Paper III

Effects of temperature on archaeal community structure and methanogenesis in peat from an Arctic wetland

Lone Høj[1†], Rolf A. Olsen[2,3], Vigdis L. Torsvik[1]

[1] Department of Biology, University of Bergen, Jahnebakken 5, P.O. Box 7800, N-5020 Bergen, Norway

[2]Department of Chemistry, Biotechnology and Food Science, Norwegian University of Life Sciences, P.O. Box 5003, N-1432 Ås, Norway

[3]The University Centre in Svalbard, P.O. Box 156, N-9171 Longyearbyen, Norway

*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

Running title: Temperature effects on Arctic peat methanogens

Keywords: *Archaea*, temperature, methane, peat, denaturing gradient gel electrophoresis, FISH, Arctic soil, isobutyrate, cell aggregates

Summary

The effects of temperature on archaeal community structures and methanogenesis were studied in slurries of Arctic peat (pH 6.8) from Spitsbergen, Norway (78°N). The overall process of methanogenic degradation of biopolymers was poorly adapted to low temperatures. The temperature response (Q_{10} values) for the linear phase of methane accumulation was 14.7 for the interval 10-20°C, and the corresponding Ratkowsky plot gave an apparent minimum temperature of 6.5°C. The methane accumulation at 1°C and 5°C corresponded to less than 0.5% of the methane accumulation at 25°C. Temperature affected the temporal development in the slurries, with conditions at 10°C approaching those seen at higher temperatures towards the end of the experiment (83 days). At 5°C and 10°C the accumulation of isobutyrate was conspicuous. Fluorescence *in situ* hybridization (FISH) analysis revealed a large (11-12% of the total counts), unidentified, active archaeal community at 1°C and 5°C. Highly ordered sphere shaped aggregates were detected by probes specific for methanogenic archaea at temperatures ≥ 10°C. 16S rRNA based community profiles showed that populations of methanogenic archaea could grow at all tested temperatures, and indicated that their dynamics was controlled by substrate availability. The results suggest that, for the studied site, field emissions of methane at low temperatures are due to a continuous supply of easily degradable substrates rather than the degradation of complex organic polymers.

Introduction

Most climate models predict that by 2080, the summer temperature in Arctic regions will increase by 4.0-7.5°C, and there will also be increased precipitation (Anisimov and Fitzharris, 2001). Studies of wetlands in Arctic Eurasia have indicated that mean

seasonal soil temperature is a major controlling factor for methane emissions from these ecosystems (Christensen *et al.*, 2003). This potentially represent a positive feedback mechanism on climate change, given that conditions such as soil moisture and the supply of methanogenic substrates do not change (Høj *et al.*, 2006; Christensen *et al.*, 2003).

Despite the demonstrated impact of temperature on methane emissions, relatively few controlled laboratory studies have specifically addressed the effect of temperature on methane production in Arctic and subarctic soils (Metje and Frenzel, 2005; Kotsyurbenko *et al.*, 2004; Kotsyurbenko *et al.*, 1996; Dunfield *et al.*, 1993; Svensson, 1984). The temperature response of methane production can be described using the Q_{10} value, which is the increase in production rate when the temperature increases 10°C. Q_{10} values from previous studies are in the range 1-35 (Bergman *et al.*, 1998), and several reasons for this large variation have been proposed. Both differences in substrate supply (Bergman *et al.*, 2000; Bergman *et al.*, 1998; Yavitt *et al.*, 1997) and the fact that the methane production phase may be different at the upper and lower temperature (van Hulzen *et al.*, 1999) might have large effects. The particular 10°C temperature intervals used in the calculations may also lead to differences in the calculated Q_{10} values (Bergman *et al.*, 1998). These factors can not explain all of the observed variation however, and some variation seems to be due the specific environmental conditions for each wetland type (Bergman *et al.*, 2000; Bergman *et al.*, 1998).

Recently, Metje and Frenzel (2005) described for the first time the effect of temperature on both methane production pathways and archaeal community composition in a northern wetland. They found that methanogenesis in slurries of acidic peat (pH 4.1) had a theoretical minimum temperature of –5°C (Ratkowsky

plot), with the production at 4°C corresponding to nearly 10% of the production at the optimum temperature for methanogenesis (25°C). In this northern wetland the archaeal community was relatively stabile in the temperature range 4 – 30°C. There was also a close correspondence between the archaeal community structure and the functioning of the system, as hydrogenotrophic methanogenesis accounted for about 80% of the total methanogenesis, and all recovered sequences that were affiliated with methanogenic archaea clustered within the order *Methanobacteriales*. These observations differ significantly from those from rice paddy soils, where the contribution of acetoclastic methanogenesis increased at low temperatures, and the archaeal community structure changed (Fey and Conrad, 2000). Detailed studies from several wetland types are therefore needed to elucidate what may cause such site-specific differences.

In a previous study we reported on *in situ* emissions of methane and CO_2 at two wetland sites on Spitsbergen over two summer seasons. The archaeal community structures at these sites were also investigated and described (Høj *et al.*, 2005). Here we report on a controlled laboratory experiment addressing the effect of temperature on microbial activity and microbial community structures in slurries of peat from the site with the highest *in situ* methane emissions, Solvatnet. Unamended slurries were incubated at ecologically relevant temperatures (1°C - 25°C) with 5°C increments. The temperature effect on microbial activities was addressed by monitoring methane production, CO_2-production, and concentrations of volatile fatty acids, and by calculating Q_{10} values and a theoretical minimum temperature for methane production (Ratkowsky plot). Temperature effects on the archaeal community structures were studied using 16S rRNA based analyses. These analyses were polymerase chain reaction (PCR) combined with denaturing gradient gel electrophoresis (DGGE), and

fluorescent *in situ* hybridization (FISH). Based on quantitative data from FISH and methane production rates, the temperature effect on specific (per cell) methane production rates was evaluated.

Results

Accumulation of methane

The unamended peat slurries showed increasing methane partial pressures with temperature (Fig. 1). On a linear scale, methane production became apparent at different incubation times, and the length of the apparent lag phase decreased with increasing temperatures (Figs. 1a and 1b). At 1°C and 5°C the methane production was so slow that when all temperatures were plotted together (Fig. 1a), the lag phase seemed to last throughout the experiment. When data for the three lowest temperatures (1– 10°C) were plotted separately (Fig. 1b) however, methane production was apparent after 35 days at 1°C and 5°C and after 18 days at 10°C. The length of the period with almost exponential methane accumulation also decreased with increasing temperature. This phase lasted throughout the experiment (83 days) at 5°C, while at temperatures ≥ 10°C the methane accumulation eventually slowed down and was followed by an almost linear increase in the methane partial pressures with time. Plotting the methane accumulation on a logarithmic scale revealed that methane production started immediately at all temperatures (Fig. 1c). The plot also revealed that for most incubation temperatures the initial fast methane production was followed by a period when the production slowed down and nearly came to a halt. The timing of the onset and duration of this phase was affected by the incubation temperature.

The temperature response (Q_{10} values) for the linear methane production was 14.7 and 4.1 for the intervals 10-20°C and 15-25°C, respectively. A Ratkowsky plot based on the methane production rates in the linear phase (temperatures ≥ 10°C) had an apparent minimum temperature of 6.5°C.

Relationship between CO_2 and CH_4 accumulation

The ratio between CO_2 and CH_4 accumulating in the headspace decreased with temperature and with time (Fig 2). In the beginning of the experiment (day 13) similar and high values were seen for the three lowest temperatures (1-10°C) (range 560-617). The ratio decreased with increasing temperature, but also the ratios at 15°C and 20°C were relatively high (334 and 71, respectively). At 25°C the ratio was much lower (14), indicating that at this temperature methanogenesis was a relatively important pathway in the anaerobic degradation already from the beginning. For all temperatures the CO_2/CH_4 ratio decreased with time. At 1°C and 5°C the lowest values were 297 and 73, respectively, while at 10°C the lowest ratio was 9.6. For the temperatures 15°C, 20°C and 25°C the lowest ratios were 2.2, 1.4, and 1.0, respectively.

Accumulation of metabolic intermediates

Hydrogen could not be detected in any of the soil slurries, and this was probably due to the relatively low sensitivity of the method used (detection limit 18 Pa). Acetate, propionate, butyrate and isobutyrate were the only volatile fatty acids that were detected in the soil slurries (Fig. 3). The temperature influenced the metabolite profiles in the slurries. At the lowest temperature (1°C) no fatty acid concentrations above the detection limits were recorded during the entire experiment. At 5°C and 10°C isobutyrate was the predominant metabolite, accumulating to 1400 µM and 800

µM respectively at the end of the experiment. Isobutyrate accumulated throughout the incubation period also at temperatures ≥ 15°C, but the concentrations at the end of the experiment were significantly lower than at 5°C and 10°C (236 µM, 136 µM, and 77 µM at 15°C, 20°C and 25°C, respectively). At 5°C no other fatty acids were detected. At 10°C especially acetate (680 µM, day 83), but also propionate, accumulated towards the end of the incubation period, and butyrate was detected transiently at day 51. At the higher temperatures (≥ 15°C) acetate, propionate and butyrate (when present) increased transiently with maximum values at day 51. At 15°C acetate was the metabolite with highest maximum concentration (612 µM), while at 20°C the maximum concentrations of acetate and propionate were more similar (284 µM and 227 µM, respectively). At 25°C propionate was the metabolite with highest metabolite concentration (327 µM), and butyrate was not detected.

Total counts and FISH specific counts

Total prokaryotic counts generally decreased by 36 – 50% between weeks 4 and 13, except at 1°C where the count remained constant at $5.8x10^{10}$ cells g dw^{-1} (Fig. 4a). In general, FISH counts with the *Bacteria*-specific probe EUB338 detected 30-50% of the total counts (PicoGreen-stained cells, Fig. 4b). An exception was seen in week 13 at 25°C, when the fraction detected with EUB338 was reduced to 21%. The probes ARCH915 and EURY498 resulted in statistically valid counts only for samples from week 4. This was probably due to the general drop in the total number of prokaryotic cells during the incubation period (Fig. 4a). In week 4, *Archaea* constituted about 11-12% of the total prokaryotic community at the two lowest temperatures (1°C and 5°C). At the higher temperatures, the fractions of *Archaea* were significantly lower and varied between 1-6%, with the lowest fraction recorded in slurries incubated at 10°C. The *Euryarchaeota*-specific probe EURY498, detected between 1 and 3% of

the total number of cells (Fig. 4b). Hence, at the two lowest temperatures a relatively large fraction of cells detected by ARCH915 was not detected by EURY498. The morphology of cells detected by the probes ARCH915 and EURY498 differed with temperature, with rods dominating the community at 1°C and 5°C, while cocci, sarcina-like clusters and highly organized spheres dominated at higher temperatures (Fig. 5). The group specific methanogen probes MSMX860 (*Methanosarcinales*) and MB310 (*Methanobacteriacea*) did not yield statistically valid counts, but positive cells were detected at temperatures ≥ 10°C. The probe MSMX860 detected sarcina-like and sphere-formed cell aggregates (Fig. 5). Both the size of the spheres and the probe signal intensity increased at temperatures ≥ 15°C relative to 10°C. The probe MB310 detected a few rods, and surprisingly a few spheres were also detected at 20°C and 25°C (Fig. 5). The spheres did not consist of autofluorescent cells since they were not observed on filters hybridized with the probe Non-EUB338.

Estimation of per cell methane production

Per cell methane production was calculated based on the production rate between days 22 and 28 and the number of cells detected by the probe EURY498 in week 4. At 1°C and 5°C the estimated per cell activities were below 0.01 fmol day^{-1} $cell^{-1}$. At 10°C the per cell activity was 0.28 fmol day^{-1} $cell^{-1}$, while at 15°C, 20°C and 25°C the specific activities were 4.3, 4.4 and 10.3 fmol day^{-1} $cell^{-1}$, respectively.

PCR-DGGE analysis of Archaea

The PCR-DGGE analysis of archaeal 16S rRNA genes showed 4-6 well defined bands per DGGE-profile (Fig. 6). The profiles were similar to those previously obtained from field samples, and the retrieved sequences were identical to sequences previously recovered (Høj *et al.*, 2005). The archaeal community composition was

relatively stable with temperature. Replacing primer 1100r with the primer 1045r (Høj *et al.*, 2006) did not change this result (data not shown). The relative intensity of some bands however, changed systematically with temperature and time (Fig. 6). The band Msae (*Methanosaeta*) increased in relative intensity with increasing temperature, and was significantly stronger in week 13 than in week 4. Band Msar1 (*Methanosarcina*) showed a different pattern. Also this band had highest relative intensity at temperatures ≥ 10°C in week 4, but in week 13 the highest relative intensity was observed at temperatures ≤ 10°C. Band Mmi3 (*Methanomicrobiales*) showed trends similar to Msar 1. Band Mmi4 (*Methanomicrobiales*) showed higher relative intensity at temperatures ≤ 10°C for both sampling days, but the trend was clearest at the end of the experiment. The band Mb (*Methanobacteriaceae*) had a relatively high intensity in all profiles. The band Cr (Group 1.3b uncultured *Crenarchaeota*) was present in all samples, but had the lowest relative intensity at 1°C on both sampling dates.

Discussion

During the first part of the experiment no fatty acids were detected, demonstrating that the studied process was methanogenic degradation of biopolymers rather than more labile carbon sources. It should be noted that the field conditions would differ from those in the slurries, since freeze-thaw cycles during spring thaw, root exudates, plant detritus and bird dropping affect the substrate supply (see below). However, since the PCR-DGGE-profiles of archaeal communities in the slurries were similar or identical to profiles of field sample communities (Høj *et al.*, 2005), it can be assumed that the observed temperature effects are ecologically relevant.

The overall process of methanogenic degradation of complex organic compounds in Solvatnet peat at temperatures ≥ 10°C was poorly adapted to low

temperatures, as demonstrated by several measures. Q_{10} values for the methane production could be reliably calculated only for temperatures ≥ 10°C, since the methane production did not reach a linear phase for temperatures ≤ 5°C during the experimental period (83 days). For the higher temperature intervals, Q_{10} values decreased with increasing temperature (10-20°C: 14.7, 15-25°C: 4.1). A Ratkowsky plot confirmed the poor temperature adaptation, with an apparent minimum temperature of 6.5°C. Finally, the methane accumulation at 1°C and 5°C corresponded to 0.5% or less of the methane accumulation at 25°C. The relevance of the calculated apparent minimum temperature (6.5°C) for the processes in the slurries was supported by two independent analyses: the observed shift in the accumulation pattern of volatile fatty acids seen at temperatures ≤ 10°C, and the differences in the active archaeal communities at temperatures below and above 10°C as determined by FISH analysis (see below). However, it must be noted that the calculation was based on the methane production rates in the linear phase at temperatures ≥ 10°C, and the calculated value is valid for the overall degradation pathways operating in this phase only (see below).

The poor temperature adaptation of the degradation of complex organic compounds in Solvatnet peat is consistent with the low degradation status of this peat as classified by the von Post's scale (H1/H2). It is however in contrast to recently published data for acidic (pH 4.1) peat from northern Finland, where the theoretical minimum temperature was -5°C and the activity at 4°C was about 10% of the activity at 25°C (Metje and Frenzel, 2005). Interestingly, the two sites differed significantly also in their community composition and in their dominant anaerobic degradation pathways. Differences in temperature adaptation of wetland microbial communities have been observed previously in functional studies (Bergman *et al.*, 2000; Bergman

et al., 1998; Yavitt *et al.*, 1997). The new studies including information on archaeal communities and metabolic intermediates suggest that such differences can in part be related to differences in the dominant degradation pathways and the involved microbial communities.

Temperature affected the temporal development in the slurries as demonstrated by the transition between the CH_4 accumulation phases, the CO_2/CH_4 fraction, the accumulation pattern of volatile fatty acids, and the development of the archaeal community (see below). The CO_2/CH_4 ratios observed in the beginning of the incubations, and throughout the experiment for the lowest temperatures, were so high that the anoxic CO_2 production can not be accounted for by the CH_4 production or by fermentation. The SO_4^{2-} and NO_3^- concentrations were relatively low for the soil used in the experiment (24 µM and 0.5 µM, respectively; Lars Egil Haugen, personal communication), and hence it can be assumed that parts of this high CO_2 production was the result of respiration with electron acceptors such as Fe^{3+} or organic matter (humins). The CO_2/CH_4 ratios and the fatty acid accumulation patterns in slurries incubated at 10°C approached the conditions seen at higher temperatures towards the end of the experiment. Similar effects of temperature on the temporal development in methanogenic slurries have been observed previously (Fey *et al.*, 2004; van Hulzen *et al.*, 1999). The interaction between temperature effects and time on the methane production is highly relevant for predicting the effect of climate change on seasonal methane emissions in ecosystems that currently experience a short summer season.

High levels of isobutyrate accumulation at low temperatures could theoretically be caused by increased formation or decreased consumption of isobutyrate at the low temperatures, or by a combination of the two. At low temperatures the Gibbs free energy of syntrophic degradation of volatile fatty acids

increases (Kotsyurbenko, 2005), and it is possible that syntrophic degradation of isobutyrate was inhibited at the low temperatures. Further studies are however needed to elucidate why isobutyrate accumulated at low incubation temperatures, and especially whether the isobutyrate production can be linked to the relatively large active non-methanogenic archaeal community demonstrated by FISH analysis (see below).

The numbers of prokaryotic cells numbers generally decreased between weeks 4 and 13. This could potentially be due to predation by protozoa or to degradation of cells not able to grow in the slurries. At 25°C, also the fraction of active bacterial cells detected with EUB338 dropped (from 40% to 21%), indicating that the more easily degradable organic matter was depleted. Consistent with the decrease in prokaryotic numbers, the archaeal community could not be quantified at the end of the experiment. The numbers of cells binding the probes ARCH915 and EURY498 were below the detection limit. This limit depended on the amount of peat that could be applied to the filters without deviating from a linear relationship between the sample size and the number of counted cells.

FISH data from week 4 showed that around 10°C there was a shift in the active archaeal communities. Different archaeal morphotypes were numerically dominant below and above 10°C, and the lowest counts were obtained at this temperature. In addition the group specific probes for methanogenic archaea MSMX860 and MB310 detected positive cells only at temperatures ≥10°C (see below). However, the DGGE-profiles did not reveal any specific archaeal populations that occurred only at low temperatures. This indicates that the archaeal populations that were abundant at low temperatures were not detected by the PCR primers used.

The fractions of archaeal cells detected at temperatures ≥10°C (1-6%) were consistent with previous reports from temperate soils (1%) (Sandaa *et al.*, 1999), Arctic marine sediments (<4%) (Ravenschlag *et al.*, 2001), and anoxic lake sediments (5-7%) (Zepp Falz *et al.*, 1999). Higher fractions (11-12%) of active *Archaea* cells were found at 1-5°C. Such high fractions were previously detected in FISH studies of marine planktonic *Archaea* in Antarctic waters, where 5.2 – 14.5 % of DAPI stained cells hybridized with a probe targeting crenarchaeotal group I (Murray *et al.*, 1998). Despite the relatively high abundance of *Archaea* observed at 1°C and 5°C, the fraction detected with the probe EURY498 was low. A reason for the relatively large fraction of the archaeal cells not detected with the probe EURY498, could be that there was a high contribution of *Crenarchaeaota*. Several studies have indicated that *Crenarchaeota* are relatively important in low temperature environments (Metje and Frenzel, 2005; Kemnitz *et al.*, 2004; Murray *et al.*, 1998), including wet soils and sediments from Spitsbergen (Høj *et al.*, 2006). Another reason could be that the true contribution of *Euryarchaeota* was probably underestimated since the probe EURY498 covers less than 50% of known euryarchaeotal sequences (Jurgens *et al.*, 2000). Our results suggest that archaeal communities might be relatively abundant in cold environments, and the phylogeny and genetic diversity of these communities should be targeted in further studies.

Sphere-formed aggregates were detected with the probes ARCH915, EURY498 and MSMX860 at temperatures ≥ 10°C, and occasionally also with MB310 at 20°C and 25°C. The nature of these aggregates should be further elucidated to determine whether the ordered structure was due to cells living in a syntrophic association. A spheric organization of cells participating in a syntrophic association

has previously been observed for anaerobic methane oxidation (Knittel *et al.*, 2005; Boetius *et al.*, 2000).

The effect of temperature on the specific (per cell) activity of methanogenic archaea was estimated by combining FISH data (counts with EURY498) with measured methane production rates. The specificity of probe EURY498 for sequences recovered from field samples from Spitsbergen soils (Høj *et al.*, 2006; Høj *et al.*, 2005) could be evaluated since the target region was included in the region used in the PCR-DGGE analyses. The probe had a full match only with sequences affiliated with known methanogenic groups and Rice Cluster II. We therefore assumed that the probe EURY498 could be used to estimate the number of methanogenic archaea belonging to known groups, rather than the entire euryarchaeotal population in samples of wet Arctic soils. It is however possible that this assumption was not valid for populations that were active at 1°C and 5°C, since the large archaeal community present at these temperatures could include unknown euryarchaeotal populations that were detected by this probe.

At 1°C and 5°C the estimated specific activities were low (< 0.01 fmol CH_4 day^{-1} $cell^{-1}$), which is consistent with the low methane production in these slurries where the accumulating methane was not yet visible on the linear scale. The estimated specific methane production at temperatures ≥ 10°C was consistent with previous estimates. At 10°C the specific activity corresponded to 0.042 µmol CH_4 h^{-1} mg of dry $cells^{-1}$, which is in the same range as the 0.029 µmol CH_4 h^{-1} mg dry $cells^{-1}$ estimated by Watanabe (2002) for specific activities in the unamended sediment of Lake Rotsee at 6°C (Zepp Falz *et al.*, 1999). It is possible that the specific activity at 10°C increased towards the end of the experiment, but this could not be determined as the abundance of the archaeal community dropped below the detection level for the

FISH assay. The per cell activity at 15°C and 20°C were similar (4.3 and 4.4 fmol CH_4 day^{-1} $cell^{-}1$, respectively), showing that the higher overall methane production rate at 20°C could primarily be explained by a higher number of active methanogenic cells. The highest specific activity (10.3 fmol CH_4 day^{-1} $cell^{-1}$) was observed at 25°C, and it corresponded to about half of the specific activity estimated for endosymbionts of the anaerobic ciliate *Metopus palaeformis* at 25°C (23.3 fmol CH_4 day^{-1} $cell^{-1}$) (Finlay and Fenchel, 1991).

Great care has to be taken when interpreting relative intensities of DGGE-bands especially when using a nested PCR protocol (Høj *et al.*, 2005). The trends seen in this experiment however, should be relatively robust since the same soil was used in all slurries. The relative intensity of the *Methanosaeta*-affiliated band (Msae) increased both with temperature and with time. These trends were consistent with data on acetate concentrations, as the transient accumulation of acetate indicated that acetate consumption increased with time. The observed temporal trend in *Methanosaeta* abundance may also reflect that these slow-growing microorganisms need time to become established in the system. The observed trends are consistent with field data, which showed that in two consecutive summers the relative intensity of the *Methanosaeta*-affiliated band (Msae) increased in the lower soil layers towards the end of the summer season (Høj *et al.*, 2005).

The trends in the relative intensity seen for the *Methanosarcina* affiliated band (Msar1) indicated that increased temperature caused the temporal development of this population to proceed faster. The absence of the *Methanosarcina* affiliated band (Msar1) in the DGGE-profiles obtained in week 4 from slurries incubated at 1°C and 5°C, was consistent with FISH data, since no cells were detected with the probe MSMX860 in these slurries. After 13 weeks however, Msar1 was present in the

profiles from the slurries incubated at low temperatures. At temperatures ≥ 15°C, the relative intensity of Msar1 decreased with time, which suggests that the abundance of this population was regulated by substrate availability rather than by temperature directly. The result is consistent with field data, where the detection of Msar1 correlated with the general bacterial activity as measured by *in situ* emissions of CO_2 (Høj *et al.*, 2005). It should be noted that changes in the relative abundance of the *Methanosarcina* population was seen for slurries where the acetate concentration never exceeded the detection limit of 67 µM (1°C and 5°C). Since known *Methanosarcina* strains capable of acetoclastic methanogenesis generally have threshold values for utilization of acetate in the range 200-1200 µM (Conrad, 1999), the population corresponding to Msar1 probably utilized other substrates in these slurries.

The DGGE-band affiliated with *Methanobacteriaceae* was relatively strong in all profiles. In contrast, in two subsequent summer seasons it was observed that the relative intensity of this band decreased in the lower soil layer profiles towards the end of the season (Høj *et al.*, 2005). Unfortunately, the data from the present experiment provide no further information on how the dynamics of this population is controlled. The probe MB310 hybridized with few cells and only at temperatures ≥ 10°C. This suggests that the *Methanobacteriaceae* cells present in Solvatnet peat either had low ribosome contents, or that the probe had mismatches with the 16S rRNA gene of the most abundant members of this family.

The relative intensity of the bands affiliated with *Methanomicrobiales* (Mmi3 and Mmi4) decreased with time at temperatures ≥ 15°,C suggesting that the substrate availability regulated their abundance. While the trends seen for Mmi3 were similar to the trends seen for Msar1 (see above), Mmi4 seemed to decrease in intensity with

increasing temperature at both time points. Based on the current experiment however, no conclusions can be made on the type of substrates used by the individual populations. No FISH probe was used that targeted specifically the order *Methanomicrobiales*, since as far as we know there is no available probe specific for this taxon, which target a highly accessible 16S rRNA sequence on intact ribosomes (Fuchs *et al.*, 1998).

In the field, significant methane emissions were recorded even on days when the soil temperature at 5 cm depth was below 5°C (Høj *et al.*, 2005). The field results could therefore seem to be in contradiction with those from the present experiment, where an apparent minimum temperature of 6.5°C was indicated for the overall methanogenic degradation. However, some of the methanogenic populations in Solvatnet peat could grow during continuous incubation at 1°C and 5°C. This suggests that a supply of more easily degradable substrates in the field from sources such as root exudates, plant detritus, bird droppings, and possibly also from freeze-thaw events (Sharma *et al.*, 2006), could circumvent the temperature limitation for methanogenesis. This is consistent with a recent study by Yavitt and Seidman-Zager (2006), which suggested that in peat soils methanogenic conditions rely on a constant supply of easily decomposable metabolic substrates. Also other studies have identified substrate supply as a major factor controlling methane production in northern wetlands (Wagner *et al.*, 2003). The conclusion is also supported by *Archaea* being specifically associated with fresh organic material in methanogenic German peat (Wachinger et al. 2000).

A consequence of such a control of methanogenesis in northern wetlands is that the effect of increased soil temperatures on methane production could potentially be additive, via effects both on the production and consumption of the easily

degradable substrate pool that is currently transformed to methane, as well as via effects on the production and consumption of more complex organic compounds that currently accumulate in northern wetlands. Further studies should give special attention to disentangling the effects of substrate and temperature on methanogenesis, to seasonal changes in the availability of methanogenic substrates and to the field conditions present in early spring. This could aid in predicting the effect of a temperature increase during the ecologically relevant time frame of an Arctic summer season.

Experimental procedures

Study site and sample collection

The site Solvatnet is located on a marine terrace just outside Ny-Ålesund (78°50'N-11°30'E) on the West-Coast of Spitsbergen. The site and peat characteristics, as well as the *in situ* archaeal community composition at two depths, have been described in detail elsewhere (Høj *et al.*, 2006; Høj *et al.*, 2005). The peat is classified as undecomposed (H1) and almost undecomposed (H2) using von Post's scale (Clymo, 1983). The peat has a high organic content (88 % of dry weight) and a high water content (>600% of dry weight), and is weakly acidic (pH 6.8 in H_2O). The site is grazed by Barnacle Geese (*Branta leucopsis*). Previously recorded CO_2-emssions were in the range 0.18-0.51 g CO_2 m^{-2} h^{-1}, and methane-emissions were in the range 93-2801 µg CH_4 m^{-2} h^{-1} (Høj *et al.*, 2005). Peat samples for this experiment were collected in mid-August 2000 using PVC corers (inner diameter 5 cm, length 10 cm), as previously described (Høj *et al.*, 2005). The cores were stored at 2-4°C until the experiment was started.

Preparation and incubation of soil slurries

Soil from the inner part (5-7 cm depth) of three cores were mixed and used to make slurries. Peat slurries were made under N_2 atmosphere by mixing 50 g wet peat (corresponding to 4.5 g dry peat) with 15 ml sterile, anoxic water in a 125 ml serum bottle. Sealed and capped slurries were gassed with N_2 and incubated in the dark without shaking in precisely controlled (± 0.01°C) water baths at temperatures 1, 5, 10, 15, 20 and 25°C. Two parallel slurries were made for each temperature. One parallel was terminated in week 4 to perform analyses of the archaeal community structure while the other parallel was terminated in week 13. Due to the labor intensity associated with sample processing, samples from no more than two temperatures were processed per day. Hence, for each sampling occasion (weeks 4 and 13) all temperatures were sampled within 5-6 days, with the highest temperatures being sampled first.

Analytical techniques

Before sampling the headspace gas, the bottles were shaken vigorously to allow equilibration between the liquid and gas phases. The methane concentrations in 1 ml of headspace gas was measured using a Shimadzu GC-14A gas chromatograph equipped with a Porapak Q stainless steel column (1.5 m x 1/8 inch) and a flame ionization detector, with N_2 as carrier gas. Injection, detection and column temperatures were 200, 240 and 45°C, respectively. The hydrogen concentration in 2 ml headspace gas was measured using a Varian Aerograph model 920 gas chromatograph equipped with a Stainless Steel Washed Molesieve (Alltech) 60/80 mesh column (2.0 m x 1/8 inch) and a thermal conductivity detector (125 mA), with Argon as carrier gas. Injection, detection and column temperatures were 100, 100 and 50°C, respectively. Carbon dioxide concentration in 2 ml head space gas was

determined using an infrared CO_2 analyzer (Binos 100, Rosemount GmbH & Co, Geschaftsbereich Analysentechnik, Hanau, Germany).

Samples of slurry water (1 ml) were stored frozen at -20°C until analysis for volatile fatty acids by a Schimadzu GC 14A gas chromatograph equipped with a flame ionization detector, with N_2 as carrier gas. Thawed samples were acidified by addition of 10 µl HCl so that C2-C5 volatile fatty acids could be separated as free acids on a 6 feet x 2 mm ID glass column packed with 10% SP-1200 and 1% H_3PO_4 on 80/100 Chromosorb W AW). Injection, detection and column temperatures were 200, 200 and 125°C, respectively.

Calculations

Calculation of methane accumulation was based on the concentration in the gas phase, as the amount of dissolve methane can be assumed to be low (Wilhelm *et al.*, 1977). The partial pressure was calculated, based on the amount of methane in the headspace, using the ideal gas law. The methane production rate in the linear accumulation phase was determined by linear regression. These rates were used for calculations of temperature responses (Q_{10}-values) and for preparing a Ratkowsky plot (Ratkowsky *et al.*, 1983). Since the linear phase of methane accumulation was reached only at temperatures $\geq$ 10°C, the Q_{10} values and the Ratkowsky plot is valid only for the methanogenesis pathways operating at temperatures $\geq$ 10°C. Linear regression of the linear part of the Ratkowsky plot was used to find the apparent minimum temperature (t_{min}) for methanogenic degradation pathways operating at temperatures $\geq$ 10°C. Per cell methane production was estimated based on the methane production rate between days 22 and 28, and the number of cells detected with the probe EURY498 in week 4. The CO_2 partial pressure of in the headspace was calculated as described above for methane.

DNA extraction from soil slurries

Samples for DNA extraction were frozen at -80°C until they were processed. DNA extraction and purification was performed with FastDNA© SPIN Kit for soil (BIO101, California, USA) in combination with a freeze-thaw lysis protocol (Høj *et al.*, 2005; Nakatsu *et al.*, 2000). 0.4 g wet peat and 1 ml lysis buffer (0.12 M NaP (pH 8), 5% SDS) were combined in a MULTIMIX 2 Tissue Matrix Tube. The tube was vortex mixed for 1 min, incubated at 65ºC for 45 minutes, followed by three quick freeze-thaw cycles (-80°C and +65°C). After this combined mechanical and freeze-thaw cycle lysis, the DNA was purified with the kit as described by the manufacturer. Two parallel extractions were performed per sample, and the resulting eluates were mixed.

PCR amplification and DGGE analysis

A nested protocol was used for amplification of the 16S rRNA gene of *Archaea* using the primers PRARCH112F and PREA1100R in the first reaction, and the primers PARCH340-GC and PARCH519R in a second touchdown reaction as previously described (Høj *et al.*, 2005). Denaturing gradient gel electrophoresis and sequencing of bands was performed as described previously (Høj *et al.*, 2005). Sequences were analyzed using the BLAST tool at the National Center for Biotechnology Information (NCBI).

Total counts and FISH analysis

Soil samples (2.5 g) were fixed in 4% paraformaldehyde (10 ml) at 4°C with shaking over night. The fixed soil was transferred to a 250 ml Waring-blender steel jar (Waring, Connecticut, USA) with 12.5 ml ice-cold 0.2 μm filtered water, and was homogenized 10 times for 1 minute, with 5 minutes cooling between each blending. After the last blending, the homogenate was allowed to sediment for 1 minute, and 2

parallels of 1 ml samples were taken with a 1 ml plastic syringe without needle. The maximum amount of sample that could be filtered while still keeping the linear relationship between bacterial numbers and the sample volume, was determined for each sample (Fægri *et al.*, 1977). Samples of appropriate dilutions were filtered onto white polycarbonate membrane filters (0.2 µm pores, 25 mm diameter; Osmonics Poretics Products, Minnesota, USA). The membrane filters were dried on filter paper and stored frozen at -20°C until use. Each filter was cut into 6 sections for total counts and hybridization with phylogenetic probes.

The DNA-specific stain PicoGreen (Molecular Probes, Invitrogen) was chosen for total prokaryotic counts, as initial experiments showed that it was superior to DAPI (Porter and Feig, 1980) in discriminating between cells and soil particles, and caused less background on white polycarbonate filters than SYBRGreen I (Molecular Probes, Invitrogen). Filter sections were stained with PicoGreen (1:100 dilution in 1 x TAE) in the dark for 15 minutes (Weinbauer *et al.*, 1998). The filter sections were gently washed in sterile milliQ water, air-dried and mounted with a drop of antifade solution (0.1% p-phenylenediamine, 50% glycerol, 0.5 x PBS) (Noble and Fuhrman, 1998).

Oligonucleotide probes were purchased with a CY3 fluorochrome in the 5' end (Interactiva Biotechnologie). The domain-specific probes EUB338 and ARCH915 (Amann *et al.*, 1990) were used for detection of *Bacteria* and *Archaea*, respectively. The probe EURY498 (Burggraf *et al.*, 1994) was used for detection of *Euryarchaeota*. The group specific probes MSMX860 and MB310 (Raskin *et al.*, 1994) were used for detection of *Methanosarcinales* and *Methanobacteriacea*, respectively. The complementary Non-EUB338 probe was used as a negative control for non-specific binding (Wallner *et al.*, 1993). Stringency conditions for the probes were controlled

using cells of *Escherichia coli*, *Archaeoglobus fulgidus*, *Methanococcus voltae*, *Methanosaeta concilii*, *Methanosarcina siciliae*, *Methanobacterium formicicum*, *Methanospirillum hungatei* and *Sulfolobus solfataricus*.

Filter sections were placed on glass slides and covered with 25 µl hybridization solution. The hybridization solution contained 0.9 M NaCl, 20 mM Tris-HCl (pH 7.4), 0.01% sodium dodecyl sulfate, 50 ng of probe and appropriate formamide concentration (15% for EURY498 probe, 25% for ARCH915 probe, 30% for EUB338 and NON338 probes, and 35% for MSMX860 and MB310 probes). Hybridizations were carried out in equilibrated chambers at 42°C over night. The filters were transferred to a vial containing 50 ml of pre-warmed (48°C) washing solution, and incubated freely floating without shaking at 48°C for 15 minutes. The washing solution contained 20 mM Tris-HCl (pH 7.4), 5 mM EDTA (at stringencies were corresponding to ≥ 20% formamide), 0.01% sodium dodecyl sulfate and appropriate NaCl concentration (318 mM for EURY498 probe, 149 mM for ARCH915 probe, 102 mM for EUB338 and NON338 probes, and 70 mM for MSMX860 and MB310 probes). Filters were dried on filter paper and mounted with a drop of antifade solution as described for total counts (Noble and Fuhrman, 1998).

Two replicate filters from each parallel dilution series were used for counting in the microscope, giving a total of four filters analysed per sample. At least 10 fields of view per filter and a total of 2500 cells were counted per sample using a Zeiss Axioplan epifluorescensce microscope (Zeiss, Jena, Germany) equipped with a 50 W high pressure mercury bulb. The specific filter set Chroma HQ 41007 (535-550 nm excitation filter, 565 nm dicroic beam splitter, and 610-675 nm emission filter; Chroma Tech. Corp., Brattleboro, Vt) was used for CY3. The Zeiss filter set 09 (450-

490 nm excitation filter, 510 nm dichroic beam splitter, and 515 nm LP barrier filter) was used for PicoGreen stained cells.

Acknowledgements

We thank Ingvild Wartianen and Lars Egil Haugen for collaboration in the field. This work was funded by The Norwegian Research Council (Grant 121458/720) and by grants from Meltzers høyskolefond and Bergen Myrdyrkningsforenings fond to L.H. The Norwegian Polar Institute is acknowledged for financial support of the field work.

Fig.1: Methane accumulation in slurries of Solvatnet peat incubated at 1°C (●), 5°C (○), 10°C (▼), 15°C (□), 20° (■), and 25°C (□). a) Accumulation of methane at all incubation temperatures on a linear scale. The accumulation curves for 1°C and 5°C could not be distinguished on this plot. b) Accumulation of methane at temperatures ≤ 10°C on a linear scale. c) Accumulation of methane at all temperatures on a logarithmic scale.

Fig. 2: Relationship between accumulation of total CO_2 and CH_4 in slurries of Solvatnet peat incubated at 1°C (●), 5°C (○), 10°C (▼), 15°C (□), 20° (■), and 25°C (□).

Fig. 3: Concentrations of volatile fatty acids detected in slurries of Solvatnet peat. The detection levels were 67 µM for acetate, 26 µM forpropionate, 22 µM for isobutyrate and 18 µM for butyrate.

Fig. 4: a) Total prokaryotic counts. b) FISH counts; fraction of total number of prokaryotes detected with the probes EUB338 (weeks 4 and 13), ARCH915 (week 4), and EURY498 (week 4), targeting *Bacteria*, *Archaea* and *Euryarchaeota*, respectively.

Fig. 5: Image showing sphere-shaped aggregate detected by the probe EURY498 at 25°C. The scale bar corresponds to 10 µm.

Fig. 6: DGGE-profiles of archaeal 16S rRNA genes amplified from Solvatnet peat slurries in weeks 4 and 13. The phylogenetic labels are consistent with labels used in

previous studies of field samples (Høj *et al.*, 2006; Høj *et al.*, 2005). Cr: *Crenarchaeota*, Mmi3-Mmi4: *Methanomicrobiales*, Mb: *Methanobacteriaceae*, Msae: *Methanosaeta*, Msar1: *Methanosarcina*.

References

Amann, R.I., Binder, B.J., Olson, R.J., Chisholm, S.W., Devereux, R. and Stahl, D.A. (1990) Combination of 16S ribosomal RNA targeted oligonucleotide probes with flow-cytometry for analyzing mixed microbial populations. *Appl. Environ. Microbiol.* 56: 1919-1925.

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, Cambridge University Press, pp. 1000

Bergman, I., Svensson, B.H. and Nilsson, M. (1998) Regulation of methane production in a Swedish acid mire by pH, temperature and substrate. *Soil Biol. Biochem.* **30**: 729-741.

Bergman, I., Klarqvist, M. and Nilsson, M. (2000) Seasonal variation in rates of methane production from peat of various botanical origins: effects of temperature and substrate quality. *FEMS Microbiol. Ecol.*. **33**: 181-189.

Boetius, A., Ravenschlag, K., Schubert, C.J., Rickert, D., Widdel, F., Gieseke, A., *et al* (2000) A marine microbial consortium apparently mediating anaerobic oxidation of methane. *Nature*. **407**: 623-626.

Burggraf, S., Mayer, T., Amann, R., Schadhauser, S., Woese, C.R. and Stetter, K.O. (1994) Identifying members of the domain *Archaea* with ribosomal-RNA-targeted oligonucleotide probes. *Appl. Environ. Microbiol.* **60**: 3112-3119.

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**: doi: 10.129/2002GLO16848.

Clymo, R.S. (1983) Peat. In *Mires: Swamp, bog, fen and moor*. Gore, A.J.P. (ed.) Amsderdam: Elsevier Scientific Publishing Company, pp. 159-224.

Conrad, R. (1999) Contribution of hydrogen to methane production and control of hydrogen concentrations in methanogenic soils and sediments. *FEMS Microbiol. Ecol.* **28**: 193-202.

Dunfield, P., Knowles, R., Dumont, R. and Moore, T.R. (1993) Methane production and consumption in temperate and subarctic peat soils: Response to temperature and pH. *Soil Biol. Biochem.* **25**: 321-326.

Fægri, A., Torsvik, V. and Goksøyr, J. (1977) Bacterial and fungal activities in soil: Separation of bacteria and fungi by a rapid fractionated centrifugation technique. *Soil Biol. Biochem.* **9**: 105-112.

Fey, A. and Conrad, R. (2000) Effect of temperature on carbon and electron flow and on the archaeal community in methanogenic rice field soil. *Appl. Environ. Microbiol.* **66**: 4790-4797.

Fey, A., Claus, P. and Conrad, R. (2004) Temporal change of ^{13}C-isotope signatures and methanogenic pathways in rice field soil incubated anoxically at different temperatures. *Geochim. Cosmochim. Acta*. **68**: 293-306.

Finlay, B.J. and Fenchel, T. (1991) An Anaerobic Protozoan, With Symbiotic Methanogens, Living In Municipal Landfill Material. *FEMS Microbiol. Ecol.* **85**: 169-179.

Fuchs, B.M., Wallner, G., Beisker, W., Schwippl, I., Ludwig, W. and Amann, R. (1998) Flow cytometric analysis of the *in situ* accessibility of *Escherichia coli* 16S rRNA for fluorescently labeled oligonucleotide probes. *Appl. Environ. Microbiol.* **64**: 4973-4982.

Høj, L., Olsen, R. and Torsvik, V. (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.

Høj, L., Rusten, M., Haugen, L.E., Olsen, R.A. and Torsvik, V.L. (2006) Effects of water regime on archaeal community composition in Arctic soils. *Environ. Microbiol.* **0**(0), ???-???. doi: 10.1111/j.1462-2920.2006.00982.x.

Jurgens, G., Glöckner, F.-O., Amann, R., Saano, A., Montonen, L., Likolammi, M. and Munster, U. (2000) Identification of novel Archaea in bacterioplankton of a boreal forest lake by phylogenetic analysis and fluorescent in situ hybridization. *FEMS Microbiol. Ecol.* **34**: 45-56.

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.

Knittel, K., Losekann, T., Boetius, A., Kort, R. and Amann, R. (2005) Diversity and distribution of methanotrophic archaea at cold seeps. *Appl. Environ. Microbiol.* **71**: 467-479.

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., Nozhevnikova, A.N., Soloviova, T.I. and Zavarzin, G.A. (1996) Methanogenesis at low temperatures by microflora of tundra wetland soil. *Anton. Leeuw. Int. J. G.* **69**: 75-86.

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.

Metje, M. and Frenzel, P. (2005) Effect of temperature on anaerobic ethanol oxidation and methanogenesis in acidic peat from a northern wetland. *Appl. Environ. Microbiol.* **71**: 8191-8200.

Murray, A.E., Preston, C.M., Massana, R., Taylor, L.T., Blakis, A., Wu, K. and DeLong, E.F. (1998) Seasonal and spatial variability of bacterial and archaeal assemblages in the coastal waters near Anvers Island, Antarctica. *Appl. Environ. Microbiol.* **64**: 2585-2595.

Nakatsu, C.H., Torsvik, V. and Øvreås, L. (2000) Soil community analysis using DGGE of 16S rDNA polymerase chain reaction products. *Soil Sci. Soc. Am. J.* **64**: 1382-1388.

Noble, R.T. and Fuhrman, J.A. (1998) Use of SYBR Green I for rapid epifluorescence counts of marine viruses and bacteria. *Aquat. Microb. Ecol.* **14**: 113-118.

Porter, K.C. and Feig, Y.S. (1980) The use of DAPI for identifying and counting aquatic microflora. *Limnol. Oceanogr.* **25**: 943-948.

Raskin, L., Stromley, J.M., Rittmann, B.E. and Stahl, D.A. (1994) Group-specific 16S rRNA hybridization probes to describe natural communities of methanogens. *Appl. Environ. Microbiol.* **60**: 1232-1240.

Ratkowsky, D.A., Lowry, R.K., McMeekin, T.A., Stokes, A.N. and Chandler, R.E. (1983) Model for bacterial culture-growth rate throughout the entire biokinetic temperature-range. *J. Bacteriol.* **154**: 1222-1226.

Ravenschlag, K., Sahm, K. and Amann, R. (2001) Quantitative molecular analysis of the microbial community in marine Arctic sediments (Svalbard). *Appl. Environ. Microbiol.* **67**: 387-395.

Sandaa, R.A., Torsvik, V., Enger, Ø., Daae, F.L., Castberg, T. and Hahn, D. (1999) Analysis of bacterial communities in heavy metal-contaminated soils at different levels of resolution. *FEMS Microbiology Ecology*. **30**: 237-251.

Sharma, S., Szele, Z., Schilling, R., Munch, J.C. and Schloter, M. (2006) Influence of freeze-thaw stress on the structure and function of microbial communities and denitrifying populations in soil. *Appl. Environ. Microbiol.* **72**: 2148-2154.

Svensson, B.H. (1984) Different temperature optima for methane formation when enrichments from acid peat are supplemented with acetate or hydrogen. *Appl. Environ. Microbiol.* **48**: 389-394.

van Hulzen, J.B., Segers, R., van Bodegom, P.M. and Leffelaar, P.A. (1999) Temperature effects on soil methane production: An explanation for observed variability. *Soil Biol. Biochem.* **31**: 1919-1929.

Wachinger, G., Fiedler, S., Zepp, K., Gattinger, A., Sommer, M., Roth, K. (2000) Variability of soil methane production on the micro-scale: spatial association with hot spots of organic material and Archaeal populations. Soil Biol. Biochem. 32: 1121-1130.

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.

Wallner, G., Amann, R. and Beisker, W. (1993) Optimizing fluorescent *in situ* hybridization with rRNA-targeted oligonucleotide probes for flow cytometric identification of microorganisms. *Cytometry*. **14**: 136-143.

Watanabe, K., Kodama, Y., Hamamura, N. and Kaku, N. (2002) Diversity, abundance, and activity of archaeal populations in oil-contaminated groundwater

accumulated at the bottom of an underground crude oil storage cavity. *Appl. Environ. Microbiol.* **68**: 3899-3907.

Weinbauer, M., Beckmann, C. and Höfle, M. (1998) Utility of green fluorescent nucleic acid dyes and aluminum oxide membrane filters for rapid epifluorescence enumeration of soil and sediment bacteria. *Appl. Environ. Microbiol.* **64**: 5000-5003.

Wilhelm, E., Battino, R. and Wilcock, R. (1977) Low-pressure solubility of gases in liquid water. *Chem. Rev.* **77**: 219-262.

Yavitt, J.B. and Seidmann-Zager, M. (2006) Methanogenic conditions in northern peat soils. *Geomicrobiol. J.* **23**: 119-127.

Yavitt, J.B., Williams, C.J. and Wieder, R.K. (1997) Production of methane and carbon dioxide in peatland ecosystems across North America: Effects of temperature, aeration, and organic chemistry of pent. *Geomicrobiol. J.* **14**: 299-316.

Zepp Falz, K., Holliger, C., Grosskopf, R., Liesack, W., Nozhevnikova, A.N., Müller, B., *et al* (1999) Vertical distribution of methanogens in the anoxic sediment of Rotsee (Switzerland). *Appl. Environ. Microbiol.* **65**: 2402-2408.

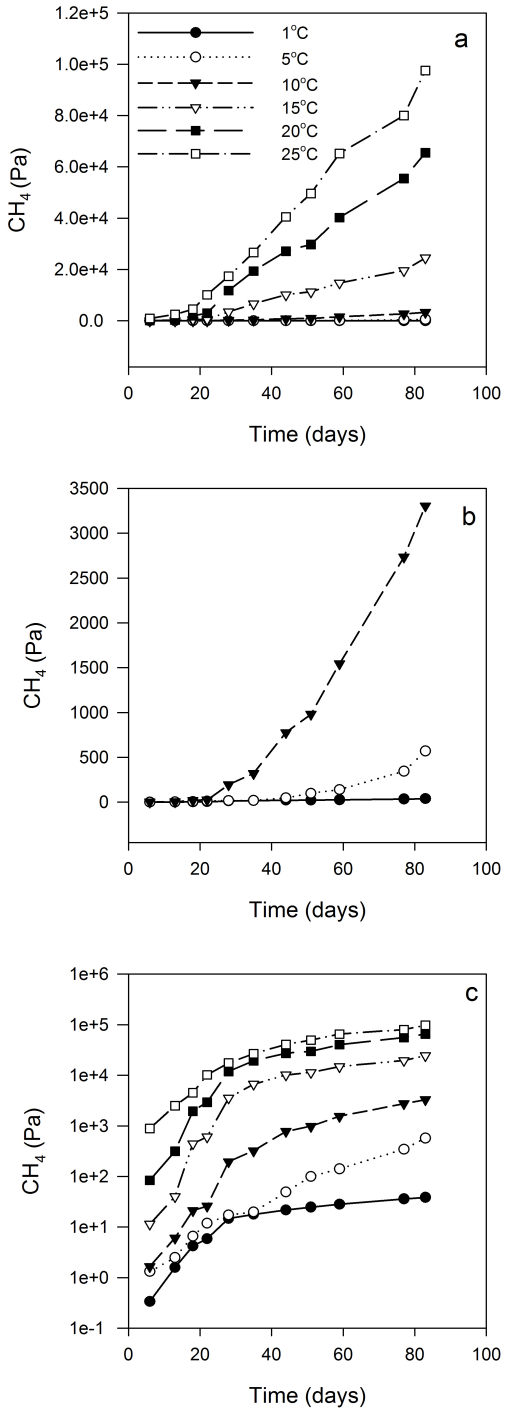

a
1°C
5°C
10°C
15°C
20°C
25°C
CH4 (Pa)
Time (days)
b
CH4 (Pa)
Time (days)
c
CH4 (Pa)
Time (days)

Fig. 1

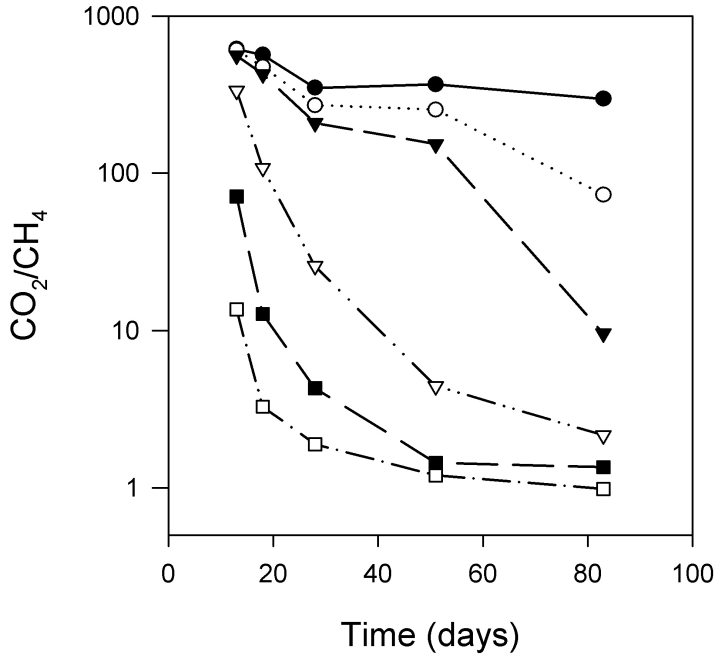


Fig. 2

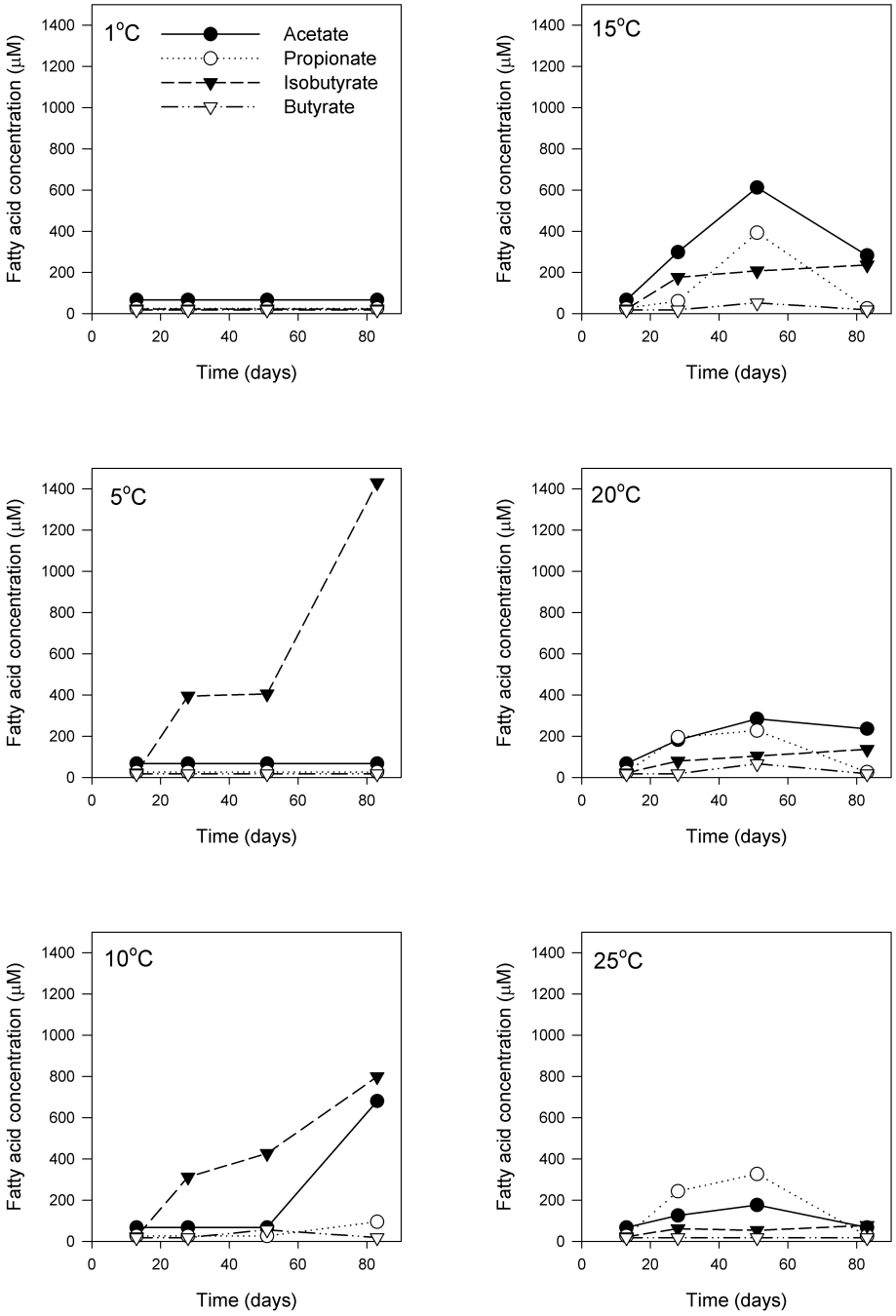
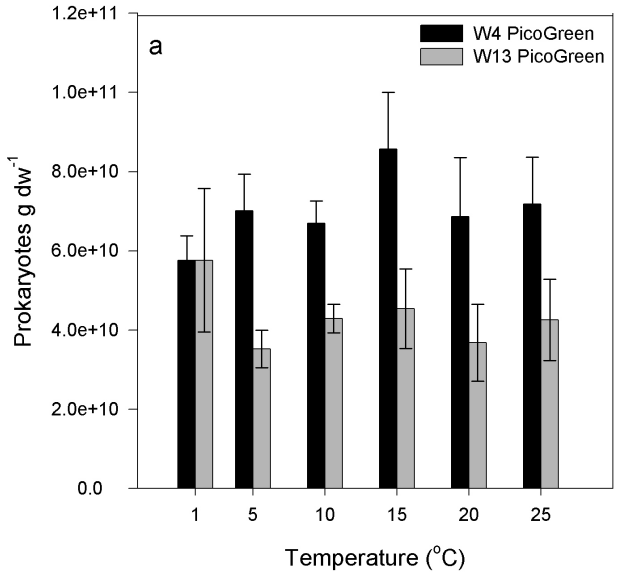

1°C
Acetate
Propionate
Isobutyrate
Butyrate
5°C
10°C
15°C
20°C
25°C
Fatty acid concentration (µM)
Time (days)
0
200
400
600
800
1000
1200
1400
0
20
40
60
80

Fig. 3


a
W4 PicoGreen
W13 PicoGreen
Prokaryotes g dw-1
1.2e+11
1.0e+11
8.0e+10
6.0e+10
4.0e+10
2.0e+10
0.0
1
5
10
15
20
25
Temperature (°C)

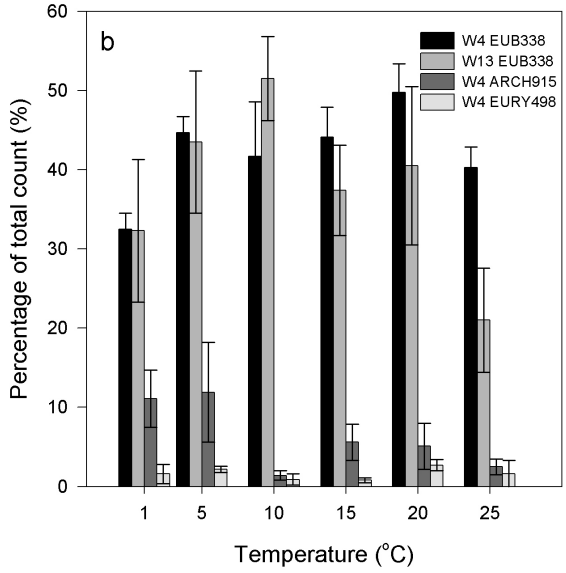

b
W4 EUB338
W13 EUB338
W4 ARCH915
W4 EURY498
Percentage of total count (%)
60
50
40
30
20
10
0
1
5
10
15
20
25
Temperature (°C)

Fig. 4

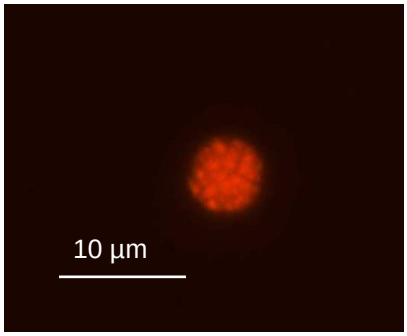


Fig. 5

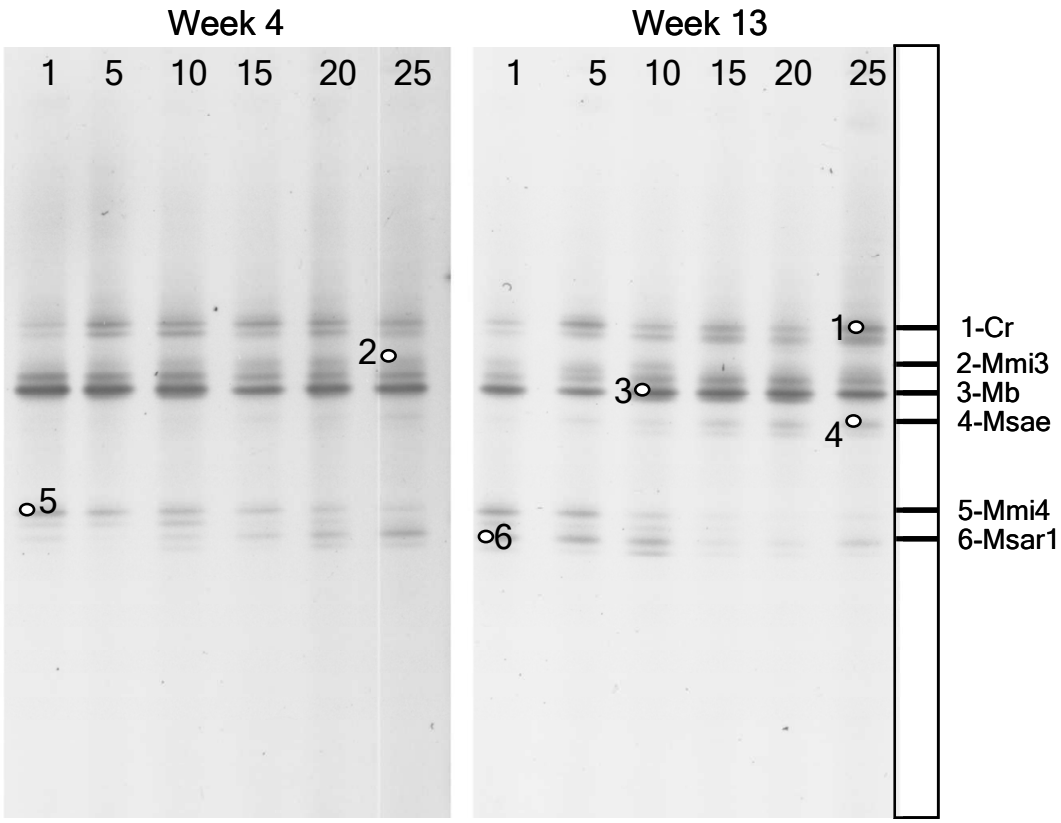

Week 4
1
5
10
15
20
25
Week 13
1
5
10
15
20
25
1
2
3
4
5
6
1-Cr
2-Mmi3
3-Mb
4-Msae
5-Mmi4
6-Msar1

Fig. 6