely emitted to the atmosphere. However, another part was trapped and stored in the frozen ground during the time of deposition, which is a reasonable explanation for the relatively high methane concentrations measured in the older permafrost deposits (2200 - 1900 cm) of Unit III.

In contrast, in the younger sediments of Unit III (1900 – 1400 cm) the methane concentrations are generally low, although the archaeol concentrations remain relatively high (Figs. 2c and 2g), implying that methanogens were still abundant and methane was still produced during that time. As discussed above (section 3.1) a change in the soil lithology in this interval might have presented less favorable conditions for trapping methane. Generally, this interval (1900 – 1400 cm depth) contains higher

proportions of sand (more permeable for gases), lower proportions of clay, and partly also lower amounts of TOC compared to the older sediments in Unit III (Figs. 2a and b). The lower TOC contents in the upper deposits of Unit III might also have contributed to lower the capability of the deposits to store methane because gases are reported to be attached to the organic matrixes (Chalmers & Bustin, 2007).

Climate conditions during the formation of Unit IVa have been interpreted as cold and rather dry (Late Weichselian (Sartan) Stadial; Wetterich*, et al.*, 2008). The decreasing amounts of archaeol (Fig. 2g) in this interval reflect a response of the archaeal communities to constant climate cooling and dry conditions. The data imply a less abundant community of methanogenic archaea and smaller amounts of methane produced and trapped during this time (Fig. 2c). Two exceptions are found in the small interval at 1200 cm and at the end of the Late Pleistocene (500-300 cm). The interval at 1200 cm is characterized by relatively high TOC and archaeol values, indicating past methane production and trapping. In contrast, the small increase of methane at the end of the Late Pleistocene, despite low archaeol concentrations, is difficult to explain. As mentioned above (section 3.2) the PLEL data and methanogenic activity experiments suggest the possibility of recent methane production in this interval. Another hypothesis is that the TOC and methane-rich Holocene sediments might have acted as a seal for gas percolating upwards from depth, leading to the accumulation of methane which was produced at a low rate over geological times in the Late Pleistocene sediments.

During the early Holocene (Unit IVb) methane and archaeol contents increase (Figs. 2c and 2g), indicating that the temperature rise during this period caused an increase in the abundance of methanogenic archaea again. The environment during this time has been described as warm and dry (Wetterich*, et al.*, 2008).

The Middle to Late Holocene deposits (Unit V) are characterized by warmer winter temperatures with dry habitats disappearing and the formation of peatlands (Wetterich*, et al.*, 2008). These changes to warm and wet

conditions triggered a dramatic increase in the abundance of methanogenic archaea, as amounts of archaeol increase remarkably in comparison to Late Pleistocene and Early Holocene deposits. This is in accordance with the highest concentrations of methane in these deposits (Fig. 2c).

Overall, our results show a climate-driven response of methanogenic archaea in terrestrial permafrost environments during the Late Pleistocene and Holocene. Warm and especially wet climatic conditions caused an increase in the abundance of methanogenic archaea; this is reflected by high amounts of the molecular marker archaeol being incorporated into the terrestrial permafrost deposits. A reason for this might be that the warm and wet climate conditions favored a landscape with flooded lowlands, causing water-saturated sediments and, therefore, suitable anaerobic conditions for the growth and activity of methanogenic archaea. Consequently, higher methane emissions to the atmosphere are suggested for these periods as reported for northern peatlands in the early Holocene (MacDonald*, et al.*, 2006). Furthermore, we show that a cold and dry climate causes a decline of the methanogenic archaeal community implying lower methane emissions for this interval. These interpretations are also supported by (Fischer*, et al.*, 2008), who demonstrated that boreal wetlands contribute enormously to methane emissions during warming events, whereas during cold climate periods these emissions decrease considerably.

2.4.5. Climatic impact on the composition of methanogenic communities in the Kurungnakh permafrost sequence

The biomarker data show a strong variability in the abundance of the archaeal community in the Kurungnakh permafrost sequence with respect to the stratigraphic units and their associated climatic conditions. Additionally, eco-molecular characterization of the methanogenic communities based on 16S rRNA gene sequence analysis revealed changes in the community composition of methanogens along the different stratigraphic units (Fig. 2i).

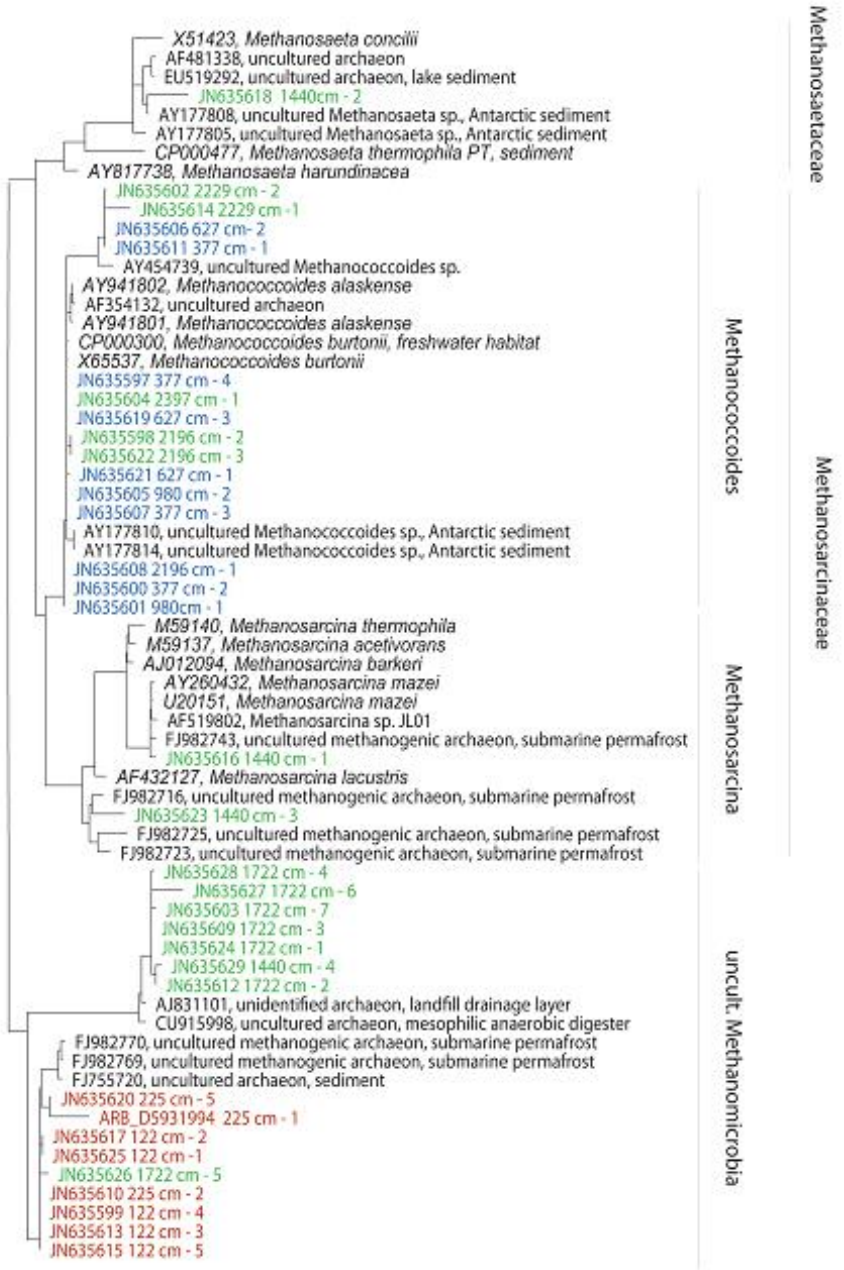

Figure 5: *Phylogenetic tree showing 16S rRNA gene sequences of methanogenic archaea and their nearest relatives detected by denaturing gradient gel electrophoresis (DGGE) analysis of samples from Kurungnakh Island. The vertical captions on the right side indicate their affiliation to known groups of methanogenic archaea. The single sequences are labeled with accession number, name of pure culture or clone, and isolation source. Sequences obtained within the scope of this work are labeled with depth and band number. Sequences that originated from the same climatic interval are marked in the same color: Late Pleistocene; Unit III (green), Unit IVa (blue). Holocene; Unit IVb and V (red).*

DNA was successfully extracted from samples throughout the whole permafrost core and 41 distinct bands of methanogenic fingerprints were obtained.

Individual Holocene (Unit V) and Late Pleistocene sediments (1440 cm and 1722 cm; Unit III) are characterized by a diverse pattern of up to 7 bands, suggesting a diverse community of methanogenic archaea. This indicates higher archaeal variability during warm and wet climate conditions. Additionally, comparatively high amounts of PLELs were detected (Figs. 2e and 2g), emphasizing possible living archaea in both intervals.

Successfully sequenced phylotypes (34 sequences) were classified as members of the phylum *Euryarchaeota* and assigned to the class *Methanomicrobia*, including the families *Methanosaetaceae* and *Methanosarcinaceae* (Fig. 5). Furthermore, sequences assigned to *Methanosarcinaceae* could be differentiated on the genus level to *Methanosarcina* and *Methanococcoides*.

Methanogenic archaeal sequences obtained from Late Pleistocene permafrost deposits were strongly affiliated with the known genera *Methanococcoides* and *Methanosarcina.* Sequences of Unit III (Fig. 5, marked in green) are widely distributed throughout the phylogenetic tree and exhibit a diverse genetic affiliation. Unit III includes the families *Methanosaetaceae* and *Methanosarcinaceae* and a cluster of sequences related to uncultivated *Methanomicrobia*. Due to the favorable warm and wet growth conditions during Unit III, a diverse and abundant community of methanogenic archaea could develop, as was also indicated by the increased amounts of archaeol (Fig. 2g).

In contrast, Unit IVa is characterized by the low amounts of archaeal life markers, low methanogenic activity in incubation experiments, low amounts of archaeol and less diverse community fingerprints of methanogenic archaea, suggesting a lower abundant and diverse methanogenic community during the cold and dry climate conditions of Unit IVa. Sequences obtained from Unit IVa sediments (Fig. 5, marked in blue) clustered solely in the genus *Methanococcoides*, showing a strong affiliation (up to 99 % similarity) to

Methanococcoides burtonii, which was isolated from the anoxic hypolimnion of Ace Lake, Antarctica (Franzmann*, et al.*, 1992) and *Methanococcoides alaskense*, isolated from marine sediments in Skan Bay, Alaska (Singh*, et al.*, 2005). The close relationship of the amplified sequences to these cold-adapted methanogenic archaea indicates a response to the cold and dry climate conditions during this period. Furthermore, given their adaption to sub-zero conditions, these organisms can potentially survive in frozen ground and retain metabolic activity.

Generally, methanogenic sequences received from Holocene sediments indicated a different phylogenetic affiliation than those from Late Pleistocene sediments. Sequences from Holocene deposits (Fig. 5, marked in red) showed the closest affiliation to sequences retrieved from submarine permafrost habitats in the Laptev Sea (FJ982770, FJ982769, FJ755720; Koch*, et al.*, 2009) and clustered as a group of so-far uncultivated *Methanomicrobia.* These sequences could not be assigned to existing groups of cultured methanogenic archaea; perhaps indicating new members of the methanogenic community that have not been described before and that differ from currently known genera. This is substantiated by studies describing new phylogenetic clusters for terrestrial and submarine permafrost deposits in the Siberian Arctic (Ganzert*, et al.*, 2007, Koch*, et al.*, 2009).

Generally, the phylogenetic analyses indicate that the terrestrial permafrost deposits of the different stratigraphic units contain unique methanogenic communities. These communities seem to have developed during the time of sedimentation and they preserve the paleo-environmental conditions in their respective genetic compositions over geologic timescales.

2.5. **Conclusion**

This new comprehensive study on Kurungnakh Island, in the Lena Delta of the Siberian Arctic investigates methane concentrations, sedimentary properties, microbial biomarkers and ecology data. Based on climate reconstruction data, we evaluate the impact of past climate variations on the

abundance, composition, and potential methane production of methanogenic archaea in a Late Pleistocene to Holocene permafrost sequence.

Microbial life marker signals (phospholipids) indicate the presence of living bacteria and archaea mainly in Holocene deposits. Furthermore, methanogenic activity experiments and the biomarker signals point to the possibility of recently-produced methane mixed with paleo-produced methane trapped within the sediments. Comparison between the phospholipid signals and archaeol suggests that the archaeol profile represents mainly past archaeal communities especially during the Late Pleistocene. Microbial ecology data reveal unique methanogenic communities in different stratigraphic units being influenced by different climatic conditions. Overall, the archaeol data imply an enhanced occurrence of methanogenic archaea during the Holocene and an interval during the Late Pleistocene that was characterized by warm and particularly wet conditions. It is suggested that considerable amounts of methane were emitted during these past warming events. In view of the recently observed and prospectively modeled temperature rise in the Arctic, our findings are of particular significance as they contribute to the understanding of potentially temperature-dependent responses and shifts of microbial communities and concomitant increase in methane emission from these environments.

2.6. **Acknowledgement**

We thank the Russian-German field parties working during the LENA 2002 expedition, especially Mikhail N. Grigoriev (Permafrost Institute, Russian Academy of Sciences, Yakutsk, Russia) and Günter “Molo” Stoof (Alfred Wegener Institute for Polar and Marine Research (AWI), Potsdam, Germany), for their assistance with drilling. Furthermore, we want to thank Dmitri V. Melnitschenko (Hydro Base Tiksi, Russia) and Waldemar Schneider (AWI) for logistic support during the expedition and Frank Günther (AWI) for drawing the map. We appreciate the technical assistance of Anke Kaminski and Cornelia Karger (GFZ German Research Centre for Geosciences) as well as the

help of Janine Görsch (AWI) and Patrick Häcker (GFZ). This manuscript benefited by the contribution of Candace O'Connor (University of Fairbanks, Alaska, USA) and Ryan Pereira (Newcastle University, UK). Finally, we thank one anonymous reviewer and Richard D. Pancost for their critical and helpful comments. This work was supported by the Deutsche Forschungsgemeinschaft (DFG) within the framework of the 'International Continental Drilling Program' by grants to D.W. (WA 1554/10) and K.M. (MA 2470/4).

3. Response of microbial communities to landscape and climatic changes in a terrestrial permafrost sequence of the El'gygytgyn crater, Far East Russian Arctic

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Final draft.

3.1. Abstract

Large areas of the Earth are affected by permanently or temporary low temperatures and furthermore affected by freeze-thaw cycles, low precipitation, low soil moisture and nutrient poor conditions. Extremophile microorganisms are able to withstand the harsh conditions and play an important role in the material- and energy cycles in these environments. However, the abundance and adaption of these extremophile microorganisms to environmental and climatic changes in terrestrial permafrost deposits in the Russian Arctic remain currently understudied. Here we present data on the distribution of bacterial and archaeal communities in deposits on the western shore of the Lake El'gygytgyn, Far

East Russian Arctic. During a lake high stand, the investigation site was flooded and after the lake level dropped, ongoing from the Allrød, exposed to subaerial conditions. Using a combined approach of microbiological and lipid biomarker analyses we can demonstrate a response of the microbial communities to these environmental changes. The composition of isoprenoid glyceroal dialkyl glycerol tetraether (GDGTs) and archaeol, as characteristic lipid markers for *Archaea*, reveal distinct patterns in the subaquatic when compared to subaerial deposits, indicating changes in the composition of the methanogenic communities. After the exposure of the investigation site to subaerial conditions, the abundance of *Bacteria* and *Archaea* increases strongly as was shown by the increasing 16S ribosomal ribonucleic acid (rRNA) gene copy numbers, GDGTs and phospholipid fatty acids (PLFAs). In particular the uppermost Holocene layers are characterised by comparably higher amounts of total organic carbon along with high microbial abundance. Our data shows the influence of the incorporation of organic material and beginning pedogenesis on the microbial communities, re-inforcing the role of the uppermost 'active layer' as hotspots of carbon cycling in permafrost environments.

3.2. **Introduction**

About 85 % of the Earth's surface is temporarily exposed to low temperatures (Kotsyurbenko, 2005, Margesin & Miteva, 2011). This causes large areas (about 25%) in the northern Hemisphere (around 23 Mio km^2) to be underlain by permafrost (Zhang*, et al.*, 2008), defined as ground (soil or rock and included ice and organic material) that remains below 0°C for at least two consecutive years (van Everdingen, 1998 revised May 2005). Permafrost can reach thicknesses of up to 600 m (Romanovskii & Hubberten, 2001) and only the uppermost few centimetres thaw during the summer months, forming the seasonally thawed 'active layer'. Extremely low temperatures, freeze-thaw cycles, low precipitation, low soil moisture and nutrient poor conditions form harsh conditions in these environments.

However, previous studies reported a diverse and abundant community of microorganisms in various Arctic and Antarctic settings that demonstrate their ability to adapt to these extreme environmental conditions (Aislabie*, et al.*, 2006, Yergeau*, et al.*, 2007, 2008, Liebner*, et al.*, 2008, 2009).

The permafrost in the crater depression surrounding Lake El'gygytgyn, northeastern Russian Arctic, represents such an extreme habitat for several reasons: the mean annual air temperatures are low (-10.3 °C Nolan & Brigham-Grette, 2007), the slope deposits in the crater largely consists of coarse-grained material (sand and gravel) and there is only a low availability of carbon from the surrounding catchment area (Schwamborn*, et al.*, 2002). Previous microbiological studies on Lake El'gygytgyn focussed on non-permafrost sediments obtained from the centre of the lake (site 5011-1, Fig. 1b), where significant amounts of bacterial and archaeal genes and lipid biomarkers were reported. A response of the microbial communities within the lake to glacial-interglacial variations was demonstrated during the Middle Pleistocene time interval investigated. The occurrence of higher numbers of microorganisms during the interglacials was most likely driven by the carbon availability in the lake (Bischoff*, et al.*, submitted). In contrast to the lake microbiological studies on the terrestrial permafrost surrounding Lake El'gygytgyn, being representative for the catchment area of the lake, are currently missing. The western shore of Lake El'gygytgyn was exposed to significant environmental and climatic changes during its formation. In younger times during the Allerød (11.4-10.73 ka) the lake level underwent several changes and finally dropped, exposing lake shore areas to subaerial conditions enabling the deposition of primarily terrestrial material (Schwamborn*, et al.*, 2012).

To investigate the abundance and composition of the microbial communities in response to the climate and environmental changes in the lake shore range, this study uses a combined approach of microbial lipid biomarkers and gene analyses. Glycerol dialkyl glycerol tetraethers (GDGTs), characteristic biomarkers for bacteria and archaea (Weijers*, et al.*, 2006) can be considered as fossil markers, as they are already partly degraded (loss of

headgroups) and they are relatively stable outside intact cells (Pease*, et al.*, 1998). Previous studies in Siberian permafrost (Bischoff*, et al.*, 2013) and El'gygytgyn Lake sediments (Bischoff*, et al.*, submitted) have shown that GDGTs and gene analyses are suitable approaches to reconstruct the response of microbial communities to climate changes in Arctic environments in up to 480 ka old sediments.

Thus, combining biogeochemical and molecular microbiological methods, this study aims to assess the compositional response of microbial communities in the lake shore area to lake level changes and with that to variations between subaerial to subaquatic conditions during the Late Pleistocene to Holocene and to characterize extremophile microorganisms the in permafrost deposits of lake El'gygytgyn in the far eastern Russian Arctic.

3.3. **Materials and Methods**

3.3.1. Study site

The El'gygytgyn impact crater (N 67°27', E 172°05' E) is located in central Chukotka, northeast Russia (Fig. 1a). A 3.6 million years old meteorite impact (Layer, 2000) caused a crater of about 18 km in diameter that is partially filled with an off-centred lake of 12 km in diameter (Fig. 1b). Today's climate in this region is characterized by cold temperatures, with mean annual air temperatures of – 10.3 °C (Nolan & Brigham-Grette, 2007); in 2003 the measured annual air temperatures ranged from 26 °C to -40 °C and the total precipitation was 180 mm a^{-1} with 70 mm of rainfall during the summer months (June to September) and 110 mm precipitated in the remainder of the year as water equivalent of snow.

Lake El'gygytgyn is located in the zone of continuous permafrost and the permafrost depth is estimated to reach a thickness of up to 350 m (Mottaghy*, et al.*, 2013), whereas there is no permafrost below the actual lake. In 2003 the active layer of the permafrost surrounding the lake was reported to be

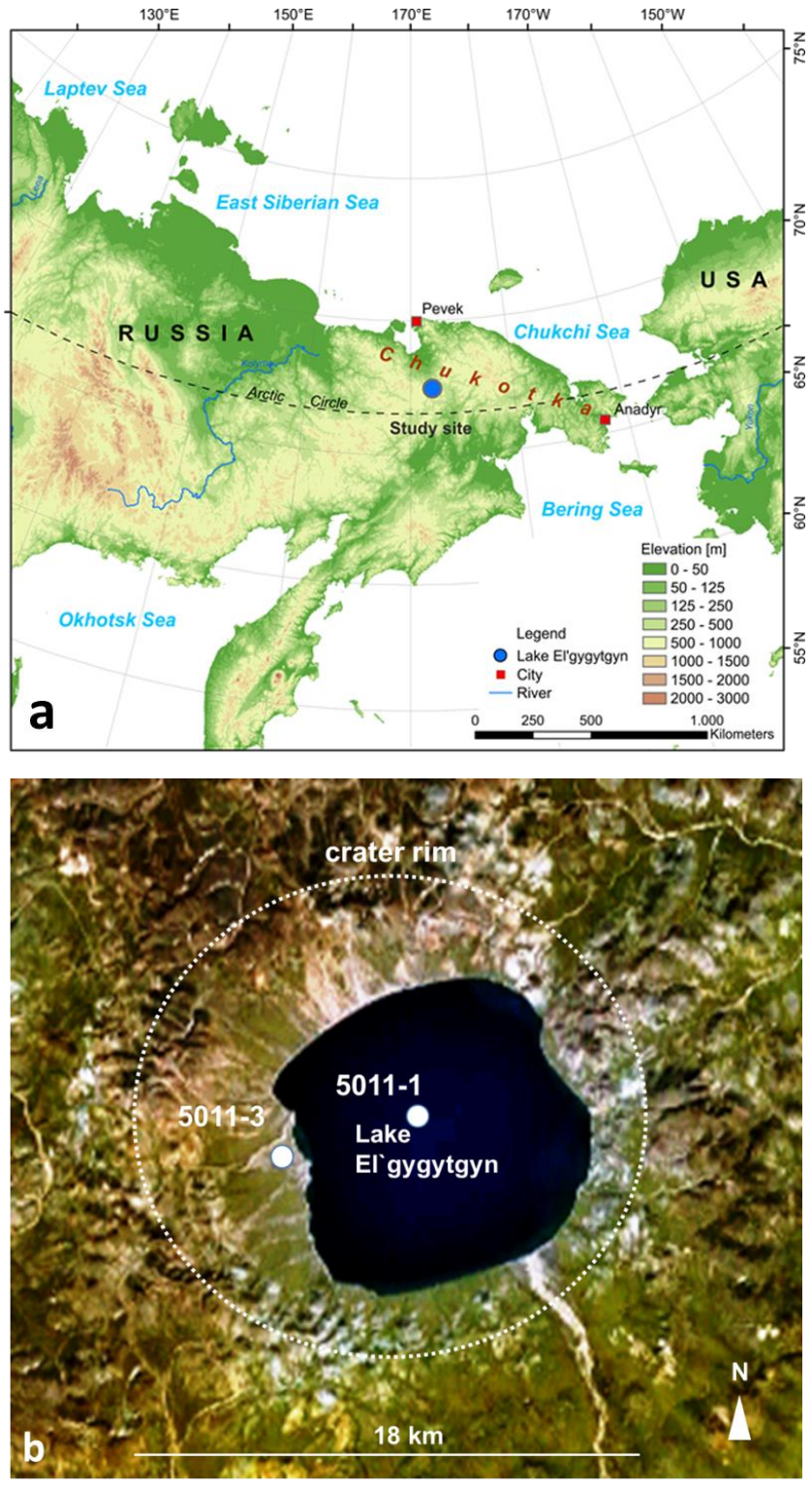

Figure 1: *(a) Location of Lake El'gygytgyn in far easternRussia and (b) Landsat image showing Lake El'gygytgyn and the drilling site 5011-3 on the western shore of the lake. Site 5011-1 marks the drilling site that recovered sediments from the centre of the lake. Figure modified after: Schwamborn, et al. (2012).*

0.4 m thick for peaty silts and 0.5 - 0.8 m deep for sand and gravel rich deposits (Schwamborn*, et al.*, 2012). Based on the sedimentological interpretations Schwamborn et al. (2012) reconstructed lake level changes of Lake El'gygytgyn. The coring site on the shore of lake El'gygytgyn was flooded during times of lake level highstand. Thus, the deposits of the study site were influenced by alternating subaerial and subaquatic conditions.

3.3.2. Drilling and sample material

From November to December 2008 the El'gygytgyn Drilling project was conducted within the scope of the 'International Scientific Continental Drilling project' (ICDP; icdp-online.org). A 141.5 m long core (5011-3, Fig 1b) of terrestrial permafrost deposits was drilled on the western shore of the lake (76°29.1'N, 171°56.7'E). For further information on the drilling operation see Melles et al. (2011, 2012) and Schwamborn*, et al.* (2012).

The shipping and storage of the samples was done under frozen conditions. The core segments were transferred to the ice laboratory of the Alfred-Wegener-Institute in Bremerhaven (Germany), cleaned and a comprehensive core description was accomplished. The sub-sampling of the uppermost 20 m of the core for this study was conducted in a climate controlled laboratory (-12°C) at the Helmholtz Centre Potsdam (GFZ) German Research Centre for Geosciences. Samples were taken almost every 0.5 m or where sediment changes occurred. The core diameter was 11 cm and the sampled sections were 15 to 20 cm long, the gross weight of each sample was 2 to 3 kg. Subsamples of about 90 g were taken from the undisturbed inner parts of these sections. Samples for biomarker analyses were stored in pre-heated brown glass jars. Samples for microbial analyses were put in sterile 2 mL tubes and frozen at -20°C for subsequent analyses. All reported core depths are the middle field depth of the homogenised sample core section.

The lithology of the upper 20 m contained two prominent sandy beds between 24.0 to 19.0 m and 9.6 to 9.1 m core depth. Both were interpreted to represent lake level highstands. The lower sandy bed (24.0 to 19.0 m) was likely formed during MIS 7 according to an age estimate based on the pollen

load (Andreev et al., 2012). During the Last Glacial Maximum the lake level was 10 m lower than today according to a reconstruction by Juschus et al. (2011). The top 9.5 m in the core represent alluvial material which has been distributed by migrating creeks transporting reworked coarse-grained material (i.e. gravelly sand) on the slope (Schwamborn et al., 2012). The upper sandy beds (9.6 to 9.1 m) have an Allerød-age (11.73-11.4 ka) estimated from the pollen load (Andreev et al., 2012). Further pollen based paleoclimate reconstructions identified 3 major intervals for the uppermost 9.5 m, including the Allerød (2.5 – 9.5 m), Younger Dryas (2.5 – 1.8 m, 10.73-9.7 ka) and Holocene (1.8 – 0 m core depth, 9.6-today) and clearly indicating the impact of changing climatic conditions on the deposits within the Lake El'gygytgyn crater (Andreev*, et al.*, 2012). Further downcore (9.5 m – 19 m) no pollen load could be observed.

3.3.3. Sediment properties

Total organic carbon (TOC) was measured with a Vario EL III element analyser on freeze-dried and homogenized samples (5 mg) that had been treated with hydrochloric acid (HCl, 10 %) at a temperature of 80 °C to remove carbonate, for further details see Schwamborn*, et al.* (2012).

3.3.4. Lipid biomarker analysis

Freeze-dried and ground deposits samples were extracted using a flow blending system with a mixture of methanol/dichloromethane/ammonium acetate buffer (pH 7.6), 2:1:0.8 (v/v), modified after Bligh and Dyer (1959). For compound quantification, internal phospholipid standards (1-palmitoyl(D_{31})-2-hydroxy-glycero-3-phosphocholine and 1,2-distearoyl(D_{70})-glycero-3-phosphatidylcholine) were added. For details on the method see (Zink & Mangelsdorf, 2004). The obtained sediment extract was separated into fractions of different polarity (low polar lipids, free fatty acids, glycolipids, and phospholipids) using a pure silica column and a florisil column in sequence; for details see Zink and Mangelsdorf (2004).

3.3.5. Detection of glycerol dialkyl glycerol tetraethers (GDGTs) and archaeol

Ground and freeze-dried lake sediments were extracted with a mixture of organic solvents and the obtained sediment extracts were separated into fractions of different polarity by column chromatography using a protocol described in Bischoff, et al. (2013). Tetraether lipids and archaeol were analysed using an HPLC-APCI (high performance liquid chromatography-atmospheric pressure chemical ionization) mass spectrometry (MS) method described in Schouten, et al. (2007). For semi-quantitative determination of tetraether compounds an external synthetic archaeol standard (AvantiPolarLipids Inc., Al, USA) was regularly measured in parallel. The detected GDGTs differ in the number of pentacyclic ring moieties ranging from 0 to 4 rings. Crenarchaeol contains in addition to 4 pentacyclic rings one hexacyclic ring. For structures of the detected GDGTs see Weijers, et al. (2006). Blanks (pre-heated sea sand) were measured regularly and did not contain any GDGTs or archaeol. For details on the applied method see Bischoff, et al. (2013).

3.3.6. Detection of phospholipid fatty acids (PLFA)

One third of the phospholipid fraction was used for alkaline hydrolysis to obtain the methylated phospholipid fatty acids (PLFA) by following the method described by Müller, *et al.* (1990). The aliquot of the PL fraction was preheated at 70°C and dissolved in 50 µl Dichlormethane: Methanol (9:1, v/v). Samples were ultrasonicated for 5 min, 50 µl of TMSH (trimethylsulfonium-hydroxid) were added and incubated at 70°C for 2 hours. The trans-esterified (methylated) PLFAs were directly analysed on a gas chromatographic system (Trace GC Ultra, Thermo Electron Corporation) coupled with a mass spectrometer (DSQ, Thermo Electron Corporation). The GC is equipped with a cold injection system operating in the splitless mode and a SGE BPX 5 fused silica capillary column (50 m length, 0.22 mm ID, 0.25 µm film thickness) using the following temperature program: injector temperature 50° C, initial temperature 50 °C, heating rate 3 °C/min to 310 °C,

held isothermal for 30 min. Helium is used as a carrier gas. For compound identification the GC is linked to a DSQ mass spectrometer operating in the electron impact mode at 70 eV. Full scan mass spectra is recorded from m/z 50 to 650 at a scan rate of 1 s per decade and an inter scan delay of 0.2 s, resulting in a scan cycle time of 1.3 s. Blanks did not contain any PLFAs.

3.3.7. Deoxyribonucleic acid (DNA) extraction and amplification

The total genomic DNA was extracted in duplicates of 0.75 g terrestrial permafrost deposit using a Power Soil™ DNA Isolation Kit (Mo Bio Laboratories Inc., USA) according to the manufacturer's protocol. The amplification of the respective small subunit (SSU) ribonucleic acid (RNA) genes was done in triplicate using an iCycler (Biorad, USA). The polymerase chain reaction (PCR) mix contained 0,25 µl of each primer (20 µM; TIB Molbiol, Germany), 5 µl Q-Solution, 5 µl PCR-Buffer, 1,5 µl 10mM dNTP-Mix, 1 U HotStar Taq™ DNA Polymerase (all Quiagen, Germany), 1 µl template and was filled up to 25 µl with PCR-clean water (Mo Bio Laboratories Inc., USA). The methanogenic SSU rRNA genes were amplified in a respective nested PCR approach, using the primer pair ArUn4F and 958 R (DeLong, 1992, Hershberger*, et al.*, 1996) followed by an amplification using GC_0357Fa and 0691 R (Watanabe*, et al.*, 2004). The PCR reaction conditions were as follow: initial denaturation at 95 °C for 5 min, 25-30 cycles with a denaturation at 95 °C for 30 s, annealing at 53°C/57°C for 20 s, elongation at 72 °C for 45 s and a final elongation at 72 °C for 10 min. PCR products were purified using HiYield™PCR Clean-up / Gel Extraction Kit (Süd-Laborbedarf, Germany) prior to any downstream applications. The denaturing gradient gel electrophoresis (DGGE) analyses were conducted as described previously by Ganzert*, et al.* (2007) and amplicons were sequenced by GATC Biotech AG (Germany).

3.3.8. Quantitative PCR analysis of archaeal and bacterial small sub unit (SSU) rRNA genes

The SSU rRNA gene copy numbers of bacteria and archaea in terrestrial permafrost deposits were determined using a real-time PCR approach. The primer pairs Uni 331F and 797R (Nadkarni*, et al.*, 2002) and A 571F and UA 1406R (Baker*, et al.*, 2003) were used to amplify fragments of 466 bp and 687 bp length from the bacterial and archaeal SSU rRNA genes, respectively. Real-time PCR reactions were conducted in triplicates in a Rotor Gene Q (Quiagen, Germany) instrument using the Rotor-Gene SYBR Green PCR Kit (Quiagen, Germany) and 0.5mM of each Primer. 3µl of purified and diluted DNA-Extract (1:10) was added as template. Cycling conditions were as follows: initial denaturation 95°C for 10 min, 40-50 cycles of denaturation at 95° for 20 s, annealing at 57°C, respectively for 20s and elongation at 72° for 45-90 s. A standard curve was generated using known dilutions (10^1 – 10^7 gene copies) of the target fragments amplified from *B. subtilis* (for *Bacteria*) and *M. vaculata* (for *Archaea*). The slope of the respective standard curve was used to calculate the gene copy numbers in the samples. After each run, melting curve analysis were driven to ensure the correct amplification of the target sequence.

3.3.9. Phylogenetic analysis

The obtained sequence data were checked for quality and length using Sequencher® Software (Version 4.7., Gene Codes, USA); when indicated, sequences were cut or edited. The sequences were aligned with full length sequences of a sufficient quality provided by the Silva rRNA database project using the online available alignment software (www.arb-silva.de; Pruesse*, et al.*, 2007). The phylogenetic analysis of partial 16S rRNA gene sequences was performed using the ARB software package (www.arb-home.de; Ludwig*, et al.*, 2004). The phylogenetic tree (Maximum likelihood) was constructed using the 'pos_var_ssu:ref:archaea' and 'termini' filter provided by ARB. For a better overview, only selected data is shown in the resulting tree.

3.4. **Results**

3.4.1. TOC-contents

Overall, the TOC content varies between <0.1 and 1.7 wt% throughout the studied El'gygytgyn permafrost core (Fig. 2a). The uppermost 0 – 1.25 m can be clearly distinguished from the rest of the core by significantly higher TOC contents with values ranging between 0.4 and 1.7 wt% TOC. Further down core, the organic carbon content decreases and is below the analytical detection limit of 0.1 % TOC below 4m depth.

3.4.2. Distribution of glycerol dialkyl glycerol tetraethers (GDGTs) and archaeol

GDGTs and archaeol were detected throughout the entire upper 20 m of the El'gygytgyn terrestrial permafrost sequence (Figs. 2b-d). Branched GDGTs, characteristic markers for bacteria (Weijers*, et al.*, 2006), were predominantly found in the uppermost 1.25 - 0 m of the deposits with concentrations of up to 316 ng g^{-1} sediment dry weight (dw) (Fig. 2b). Throughout the remaining core, the amount of total branched GDGTs decreases below 10 ng g^{-1} sediment dw. Isoprenoid GDGTs, a characteristic markers for archaea (Weijers*, et al.*, 2006), were less abundant with total amounts, ranging from 0 to 2 ng g-1 sediment dw throughout the core (Fig. 2c). Highest values were observed in the uppermost layers (1.25 – 0 m), but also in an interval between 3.4 to 3 m depth with up to 1.5 ng g^{-1} sediment dw. Below 7.3 m the concentration of isoprenoid GDGTs decreased to values of 0.1 ng g^{-1} sediment dw. Archaeol, a characteristic marker for methanogenic archaea (Pancost*, et al.*, 2011) is abundant throughout the uppermost 7 m of the analysed core, with concentrations ranging from 0.1 to 10.3 ng g^{-1} sediment dw (Fig. 2d). Highest amounts of archaeol were detected in intervals between 6.6 – 4.25 and 1 – 0.3 m depth.

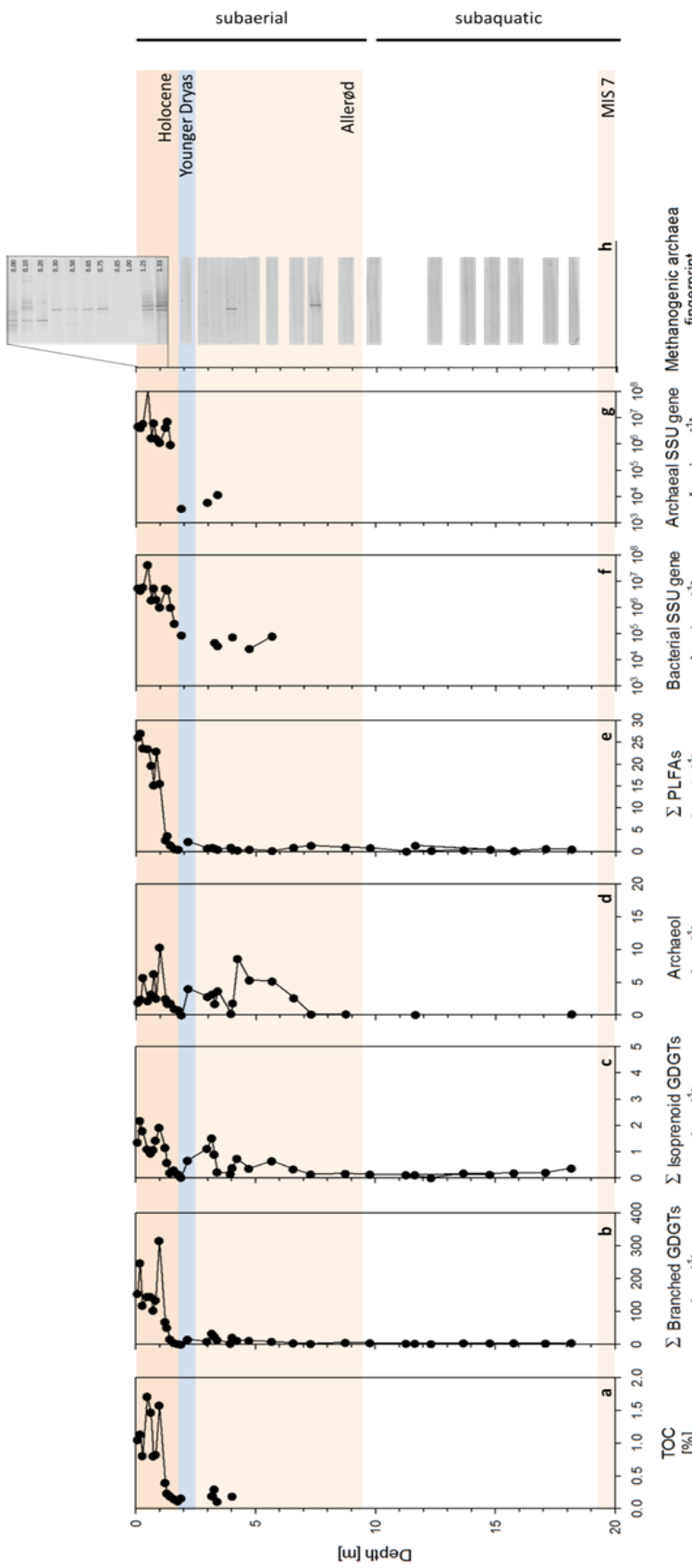

Figure 2: *Vertical profile of El'gygytgyn terrestrial permafrost sequences comprising deposits of MIS 7 to Holocene, showing (a) total organic carbon [%] in sediments, (b) sum of branched glycerol dialkyl glycerol tetraether (GDGTs) [ng g^{-1} sediment], (c) sum of isoprenoid GDGTs [ng g^{-1} sediment], (d) archaeol [ng g^{-1} sediment], (e) sum of phospholipid fatty acids [nmol g^{-1} sediment], (f) abundance of bacterial SSU RNA genes [copies g^{-1} sediment], (g) abundance of archaeal SSU RNA genes [copies g^{-1} sediment] and (h) methanogenic fingerprints. The underlining paleoclimate reconstruction is based on Andreev, et al. (2012)and the lake level reconstruction according to Schwamborn, et al. (2012).*

3.4.3. Distribution of phospholipid fatty acids (PLFA)

Total amounts of PLFA were highest in the uppermost 1.25 m of the permafrost sequence with up to 27 nmol g^{-1} sediment dw (Fig. 2e). Below, the amounts of total PLFA remained rather stable with comparably low values of 1 nmol PLFA g^{-1} sediment dw.

3.4.4. Composition of archaeol and isoprenoid GDGTs

The relative percentage proportion of archaeol and all isoprenoid GDGTs are shown in Figure 3. Sediments from 20 to 8.5 m depth are characterised by high relative abundances of GDGT-0 (m/z = 1302), which accounts for about 50% of all detected isoprenoid GDGTs. Additionally, crenarchaeol (m/z = 1292) was abundant in the same sediment interval with on average 25% of the total isoprenoid GDGTs. Archaeol was not detectable in most of the samples in the 20 to 8.5 m depth interval, with the exception of 18.2, 11.7 and 8.8 m depth. The composition of isoprenoid GDGTs in sediment interval from 8.5 to 0 m depth are characterized by an increase in the relative abundance of archaeol, accounting for on average 80% of the total isoprenoid GDGTs, whereas amounts of crenarchaeol and GDGT-0 decrease below 10%. The abundance of GDGT-2 (m/z=1298) and GDGT-3 (m/z=1296) is limited to the uppermost 8 m of the core, with GDGT-3 being exclusively found in the uppermost layers (1.3 to 0 m depth). GDGT-1 (m/z=1300) and GDGT-4 (m/z=1294) are abundant throughout all analysed samples, but highest amounts with on average 5% of all isoprenoid GDGTs were detected at the uppermost 4 to 0 m depth and further downcore at 18.20 to 13.7 m depth with on average 13 % of all isoprenoid GDGTs.

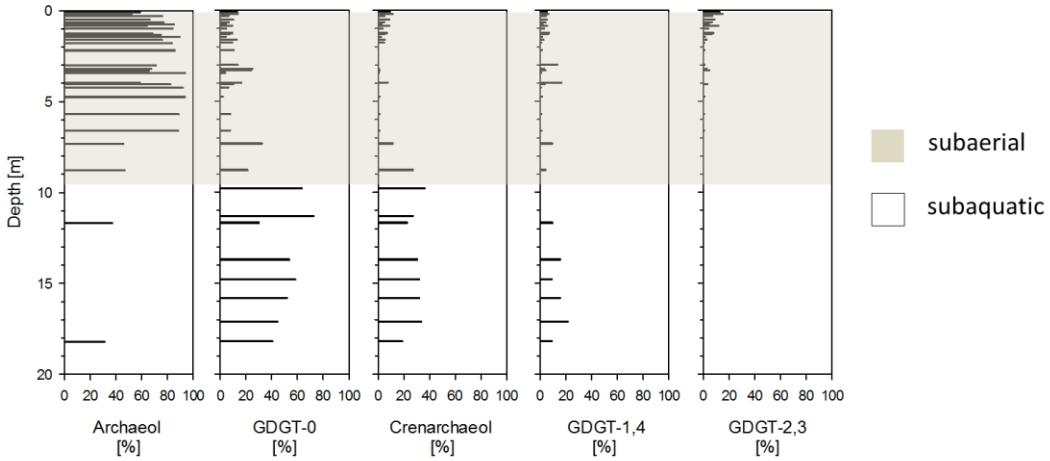

Figure 3: *Relative composition of isoprenoid glycerol dialkyl glycerol tetraethers (GDGTs) and archaeol in the Lake El'gygytgyn shore permafrost deposits with depth. The environmental reconstruction is according to Schwamborn, et al. (2012).*

3.4.5. Quantification of bacterial and archaeal genes

Bacterial and archaeal SSU rRNA genes were quantified throughout the sequence (Figs. 2f and g). The gene copy numbers are highest in the uppermost 2 m with 4.21 10^7 and 1.16 10^8 copies g^{-1} sediment for bacterial and archaeal genes, respectively. Downcore, these values decrease considerably and no gene copy numbers could be calculated for samples below 5.7 m for *Bacteria* and 3.4 m depth for *Archaea* due to the analytical detection limit.

3.4.6. Analysis of methanogenic community fingerprints

DGGE analyses were conducted to obtain a genetic fingerprint profile of methanogenic archaea throughout the El'gygytgyn terrestrial permafrost sequence. In total, 45 distinct bands were obtained, indicating microbial community changes with increasing depth (Fig. 2h).

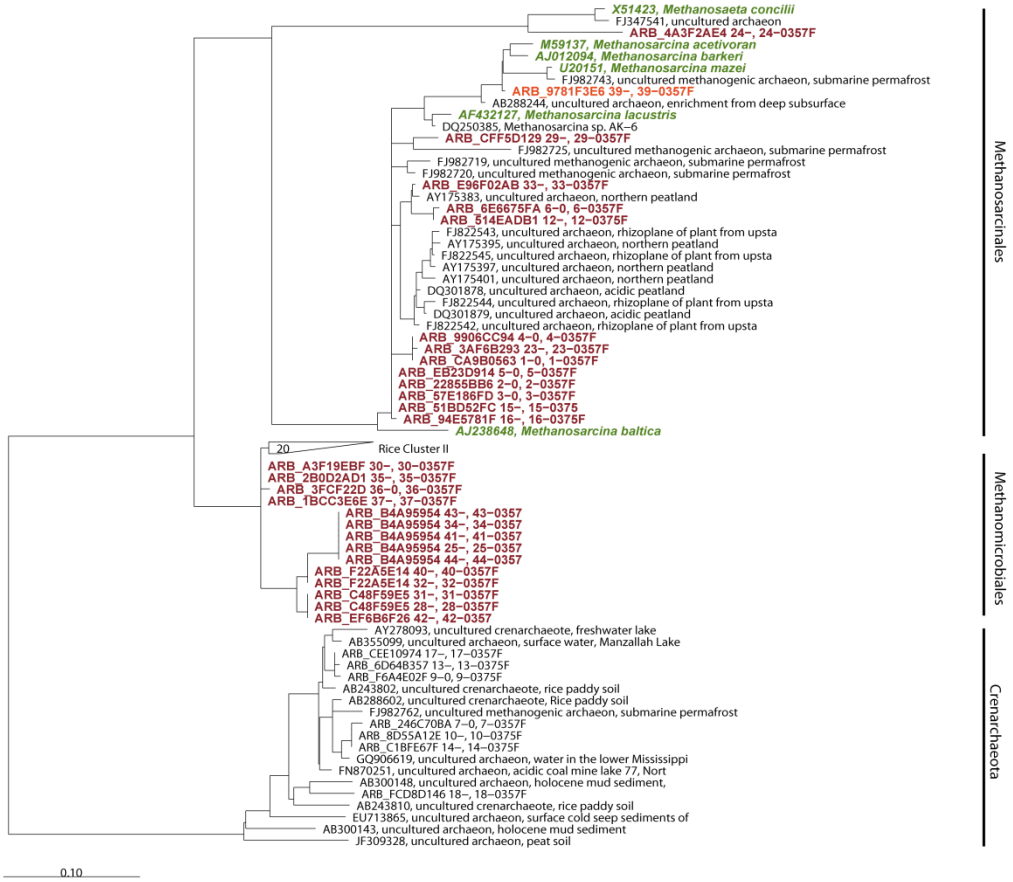

***Figure 4**: Phylogenetic tree showing the relation of 16S rRNA gene sequences of methanogenic archaea obtained from terrestrial permafrost deposits of Lake El'gygytgyn. The tree represents a maximum parsimony tree based on sequence data provided by Silva Database, but due to only selected data is shown. Sequences obtained in this work are colored red.*

Overall, the amplification of methanogenic SSU genes was limited to the uppermost 1.3 m. Further downcore (from 1.3 to 18.3 m depth), the abundance of microbial DNA decreases strongly and phylogenetic analyses were not possible. The highest variety of bands could be detected in surface layers (3-5-bands, 0.2 to 0 m depth). From 0.2 to 0.75 m depth a lower variety of bands could be detected with only one distinct band. Between 0.85 and 1.0 m no bands could be obtained. In contrast, in the interval between 1.25 -1.33 m depth up to 5 bands were detected. Subsequently, only one distinct band could be detected at 3.98 and 7.33 m depth, respectively. No bands occur below 8m depth. In total, 8 sequences were excluded from further analyses because of insufficient quality or length of the obtained DNA sequence. Successfully sequenced phylotypes (35 sequences) were classified

as members of the phylum *Crenarchaeota* (7) and *Euryarchaeota* (28). These were successively assigned to the class *Methanomicrobia*, including the order *Methanosarcinales* and *Methanomicrobiales* (Fig. 4).

3.5. **Discussion**

The study site on the western shore of Lake El'gygytgyn records the depositional history influenced by major lake level changes (Juschus*, et al.*, 2011). The lower part of the core (20 – 8.5 m) was deposited under subaquatic condition, as evidenced by Schwamborn et al. (2008), indicating that the lake level was higher than today. During the Allerød (11.4-10.73 ka) the lake level underwent several fluctuations before it finally dropped, exposing lake shore areas permanently to subaerial conditions and allowing the deposition of only terrestrial material, as was shown by sedimentary analyses of grain sizes, pH and lithological descriptions (Schwamborn*, et al.*, 2012). This general change in the depositional environment forced the microbial communities to adapt consistent with the development of unique and diverse microbial communities in both parts of the section.

3.5.1. Microbial communities in subaquatic deposits

Sediments, deposited under subaquatic conditions (20 – 9.6 m), are generally characterized by low concentrations of branched (<5 ng per g sediment) and isoprenoid GDGTs (<1 ng per g sediment), indicating a very limited number of microorganisms in these sediments (Figs. 2b and c). This is also supported by the low amounts of PLFAs (<1 ng per g sediment), indicating only low numbers of living bacteria (Fig. 2e). The absolute amounts of branched and isoprenoid GDGTs, reported for sediments in the centre of Lake El'gygytgyn during previous glacial interglacial cycles (MIS (Marine Isotope Stage) 13 - 9), were considerably higher with values between 200 to 3400 ng branched GDGTs and 20 - 870 ng isoprenoid GDGTs per g sediment (Bischoff*, et al.*, submitted).

The low numbers of microbial lipid markers in the subaquatic interval (20 – 9.6 m) of the lake El'gygytgyn margin site reflect the low abundance of microorganisms during the time of deposition. This is likely due to the low total organic carbon content in these sediments (TOC below detection limit) (Fig. 2a) resulting in a lower substrate availability for the indigenous microbial communities at the lake shore. In contrary, the glacial and interglacial sediments from the centre of the lake contain higher TOC contents (TOC ~ 0.5%) and microbial abundances likely driven by an intra-lake production of algea biomass (Bischoff*, et al.*, submitted). A reason for the low organic matter deposition under subaquatic conditions at the lake margin site might be found in the sedimentary analyses of the subaquatic interval of the core revealing a dominance of coarse grain material such as gravel and sand. High-energy conditions, belonging to a typical progradational fan delta with alluvial transport and slope processes are, therefore, suggested to prevent clay and pollen from deposition (Schwamborn*, et al.*, 2012). This scenario would cause finer proportions of the sediment load including organic matter particles to be transported and deposited further downslope in the deeper part of the lake. As a consequence of the low organic matter contents the abundance of microbial communities are rather small.

Despite the low abundance of isoprenoid microbial lipid markers, the relative composition of the GDGTs with different numbers of rings and archaeol reveals an indicative signature for the subaquatic deposition (Fig. 3). Generally, the subaquatic deposits contain a high proportion of GDGT-0 accounting for on average 50% and crenarchaeol accounting for on average 25% of all analysed isoprenoid GDGTs. Archaeol is only present in two samples. GDGT-0 is a characteristic biomarker for methanogenic archaea (de Rosa et al., 1986), indicating that the limited archaeal community found in the subaquatic interval of the analysed core contained a significant proportion of methanogenic archaea. Methanogenic archaea were previously found in the central lake sediments of Lake El'gygytgyn (Bischoff et al., submitted) and other cold lakes, such as Lake Fryxell (Karr*, et al.*, 2006) and

Ace Lake (Coolen*, et al.*, 2004) on Antarctica. Furthermore, methanogenic archaea are described to be able to withstand harsh environmental conditions such as low temperatures and starvation (Morozova & Wagner, 2007, Wagner*, et al.*, 2013) and are, therefore, not unexpected for this environment. Despite the abundance of GDGT-0, as a characteristic biomarker for methanogens, analyses of methanogenic fingerprints for this interval were not successful and, therefore, a phylogenetic classification not possible. A further indicative marker for the subaquatic conditions during the time of deposition of the lower part of the core is the high relative proportion of crenarchaeol (up to 25% of all isoprenoid GDGTs). Crenarchaeol is described a characteristic biomarker for *Crenarchaeota* group I in marine environments (Damste*, et al.*, 2002), but more recently found in high proportions of up to 49% of all analysed GDGTs in the cold, lacustrine sediments in northern Scandinavia (Blaga*, et al.*, 2009), the Swiss Alps (Bechtel*, et al.*, 2010) and in Siberian arctic terrestrial environments (Bischoff*, et al.*, 2013). Further research is necessary to better constrain the source organisms of this ubiquitous biomarker.

3.5.2. Microbial communities in subaerial deposits

The decrease of the Lake El'gygytgyn water level during the Allerød to Holocene exposed the lake margin site on the western shore of the lake to subaerial conditions and mirrors the present surface conditions in a channel environment on the delta plain (Schwamborn*, et al.*, 2012). Although there is only a slow increase in the abundance of bacterial and archaeal GDGTs, not starting before 7.5 m depth (Figs. 2b and c), the environmental change is reflected in the relative composition of isoprenoid GDGTs (Fig. 3) that show a clear distinction between subaquatic-lake and subaerial-terrestrial sediments. GDGT-0 and crenarchaeol that dominate the relative proportions of GDGTs under subaquatic conditions decrease significantly and in the upper 9.5 m of the core archaeol becomes the most prominent isoprenoid compound (up to 80%).

Archaeol is also considered as a characteristic lipid marker for methanogenic archaea (De Rosa & Gambacorta, 1988). However, the relative abundance of GDGT-0 and archaeol differ throughout different methanogenic genera (Koga & Morii, 2006). Therefore, the shift from GDGT-0 to archaeol at the transition from subaquatic to subaerial conditions reveals most likely a change in the composition of the methanogenic community in response to the changing lake level. As reported before, gene analysis was only possible in the uppermost subaerial layers showing high abundance of *Methanosarcinaceae*, *Methanosaetaceae* and *Methanococcaceae* in the terrestrial deposits (Figure 4). All of these genera contain archaeol. In contrast, GDGT-0 is only minor abundant in *Methanosarcinaceae*, *Methanosaetaceae* and occurs only in some species within *Methanococcaceae* (Koga et al., 1998). Thus, the gene analysis data coincide with the high abundance of archaeol and the low occurrence of GDGT-0 in the subaerial section. Since DGGE fingerprint analysis was not successful below 8 m depth no further information on the archaeal community in the subaquatic section can be obtained.

Methanosarcina-related sequences were previously found in permafrost-affected soils and sediments (Ganzert*, et al.*, 2007, Koch*, et al.*, 2009, Bischoff*, et al.*, 2013) in temporarily or permanently cold environments and new *Methanosarcina* strains could be isolated (Simankova*, et al.*, 2001, Wagner*, et al.*, 2013) from Russian Arctic soils. In addition to the relatives of *Methanosarcina*, a number of sequences isolated from the shore of Lake El'gygytgyn were affiliated to the Rice Cluster II belonging to the order *Methanomicrobiales* (Fig. 4). The analyses of the microbial communities within the centre of Lake El'gygytgyn (Bischoff*, et al.*, submitted) show that affiliates of *Methanosarcina* and *Methanomicrobiales* (Rice Cluster II) are both abundant in glacial and interglacial sediments in the central lake, which might provide evidence of transport processes from the surrounding permafrost deposits into the lake.

3.5.3. Microbial succession in the Holocene sequence of Lake El'gygytgyn permafrost

The Holocene permafrost sequence of the subaerial deposits on the western shore of Lake El'gygytgyn can be clearly distinguished from the underlying deposits (Figs. 2a-g). This uppermost layer contain the seasonally thawed 'active layer' (Schwamborn*, et al.*, 2012) and, as shown in Figure 2, are characterized by an increase in TOC and microbial abundance and activity. In addition to increased bacterial and archaeal GDGT lipid markers, high bacterial and archaeal SSU rRNA gene copy numbers together with divers fingerprint pattern of methanogenic archaea (Fig. 2) indicate the establishment of an abundant microbial community during the Holocene, especially, compared to the previous subaerial and subaquatic core sections. The high number of gene copies and different DNA bands in the DGGE analyses suggest the abundance of living microorganisms. This was supported by a significant increase in the PLFA amounts (Fig. 2e) that was solely shown for the near surface layers. PLFAs are considered to indicate living bacterial cells, as these markers degrade relatively rapidly after the death of the microorganisms (White*, et al.*, 1979, Harvey*, et al.*, 1986).

We suggest that with the beginning of the Holocene warming soil formation processes started leading to the incorporation of organic matter by slope input or plant detritus as was previously shown for Lake El'gygytgyn in the uppermost layers in (Schwamborn*, et al.*, 2012). ^{14}C dating of plant remains show modern ages (Andreev*, et al.*, 2012) for the incorporated organic matter. Soils in polar regions are often poorly developed and contain low carbon and nitrogen values as the cold temperatures restrict the physical, chemical and biological processes that are involved in the soil formation (Chapin*, et al.*, 1994). However, microorganisms have been demonstrated to contribute to primary succession and pedogenesis (Schuette*, et al.*, 2009) with an ability to support chemical and mechanical bedrock degradation (Frey*, et al.*, 2010) in cold settings. It is suggested that similar processes are taking place in the uppermost active layers of the permafrost deposits in the shore of Lake El'gygytgyn during the Holocene supporting the development

of a relative abundant microbial communities in the Holocene deposits of Lake El'gygytgyn despite of the harsh environmental condition of this permafrost affected region. These microbial communities are likely fundamental to the formation of soil but also respond to the presence of these initial soils in a positive feedback mechanism allowing the development of substantial microbial communities in the uppermost layers. The uppermost active layer of permafrost deposits are, therefore, of central importance for carbon cycling in arctic permafrost.

3.6. **Conclusion**

In the current paper new data on the distribution of bacterial and archaeal communities in deposits on the western shore of Lake El'gygytgyn in the far east Russian Arctic are presented. Using lipid biomarkers as characteristic markers for bacteria and archaea, it was demonstrated that lake level changes during the deposition of the permafrost deposits affected the composition of the indigenous microbial community. The biomarker composition shows a boundary at around 9.5 m core depth (Allerød) indicating the transition between deposits that have been accumulated under subaquatic conditions during lake level highstand and the overlying deposits being formed under subaerial conditions during lake level lowstand. Especially, changes in the relative proportion of archaeal biomarkers suggest a change in the archaeal community at this transition. Compared to the sediment below the boundary, in the deposits being exposed to subaerial conditions the abundance of microorganisms slowly rises as indicated by the increase of the microbial GDGT and PLFA biomarkers as well as the bacterial and archaeal SSU rRNA gene copy numbers. The increase is particularly pronounced in the Holocene sequence, along with a significantly increase of the TOC content. The beginning of soil formation is suggested in the Holocene sequence stimulating microbial growth during the pedogenetic processes. The data show that the Holocene deposits at the western shore of Lake

El'gygytgyn contain relative abundant and active microbial communities. Gene analyses reveal that methanogens belonging to *Methanosarcinales* and *Methanomicrobiales* are part of the microbial community which are important members for the cycling of organic carbon in the uppermost permafrost layers. This is of central importance when considering the observed temperature rise in ongoing climate change will be most pronounced in the Arctic as a rise in the atmospheric temperature will in particular affect the uppermost layers of permafrost.

3.7. **Acknowledgements**

Funding for this research was provided by ICDP, the US National Science Foundation (NSF), the German Federal Ministry of Education and Research (BMBF), the Alfred Wegener Institute for Polar and Marine Research (AWI) and GeoForschungsZentrum Potsdam (GFZ), the Russian Academy of Sciences Far East Branch (RAS FEB), the Russian Foundation for Basic Research (RFBR), and the Austrian Federal Ministry of Science and Research (BMWF).

We highly appreciate the technical assistance and help of Anke Kaminski, Cornelia Karger, Janine Görsch, Patrick Häcker and Frederik Büks. This work was supported by the Deutsche Forschungsgemeinschaft (DFG) in the framework of the priority program 'International Continental Scientific Drilling Program' by a grant to D.W. (WA 1554/10) and K.M. (MA 2470/4).

4. Glacial-interglacial microbial community dynamics in Middle Pleistocene sediments in the Lake El'gygytgyn, Far East Russian Arctic

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Under review for publication in *the ISME Journal*.

4.1. Abstract

Arctic lakes represent an important source for the greenhouse gas methane and are particularly sensitive to global warming. Microbial communities in arctic lakes, especially their changes in abundance and composition in response to climate change are poorly understood. Here we show changing microbial abundances in response to glacial-interglacial variations in Middle Pleistocene sediments from Lake El'gygytgyn, NE Russian Arctic. A combined microbiological and organic geochemical approach revealed high abundances of bacterial and archaeal markers during interglacial intervals. This increase in microbial community size was most likely driven by a climatically induced increase in organic matter supply into the lake sediments. In particular, there

was a substantial increase of algae-derived, autochthonous organic matter during interglacial periods. The increase in abundance of archaea is accompanied by a higher diversity of methanogens and concomitant with high methane production potentials, suggesting elevated methane production from the lake during interglacial periods. Our findings demonstrate a previously unrecognized climate-driven impact on the microbial communities via changing organic matter supply and the significance of microorganisms for the carbon turnover in arctic lake sediments.

4.2. **Introduction**

Arctic regions are key areas which are sensitive to the on-going climate change (McGuire*, et al.*, 2009). Especially, arctic lake systems are suggested to be particularly vulnerable to climate changes. Small increases in the water temperature have been demonstrated to have a high influence on the ice coverage and water mixing (Vincent & Laybourn-Parry, 2008). These changes influence several other important environmental parameters such as bioproductivity, water mass stratification, exchange of greenhouse gases and, therefore, causing subsequent climate feedback processes on a spatial and larger time scale (ACIA, 2005). Given the large number of lakes in the Northern Hemisphere, about 47 % of all lakes world-wide are located north of 54 degrees north latitude (Lehner & Doll, 2004), it is essential to understand the dynamics of microbial communities that are involved in the cycling of carbon and their response to climate changes in lake systems of the Arctic.

Lake sediments contain buried organic matter (OM) that was either produced in the overlying water (autochthonous OM) or transported from the surrounding catchment area (allochthonous OM) and, therefore, provide the substrates for a wide variety of metabolically active microorganisms. This OM has been shown to degrade to acetate, hydrogen (H_2) and carbon dioxide (CO_2) in a cascade of processes by heterotrophic microorganisms and subsequently can be metabolized to methane by methanogenic archaea

(Conrad, *et al.*, 1989). Methanogenesis is considered as the most important terminal step in the degradation of OM in lake sediments and the produced methane is released by ebullition or diffusion (Glissmann, *et al.*, 2004). Lakes represent an important natural source of methane, contributing with 6-16 % (8-48 Tg CH4 a 1) to the total methane emission (Bastviken, *et al.*, 2004). Recent studies report ebullition of enormous amounts of methane from arctic thaw lakes, contributing significantly to the methane emission of arctic wetlands with 3.8 Tg CH4 per year (Walter, *et al.*, 2006).

Several studies characterised methanogenic archaea in lake sediments of temperate (Zepp Falz, *et al.*, 1999, Nusslein, *et al.*, 2001) and tropic (Conrad, *et al.*, 2010) environments. Studies on polar settings are limited to Antarctic sites, including Lake Fryxell (Karr, *et al.*, 2006) and Ace Lake (Coolen, *et al.*, 2004), where novel psychrophilic methanogenic species such as *Methanococcoides burtonii* and *Methanogenium frigidum* were isolated (Franzmann, *et al.*, 1992, Franzmann, *et al.*, 1997). However, little information is available on the response of the microbial communities in Arctic lakes, especially, to past climate changes and on the structure of methanogenic communities in deeper parts of the lake sediments. Furthermore, the question of intact and, thus, living and potentially metabolic active cells in this habitat remains poorly constrained.

The El'gygytgyn crater lake in Chukotka, far eastern Russia provides an excellent opportunity to reconstruct the effects of past climate conditions and changes of the microbial ecosystem in an Arctic lake. The crater lake is one of the best conserved impact structures on Earth and represents a unique climate archive in high latitudes due to its age, size and continuous sedimentation history (Layer, 2000, Melles, *et al.*, 2011). The lake sediments were drilled in the scope of the 'Lake El'gygytgyn Scientific Drilling Project' (Melles, *et al.*, 2012) in 2009 as part of the International Continental Scientific Drilling Programme (ICDP). The recovered El'gygytgyn lake sediments allow qualitative and quantitative investigation of the microbial ecosystem in response to glacial-interglacial changes in the Middle Pleistocene. In the present study, deposits comprising the marine isotope stages (MIS) 12 to 9

(ca. 480 ka to 300 ka BP) were examined using a broad set of molecular ecological and organic biogeochemical methods. A specific focus was placed on the dynamics of methanogenic communities being responsible for the biogenic production of the greenhouse gas methane.

4.3. **Materials and Methods**

4.3.1. Study site

The El'gygtgyn Crater Lake (N 67°30', E 172°05' E) is located in central Chukotka, far eastern Russia. A meteorite impact 3.6 million years ago (Layer, 2000) caused a crater of about 18 km in diameter that is partially filled with an off-centred lake of 12 km in diameter and a maximum water depth of 170 m (Melles*, et al.*, 2011). Modern climate in this region is characterised by cold temperatures with mean annual air temperatures of -10.3°C, extreme temperatures of 26°C in summer and -40°C in winter as well as an annual total precipitation of about 200 mm (Nolan & Brigham-Grette, 2007).

4.3.2. Drilling and sample preparation

Drilling of lake sediments was conducted in scope of the ICDP Lake El'gygytgyn Drilling project in 2009 (Melles*, et al.*, 2011). For further information on the drilling and processing of the cores see Melles, *et al.* (2011, 2012). Sampling of the cores was conducted at the University of Cologne. The samples were taken from inner parts of the core, to avoid potentially contaminated outer core sections. The subsamples for biomarker analyses were transferred in pre-heated brown glass jars. Samples for microbial analyses were put into sterile 15 mL tubes. All samples were frozen at -20°C for later analyses. For the current study 28 samples representing Middle Pleistocene deposits from MIS 12 to 9 were selected. Herein, the corrected middle field depth is referred to as 'depth'.

4.3.3. Sediment properties

Quantification of total organic carbon (TOC) was done on freeze-dried, homogenized lake sediments using an element analyzer (Elementar Vario max C, Germany). The TOC content was measured on samples after HCl (10%) acid digestion to remove the carbonate. The biogenic silica content was determined using a fourier transform infrared spectroscopy (FTIRS) approach, as described in Rosen*, et al.* (2010). The potential methane production rates in different depth horizons were determined on freshly thawed lake sediment samples as described previously in Bischoff*, et al.* (2013).

4.3.4. Lipid biomarker analyses

Ground and freeze-dried lake sediments were extracted with a mixture of organic solvents and the obtained sediment extracts were separated into fractions of different polarity by column chromatography using a protocol described in Bischoff*, et al.* (2013). Tetraether lipids and archaeol were analysed using an HPLC-APCI (high performance liquid chromatography-atmospheric pressure chemical ionization) mass spectrometry (MS) method described in Schouten*, et al.* (2007). For semi-quantitative determination of tetraether compounds an external synthetic archaeol standard (AvantiPolarLipids Inc., Al, USA) was regularly measured in parallel. Blanks (pre-heated sea sand) were measured regularly and did not contain any GDGTs or archaeol. For details on the applied method see Bischoff*, et al.* (2013).

4.3.5. Deoxyribonucleic acid (DNA) extraction and quantitative polymerase chain reaction (qPCR)

The total genomic microbial DNA was extracted in triplicates of 0.5 g lake sediment samples using a Power Soil™ DNA Isolation Kit (Mo Bio Laboratories Inc., USA) according to the manufacturer's protocol, modified with an additional step using a bead-beating system (FastPrep, MP biomedicals, US) for 20 s at 4 m s^{-1}. Afterwards triplicates were pooled for all

succeeding procedures. The SSU rRNA (16S) gene copy numbers of *Bacteria* and *Archaea* in the lake sediment samples were determined using qPCR analyses in triplicates. The primer pairs Uni331F/Uni797R (Nadkarni, *et al.*, 2002) and A571F/UA1406R (Baker, *et al.*, 2003) were used to amplify SSU rRNA gene fragments of *Bacteria* and *Archaea*, respectively. The qPCR reactions were carried out in an iQ5 qPCR Cycler (Biorad, USA) and a Rotor Gene Q cycler (Qiagen, Germany) using a Power Sybr Green PCR Master Mix Kit (Applied Biosystems, US; for bacteria) or Rotor-Gene SYBR Green PCR Kit (Qiagen, Germany; for archaea), 0.5 mM of each primer, and 1 µl of purified and diluted DNA-Extract (1:10). The qPCR conditions were as follows: initial denaturation 95 °C for 10 min, 40-50 cycles of denaturation at 95 °C for 20 s, annealing at 57 °C for 20 s and elongation at 72 °C for 45-90 s. Melting curve analyses ensured the correct amplification of the target sequences. The respective standard curves were generated using known dilutions (10^1 – 10^7 gene copies) of the target fragment amplified from *Bacillus subtilis* (DSM 10) and *Methanosarcina vacuolata* (DSM 1232).

4.3.6. PCR amplification of methanogenic SSU rRNA genes

The amplifications of archaeal SSU rRNA genes were conducted in triplicates using a nested approach of a first amplification with the universal archaeal primers ArUn4F (Hershberger, *et al.*, 1996) plus Ar958R (DeLong, 1992). This was followed by a second amplification with the methanogenic archaea specific primer set GC_0357F/0691R (Watanabe, *et al.*, 2004) for the denaturing gradient gel electrophoresis (DGGE) analysis. For further details on the PCR/DGGE methods and the phylogenetic analyses see Bischoff, *et al.* (2013).

4.4. **Results**

4.4.1. Sedimentary TOC and biogenic silica concentration

The TOC concentrations ranged between 0.1 and 3.4 wt% throughout the selected lake sediments (Fig. 1a). In 5 samples the TOC contents were below the detection limit (<0.1%). The highest TOC concentrations were measured in an interval from 18.9 to 18.1 m depth with up to 3.4 wt%. TOC concentrations were also high at around 21 m and between 16.4 - 15.6 m depth with about 1 wt%. In the remaining core the average TOC concentrations were not above 0.4 wt%. The concentration of biogenic silica ranged from 7-43 % throughout the selected lake sediment sequence (Fig. 1a). The highest amounts were determined for the interval between 19.1 and 18.1 m as well as 16.4 and 15.6 m depth. The remaining core exhibited average values of biogenic silica of around 9%.

4.4.2. Quantification of bacterial and archaeal genes

Archaeal and bacterial SSU RNA genes were quantified for all depths of the selected lake sediments (Fig. 1b). Bacterial gene copy numbers varied between 7.9 x 10^3 and 2.3 x 10^7 copies g^{-1} sediment (Fig. 1b). Highest numbers were measured in two intervals between 18.9-18.1 m and 16.4-15.6 m depth, where gene copy numbers of 10^6-10^7 copies g^{-1} sediment were determined. Throughout the remaining depths, bacterial gene copy numbers were rather stable with on average 10^4 copies g^{-1} sediment. Archaeal gene copy numbers ranged from 8.5 x 10^2 to 3.7 x 10^6 copies g^{-1} sediment (Fig. 1b). Highest gene copy numbers (10^5-10^6 copies g^{-1} sediment) were determined in 18.9-18.1 m and 16.6-15.6 m depth. Additionally, one sample at 21 m was

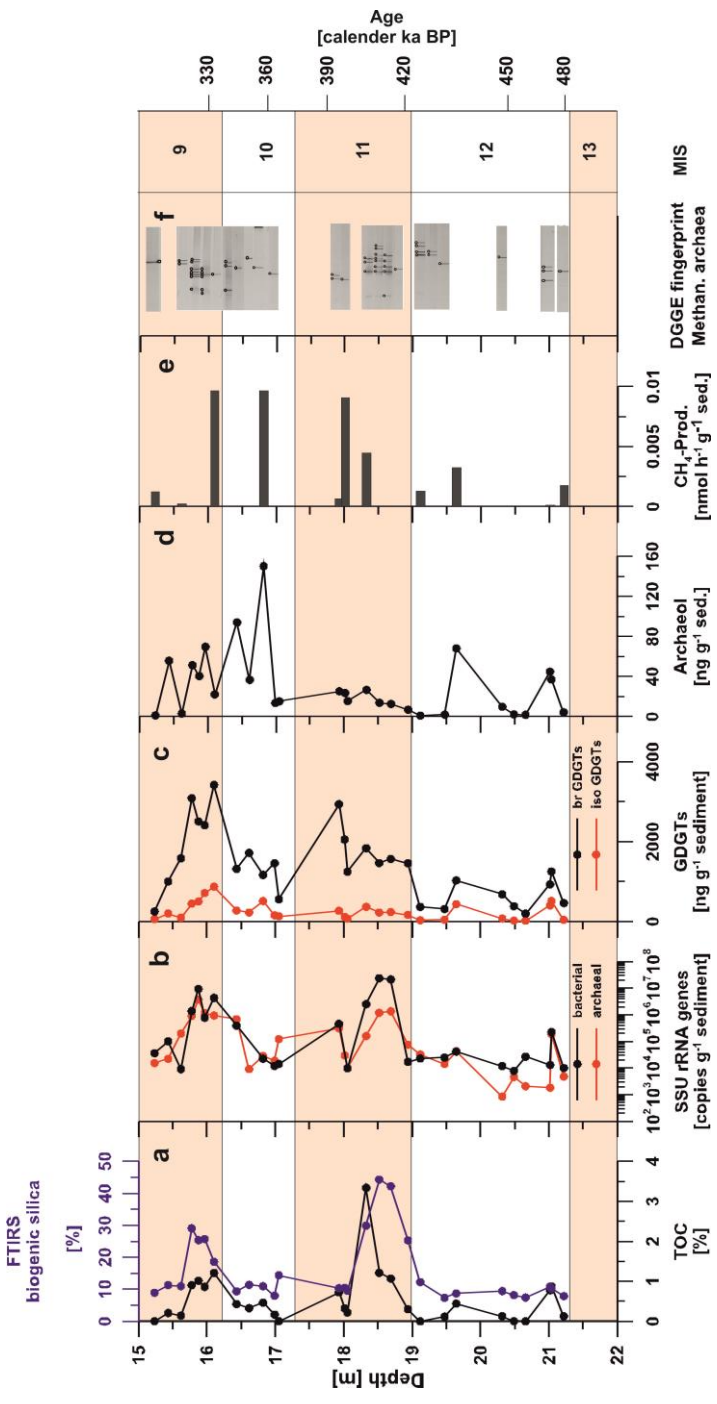

Figure 1 *Vertical profile of El'gygytgyn Lake sediments from 21-15 m depth showing (a) the total organic carbon (TOC) and biogenic silica concentration in wt%, (b) the abundance of bacterial (black line) and archaeal (red line) small subunit ribonucleic acid (SSU RNA) genes in copies g-1 sediment, (c) the total branched (= bacterial lipid markers, black line) and isoprenoid (= archaeal lipid markers, red line) glycerol dialkyl glycerol tetraether (GDGT) in ng g-1 sediment, (d) the concentration of archaeol in ng g-1 sediment, (e) the potential methane production in nmol CH4 h-1 g-1 sediment, and (f) the fingerprint pattern of methanogenic archaea retrieved from denaturizing gradient gel electrophoresis (DGGE) with depth. The underlining reconstruction of marine isotope stages is defined according to Melles, et al. (2012), whereas inter-glacial periods are highlighted in orange.*

characterised by an increase of both bacterial and archaeal gene copy numbers.

4.4.3. Quantification and composition of lipid biomarkers

Branched and isoprenoid glycerol dialkyl glycerol tetraethers (GDGTs) are microbial lipid biomarkers that were used as characteristic markers for bacteria and archaea, respectively (Weijers*, et al.*, 2006). Both were extractable throughout the whole selected El'gygytgyn lake sediments, but their individual abundances varied with depth (Fig. 1c). Overall, the amounts of total branched GDGTs ranged from ca. 200-3400 ng g^{-1} sediment (Fig. 1c) and account for 72-95 % of the total GDGTs. The sediments between 21.1-19.1 m depths are characterized by lowest amounts (< 1000 ng g^{-1} sediment) of branched GDGTs compared to the remaining core interval. Overall, high amounts of total branched GDGTs with 2000-3400 ng g^{-1} sediment were detected in the intervals between 18.9-17.1 m and 16.4-15.1 m depth. The amounts of total isoprenoid GDGTs ranged from ca. 20-870 ng g^{-1} sediment throughout the sequence (Fig. 1c) and account for 6-20 % of the total GDGTs. The profile of the isoprenoid GDGTs mimics the branched GDGT curve although with a generally lower abundance. The concentration of archaeol (Fig. 1d) varied widely throughout the analyzed lake sediment sequence with values from 1 to 150 ng g^{-1} sediment. In particular the intervals from 21 to 19.6 m and 16.8 to 15.8 m depth were characterized by high concentrations of archaeol. In the remaining depth the amount of archaeol was rather low with a mean value of 18 ng g^{-1} sediment.

4.4.4. Potential methane production

The methane production potential was determined for 11 samples from the selected El'gygytgyn lake sediments (Fig. 1e). The potential to produce methane varied throughout the core, indicating intervals of almost no methane production potential (< 0.001 nmol CH4 g^{-1} h^{-1}) and intervals with a comparatively higher potential to produce methane with a rate of 0.01 nmol CH4 g^{-1} h^{-1}. Methane production rates were low in sediments of 21.2-19.1 m

depth, but increase towards the top of the sediments with highest rates observed at 18.0 m, 16.8 m and 16.1 m depth.

4.4.5. Methanogenic community composition

The 16S SSU genes of methanogenic archaea could be amplified in 22 samples from different depth horizons of the lake sediments. Subsequently, DNA fingerprint analyses using DGGE were conducted and resulted in a variable methanogen fingerprint pattern along the sequence (Fig. 1f). In total, 67 distinct bands could be recovered; whereas 56 successfully sequenced DNA bands of sufficient length (> 200 basepairs) were obtained. The occurrence of DNA from methanogenic archaea could be shown in all investigated deposits along the sequence with an age back to 480 ka BP. Generally, the highest numbers of up to 7 bands were detected in the intervals from 19.1-18.3 m and 16.1-15.8 m depth (Fig. 1f). Throughout the remaining depths the number of bands remains rather low with only one band, with the exception of 21.0, 19.5, 17.9 and 15.6 m depth, were two bands, respectively, are apparent. 92 % of the successfully sequenced phylotypes were classified as *Archaea*. Among these sequences 33 could be assigned to *Euryarchaeota* and additionally to *Methanomicrobia* and another 23 sequences were assigned to *Crenarchaeota*. Methanogenic archaea sequences recovered from the lake sediments could be classified further as members of *Methanosarcinales, Methanocellales* and *Methanomicrobiales*. A phylogenetic tree shows the affiliation of the single sequences received from DGGE analyses to known methanogenic archaea (Figure 2).

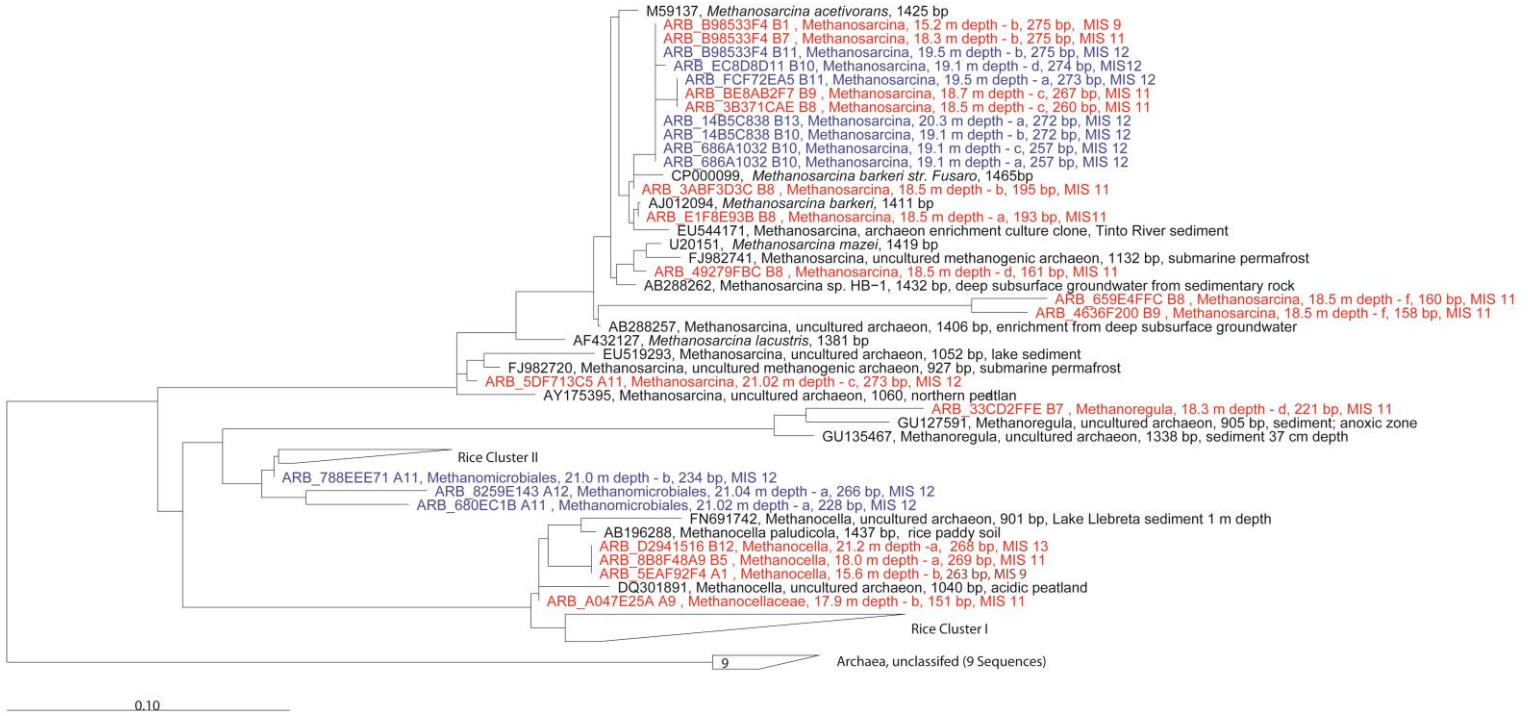

Figure 2: *Phylogenetic tree showing the relation of 16S rRNA gene sequences of methanogenic archaea obtained from El'gygytgyn lake sediments. The tree represents a maximum parsimony tree based on sequence data provided by Silva Database, but due to a better overview only selected data are shown. The sequences are labelled with accession number, name of pure culture or clone and isolation source. The sequences obtained within this study are labelled with depth and band number, length of amplified fragment in basepairs (bp), respectively. Furthermore, a colour code was added indicating sequences retrieved from glacial (blue) and interglacial (red) deposits.*

4.5. **Discussion**

Extensive microbial communities could be shown for sediments deposited during interglacial periods MIS 11 and 9 in contrast to the glacial periods MIS 12 and 10, as indicated by the increased amounts of archaeal and bacterial SSU RNA gene copies in the respective sediments. A comparable distribution of the qPCR data is reflected in the microbial biomarker profiles with increased amounts of microbial markers being found distinctively in MIS 11 and 9, when compared to MIS 12 and 10. Branched and isoprenoid GDGTs were used as characteristic biomarkers for bacteria and archaea, respectively. These tetraetherlipids were identified to be solely of either bacterial (=branched) or archaeal (=isoprenoid) origin due to their chain architecture and the stereo configuration of the glycerol backbone (Weijers, *et al.*, 2006). In our study these lipid compounds were measured as core lipids that remain stable outside intact cells over geological times. Thus, the abundance of these respective biomarkers does not solely indicate the amount of recent microorganisms, but a signal that accumulated during the last 480 ka. Therefore, these compounds are suitable to reconstruct past microbial communities and their responses to climate changes. An increase in the abundance of branched and isoprenoid GDGTs in the respective sediments can, thus, be interpreted as an increase in the abundance of microorganisms during these times. Generally, the archaeal (=isoprenoid) GDGT profile shows the same variations as the bacterial (=branched) GDGT curve, although in a much lower abundance.

Temperature changes during the glacial-interglacial variations have been demonstrated to be a possible driver for variations in the abundance of microbial communities. Bischoff, *et al.* (2013) for instance observed previously for Siberian permafrost deposits that temperature changes in the scope of glacial-interglacial variation had a direct impact on the abundance and composition of the methanogenic community in a Late Pleistocene to Holocene permafrost deposits. Warm and wet periods are characterized by a higher abundance and diversity of methanogens and the composition of the

methanogenic species shows an adaptation to the prevailing temperature conditions. However, in lake sediments a direct effect of the temperature conditions on the lake sediments and, therefore, on the microbial community is not expected, due to the buffering effect of the overlying lake water with the lake bottom temperatures being suggested to remain relatively constant during interglacial and glacial times. The lake sediments contain no permafrost. Thus, when the direct effect of the temperature change on the microorganisms in the lake sediments is only minor, the question arises, why is there a glacial-interglacial variability preserved in the microbial community data?

A reason for this can be found in the quantity and quality of the available carbon sources. As indicated by the TOC profile, interglacial intervals with high bacterial and archaeal gene copy numbers are also characterized by high TOC contents. Generally, there are two conceivable sources of the organic matter (OM) in the El'gygytgyn lake sediments. First it originates from production within the overlying water (autochthonous OM) and second it is transported from the surrounding catchment area (allochthonous OM) into the lake.

Diatoms are considered to be the major contributors to the intra-lake bio-production (Cherapanova*, et al.*, 2007). Thus, the percentage of biogenic silica, mostly derived from diatoms, is taken as a proxy for the bioproductivity in Lake El'gygytgyn. We can show significant proportions of biogenic silica in particular for the interglacial periods MIS 11 and 9, indicating a large contribution of OM provided by algae to the carbon pool of the lake during these times. Additionally, high algae productivity of dinoflagellates and eustigmatophyte algae during these interglacials was shown by D'Anjou*, et al.* (2013) using an algae biomarker approach. Bioproduction and TOC content are highest in MIS 11. This period is considered as the longest and warmest interglacial during the last 500 ka (Howard, 1997), characterized by overall warm sea-surface temperatures in high latitudes, strong thermohaline circulation, unusual blooms of calcareous plankton in high latitudes, higher sea level than the present and coral reef

expansion. Thus, high amounts of OM were produced in the water column of Lake El'gygytgyn during the interglacials and provided substrates for the indigenous microorganisms. The OM produced by algae in the water column is described to be of high compositional quality and high biological availability (Nguyen*, et al.*, 2005), explaining the distinct and noticeable effect on the microbial community.

Additionally, allochthonous sources of OM have to be considered. Although branched GDGTs were previously considered to be of terrestrial origin and, therefore, used as a marker for the input of terrestrial matter into aquatic systems (Hopmans*, et al.*, 2004), more recent studies clearly indicate a possibility of branched GDGTs being produced in lakes (Tierney & Russell, 2009). Thus, the branched GDGT signal in the lake sediments might be a mixture from both autochthonous and allochthonous sources. The good correlation between amounts of branched GDGTs and bacterial SSU RNA gene copies suggests that a significant part of the branched GDGTs was produced within the lake system. However, a supply of terrestrial GDGTs might be an explanation for some of the differences between the branched GDGT biomarker and qPCR profiles, especially during the MIS 11.

The impact of glacial-interglacial cycles on the microbial communities was also shown for methanogenic archaea, which are the major drivers of OM degradation under anoxic conditions. The archaeal qPCR and isoprenoid GDGT biomarker data indicate a higher abundance of archaeal communities during certain intervals in the interglacial periods (MIS 11 and 9). Additionally, we demonstrate that the variety in the community of methanogenic archaea is higher during interglacial periods as can be seen in the pronounced DGGE fingerprint band pattern in sediments of MIS 11 and 9. The development of a more diverse methanogenic community was likely possible due to higher substrate availability during interglacial periods. Experiments of the methane production potential revealed high potential for methane generation during the interglacials. Overall, the data indicate

production and subsequent release of methane from the lake during interglacial periods.

In contrast to the warmer periods, in sediments deposited during glacial times (MIS 12 and 10) a lower number of methanogenic DNA bands could be recovered and with one exception also showed a lower methane production potential. This might be related to reduced substrate availability, as indicated by lower TOC and biogenic silica contents. However, archaeol, a biomarker for methanogens (Pancost*, et al.*, 2011), shows also some intervals of higher abundances during the glacial periods MIS 12 and especially MIS 10. Additionally, these high archaeol signals are accompanied by significant methane production potentials. This indicates that in the El'gygytgyn Lake sediments methane might also be produced during glacial periods. This makes sense since the effect of colder air temperatures on the lake sediments is buffered by the lake water. If there is still a sufficient amount of OM in the glacial sediment, which can be degraded, production of methane is conceivable. This assumption is supported by preliminary studies on the winter methane fluxes which showed elevated methane concentrations in the El'gygytgyn Lake ice (Melles*, et al.*, 2005). This is also in accordance with recent studies on lipid biomarkers as indicators for key sources of organic matter showing a number of productive intervals during cold glacial periods (Holland*, et al.*, 2013).

As indicated before, the strictly anaerobic methanogenic archaea are responsible for the biological methane production. Their community composition and ecological function, particularly in deeper lake sediments is poorly constrained. Our data provide a first phylogenetic fingerprint characterization of methanogens in selected El'gygytgyn Lake sediments and shows the abundance of three different orders of methanogenic archaea in up to 480 ka old sediments: *Methanosarcinales, Methanocellales* and *Methanomicrobiales*. The results show that the abundance of these orders changes with depth. Closest relatives to *Methanosarcina* were detected throughout the whole investigated lake sediment profile, while sequences

related to *Methanocellales* and *Methanomicrobiales* were only found in certain horizons of the sediment.

Methanosarcina species in general were isolated from a broad range of environments, including several psychrotolerant isolates from temporarily or permanently cold environments such as permafrost-affected soils (Wagner, *et al.*, 2013), Holocene and older permafrost deposits (Krivushin, *et al.*, 2010, Shcherbakova, *et al.*, 2011) and anoxic, cold lake sediments (Simankova, *et al.*, 2001). *Methanosarcina*-related sequences were also regularly found in permafrost-affected soils and sediments (Ganzert, *et al.*, 2007, Koch, *et al.*, 2009, Bischoff, *et al.*, 2013). This is in accordance with our results indicating that affiliates of *Methanosarcina* species are ubiquitous in the El'gygytgyn Lake sediments. This shows that *Methanosarcina*-related microorganisms are obviously less influenced by chemical and physical changes in the lake system throughout the glacial-interglacial variations in contrast to the relatives of *Methanocellales* and *Methanomicrobiales* that were also found. One reason for this tolerance might be the potential of *Methanosarcina*-like species to use all three metabolic pathways with a broad range of substrates, e.g. acetate, methanol and hydrogen (Conrad, 2005), giving them a high flexibility to react on changing substrate concentrations. Homoacetogenesis for instance is a well-known syntrophic process, which dominates in low temperature ecosystems such as lake sediments (Conrad, *et al.*, 1989). Another reason might be the specific tolerance and resistance of *Methanosarcina* species against several abiotic and biotic factors, as recently shown for the new strain *Methanosarcina soligelidi* (Morozova & Wagner, 2007, Wagner, *et al.*, 2013).

In contrast, members of *Methanocellales* and *Methanomicrobiales* show a more sensitive response to glacial-interglacial variations and the consequent chemical and physical changes throughout the lake. *Methanocellales* represent the new order for the formerly called 'Rice Cluster I' lineage of methanogenic archaea (Sakai, *et al.*, 2008). This archaeal group plays a key role in the methane production in rice fields (Conrad, *et al.*, 2006) and, as far as we know, had never been detected in permafrost environments (Ganzert,

et al., 2007, Koch*, et al.*, 2009), which is the catchment for the El'gygytgyn Lake. In contrast to *Methanosarcina* species all three known isolates of *Methanocella* can utilize H_2/CO_2 and formate for growth and methane production with highest activity under low hydrogen partial pressure and temperatures between 36°C and 55°C (Sakai*, et al.*, 2008, Sakai*, et al.*, 2010, Lu & Lu, 2012). The usually adaptation of these organisms to moderately high temperatures might be the reason why *Methanocellales*-related sequences in this study were predominantly found in sediments deposited in interglacial stages MIS 11 and MIS 9. Especially during the warm interglacial MIS 11, which was also characterized by the highest OM contents and highest archaeal gene copy numbers, representatives of *Methanocellales* might play an important role for the methane production within the lake sediments.

Finally, *Methanomicrobiales*-related sequences were mainly found in the colder glacial period MIS 12 (481-424 ka BP). Members of the order *Methanomicrobiales* are characterized by few unique properties, such as prossessing unique membrane lipids (Dworkin & Falkow, 2006) and using secondary alcohols as electron donors among growth on H_2/CO_2 and formate (Zellner & Winter, 1987). They are also known to be involved in degrading particularly complex organic compounds (Qiu*, et al.*, 2004). Lower temperatures lead to an alteration of the carbon and electron flow in the methanogenic degradation pathway of organic matter (Chin & Conrad, 1995). Thus, the shift to lower temperature resulted in a decrease in the steady-state H_2 partial pressure and a transient accumulation of acetate, propionate, caproate, lactate, and isopropanol. Because of the metabolic versatility of psychrophilc homoacetogenic bacteria, they are strong competitors of fermenting bacteria and hydrogenotrophic methanogenic archaea (Drake*, et al.*, 1997). Secondary alcohols can be, therefore, an alternative substrate for methanogens like species of the order *Methanomicrobiales*, which can explain their dominance in the respective glacial period and show their significance for methane production under colder conditions.

The presented results provide comprehensive evidence of the reaction of microbial communities in the El'gygytgyn Lake sediments to glacial-interglacial variations. In contrast to permafrost deposits, where a direct climatic impact on the microbial communities was demonstrated (Bischoff*, et al.*, 2013), in the studied lake sediments the abundance of the microbial communities is most likely indirectly coupled to glacial-interglacial cycles via the autochthonous OM production in the lake. This OM input stimulated the growth of microbial communities especially during interglacial periods and an increased production and release of the greenhouse gas methane can be expected during this period. Furthermore, the current data on the sediments in Lake El'gygytgyn shows the potential for methane emissions also during cold periods and give first evidence for the involved microorganisms. This underlines the potential of arctic lakes as significant sources for climate relevant trace gases not only during warmer interglacials but also during colder glacial times.

4.6. **Acknowledgements**

The El'gygytgyn Lake sediments were recovered in the scope of the ICDP project "Scientific Drilling at El'gygytgyn Crater Lake" in 2009. Funding for this project was provided by the ICDP, the US National Science Foundation (NSF), the German Federal Ministry of Education and Research (BMBF), the Alfred Wegener Institute for Polar and Marine Research (AWI) and the GeoForschungsZentrum Potsdam (GFZ), the Russian Academy of Sciences Far East Branch (RAS FEB), the Russian Foundation for Basic Research (RFBR), and the Austrian Federal Ministry of Science and Research (BMWF). The authors wish to thank the El'gygytgyn field parties, especially Martin Melles (University of Cologne), Julie Brigham-Grette (University of Massachusetts Amherst), Pavel Minyuk (Northeast Interdisciplinary Scientific Research Institute) and Christian Koerberl (University of Vienna). The Russian GLAD 800 drilling system was developed and operated by DOSECC Inc., the

downhole logging was performed by the ICDP-OSG, and LacCore, at the University of Minnesota, handled core curation." We highly appreciate the technical assistance of Anke Kaminsky, Cornelia Karger, Oliver Burckhardt (all GFZ) and the laboratory help of Patrick Häcker and Frederik Büks. This work was supported by the Deutsche Forschungsgemeinschaft (DFG) in the framework of the priority program 'International Scientific Continental Drilling Program' by a grant given to DW (WA 1554/10) and KM (MA 2470/4).

5. **Synthesis**

The Arctic is a globally significant carbon store containing about 1672 Pg (10^{15} g) of carbon, which is twice the amount of carbon that is currently in the atmosphere (Tarnocai*, et al.*, 2009). A large proportion of this carbon, estimated to be around 50% of the global soil carbon, is stored below the ground in Arctic permafrost. This permafrost is known to respond particularly sensitive to an increase in global temperatures and is therefore a focal point of on-going climate change research. Rising temperatures in the Arctic, amongst other severe consequences, cause progressive deepening and duration of permafrost thawing during the arctic summer, creating an 'active layer' with high bioavailability of nutrients and labile carbon for microbial consumption. The microbial mineralization of permafrost carbon creates large amounts of greenhouse gases, including carbon dioxide and methane, which can be released to the atmosphere, creating a positive feedback to global warming. However, to date, the microbial communities that drive the overall carbon cycle and specifically methane production in the Arctic are poorly constrained.

To contribute to the elucidation which and how arctic microbial soil and sediment communities respond to changing environmental conditions, this study utilised three cores from different arctic settings: permafrost, lake shore and non-permafrost lake deposits from Siberia and Chukotka in the Russian Arctic (Table 1). Using a combination of microbial and molecular organic geochemical techniques, these studies characterise and identify the climate response of microbial communities involved in past carbon cycling.

Table 1: Study sites and age of collected deposits.

Location	Type	Geological period
Kurungnakh Island, central Lena Delta	Terrestrial permafrost deposits	Late Pleistocene - Holocene
Lake El'gygytgyn, Chukotka	Lake shore terrestrial permafrost deposits	Allerød - Holocene
Lake El'gygytgyn, Chukotka	Lake sediments	Middle Pleistocene

The following synthesis highlights the key outcomes of this study, namely (1) the reaction of microbial communities to past climate change and (2) the response of microbial communities to carbon composition and availability. The scientific evidences for this synthesis is based on three manuscripts (Bischoff*, et al.*, 2013, Bischoff*, et al.*, draft, Bischoff*, et al.*, submitted) focussing on the three different study sites and which are presented in chapters 2, 3 and 4. Finally, the implications of this research, the questions raised and possible future approaches for further research are summarized.

5.1. **The reaction of microbial communities to past climatic change in the Arctic**

With the Arctic region being most strongly affected by on-going and future predicted global temperature increases, the question arises *how microbial communities involved in the cycling of carbon will respond?* To better understand and constrain the potential future development of microbial communities in the changing Artic, this PhD thesis investigated in great detail the response of microbial communities, particularly methanogenic archaea to environmental and climate variability in the past.

This study for the first time demonstrates the responses of the abundance and composition of microbial communities to Middle Pleistocene and Late Pleistocene to Holocene climate variability in Arctic permafrost and lake sediments.

Previous microbiological studies have mainly focussed on surface or near-surface samples of the active layer in the Arctic (e.g. Høj*, et al.*, 2005, Ganzert*, et al.*, 2007, Yergeau*, et al.*, 2010). By using deposits of Middle Pleistocene to Holocene age, this study substantially extends the depth and stratigraphic range of previous work. Unique methanogenic community records from gene and lipid biomarkers in different stratigraphic units could be reconstructed that developed in response to the full temperature range between past glacial and modern interglacial conditions.

This study provides evidence that previous warmer periods were associated with an expansion of bacterial and archaeal communities in the Russian Arctic, similar to present day conditions. Different from this situation, past glacial and stadial periods experienced a substantial decrease in the abundance of *Bacteria* and *Archaea,* with broad implications for the cycling of carbon and the production and likely the subsequent emission of greenhouse gases.

The climate-driven response of methanogenic archaea is evident in terrestrial permafrost deposits that archive the transition of temperature changes in the Late Pleistocene and Holocene (Bischoff*, et al.*, 2013). Records demonstrate high amounts of methanogenic archaea in sediments from past warm periods, such as the Middle Weichselian (Kargin) interstadial and the Holocene (Wetterich*, et al.*, 2008) were associated with high concentrations of archaeol, a diagnostic biomarker for methanogenic archaea (Pancost*, et al.*, 2011). In contrast, periods of colder and generally drier climate show less abundant communities of methanogenic archaea and decreasing concentrations of archaeol. These findings represent the first record of archaeol in permafrost deposits of the Russian Arctic combined with detailed information on the abundance and composition of past methanogenic communities (Bischoff*, et al.*, 2013). DNA-based analyses confirm a higher methanogenic variety during warm periods, when compared to colder periods. A likely reason for this contrasting pattern is that warm and humid interglacial and interstadial climate conditions favored extended flooded lowlands with water-saturated sediments, which would have supported widespread anaerobic conditions and the growth of methanogenic archaea. Support for this concept comes from new evidence from terrestrial permafrost deposits on the western shore of Lake El'gygytgyn (Bischoff*, et al.*, draft). Here, a similar feedback of warm climate on microbial communities was observed for the transition of Allerød - Younger Dryas – Holocene.

Similar to the observations from permafrost environments, microbial communities in sediments of Lake El'gygytgyn also demonstrate changing abundances in response to glacial-interglacial variations during the Middle

Pleistocene. Within this study the first record of a climate-driven microbial response for any Arctic lake setting (Bischoff*, et al.*, submitted) was presented. The data suggest that extensive microbial communities developed in the water column and in the uppermost lake sediments during interglacial periods, but they declined as climate cooled towards glacial conditions. Remarkably, this characteristic pattern of high and low microbial abundance is still preserved in a variety of gene and lipid markers remnants of microbial communities, demonstrating the unique preservation potential of this microbial information in Arctic sediments. These results from the different Arctic settings (permafrost and lake) support the conclusion that the observed climate-microbiology relationships are of wider, possibly even Pan-Arctic, relevance.

This study demonstrates that climate fluctuations changes in the composition of the methanogenic community throughout the whole Russian Arctic.

The adaption potential of the methanogenic community to changing environmental and climate conditions was discussed based on data from the terrestrial permafrost sequence on Kurungnakh Island (Bischoff*, et al.*, 2013). It was found that Late Pleistocene deposits contained methanogenic sequences that were strongly affiliated with the known genera *Methanococcoides* and *Methanosarcina* and exhibit a diverse genetic affiliation. Due to the favorable warm and wet growth conditions during the Middle Weichselian Interstadial; Kargin (Wetterich*, et al.*, 2008), a diverse and abundant community of methanogenic archaea could develop. Instead, during colder climate conditions (Late Weichselian Stadial, Sartan; Wetterich*, et al.*, 2008) a dominance of sequences affiliated to the psychrophilic microorganism *Methanococcoides burtonii* isolated from the anoxic hypolimnion of Ace Lake, Antarctica *(Franzmann, et al., 1992) and Methanococcoides alaskense*, isolated from marine sediments in Skan Bay, Alaska (Singh*, et al.*, 2005), was found. This evidence is consistent with an overall reduction in the abundance of methanogenic communities in response to cold and dry climate conditions.

These new findings confirm that past climate conditions are conserved in the composition of methanogenic archaea, providing a possibility for future studies to deduce climate conditions from the abundance and composition of methanogenic archaea.

Also in the sediments of Lake El'gygytgyn comparable adaptions within the methanogenic community were confirmed for glacial-interglacial variations (Bischoff*, et al.*, submitted). Members of *Methanocellales* and *Methanomicrobiales* show a sensitive response to general climate variations and the consequent chemical and physical changes throughout the lake. *Methanocellales*-related sequences in this study were predominantly found in sediments deposited in interglacial periods. In contrast, *Methanomicrobiales*-related sequences were mainly found in sediments deposited during glacial period. These findings support the conclusion that different methanogenic archaea in Lake El'gygytgyn contributed to methane production and possibly emissions during warmer or colder periods. Despite these temperature adaptions, this PhD thesis also identified methanogenic archaea that seem to be less influenced by climatic and environmental changes.

***Methanosarcinales*–related members of methanogenic archaea can adapt to changes in their environments and are less susceptible to changing climate conditions.**

In particular, sequences assigned to the order *Methanosarcinales*, family *Methanosarcinaceae* could be detected throughout the analysed permafrost as well as lake sediment samples that were deposited under cold and warm climate conditions (Bischoff*, et al.*, 2013, Bischoff*, et al.*, draft, Bischoff*, et al.*, submitted). The reason why only some methanogenic archaea are ubitiquiously abundant and able to adapt to changing climate conditions is likely related to their genetic constitution and potential to adapt to changing abiotic and biotic factors, e.g. by using different biochemical pathways or withstand changing environmental conditions. Recently, a *Methanosarcina solegelidi* species, isolated from Siberian permafrost was described to have a high resistance against various stress factors (Morozova & Wagner, 2007,

Wagner*, et al.*, 2013) and metabolize different organic substrates by using all known methanogenic pathways (Conrad, 2005).

5.2. The response of microbial communities to carbon composition and availability

Given the huge amounts of carbon stored in Arctic permafrost areas (Tarnocai*, et al.*, 2009), this study specifically addressed the influence of organic carbon quantity and quality on microbial communities in the Russian Arctic.

This study shows that the availability of soil organic carbon is a critical factor in the abundance and diversity of microorganisms throughout all analysed study sites.

Extensive abundances of microbial communities were demonstrated during intervals of high organic matter content in sediments from Lake El'gygytgyn (Bischoff*, et al.*, submitted), from terrestrial permafrost deposits in Siberia (Bischoff*, et al.*, 2013, Bischoff*, et al.*, draft) and – notably – also for the TOC-limited gravel-rich permafrost site at the western shore of Lake El'gygytgyn (Bischoff*, et al.*, draft). Evidence is presented that demonstrates an up to 10-fold increase in the abundance of *Archaea* and a 10^3-fold increase in the *Bacteria* when increased organic substrates are present in the deposit. Additionally, increased amounts of carbon substantially increase the variety of methanogenic archaea. These observations from three different Arctic settings imply that sedimentary organic carbon acts as critical substrate that is metabolized in a cascade of microbial processes by a broad consortium of indigenous heterotrophic bacterial communities, including methanogenic archaea.

Changes in the organic matter quality, determined by climate boundary conditions, affect the abundance of microbial communities in lake sediments and terrestrial permafrost.

Initial data on the characteristic of organic matter by Rock Eval Pyrolyses throughout the terrestrial permafrost deposits on Kurungnakh Island, reveal

compositional differences between the organic matter based on changes in the Hydrogen Index from low values (~ 200 mgHC/g TOC) in the underlying, older Ice complex in comparison to high values (~450 mgHC/g TOC) in the less altered organic matter in the uppermost Holocene layers (Bischoff*, et al.*, 2013). High microbial abundances and diversities, in particular in the uppermost soil layers from warm climatic conditions, support a high bioavailability of younger carbon for microbial turnover. A similar impact of 'fresh', younger carbon on microbial abundances and diversities in relation to warm climate could also be shown for Lake El'gygytgyn (Bischoff*, et al.*, submitted). During interglacial periods the lake experienced significant production of autochthonous organic matter, as evidenced by the abundance of biogenic silica, a proxy for the presence of diatoms (Rosen*, et al.*, 2010, Melles*, et al.*, 2012) This increase of autochthonous organic matter was directly coupled to extensive microbial growth, suggesting that algae-derived organic carbon is largely available for the microbial communities in this lake system (Bischoff*, et al.*, submitted). If correct, this argues that algae-derived organic matter is metabolised by a network of heterotrophic bacteria, providing substrates for the growth of methanogenic archaea. This would then allow large amounts of methane to be produced during interglacial periods and likely emitted to the atmosphere. This carbon-mediated response of the intra-lake bacterial and archaeal communities to glacial-interglacial changes has not been shown for an Arctic lake before and thus this study presents first evidence of the response of microbial communities in an Arctic lake setting to long-term climate change.

5.3. **Implications from this study for future research.**

From year to year, natural climate variability influences Arctic temperatures, with both cold and warm periods. However, long-term observations clearly show the rising temperatures in the Arctic. For example from 2001 – 2011 no part of the Arctic was colder than the long-term average (ACIA, 2012).

Furthermore, current models suggest that the Arctic warms twice as much as the rest of the Earth (IPCC, 2007).

This PhD thesis provides first evidence that past climatic warming led to an increased abundance of microbial communities in the Arctic, closely linked to the cycling of carbon and methane production.

With predicted climate warming, it may, therefore, be anticipated that extensive amounts of microbial communities will develop. Increasing temperatures in the Arctic will particularly affect the temperature sensitive parts of the current microbiological communities, possibly leading to an extinction/suppression of cold adapted species and the prevalence of methanogenic archaea that tolerate or adapt to the increasing temperature. These changes in the composition of methanogenic archaea will likely increase the methane production potential of high latitude terrestrial regions. However, the interrelationship between microbial communities, carbon cycling and the resulting methane and carbon dioxide emissions in a warming Arctic need to be further constraint.

The increase in atmospheric and ground temperature will intensify the destabilization of currently permanently frozen deposits (IPCC, 2007). Recent predictions estimate that by the end of this century 61 to 399 Pg of permafrost stored carbon will thaw and, thus, represent a large pool of organic carbon that will become available for microbial consumption (Harden, et al. 2012). The observations from this study suggest that an increase in the bioavailability of labile organic carbon in near surface deposits or within Arctic lakes would provide organic substrates for the growth of heterotrophic and methanogenic microorganisms. This will likely enhance the decomposition rate of young and old carbon storages and consequently increase emissions of carbon dioxide and methane to the environment. This scenario is supported by models, suggesting that the Arctic will gradually change from a carbon sink to a carbon source by 2020 (Schaefer, et al., 2011).

To fully understand the methane and carbon dioxide emissions from the Arctic, it is necessary to constrain the entire cycle of carbon consumption and

production. This PhD thesis contributes to this extremely broad question by providing a range of fundamental insights into the microbial driven processes and relationships within the methane production. However, future work must include methane oxidizing processes, as this is the biggest terrestrial surface sink for methane (McGuire*, et al.*, 2009). Furthermore, this work provides first insight into question on how the quality of organic matter influences the abundance and composition of microbial communities through different microbial pathways. However, future work will need to include detailed characterisation of the carbon sources currently stored in the frozen deposits, to constrain its decomposition rates and availability for microbial consumption. The vulnerability of the Arctic and its importance for the global carbon cycle can only be established using a multi-disciplinary scientific approach that incorporates microbiology, organic geochemistry and geology. This will provide the mechanisms behind current observations in these unique environments that will allow for estimates for net carbon dioxide and methane production and emissions for the Arctic in response to changing climatic conditions.

6. Data collection

6.1. Manuscript I: Response of methanogenic archaea to Late Pleistocene and Holocene climate changes in the Siberian Arctic

6.1.1. Sediment properties

Depth (middle)	Depth Interval	Sand 63 -2000 µm	Silt 2-63 µm	Clay < 2 µm	TOC	H_20 content	Methane	Potential methane-production
[cm]	[cm]	[%]	[%]	[%]	[%]	[%]	[nmol CH_4 g^{-1}]	[nmol CH_4 h^{-1} g^{-1}]
34	28-40	21.8	55.1	23.1	3.1	6.2	8	0.0055
44	40-48	16.9	63.8	19.3	16.6	15.6	113	0.0887
57	48-66	13.2	68.5	18.3	5.3	30.8	1883	
72	66-78	36.6	41.5	21.9	4.0	22.9	339	
87	78-86	13.6	62.0	24.4	6.4	38.8	3534	
98	86-110	19.8	65.3	14.9	12.1	32.9	3508	0.0080
113	110-116	34.2	43.6	22.2	6.0	32.6	2240	
122.5	116-129	10.6	66.1	23.3	11.7	30.9	2086	0.0056
136	129-143	8.7	63.5	27.8	16.7	28.8	3633	
152.5	143-162	10.9	62.5	26.5	6.7	31.9	2461	
171.5	162-182	6.9	77.5	15.6	16.4	34.9	2263	
191.5	182-201	55.7	22.1	22.2	4.8	27.5	625	
225	201-248	9.6	69.6	20.8	14.5	31.7	2679	0.0214
257.5	249-266	85.3	4.1	10.6	26.5	31.1	2301	
280	266-294	17.4	57.0	25.7	5.5	34.0	425	
299.5	294-305	31.4	40.4	28.2	13.3	26.6	687	
323.5	305-342	n.d.	n.d.	n.d.	1.4	25.0	625	0.0021
350	342-358	58.0	0.0	42.0	1.7	24.0	824	
363.5	358-369	12.3	74.3	13.3	1.8	30.7	1531	
377	369-385	34.9	53.6	11.5	1.9	22.4	586	0.0145
399	385-387	15.4	72.1	12.4	1.9	25.4	1629	
396.5	387-397	28.4	55.6	16.1	2.0	26.3	576	
404	397-425	4.2	76.1	19.7	2.0	22.6	631	0.0026
442.5	425-460	36.8	47.0	16.2	2.4	32.2	669	
477.5	460-495	57.8	26.1	16.1	2.4	19.1	412	
506	495-517	45.0	40.5	14.6	2.8	28.5	37	
530.5	517-544	43.7	39.5	16.8	2.1	20.3	41	
559	544-574	33.9	55.1	11.0	1.4	32.9	16	
590.5	574-607	49.6	38.6	11.8	1.8	25.4	12	
627	607-647	n.d.	n.d.	n.d.	1.5	32.7	16	0.0023
658	647-669	33.6	50.3	16.1	1.9	28.6	8	
682	669-695	23.2	59.8	17.0	2.1	27.1	6	
711.5	695-728	29.4	54.9	15.7	2.2	23.4	8	
736	728-744	35.8	51.7	12.5	1.5	20.3	5	
753.5	744-763	31.9	55.4	12.6	1.7	23.9	6	
780.5	763-798	22.7	59.7	17.6	1.9	24.6	5	0.0015
815	798-832	21.4	59.3	19.3	1.7	31.7	5	
846.5	832-861	35.4	47.6	17.0	1.8	24.4	10	
880.5	861-900	41.9	38.1	20.0	1.6	26.0	6	
928	900-956	49.8	34.8	15.3	2.5	23.0	7	
980.5	956-1005	11.8	69.2	19.0	2.1	31.7	7	0.0121
1014.5	1005-1024	7.4	73.1	19.6	3.2	21.3	7	

Depth (middle)	Depth Interval	Sand 63 -2000 µm	Silt 2-63 µm	Clay < 2 µm	TOC	H_20 content	Methane	Potential methane-production
[cm]	[cm]	[%]	[%]	[%]	[%]	[%]	[nmol CH_4 g^{-1}]	[nmol CH_4 h^{-1} g^{-1}]
1057.5	1024-1091	14.0	64.2	21.7	4.3	35.4	20	
1130	1091-1169	13.2	63.5	23.3	6.0	27.8	149	
1184	1169-1199	14.0	62.3	23.7	5.6	30.0	857	
1201.5	1199-1204	n.d.	n.d.	n.d.	2.1	32.0	780	
1311	1291-1331	9.2	62.9	27.9	2.2	22.0	94	
1351	1331-1371	22.7	61.6	15.7	1.7	34.9	8	
1390.5	1371-1410	29.0	57.4	13.5	2.7	26.2	6	
1440.5	1410-1471	28.3	55.2	16.5	2.4	25.8	6	0.0023
1495.5	1471-1520	53.9	37.7	8.4	4.5	19.4	5	
1548	1520-1576	n.d.	n.d.	n.d.	2.7	22.0	11	
1595.5	1576-1615	40.2	45.6	14.2	2.2	23.0	6	0.0026
1630.5	1615-1646	41.2	43.4	15.5	2.3	30.8	6	
1625.5	1646-1659	32.6	54.0	13.5	2.3	27.6	7	
1684.5	1659-1710	22.1	62.8	15.0	2.2	30.5	6	
1722	1710-1734	32.8	54.2	13.0	1.9	23.5	253	
1764	1734-1794	33.6	54.4	12.0	0.9	21.3	6	
1820.5	1794-1847	48.5	37.9	13.6	4.4	25.3	5	
1860	1847-1873	65.3	27.3	7.4	6.1	20.0	5	
1879	1873-1885	21.4	58.5	20.0	4.1	24.2	36	
1901.5	1885-1918	20.4	58.2	21.4	3.4	25.2	174	
1927.5	1918-1937	16.0	63.9	20.1	3.3	36.4	37	0.0382
1947.5	1937-1958	30.8	53.8	15.5	4.7	19.4	7	
1985.5	1958-2013	25.1	56.9	18.0	5.0	28.6	7	
2019.5	2013-2026	21.0	57.3	21.7	3.6	27.1	886	
2043	2026-2060	11.4	64.2	24.5	5.3	36.6	99	
2067	2060-2074	30.7	49.9	19.4	6.3	27.4	1239	
2086	2074-2098	13.3	63.0	23.7	4.3	31.0	1335	0.0011
2132	2098-2166	13.2	63.1	23.7	0.7	29.5	1059	
2196	2166-2226	62.7	27.1	10.1	4.3	29.8	485	
2229.5	2226-2233	n.d.	n.d.	n.d.	4.9	11.8	1801	
2243	2233-2253	90.9	6.0	3.1	0.2	12.5	279	
2266.5	2253-2380	18.4	59.0	22.5	4.2	36.3	450	
2397	2380-2414	19.3	61.1	19.6	6.3	n.d.	n.d.	0.0040
2417.5	2414-2421	n.d.	n.d.	n.d.	0.2	n.d.	n.d.	
2427.5	2421-2434	97.4	2.0	0.6	0.6	n.d.	n.d.	
2455	2434-3476	96.5	2.3	1.1	n.d.	n.d.	n.d.	

6.1.2. Isoprenoid glycerol dialkyl glycerol tetraethers and archaeol

		m/z						
Depth (middle)	**Internal sample ID GFZ**	**654**	**1302**	**1300**	**1298**	**1296**	**1294**	**1292**
[cm]		[ng g^{-1}]						
34	G007832	22.9	4.3	0.6	0.3			0.0
44	G007550	64.9	0.9	0.0	0.1	0.0	0.0	0.0
98	G007551	27.2	7.1	1.0	1.3	0.1		
122.5	G006513	62.6	0.1	0.2	0.7	0.2	0.0	0.0
171.5	G007552	66.7	2.7	0.5	0.5			
225	G006514	14.4	0.3	0.2	0.2	0.0	0.0	0.2
323.5	G007553	13.0	1.4	0.2	0.1	0.1	0.2	0.5
350	G007833	0.3	0.3	1.9	1.1	0.6	2.5	7.5
377	G006515	5.1	0.7	0.8	1.0	0.6	1.2	1.3
404	G007829	4.0	3.9	2.8	1.4	0.9	3.4	10.5
477.5	G007554	7.5	1.2				0.9	2.8
627	G006516	4.8	0.6	0.7	0.5	0.2	0.5	1.2
780.5	G007555	9.2	3.5	1.2	1.0	0.3	1.4	4.4
980.5	G006517	3.8	1.1	0.8	0.7	0.4	0.9	2.1
1184	G006518	11.4	1.8	0.8	0.3	0.3	0.9	2.3
1440.5	G006519	23.2	1.5	0.5	0.4	0.1	0.4	1.4
1595.5	G007556	28.0	5.2	1.0	0.6	0.1	0.6	1.6
1722	G006520	29.9	2.0	0.5	0.4	0.1	0.3	0.8
1901.5	G007830	8.4	7.1	2.0	1.0	0.3	1.9	5.8
1927.5	G007557	34.3	5.6	2.0	0.9	0.4	1.0	3.0
1985.5	G007831	9.6	3.3	1.2	0.6	0.2	0.9	2.8
2086	G006521	5.3	0.3	0.2	0.2	0.1	0.2	0.6
2132	G007558	22.8	1.1	0.6	0.4	0.2	0.5	1.7
2196	G006522	7.4	0.4	0.2	0.0	0.0	0.3	0.8
2229.5	G006523	13.7	1.3	0.4	0.6	0.0	0.2	0.5
2243	G007559	7.5	1.4	0.3	0.2		0.2	0.7
2397	G006524	18.3	1.3	0.3	0.0	0.0	0.1	0.3
2417.5	G007560	6.4	0.4	0.3	0.2		0.1	0.3
2455	G007561	1.5	0.1		0.0			0.1

6.1.3. *Branched glycerol dialkyl glycerol tetraethers*

		m/z								
Depth (middle)	**Internal sample ID GFZ**	**1050**	**1048**	**1046**	**1036**	**1034**	**1032**	**1022**	**1020**	**1018**
[cm]		[ng g^{-1}]								
34	G007832	348.6	4.0	1.3	261.2	4.9	2.2	72.0	5.6	2.6
44	G007550	220.1			214.3			82.7	1.1	0.4
98	G007551	632.3	5.6	1.0	564.6	6.3	1.4	177.6	5.1	4.4
122.5	G006513	202.5	3.8	0.0	159.5	2.4	1.5	60.2	2.1	0.3
171.5	G007552	261.5			240.6			112.6	2.2	4.2
225	G006514	62.9	0.6	0.0	51.9	0.9	0.7	27.3	0.8	0.0
323.5	G007553	49.3	1.0		15.5	0.8		12.0	0.6	0.4
350	G007833	62.6	0.7	0.3	22.6	3.4		12.1	1.5	2.0
377	G006515	8.6	0.5	0.1	7.6	2.5	0.1	2.4	0.9	0.1
404	G007829	47.4	2.6	0.6	38.8	19.7		11.2	10.9	7.4
477.5	G007554	42.6	1.3		37.6	15.9	0.6	11.8	6.3	2.5
627	G006516	9.3	0.5	0.0	6.8	3.4	0.2	2.0	1.0	0.1
780.5	G007555	36.0	1.5		31.4	13.0	0.3	10.9	5.5	1.1
980.5	G006517	6.5	0.3	0.0	5.6	2.1	0.1	2.2	0.7	0.1
1184	G006518	18.1	1.0	0.0	17.4	5.3	0.1	6.3	2.4	0.1
1440.5	G006519	22.4	0.2	0.0	12.5	3.1	0.1	2.8	1.2	0.1
1595.5	G007556	58.1	1.0		35.2	9.4		8.0	4.4	0.8
1722	G006520	23.0	0.5	0.0	11.9	3.1	0.0	0.0	0.0	0.0
1901.5	G007830	166.8	2.6	0.7	110.8	30.2	0.6	28.5	1.6	1.6
1927.5	G007557	239.5	1.7	0.6	93.1	24.0	0.7	22.1	10.6	1.6
1985.5	G007831	135.8	1.5		68.5	15.7	0.3	17.7	7.7	0.7
2086	G006521	12.8	0.4	0.1	11.0	2.7	0.2	3.6	1.1	0.1
2132	G007558	118.6	2.8	0.5	93.3	21.4	0.4	25.8	11.3	3.2
2196	G006522	24.5	0.5	0.1	23.2	4.2	0.4	6.6	2.2	0.2
2229.5	G006523	87.0	3.1	0.4	74.1	11.4	0.9	23.0	6.1	0.6
2243	G007559	25.7	0.9		38.5	8.0		11.6	3.8	0.5
2397	G006524	197.0	4.3	0.6	131.0	7.7	1.9	41.5	4.7	0.6
2417.5	G007560	62.1			45.3			15.6	2.9	0.7
2455	G007561	3.6			3.2			1.0		

6.1.4. Phospholipid ester and ether lipids (summary)

Depth (middle)	**Depth**	**Internal sample ID Munich**	**PLFA total**	**PLFA total**	**PLEL total**	**PLEL total**
[cm]	[cm]		[nmol g^{-1}]	[ng g^{-1}]	[nmol g^{-1}]	[ng g^{-1}]
34	28-40	721	23.84	6676.64	1.43	442.07
98	86-110	729	101.97	27760.07	7.82	2567.83
122.5	116-129	730	33.03	9407.43	7.54	2608.12
171.5	162-182	731	39.17	10905.08	3.78	1253.81
225	201-248	732	30.81	8517.95	1.34	448.57
280	266-294	733	15.53	4299.40	1.75	567.16
299.5	294-305	722	32.55	9287.70	2.65	877.18
363.5	358-369	734	5.16	1511.71	0.22	96.15
377	369-385	723	3.63	993.57	0.19	95.58
399	385-387	724	5.02	1404.62	0.45	148.55
477.5	460-495	735	1.63	461.76	0.06	25.14
780.5	763-798	736	2.29	627.39	0.07	22.71
980.5	956-1005	737	2.82	805.17	0.14	51.68
1130	1091-1169	738	2.95	836.91	0.19	68.33
1184	1169-1199	739	4.74	1338.80	0.32	110.85
1495.5	1471-1520	740	1.81	503.03	0.31	100.05
1684.5	1659-1710	741	5.45	1492.18	1.70	531.14
1722	1710-1734	725	6.12	1643.40	0.70	229.90
1901.5	1885-1918	742	5.44	1488.97	0.53	178.06
2019.5	2013-2026	726	4.56	1258.89	0.27	76.11
2043	2026-2060	743	5.85	1596.38	0.39	131.52
2067	2060-2074	727	4.90	1329.53	0.26	84.76
2086	2074-2098	744	3.30	918.43	0.16	55.48
2196	2166-2226	745	5.82	1608.21	0.28	88.07
2229.5	2226-2233	746	8.79	2419.54	0.37	117.91
2266.5	2253-2380	728	8.00	2173.84	0.54	176.51

6.2. **Manuscript II: Response of microbial communities to landscape and climatic changes in a terrestrial permafrost sequence of the El'gygytgyn crater, Far East Russian Arctic**

6.2.1. Sediment properties and gene quantifications

Depth ( ) [m]	Depth interval [m]	TOC [%]	H_20 content [%]	Bacteria 16S RNA gene (SSU) [copies g^{-1}]	Archaea 16S RNA gene (SSU) [copies g^{-1}]
0.1	0-0,4	1.053	7.1	1.4E+07	1.3E+07
0.2	0-0,4	1.137	6.3	4.5E+06	4.2E+06
0.3	0-0,4	0.809	5.3	5.9E+06	6.0E+06
0.5	0,4-0,6	1.713	11.5	4.2E+07	1.2E+08
0.7	0,6-0,7	1.471	6.4	1.9E+06	1.7E+06
0.8	0,7-1	0.806	6.2	5.4E+06	6.4E+06
0.9	0,7-1	0.827	5.0	2.0E+06	1.6E+06
1.0	1-1,25	1.579	6.5	1.0E+06	1.1E+06
1.3	1-1,25	0.399	4.6	5.3E+06	4.3E+06
1.3	1,25-1,4	0.239	3.4	4.6E+06	7.3E+06
1.5	1,4-1,5	0.194	2.6	9.9E+05	9.4E+05
1.6	1,5-1,73	0.151	10.6	2.4E+05	2.1E+03
1.8	1,73-1,83	0.116	17.4	2.0E+04	7.8E+01
1.9	1,83-2	0.161	21.8	8.8E+04	3.5E+03
2.2	2,08-2,3	< d.l.	3.6		
3.0	2,95-3,05	< d.l.	9.0	1.4E+04	6.0E+03
3.2	3,15-3,25	0.196	6.2	1.6E+03	2.0E+02
3.3	3,25-3,35	0.299	5.3	4.6E+04	5.5E+03
3.4	3,35-3,5	0.112	3.7	3.4E+04	1.2E+04
4.0	3,95-4,0	< d.l.	8.9	3.0E+04	7.4E+01
4.1	4-4,1	0.191	7.7	7.5E+04	7.0E+03
4.3	4,2-4,3	< d.l.	6.5	1.5E+04	7.1E+03
4.8	4,5-5	< d.l.	4.3	2.7E+04	8.3E+03
5.7	5,3-6,1	< d.l.	9.9	8.0E+04	1.8E+02
6.6	6,3-6,9	< d.l.	9.1	2.2E+04	1.0E+02
7.3	7-7,65	< d.l.	9.3	2.5E+04	2.6E+01
8.8	8,45-9,1	< d.l.	8.0	1.9E+04	1.4E+02
9.8	9,6-10	< d.l.	7.0	1.9E+04	1.2E+03
11.3	11-11,6	< d.l.	7.8	7.4E+03	2.9E+02
12.4	12-12,7	< d.l.	6.8	8.1E+03	2.6E+02
13.7	13,2-14,2	< d.l.	7.9	1.5E+04	1.3E+03
14.8	14,4-15,2	< d.l.	7.0	2.6E+04	1.1E+02
15.7	15,4-15,65	< d.l.	10.9	1.8E+04	7.7E+01
15.8	15,65-15,95	< d.l.	7.9	8.3E+03	6.5E+00
17.1	16,95-17,3	< d.l.	13.2	2.2E+04	3.5E+02
18.2	18-18,4	< d.l.	10.3	1.1E+03	2.9E+02

< d.l. – value below the detection limit (0.1 wt% TOC)

6.2.2. *Phospholipid fatty acids composition*

Depth (middle)	Internal sample ID GFZ	14:0iso	14:0	iso15:0	anteiso15:0	15:0	iso16:0	16:1w7	16:1w5
[m]		[nmol g^{-1}]							
0.1	G007945	0.16	0.38	1.54	1.09	0.33	0.86	1.99	0.61
0.2	G007946	0.20	0.40	1.71	1.24	0.35	0.86	1.93	0.79
0.3	G007947	0.14	0.36	1.51	1.13	0.27	0.71	1.72	0.60
0.5	G007948	0.18	0.53	1.79	1.45	0.44	0.91	1.93	0.57
0.7	G007949	0.26	0.50	1.85	1.32	0.38	0.45	2.33	0.58
0.8	G007950	0.13	0.30	1.32	0.97	0.24	0.40	1.50	0.41
0.9	G007951	0.23	0.54	2.13	1.38	0.38	0.76	2.06	0.62
1	G007952	0.22	0.42	1.46	1.01	0.29	0.37	1.87	0.48
1.3	G007953	0.00	0.09	0.21	0.21	0.04	0.06	0.18	0.00
1.3	G007954	0.00	0.15	0.48	0.38	0.13	0.08	0.32	0.04
1.5	G007955	0.00	0.08	0.17	0.13	0.05	0.04	0.00	0.00
1.6	G007956	0.00	0.03	0.11	0.06	0.02	0.02	0.00	0.00
1.8	G007957	0.01	0.04	0.02	0.02	0.02	0.02	0.00	0.00
1.9	G007958								
2.2	G007959	0.00	0.25	0.06	0.06	0.17	0.03	0.00	0.00
3	G007960	0.00	0.09	0.03	0.03	0.06	0.01	0.00	0.00
3.2	G007961	0.00	0.03	0.04	0.02	0.02	0.03	0.00	0.00
3.3	G007962	0.00	0.06	0.07	0.04	0.05	0.03	0.00	0.00
3.4	G007963	0.00	0.02	0.02	0.01	0.03	0.00	0.00	0.00
4	G007964	0.00	0.10	0.01	0.02	0.06	0.01	0.00	0.00
4.1	G007965	0.00	0.04	0.02	0.01	0.06	0.00	0.00	0.00
4.3	G007966	0.00	0.03	0.01	0.01	0.00	0.02	0.00	0.00
4.8	G007967	0.00	0.00	0.00	0.00	0.00	0.03	0.00	0.00
5.7	G007968	0.00	0.03	0.01	0.00	0.00	0.03	0.00	0.00
6.6	G007969	0.00	0.11	0.02	0.02	0.07	0.00	0.00	0.00
7.3	G007970	0.00	0.03	0.01	0.01	0.03	0.00	0.00	0.00
8.8	G007971	0.00	0.03	0.00	0.00	0.01	0.00	0.00	0.00
9.8	G007972	0.01	0.07	0.00	0.00	0.05	0.00	0.00	0.00
11.3	G007973	0.00	0.02	0.00	0.00	0.01	0.00	0.00	0.00
12.4	G007974	0.00	0.02	0.00	0.00	0.01	0.00	0.00	0.00
13.7	G007975	0.00	0.02	0.00	0.00	0.01	0.00	0.00	0.00
14.8	G007976	0.00	0.07	0.01	0.02	0.05	0.00	0.00	0.00
11.7	G007977	0.02	0.13	0.02	0.02	0.09	0.00	0.00	0.00
15.8	G007978	0.00	0.01	0.00	0.00	0.01	0.00	0.00	0.00
17.1	G007979	0.00	0.06	0.01	0.01	0.03	0.00	0.00	0.00
18.2	G007980	0.00	0.03	0.00	0.00	0.02	0.00	0.00	0.00

Depth (middle)	Internal sample ID GFZ	16:0	10 Me 16:0	iso17:0	anteiso17:0	17:1w7	17:1 cyclo	17:0	18:0 iso
[m]		[nmol g^{-1}]							
0.1	G007945	4.83	1.28	0.51	0.47	0.00	1.11	0.59	0.00
0.2	G007946	4.85	1.35	0.53	0.45	0.00	1.10	0.63	0.00
0.3	G007947	4.03	1.17	0.45	0.40	0.00	1.18	0.58	0.00
0.5	G007948	3.74	1.38	0.00	0.53	0.00	1.18	0.80	0.00
0.7	G007949	2.98	1.16	0.36	0.40	0.00	1.29	0.53	0.00
0.8	G007950	2.38	0.92	0.00	0.28	0.00	0.85	0.43	0.00
0.9	G007951	3.71	1.47	0.57	0.55	0.00	1.03	0.76	0.00
1	G007952	2.28	0.82	0.32	0.30	0.23	0.97	0.40	0.00
1.3	G007953	0.41	0.12	0.04	0.04	0.03	0.11	0.04	0.00
1.3	G007954	0.82	0.12	0.05	0.07	0.00	0.17	0.08	0.02
1.5	G007955	0.36	0.06	0.02	0.02	0.00	0.03	0.03	0.00
1.6	G007956	0.13	0.00	0.02	0.01	0.01	0.01	0.01	0.00
1.8	G007957	0.15	0.00	0.00	0.00	0.00	0.00	0.01	0.01
1.9	G007958								
2.2	G007959	0.81	0.00	0.04	0.02	0.07	0.00	0.05	0.00
3	G007960	0.00	0.24	0.00	0.00	0.00	0.00	0.00	0.00
3.2	G007961	0.00	0.11	0.00	0.00	0.00	0.00	0.00	0.00
3.3	G007962	0.00	0.19	0.00	0.00	0.00	0.00	0.01	0.00
3.4	G007963	0.00	0.06	0.00	0.00	0.00	0.00	0.00	0.00
4	G007964	0.00	0.35	0.00	0.00	0.00	0.00	0.00	0.00
4.1	G007965	0.00	0.11	0.00	0.00	0.00	0.00	0.00	0.00
4.3	G007966	0.00	0.05	0.00	0.00	0.00	0.00	0.00	0.00
4.8	G007967	0.00	0.15	0.00	0.00	0.00	0.00	0.00	0.00
5.7	G007968	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
6.6	G007969	0.32	0.00	0.01	0.01	0.02	0.00	0.02	0.00
7.3	G007970	0.13	0.00	0.00	0.00	0.00	0.00	0.00	0.00
8.8	G007971	0.13	0.00	0.00	0.00	0.00	0.00	0.03	0.00
9.8	G007972	0.27	0.00	0.00	0.00	0.01	0.00	0.02	0.00
11.3	G007973	0.06	0.00	0.00	0.00	0.00	0.00	0.00	0.00
12.4	G007974	0.07	0.00	0.00	0.00	0.00	0.00	0.01	0.00
13.7	G007975	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
14.8	G007976	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
11.7	G007977	0.46	0.00	0.00	0.00	0.02	0.00	0.02	0.00
15.8	G007978	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
17.1	G007979	0.23	0.00	0.00	0.00	0.00	0.00	0.00	0.00
18.2	G007980	0.09	0.00	0.00	0.00	0.00	0.01	0.00	0.00

Depth (middle)	Internal sample ID GFZ	18:0 anteiso	18:1w9	18:1w7	18:0	20:0	21:0	22:0	23:0
[m]		[nmol g^{-1}]							
0.1	G007945	2.93	2.68	3.68	0.68	0.00	0.08	0.11	0.05
0.2	G007946	3.18	2.51	3.96	0.64	0.11	0.00	0.09	0.00
0.3	G007947	2.02	2.18	4.25	0.59	0.09	0.05	0.08	0.04
0.5	G007948	1.44	2.13	3.28	0.69	0.00	0.11	0.10	0.05
0.7	G007949	0.58	1.32	2.60	0.45	0.12	0.00	0.09	0.00
0.8	G007950	0.66	1.36	2.47	0.34	0.07	0.04	0.05	0.02
0.9	G007951	0.56	1.63	2.83	0.72	0.23	0.14	0.16	0.07
1	G007952	0.39	1.20	1.76	0.36	0.11	0.13	0.06	0.02
1.3	G007953	0.00	0.07	0.09	0.10	0.06	0.05	0.14	0.05
1.3	G007954	0.04	0.14	0.13	0.21	0.05	0.04	0.04	0.02
1.5	G007955	0.03	0.07	0.09	0.12	0.03	0.01	0.05	0.02
1.6	G007956	0.00	0.02	0.00	0.05	0.01	0.01	0.02	0.01
1.8	G007957	0.00	0.02	0.02	0.07	0.01	0.01	0.02	0.01
1.9	G007958								
2.2	G007959	0.32	0.04	0.04	0.23	0.02	0.00	0.02	0.01
3	G007960	0.00	0.06	0.03	0.10	0.02	0.01	0.03	0.02
3.2	G007961	0.00	0.01	0.03	0.05	0.03	0.01	0.10	0.05
3.3	G007962	0.00	0.04	0.00	0.08	0.02	0.01	0.03	0.03
3.4	G007963	0.00	0.00	0.00	0.03	0.01	0.01	0.04	0.03
4	G007964	0.00	0.10	0.00	0.10	0.00	0.00	0.02	0.02
4.1	G007965	0.00	0.02	0.04	0.05	0.01	0.01	0.03	0.03
4.3	G007966	0.00	0.00	0.00	0.02	0.00	0.00	0.02	0.01
4.8	G007967	0.00	0.00	0.00	0.04	0.01	0.00	0.03	0.02
5.7	G007968	0.00	0.00	0.05	0.04	0.00	0.00	0.01	0.00
6.6	G007969	0.00	0.10	0.00	0.10	0.01	0.00	0.02	0.01
7.3	G007970	0.00	0.03	0.06	0.08	0.02	0.01	0.08	0.07
8.8	G007971	0.00	0.00	0.23	0.05	0.01	0.00	0.04	0.04
9.8	G007972	0.00	0.08	0.12	0.08	0.01	0.00	0.02	0.01
11.3	G007973	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
12.4	G007974	0.00	0.02	0.03	0.04	0.00	0.00	0.00	0.00
13.7	G007975	0.00	0.02	0.04	0.04	0.01	0.00	0.01	0.01
14.8	G007976	0.00	0.06	0.08	0.07	0.01	0.02	0.00	0.00
11.7	G007977	0.00	0.14	0.05	0.12	0.01	0.00	0.02	0.01
15.8	G007978	0.00	0.00	0.01	0.03	0.01	0.00	0.00	0.00
17.1	G007979	0.00	0.08	0.05	0.08	0.01	0.00	0.01	0.01
18.2	G007980	0.00	0.02	0.03	0.05	0.01	0.00	0.02	0.01

Depth (middle)	Internal sample ID GFZ	24:0	25:0	26:0	28:0	30:0
[m]		[nmol g^{-1}]				
0.1	G007945	0.11	0.02	0.03	0.02	0.00
0.2	G007946	0.11	0.02	0.04	0.00	0.00
0.3	G007947	0.09	0.02	0.03	0.01	0.00
0.5	G007948	0.13	0.03	0.05	0.04	0.01
0.7	G007949	0.10	0.02	0.06	0.00	0.00
0.8	G007950	0.04	0.00	0.01	0.00	0.00
0.9	G007951	0.17	0.04	0.11	0.10	0.02
1	G007952	0.06	0.01	0.02	0.01	0.00
1.3	G007953	0.19	0.03	0.15	0.10	0.00
1.3	G007954	0.03	0.00	0.00	0.00	0.00
1.5	G007955	0.04	0.02	0.01	0.00	0.00
1.6	G007956	0.03	0.01	0.02	0.02	0.01
1.8	G007957	0.02	0.01	0.01	0.01	0.00
1.9	G007958					
2.2	G007959	0.03	0.00	0.01	0.00	0.00
3	G007960	0.03	0.01	0.01	0.00	0.00
3.2	G007961	0.15	0.04	0.12	0.08	0.00
3.3	G007962	0.03	0.01	0.01	0.01	0.00
3.4	G007963	0.07	0.03	0.07	0.05	0.01
4	G007964	0.05	0.02	0.05	0.03	0.01
4.1	G007965	0.03	0.01	0.02	0.02	0.01
4.3	G007966	0.04	0.02	0.03	0.03	0.01
4.8	G007967	0.08	0.02	0.07	0.04	0.00
5.7	G007968	0.01	0.00	0.01	0.01	0.00
6.6	G007969	0.04	0.01	0.03	0.02	0.00
7.3	G007970	0.25	0.11	0.27	0.17	0.03
8.8	G007971	0.12	0.06	0.11	0.08	0.02
9.8	G007972	0.04	0.02	0.03	0.02	0.01
11.3	G007973	0.00	0.00	0.00	0.00	0.00
12.4	G007974	0.01	0.00	0.01	0.00	0.00
13.7	G007975	0.06	0.02	0.06	0.03	0.01
14.8	G007976	0.03	0.01	0.03	0.02	0.00
11.7	G007977	0.06	0.03	0.07	0.06	0.03
15.8	G007978	0.00	0.00	0.07	0.00	0.00
17.1	G007979	0.02	0.01	0.03	0.02	0.01
18.2	G007980	0.06	0.03	0.08	0.04	0.02

6.2.3. *Isoprenoid glycerol dialkyl glycerol tetraethers and archaeol*

		m/z						
Depth (middle)	**Internal sample ID GFZ**	**654**	**1302**	**1300**	**1298**	**1296**	**1294**	**1292**
[m]					[ng g^{-1}]			
0.1	G007945	1.94	0.45	0.07	0.38	0.04	0.10	0.31
0.2	G007946	2.41	0.64	0.12	0.61	0.08	0.19	0.53
0.3	G007947	5.73	0.51	0.18	0.48	0.06	0.17	0.39
0.5	G007948	2.18	0.34	0.05	0.26	0.03	0.12	0.29
0.7	G007949	3.19	0.29	0.10	0.28		0.07	0.20
0.8	G007950	6.28	0.35	0.09	0.30	0.03	0.08	0.22
0.9	G007951	2.56	0.38	0.11	0.43	0.04	0.12	0.35
1.0	G007952	10.33	0.59	0.19	0.41	0.08	0.20	0.45
1.3	G007953	2.50	0.36	0.18	0.25	0.03	0.08	0.25
1.3	G007954	1.76	0.18	0.09	0.11	0.04	0.04	0.12
1.5	G007955	1.77	0.09	0.04	0.03			0.05
1.6	G007956	0.95	0.16	0.02	0.03		0.02	0.07
1.8	G007957	0.77	0.09	0.01	0.01			0.04
1.9	G007958	0.00	0.02					
2.2	G007959	4.02	0.51	0.08	0.06			0.02
3.0	G007960	2.80	0.54	0.54	0.04			
3.2	G007961	3.19	1.20	0.16	0.13			0.03
3.3	G007962	1.72	0.63	0.10	0.13		0.01	0.03
3.4	G007963	3.67	0.16	0.04				0.03
4.0	G007964	0.22	0.06	0.06				0.03
4.1	G007965	1.81	0.23	0.09	0.07			
4.3	G007966	8.61	0.62	0.08	0.00			0.04
4.8	G007967	5.34	0.13	0.08	0.05		0.02	0.07
5.7	G007968	5.16	0.47	0.05	0.04		0.02	0.07
6.6	G007969	2.61	0.23	0.03	0.02		0.01	0.04
7.3	G007970	0.12	0.09	0.02			0.01	0.03
8.8	G007971	0.15	0.07				0.01	0.09
9.8	G007972		0.09					0.05
11.3	G007973		0.08					0.03
12.4	G007974							
13.7	G007975		0.10	0.01			0.01	0.06
14.8	G007976		0.08				0.01	0.04
11.7	G007977	0.07	0.06	0.01			0.01	0.04
15.8	G007978		0.10	0.01			0.02	0.06
17.1	G007979		0.09	0.02			0.03	0.07
18.2	G007980	0.17	0.22	0.03			0.02	0.10

6.2.4. Branched glycerol dialkyl glycerol tetraethers

		m/z								
Depth (middle)	**Internal sample ID GFZ**	**1050**	**1048**	**1046**	**1036**	**1034**	**1032**	**1022**	**1020**	**1018**
[m]		[ng g^{-1}]								
0.1	G007945	48.28	0.64	0.18	70.71	1.09	0.27	32.90	0.82	0.15
0.2	G007946	90.65			105.07			51.47	1.09	0.16
0.3	G007947	30.45	0.67	0.21	51.82	0.84	0.23	33.13	0.91	0.27
0.5	G007948	41.08	0.22	0.12	66.45	0.79	0.19	36.09	0.69	0.16
0.7	G007949	58.14	0.61	0.08	55.41	0.65	0.26	29.91	0.43	0.14
0.8	G007950	33.72	0.25		45.31	0.48		23.38	0.51	0.21
0.9	G007951	46.60	0.66	0.12	57.03	1.13	0.14	27.74	0.92	0.28
1.0	G007952	110.17	1.61	0.22	126.32	2.03	0.24	74.53	1.51	0.00
1.3	G007953	29.72	0.81		24.76	0.77		12.39	0.39	0.10
1.3	G007954	24.85	0.44		17.71	0.17		7.54	0.40	0.08
1.5	G007955	7.34			5.78			2.94	0.18	
1.6	G007956	1.64			2.05	0.07		1.18	0.12	0.07
1.8	G007957	0.65			0.81			0.41		
1.9	G007958	0.16			0.03					
2.2	G007959	6.33	0.04		5.57	0.34		2.52	0.33	0.03
3.0	G007960	3.33	0.17		3.42	0.17		1.76	0.18	
3.2	G007961	12.61	0.13		12.56	0.69		7.08	0.64	
3.3	G007962	8.38			10.44	0.36	0.02	5.60	0.10	0.04
3.4	G007963	4.43	0.09		5.24	0.22		3.53	0.25	0.06
4.0	G007964	1.15	0.16	0.04	0.85	0.73		0.03	0.03	0.02
4.1	G007965	6.91	0.11	0.00	6.66	0.28	0.04	7.04	0.45	0.00
4.3	G007966	4.58	0.09	0.00	4.06	0.26	0.03	2.38	0.32	0.00
4.8	G007967	4.82	0.09	0.03	4.12	0.17	0.03	2.20	0.14	0.00
5.7	G007968	3.39	0.07	0.01	3.11	0.15	0.03	1.68	0.12	0.02
6.6	G007969	1.89	0.05	0.02	1.53	0.08	0.01	0.78	0.06	0.01
7.3	G007970	0.80	0.04	0.01	0.76	0.06	0.01	0.35	0.05	0.00
8.8	G007971	2.16	0.16	0.04	1.72	0.14	0.03	1.35	0.14	0.01
9.8	G007972	0.15	0.25	0.06	2.15	0.20	0.06	1.44	0.15	0.00
11.3	G007973	1.02	0.09	0.02	0.94	0.06	0.03	0.63	0.04	0.02
12.4	G007974	0.79	0.00	0.00	0.58	0.00	0.00	0.39	0.00	0.00
13.7	G007975	1.49	0.17	0.04	1.19	0.14	0.05	0.92	0.08	0.04
14.8	G007976	1.41	0.20	0.06	1.06	0.13	0.04	0.81	0.09	0.05
11.7	G007977	1.03	0.13	0.03	0.73	0.11	0.04	0.59	0.06	0.04
15.8	G007978	1.35	0.27	0.08	1.14	0.15	0.06	1.05	0.14	0.07
17.1	G007979	1.15	0.21	0.06	0.96	0.14	0.05	0.76	0.07	0.04
18.2	G007980	1.67	0.29	0.08	1.33	0.20	0.07	1.17	0.17	0.04

6.3. **Manuscript III: Glacial-interglacial microbial community dynamics in Middle Pleistocene sediments in the Lake El'gygytgyn, Far East Russian Arctic**

6.3.1. Sediment properties and gene quantifications

Depth (middle)	TOC	FTIRS-BSi	Bacteria 16S RNA gene (SSU)	Archaea 16S RNA gene (SSU)	Potent. CH4 production
[m]	[%]	[%]	[copies g^{-1}]	[copies g^{-1}]	[nMol CH_4 h^{-1} g^{-1}]
15.2	0.00	8.92	3.63E+04	1.55E+04	0.0013
15.4	0.21	11.31	1.01E+05	2.24E+04	
15.6	0.14	10.99	9.13E+03	2.00E+05	0.0002
15.8	0.90	29.09	1.37E+06	9.02E+05	
15.9	1.01	25.35	9.28E+06	3.75E+06	
16.0	0.86	25.72	7.80E+05	1.15E+06	
16.1	1.21	18.63	4.31E+06	9.38E+05	0.0096
16.4	0.43	9.36	3.93E+05	6.82E+05	
16.6	0.33	11.41		9.19E+03	
16.8	0.47	10.97	2.25E+04	2.86E+04	0.0096
17.0	0.17	7.99	1.20E+04	1.90E+04	
17.1	0.00	14.37	1.42E+04	1.23E+05	
17.9	0.72	10.35	4.57E+05	3.14E+05	0.0007
18.0	0.33	10.46		2.99E+04	0.0091
18.1	0.22	9.52	9767.333	9970.666667	
18.3	3.34	29.89	2.51E+06	1.59E+05	0.0045
18.5	1.21	44.31	2.34E+07	1.19E+06	
18.7	1.07	42.21	2.12E+07	1.35E+06	
18.9	0.30	25.31	1.71E+04	7.56E+04	
19.1	0.00	12.25	2.31E+04	3.25E+04	0.0013
19.5	0.12	7.36	2.49E+04	1.42E+04	
19.6	0.44	8.70	4.17E+04	4.30E+04	0.0032
20.3	0.13	9.44	1.19E+04	8.47E+02	
20.5	0.00	8.15	7.89E+03	4.64E+03	
20.7	0.00	7.42	2.67E+04	2.09E+03	
21.0	0.77	10.84	1.31E+04	1.83E+03	0.0001
21.0	0.87	10.25	2.27E+05	1.85E+05	
21.2	0.13	7.86	1.01E+04	4.82E+03	0.0018

6.3.2. Isoprenoid glycerol dialkyl glycerol tetraethers and archaeol

		m/z						
Depth (middle)	**Internal sample ID GFZ**	**654**	**1302**	**1300**	**1298**	**1296**	**1294**	**1292**
[m]		[ng g^{-1}]						
15.2	G009449	1.11	36.10	9.22	4.35	0.70	2.13	7.06
15.4	G009450	55.77	83.96	31.97	7.81	2.14	3.86	14.10
15.6	G009437	3.04	74.63	9.74	0.00	0.28	1.12	3.51
15.8	G009438	51.37	212.89	94.72	14.64	4.39	13.51	55.33
15.9	G009439	40.45	231.41	86.31	21.27	8.40	20.58	90.99
16.0	G009440	69.67	310.48	149.40	32.60	6.41	27.50	114.27
16.1	G009451	22.11	418.62	113.98	17.42	10.24	50.96	234.50
16.4	G009452	94.08	114.91	33.47	5.67	0.53	5.45	21.12
16.6	G009441	36.65	101.84	36.08	10.28	2.32	7.44	31.44
16.8	G009442	150.23	223.51	101.74	11.67	0.80	5.17	18.60
17.0	G009443	13.57	107.12	25.64	4.08	0.67	1.14	3.65
17.1	G009444	15.21	68.89	26.70	14.43	3.63	0.81	1.18
17.9	G009445	25.32	145.78	42.23	0.00	4.09	10.71	41.20
18.0	G009453	23.51	61.87	22.41	4.69	0.37	1.01	4.41
18.1	G009454	15.51	39.66	13.54	0.00	0.00	0.63	2.84
18.3	G009455	26.63	210.43	64.17	13.89	3.69	9.84	40.35
18.5	G009456	13.79	149.31	24.42	8.95	1.08	5.54	18.88
18.7	G009457	12.58	118.76	60.51	13.77	0.70	6.19	23.05
18.9	G009446	6.81	111.51	28.26	4.94	1.58	2.87	10.49
19.1	G009458	0.98	23.92	4.14	0.99	0.35	0.46	2.13
19.5	G009459	1.98	27.63	7.75	3.38	0.57	0.51	1.51
19.6	G009460	68.10	201.92	32.26	8.70	4.04	21.18	98.87
20.3	G009461	9.80	49.81	4.55	1.04	0.34	1.29	5.48
20.5	G009462	2.33	15.90	1.73	0.43	0.10	0.43	1.92
20.7	G009463	1.77	11.82	2.94	1.66	0.55	0.86	3.41
21.0	G009447	44.85	142.18	35.08	20.49	8.60	29.22	122.54
21.0	G009448	37.02	223.52	62.75	23.67	11.74	29.81	125.40
21.2	G009464	4.23	27.23	6.45	1.70	0.18	0.57	2.00

6.3.3. *Branched glycerol dialkyl glycerol tetraethers*

Depth (middle)	Internal sample ID GFZ	m/z								
		1050	**1048**	**1046**	**1036**	**1034**	**1032**	**1022**	**1020**	**1018**
[m]		[ng g^{-1}]								
15.2	G009449	99.48	4.96	3.36	75.35	7.15	0.61	50.08	6.68	0.61
15.4	G009450	464.66	44.01	19.32	212.31	51.10	11.69	152.11	43.47	4.57
15.6	G009437	712.85	71.30	32.11	350.40	100.13	12.79	229.73	69.92	4.96
15.8	G009438	1602.62	71.40	43.45	655.04	198.05	33.43	353.30	101.09	26.83
15.9	G009439	1284.03	58.97	36.10	537.57	173.50	38.90	261.80	88.00	25.98
16.0	G009440	1105.81	75.83	17.46	498.13	211.41	38.82	297.57	129.60	31.67
16.1	G009451	1662.29	71.38	41.13	685.80	274.79	45.75	409.50	188.75	34.80
16.4	G009452	729.50	29.73	21.36	269.63	53.19	13.21	163.23	30.30	6.28
16.6	G009441	666.44	38.21	13.35	463.71	135.61	12.93	263.54	110.37	10.80
16.8	G009442	581.95	45.67	28.89	231.81	51.21	18.39	152.07	44.50	10.31
17.0	G009443	613.12	49.55	24.77	309.05	99.89	14.28	230.65	109.26	6.96
17.1	G009444	258.31	16.07	10.15	127.24	43.20	9.44	72.35	16.99	2.84
17.9	G009445	1315.81	53.50	37.70	731.77	161.34	26.16	464.44	132.23	11.54
18.0	G009453	839.66	62.20	33.21	520.03	125.71	23.89	321.76	111.13	12.76
18.1	G009454	575.21	4.93	6.20	342.66	47.50	5.50	216.27	42.13	4.93
18.3	G009455	915.55	36.55	13.86	402.77	144.42	30.56	189.72	76.14	22.77
18.5	G009456	660.71	54.42	8.57	269.70	160.36	30.31	189.28	59.41	29.79
18.7	G009457	698.68	56.18	11.39	342.25	130.82	30.63	220.94	48.30	26.08
18.9	G009446	428.64	38.35	9.39	335.20	194.99	10.00	241.98	183.30	12.26
19.1	G009458	156.77	5.86	2.63	84.70	18.59	2.29	69.57	20.84	2.12
19.5	G009459	150.55	16.22	8.60	72.64	19.99	5.39	27.62	11.60	1.60
19.6	G009460	570.83	24.86	16.84	223.55	32.17	6.59	127.59	20.06	4.22
20.3	G009461	315.15	25.45	10.77	159.81	51.08	5.06	75.04	34.90	2.87
20.5	G009462	192.52	12.81	6.85	101.07	9.79	1.88	50.47	7.85	1.06
20.7	G009463	75.74	10.33	6.55	56.52	10.39	2.74	27.04	7.65	1.38
21.0	G009447	511.36	17.83	1.13	245.93	34.81	1.89	101.16	12.21	1.98
21.0	G009448	704.77	20.27	1.75	321.10	33.51	2.99	146.56	16.84	3.51
21.2	G009464	209.37	12.64	3.13	98.71	26.14	1.08	71.78	35.05	2.16

7. References

ACIA (2005) Arctic Climate Impact Assessment - Scientific Report. Cambridge University Press.

Aislabie JM, Jordan S & Barker GM (2008) Relation between soil classification and bacterial diversity in soils of the Ross Sea region, Antarctica. Geoderma **144**: 9-20.

Aislabie JM, Chhour K-L, Saul DJ, Miyauchi S, Ayton J, Paetzold RF & Balks MR (2006) Dominant bacteria in soils of Marble Point and Wright Valley, Victoria Land, Antarctica. Soil Biology & Biochemistry **38**: 3041-3056.

Andreev AA, Morozova E, Fedorov G, Schirrmeister L, Bobrov AA, Kienast F & Schwamborn G (2012) Vegetation history of central Chukotka deduced from permafrost paleoenvironmental records of the El'gygytgyn Impact Crater. Clim. Past **8**: 1287-1300.

Baker GC, Smith JJ & Cowan DA (2003) Review and re-analysis of domain-specific 16S primers. Journal of Microbiological Methods **55**: 541-555.

Bartlett KB & Harriss RC (1993) Review and assessment of methane emissions from wetlands. Chemosphere **26**: 261-320.

Bastviken D, Cole J, Pace M & Tranvik L (2004) Methane emissions from lakes: Dependence of lake characteristics, two regional assessments, and a global estimate. Global Biogeochemical Cycles **18**.

Bechtel A, Smittenberg RH, Bernasconi SM & Schubert CJ (2010) Distribution of branched and isoprenoid tetraether lipids in an oligotrophic and a eutrophic Swiss lake: Insights into sources and GDGT-based proxies. Organic Geochemistry **41**: 822-832.

Bischoff J, Mangelsdorf K, Schwamborn G & Wagner D (draft) Response of microbial communities to landscape and climatic changes in a terrestrial permafrost sequence of the El'gygytgyn crater, Far East Russian Arctic.

Bischoff J, Mangelsdorf K, Gattinger A, Schloter M, Kurchatova AN, Herzschuh U & Wagner D (2013) Response of methanogenic archaea to Late Pleistocene

and Holocene climate changes in the Siberian Arctic. Global Biogeochemical Cycles **in press**.

Bischoff J, Mangelsdorf K, Goersch J, Lam P, Rosen P, Wennrich V & Wagner D (submitted) Glacial-interglacial microbial community dynamics in Middle to Late Pleistocene sediments in the Lake El'gygytgyn, Far East Russian Arctic. submitted.

Blaga CI, Reichart G-J, Heiri O & Damste JSS (2009) Tetraether membrane lipid distributions in water-column particulate matter and sediments: a study of 47 European lakes along a north-south transect. Journal of Paleolimnology **41**: 523-540.

Bligh EG & Dyer WJ (1959) A RAPID METHOD OF TOTAL LIPID EXTRACTION AND PURIFICATION. Canadian Journal of Biochemistry and Physiology **37**: 911-917.

Boike J, Langer M, Lantuit H, et al. (2012) Permafrost-Physical Aspects, Carbon Cycling, Databases and Uncertainties. Recarbonization of the Biosphere: Ecosystems and the Global Carbon Cycle

(Ral R, ed.^eds.), p.^pp. Springer.

Brown J, O Ferrians J & Heginbottom JA (1997) Circum-Arctic map of permafrost and ground-ice conditions. ed.^eds.), p.^pp. Digital Media, Boulder, National Snow and Ice Data Center/World Data Center for Glaciology.

Brown J, Hinkel KM & Nelson FE (2000) The circumpolar active layer monitoring (calm) program: Research designs and initial results 1. Polar Geography **24**: 166-258.

Cao M, Gregson K & Marshall S (1998) Global methane emission from wetlands and its sensitivity to climate change. Atmospheric Environment **32**: 3293-3299.

Chalmers GRL & Bustin RM (2007) The organic matter distribution and methane capacity of the Lower Cretaceous strata of Northeastern British Columbia, Canada. International Journal of Coal Geology **70**: 223-239.

Chapin FS, Walker LR, Fastie CL & Sharman LC (1994) MECHANISMS OF PRIMARY SUCCESSION FOLLOWING DEGLACIATION AT GLACIER BAY, ALASKA. Ecological Monographs **64**: 149-175.

Cherapanova MV, Snyder JA & Brigham-Grette J (2007) Diatom stratigraphy of the last 250 ka at Lake El'gygytgyn, northeast Siberia. Journal of Paleolimnology **37**: 155-162.

Chin KJ & Conrad R (1995) INTERMEDIARY METABOLISM IN METHANOGENIC PADDY SOIL AND THE INFLUENCE OF TEMPERATURE. Fems Microbiology Ecology **18**: 85-102.

Colwell FS, T. Nunoura, M. E. Delwiche, S. Boyd, R. Bolton, D. Reed, K. Takai, R. M. Lehman, K. Horikoshi, D. A. Elias, and T. J. P. (2005) Evidence of minimal methanogenic numbers and activity in sediments collected from the Mallik 5L-38 gas hydrate research well. Vol. 585 (Collett SRDaTS, ed.^eds.), p.^pp. Geological Survey of Canada, Ottawa, Ontario.

Conrad R (2005) Quantification of methanogenic pathways using stable carbon isotopic signatures: a review and a proposal. Organic Geochemistry **36**: 739-752.

Conrad R, Erkel C & Liesack W (2006) Rice Cluster I methanogens, an important group of Archaea producing greenhouse gas in soil. Current Opinion in Biotechnology **17**: 262-267.

Conrad R, Klose M, Claus P & Enrich-Prast A (2010) Methanogenic pathway, C-13 isotope fractionation, and archaeal community composition in the sediment of two clear-water lakes of Amazonia. Limnology and Oceanography **55**: 689-702.

Conrad R, Bak F, Seitz HJ, Thebrath B, Mayer HP & Schutz H (1989) HYDROGEN TURNOVER BY PSYCHROTROPHIC HOMOACETOGENIC AND MESOPHILIC METHANOGENIC BACTERIA IN ANOXIC PADDY SOIL AND LAKE SEDIMENT. Fems Microbiology Ecology **62**: 285-294.

Coolen MJL, Hopmans EC, Rijpstra WIC, Muyzer G, Schouten S, Volkman JK & Sinninghe Damsté JS (2004) Evolution of the methane cycle in Ace Lake (Antarctica) during the Holocene: response of methanogens and

methanotrophs to environmental change. Organic Geochemistry **35**: 1151-1167.

D'Anjou RM, Wei JH, Castaneda IS, Brigham-Grette J, Petsch ST & Finkelstein DB (2013) High-latitude environmental change during MIS 9 and 11: biogeochemical evidence from Lake El'gygytgyn, Far East Russia. Clim. Past **9**.

Damste JSS, Schouten S, Hopmans EC, van Duin ACT & Geenevasen JAJ (2002) Crenarchaeol: the characteristic core glycerol dibiphytanyl glycerol tetraether membrane lipid of cosmopolitan pelagic crenarchaeota. Journal of Lipid Research **43**: 1641-1651.

Davidson EA & Janssens IA (2006) Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. Nature **440**: 165-173.

De Rosa M & Gambacorta A (1988) The lipids of archaebacteria. Progress in Lipid Research **27**: 153-175.

DeLong EF (1992) Archaea in coastal marine environments. Proceedings of the National Academy of Sciences **89**: 5685-5689.

Denman KL, G. Brasseur, A. Chidthaisong, P. Ciais, P.M. Cox, R.E. Dickinson, D. Hauglustaine, C. Heinze, E. Holland, D. Jacob, U. Lohmann, S Ramachandran, P.L. da Silva Dias, S.C. Wofsy and X. Zhang (2007) Couplings Between Changes in the Climate System and Biogeochemistry Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change

(Solomon S, D. Qin, M. Manning, Z. Chen, M. Marquis, K.B. Averyt, M.Tignor and H.L. Miller (eds.), ed.^eds.), p.^pp. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

Drake HL, Daniel SL, Kusel K, Matthies C, Kuhner C & Braus-Stromeyer S (1997) Acetogenic bacteria: what are the in situ consequences of their diverse metabolic versatilities? Biofactors **6**: 13-24.

Dutta K, Schuur EAG, Neff JC & Zimov SA (2006) Potential carbon release from permafrost soils of Northeastern Siberia. Global Change Biology **12**: 2336-2351.

Dworkin M & Falkow S (2006) The Prokaryotes: Vol. 3: Archaea. Bacteria: Firmicutes, Actinomycetes. Springer.

Fischer H, Behrens M, Bock M, et al. (2008) Changing boreal methane sources and constant biomass burning during the last termination. Nature **452**: 864-867.

Franzmann PD, Springer N, Ludwig W, Conway De Macario E & Rohde M (1992) A Methanogenic Archaeon from Ace Lake, Antarctica: Methanococcoides burtonii sp. nov. Systematic and Applied Microbiology **15**: 573-581.

Franzmann PD, Liu YT, Balkwill DL, Aldrich HC, deMacario EC & Boone DR (1997) Methanogenium frigidum sp. nov., a psychrophilic, H-2-using methanogen from Ace Lake, Antarctica. International Journal of Systematic Bacteriology **47**: 1068-1072.

Frauenfeld OW, Zhang TJ, Barry RG & Gilichinsky D (2004) Interdecadal changes in seasonal freeze and thaw depths in Russia. Journal of Geophysical Research-Atmospheres **109**.

Frey B, Rieder SR, Brunner I, et al. (2010) Weathering-Associated Bacteria from the Damma Glacier Forefield: Physiological Capabilities and Impact on Granite Dissolution. Applied and Environmental Microbiology **76**: 4788-4796.

Ganzert L, Jurgens G, Münster U & Wagner D (2007) Methanogenic communities in permafrost-affected soils of the Laptev Sea coast, Siberian Arctic, characterized by 16S rRNA gene fingerprints. FEMS Microbiology Ecology **59**: 476-488.

Gattinger A, Günthner A, Schloter M & Munch JC (2003) Characterisation of Archaea in Soils by Polar Lipid Analysis. Acta Biotechnologica **23**: 21-28.

Glissmann K, Chin KJ, Casper P & Conrad R (2004) Methanogenic pathway and archaeal community structure in the sediment of eutrophic Lake Dagow: Effect of temperature. Microbial Ecology **48**: 389-399.

Glushkova OY (2001) Geomorphological correlation of Late Pleistocene glacial complexes of Western and Eastern Beringia. Quaternary Science Reviews **20**: 405-417.

Grigoriev M (2003) Russian-German cooperation SYSTEM LAPTEV SEA : the expedition LENA 2002. (Bolshiyanov VRaD, ed.^eds.), p.^pp. 341.

Hanson RS & Hanson TE (1996) Methanotrophic bacteria. Microbiological Reviews **60**: 439-471.

Harden JW, Koven CD, Ping CL, et al. (2012) Field information links permafrost carbon to physical vulnerabilities of thawing. Geophysical Research Letters **39**.

Harvey HR, Fallon RD & Patton JS (1986) The effect of organic matter and oxygen on the degradation of bacterial membrane lipids in marine sediments. Geochimica et Cosmochimica Acta **50**: 795-804.

Hedderich R, and W. Whitman (2006) The Prokaryotes. Springer New York.

Hershberger KL, Barns SM, Reysenbach A-L, Dawson SC & Pace NR (1996) Wide diversity of Crenarchaeota. Nature **384**: 420-420.

Høj L, Olsen RA & Torsvik VL (2005) Archaeal communities in High Arctic wetlands at Spitsbergen, Norway (78°N) as characterized by 16S rRNA gene fingerprinting. FEMS Microbiology Ecology **53**: 89-101.

Holland AR, Petsch ST, Castaneda IS, Wilkie KM, Burns SJ & Brigham-Grette J (2013) A biomarker record of Lake El'gygytgyn, Far East Russian Arctic: investigating sources of organic matter and carbon cycling during marine isotope stages 1 and 3. Clim. Past **9**.

Hopmans EC, Schouten S, Pancost RD, van der Meer MTJ & Sinninghe Damsté JS (2000) Analysis of intact tetraether lipids in archaeal cell material and sediments by high performance liquid chromatography/atmospheric pressure chemical ionization mass spectrometry. Rapid Communications in Mass Spectrometry **14**: 585-589.

Hopmans EC, Weijers JWH, Schefuss E, Herfort L, Damste JSS & Schouten S (2004) A novel proxy for terrestrial organic matter in sediments based on branched and isoprenoid tetraether lipids. Earth and Planetary Science Letters **224**: 107-116.

Howard WR (1997) Palaeoclimatology - A warm future in the past. Nature **388**: 418-419.

IPCC (2007) IPCC Fourth Assessment Report: Climate Change 2007. ed.^eds.), p.^pp. Cambridge University Press.

Juschus O, Pavlov M, Schwamborn G, Preusser F, Fedorov G & Melles M (2011) Late Quaternary lake-level changes of Lake El'gygytgyn, NE Siberia. Quaternary Research **76**: 441-451.

Karr EA, Ng JM, Belchik SM, Sattley WM, Madigan MT & Achenbach LA (2006) Biodiversity of methanogenic and other Archaea in the permanently frozen Lake Fryxell, Antarctica. Applied and Environmental Microbiology **72**: 1663-1666.

Kobabe S, Wagner D & Pfeiffer E-M (2004) Characterisation of microbial community composition of a Siberian tundra soil by fluorescence in situ hybridisation. FEMS Microbiology Ecology **50**: 13-23.

Koch K, Knoblauch C & Wagner D (2009) Methanogenic community composition and anaerobic carbon turnover in submarine permafrost sediments of the Siberian Laptev Sea. Environmental Microbiology **11**: 657-668.

Koga Y & Morii H (2006) Special methods for the analysis of ether lipid structure and metabolism in archaea. Analytical Biochemistry **348**: 1-14.

Kotsyurbenko OR (2005) Trophic interactions in the methanogenic microbial community of low-temperature terrestrial ecosystems. Fems Microbiology Ecology **53**: 3-13.

Krivushin KV, Shcherbakova VA, Petrovskaya LE & Rivkina EM (2010) Methanobacterium veterum sp nov., from ancient Siberian permafrost. International Journal of Systematic and Evolutionary Microbiology **60**: 455-459.

Layer PW (2000) Argon-40/argon-39 age of the El'gygytgyn impact event, Chukotka, Russia. Meteoritics & Planetary Science **35**: 591-599.

Lehner B & Doll P (2004) Development and validation of a global database of lakes, reservoirs and wetlands. Journal of Hydrology **296**: 1-22.

Leininger S, Urich T, Schloter M, et al. (2006) Archaea predominate among ammonia-oxidizing prokaryotes in soils. Nature **442**: 806-809.

Liebner S, Harder J & Wagner D (2008) Bacterial diversity and community structure in polygonal tundra soils from Samoylov Island, Lena Delta, Siberia. International Microbiology **11**: 195-202.

Lu Z & Lu Y (2012) Methanocella conradii sp nov., a Thermophilic, Obligate Hydrogenotrophic Methanogen, Isolated from Chinese Rice Field Soil. Plos One **7**.

Ludwig W, Strunk O, Westram R, et al. (2004) ARB: a software environment for sequence data. Nucleic Acids Research **32**: 1363-1371.

MacDonald GM, Beilman DW, Kremenetski KV, Sheng Y, Smith LC & Velichko AA (2006) Rapid Early Development of Circumarctic Peatlands and Atmospheric CH4 and CO2 Variations. Science **314**: 285-288.

Mangelsdorf K (2005) Molecular indicators for the occurrence of deep microbial communities at the JAPEX/JNOC/GSC et al. Mallik 5L-38 gas hydrate production research well. ed.^eds.), p.^pp. Geological Survey of Canada.

Margesin R (2009) Permafrost Soils. Springer London, Limited.

Margesin R & Miteva V (2011) Diversity and ecology of psychrophilic microorganisms. Research in Microbiology **162**: 346-361.

McGuire AD, Anderson LG, Christensen TR, et al. (2009) Sensitivity of the carbon cycle in the Arctic to climate change. Ecological Monographs **79**: 523-555.

Melles M, Minyuk PS, Brigham-Grette J & Juschus O (2005) The expedition El'gygytgyn Lake 2003. Vol. 509 ed.^eds.), p.^pp. 139.

Melles M, Brigham-Grette J, Minyuk PS, Koeberl C, Andreev AA & Cook TL (2011) The Lake El'gygytgyn Scientific Drilling Project – Conquering Arctic Challenges through Continental Drilling. Scientific Drilling **11**: 29-40.

Melles M, Brigham-Grette J, Minyuk PS, et al. (2012) 2.8 Million Years of Arctic Climate Change from Lake El'gygytgyn, NE Russia. Science **337**: 315-320.

Metje M & Frenzel P (2007) Methanogenesis and methanogenic pathways in a peat from subarctic permafrost. Environmental Microbiology **9**: 954-964.

Morozova D & Wagner D (2007) Stress response of methanogenic archaea from Siberian permafrost compared with methanogens from nonpermafrost habitats. Fems Microbiology Ecology **61**: 16-25.

Mottaghy D, Schwamborn G & Rath V (2013) Past climate changes and permafrost depth at the Lake El'gygytgyn site: implications from data and thermal modeling. Clim. Past **9**: 119-133.

Müller K-D, Husmann H & Nalik HP (1990) A New and Rapid Method for the Assay of Bacterial Fatty Acids Using High Resolution Capillary Gas Chromatography and Trimethylsulfonium Hydroxide. Zentralblatt für Bakteriologie **274**: 174-182.

Nadkarni MA, Martin FE, Jacques NA & Hunter N (2002) Determination of bacterial load by real-time PCR using a broad-range (universal) probe and primers set. Microbiology-Sgm **148**: 257-266.

Nguyen ML, Westerhoff P, Baker L, Hu Q, Esparza-Soto M & Sommerfeld M (2005) Characteristics and reactivity of algae-produced dissolved organic carbon. Journal of Environmental Engineering-Asce **131**: 1574-1582.

Nolan M & Brigham-Grette J (2007) Basic hydrology, limnology, and meteorology of modern Lake El'gygytgyn, Siberia. Journal of Paleolimnology **37**: 17-35.

Nusslein B, Eckert W & Conrad R (2003) Stable isotope biogeochemistry of methane formation in profundal sediments of Lake Kinneret (Israel). Limnology and Oceanography **48**: 1439-1446.

Nusslein B, Chin KJ, Eckert W & Conrad R (2001) Evidence for anaerobic syntrophic acetate oxidation during methane production in the profundal sediment of subtropical Lake Kinneret (Israel). Environmental Microbiology **3**: 460-470.

Oechel WC, Hastings SJ, Vourlrtis G, Jenkins M, Riechers G & Grulke N (1993) Recent change of Arctic tundra ecosystems from a net carbon dioxide sink to a source. Nature **361**: 520-523.

Pancost RD, McClymont EL, Bingham EM, et al. (2011) Archaeol as a methanogen biomarker in ombrotrophic bogs. Organic Geochemistry **42**: 1279-1287.

Panikov NS & Sizova MV (2007) Growth kinetics of microorganisms isolated from Alaskan soil and permafrost in solid media frozen down to −35°C. FEMS Microbiology Ecology **59**: 500-512.

Pease TK, Van Vleet ES, Barre JS & Dickins HD (1998) Simulated degradation of glyceryl ethers by hydrous and flash pyrolysis. Organic Geochemistry **29**: 979-988.

Pitcher A, Rychlik N, Hopmans EC, et al. (2010) Crenarchaeol dominates the membrane lipids of Candidatus Nitrososphaera gargensis, a thermophilic Group I.1b Archaeon. ISME J **4**: 542-552.

Pruesse E, Quast C, Knittel K, Fuchs BM, Ludwig W, Peplies J & Glöckner FO (2007) SILVA: a comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB. Nucleic Acids Research **35**: 7188-7196.

Qiu YL, Sekiguchi Y, Imachi H, et al. (2004) Identification and isolation of anaerobic, syntrophic phthalate isomer-degrading microbes from methanogenic sludges treating wastewater from terephthalate manufacturing. Applied and Environmental Microbiology **70**: 1617-1626.

Radke M, Willsch H & Welte DH (1980) Preparative hydrocarbon group type determination by automated medium pressure liquid chromatography. Analytical Chemistry **52**: 406-411.

Ringelberg DB, Sutton S & White DC (1997) Biomass, bioactivity and biodiversity: microbial ecology of the deep subsurface: analysis of ester-linked phospholipid fatty acids. FEMS Microbiology Reviews **20**: 371-377.

Rivkina E & Gilichinsky D (1996) Methane as a Paleoindicator of the Dynamics of Permafrost Deposits. Lithology and Mineral Resources - Translated from Litologiya i Poleznye Iskopaemye **31**: 396-399.

Rivkina E, Laurinavichius K, McGrath J, Tiedje J, Shcherbakova V & Gilichinsky D (2004) Microbial life in permafrost. Advances in Space Research **33**: 1215-1221.

Rivkina E, Shcherbakova V, Laurinavichius K, et al. (2007) Biogeochemistry of methane and methanogenic archaea in permafrost. FEMS Microbiology Ecology **61**: 1-15.

Romanovskii NN & Hubberten HW (2001) Results of permafrost modelling of the lowlands and shelf of the Laptev Sea region, Russia. Permafrost and Periglacial Processes **12**: 191-202.

Romanovsky VE, Smith SL & Christiansen HH (2010) Permafrost Thermal State in the Polar Northern Hemisphere during the International Polar Year 2007-2009: a Synthesis. Permafrost and Periglacial Processes **21**: 106-116.

Romanovsky VE, Drozdov DS, Oberman NG, et al. (2010) Thermal state of permafrost in Russia. Permafrost and Periglacial Processes **21**: 136-155.

Rosen P, Vogel H, Cunningham L, Reuss N, Conley DJ & Persson P (2010) Fourier transform infrared spectroscopy, a new method for rapid determination of total organic and inorganic carbon and biogenic silica concentration in lake sediments. Journal of Paleolimnology **43**: 247-259.

Sakai S, Conrad R, Liesack W & Imachi H (2010) Methanocella arvoryzae sp nov., a hydrogenotrophic methanogen isolated from rice field soil. International Journal of Systematic and Evolutionary Microbiology **60**: 2918-2923.

Sakai S, Imachi H, Hanada S, Ohashi A, Harada H & Kamagata Y (2008) Methanocella paludicola gen. nov., sp nov., a methane-producing archaeon, the first isolate of the lineage 'Rice Cluster I', and proposal of the new

archaeal order Methanocellales ord. nov. International Journal of Systematic and Evolutionary Microbiology **58**: 929-936.

Schaefer K, Zhang T, Bruhwiler L & Barrett AP (2011) Amount and timing of permafrost carbon release in response to climate warming. Tellus Series B-Chemical and Physical Meteorology **63**: 165-180.

Schirrmeister L, Grosse G, Schwamborn G, et al. (2003) Late Quaternary History of the Accumulation Plain North of the Chekanovsky Ridge (Lena Delta, Russia): A Multidisciplinary Approach. Polar Geography **27**: 277-319.

Schneider J, Grosse G & Wagner D (2009) Land cover classification of tundra environments in the Arctic Lena Delta based on Landsat 7 ETM+ data and its application for upscaling of methane emissions. Remote Sensing of Environment **113**: 380-391.

Schouten S, Huguet C, Hopmans EC, Kienhuis MVM & Sinninghe Damsté JS (2007) Analytical Methodology for TEX86 Paleothermometry by High-Performance Liquid Chromatography/Atmospheric Pressure Chemical Ionization-Mass Spectrometry. Analytical Chemistry **79**: 2940-2944.

Schuette UME, Abdo Z, Bent SJ, Williams CJ, Schneider GM, Solheim B & Forney LJ (2009) Bacterial succession in a glacier foreland of the High Arctic. Isme Journal **3**: 1258-1268.

Schuur EAG, Bockheim J, Canadell JG, et al. (2008) Vulnerability of permafrost carbon to climate change: Implications for the global carbon cycle. Bioscience **58**: 701-714.

Schwamborn G, Rachold V & Grigoriev MN (2002) Late Quaternary sedimentation history of the Lena Delta. Quaternary International **89**: 119-134.

Schwamborn G, Fedorov G, Schirrmeister L, Meyer H & Hubberten H-W (2008) Periglacial sediment variations controlled by late Quaternary climate and lake level change at Elgygytgyn Crater, Arctic Siberia. Boreas **37**: 55-65.

Schwamborn G, Fedorov G, Ostanin N, Schirrmeister L, Andreev A & the El'gygytgyn Scientific P (2012) Depositional dynamics in the El'gygytgyn

Crater margin: implications for the 3.6 Ma old sediment archive. Clim. Past **8**: 1897-1911.

Shcherbakova V, Rivkina E, Pecheritsyna S, Laurinavichius K, Suzina N & Gilichinsky D (2011) Methanobacterium arcticum sp. nov., a methanogenic archaeon from Holocene Arctic permafrost. International Journal of Systematic and Evolutionary Microbiology **61**: 144-147.

Simankova MV, Parshina SN, Tourova TP, Kolganova TV, Zehnder AJB & Nozhevnikova AN (2001) Methanosarcina lacustris sp nov., a new psychrotolerant methanogenic archaeon from anoxic lake sediments. Systematic and Applied Microbiology **24**: 362-367.

Singh N, Kendall MM, Liu Y & Boone DR (2005) Isolation and characterization of methylotrophic methanogens from anoxic marine sediments in Skan Bay, Alaska: description of Methanococcoides alaskense sp. nov., and emended description of Methanosarcina baltica. International Journal of Systematic and Evolutionary Microbiology **55**: 2531-2538.

Smith SL, Romanovsky VE, Lewkowicz AG, et al. (2010) Thermal state of permafrost in North America: a contribution to the international polar year. Permafrost and Periglacial Processes **21**: 117-135.

Steven B, Briggs G, McKay CP, Pollard WH, Greer CW & Whyte LG (2007) Characterization of the microbial diversity in a permafrost sample from the Canadian high Arctic using culture-dependent and culture-independent methods. FEMS Microbiology Ecology **59**: 513-523.

Striegl RG, Aiken GR, Dornblaser MM, Raymond PA & Wickland KP (2005) A decrease in discharge-normalized DOC export by the Yukon River during summer through autumn. Geophysical Research Letters **32**: n/a-n/a.

Tarnocai C, Canadell JG, Schuur EAG, Kuhry P, Mazhitova G & Zimov S (2009) Soil organic carbon pools in the northern circumpolar permafrost region. Global Biogeochem. Cycles **23**: GB2023.

Thauer RK, Kaster A-K, Seedorf H, Buckel W & Hedderich R (2008) Methanogenic archaea: ecologically relevant differences in energy conservation. Nature Reviews Microbiology **6**: 579-591.

Tierney JE & Russell JM (2009) Distributions of branched GDGTs in a tropical lake system: Implications for lacustrine application of the MBT/CBT paleoproxy. Organic Geochemistry **40**: 1032-1036.

van Everdingen R (1998 revised May 2005) Multi-language glossary of permafrost and related ground-ice terms. ed.^eds.), p.^pp. Boulder, CO: National Snow and Ice Data Center.

Vincent WF & Laybourn-Parry J (2008) Polar Lakes and Rivers: Limnology of Arctic and Antarctic Aquatic Ecosystems. OUP Oxford.

Wagner D, Lipski A, Embacher A & Gattinger A (2005) Methane fluxes in permafrost habitats of the Lena Delta: effects of microbial community structure and organic matter quality. Environmental Microbiology **7**: 1582-1592.

Wagner D, Schirmack J, Ganzert L, Morozova D & Mangelsdorf K (2013) Methanosarcina soligelidi sp. nov., a desiccation and freeze-thaw resistant methanogenic archaeon isolated from a Siberian permafrost-affected soil. Int J Syst Evol Microbiol **in press**.

Wagner D, Gattinger A, Embacher A, Pfeiffer E-M, Schloter M & Lipski A (2007) Methanogenic activity and biomass in Holocene permafrost deposits of the Lena Delta, Siberian Arctic and its implication for the global methane budget. Global Change Biology **13**: 1089-1099.

Walter KM, Zimov SA, Chanton JP, Verbyla D & Chapin FS (2006) Methane bubbling from Siberian thaw lakes as a positive feedback to climate warming. Nature **443**: 71-75.

Wang ZP, DeLaune RD, Patrick WH & Masscheleyn PH (1993) Soil Redox and pH Effects on Methane Production in a Flooded Rice Soil. Soil Sci. Soc. Am. J. **57**: 382-385.

Washburn AL (1980) Permafrost features as evidence of climatic change. Earth-Science Reviews **15**: 327-402.

Watanabe T, Asakawa S, Nakamura A, Nagaoka K & Kimura M (2004) DGGE method for analyzing 16S rDNA of methanogenic archaeal community in paddy field soil. FEMS Microbiology Letters **232**: 153-163.

Weijers JWH, Schouten S, van der Linden M, van Geel B & Sinninghe Damsté JS (2004) Water table related variations in the abundance of intact archaeal membrane lipids in a Swedish peat bog. FEMS Microbiology Letters **239**: 51-56.

Weijers JWH, Schouten S, Hopmans EC, et al. (2006) Membrane lipids of mesophilic anaerobic bacteria thriving in peats have typical archaeal traits. Environmental Microbiology **8**: 648-657.

Wetterich S, Kuzmina S, Andreev AA, et al. (2008) Palaeoenvironmental dynamics inferred from late Quaternary permafrost deposits on Kurungnakh Island, Lena Delta, Northeast Siberia, Russia. Quaternary Science Reviews **27**: 1523-1540.

White DC, Davis WM, Nickels JS, King JD & Bobbie RJ (1979) Determination of the sedimentary microbial biomass by extractible lipid phosphate. Oecologia **40**: 51-62.

Yergeau E, Newsham KK, Pearce DA & Kowalchuk GA (2007) Patterns of bacterial diversity across a range of Antarctic terrestrial habitats. Environmental Microbiology **9**: 2670-2682.

Yergeau E, Hogues H, Whyte LG & Greer CW (2010) The functional potential of high Arctic permafrost revealed by metagenomic sequencing, qPCR and microarray analyses. Isme Journal **4**: 1206-1214.

Yergeau E, Schoondermark-Stolk SA, Brodie EL, et al. (2009) Environmental microarray analyses of Antarctic soil microbial communities. Isme Journal **3**: 340-351.

Yershov ED & Williams PJ (2004) General Geocryology. Cambridge University Press.

Zelles L (1999) Fatty acid patterns of phospholipids and lipopolysaccharides in the characterisation of microbial communities in soil: a review. Biology and Fertility of Soils **29**: 111-129.

Zelles L & Bai QY (1993) Fractionation of fatty acids derived from soil lipids by solid phase extraction and their quantitative analysis by GC-MS. Soil Biology and Biochemistry **25**: 495-507.

Zellner G & Winter J (1987) SECONDARY ALCOHOLS AS HYDROGEN DONORS FOR CO2-REDUCTION BY METHANOGENS. Fems Microbiology Letters **44**: 323-328.

Zepp Falz K, Holliger C, Grosskopf R, et al. (1999) Vertical distribution of methanogens in the anoxic sediment of Rotsee (Switzerland). Applied and environmental microbiology **65**.

Zhang T, Barry RG, Knowles K, Heginbottom JA & Brown J (1999) Statistics and characteristics of permafrost and ground-ice distribution in the Northern Hemisphere 1. Polar Geography **23**: 132-154.

Zhang T, Barry RG, Knowles K, Ling F & Armstrong RL (2003) Distribution of seasonally and perennially frozen ground in the Northern Hemisphere.

Zhang T, Barry RG, Knowles K, Heginbottom JA & Brown J (2008) Statistics and characteristics of permafrost and ground-ice distribution in the Northern Hemisphere. Polar Geography **31**: 47-68.

Zhang TJ (2005) Influence of the seasonal snow cover on the ground thermal regime: An overview. Reviews of Geophysics **43**.

Zimov SA, Schuur EAG & Chapin FS (2006) Permafrost and the Global Carbon Budget. Science **312**: 1612-1613.

Zink K-G & Mangelsdorf K (2004) Efficient and rapid method for extraction of intact phospholipids from sediments combined with molecular structure elucidation using LC–ESI-MS–MS analysis. Analytical and Bioanalytical Chemistry **380**: 798-812.

8. Final thoughts and acknowledgements

This PhD thesis can provide only initial datasets on microbial communities in deeper and older deposits of the Russian Arctic, a widely understudied environment. I recognize that three cores from three different locations are limited in their ability to spatially represent the wider area due to local heterogeneities... However, these pioneering samples required a huge personal, technical and financial effort with several years of planning, project writing, drilling, sample collection, data acquisition, another round of proposal writing, more data acquisition and interpretation, which finally led to this PhD thesis.

This work was only possible due to the visions, efforts and money of all the people involved...

I would like to thank all the people from the **AWI** and **GFZ** that have contributed to this work - this work would not have been possible without all of you. In particular, I want to thank:

Dirk Wagner for your support throughout the last eight years, for always having an open door, your ambition and belief in me and this study.
Kai Mangelsdorf for your patience, encouragement and for introducing me to the wonderful world of organic geochemistry. Thank you for recommending this course on the little island in the Dutch North Sea … the implications for my professional and private life are much appreciated.
Hans-Wolfgang Hubberten for giving me the opportunity to conduct my work at the AWI Potsdam, enabling my participation in the expedition LENA 2010 and supporting my attendances of several national and international conferences.
Ute Bastian, Cornelia Karger, Anke Kaminski and **Oliver Burckhardt** for your day-to-day support with everything and more … Without you, I would have been lost while searching for samples, glassware, labels and HIWIs …

Christine Litz, Sigrun Gräning und **Dirk Holm** für die Hilfe im Verwaltungs-Dschungel und dass letztendlich doch immer alles irgendwie ging…

Heiko Gericke und **Tobias Schmidt** für wenn am Computer mal wieder gar nichts mehr ging …

Felizitas Bajerski for your support, help and friendship at work and beyond. Actually, sometimes it was just about a hug, a piece of chocolate and a snooze on your sofa.

Janine Görsch for your wonderful and ambitious work. I hope you enjoy the possibilities and responsibilities in your PhD. I wish you all the best.

Georg Schwamborn – Es lebe der Gygy!!!

Beatrice Barbier, Janosch Schirrmack, Lars Ganzert and all the other AWI Potsdam PhD students because working with so many friends compensates fighting the obstacles of a PhD. Thanks for providing useful comments, laughter and distraction.

Günther 'Molo' Stoof, **Waldemar Schneider, Inken Preuss** and everyone else involved in the expedition LENA 2010 for a wonderful arctic summer.

Etienne, meinen Eltern und meiner Familie– Danke für die unglaubliche Liebe und Unterstützung, beides ist in Worte nicht auszudrücken.

Ryan – We went from sharing live windows to sharing a life… Thank you for believing in me and us. You inspire me in so many ways… .

9. Curriculum vitae

Personal Information

Name	Juliane Bischoff
Date of birth	26th May, 1982
Address	-
Contact	Juliane.Bischoff@ncl.ac.uk

Research Interests

Carbon cycling in Arctic environments

- **Microbial methane dynamics in permafrost regions – including methanogenic and methanotrophic processes**
- **Reconstruction of recent and fossil microbial communities based on lipid biomarker analysis and genetic information**
- **Monitoring of microbial processes in a changing Arctic**

Academic Record

May 2012 - present	**Research Associate** Newcastle University, School of Civil Engineering and Geosciences. 'The transport and fate of terrestrial organic matter in the Arctic Ocean' (NERC, UK)
Dec 2008 – Apr 2012	**PhD Researcher** Alfred Wegener Institute for Polar and Marine Research (AWI), Research Unit Potsdam *and* German Research Centre for Geosciences (GFZ) 'Microbial communities and their response to Pleistocene and Holocene climate changes'
Oct 2001 – Aug 2008	**Diploma of Biology**, University of Potsdam; Grade 1.1 (with distinction) Major: Molecular biology; Minor: Microbiology, Thesis: 'Cultivation and molecular-ecological characterization of methanotrophic enrichments from Siberian permafrost-affected soils'

List of publications

Bischoff J, Mangelsdorf K, Gattinger A, Schloter M, Kurchatova AN, Herzschuh U & Wagner D (2013) Response of methanogenic archaea to Late Pleistocene and Holocene climate changes in the Siberian Arctic. *Global Biogeochemical Cycles.*

in press.

Bischoff J, Mangelsdorf K, Goersch J, Lam P, Rosen P, Wennrich V & Wagner D, Glacial-interglacial microbial community dynamics in Middle Pleistocene sediments in the Lake El'gygytgyn, Far East Russian Arctic.

***Under review in the ISME Journal*.**

Bischoff J, Mangelsdorf K, Schwamborn G & Wagner D, Response of microbial communities to landscape and climatic changes in a terrestrial permafrost sequence of the El'gygytgyn crater, Far East Russian Arctic. **Draft.**

Supervising/Teaching Experience

MSc Project Mentor:

Janine Goersch, University of Potsdam. 'Composition of methanogenic archaea communities in the El' gygytgyn crater lake, NE Siberia'. Feb –Oct 2011.

Student Internships/Student Assistants Supervisor:

Patrick Häcker, University of Potsdam; Sep 2009 – Oct 2011

Frederik Bücks, Technical University of Berlin; Feb 2011 – June 2011.

Courses

'Recent trends in microbiology'

Weekly MSc semester series (10 weeks) 2009, University of Potsdam.

'Ecological Microbiology'

Two day MSc block module 2010, AWI/University of Potsdam.

'Eco-molecular analyses and physiological characterization of microorganisms

Ten day MSc block module 2011, AWI/University of Potsdam.

Attended Courses

Molecular Organic Biogeochemistry

One week 2010. NIOZ (ECOLMAS, NEBROC), Texel, the Netherlands.

ARB/Silva Workshop

One week 2010. Max Planck Institute for Marine Microbiology, Bremen, Germany.

Field Experience

Expedition LENA 2010

Five weeks, Summer 2010; Samoylov Station, Siberia, Russia.

Responsibilities: Preparation of field campaign, permafrost active layer sampling, trace gas emission measurements, maintenance of long-term study site.

Funds

Travel grant POGS (Potsdam Graduate School) 2010

Travel grant POLMAR (Helmholtz Graduate School for Polar and Marine Research) 2010

Travel grants VAAM (Association for general and applied microbiology)2009, 2010

Languages

German (native)

English (fluent)

Skills

Driving license (EU) full clean

First Aid (2009, 2010, 2013)

Juliane Bischoff Newcastle upon Tyne, 23/05/2013

10. **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.

Juliane Bischoff Newcastle upon Tyne, 23/05/2013