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Archaeal communities in High Arctic wetlands at Spitsbergen, Norway (78°N) as characterized by 16S rRNA gene fingerprinting

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Abstract

Emissions of the greenhouse gas methane from Arctic wetlands have been studied extensively, though little is known about the ecology and community structure of methanogenic archaea that catalyze the methane production. As part of a project addressing microbial transformations of methane in Arctic wetlands, we studied archaeal communities in two wetlands (Solvatnet and Stuphallet) at Spitsbergen, Norway (78°N) during two summer seasons. Directly extracted peat community DNA and enrichment cultures of methanogenic archaea were analyzed by nested PCR combined with denaturing gradient gel electrophoresis and subsequent sequencing of 16S rRNA gene fragments. Sequences affiliated with *Methanomicrobiales*, *Methanobacteriaceae*, *Methanosaeta* and Group I.3b of the uncultured crenarchaeota were detected at both sites. Sequences affiliated with *Methanosarcina* were recovered only from the site Solvatnet, while sequences affiliated with the euryarchaeotal clusters Rice Cluster II and Sediment 1 were detected only at the site Stuphallet. The phylogenetic affiliation of the recovered sequences suggested a potential of both hydrogenotrophic and acetoclastic methanogenesis at both sites. At Solvatnet, there were clear temporal trends in the archaeal community structure over the Arctic summer season. The archaeal community composition was significantly affected by factors influencing the activity of the overall bacterial community, as measured by in situ emissions of CO_2. Methane emissions at both sites were influenced more by peat temperatures and thaw depth than by the archaeal community structure. Enrichment cultures for methanogenic archaea determined that most of the methanogens detected directly in peat could grow in culture at 10 °C. Culture based biases were indicated in later enrichment steps by the abundant growth of a *Methanosarcina* strain that was not detected directly in peat samples.

Keywords: Archaea; Community analysis; 16S rRNA gene; Denaturing gradient gel electrophoresis; Methane; Arctic wetland

1. Introduction

Tundra wetlands are generally net carbon sinks, since waterlogged, anoxic and cold conditions reduce decomposition rates and favor peat formation. Methane emissions from Arctic wetlands are estimated to about 35 Tg $year^{-1}$ [1], which correspond to nearly 6% of the total global methane sources [2]. Almost all climate models predict substantial warming and increased precipitation for polar regions in the next century [3], and there is concern that this may increase the methane emissions from this region [4,5]. On this background, numerous studies of methane fluxes from subarctic and Arctic tundra have been performed [6–8]. However, the control

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mechanisms for methane production, consumption and emissions from Arctic wetlands are still not fully understood [4,9].

A few previous studies have indicated the existence of ecosystem-scale differences in the functioning of methanogen communities [10]. For instance, the effect of pH on methane production differed between neutral and acidic peat in Canada, and reported values for the temperature response of methane production vary widely. This indicates that knowledge of microbial community structures can improve the understanding of the complex control mechanisms of methane emissions in Arctic wetlands. To the best of our knowledge, there have been no reported molecular studies of the archaeal community composition in Arctic wetlands. However, archaeal community composition in several northern acidic bog and mires have been reported [11–16]. In these ecosystems, ribosomal RNA (16S rRNA) and methyl coenzyme M reductase (mcrA) gene sequences characteristic for *Methanosarcinaceae*, *Methanosaetaceae*, *Methanobacteriaceae*, and *Methanomicrobiales* have frequently been detected. In addition, 16S rRNA analysis has revealed crenarchaeotal and euryarchaeotal sequences with no close relatives among cultured archaea.

The present investigation was part of a project addressing microbial transformations of methane in Arctic wetlands. Studies of the methanotrophic diversity in the studied sites have been published elsewhere [17]. The overall aim of this study was to obtain basic knowledge about the community composition of methanogenic archaea in two weakly acidic, high arctic wetlands at Spitsbergen. Specific objectives were: (1) to investigate if there were site-related differences in the community composition, (2) to investigate if the community composition were different in early and middle to late phases of the short Arctic summer season, (3) to elucidate the relationship between community composition, environmental parameters and CO_2 and methane emissions, and (4) to evaluate the feasibility of culture-based approaches. To answer these questions, samples were collected in two consecutive years. DNA was extracted from two peat depths and enrichment cultures for methanogenic archaea were established with five different carbon sources (H_2/CO_2, formate, acetate, methanol, and trimethylamine). The composition of the archaeal communities in situ and in the enrichments was monitored and compared by PCR amplification and denaturing gradient gel electrophoresis (DGGE) separation and sequencing of amplicons. Environmental parameters and in situ emissions of CO_2 and methane were measured in conjunction with each sampling, and the relationships between community data, environmental parameters and emissions of CO_2 and methane were investigated using ordination methods.

2. Materials and methods

2.1. Field sites

The investigated sites were located near Ny-Ålesund, Spitsbergen (78°50′N–11°30′E). The site Solvatnet was located on a marine terrace just outside the settlement, while the site Stuphallet was located approximately 10 km northwest of Ny-Ålesund (78°57′N–11°40′E), in a large depression 500 m away from the base of a bird cliff. The vegetation was dominated by mosses at both sites [17,18]. The ecosystem at Solvatnet was influenced by grazing by Barnacle Geese (*Branta leucopsis*) and Svalbard reindeer (*Rangifer tarandus plathyrynchus*) [18]. At both sites, the peat was classified as undecomposed (H1) and almost undecomposed (H2) using von Post's scale [19].

2.2. Sample collection

Field studies and sampling were performed twice each year to obtain samples representing both early (July 1998 and 1999) and middle to late in the growing season (August 1999 and September 1998). Samples were taken with PVC corers sharpened in one end (inner diameter 4.5 cm, length 20 cm). To reduce compression, a knife was used to cut around the edge as the corer was pressed carefully down. Cores were sealed with rubber stoppers and metal tape, and stored at 4 °C. Cores were taken near the same point at each sampling date, and this point was 2–4 m away from the transect used for measuring emissions of CO_2 and CH_4 at each site. One core was used for soil characterizations, while another core was used for the molecular studies and as inoculum for enrichment cultures. The samples were processed upon arrival in the laboratory after 2–6 days, except in September 1998 when the samples were stored for 15 days due to logistic problems. Solvatnet cores were cut at a visible transition border between upper and lower peat. The upper peat layer was light brown with visible roots, while the lower peat layer was darker brown and more compact. Stuphallet cores were cut into two at the middle, since there were no clear layers in the peat.

2.3. Soil characteristics

Air and peat temperatures were measured using a hand-held digital thermometer equipped with a 50 cm long probe. Permafrost depth was measured by inserting the thermometer probe down to the frozen layer. Mean peat temperature was calculated by averaging temperature data measured at 5 cm intervals down to the permafrost. Water content and organic content were determined gravimetrically by drying 10 g of peat overnight at 105 °C, followed by combustion overnight at 450 °C. Peat pH was measured in 0.01 M $CaCl_2$ [20].

Total prokaryotic counts were determined by filtering appropriate dilutions of Waring-blended peat homogenates onto 25 mm diameter black polycarbonate filters with 0.2 μm pore size (Osmonics Poretics Products, Minnesota, USA). The filters were stained with 4′,6-diamidino-2-phenylindole (DAPI) [21] and the cells were counted with a fluorescence microscope (DM RBE, Leica, Germany) equipped with filter cube A (Leica, Germany). For each filter, a total of at least 200 cells were counted.

2.4. Methane and CO_2 emissions

Methane emission rates were measured using static stainless steel chambers of circular shape (base area 100 cm^2, volume 1160 ml) with a butyl rubber septum closely fitted into an opening on the top of each chamber. The chambers were placed at 15 points forming an L-shaped transect, with a distance of 1 m between each chamber. Care was taken to avoid compression of the peat in the immediate proximity of each chamber. A sub-sample (11 ml) of the chamber gas was withdrawn using a 20 ml plastic syringe, at time zero when the chambers were sealed and after one and two hours. The syringe was flushed 8–10 times with the chamber air before a subsample was withdrawn and immediately injected into glass vials (12 ml) sealed with a butyl rubber septum. In the laboratory, methane concentrations were measured using a gas chromatograph (Shimadzu GC-14A) equipped with a Porapak Q stainless steel column (1.8″, 1.5 m) and a Flame Ionization detector. Injection, detection and column temperatures were 200, 240, and 45 °C, respectively. Nitrogen was used as carrier gas at a flow rate of 50 ml min^{-1}. Methane fluxes were calculated from the linear increase in methane concentration in the chambers.

CO_2 emission rates were measured using a portable infrared analyzer (EGM-1, PP Systems). Three replicate measurements were performed at 8–10 of the transect points that were also used for methane emission measurements.

2.5. Enrichment cultures

Methanogenic archaea were enriched using a modified Medium 2 from Whitman et al. [22]. The basic medium contained (per liter) $CaCl_2 \cdot 2H_2O$ 0.14 g; KCl 0.34 g; NH_4Cl 0.5 g; $MgCl_2 \cdot 6H_2O$ 6.2 g; $K_2HPO_4 \cdot 3H_2O$ 0.14 g; iron stock solution (see below) 5 ml, NaCl 2.93 g; casamino acids (Difco 0230-01-01, Becton–Dickinson and Company, NJ, USA) 1 g; 0.1% (w/v) resazurin 2 ml; trace element solution (SL-10) [23] 10 ml; vitamin solution [24] (modified by adding 10 mg vitamin B_{12} per liter) 10 ml; 1 M $NaHCO_3$ 60 ml; and 0.5 M Na_2S $9H_20$ 4.5 ml. The iron stock solution was made by adding 0.2 g $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$ to a screw-top bottle and adding 0.1 ml HCl (conc.) followed by 100 ml H_2O. This solution was made new every 4 weeks. Sterile anaerobic solutions of vitamins, $NaHCO_3$, and $Na_2S \cdot 9H_2O$ were added after autoclaving, but before pH was adjusted to 7 with sterile, anaerobic HCl. The medium was then transferred to sterile serum bottles. Prior to inoculation, carbon sources from sterile, anoxic stock solutions were added to individual bottles with 30 ml basic medium to the following end concentrations: carbonate (120 mM, i.e., additional 60 mM compared to basic medium), formate (80 mM), acetate (50 mM), methanol (50 mM) and trimethylamine (TMA) (40 mM). N_2:CO_2 (80:20, v/v) was used as headspace gas for some enrichments with acetate and formate. For all other enrichments, the headspace gas was H_2:CO_2 (80:20, v/v). Enrichment cultures with (H_2/CO_2) were made in 100 ml bottles, while 50 ml bottles were used for other carbon sources.

Peat from the lower part of the cores (about 6 ml, corresponding to approximately 0.7 g dry peat) was transferred anaerobically to 100 ml serum bottles containing acid-washed glass beads and 50 ml anaerobic medium without carbon source. Bottles were left at 10 °C without shaking overnight, before peat suspensions were made by gentle shaking. The suspensions were used as inoculum (10%, v/v) for enrichment cultures. Two parallel cultures were made for each carbon- and energy-source. They were incubated at 10 °C without shaking. Bottles were re-pressurized periodically throughout the growth period. Cells were harvested from 1 ml culture (0.5 ml from each parallel) for molecular analysis. For the first enrichment step cells were harvested after 7 weeks, while for further enrichment steps cells were harvested when the cultures became turbid.

2.6. DNA extraction

Extraction and purification of peat community DNA was performed with FastDNA© SPIN Kit for soil (BIO101, CA, USA) in combination with a freeze–thaw lysis protocol [25]. Due to low density of the peat, the silica beads from the MULTIMIX 2 Tissue Matrix Tube of the kit were transferred to a sterile 15 ml Falcon tube, and 0.5 g wet peat and 1 ml lysis buffer (0.12 M sodium phosphate buffer (pH 8), 5% SDS) were added. The tube was vortexed for 1 min, incubated at 65 °C for 75 min, followed by three quick freeze–thaw cycles (−80 and +65 °C). After this combined mechanical and chemical lysis, the Falcon tube was centrifuged at 2300*g* at 15 °C for 20 min. The supernatant was then transferred to a sterile 1.5 ml Eppendorf tube together with as much of the peat material as possible. After centrifugation at 14,000*g* for 15 min, DNA in the supernatant was purified as described by the manufacturer. DNA was eluted in 100 μl DES supplied with the kit. The quality and

amount of DNA was checked on 0.7% agarose gels with ethidium bromide staining.

2.7. PCR amplification

Archaeal 16S rRNA genes were amplified from DNA extracted from peat and from cell pellets harvested from enrichment cultures, using a modification of the nested PCR protocol described by Øvreås et al. [26]. One new primer was designed for this work, designated PRARCH112F (5′GCTCAITAA/TCACGTGG3′). This primer was used in the first round of PCR amplification in combination with PREA1100R. Dilutions of the PCR products from the first amplification were used as template in a second amplification with the primers PARCH340F-GC and PARCH519R. In both amplifications, the optimal template concentration was found using tenfold serial dilutions so that final products of similar concentration were obtained from each sample. The reaction mixture of 50 μl contained 5 μl template, 0.5 μM of each primer, 100 μM of each dNTP, 0.1% (w/v) bovine serum albumin, PCR buffer 1× (Tris–Cl, KCl, $(NH_4)_2SO_4$, 1.5 mM $MgCl_2$, pH 8.7) (Qiagen, Hilden, Germany), and 1.25 U HotStarTaq DNA Polymerase (Qiagen, Hilden, Germany). In the first reaction, 1× ''Q-solution'' (Qiagen, Hilden, Germany) was added to the mix. Amplification with the primers PARCH112f and PREA1100R was performed using the following protocol: 15 min of initial enzyme activation (95 °C), followed by 35 cycles of 30 s at 94 °C, 30 s at 51 °C, and 60 s at 72 °C, with a final elongation step of 10 min at 72 °C. Amplification with the primers PARCH340F-GC and PARCH519R was performed using a touchdown protocol of 15 min initial enzyme activation (95 °C), followed by 20 cycles of 30 s at 94 °C, 30 s at 72 °C (decreasing in each cycle by 0.5 °C), and 60 s at 72 °C. Another 20 cycles followed with 30 s at 94 °C, 30 s at 62 °C, and 60 s at 72 °C. Finally, there was an elongation step of 10 min at 72 °C. PCR products were analyzed by electrophoresis in 2% agarose gels stained with ethidium bromide.

Stringency conditions for PCR were optimized using cells of *Escherichia coli*, *Clostridium perfringens*, *Halococcus morrhuae*, *Archaeoglobus fulgidus*, *Methanococcus voltae* and *Methanococcoides methylutens*. The choice of controls was based on theoretical analysis of primer specificity using the Probe Match Program from the Ribosomal Database Project (http://www.cme.msu.edu/RDP).

2.8. Denaturing gradient gel electrophoresis and sequencing

Similar amounts of amplified fragments (approximately 190 bp) were separated on gels containing 8% polyacrylamide, 1× TAE buffer and linear gradients of 45–70% denaturant (100% denaturant consisted of 7 M urea and 40% (v/v) formamide). Electrophoresis was performed at 60 °C and 20 V for 10 min, followed by 60 V for 20 h. After electrophoresis, the gels were stained with SYBR Gold (1:10,000 dilution, Molecular Probes Inc.) for 1 h, rinsed with distilled water and photographed under UV illumination with Polaroid Type 665 positive/negative films.

DNA from dominating and unique bands were punched out with a sterile pipette tip and transferred to sterile 1.5 ml Eppendorf tubes with 20 μl of double sterilized milliQ water. DNA eluted passively at 4 °C overnight and the eluate was used as template for reamplification. Reamplified products with the expected electrophoretic mobility (as controlled on a new DGGE gel) were purified by QIAquick PCR Purification Kit (Qiagen, Hilden, Germany). Sequencing reactions were performed using ABI PRISM™ Big Dye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, CA, USA) as recommended by the manufacturer. Sequences used in phylogenetic analysis were sequenced in both directions with PARCH340 and PARCH519 as sequencing primers in the forward and reverse reaction, respectively.

2.9. Phylogenetic analysis of sequences

The possibility that the sequences could be chimeric was tested using CHIMERA_CHECK (v. 2.7) from The Ribosomal Database Project (RDP) (http://35.8.164.52/cgis/chimera.cgi?su=SSU). Partial sequences were aligned with 16S rRNA sequences of the closest relatives using the BLAST search facility of the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/blast/). Our sequences and sequences with high BLAST scores were added to a pre-aligned database of 2720 complete and partial archaeal 16S rRNA gene sequences [27], (http://honeybee.helsinki.fi/users/gjurgens/Arb/arb_page.htm) using the aligning tools from the ARB program package (Technical University of Munich, Munich, Germany; http://www.arb-home.de). Sequences were added to preexisting maximum likelihood trees [27] without affecting the initial tree topology using a special ARB parsimony tool. Nodes not used in the presentation were removed from the resulting tree using the Remove Species From Tree tool. To evaluate the resolution of the hypervariable V3-region for each group, we constructed trees based on the amplified region only using Phylip Distance methods (FITCH with Jukes-Cantor correction). The partial 16S rRNA gene sequences obtained in this study are available in the EMBL/GenBank/DDBJ nucleotide sequences database under Accession Nos. AJ749951–AJ749963.

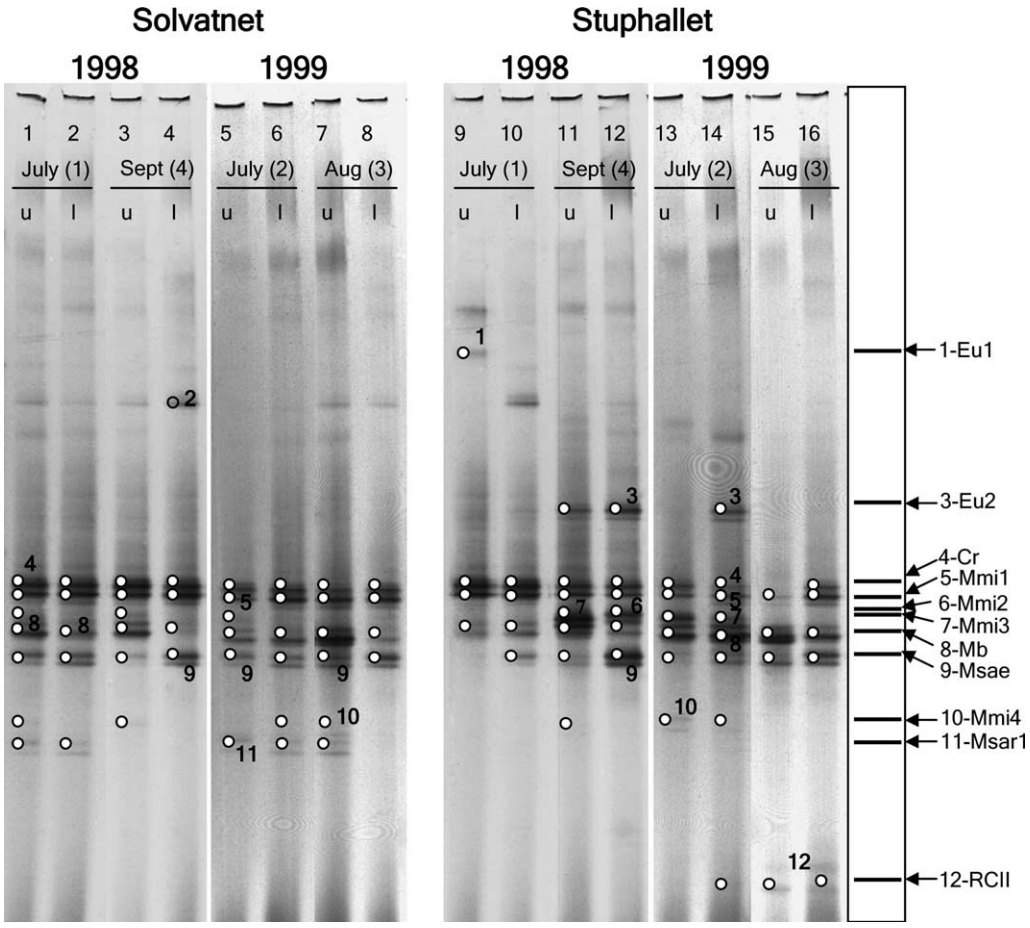

Fig. 1. DGGE profiles of 16S rRNA genes amplified from peat community DNA. Bands with identical sequences were given identical numbers. Bands marked with white circles were used in the binary matrix. Band 2 represented an artifact and was not sequenced or used in statistical analyses. The presence or absence of bands was decided based on replicate gels of the same samples. The phylogenetic affiliation of each band is indicated beside the gel figure (see Fig. 4). Abbreviations: u: upper peat, l: lower peat, Eu1-2: euryarchaeotal cluster Sediment 1, Cr: crenarchaeotal cluster Group 1.3b, Mmi1-4: *Methanomicrobiales*, Mb: *Methanobacteriaceae*, Msae: *Methanosaeta*, Msar1: *Methanosarcina*, RCII: Rice Cluster II.

2.10. Statistical analysis

Spatial and temporal variation in peat characteristics and emissions of CO_2 and methane were analyzed using Student's *t* test (confidence level 0.05).

The gels were analyzed by ordination methods based on a binary matrix of the DGGE bands marked with white circles in Fig. 1. Results from at least two separate PCR-products and at least three DGGE gels were used to ensure the robustness of the matrix. Ordination analyses were performed using the computer program CANOCO for Windows (version 4.5) and ordination results were graphically analyzed using CANODRAW (version 4.0) [28]. Correspondence analysis (CA) and canonical correspondence analysis (CCA) are adapted to presence/absence of data and were used for unconstrained and constrained analyses, respectively [29]. In general, default settings were used, except that rare "species" (DGGE bands) were down-weighted. Significance of constrained ordination models (CCA) was tested using Monte Carlo permutation tests with 999 iterations under the reduced model.

3. Results

3.1. Environmental variables

The physicochemical environment of the peat as well as climatic parameters can potentially affect both microbial communities and emissions of CO_2 and methane. We therefore characterized the peat at each sampling time (Table 1) and recorded peat temperatures and thaw depth (Table 2). At both sites, the peat had relatively high values for water content, organic content and pH (Table 1). Distinct layers were visible only in Solvatnet peat and decreasing organic content with depth was a clear trend only at this site. The micro-climatic conditions were slightly different between the sites, with a delayed spring thaw at Stuphallet compared to Solvatnet,

Table 1
Characteristics of upper and lower peat[a] from Solvatnet and Stuphallet at each sampling date

Site	Sampling date	Water content (% of dw)[b]		Organic content (% of dw)[b]		pH[b]		Total prokaryotic count (g dw)$^{-1}$ × 10^9	
		Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower
Solvatnet	08.07.1998	600	390	88	68	5.9	5.7	40	20
	02.09.1998	>800	680	90	79	6.0	5.8	20	4
	05.07.1999	600	440	86	72	6.0	5.8	20	8
	09.08.1999	680	490	87	75	6.0	5.9	10	8
Stuphallet	07.07.1998	>800	>800	79	79	6.4	6.1	20	4
	03.09.1998	>800	550	91	72	5.7	5.2	20	3
	06.07.1999	>800	730	82	81	6.2	6.0	10	2
	10.08.1999	>800	690	81	80	6.7	5.8	10	10

dw: dry weight.
[a] At Solvatnet, the distinction between upper and lower peat was based on a visible transition border. At Stuphallet, there were no visible layers and the cores were cut in half.
[b] Physicochemical parameters were determined with three parallels. Mean values are shown.

Table 2
Temperature and activity-related parameters at each sampling date

Site	Sampling date	Thaw depth (cm)	Mean peat temperature	Temperature at 5 cm depth	CH_4 emission[a] (μg CH_4 m^2 h^{-1})	CO_2 emission[a] (g CO_2 m^2 h^{-1})
Solvatnet	08.07.1998	28	7.1	14.8	2801 (1030)	0.51 (0.04)
	02.09.1998	31	1.5	2.9	706 (417)	0.18 (0.01)
	05.07.1999	25	3.3	8.1	93 (14)	0.36 (0.05)
	09.08.1999	40	2.4	4.8	955 (219)	0.34 (0.04)
Stuphallet	07.07.1998	15	6.6	9.3	605 (114)	<0.05
	03.09.1998	31	1.5	2.6	576 (146)	0.05 (0.01)
	06.07.1999	20	3.9	8.1	40 (14)	0.15 (0.02)
	10.08.1999	34	2.0	4.0	93 (22)	0.14 (0.02)

[a] Median values and standard errors are presented.

as reflected in the thaw depths measured in July (Table 2).

3.2. Emissions of methane and CO_2

At the studied sites, aerobic methane oxidation potential was not detectable in upper peat (<0.1 ppm per 48 h, data not shown), and hence it can be assumed that methane emissions were not significantly affected by the activity of methane oxidizing bacteria. The measured emissions were therefore primarily a function of methane produced by methanogenic archaea and methane stored in the peat. The methane emissions were relatively low at both sites, but were within the previously reported ranges for other northern wetland and tundra habitats (reviewed in [7]). Median values of recorded methane emissions were in general higher at Solvatnet than at Stuphallet, but due to high standard errors the difference was significant only in July 1998 and in August 1999 ($p < 0.05$) (Table 2). We recorded relatively low methane emissions (<100 μg CH_4 m^2 h^{-1}) at both sites in July 1999 when both peat temperatures and thaw depths were low (<5 °C and = 25 cm, respectively). In contrast, methane emissions were in general higher than 500 μg CH_4 m^2 h^{-1} for dates that had higher values for at least one of these variables, except at Stuphallet in August 1999.

Respiration in the moss-layer constitutes a minor part of the total ecosystem respiration [30], and hence the measured CO_2 emissions (Table 2) largely represented the microbial respiration in the peat. The CO_2-emissions were higher at Solvatnet than at Stuphallet at all sampling dates ($p < 0.001$), even in September 1998 when the CO_2-emissions at Solvatnet were lower than at the other dates ($p < 0.001$). The largest emissions of both CO_2 and methane were recorded at Solvatnet in July 1998, coinciding with the highest peat temperatures (Table 2), and the highest prokaryotic counts both in the upper and lower peat layers (Table 1).

3.3. DGGE analysis of peat samples

Archaeal communities at two peat depths (Section 2.2) of the Arctic wetlands, Solvatnet and Stuphallet, were compared using DGGE fingerprinting (Fig. 1). The DGGE profiles revealed the presence of four to

eight well-defined (see below) bands for each sample. Four bands (bands 4, 5, 8, and 9) were present in nearly all profiles irrespective of site, peat depth and sampling date. In addition, two other bands (bands 7 and 10) were detected at both sites. In contrast, band 11 was only detected in Solvatnet profiles, and bands 1, 3, 6, and 12 were only detected in Stuphallet profiles.

Several measures were taken to ensure that the interpretation of the DGGE gels was not biased by PCR-artifacts. Firstly, we merged double bands, i.e., bands that could not be separated by repeated excisions and re-amplifications and which yielded pure sequences. Secondly, only bands in the same position as those yielding good sequences were included in the binary matrix. For instance, we excluded some bands in the upper part of the gel (i.e., band 2 and bands with similar migration, Fig. 1), which upon reamplification repeatedly resulted in nearly full DGGE profiles instead of a band with the expected migration.

A higher variability of DGGE profiles from Stuphallet as compared to Solvatnet was indicated by CA analysis, showing a larger spread of the samples from this site (Fig. 2). Trends with time and depth were seen only in Solvatnet DGGE profiles (Figs. 1 and 2). When looking at bands 8 (Mb) and 9 (Msae), no depth-related differences in the relative band intensities could be seen in samples from July (Fig. 1, lanes 1–2 and 5–6). However, later in the season depth related differences were observed (September 1998 and August 1999; Fig. 1, lanes 3–4 and 7–8). Then, band 8 was relatively stronger in upper peat and band 9 relatively stronger in lower peat. Also, the presence of band 11 (Msar1) varied. In the July samples, this band was detected in both the upper and lower peat profiles (Fig. 1, lanes 1–2 and 5–6), but it was only present in the upper peat profile in August 1999 (Fig. 1, lanes 7–8), and it was not detected in September 1998 (Fig. 1, lanes 3–4). The three DGGE profiles from Solvatnet samples that did not contain the Msar1 band (Sv3l, Sv4u, and Sv4l) appeared to be relatively similar to profiles from two of the early Stuphallet samples (St2u and St1l) (Fig. 2).

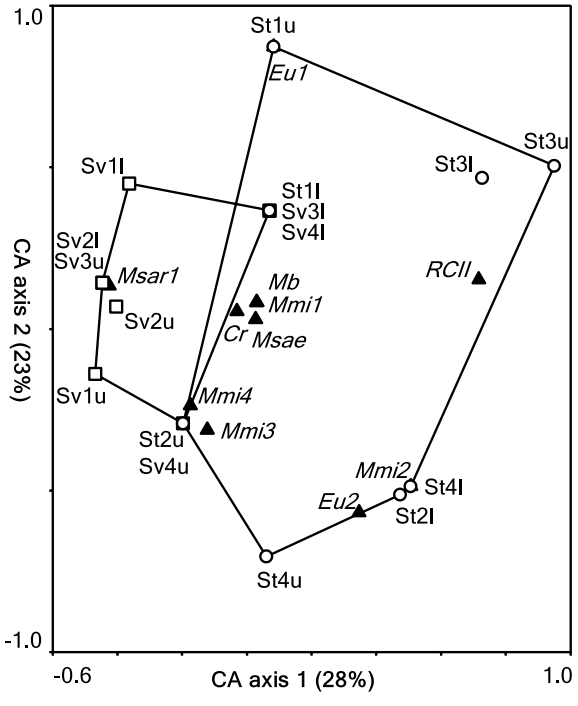

Fig. 2. CA analysis of binary DGGE data. Squares mark samples from Solvatnet and circles mark samples from Stuphallet. The score of the individual bands are marked with triangles and the corresponding band label is shown in italics. Where the triangle cannot bee seen, it is covered by the symbol for the sample; in such cases, the band was present in only one sample. Abbreviations: Sv: Solvatnet, St: Stuphallet, 1: July 1998, 2: July 1999, 3: August 1999, 4: September 1998, u: upper peat, l: lower peat. The phylogenetic labels are as described in the legend of Fig. 1.

Constricted canonical analysis was used to investigate whether any of the environmental variables (Tables 1 and 2) or the CO_2 emissions (Table 2) individually could explain a significant ($p < 0.05$) part of the variation in the binary DGGE data. Only CO_2 emission contributed significantly ($p < 0.01$) when used as the only explanatory variable, explaining about 18% of the total inertia (0.731). To test the robustness of the statistical analyses, we tested the effect of removing the three bands that were in some cases uncertain (bands 6, 7, and 10) from the binary matrix. This had no significant effect on the conclusions that could be drawn from the CA and CCA analyses.

3.4. DGGE analysis of enrichment cultures

Enrichment cultures for methanogenic archaea were made to see if the methanogens detected directly in peat by molecular methods could be cultivated in the laboratory, and if there were additional methanogens that could be detected by cultivation. Fig. 3 shows a DGGE gel of primary enrichments with Solvatnet peat from 1999 to illustrate typical results. Criteria indicating growth in enrichment cultures were that the corresponding band yielded an identical sequence and was relatively strong, that the DGGE profile clearly differed from the profile of the peat used as inoculum, and further that methane production could be confirmed by gas chromatography. Based on these criteria, growth in culture at 10 °C was indicated for most of the methanogens detected in peat community DNA (full dataset not shown). Exceptions were bands 7 (Mmi3) and 11 (Msar1) that were not detected in enrichment cultures, and growth of archaea corresponding to band 5 (Mmi1) could not be confirmed unambiguously.

Bands in enrichment profiles were in general present also in peat profiles, and the recovered sequences were identical. However, two sequences, Mmi5 (Band 13) and Msar2 (Band 14), were recovered only from enrich-

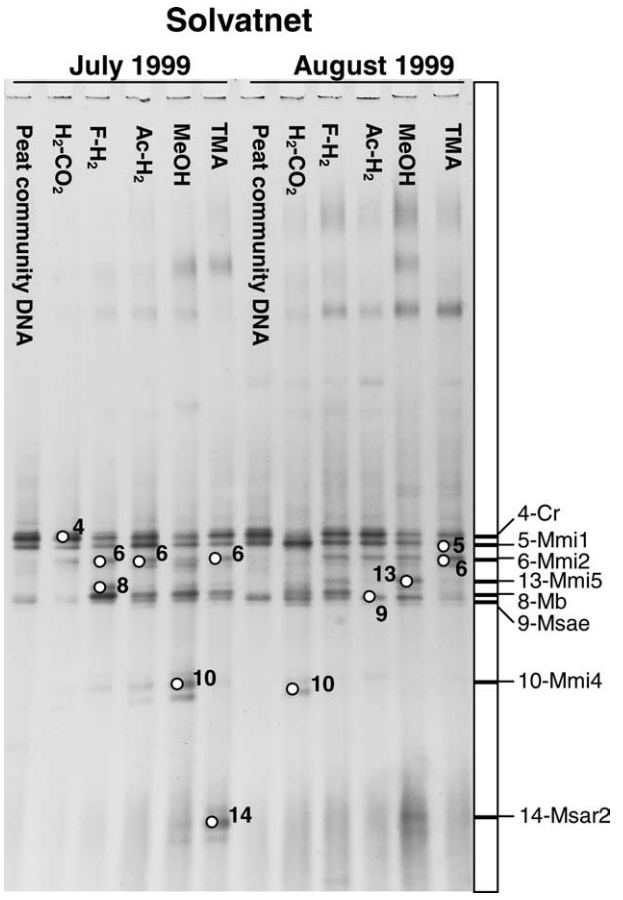

Fig. 3. DGGE profiles of 16S rRNA genes amplified from peat community DNA and enrichment cultures of Solvatnet peat from 1999. Bands with identical sequences were given identical numbers. The phylogenetic affiliation of each band is indicated on the side of the gel figure (see Fig. 4). Abbreviations: H_2–CO_2: carbonate (headspace H_2:CO_2), F–H_2: formate (headspace N_2:CO_2), Ac–H_2: acetate (headspace N_2:CO_2), MeOH: methanol (headspace H_2:CO_2), TMA: trimethylamine (headspace H_2:CO_2).

ment cultures (Figs. 3 and 4). The sequence Mmi5 was recovered from enrichments from both sites, while the sequence Msar2 was only recovered from enrichments of Solvatnet peat. Mmi5 was recovered from the enrichment with methanol (Fig. 3). Since no known member of the order *Methanomicrobiales* can grow with methanol, it seems likely that the corresponding strain was utilizing H_2 and CO_2 from the headspace gas. Msar2 was recovered from a relatively strong band in the DGGE profiles from first-step enrichment cultures with methanol and trimethylamine. After several transfers, this band became relatively strong in profiles from enrichment cultures with all tested carbon sources (data not shown).

3.5. Phylogenetic analysis of detected sequences

In total, 44 DGGE bands from profiles of peat community DNA (Fig. 1) and enrichment cultures (Fig. 3) were sequenced, and in total 13 different sequences were obtained. Nine sequences were affiliated with known methanogenic groups (Fig. 4). These sequences showed 97–100% homology to sequences in the database, when omitting the degenerate primer positions from the calculations. The allocation of each sequence to a phylogenetic group was based on the addition of the sequences to phylogenetic trees of nearly full-length 16S rRNA gene sequences and on the generation of trees based on the amplified region only. The phylogenetic affiliation could be determined to the genus-level for sequences affiliated with *Methanosarcina* and *Methanosaeta*, while the sequence affiliated with the family *Methanobacteriaceae* could not unambiguously be assigned to one of the recognized genera (data not shown). Sequences affiliated with the order *Methanomicrobiales* could not be more precisely allocated, since they did not cluster closely with sequences in the recognized families (data not shown).

Two sequences (Mmi5 and Msar2) were detected only in enrichment cultures of Solvatnet peat. These sequences had only one mismatch to the closely related sequences Mmi2 and Msar1, respectively. The closely related sequences migrated differently in DGGE gels, showing that the mismatch was not a result of sequencing error.

Four sequences grouped with clusters that currently contain no cultured members. The sequence RCII was affiliated with the euryarchaeotal cluster Rice Cluster II [39], with 99% homology to the closest relative. One sequence (Cr) was affiliated with Group 1.3b of uncultured crenarchaeota [31], and identical sequences have previously been deposited in the database. The two last sequences (Eu1 and Eu2) both clustered with sequences in the euryarchaeotal cluster Sediment 1 [27] when added to stabile backbone trees (Fig. 4), but were not closely affiliated with any database sequences. Eu1 had 86% homology to its closest relative over the amplified 151 bp, while Eu2 had 88% in the 69 bp long fragment that BLAST used in the best alignment (of 147 bp submitted). Eu2 was recovered from several samples as well as from an enrichment culture, indicating that this sequence was not chimeric. This was supported by the CHIMERA_CHECK analysis. The sequence Eu1, however, was recovered from only one sample. The CHIMERA_CHECK analysis indicated that the sequence could possibly be chimeric, but since the sequence was short and all its subsequences had low homology to known sequences, this was difficult to determine.

4. Discussion

We used a nested PCR protocol combined with DGGE and subsequent sequencing of bands to obtain basic information on the structure and variability of the archaeal communities in two High Arctic wetlands.

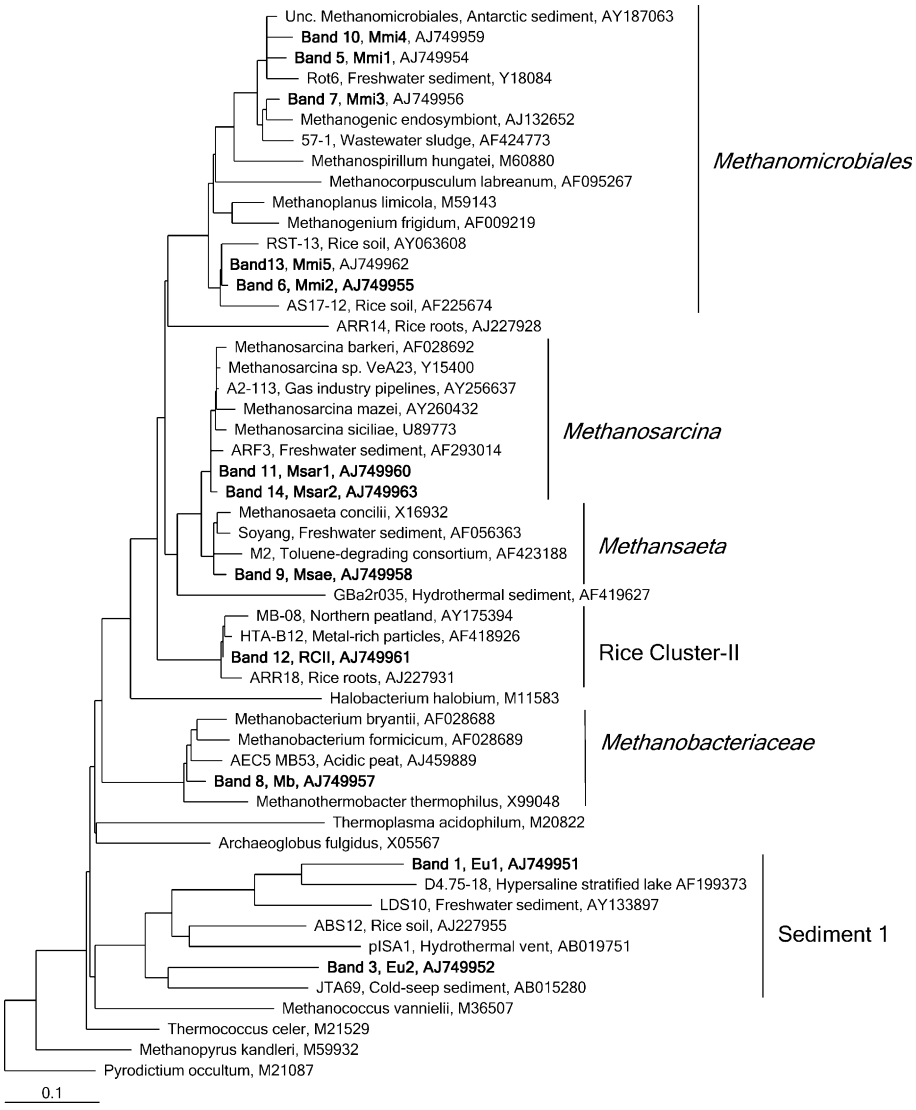

Fig. 4. Phylogenetic tree illustrating the phylogenetic affiliation of euryarchaeotal partial 16S rRNA gene sequences from reamplified DGGE bands. Novel sequences and close relatives were added to a maximum likelihood tree based on nearly full-length sequences without affecting its topology. The scale bar represents 0.1 changes per nucleotide. Sequences from the present study are indicated in bold, and band number, phylogenetic label (Figs. 1 and 2), and accession numbers are shown. A sequence affiliated with Group 1.3b of uncultured crenarchaeota (band 4, Cr, AJ749953) was not included in the tree.

Ordination analyses were used to elucidate the relationships between the archaeal community composition, peat characteristics and emissions of CO_2 and CH_4. Nested PCR was used to ensure amplification of archaeal 16S rRNA genes also from samples with low archaeal cell numbers. This approach increases the sensitivity and the specificity of the reaction, but may also increase PCR biases [32,33]. Several measures were taken to avoid inclusion of PCR artifacts in the interpretation of the gels. Since optimal amplification was ensured (within tenfold dilutions) at each amplification step, the DGGE profiles could be used as qualitative fingerprints of the most abundant archaeal rRNA sequence types in each sample. Only in one case did we interpret band intensities semi-quantitatively (see below), and we assume that this trend was robust since it was seen in both studied years. The formation of double bands in the DGGE gels (Section 3.3) could be related to degenerate positions in the primers [34], to formation of heteroduplexes [35] or to the used GC-clamp, which has the potential of forming stabile dimers and hairpins [36,37]. The gels could be interpreted despite this problem, since we reamplified nearly all bands and thoroughly investigated the migration of each reamplified product, and this was combined with an extensive sequencing effort.

Bands affiliated with *Methanomicrobiales*, *Methanobacteriaceae*, *Methanosaeta*, and Group 1.3b of uncultured crenarchaeota were present in the DGGE profiles of nearly all samples. Sequences characteristic

for the order *Methanomicrobiales* have been detected in most previous studies of northern wetlands [11–16]. Both *Methanosaetaceae*- and *Methanobacteriaceae*-related ribotypes were detected in two peatlands in New York State [15] and in the sediment of a bog lake [38]. In addition, *Methanosaetaceae* was detected in a Siberian wetland [12], while *Methanobacteriaceae* was detected in an acidic bog in Germany [13]. One sequence (Cr) was affiliated with Group 1.3b of uncultured crenarchaeota [31], which includes sequences assigned to Rice cluster IV [39]. This cluster has also previously been detected in northern wetlands [12,14,16]. Since the cluster has no cultured members, the physiology of the organisms is unknown.

At Solvatnet we also detected sequences corresponding to the genus *Methanosarcina*, a group previously detected in many studies of northern peatlands [12–16]. At Stuphallet, we detected three sequences that were all affiliated with clusters with no cultured members. One sequence (RCII) was affiliated with Rice Cluster II, a cluster which has also previously been detected [11,15,16] in northern peatlands. A methanogenic physiology is suggested for members of this cluster due to its phylogenetic placement close to *Methanosarcinales* and *Methanomicrobiales* [39], but this has yet to be demonstrated. Two sequences (Eu1 and Eu2) had low homology to sequences in the database, but clustered with sequences in the euryarchaeotal cluster Sediment 1. This cluster contains sequences assigned to Rice Cluster V [39] and sequences recovered from various sediment systems. The physiological characteristics of the members of this cluster are unknown. Sequences in this cluster have been retrieved from two previous studies of northern wetlands [14,16]. Sequences in euryarchaeotal cluster Sediment 1 are not closely related, indicating that they potentially represent a large currently unknown diversity. As a result, the precise positions of Eu1 and Eu2 within this cluster and their placement in the phylogenetic tree (Fig. 4) may be uncertain.

Our results demonstrated the potential for both hydrogenotrophic and acetoclastic methanogenesis at the studied sites. While known members of *Methanomicrobiales* and *Methanobacteriaceae* grow exclusively by the CO_2-reduction pathway, using one or more of the substrates H_2/CO_2, formate and short-chained alcohols, known members of *Methanosaeta* grow exclusively using acetate as the energy source [40]. The detection of hydrogenotrophic methanogens in Arctic wetlands is an important observation, since several studies have suggested that acetate is relatively more important as methanogenic substrate in cold compared to temperate environments [41,42]. Kotsyurbenko et al. [16] recently suggested that this may be valid only in environments where most of the organic matter is made up of polysaccharides (e.g., lake sediments and rice paddy soil), and hence may not be valid for peat. Further studies that address the effect of temperature on both the methanogenic carbon flow and the archaeal community structure in Arctic wetlands should be undertaken.

Trends with time and depth were indicated in the DGGE profiles of Solvatnet only. The relative band intensity of *Methanobacteriaceae* (band 8, Mb) increased in upper peat, while the relative band intensity of *Methanosaeta* (band 9, Msae) increased in lower peat towards the end of the summer season. Controlled laboratory studies are needed in order to link these observations to seasonal changes in specific environmental parameters. The temporal trend seen in the detection of *Methanosarcina* (band 11, Msar1) was reflected in the binary matrix and the possible relationship to changes in the measured environmental variables is discussed below. DGGE profiles from Stuphallet were variable, and no trends with depth and time were indicated. The reasons for this site difference are currently not clear, but it could be related to the fact that Stuphallet peat did not have visible layers, and the peat organic content did generally not decrease with depth.

CO_2 emissions contributed significantly to the separation of samples based on binary DGGE data of peat community DNA. This was probably related to the significant difference in the CO_2-emission levels between the two sites, and to the detection of the band Msar1 only in Solvatnet DGGE profiles from early- and mid-season, when the CO_2 emissions were high (>0.30 g CO_2 m^2 h^{-1}). Although somewhat speculative, we hypothesize that the detection of the *Methanosarcina*-affiliated band Msar1 was related to a relatively high availability of methanogenic substrates, which is linked to the activity of the bacterial community [43,44]. This hypothesis is supported by the fact that characterized species of *Methanosarcina* require relatively high substrate concentrations for growth [45]. Also, previous studies of rice soils have demonstrated that the relative abundance of *Methanosarcina*-affiliated sequences can change in response to environmental factors such as substrate concentrations and temperature, with substrate concentration apparently being the primary controlling factor [46]. The observed seasonal pattern is also consistent with previous studies of seasonal changes in dissolved organic carbon [47] and acetate [48]. Some of the other measured parameters may also influence the archaeal community structure in Arctic wetlands. However, the wetlands in this study may have been too similar to reveal such relationships, and further studies are needed.

The measured methane emissions seemed to be influenced by mean peat temperature and thaw depth. Peat temperature could have affected the activity of the methanogenic community either directly, or indirectly through effects on the supply of methanogenic substrates [49], while thaw depth could have affected the emissions through increasing the volume of peat with

methanogenic activity [50,51]. The significance of thaw depth could also be related to other factors that change through the summer season, such as for instance plant growth variables [8], but untangling of such relationships was beyond the scope of this study. The methane emission measured at Stuphallet in August 1999 was surprisingly low (<100 μg CH_4 m^2 h^{-1}) considering the relatively large thaw depth (34 cm). It is currently unknown whether this was linked, either directly or indirectly, to the fact that the archaeal community composition at this sampling date differed from the other dates.

Studies of enrichment cultures showed that most methanogens detected in peat community DNA could grow in enrichment cultures at 10 °C, and only two novel sequences were recovered from the cultures as compared to the inoculum peat. However, the results indicated that one *Methanosarcina*-affiliated strain (corresponding to Msar1) was favored in situ, while another (corresponding to Msar2) was favored in culture. The physiological differences causing this are not known, but substrate affinities and temperature adaptation could be involved. Known members of the genus *Methanosarcina* can all grow on methylated compounds, and some can also grow on either H_2/CO_2 or acetate or both [40]. A broad substrate range was suggested for the strain corresponding to Msar2, since this band became dominant after several transfers with all tested carbon sources. It is currently unknown whether Msar2 originated in an ecologically important strain, or whether growth of this strain in enrichment cultures could reflect culture-based bias. This emphasizes that in culture-based studies the community composition in the cultures should be monitored and compared with the in situ community structure. This is especially important if ecologically relevant conclusions about physiological characteristics are to be evaluated.

In conclusion, this study has provided knowledge of the archaeal community structures in two High Arctic wetlands. The results demonstrated the presence of methanogens capable of hydrogenotrophic and acetoclastic methanogenesis at both sites, and the range of detected archaeal groups was consistent with previous studies of northern wetlands. At Solvatnet, there were clear temporal trends in the archaeal community structure over the Arctic summer season. The results suggested that within the studied sites the archaeal community composition was affected by factors affecting the CO_2 production, and we hypothesize that this was caused by changes in the availability of methanogenic substrates. The overall methane emissions appeared to be influenced by peat temperature and thaw depth rather than by the archaeal community structure. However, the methane production potential of peat with different archaeal communities should be addressed in further studies. In combination with controlled laboratory studies, the presented knowledge can improve the understanding of how methane emissions from Arctic wetlands are controlled.

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