 doi:10.1111/j.1462-2920.2005.00982.x

Effects of water regime on archaeal community composition in Arctic soils

Lone Høj,[1*†] Marte Rusten,[1‡] Lars Egil Haugen,[2] Rolf A. Olsen[3,4] and Vigdis L. Torsvik[1]
[1]*Department of Biology, University of Bergen, Jahnebakken 5, N-5020 Bergen, Norway. Departments of* [2]*Plant and Environmental Sciences and* [3]*Chemistry, Biotechnology and Food Science, Norwegian University of Life Sciences, PO Box 5003, N-1432 Ås, Norway.* [4]*The University Centre in Svalbard, PO Box 156, N-9171 Longyearbyen, Norway.*

Summary

Effects of water regime on archaeal communities in Arctic soils from Spitsbergen were studied using denaturing gradient gel electrophoresis (DGGE) of amplified 16S rRNA genes, with subsequent sequencing of amplicons and ordination analysis of binary DGGE data. Samples with major differences in soil water regime showed significant differences in their archaeal community profiles. *Methanomicrobiales*, *Methanobacteriaceae* and *Methanosaeta* were detectable only in environments that were wet during most of the growth season, while a novel euryarchaeotal cluster was detected only in less reduced solifluction material. Group 1.3b of *Crenarchaeota* had a high relative abundance within the archaeal community in a wide range of wet soils. Along a natural soil moisture gradient, changes in archaeal community composition were observed only in upper soil layers. The results indicated that members of *Methanomicrobiales* were relatively tolerant to soil aeration. Differences in archaeal community composition associated with soil water regime were predominant over regional and seasonal variation, and over differences between individual wetlands. The results suggest that the observed 'on–off switch' mechanism of soil hydrology for large-scale variations in methane emissions from northern wetlands is at least partly caused by differences in the community structure of organisms involved in methane production.

Received 18 November, 2005; accepted 23 November, 2005. *For correspondence. E-mail lhoj@aims.gov.au; Tel. (+61) 7 4753 4364; Fax (+61) 7 4772 5852. Present address: †Australian Institute of Marine Science, PMB No. 3, Townsville MC, Queensland 4810, Australia; ‡Department of Biomedicine, Section of Anatomy and Cell Biology, Jonas Lies vei 91, N-5009 Bergen, Norway.

Introduction

Most models predict climate change to be most pronounced in polar areas and substantial changes in Arctic ecosystems have already been reported (Anisimov and Fitzharris, 2001). Changes in permafrost distribution could affect the water regime for large volumes of Arctic soils through effects on soil drainage, and thereby influence the balance between aerobic and anaerobic soil processes. Several functional studies have demonstrated the importance of soil water conditions on methane emissions from Arctic tundra (Bubier *et al.*, 1993; Vourlitis *et al.*, 1993; Christensen *et al.*, 1995; 2003). Christensen and colleagues (2003) suggested that soil hydrology acts as an 'on–off switch' for large-scale variations in methane emissions, with a dramatic decrease in emissions where the water table is below a certain threshold. Other factors such as temperature and availability of methanogenic substrates control seasonal dynamics where water table levels are higher (Christensen *et al.*, 1995; 2003).

Soil water regime affects microbial communities in several ways. Soils with stagnant water develop anoxic conditions due to a low diffusion rate of oxygen in water, and soil aeration is a key factor in controlling gradients also of other electron acceptors [nitrate, iron(III) and sulfate] (Liesack *et al.*, 2000). The availability of electron acceptors affects both the redox potential of the soil and the competition for substrates between functional groups such as aerobic heterotrophs, nitrate-reducing bacteria, iron-reducing bacteria, sulfate-reducing bacteria and methanogens. In addition, effects of soil water regime on the vegetation distribution (Bubier, 1995) can indirectly affect the microbial community through O_2 leaked from roots (Watson *et al.*, 1997) and by the amount and quality of litter and root exudates which act as substrate for the microbial community (Nilsson and Bohlin, 1993; Joabsson and Christensen, 2001).

Methane production in soils with different water regimes (Mayer and Conrad, 1990; Boon *et al.*, 1997; Wagner *et al.*, 2003) and in soils that have been subjected to drying, wetting or fluctuations in the water table level (Mayer and Conrad, 1990; Moore and Dalva, 1993; Peters and Conrad, 1996; Kettunen *et al.*, 1999) have been studied extensively. There is, however, limited information on how soil water regime affects the archaeal community structure, including methanogenic archaea that catalyse the methane production step. In rice soil, the presumably obligatory anaerobic

methanogenic community shows good survival during dry periods, and the biomass of methanogens is not limiting for the onset of methanogenesis after flooding (Mayer and Conrad, 1990; Roy *et al.*, 1997). In riparian soils, however, methanogenic sequences were abundant only in soils that were flooded permanently or frequently (Kemnitz *et al.*, 2004). The archaeal community dynamics also seemed to vary in these two systems (Lueders and Friedrich, 2000; Kemnitz *et al.*, 2004). The apparent differences between the systems emphasize that the effects of water regime on archaeal communities in seasonally flooded environments are still poorly understood and further studies are warranted.

Archaeal communities in northern wetlands have been studied by molecular methods only in the last decade (Hales *et al.*, 1996; Basiliko *et al.*, 2003; Galand *et al.*, 2003; Utsumi *et al.*, 2003; Kotsyurbenko *et al.*, 2004; Høj *et al.*, 2005; Juottonen *et al.*, 2005) and as far as we know only one study has included Arctic sites (Høj *et al.*, 2005). Sequences affiliated with *Methanomicrobiaceae*, *Methanosarcinaceae*, *Methanosaetaceae*, *Methanobacteriaceae*, as well as euryarchaeaotal and crenarchaeotal clusters that currently contain no cultured members have repeatedly been detected, although not all groups were detected in all studied samples. The studies have demonstrated site-specific differences in the archaeal communities, as well as differences associated with mire nutrition status (Juottonen *et al.*, 2005), soil depth (Galand *et al.*, 2002; 2003; Høj *et al.*, 2005) and seasonal trends (Høj *et al.*, 2005).

We hypothesized that archaeal communities in Arctic soils are significantly affected by soil water regime and that this effect is predominant over differences between individual wet sites and seasonal variation within a site. To test this hypothesis, we investigated the archaeal community composition in soils and lake sediments from Spitsbergen, Norway (78°N). Samples differed in soil water regime and included lake sediments, fluvial deposits, peat soils, solifluction material and till soils. Community profiles were obtained using denaturing gradient gel electrophoresis (DGGE) separation of polymerase chain reaction (PCR)-amplified 16S rRNA gene fragments, with subsequent sequencing of bands. The variation in the detected archaeal communities was analysed using non-metric multidimensional scaling (NMDS) of binary DGGE data. Statistical analysis of a combined data set, including data from a previously reported 2-year study of the Arctic wetlands at Solvatnet and Stuphallet (Høj *et al.*, 2005), was used to evaluate whether variation associated with water regime was predominant over seasonal trends and variations between individual wetlands.

Results

Soil and sediment characteristics

Sampling sites (Fig. 1) were selected based primarily on differences in soil surface material, soil water regime, and dominating vegetation (Table 1). Peat was sampled from three wetlands, two located at the Brøgger Peninsula (Stuphallet: St-pe; Solvatnet: Sv-pe; Fig. 1A) and one in the Sassen Valley (Sa-pe; Fig. 1B). These sites were permanently wet and the vegetation was dominated by mosses (Table 1). Lake sediments were sampled from two

Table 1. Characteristics of peat, soil and sediment samples.

Site	Surface material	Dominating vegetation	Depth (cm)	Water regime	Temperature range (°C)	pH in H_2O	Organic content (% dw)	Water content (% dw)	Air filled pores (% vol)
Sv-sed	Lake sediment	N.a.	0–5	Inundated	N.m.	7.1	74	> 800	W.s.
Sa-sed	Lake sediment	N.a.	0–5	Inundated	N.m.	7.2	35	646	W.s.
Ad1-flu	Fluvial deposit	Sedges/grasses	5–10[b]	Seasonally flooded	4.0–5.1	6.4	11	42	N.m.
Ad2-flu	Fluvial deposit	Sedges/grasses	10–15	Seasonally flooded	4.6–5.9	5.8	10	56	N.m.
St-pe	Peat	Mosses	10–15	Permanently wet	3.5–6.9	6.4	78	> 800	10
Sv-pe	Peat	Mosses	10–15	Permanently wet	4.9–6.7	6.8	82	641	8
Sa-pe	Peat	Mosses	10–15	Permanently wet	N.m.	6.8	55	762	N.m.
Sa-sol	Solifluction material	Mixed	10–15	Seasonally wet	N.m.	6.4	15	91	N.m.
Lb1-sol	Solifluction material	Mixed	5–10[b]	Seasonally wet	3.2–5.2	7.4	30	106	N.m.
Lb2-sol	Solifluction material	Mixed	5–10[b]	Seasonally wet	2.0–3.2	6.7	35	170	N.m.
Au[a]	Till	Mixed	2–7	Seasonally wet	2.2–10.1	4.7	22	117	35
Al[a]	Till	N.a.	10–15	Permanently wet	1.4–6.0	4.6	12	67	W.s.[c]
Bu[a]	Till	Mixed	2–7	Seasonally wet	3.2–9.9	5.6	31	203	25
Bl[a]	Till	N.a.	10–15	Permanently wet	2.3–8.2	5.7	12	77	W.s.
Cu[a]	Peat	Mosses	2–7	Permanently wet	3.4–9.0	5.7	37	558	14
Cl[a]	Till/peat	N.a.	10–15	Permanently wet	2.3–7.4	5.7	19	188	W.s.

a. Values based on average of two to four sampling dates.
b. Lower part of core dominated by gravel.
c. Calculated based on volumetric data for 15–20 cm depth.
dw, dry weight; N.a., not applicable; W.s., water saturated; N.m., not measured.

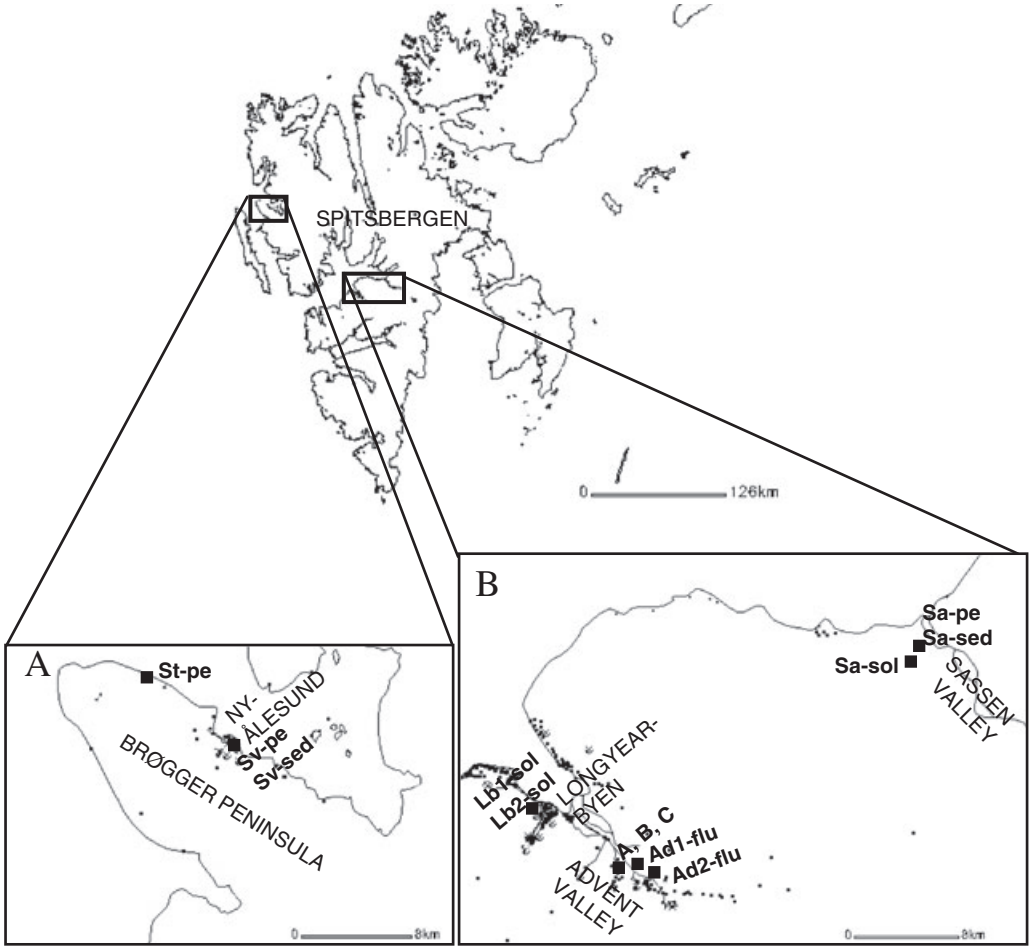

Fig. 1. Maps showing the localization of sampling areas on the West Coast of Spitsbergen.
A. Sites located at the Brøgger Peninsula.
B. Sites located in Longyearbyen, the Advent Valley and the Sassen Valley.

small lakes that were located adjacent to two of the peat sampling sites (Sv-sed, Fig. 1A; Sa-sed, Fig. 1B). Fluvial deposits were sampled from two seasonally flooded sites in the Advent Valley (Ad1-flu, Ad2-flu; Fig. 1B) where the vegetation was dominated by sedges and grasses (Table 1). Solifluction material was sampled from two sites in Longyearbyen (Lb1-sol, Lb2-sol; Fig. 1B), and one site in the Sassen Valley (Sa-sol; Fig. 1B) where the vegetation was mixed, comprising mosses, sedges, grasses and dicotyledons (Table 1). In the Advent Valley, till soils were sampled from three positions (A, B, C; Fig. 1B) along a natural soil moisture gradient, with samples taken from both upper (Au, Bu, Cu) and lower (Al, Bl, Cl) soil layers (2–7 cm and 10–15 cm respectively). The vegetation at the wettest position (C) was dominated by mosses, while mixed vegetation was found at the other two positions (A and B) (Table 1). Soil temperature in the sampled layers varied from 1.4°C to 10.1°C, soil pH values (in H_2O) were in the range 4.6–7.4 and the organic content varied from 10 to 82% (Table 1). Gravimetric water content was correlated with the organic content ($r = 0.885$, $P < 0.01$), as can be expected due to the lower density of organic material as compared with minerals.

The degree of soil aeration (Table 1) and oxygen concentrations in soil water (Fig. 2) were determined for positions along the natural gradient (A, B, C). The volume of air-filled pores in the upper layer (2–7 cm depth) decreased from the driest (Au) to the wettest position (Cu) (Table 1). At the driest position (A), no oxygen measurements were obtained in the upper layer due to very little free water, but it was demonstrated that oxygen penetrated deeper at the intermediate position (B) as compared with the wettest position (C) (Fig. 2). For the lower soil layer (10–15 cm), the calculated volume of air filled pores indicated water saturation at all positions along the gradient (Al, Bl, Cl) (Table 1). This was consistent with no detectable oxygen at this depth (Fig. 2). It is, however, possible that soil aeration was underestimated for the driest position (Al) as the calculation was based on soil density at 15–20 cm rather than 10–15 cm (Table 1).

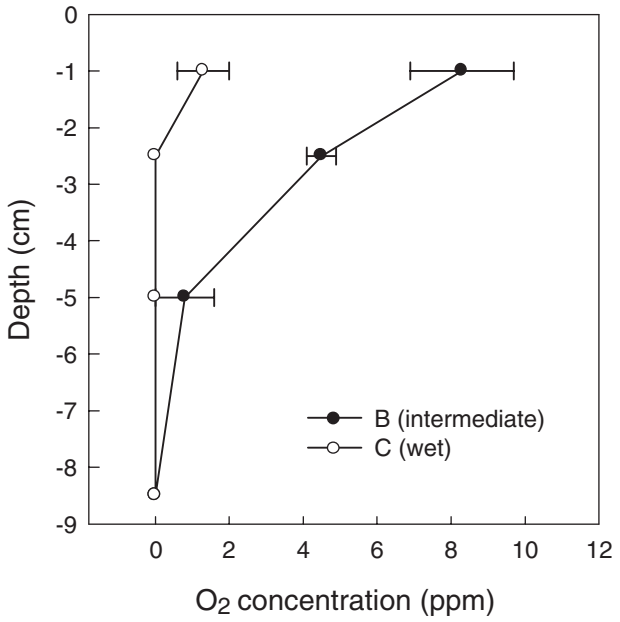

Fig. 2. Depth profiles of O_2 concentrations (p.p.m.) in soil water at the intermediate (B) and wet (C) positions along a natural soil moisture gradient.

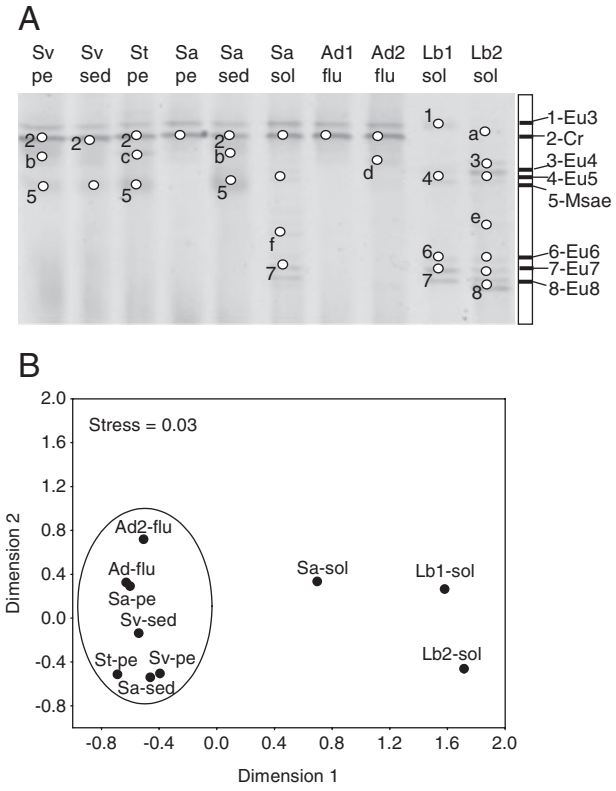

Fig. 3. A. The PCR-DGGE analysis of archaeal 16S rRNA genes from lake sediments, fluvial deposits, peat soils and solifluction material from four areas on the West Coast of Spitsbergen. Bands that were successfully sequenced are indicated by numbers, and their phylogenetic affiliations are indicated by phylogenetic labels on the side of the gel picture (see also Fig. 4). The phylogenetic labels are consistent with a previously published survey of archaeal communities in Arctic wetlands (Høj *et al.*, 2005). Bands marked with white circles were included in the binary matrix.
B. Non-metric multidimensional scaling (NMDS) analysis of the binary DGGE data.
Sv, Solvatnet; St, Stuphallet; Sa, Sassen Valley; Ad, Advent Valley; Lb, Longyearbyen; pe, peat; sed, lake sediment; sol, solifluction material; flu, fluvial deposits, Eu3-Eu8, novel euryarchaeotal cluster; Cr, Group 1.3b of *Crenarchaeota*; Msae, *Methanosaeta*.

Methane emission rates were measured for the three positions along the soil moisture gradient (A, B, C) (Table 2). Due to high temporal variation in emissions, data for five sampling dates are included. An overall trend of increasing methane emissions from the driest (A) towards the wettest (C) position was indicated. At the driest position (A), net oxidation was recorded for two of the sampling dates (23.07.2000 and 27.07.2002) and the emissions were in the range from –2.6 (± 0.3) to 40 (± 25) μg m^{-2} h^{-1}. At the intermediate position (B), emissions varied from 33 (± 10) to 5870 (± 1620) μg m^{-2} h^{-1}, and at the wettest position (C) from 96 (± 40) to 91 600 (± 31 400) μg m^{-2} h^{-1}.

Archaeal communities in soils and lake sediments

In an initial survey of archaeal communities at Spitsbergen, peat soils (pe), fluvial deposits (flu), solifluction material (sol) and lake sediments (sed) were sampled in July 1997. The archaeal communities in these samples were investigated using DGGE community profiling (Fig. 3) and sequencing of bands. As previously described (Høj *et al.*, 2005), care was taken to avoid inclusion of PCR artefacts when interpreting the community profiles. However, six bands (denoted a–f) were included in the community analysis although they did not yield pure sequences. We judged that problems with obtaining pure sequences from these bands were not PCR artefacts but rather due to co-

Table 2. Methane emission rates (μg m^{-2} h^{-1}) measured at three positions (A, B, C) along a soil moisture gradient.

Position	07.07.1999	20.07.1999	11.08.1999	23.07.2000	27.07.2002
A	40 (25)	2.7 (0.8)	18 (8)	–2.0 (0.1)	–2.6 (0.3)
B	151 (63)	33 (10)	5 870 (1620)	1250 (1130)	150 (86)
C	96 (40)	530 (109)	91 600 (31 400)	5430 (5000)	508 (146)

Average values and standard errors are shown.

migration of fragments amplified from different organisms. Each DGGE profile showed one to seven well-defined bands (Fig. 3A). Band 2 (Cr) was the only band detected in all sample types. It was present in all profiles of peat soils, fluvial deposits and lake sediments, and in one sample of solifluction material (Sa-sol). Three bands, namely 5 (Msae), b and c, were detected only in peat and lake sediment samples (Sv-pe: b and 5; Sv-sed: 5; St-pe: c and 5; Sa-sed: b and 5). The following nine bands, 1 (Eu3), 3 (Eu4), 4 (Eu5), 6 (Eu6), 7 (Eu7), 8 (Eu8), a, e and f, were detected only in solifluction material (Sa-sol: 4, 7, f; Lb1-sol: 1, 4, 6, 7; Lb2-sol: 3, 4, 6, 7, 8, a, e). Non-metric multidimensional scaling analysis of binary DGGE data separated the three solifluction samples (Sa-sol, Lb1-sol, Lb2-sol) from all other samples (Fig. 3B). These three samples were well separated and the distances between them and other samples were significantly larger than the internal distances between the other samples (*t*-test, $P < 0.001$).

A total of 14 bands yielded pure sequences, resulting in eight different partial 16S rRNA sequences (Figs 3A and 4). The sequence Cr was recovered from band 2, which represented the dominant amplicon in samples where it was present. This sequence (Cr) was affiliated with Group 1.3b of *Crenarchaeota* (Ochsenreiter *et al*., 2003) (Fig. 4) and was identical to a sequence detected in our previously reported study (Høj *et al*., 2005). The sequence recovered from band 5 (Msae; Fig. 3A) was identical to the *Methanosaeta*-affiliated sequence (Fig. 4) reported previously for peat from Solvatnet and Stuphallet (Høj *et al*., 2005). Sequences recovered only from solifluction material (Eu3-Eu8; Fig. 3A) showed 92–99% similarity to sequences in the database. These sequences clustered together in a euryarchaeotal cluster that currently contain only a few environmental sequences and no cultured organisms (Fig. 4).

To increase the representation of amplicons from other archaea relative to the apparently dominant Group 1.3b of *Crenarchaeota*, we exchanged one primer used in the first PCR reaction with a primer that had one mismatch with sequences in this cluster. The modified protocol was used to investigate the archaeal communities along a soil moisture gradient.

Archaeal communities along a soil moisture gradient

The effect of soil water regime on archaeal communities was studied in more detail at two depths (2–7 cm and 10–15 cm) at three positions along a natural soil moisture gradient. Denaturing gradient gel electrophoresis profiles of samples collected in July 1998 are presented in Fig. 5A. Additional profiles that were obtained for samples collected in September 1998, July 1999 and August 1999 showed only few deviations from the presented profiles and these are mentioned specifically below. Denaturing gradient gel electrophoresis profiles of upper soil at the wettest position (Cu) and all the lower soil samples (Al, Bl, Cl) included bands 2 and 10 (Cr and Mb respectively). In contrast, these bands were not present in profiles of upper soil from the intermediate (Bu) position. The DGGE profile of upper soil from the driest position (Au) comprised only one band, which migrated similarly to band 2 (Cr). Band 11 (Mmi6) and band 12 (Mmi4) were detected in both upper and lower soil layers from the intermediate (B) and wet (C) positions. Only these bands showed temporal variations, and in some cases one (band 11) or both were missing from the profile. However, no seasonal trends for the presence or absence of these bands were apparent. Band 9 (Mmi3) was exclusively detected in upper soil from the intermediate position (Bu).

The same PCR-DGGE protocol was used for samples from the gradient as previously reported for studies of peat from Solvatnet and Stuphallet (Høj *et al*., 2005) making the results directly comparable. Binary DGGE data from the two studies were combined for statistical analysis in order to evaluate whether the variation associated with water regime was more prominent than seasonal and regional variation. Three-dimensional NMDS analysis showed that DGGE profiles of upper soil layers (2–7 cm) from the dry (A) and intermediate (B) positions were clearly separated both from each other and from all other profiles (Fig. 5B). The distances between these samples and the other samples were statistically larger than the internal distances between all other samples (*t*-test, $P < 0.001$).

A total of eight bands from DGGE profiles of the soil moisture gradient samples were sequenced (Fig. 5A), resulting in five different sequences (Cr, Mb, Mmi3, Mmi4, Mmi6; Figs 4 and 5A). The sequence Cr (band 2 in Figs 4 and 5A) was identical to the sequence Cr recovered in the initial survey and in the previously reported study (Høj *et al*., 2005). The sequence Mb (band 10, Figs 4 and 5A) was affiliated with the methanogenic family *Methanobacteriaceae*, and was identical to a sequence recovered from the peatlands Solvatnet and Stuphallet (Høj *et al*., 2005). The sequences Mmi3, Mmi4 and Mmi6 (bands 9, 12 and 11; Figs 4 and 5A) were all affiliated with the methanogenic order *Methanomicrobiales*. Mmi3 and Mmi4 were identical to sequences recovered from DGGE profiles of peat from Solvatnet and Stuphallet (Høj *et al*., 2005), and Mmi6 had 99% homology to the sequence Mmi3.

Discussion

Denaturing gradient gel electrophoresis profiling with subsequent ordination analysis of binary DGGE data was used to analyse and compare archaeal communities in

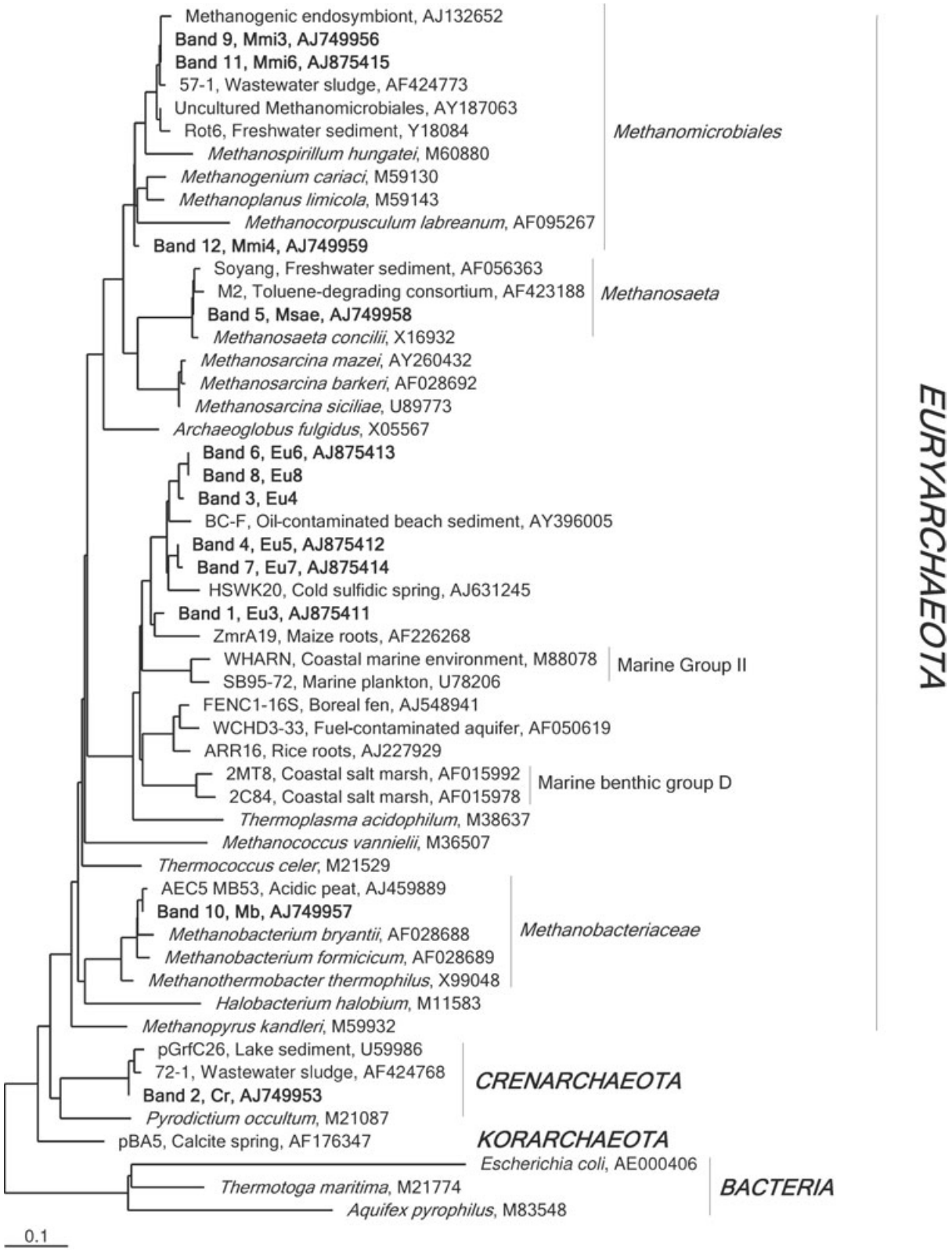

Fig. 4. Phylogenetic tree illustrating the affiliation of partial 16S rRNA gene sequences from re-amplified DGGE bands. Band numbers and phylogenetic labels used in Figs 3 and 5 are shown. Novel sequences and close relatives were added to a maximum likelihood tree based on nearly full-length sequences without affecting its topology. The scale bar represents 0.1 changes per nucleotide. Sequences from the present study are indicated in bold. The sequences Eu4 and Eu8 have no accession numbers as they each had two ambiguous bases (N).

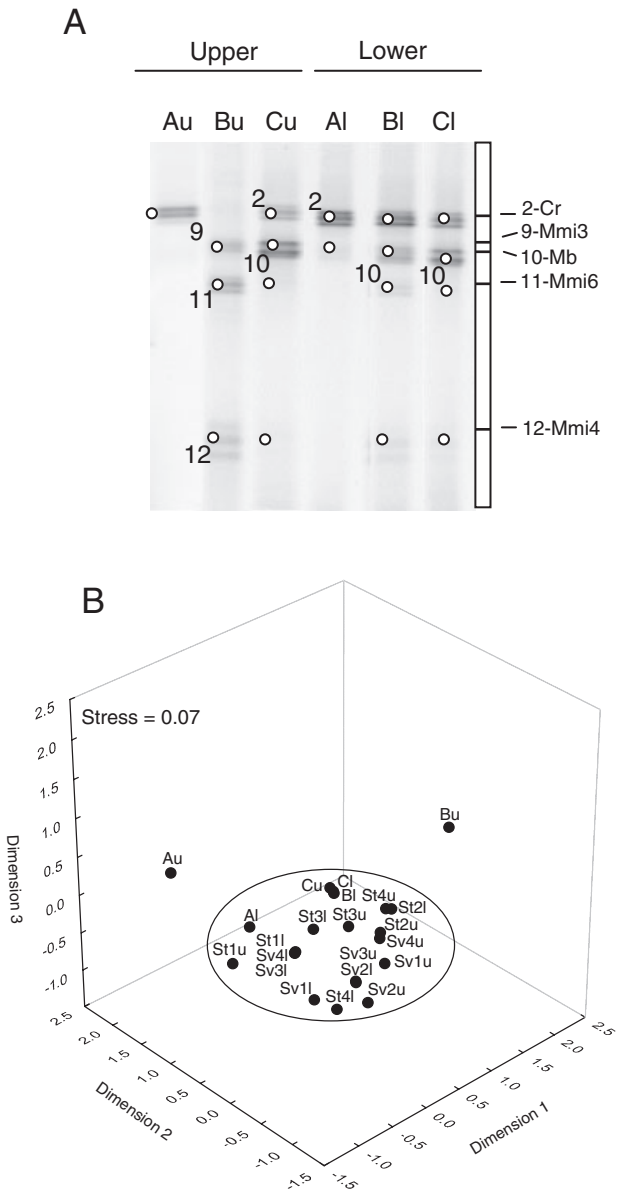

Fig. 5. A. The PCR-DGGE analysis of archaeal 16S rRNA genes from a natural soil moisture gradient. Bands that were successfully sequenced are indicated by numbers, and their phylogenetic affiliations are indicated by phylogenetic labels on the side of the gel picture (see also Fig. 4). The band numbering is consistent with Fig. 3, and the phylogenetic labels are consistent with Fig. 3 and a previous study (Høj *et al.*, 2005). Bands marked with white circles were included in the binary matrix.

B. Non-metric multidimensional scaling (NMDS) analysis of combined binary DGGE data from a soil moisture gradient and from a detailed study of the Arctic wetlands Solvatnet and Stuphallet (Høj *et al.*, 2005).

A, driest position of gradient; B, intermediate position of gradient; C, wettest position of gradient; u, upper soil layer; l, lower soil layer; Cr, Group 1.3b of *Crenarchaeota*; Mmi3, Mmi4 and Mmi6, *Methanomicrobiales*; Mb, *Methanobacteriaceae*; Sv, Solvatnet; St, Stuphallet; 1, Sample from July 1998; 2, Sample from July 1999; 3, Sample from August 1999; 4, Sample from September 1998.

soils and sediments with varying water regime and surface material. The use of a nested PCR protocol ensured a high sensitivity of the analysis; however, nested PCR protocols increase the potential for PCR biases and great care must be taken when interpreting the DGGE profiles. Methodological considerations regarding the use of a nested PCR protocol in combination with DGGE have previously been discussed in detail (Høj *et al.*, 2005). As the amount of template used in PCR amplifications was optimized for each individual sample at each step of the nested PCR reaction (within 10-fold dilutions), the resulting profiles could be used as qualitative fingerprints of the archaeal communities in each sample. Band intensities were generally not interpreted, with the exception of the apparent dominance of Group 1.3b of *Crenarchaeota* in most profiles (see below).

This study has shown that differences in soil water regime were correlated with major differences in the archaeal community composition. Bands affiliated with methanogenic archaea and Group 1.3b of *Crenarchaeota* were detected in most profiles of poorly drained soils and lake sediments. No DGGE bands affiliated with methanogenic archaea were detected for better-drained solifluction soils or the driest sample from a natural soil moisture gradient, while a novel euryarchaeotal cluster was detected only in the relatively well-drained solifluction samples (see below). Soil water regime was related both to aeration of the upper soil layer, O_2 concentrations in soil water, and to the dominating vegetation, with mosses or sedges and grasses dominating at sites with poor drainage. In enrichment experiments, where methanogenic medium was inoculated with soil and sediment samples, only solifluction material caused an initial colour change of the redox indicator resazurin (data not shown). This indicates that the redox potential in the solifluction samples was higher than in the other samples. The current study can, however, not disclose whether the observed effects of water regime on archaeal community composition were predominantly caused by redox effects, substrate availability or a combination of these two factors and further studies are needed.

Functional studies have identified a range of physicochemical parameters other than hydrology that can be correlated with methane emissions. These include soil temperature (Christensen *et al.*, 1995), pH (Walker *et al.*, 1998) and organic content (Christensen *et al.*, 1995). A thorough discussion of additional environmental factors that could have caused the observed variability in community composition is therefore warranted. In the initial survey, recorded temperatures for the sampled soil depths were in the range 2.0–6.9°C and two samples with significantly different DGGE profiles had similar soil temperatures at the time of sampling (St-pe: 3.5–6.9°C and Lb1-sol: 3.2–5.2°C). Similarly, temperatures recorded for

upper soil layers did not vary much along the gradient, while the community composition showed significant differences. Hence, the major trends described above could not be explained by differences in soil temperature. Soil pH did not seem to control the observed variability either, as samples separated from the main groups in NMDS plots (Sa-sol, Lb1-sol, Lb2-sol in Fig. 3, Au and Bu in Fig. 5) had pH values covering the entire range of detected values (Au: 4.7; Bu: 5.6; Sa-sol: 6.4; Lb2-sol: 6.7; Lb1-sol: 7.4). Soil organic content could be ruled out as a controlling factor as mineral-rich samples grouped together with highly organic samples in both surveys.

Despite using a highly sensitive nested PCR protocol, no methanogenic archaea were detected in Arctic soils that were wet only during spring melt. Therefore, methanogens were probably not abundant in such soils. This observation is consistent with the hypothesis that soil hydrology serves as an 'on-off switch' regulating large-scale methane emissions from northern wetlands (Christensen *et al*., 2003). It suggests that the dramatic decrease in methane emissions when the water table level drops below a certain threshold could be related to low sustainability of methanogenic archaea under such conditions (see below). In the present investigations the variation in archaeal community composition associated with water regime was predominant over variation within and between wetlands. This shows that there is a hierarchy in how environmental factors affect the archaeal community composition, with long-term water regime being predominant over other parameters that may differ between wet sites or change seasonally. The data are consistent with a study of river floodplains (Kemnitz *et al*., 2004), where methanogenic archaea were detected only in soils that were flooded frequently or permanently. Together these studies support the concept that the biomass of methanogenic archaea could be significant for the onset of methanogenesis in some natural systems. The inclusion of this parameter in methane emission models could improve their precision for predicting consequences of changes in substrate supply and water table fluctuations (Kettunen, 2003).

In the Arctic, permafrost impedes drainage and large volumes of soil are water logged, especially during spring melt. The abundance of methanogenic archaea in soils that are seasonally wet is likely to be dependent on: (i) the length of the period when soil conditions (i.e. redox potential and substrate availability) permit growth, (ii) the growth rate under the prevailing environmental conditions (e.g. soil temperature) and (iii) the die-off of methanogenic archaea during dry periods and freeze–thaw cycles. There are indications that anaerobic psychrophilic and psychrotrophic microorganisms are especially sensitive to even low concentrations of oxygen. Furthermore, the accumulation of methanogen biomass is often slow in cold environments, and a long time period is required both for the formation of syntrophic aggregates with hydrogenotrophic methanogens as the H_2-consuming partner, and for the build-up of biomass of slow-growing acetoclastic methanogens (Kotsyurbenko, 2005). Due to the low soil temperatures and freeze–thaw cycles in Arctic soils, the time period with wet or flooded conditions required for establishing ample populations of methanogenic archaea might be different from that of temperate regions. Due to the relevance for climate change studies, the effect of temperature on the dynamics of methanogenic communities exposed to freeze–thaw cycles and wetting and drying should be targeted in future studies. It is also necessary to consider potential effects of a prolonged summer season on the community dynamics.

Methane emissions were recorded for the three sampling positions along a natural soil moisture gradient in order to confirm that emissions varied between positions. Due to the steepness of the gradient and the consequent close proximity of the sampling positions only three chambers were used for each position, as chambers further away from the sampling position would confuse rather than resolve any trends along the gradient. The values for the measured emissions should therefore be handled with care. However, despite these methodological constraints increasing emissions along the gradient were confirmed. The archaeal community composition changed in the upper soil layer along the gradient, while only small effects were seen for deeper soil layers. We note that only *Methanomicrobiales*-affiliated bands were present in profiles from upper soil at the intermediate position (B). This order has previously been shown to be dominant in upper layers of hummocky peat (Galand *et al*., 2003). The observations suggest that members of this order are relatively tolerant to environments with less reduced conditions, or that they are relatively effective competitors for substrate in such environments. Temporal variation in the detection of two *Methanomicrobiales*-affiliated bands could not be explained by the measured environmental parameters or seasonal changes. Elucidation of how the competition between different genera of hydrogenotrophic methanogens is regulated was beyond the scope of this field study and further investigations with controlled systems are needed. A higher spatial variation in methanogenic community composition in upper soil as compared with lower soil has previously been reported for boreal oligotrophic fen (Galand *et al*., 2003). In general, microbial communities in upper soil layers are more influenced both by the water regime of the site and by the vegetation than communities in lower soil layers (Galand *et al*., 2003).

All methanogen-affiliated sequences detected in this study were identical or closely related to sequences detected in a detailed study of archaeal community composition in the wetlands Solvatnet and Stuphallet (Høj

et al., 2005). Nevertheless, regional differences in the methanogenic community composition were revealed, although these trends were less prominent than trends related to water regime (see above). The genus *Methanosaeta* was detected only in peat from Solvatnet (Sv-pe) and Stuphallet (St-pe), as well as in the sediments of lake Solvatnet (Sv-sed) and a lake in the Sassen Valley (Sa-sed). The genus *Methanosarcina*, which includes many strains that can grow acetoclastically (Kendall and Boone, 2004) was detected only in DGGE profiles of peat from Solvatnet (Høj *et al*., 2005). In contrast, methanogenic archaea with potential for acetoclastic methanogenesis were not detected in soil from the moisture gradient despite sampling at different depths and times during the summer season. Also previous studies have indicated that the presence of methanogenic archaea capable of acetoclastic methanogenesis may vary between sites (Galand *et al*., 2005; Juottonen *et al*., 2005). The literature suggests that hydrogenotrophic methanogenesis is favoured by a low supply of labile carbon for methanogenesis (Schoell, 1988; Hornibrook *et al*., 2000) and by low pH values (Horn *et al*., 2003; Kotsyurbenko, 2005).

The PCR-DGGE protocol used for the initial survey of wet soils and sediments was modified by exchanging one of the primers in the first amplification step and further optimizing the DGGE conditions. The modified protocol was used for studies along a soil moisture gradient and more detailed studies in the peatlands Solvatnet and Stuphallet (Høj *et al*., 2005). The modifications improved the detection of methanogen populations, as a wide range of methanogen-affiliated sequences could be recovered from profiles of peat from Stuphallet and Solvatnet with the modified PCR-DGGE protocol (Høj *et al*., 2005), while only one methanogen-affiliated sequence (Msae) was recovered from the corresponding profiles in the initial survey. The difference was probably in part due to the exchanged primer and in part due to improved separation of fragments with similar melting behaviour in the DGGE gels. The change in the protocol makes direct comparison between the initial survey and the later studies more difficult. However, the two sites that were analysed by both protocols (Solvatnet and Stuphallet) provided good references, and theoretical primer analysis was used to assist in the interpretation (see below). The conclusions from this study are strengthened by the fact that data obtained by two different protocols supported the same trend.

In the initial survey the most dominating amplicon was affiliated with Group 1.3b of *Crenarchaeota*. The same amplicon was detected also in subsequent studies using a primer with one mismatch to sequences in this cluster, confirming the that this group is relatively abundant within the archaeal community in wet Arctic soils. Interestingly, a previous study of river sediments in the Netherlands showed that during winter (December) approximately 50% of the amplicons from the most frequently flooded site were affiliated with this crenarchaeotal cluster (Kemnitz *et al*., 2004). Hence, at least some members of this cluster seem to have a competitive advantage in cold and wet terrestrial environments; however, details of the physiology of these microorganisms are currently not available.

Sequences affiliated with a novel euryarchaeotal cluster (Eu3-Eu8) were recovered from solifluction material. This cluster is related to the clusters Marine Group II and Marine benthic group D, and also to fen-cluster III (Galand *et al*., 2003) (Fig. 4). As no members of these clusters are isolated, their physiological characteristics are unknown. Sequences affiliated with this cluster were only detected in the initial survey and this could be related to the modification of the PCR protocol. Theoretical primer analysis showed that the new primer had one or two mismatches with sequences in this cluster. Hence, an underestimation of the abundance of this cluster was possible in the later studies.

In conclusion, this study has demonstrated that water regime has a major effect on the archaeal community composition in Arctic terrestrial systems. Further, the variation associated with soil water conditions was predominant over regional and seasonal variations and over differences between several types of poorly drained soils. Most notably, the water regime was essential for whether populations of methanogenic archaea were abundant. This suggests that the observed 'on–off switch' mechanism of soil hydrology for methane emissions from northern wetlands (Christensen *et al*., 2003) is at least partly caused by associated differences in the community structure of organisms involved in methane production.

Experimental procedures

Sampling sites

Peat, soil and sediment samples were collected from sites on the West Coast of Spitsbergen (Fig. 1). Due to the Gulf Stream the coastal climate is relatively mild considering the high latitude, with yearly average temperatures ranging from –7°C to –5°C. July is the warmest month of the year, with mean temperatures in the range 4–7°C (Førland *et al*., 1997). The samples were classified based on geomorphological characteristics (Tolgensbekk *et al*., 2000), water regime and the dominating vegetation (Table 1).

Peat samples were collected from Stuphallet (St-pe; Fig. 1A), Solvatnet (Sv-pe; Fig. 1A) and the Sassen Valley (Sa-pe; Fig. 1B). The site Stuphallet was located in a large depression with lateral inflow of melt and ground water during the whole summer period. The vegetation was dominated by brown mosses such as *Bryum* sp., *Bryum pseudotricuetum*, *Aulacomnium palustre* and *Tomentypnum nitens* (Wartiainen *et al*., 2003). Lake Solvatnet is a small lake located just outside Ny-Ålesund. Peat samples were collected from the lake shore, which was covered by a dense carpet of mosses such

as *Calliergon richardsonii*, *Drepanocladus uncinatus*, *Bryum pseudotricuetum* and *Bryum* sp. (van der Waal and Loonen, 1998; Wartiainen *et al*., 2003). Peat samples from Solvatnet and Stuphallet were collected from the same sampling points as used in a previously described study (Høj *et al*., 2005). In the Sassen Valley peat was collected from the shore of a small lake. The vegetation at this site was dominated by yellow and green mosses (not identified). All peat samples were classified as undecomposed (H_1) and almost undecomposed (H_2) using von Post's scale (Clymo, 1983). Lake sediments (Sv-sed; Fig. 1A, Sa-sed; Fig. 1B) were sampled from the lakes mentioned above. Both lake sediments consisted mainly of poorly decomposed organic material.

Fluvial deposits were collected from the Advent Valley (Ad1-flu, Ad2-flu; Fig. 1B). The soils were waterlogged at the time of sampling, and a blue-grey colour indicated anoxic conditions. Grasses and sedges dominated the vegetation at these sites. Solifluction materials were sampled from Longyearbyen and from the Sassen Valley. Solifluction material is found on slopes where drainage is impeded due to permafrost, and the relatively wet soil is exposed to a slow gravitational downslope movement called solifluction. In Longyearbyen (Lb1-sol, Lb2-sol; Fig. 1B) samples were collected from two shallow depressions that were wetter than the surrounding areas; however, these soils were not waterlogged at the time of sampling. In the Sassen Valley (Sa-sol; Fig. 1B), samples were collected at the edge of a puddle, the size of which decreased substantially during the next few weeks after sample collection. The vegetation at these sites included a mixture of mosses, sedges, grasses and dicotyledons. Samples from all these sites were collected in July 1997.

A natural soil moisture gradient (A, B, C; Fig. 1B) was selected for more detailed studies of the effect of water regime on archaeal community composition. Three positions were sampled along a transect from the top of a hummock (A) and down to a pond (C). The surface material along the gradient consisted of till, which was covered by a 10–15 cm thick layer of poorly decomposed mosses at the wettest position (C). The hummock position (A) was approximately 70 cm higher, and the intermediate position (B) approximately 20 cm higher than the edge of the pond (C). The lateral distance between positions A and B was approximately 4 m, while the distance between positions B and C was approximately 10 m. The vegetation at the driest position (A) included monocotyledons (*Dupontia fisheri*, *Alopecurus borealis*, *Luzula arcuata* ssp. *confusa*), dicotyledons (*Polygonum viviparum*, *Salix polaris*) and horsetails (*Equisetum arvense*). In contrast, the vegetation at the wettest position (C) was dominated by mosses (*Calliergon* spp. and *Drepanocladus* sp.). At the intermediate point (B) the vegetation was greener than at A, and was dominated by monocotyledons. The sedge *Eriophorum scheuchzeri* was found sparsely at positions B and C.

Sampling procedure

Soil and sediment samples were taken with PVC corers (inner diameter 4.5 cm, length 20 cm), which were sealed with rubber stoppers and metal tape and stored at 4°C until processing (Høj *et al*., 2005). One core was used for physicochemical characterizations, while a parallel core from the same position was used for molecular analysis. Along the soil moisture gradient additional samples were taken with 100 cm^3 steel corers for determination of dry bulk density and pore volume (see below). These samples were taken in triplicate at two depths [2–7 cm (A, B, C) and 15–20 cm (A) or 10–15 cm (B, C)].

Physicochemical parameters

Gravimetric water content was determined by drying 10 g of sample overnight at 105°C, and organic content (ignition loss) was determined by combustion overnight at 450°C. Soil and sediment pH was measured in distilled water. Dry bulk density and pore volume were determined using undisturbed soil samples (100 cm^3). Average volumetric water content was calculated from the measured average gravimetric water content multiplied by the dry bulk density of the soil. The average volume of air-filled pores was calculated by subtracting the average volumetric water content from the pore volume, which corresponds to the volumetric water content at saturation. O_2 in soil water was measured in the field by cutting a soil profile and inserting an O_2 probe (HI9143, Hannah Instruments) at specific depths. Two parallels were used for all measurements in the initial survey, while three parallels were used for samples from the soil moisture gradient.

Methane emissions

Methane emission rates were measured at each sampling position along the gradient using static stainless steel flux 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. Care was taken to avoid compression of the peat in the immediate proximity of each chamber. Due to the close proximity between sampling positions only three chambers were used for each point along the gradient. Subsamples (11 ml) were withdrawn with a 20 ml plastic syringe when the chambers were sealed, and after 2 h. The syringe was flushed 8–10 times with 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 (Høj *et al*., 2005). Injection, detection and column temperatures were 200°C, 240°C 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 increase in methane concentration in the chambers during the incubation period.

Molecular analyses

DNA was extracted from soils and sediments using FastDNA© SPIN Kit for soil (Bio101, CA, USA) in combination with a freeze–thaw lysis protocol (Høj *et al*., 2005). Archaeal 16S rDNA was amplified in a nested PCR protocol and analysed by DGGE using a protocol modified from Øvreås and

colleagues (1997). The amount of template used in the PCR amplifications was optimized for each individual sample at each step (within 10-fold dilutions). In the initial survey, the primers PRARCH112f (Høj *et al*., 2005) and PRARCH1045r (5′-GGCCATGCACCWCCTC-3′) were used in the first amplification, and the primers PARCH340-GC and PARCH519r (Øvreås *et al*., 1997) were used in the second amplification. The reaction mixture of 50 μl contained 5 μl of template, 0.5 μM of each primer, 0.1% (w/v) bovine serum albumine, 1× PCR buffer (final concentrations 10 mM Tris-HCl, pH 8.3, 50 mM KCl, Applied Biosystems, CA, USA), 1.5 mM $MgCl_2$, 100 μM of each dNTP and 1.25 U AmpliTaqGold™ (Applied Biosystems, CA, USA). In the first reaction the mixture contained 5% acetamide (Reysenbach *et al*., 1992). The following PCR cycle was used for both amplifications: 94°C in 9 min followed by 30 cycles of 94°C for 30 s, 53.5°C for 30 s and 72°C for 1 min, followed by a final extension at 72°C for 7 min. Amplified fragments were separated on 8% polyacrylamide gels in 0.5× TAE with a linear gradient of 20–65% [100% denaurant was 7 M urea and 40% (v/v) formamide]. Electrophoresis was performed at 60°C and 10 V for 10 min, followed by 200 V for 3 h. Gels were stained with SYBRGreen II (1:10 000 dilution, Molecular Probes) for 1 h, rinsed with distilled water and photographed under UV illumination. Denaturing gradient gel electrophoresis bands were cut from the gel using a sterile pipette tip, re-amplified and sequenced as previously described (Høj *et al*., 2005).

The nested PCR protocol used for samples collected in 1997 was modified and improved for further studies. The modified protocol has been described in detail previously (Høj *et al*., 2005). The primer PREA1100r (Øvreås *et al*., 1997) was used instead of primer PRARCH1045r in the first reaction, and a touchdown protocol was used for the second reaction. The primer PREA1100r had one mismatch with crenarchaeotal sequences in GenBank that were closely related to the crenarchaeotal sequence obtained in this study. In the modified protocol the DGGE analysis was performed using 8% polyacrylamide gels in 1× TAE with a linear gradient of 45–70%. The electrophoretic conditions were 60 V for 20 h at 60°C, and the gels were stained with SYBR Gold (1:10 000 dilution, Molecular Probes).

Phylogenetic analysis

Partial sequences from re-amplified DGGE bands were analysed using the BLAST tool at the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/blast/). Sequences from DGGE bands and sequences with high BLAST scores were added to a pre-aligned database (ssu_jan04.arb) using the aligning tools in the ARB program package (Technical University of Munich, Munich, Germany; http://www.arb-home.de). Sequences were added to maximum likelihood trees with sequences >1000 bp without affecting the initial tree topology using a special ARB parsimony tool. The original maximum likelihood tree was supplied with the database (Jul04_1000). To evaluate the resolution of the hypervariable V3 region of 16S rRNA for each phylogenetic group we constructed trees based solely on the amplified region using Phylip Distance methods (FITCH with Jukes-Cantor correction). The partial 16S rRNA gene sequences are available in the EMBL/GenBank/DDBJ nucleotide sequences database under Accession No. AJ749953, AJ749956–AJ749959 and AJ875411–AJ875415.

Statistical analyses

The statistical significance of trends in the physicochemical data and methane emission data were analysed using Student's *t*-test ($P = 0.05$), and correlations were analysed by Pearson product moment correlation. Binary matrices for DGGE data were constructed as described before (Høj *et al*., 2005). Each matrix was used to calculate a distance matrix (Dice), which was analysed by NMDS (Statistica, v. 6.0). The stress value was used to evaluate how well the distances in the ordination diagram corresponded to the dissimilarity values, with values < 0.1 meaning that the plot represented the original data reasonably well (van Hannen *et al*., 1999). Distances between outgroups and the main sample groups were compared with distances within the main sample group using Student's *t*-test ($P = 0.05$).

Acknowledgements

We thank Mette M. Svenning, Ingvild Wartianen and Anne Grethe Hestnes for collaboration in the field and with characterization of soil samples. David G. Bourne is thanked for critically reading the article. This work was funded by The Norwegian Research Council (Grant 121458/720). The Norwegian Polar Institute is acknowledged for financial support of the field work.

References

Anisimov, O., and Fitzharris, B. (2001) Polar regions (Arctic and Antarctic). In *Climate Change 2001: Impacts, Adaptation, and Vulnerability*. McCarthy, J.J., Canziani, O.F., Leary, N.A., Dokken, D.J., and White, K.S. (eds). Cambridge, UK: Intergovernmental Panel on Climate Change, pp. 801–841.

Basiliko, N., Yavitt, J.B., Dees, P.M., and Merkel, S.M. (2003) Methane biogeochemistry and methanogen communities in two northern peatland ecosystems, New York State. *Geomicrobiol J* **20:** 563–577.

Boon, P.I., Mitchell, A., and Lee, K. (1997) Effects of wetting and drying on methane emissions from ephemeral floodplain wetlands in south-eastern Australia. *Hydrobiologia* **357:** 73–87.

Bubier, J.L. (1995) The relationship of vegetation to methane emission and hydrochemical gradients in northern peatlands. *J Ecol* **83:** 403–420.

Bubier, J.L., Moore, T.R., and Roulet, N.T. (1993) Methane emissions from wetlands in the midboreal region of Northern Ontario, Canada. *Ecology* **74:** 2240–2254.

Christensen, T.R., Jonasson, S., Callaghan, T.V., and Havström, M. (1995) Spatial variation in high-latitude methane flux along a transect across Siberian and European tundra environments. *J Geophys Res* **100:** 21035–21045.

Christensen, T.R., Ekberg, A., Ström, L., Mastepanov, M., Panikov, N., Öquist, M., *et al.* (2003) Factors controlling large scale variations in methane emissions from wetlands. *Geophys Res Lett* **30:** 67-1–67-4. 10.129/2002GLO16848

Clymo, R.S. (1983) Peat. In *Mires: Swamp, Bog, Fen and Moor*. Gore, A.J.P. (ed.). Amsterdam, the Netherlands: Elsevier Scientific Publishing, pp. 159–224.

Førland, E.J., Hanssen-Bauer, I., and Nordli, P.Ø. (1997) *Climate Statistics and Long-Term Series of Temperature and Precipitation at Svalbard and Jan Mayen.* Oslo, Norway: Norwegian Meteorological Institute.

Galand, P.E., Saarnio, S., Fritze, H., and Yrjälä, K. (2002) Depth related diversity of methanogen Archaea in Finnish oligotrophic fen. *FEMS Microbiol Ecol* **42:** 441–449.

Galand, P.E., Fritze, H., and Yrjälä, K. (2003) Microsite-dependent changes in methanogenic populations in a boreal oligotrophic fen. *Environ Microbiol* **5:** 1133–1143.

Galand, P.E., Fritze, H., Conrad, R., and Yrjälä, K. (2005) Pathways for methanogenesis and diversity of methanogenic archaea in three boreal peatland ecosystems. *Appl Environ Microbiol* **71:** 2195–2198.

Hales, B.A., Edwards, C., Ritchie, D.A., Hall, G., Pickup, R.W., and Saunders, J.R. (1996) Isolation and identification of methanogen-specific DNA from blanket bog peat by PCR amplification and sequence analysis. *Appl Environ Microbiol* **62:** 668–675.

van Hannen, E.J., Zwart, G., van Agterveld, M.P., Gons, H.J., Ebert, J., and Laanbroek, H.J. (1999) Changes in bacterial and eukaryotic community structure after mass lysis of filamentous cyanobacteria associated with viruses. *Appl Environ Microbiol* **65:** 795–801.

Høj, L., Olsen, R.A., and Torsvik, V.L. (2005) Archaeal communities in High Arctic wetlands at Spitsbergen, Norway (78°N) as characterized by 16S rRNA gene fingerprinting. *FEMS Microbiol Ecol* **53:** 89–101.

Horn, M.A., Matthies, C., Küsel, K., Schramm, A., and Drake, H.L. (2003) Hydrogenotrophic methanogenesis by moderately acid-tolerant methanogens of a methane-emitting acidic peat. *Appl Environ Microbiol* **69:** 74–83.

Hornibrook, E.R.C., Longstaffe, F.J., and Fyfe, W.S. (2000) Evolution of stable carbon isotope compositions for methane and carbon dioxide in freshwater wetlands and other anaerobic environments. *Geochim Cosmochim Acta* **64:** 1013–1027.

Joabsson, A., and Christensen, T.R. (2001) Methane emissions from wetlands and their relationship with vascular plants: an Arctic example. *Glob Change Biol* **7:** 919–932.

Juottonen, H., Galand, P.E., Tuittila, E.-S., Laine, J., Fritze, H., and Yrjälä, K. (2005) Methanogen communities and *Bacteria* along an ecohydrological gradient in a northern raised bog complex. *Environ Microbiol* **7:** 1547–1557.

Kemnitz, D., Chin, K.J., Bodelier, P., and Conrad, R. (2004) Community analysis of methanogenic archaea within a riparian flooding gradient. *Environ Microbiol* **6:** 449–461.

Kendall, M.M., and Boone, D.R. (2004) The order *Methanosarcinales*. In *The Prokaryotes: An Evolving Electronic Resource for the Microbiological Community*. Dworkin, M. (ed.). New York, USA: Springer-Verlag [WWW document]. URL http://link.springer-ny.com/link/service/books/10125/

Kettunen, A. (2003) Connecting methane fluxes to vegetation cover and water table fluctuations at microsite level: a modeling study. *Global Biogeochem Cy* **17:** 20-1–20-19.

Kettunen, A., Kaitala, V., Lehtinen, A., Lohila, A., Alm, J., Silvola, J., and Martikainen, P.J. (1999) Methane production and oxidation potentials in relation to water table fluctuations in two boreal mires. *Soil Biol Biochem* **31:** 1741–1749.

Kotsyurbenko, O.R. (2005) Trophic interactions in the methanogenic microbial community of low-temperature terrestrial ecosystems. *FEMS Microbiol Ecol* **53:** 3–13.

Kotsyurbenko, O.R., Chin, K.J., Glagolev, M.V., Stubner, S., Simankova, M.V., Nozhevnikova, A.N., and Conrad, R. (2004) Acetoclastic and hydrogenotrophic methane production and methanogenic populations in an acidic West-Siberian peat bog. *Environ Microbiol* **6:** 1159–1173.

Liesack, W., Schnell, S., and Revsbech, N.P. (2000) Microbiology of flooded rice paddies. *FEMS Microbiol Rev* **24:** 625–645.

Lueders, T., and Friedrich, M. (2000) Archaeal population dynamics during sequential reduction processes in rice field soil. *Appl Environ Microbiol* **66:** 2732–2742.

Mayer, H.P., and Conrad, R. (1990) Factors influencing the population of methanogenic bacteria and the initiation of methane production upon flooding of paddy soil. *FEMS Microbiol Ecol* **73:** 103–112.

Moore, T.R., and Dalva, M. (1993) The influence of temperature and water-table position on carbon-dioxide and methane emissions from laboratory columns of peatland soils. *J Soil Sci* **44:** 651–664.

Nilsson, M., and Bohlin, E. (1993) Methane and carbon dioxide concentrations in bogs and fens – with special reference to the effects of the botanical composition of the peat. *J Ecol* **81:** 615–625.

Ochsenreiter, T., Selezi, D., Quaiser, A., Bonch-Osmolovskaya, L., and Schleper, C. (2003) Diversity and abundance of *Crenarchaeota* in terrestrial habitats studied by 16S RNA surveys and real time PCR. *Environ Microbiol* **5:** 787–797.

Øvreås, L., Forney, L., Daae, F.L., and Torsvik, V. (1997) Distribution of bacterioplankton in meromictic lake Sælenvannet, as determined by denaturing gradient gel electrophoresis of PCR-amplified gene fragments coding for 16S rRNA. *Appl Environ Microbiol* **63:** 3367–3373.

Peters, V., and Conrad, R. (1996) Sequential reduction process and initiation of CH_4 production upon flooding of oxic upland soils. *Soil Biol Biochem* **28:** 371–382.

Reysenbach, A.-L., Giver, L.J., Wickham, G.S., and Pace, N.R. (1992) Differential amplification of rRNA genes by polymerase chain reaction. *Appl Environ Microbiol* **58:** 3417–3418.

Roy, R., Klüber, H.D., and Conrad, R. (1997) Early initiation of methane production in anoxic rice soil despite the presence of oxidants. *FEMS Microbiol Ecol* **24:** 311–320.

Schoell, M. (1988) Multiple origins of methane in the earth. *Chem Geol* **71:** 1–10.

Tolgensbekk, J., Sørbøl, L., and Høgvard, K. (2000) Adventdalen, geomorphological and quaternary geological map, Svalbard 1:100,000, Spitsbergen sheet C9Q. In *Norsk Polarinstitutt Temakart nr. 32.* Tromsø, Norway: Norsk Polarinstitutt.

Utsumi, M., Belova, S.E., King, G.M., and Uchiyama, H. (2003) Phylogenetic comparison of methanogen diversity in different wetland soils. *J Gen Appl Microbiol* **49:** 75–83.

Vourlitis, G.L., Oechel, W.C., Hastings, S.J., and Jenkins,

M.A. (1993) The effect of soil moisture and thaw depth on CH_4 flux from wet coastal tundra ecosystems on the north slope of Alaska. *Chemosphere* **26:** 329–337.

van der Waal, R., and Loonen, M.J.J.E. (1998) Goose droppings as food for reindeer. *Can J Zool* **76:** 1117–1122.

Wagner, D., Kobabe, S., Pfeiffer, E.-M., and Hubberten, H.-W. (2003) Microbial controls on methane fluxes from a polygonal tundra of the Lena Delta, Siberia. *Permafrost Periglac* **14:** 173–185.

Walker, D.A., Auerbach, N.A., Bockheim, J.G., Chapin,F.S., III, Eugster, W., King, J.Y., *et al.* (1998) Energy and trace-gas fluxes across a soil pH boundary in the Arctic. *Nature* **394:** 469–472.

Wartiainen, I., Hestnes, A.G., and Svenning, M.M. (2003) Methanotrophic diversity in high arctic wetlands on the islands of Svalbard (Norway) – denaturing gradient gel electrophoresis analysis of soil DNA and enrichment cultures. *Can J Microbiol* **49:** 602–612.

Watson, A.K.D.S., Nedwell, D.B., and Arah, J.R.M. (1997) Oxidation of methane in peat: kinetics of CH_4 and O_2 removal and the role of plant roots. *Soil Biol Biochem* **29:** 1257–1267.