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

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[1] 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 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, 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 during 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.

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1. Introduction

[2] 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 (NH); both long-term measurements and future climate models indicate a significant increase in atmospheric and ground temperature for this particular environment [*ACIA*, 2005; *IPCC*, 2007]. Permafrost, defined as ground remaining below 0 °C for 2 consecutive years [*Washburn*, 1980 and references therein], is widely distributed in the Arctic and covers about 25% of the exposed land surface of the NH [*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].

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[3] Methane is a significant trace gas that contributes with ~20% to the total greenhouse gases impacting on global warming [*Denman et al*., 2007]. Presently, methane emission estimates from northern, high latitude, terrestrial environments range from 10 to 51 Tg a^{-1} (10^{12} g/yr), which accounts for up to 8.6% of the global, natural methane emissions [*Bartlett and 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.

[4] 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 and Whitman*, 2006]. Subsequently, methane is released to the atmosphere by ebullition, plant-mediated transport by 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* [*Hanson and Hanson*, 1996].

[5] 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 3×10^8 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 values 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, 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 et al*., 2005; *Mangelsdorf et al*., 2005]. However, lipid biomarker studies in the Siberian Arctic on methanogenic communities in older permafrost deposits are currently missing.

[6] Here we present a combined stratigraphic analysis using microbial lipid markers and ribosomal ribonucleic acid (rRNA) genes on a permafrost sequence of Late Pleistocene and Holocene deposits from the Lena River Delta in Siberia, Russia. The aim of this study 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. Materials and Methods

2.1. Study Site

[7] The Lena River Delta (Figure 1a) is the largest Arctic delta, with an area of about 29×10^3 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 and Hubberten*, 2001]. The fieldwork for this study was conducted on Kurungnakh Island, which is located in the southern part of the Lena River Delta close to the Olenyekskaya Channel (Figure 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.

[8] During the Late Pleistocene and Holocene, Kurungnakh Island had undergone several climatic stages. This was indicated by the paleoclimatic and -environmental 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 macrofossils, 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 (Figure 2). Late Pleistocene deposits were divided into a cold and dry Unit II (2400–2476 cm; Early Weichselian Stadial, Zyryan), a warm and wet Unit III (1400–2400 cm; Middle Weichselian Interstadial, Kargin), and a cold and dry Unit IVa (300–1400 cm; Late Weichselian Stadial, Sartan). Holocene deposits were sectioned into a warm and dry Unit IVb (100–300 cm; Early Holocene) and a warm and wet Unit V (0–100 cm; Middle and Late Holocene).

2.2. Permafrost Drilling and Sample Preparation

[9] 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 (72°20'N, 126°17'E; Figure 1c) [*Grigoriev et al*., 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

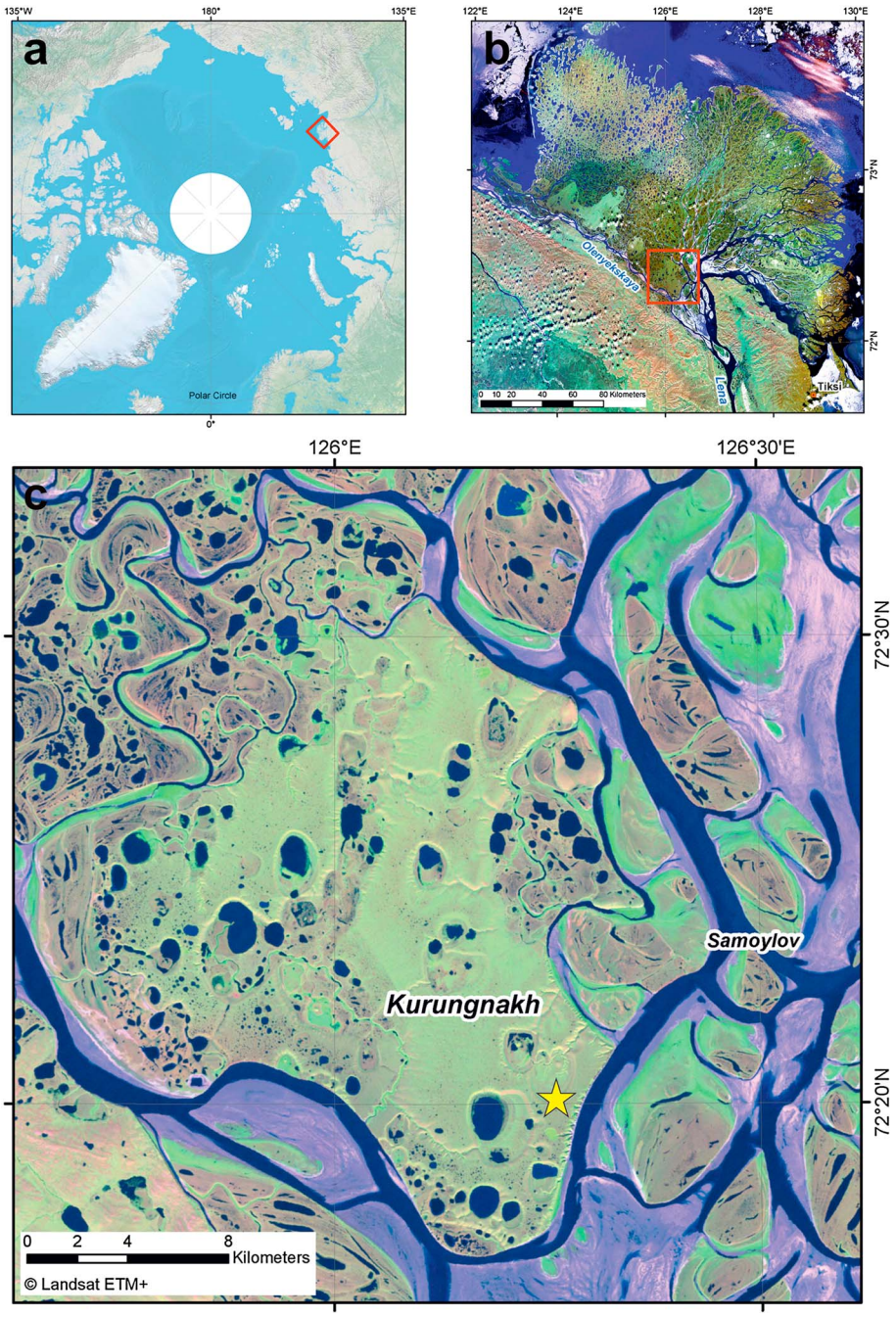

Figure 1. Location of the Lena River Delta in the Russian Arctic (a). Kurungnakh Island study site in the Lena River Delta (b), and location of the drill site, marked with symbol (c). (ESRI data, Landsat ETM+).

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).

[10] 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 (Bremerhaven, Germany).

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

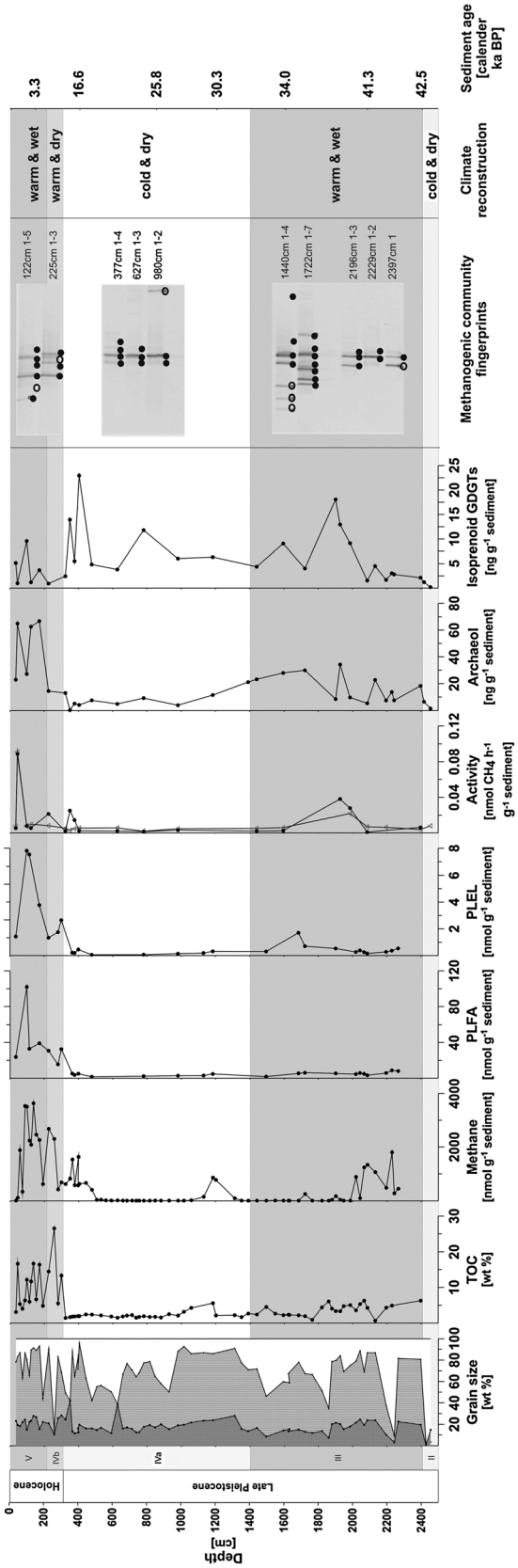

Figure 2. Abiotic and biotic parameters of the permafrost deposits from Kurungnakh Island, Lena Delta (Siberian Arctic), presented with respect to sediment depth (left axis), sediment age in calendar years BP (right axis), and the underlining paleoclimate reconstruction provided by *Wetterich et al.* [2008]. The vertical profile shows: a. grain size distribution in wt% of clay (black), silt (grey), and sand (white); b. TOC content in wt%; c. methane content in nmol CH_4 g^{-1} sediment; d. abundance of PLFAs in nmol g^{-1} sediment dw and µg g^{-1} sediment dw; e. abundance of PLELs 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 GDGTs in ng g^{-1} sediment dw; and i. 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.

2.3. Sediment Properties

[12] The TOC and total carbon contents of the freeze-dried and homogenized permafrost samples were determined as described previously in *Wagner et al*. [2007].

[13] 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.

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

[15] 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).

[16] In order to obtain a vertical distribution of methane with depth, methane concentrations were analyzed by putting a slice (approximately 5 g) of frozen sediment in a glass bottle and immediately sealing the bottle gas-tight with a butyl-rubber stopper. After the 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, Bonaduz, Switzerland). Gas analysis was performed using a gas chromatograph (Agilent 6890; Agilent Technologies, Santa Clara, California, USA) equipped with a bonded polystyrene-divinylbenzene–based column (HP-Plot-Q 15 m, 0.53 mm, 40 μm) and a flame ionization detector at operating conditions described previously [*Koch et al*., 2009].

2.4. Potential Methane Production Rates

[17] 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 ultrapure N_2/CO_2. Afterwards, the samples were supplemented with 0.1 mL sterile and anoxic tap water (tap water flushed with ultrapure 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 semiweekly over a period of 3 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.

2.5. Lipid Biomarker Analysis

[18] 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 reextracted two times with dichloromethane. For details on the method, see *Zink and Mangelsdorf* [2004].

[19] 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 (preheated sea sand) were measured regularly and no phospholipids or glycerol dialkyl glycerol tetraethers (GDGTs) were detected.

2.6. Detection of Archaeol and Isoprenoid GDGTs

[20] 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.

[21] The NSO fraction was investigated for tetraether lipids using an high performance liquid chromatography/atmospheric pressure chemical ionization (APCI) 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 × 250 mm, 5 μm; Altech). The mobile phase consisted of *n*-hexane A and isopropanol B (5 minutes, 99% A, 1% B) linear gradient to 1.4% B within 22.5 minutes, in 1 minute to 10% B, holding 5 minutes to clean the column and back to initial conditions in 1 minute, held for 6 minutes for equilibration. The flow rate was set to 200 μl/min, and injection was performed by 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, in the positive ion mode. The multiplier voltage was 1500 V, and the scan rate was 1 second. For semiquantitative determination of tetraether compounds, an external synthetic archaeol standard (Avanti Polar Lipids, Inc., Alabaster, Alabama, USA) was regularly measured in parallel. Blanks did not contain any GDGTs.

2.7. Detection of Phospholipid Fatty Acid and Phospholipid Ether Lipids

[22] 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, California, USA) by elution with chloroform, acetone, and methanol, respectively. One half of the phospholipid fraction was used for phospholipid fatty acid (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 kinds of environmental samples and sample matrices. After mild alkaline hydrolysis, the resulting fatty acid methylesters were measured by gas chromatography (GC)/MS (5973 MSD; Agilent Technologies, Palo Alto, California, USA) at operating conditions described elsewhere [*Zelles and Bai*, 1993]. The detected PLFAs comprise 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}), monounsaturated (*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 polyunsaturated (*n*-$C_{18:2}\Delta^{9,12}$, 18:3) fatty acids. The sum of all detected PLFAs resulted in a PLFA parameter.

[23] The other half of the phospholipid extracts were subjected to phospholipid ether lipid (PLEL) analysis, according to the method described by *Gattinger et al*. [2003]. After the formation of ether core lipids, ether-linked 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 comprise phytane and biphytane, and the sum of these compounds resulted in a PLEL parameter.

2.8. DNA Extraction and Polymerase Chain Reaction Amplification

[24] Total genomic DNA was extracted using a Power Soil™ DNA Isolation Kit (Mo Bio Laboratories Inc., Carlsbad, California, 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 0357 F 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.

[25] The polymerase chain reaction (PCR) reaction mix contained 12.5 µl of premixed SAHARA-Mix™, including a PCR buffer, deoxyucleoside triphosphates, and polymerase (Bioline GmbH, Luckenwalde, 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., Carlsbad, California, USA). PCR was performed using an iCycler (Bio-Rad, Hercules, California, USA) and the following PCR cycle: 95 °C for 7 minutes, 30 cycles of 1 minute at 95 °C, 1 minute at 56 or 53 °C, 1 minute at 72 °C, and a final elongation of 72 °C for 10 minutes. The amplified samples and positive and negative controls were checked by electrophoresis on a 1.5% agarose gel in 0.5 × TAE (diluted from 50 × TAE; 5 PRIME, Hamburg, Germany), which was prestained with GelRed™ (Biotium, Inc., Hayward, California, USA). The PCR products were purified using a HiYield™PCR Clean-up/Gel Extraction Kit (Süd-Laborbedarf, Gauting, Germany). Subsequently, DGGE analyses were conducted as described in *Ganzert et al*. [2007], and sequence analyses were conducted by GATC Biotech AG (Konstanz, Germany).

2.9. Phylogenetic Analysis

[26] Sequence data obtained after DGGE analyses were checked for quality using the Sequencher® software (Version 4.7; Gene Codes Corporation, Ann Arbor, Michigan, 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 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).

3. Results and Discussion

3.1. Methane Profile of the Kurungnakh Permafrost Sequence

[27] 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 (Figure 2c). Three intervals of increased methane concentration were identified in sediments at (i) 2000–2300 cm, (ii) around 1200 cm, and (iii) 100–500 cm depth. Overall, the highest methane concentrations were determined in Holocene deposits (100–300 cm depth).

[28] 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 (Figure 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 (0.78–1.8 Ma BP), but were not able to detect methane in Late Pleistocene ice complex deposits (11.5–130 ka BP).

[29] The observed methane profile in the Kurungnakh core corresponds to the distribution of the sedimentary organic matter (TOC; Figure 2b). This implies that sedimentary organic carbon can be metabolized in a cascade of microbial processes by a broad consortium of heterotrophic bacterial communities. This provides appropriate substrates (e.g., hydrogen, carbon

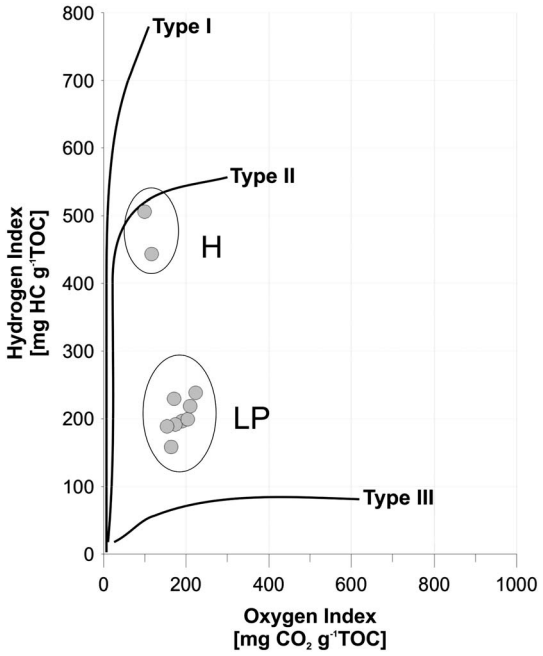

Figure 3. Hydrogen index versus oxygen index plot from Rock Eval pyrolysis data characterizing the sediment organic matter of terrestrial permafrost deposits. The plot indicates differences in the quality of organic matter. Holocene samples (marked with "H") cluster separately from Late Pleistocene (marked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

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 (Figures 2b and 2c). Interestingly, the Late Pleistocene and Holocene organic matter show different compositions revealed by Rock-Eval pyrolysis (Figure 3). 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.

[30] 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 and 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.

[31] 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 (Figure 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 (1400–1800 cm) and IVa (400–1000 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.

[32] 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 and 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.

3.2. Signals of Living Microbial Communities in the Kurungnakh Permafrost Sequence

[33] 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 and 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 the death of the source microorganisms [*White et al*., 1979; *Harvey et al*., 1986]. PLFAs are used as markers for viable *Bacteria* and *Eukarya* [*Ringelberg et al*., 1997; *Zelles*, 1999], and PLELs are characteristic markers for living archaeal communities in sediments [*Zelles and Bai*, 1993].

[34] In the Late Pleistocene, deposits in the amounts of PLFA and PLEL are comparatively lower than in Holocene deposits (Figures 2d and 2e), with the exception of a small interval at 1500–1700 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 PLELs 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 (Figure 2i) reveal a higher variability, compared with other core sections. This indicates a diverse methanogenic community and implies the possibility of living methanogenic archaea.

[35] Additionally, methanogenic activity incubation experiments with hydrogen and without any additional substrate (Figure 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 (350–377 cm) and in the middle of Unit III (1927–1985 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].

[36] Overall, in the Holocene sequence, the PLFA and PLEL life-marker profiles (Figures 2d and 2e) and the methanogenic activity experiments (Figure 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 (Figure 2c) might have been recently produced.

[37] 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 (Figures 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.

[38] The higher methane concentrations detected in the older sediments of the Late Pleistocene Unit III (Figure 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.

3.3. Reconstruction of Past Microbial Communities in the Kurungnakh Permafrost Sequence

[39] 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 and Gambacorta*, 1988; *Koga and 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 (Figures 2g and 2h) with the previously discussed life marker profiles (Figures 2d and 2e).

[40] Archaeol was detected throughout the whole Kurungnakh permafrost sequence and varied between 0.3 and 66.7 ng g^{-1} sediment dw (Figure 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.

[41] Isoprenoid GDGTs (Figure 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.

[42] An interesting variability in the composition of the archaeal community is indicated by the archaeol/crenarchaeol ratio (Figure 4). Archaeol is the dominating compound throughout the whole permafrost sequence. However, the archaeol/crenarchaeol ratio shows that enhanced amounts of crenarchaeol (Figure 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]. AOA have been identified in various soils and in arctic environments [*Leininger et al*., 2006; *Yergeau et al*., 2010].

3.4. Climate Impact on the Distribution of Microbial Communities in the Kurungnakh Permafrost Sequence

[43] 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].

[44] 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 32 and 40 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 (Figures 2c and 2g), indicating an increase in the methanogenic community during this period. Thus, an

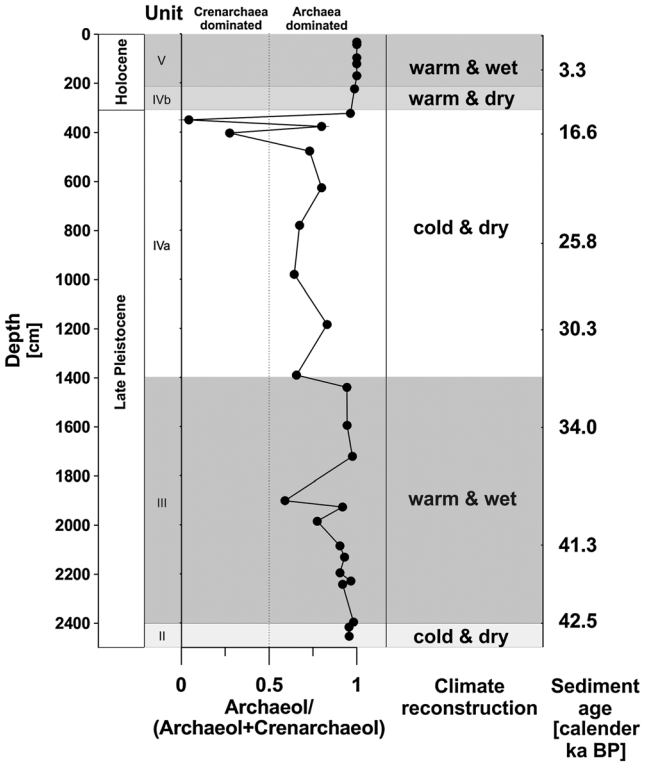

Figure 4. Ratio of the relative abundances of archaeol and crenarchaeol along the Kurungnakh permafrost sequence.

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 (1900–2200 cm) of Unit III.

[45] In contrast, in the younger sediments of Unit III (1400–1900 cm) the methane concentrations are generally low, although the archaeol concentrations remain relatively high (Figures 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 (1400–1900 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 (Figures 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 and Bustin*, 2007].

[46] 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 (Figure 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 (Figure 2c). Two exceptions are found in the small interval at 1200 cm and at the end of the Late Pleistocene (300–500 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.

[47] During the early Holocene (Unit IVb), methane and archaeol contents increased (Figures 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].

[48] 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 increased remarkably, in comparison to Late Pleistocene and Early Holocene deposits. This is in accordance with the highest concentrations of methane in these deposits (Figure 2c).

[49] 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.

3.5. Climatic Impact on the Composition of Methanogenic Communities in the Kurungnakh Permafrost Sequence

[50] 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, ecomolecular 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 (Figure 2i). DNA was successfully extracted from samples throughout the

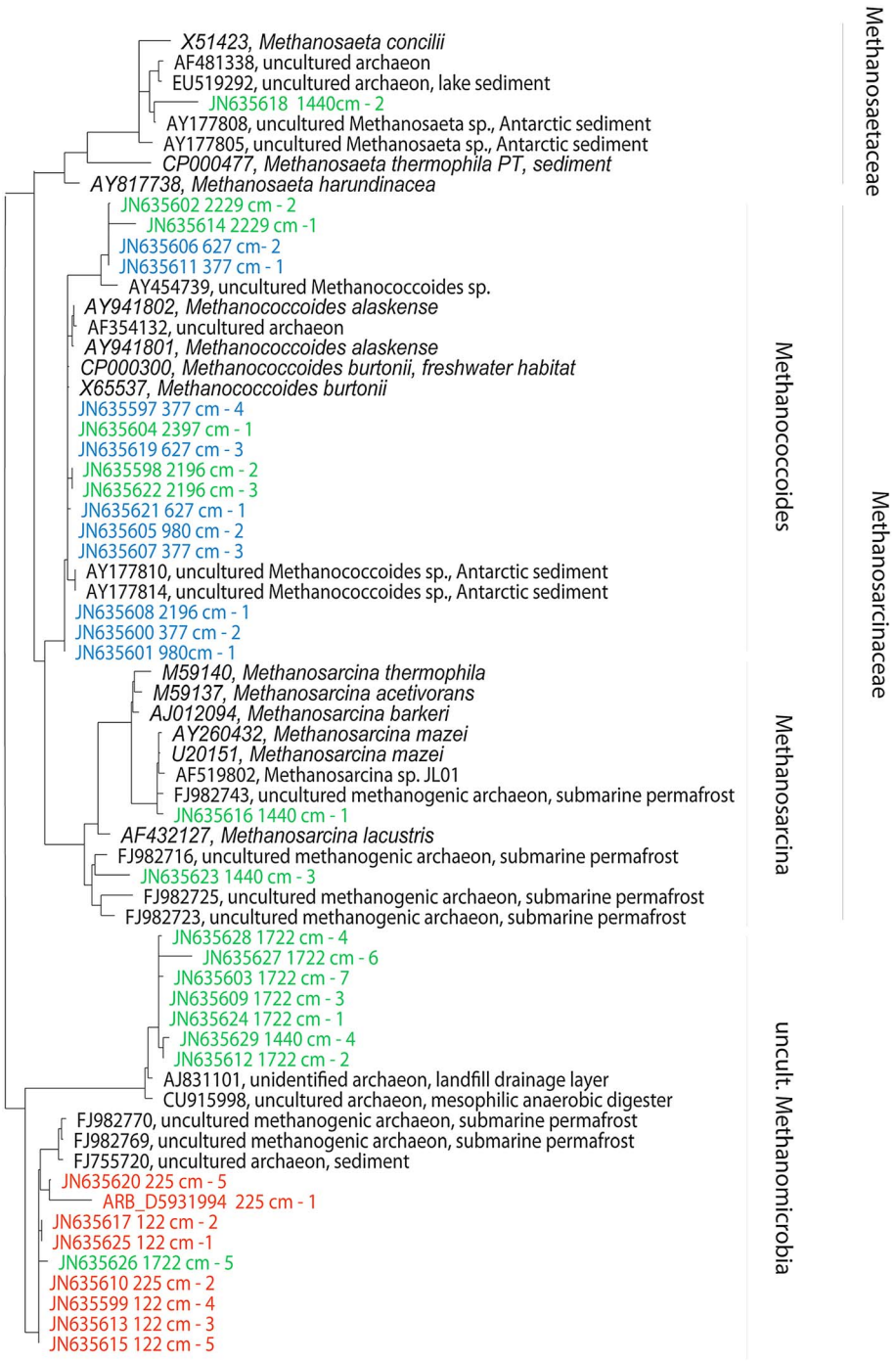

Figure 5. Phylogenetic tree showing 16S rRNA gene sequences of methanogenic archaea and their nearest relatives detected by DGGE analysis of samples from Kurungnakh Island. The vertical captions on the right side indicate their affiliation to known groups of methanogenic archaea. The single sequences 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) and Unit IVa (blue); Holocene: Unit IVb and V (red).

whole permafrost core and 41 distinct bands of methanogenic fingerprints were obtained.

[51] Individual Holocene (Unit V) and Late Pleistocene sediments (1440 and 1722 cm; Unit III) are characterized by a diverse pattern of up to seven 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 (Figures 2e and 2g), emphasizing possible living archaea in both intervals.

[52] 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* (Figure 5). Furthermore, sequences assigned to *Methanosarcinaceae* could be differentiated on the genus level to *Methanosarcina* and *Methanococcoides*.

[53] Methanogenic archaeal sequences obtained from Late Pleistocene permafrost deposits were strongly affiliated with the known genera *Methanococcoides* and *Methanosarcina*. Sequences of Unit III (Figure 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 (Figure 2g).

[54] 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 (Figure 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 subzero conditions, these organisms can potentially survive in frozen ground and retain metabolic activity.

[55] Generally, methanogenic sequences received from Holocene sediments indicated a different phylogenetic affiliation than those from Late Pleistocene sediments. Sequences from Holocene deposits (Figure 5, marked in red) showed the closest affiliation to sequences retrieved from submarine permafrost habitats in the Laptev Sea (FJ982770, FJ982769, and 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].

[56] 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 paleoenvironmental conditions in their respective genetic compositions over geologic timescales.

4. Conclusion

[57] This new comprehensive study on Kurungnakh Island, in the Lena Delta of the Siberian Arctic, investigated methane concentrations, sedimentary properties, microbial biomarkers, and ecology data. Based on climate reconstruction data, we evaluated 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.

[58] 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 the associated increase in methane emission from these environments.

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