

Methanogenic archaea in Arctic soils from Spitsbergen, Norway (78°N)

Lone Høj



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ABBREVIATIONS

16S rDNA:	gene coding for 16S rRNA (see below)
16S rRNA:	molecule in the 30S subunit of the bacterial (70S) ribosome
bp:	base pairs
DNA:	deoxyribonucleic acid
DGGE:	denaturing gradient gel electrophoresis
F ₄₂₀ :	coenzyme F ₄₂₀ , a fluorescent 5-diazaflavin derivative which acts as an electron carrier in the last step of methanogenesis
FISH:	fluorescence <i>in situ</i> hybridization
IPY:	International Polar Year
<i>mcrA</i> :	gene that encodes the α subunit of methyl coenzyme M reductase, a key enzyme for methanogenesis.
PCR:	polymerase chain reaction
p.p.m.:	parts per million
RNA:	ribonucleic acid
T-RFLP:	terminal restriction fragment length polymorphism
Q ₁₀ :	the increase in production rate when the temperature increases 10°C
V3:	a hypervariable region in the 16S rRNA molecule
VFA:	volatile fatty acids

LIST OF PAPERS

Paper I: 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 (1): 89-101.

Paper II: 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.

Paper III: Høj L., Olsen R.A. and Torsvik V.L. (2006). Effects of temperature on archaeal community structure and methanogenesis in peat from an Arctic wetland. Manuscript submitted to *Environmental Microbiology*.

SUMMARY

The potential feedback between climate change and methane emissions from wet Arctic soils is poorly understood due to lacking information on how the underlying microbial processes are controlled. Methane emissions are a function of the balance between methane production by methanogenic archaea and methane consumption by methanotrophic bacteria. The papers in this thesis address topics such as seasonal changes, site variations and depth variations in the archaeal community, effects of soil water regime on the community composition, and effects of temperature on archaeal community structure and methane production.

DNA extracted from field samples, soil slurries and enrichment cultures of methanogenic archaea were analysed by nested polymerase chain reaction (PCR) combined with denaturing gradient gel electrophoresis (DGGE) and subsequent sequencing of 16S rDNA fragments. Ordination analysis of binary DGGE data was used to evaluate trends in the community profiles and to analyse the relationship between the community profiles and environmental parameters. In addition, fluorescence *in situ* hybridization (FISH) was used for quantitative analyses of the microbial communities in soil slurries incubated at different temperatures.

The results showed that the range of archaeal groups present in Spitsbergen soils is consistent with previous studies of wet soils and sediments, both from temperate and northern regions. The recovered sequences were affiliated with *Methanomicrobiales*, *Methanobacteriaceae*, *Methanosarcina*, *Methanosaeta*, Rice Cluster II, Sediment 1, a new euryarchaeotal cluster, and Group 1.3b of *Crenarchaeota*. Studies of enrichment cultures demonstrated that most populations detected by PCR-DGGE in field samples could grow in enrichment cultures at 10°C. The studies demonstrated however that culture based studies should include monitoring of the methanogenic populations to evaluate whether the results are ecologically relevant. Soil water regime was found to be critical for the presence of abundant methanogenic populations in Spitsbergen soils. The variability associated with soil water regime was predominant over variability between different wet sites and seasonal variations. Differences in the archaeal community composition between wet sites were however also demonstrated.

Especially, differences in the occurrence of methanogens capable of acetoclastic methanogenesis were conspicuous. Seasonal changes in the archaeal community composition were detected at some sites, while other sites had more stable archaeal communities. Depth related changes were observed only at sites with significant changes in the physicochemical conditions between the tested soil depths.

The overall process of methanogenic degradation of complex organic compounds in peat from Solvatnet 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 accumulating at 1°C and 5°C corresponded to less than 0.5% of the methane accumulating at 25°C. Temperature affected the temporal development in the slurries as seen for the transition between methane accumulation phases, the CO_2/CH_4 ratio, the accumulation of volatile fatty acids and the development of the archaeal communities. At 5°C and 10°C the accumulation of isobutyrate was conspicuous. FISH analysis revealed a large (11-12% of the total count), unidentified, active archaeal community at low temperatures (1°C and 5°C). DGGE-profiling showed that populations of methanogenic archaea could grow at all tested temperatures, and indicated that their dynamics was primarily controlled by substrate availability. The results suggest that, at 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.

The studies presented in this thesis have provided new information on the archaeal community composition in Arctic soils, with special emphasis on methanogenic populations. Although the studies do not provide mechanistic explanations for observed trends, they provide a basis for setting up hypotheses that can be tested in further experiments.

1. INTRODUCTION

The Arctic is likely to respond rapidly and more severely to a global climate change than any other area due to a variety of positive feedback mechanisms (Anisimov and Fitzharris, 2001). Increased snowfall and higher temperatures are expected in winter, and summer could be much warmer and wetter than at present. Changes in temperature, precipitation and permafrost coverage are already taking place, but the existing data demonstrate differences between the Arctic regions (Anisimov and Fitzharris, 2001; Weller, 1998).

Arctic soils contain a significant portion (14%) of the global soil organic carbon (Post *et al.*, 1982). The accumulation of organic matter is a result of low decomposition rates due to a combination of low temperatures and large areas where permafrost impedes draining, resulting in wet and anaerobic soil conditions. In such anaerobic systems, where the concentrations of external electron-acceptors such as NO_3^- and SO_4^{2-} are relatively low, methane production is the dominant terminal process in microbial degradation of organic material (Hedderich and Whitman, 2005). Current methane emissions from northern wetlands and tundra are estimated to be approximately 35 Tg yr^{-1} (Reeburgh and Whalen, 1992), corresponding to nearly 6% of the total global methane source (Ehhalt *et al.*, 2001), and nearly 18% of emissions from sources not related to human activities (Lelieveld *et al.*, 1998). Due to the high effectiveness of methane as a greenhouse gas, Arctic wetlands are currently net sources of radiative forcing despite being overall carbon sinks (Friborg *et al.*, 2003).

The effect of a climate change on the net balance of CO_2 and methane in Arctic tundra is expected to depend largely on the magnitude and direction of hydrological changes, vegetation and plant primary production changes, and on the temperature response of microbial decomposition (Anisimov and Fitzharris, 2001; Christensen, 1999; Christensen *et al.*, 1999; Oechel *et al.*, 1993). Climate change can affect soil processes by altering the functioning of the soil system (carbon and electron flow), by affecting metabolic rates of existing organisms and by altering the community structure (Schimel and Gullledge, 1998). Previous studies of the effects of environmental

variables on methane production and consumption in northern wetlands have primarily been process related, and little attention has been given to the involved microbial communities. One reason for this is that it has been assumed that microbial community structure has little relevance to large-scale biogeochemical models (Schimel, 1995). Another reason is that the methods needed to investigate diversity and structure of microbial communities and their control, have only been available during the last decade. Some functional studies have however shown that the response of methane production to e.g. pH and temperature may vary between systems (Bergman *et al.*, 2000; Bergman *et al.*, 1998; Yavitt *et al.*, 1997), indicating that the microbial communities may vary between sites. Bergman and coworkers (2000) have also demonstrated seasonal changes in the functioning of the microbial community (Bergman *et al.*, 2000). Such observations suggest that information on the diversity and structure of microbial communities involved in methane transformations can potentially improve the understanding of how methane emissions are controlled.

The work presented in this thesis was part of project called “Microbial production and consumption of methane in Arctic ecosystems”. The project was financed by the Norwegian Research Council on the program Arctic Light and Heat. The work relating to methane oxidation and methane oxidizing bacteria has been presented previously (Wartiainen *et al.*, 2006; Svenning *et al.*, 2003; Wartiaainen *et al.*, 2003; Wartiaainen, 2002), and will not be discussed in detail here. In summary, the performed studies showed that both type I and type II methanotrophs are present in Spitsbergen soils. A higher diversity of type I versus type II methanotrophs was indicated both by PCR-DGGE analysis (Wartiainen *et al.*, 2003) and by combining micro colony growth with fluorescence *in situ* hybridization (Wartiainen, 2002). Sequences recovered from DGGE bands were affiliated with members of the genera *Methylobacter* (type I) and *Methylocystis*/*Methylosinus* (type II) (Wartiainen *et al.*, 2003). Enumeration of methanotrophic bacteria using microcolony growth and FISH analysis with methanotroph specific oligonucleotide probes showed a population size of $1.1 \times 10^5 - 5.2 \times 10^5$ methanotrophs per g wet soil (Wartiainen, 2002). Two strains of methanotrophic bacteria were isolated (Wartiainen, 2002); one representing a new species of *Methylocystis*, *Methylocystis rosea* (Wartiainen *et al.*, 2006a) and the other representing a new species of *Methylobacter*, *Methylobacter tundripaludum*

(Wartiainen *et al.*, 2006b). Due to the high water table at current climatic conditions, the methanotrophic activity is restricted to a limited area and appears not to be significant for the regulation of methane emissions from the studied sites (Wartiainen, 2002; **paper I**). However, the temperature characteristics of the two isolates show that climate-related changes in water conditions and increased soil temperatures may cause a substantial increase in both the abundance and activity of methanotrophic bacteria in Spitsbergen soils (Wartiainen, 2002).

The included papers all relate to the composition of archaeal communities in Arctic soils. The archaeal community include the methanogenic archaea, which perform the methane-production step in anaerobic degradation of organic matter. Individual papers address topics such as seasonal changes, site and depth variations, and effects of soil water regime on the archaeal communities, as well as effects of temperature on archaeal community structure and methane production.

2. AIMS

The overall aim of this study was to obtain knowledge about the archaeal communities in wet soils from Spitsbergen, with special emphasis on the methanogenic archaea.

Specific objectives were to

1. Obtain basic knowledge about the archaeal community composition in wet Spitsbergen soils
2. Evaluate the culturability of methanogenic archaea that were detected in field samples, and the potential ecological relevance of culture-based studies.
3. Investigate the effects of soil water regime on archaeal community composition
4. Investigate the distribution of methanogenic populations in space and time
5. Investigate the effects of temperature on archaeal community structures and methanogenesis.

3. BACKGROUND

3.1 Svalbard - a natural area for studying global change

Svalbard is an island group located between 74-81°N and 10-35°E with a total land area of 62,700 km². Spitsbergen is the largest island, with an area of 39,000 km² (Fig. 1). The light regime reflects the high latitude, with midnight sun from April 21 to August 21, and polar night from October 28 to February 14 at 78°N (Hisdal, 1998). The climate on the West-Coast of Spitsbergen is affected by the Gulf Stream and is therefore relatively mild considering the high latitude, with yearly average temperatures ranging from -7°C to -4°C. January, February and March are the coldest months, with average temperatures in the range -16°C to -11°C, while July is the warmest month with mean temperatures in the range 4-6°C. The yearly precipitation is 190-525 mm, and the period from April to June are the driest months (Førland *et al.*, 1997). The permafrost at Svalbard is continuous and ranges from less than 100 m thick in coastal areas to 400-500 m thick in mountainous areas (Humlum *et al.*, 2003). The depth of the upper soil layer that thaws during summer (the active layer), is generally in the range 35-150 cm (Brown *et al.*, 2000), and the plant growing season lasts approximately 2 months.

Through recent years Svalbard has been established as an internationally important base for Arctic research. Current projects cover a broad range of disciplines in biology, geology, geophysics and others (Hansen and Bjørdal, 2004). Due to the relatively high accessibility and good logistic support in this polar region, it is expected that Svalbard will be included as a site in a number of projects in the coming International Polar Year (IPY) (March 2007 – March 2009). Studies that target potential effects of climate change and potential interactions between the atmosphere and the biosphere are intrinsically complex, and interdisciplinary research including vegetation ecology, zoology, microbiology, soil sciences, meteorology, and mathematical modelling is required. However, knowledge from smaller projects such as the one reported in this thesis is important to set up good working hypotheses that can subsequently be tested within the framework of multidisciplinary collaboration projects, potentially under the IPY umbrella.



Fig.1. Maps of the Svalbard archipelago. Top: The localization of the archipelago. Bottom: The islands of Svalbard. From The Norwegian Polar Institute.

3.2 Methane as a greenhouse gas

Methane has the second largest effect on the global radiative balance after CO₂, and increased atmospheric methane concentrations are currently responsible for approximately 20% of the direct radiative forcing of the climate (Albritton *et al.*, 2001). Since 1750 the atmospheric methane concentrations have increased by approximately 150%, reaching a concentration of 1.745 p.p.m. in 1998. In comparison, the atmospheric CO₂ concentrations have increased by approximately 25% in the same period, from about 280 p.p.m. to 365 p.p.m. Methane absorbs infrared radiation from Earth more effectively than CO₂, and is estimated to be 23 times more effective (per kg) as a greenhouse gas on a 100 years time horizon (Albritton *et al.*, 2001). Hence, a small increase in the emissions from major methane

sources (Table 1) can potentially have a significant effect on the global climate system.

Table 1: Potential annual methane emissions from identified sources (Tg of CH₄ year⁻¹). Based on Lelieveld and coworkers (1998).

Microbial		Thermogenic		Total	
Natural		Natural			
Wild ruminants	5	Methane hydrates*		10	
Termites	20				
Wetlands	145				
Oceans	10				
Freshwaters	5				
Total natural microbial	185	Total natural thermogenic	10	Total natural	195
Anthropogenic		Anthropogenic			
Domestic ruminants	80	Energy use		110	
Rice paddies	80	Biomass burning		40	
Animal wastes	30				
Landfills	40				
Wastewater	25				
Total anthropogenic microbial	255	Total anthropogenic thermogenic	150	Total anthropogenic	405
Total microbial	440	Total thermogenic	160	Subtotal sources	600

*: Methane in hydrates can be biogenic or thermogenic (Tyler, 1991).

** : Animal waste include waste both from wild and domestic animals.

3.3 The global methane budget

Currently the total global methane emissions are estimated to approximately 600 Tg yr⁻¹ (1Tg = 10¹² g) (Ehhalt *et al.*, 2001; Lelieveld *et al.*, 1998), of which about two-thirds is related to human activity (Table 1). The most important anthropogenic sources are rice fields and domesticated ruminants, each being responsible for approximately 13% of the total emissions. Natural wetlands are the largest natural methane source, contributing to approximately 24% of the total emissions. Other natural sources are termites, oceans, methane hydrates, freshwater sediments and wild ruminants. According to the current paradigm about 95% of the naturally produced methane is produced microbially, while for the anthropogenic sources about 63% is produced microbially, with the rest originating from biomass burning and fossil fuels (Table 1). However, recently Keppler *et al* (2006) reported that methane can be produced non-microbially by terrestrial plants and plant litter under aerobic

conditions. They estimated a methane source strength of 62-236 Tg yr⁻¹ for living plants and 1-7 Tg yr⁻¹ for plant litter, which corresponds to 10-30% of the present annual source strength. It is possible that this source may overlap with production previously assigned to wetlands and rice fields (Keppler *et al.*, 2006), and this new information suggests that further studies should account for methane production both directly from plant material and from microbial communities associated with plants, plant roots and soil.

3.4 Methane emissions from northern wetlands

Numerous studies of methane fluxes from Arctic and subarctic wetlands have been performed in the last 30 years (Christensen *et al.*, 2000; Christensen *et al.*, 1995; Morrissey and Livingston, 1992; Sebacher *et al.*, 1986; Svensson, 1980; Svensson, 1975). For detailed reviews on environmental controls on methane production, consumption and emissions in Arctic and subarctic soils, see Christensen and coworkers (2003), Christensen (1999), Segers (1998), Bubier and Moore (1994), and Svensson and Sundh (1992). Large spatial and temporal variability in emissions have repeatedly been observed, and this seems to be the normal situation (Morrissey and Livingston, 1992; Whalen and Reeburgh, 1988). A wide range of parameters have been found to correlate with methane emissions. These include soil temperature, soil moisture and water table depth, thaw depth, pH, substrate quality and availability, vegetation types, plant production and net ecosystem exchange. Many of these variables are co-regulated and interdependent (Christensen *et al.*, 2000; Bergman *et al.*, 1998; Svensson and Sundh, 1992; Svensson and Rosswall, 1984), complicating the interpretation of the observed correlations. In addition, both the temporal and the spatial scale of the study influences which parameters that are correlated with methane emissions (Friborg *et al.*, 2000; Roulet *et al.*, 1994). Some recent publications compile data from several areas and several years, and based on these larger datasets the authors conclude that average seasonal methane emissions can be explained primarily by a combination of soil temperature and the availability of methanogenic substrates, with water table acting as an overruling parameter (Christensen *et al.*, 2003).

3.5 Methane production potentials in northern wetland soils

The potential methane production in northern wetlands has been found to depend mainly on the quality of organic material available for anaerobic degradation (Wagner *et al.*, 2003; Bergman *et al.*, 2000; Bergman *et al.*, 1998). The maximum production potentials are found in the upper anoxic peat layers where most of the anaerobic degradation takes place. In the upper anoxic layers the oxygen concentration is low enough to permit methanogenesis, and the amount of methanogenic substrates is sufficient for methanogenesis. It should be noted that the maximum methane production from any soil is modulated by temperature, but the temperature response vary between soil types (see section 4.5).

3.6 Trophic interactions in cold methanogenic systems

Anaerobic degradation of organic matter involves several microbial groups which interact in a complex trophic network. The trophic interactions between the different groups determine the element and energy flow in the system. Several studies have targeted the effect of low temperature on the trophic interactions in methanogenic systems. These studies were recently reviewed by Kotsyurbenko (2005). The principal outline of trophic interactions in a methanogenic microbial community developing at low temperature is shown in Fig. 2. A detailed discussion of each step was given by Kotsyurbenko (2005), and only a general outline will be presented here.

The first step in anaerobic degradation of organic matter is hydrolysis of polymers to monomers. This step is mediated by extracellular enzymes from fermenting bacteria. The degradation of monomers can then proceed by two alternative pathways.

In the first pathway, the monomers serve as substrate for primary fermenters, which produce H₂, CO₂, various fatty acids and alcohols. The fatty acids can be further utilized by syntrophic bacteria to acetate, H₂ and CO₂. As indicated in Fig. 2, the syntrophic step is of reduced importance at low temperatures. Syntrophic reactions are thermodynamically less favourable at low temperature, and formation of syntrophic aggregates may be needed in order to facilitate diffusional transfer of H₂, formate or acetate. In addition, at low temperature the formation of the syntrophic associations may take a long time.

In the alternative pathway, the monomers are catabolized to acetate by homoacetogenic bacteria. This pathway is generally enhanced at low temperature, and hence the relative contribution of acetoclastic methanogenesis tends to increase to above 67% (see section 3.7) (Kotsyurbenko, 2005; Fey and Conrad, 2000; Schulz *et al.*, 1997; Kotsyurbenko *et al.*, 1996). In some cold systems however, the contribution of acetoclastic methanogenesis is lower than 67%. This can be explained by additional sources or sinks of either H₂ or acetate (Conrad, 1999). It has also been suggested that the contribution of hydrogenotrophic methanogenesis (see section 3.7) is favoured by a low supply of labile organic carbon (Hornibrook *et al.*, 2000; Schoell, 1988), and by acidic conditions (Kotsyurbenko, 2005; Brauer *et al.*, 2004; Horn *et al.*, 2003). However, the high contribution of hydrogenotrophic methanogenesis in for instance peat bogs is still poorly understood (Kotsyurbenko *et al.*, 2004; Conrad, 1999).

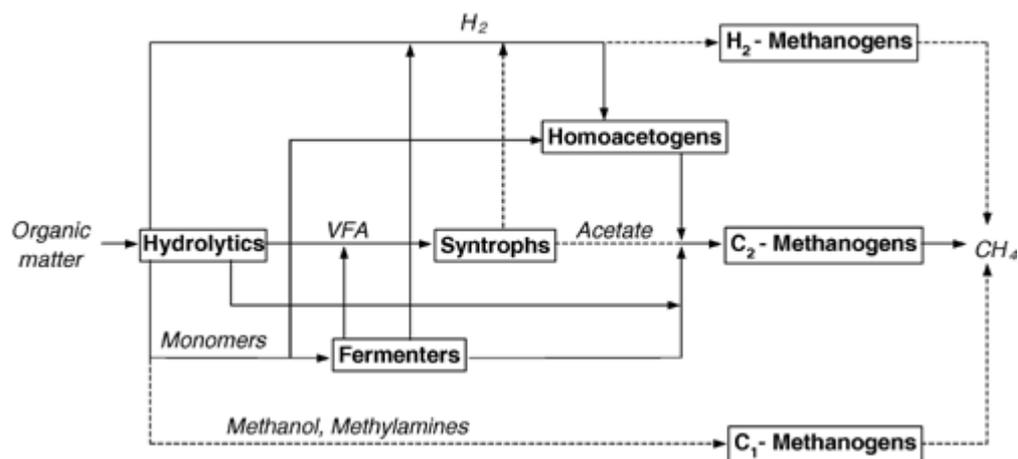


Fig. 2: The general scheme of trophic interactions in the methanogenic microbial community developing at low temperature. Solid arrows: main pathways; dotted arrows: reduced pathways. From Kotsyurbenko (2005). VFA: Volatile fatty acids.

3.7 Methanogenic archaea

Methanogenesis is the energy-yielding process of methanogenic archaea, all of which are members of the archaeal phylum *Euryarchaeota*. Five orders are currently recognized: *Methanobacteriales*, *Methanococcales*, *Methanomicrobiales*, *Methanosarcinales* and *Methanopyrales*, with a total of 10 families and 28 genera

(Hedderich and Whitman, 2005). Only a few low-molecular weight compounds can be used as energy sources for methanogenesis. The substrates can be divided in three classes based on the methanogenic catabolic pathway: CO₂-reduction (hydrogen/carbon dioxide, formate, short-chained alcohols), acetoclastic (acetate) and methylotrophic (methanol, methylated amines and sulfides, tetramethylammonium, methanethiol) methanogenesis (Hedderich and Whitman, 2005). Members of the orders *Methanobacteriales*, *Methanococcales*, *Methanomicrobiales* and *Methanopyrales* can only grow by the CO₂-reduction pathway, with the exception of *Methanosphaera* (*Methanobacteriales*), which can use H₂ to reduce methanol to methane. The last order, *Methanosarcinales*, has a larger physiological diversity, and with the exception mentioned above all methanogens capable of acetoclastic and/or methylotrophic methanogenesis are members of this order. Some members are versatile and can grow on a range of substrates, e.g. some *Methanosarcina* strains can use all metabolic pathways described above. Other members are specialists, such as all described members of the genus *Methanosaeta*, which can only grow on acetate, but can utilize very low acetate concentrations. In nature, acetate and H₂/CO₂ (or formate) are considered the most important substrates for methanogenesis, and normally contribute to > 67% and < 33% to the methane production, respectively (Conrad, 1999; Zinder, 1993). Detailed descriptions of the phylogeny, taxonomy, and physiology of methanogenic archaea can be found in Bergey's manual of Systematic Bacteriology (Garrity and Bergey, 2001) and in The Prokaryotes (Dworkin, 2005).

3.8 Methanogenic archaea in northern wetlands

Methanogenic populations in wetland soils have been studied by molecular methods only in the last decade, irrespective of climate regions (Galand *et al.*, 2003; Horn *et al.*, 2003; Galand *et al.*, 2002; Edwards *et al.*, 1998; Grosskopf *et al.*, 1998b; Kudo *et al.*, 1997). This approach has greatly facilitated the study of this functional group in northern wetlands, since they are relatively difficult to cultivate (see below). The molecular methods used to study methanogenic archaea in northern wetlands include PCR, cloning, community profiling methods such as terminal restriction fragment length polymorphism (T-RFLP) and DGGE, sequencing, oligonucleotide probing and FISH. The different methods vary in their level of resolution and in the number of samples that can be conveniently processed and compared. Two major molecular

markers have been used in the analyses: 16S rRNA and the corresponding gene, and the functional gene *mcrA*. The ribosomal sequences have the advantage that a high number of sequences are available in public databases, and the gene contains both conserved and variable regions. The gene *mcrA* encodes the α subunit of methyl coenzyme M reductase, a key enzyme for methanogenesis. This functional gene is present in all methanogenic archaea and is unique to this functional group; hence the detection of this gene demonstrates the presence of methanogenic archaea. However, until recently public databases contained only a limited number of *mcrA* sequences, and this previously restricted the usefulness of this approach.

Molecular studies of archaeal communities in boreal and temperate peatlands have repeatedly demonstrated the presence of sequences affiliated with the order *Methanomicrobiales*, and the families *Methanosarcinaceae*, *Methanosaetaceae*, and *Methanobacteriaceae*, although not all groups were detected in all studied samples (Merilä *et al.*, 2006; Galand *et al.*, 2005b; Juottonen *et al.*, 2005; Metje and Frenzel, 2005; Kotsyurbenko *et al.*, 2004; Basiliko *et al.*, 2003; Galand *et al.*, 2003; Utsumi *et al.*, 2003). In addition, the family *Methanococcaceae* was detected in studies of two bogs in the UK (Upton *et al.*, 2000; McDonald *et al.*, 1999). Other archaeal sequences that are affiliated with clusters that currently contain no cultured members have also repeatedly been detected. The phylogeny and diversity of the archaeal communities detected in previous studies is discussed in more detail in the general discussion.

Archaeal sequences recovered from cold and wet soils do not form separate clusters in phylogenetic trees. This is consistent with recent reports on psychrotolerant methanogens isolated from cold terrestrial environments (Simankova *et al.*, 2003). Some of them have nearly identical 16S rRNA gene and *mcrA* sequences to the mesophilic type strain but was classified as new ecotypes based on their temperature characteristics (see below). The fact that adaptation to low temperatures is seen in several methanogen groups indicate that the cold adaptation have been effective for groups with various phenotypic characteristics.

Culture based studies of these organisms have been hampered by their oxygen sensitivity, low growth rates at *in situ* temperatures and the often close association with syntrophic partners (Kotsyurbenko, 2005; Sizova *et al.*, 2003). The only

published methanogenic isolate from northern wetlands is a novel ecotype of *Methanosarcina mazei* isolated from weakly acidic (pH 6.1) tundra wetland soil in Polar Ural, Russia (Simankova *et al.*, 2003; Nozhevnikova *et al.*, 2001). The strain was better adapted to growth at low temperatures than previously characterized strains of this species, with a temperature range for growth from 5°C to 40°C. However, the temperature optimum was relatively high (35°C). The strain was metabolically versatile, and could grow on H₂/CO₂, methylamines and acetate. The ecological relevance of the isolated strain however has not yet been demonstrated (Simankova *et al.*, 2003). Two highly enriched methanogenic consortia have been isolated from an acidic Siberian Sphagnum peat bog (Sizova *et al.*, 2003). One of these consortia contained a *Methanobacterium* sp. as the only archaea, while the other contained a *Methanomicrobiales*-affiliated strain as a minor archaeal component and a strain affiliated with Rice Cluster I as the dominant archaeal component. Both enrichment cultures contained less than 10% of bacterial satellites.

4. GENERAL DISCUSSION

The studies included in this thesis have been discussed in detail in the corresponding papers (**papers I, II and III**). The aim of this general discussion is to present a synthesis of the results and discuss the overall trends in the datasets in relation to previous reports from both temperate and northern wetlands. In addition, some topics that were not addressed in the papers will be discussed.

4.1 Archaeal community composition in wet Spitsbergen soils

Methanogenic archaea in northern wetlands have been studied by molecular methods only in the last decade. The most commonly detected groups are *Methanomicrobiales*, *Methanobacteriaceae*, *Methanosarcinaceae* and *Methanosaetaceae* (Merilä *et al.*, 2006; Galand *et al.*, 2005b; Galand *et al.*, 2005a; Juottonen *et al.*, 2005; Metje and Frenzel, 2005; Kotsyurbenko *et al.*, 2004; Basiliko *et al.*, 2003; Galand *et al.*, 2003; Utsumi *et al.*, 2003; Galand *et al.*, 2002). Using 16S rRNA as a phylogenetic marker, representatives of all these groups were detected in Spitsbergen soils and sediments (**papers I and II**). A total of 8 different sequences affiliated with known methanogenic archaea were recovered from environmental samples (Fig. 3, red print). Two additional sequences were recovered only from enrichment cultures of Solvatnet peat (Fig. 3, green print) (see also section 4.2). These sequences showed 97-100% homology to sequences in the public databases. Nevertheless, the methanogenic archaea detected in Svalbard soils and sediments (**papers I, II and III**) could theoretically represent new ecotypes that are better adapted to growth at low temperatures, since psychroactive methanogenic archaea may have 16S rRNA gene sequences nearly identical to their close temperate relatives (Simankova *et al.*, 2003).

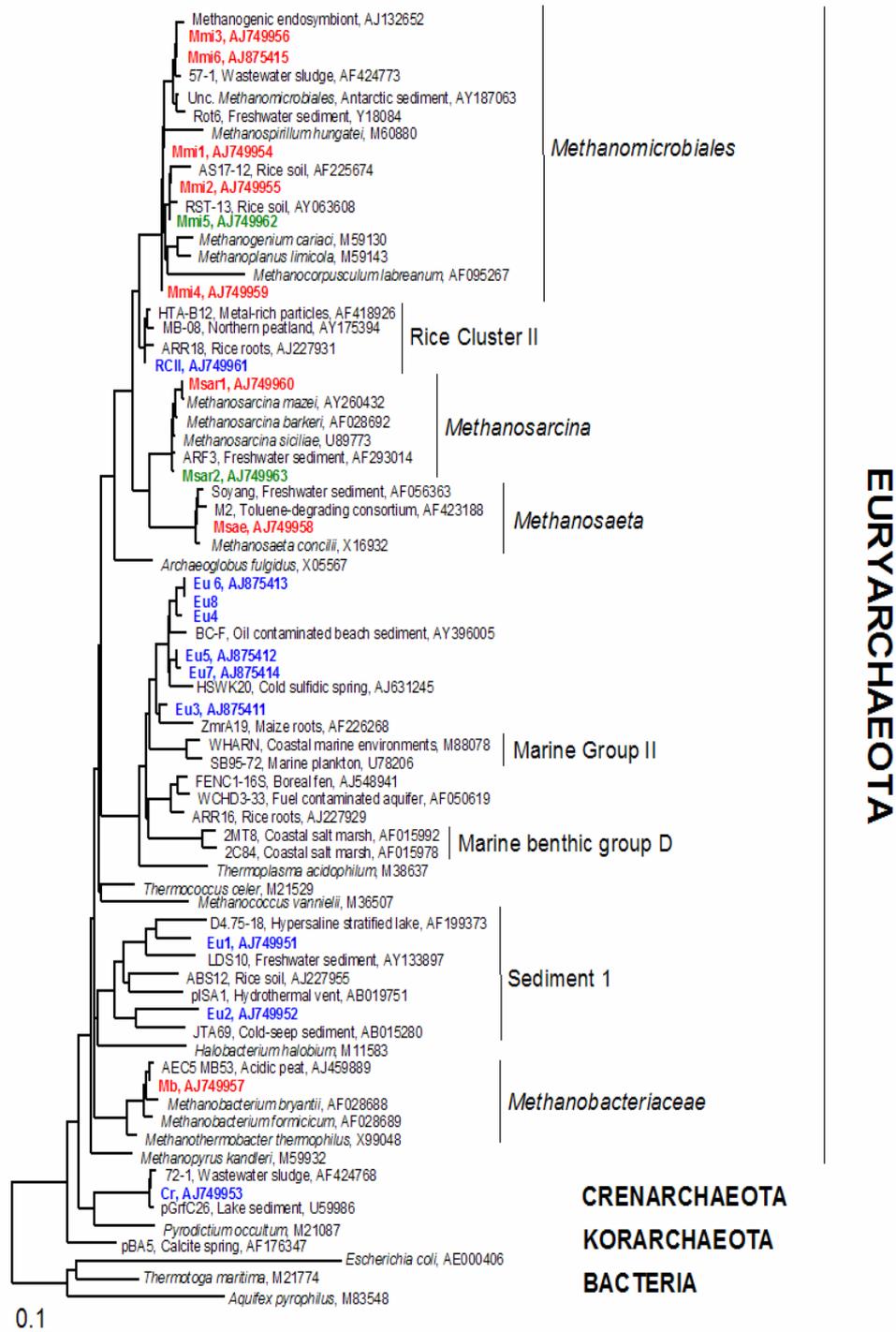


Fig. 3: Phylogenetic tree illustrating the affiliation of partial 16S rRNA gene sequences from reamplified DGGE bands. Phylogenetic labels as used in papers I, II and III are shown. Novel sequences and close relatives were added to a Maximum Likelihood tree based on nearly full-length sequences without 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).

While the identity and number of detectable groups were comparable to previous studies of wet soils and lake sediments, fewer closely related sequences were detected as compared to most cloning studies from northern wetlands (Kotsyurbenko *et al.*, 2004; Galand *et al.*, 2003). A cloning study of soil community DNA from the Svalbard soils and sediments would be required to determine whether the lower microheterogeneity was real or was caused by methodological bias. Methodological limitations that could potentially have contributed to an underestimation of diversity include the short (147-152 bp) sequences analysed, potential biases in the PCR-amplification, and similar melting behaviour of fragments with different sequences.

The phylogenetic analysis was restricted by the relatively short sequences recovered from reamplified DGGE-bands (Ludwig and Schleifer, 1994). However, the precision level for the assignment of each sequence to a phylogenetic group could be tested by constructing trees based on the amplified hypervariable V3-region of 16S rRNA only (**papers I and II**). A tree based on the V3-region with focus on the order *Methanosarcinales* showed that the genus *Methanosarcina* was clearly separated from other genera in the family *Methanosarcinaceae*, and the new sequences Msar1, Msar2 clustered with sequences in this genus. The new sequence Msae clustered tightly with sequences in the genus *Methanosaeta*, which is the only recognized genus in the family *Methanoseataceae*. Hence, the phylogenetic affiliation for the sequences Msar1, Msar2 and Msae could be determined to the genus-level. A tree with focus on *Methanobacteriales* showed that the V3 region could not be used to resolve genera in the family *Methanobacteriaceae*. Hence, the phylogenetic affiliation of the sequence Mb could only be determined to the family level. Sequences affiliated with the order *Methanomicrobiales* (Mmi1-Mmi6) could not be more precisely allocated since they did not cluster closely with sequences in the currently recognized families.

Two *Methanomicrobiales*-affiliated sequences (Mmi3, Mmi6) had high homology to sequences from endosymbionts of anaerobic ciliates. Closely related sequences have previously been detected in peat bog (Hales *et al.*, 1996), rice soil (Grosskopf *et al.*, 1998b) and lake sediment (Zepp Falz *et al.*, 1999). It is however unclear whether these environmental sequences originated in endosymbiotic or free-living methanogens. Recently, Schwarz and Frenzel (2003, 2005) demonstrated the presence of anaerobic ciliates with methanogenic endosymbionts in rice soil, and showed that

the endosymbiotic methanogens contribute significantly to the methane production during the first days of flooding of rice paddies. To the best of our knowledge, there exists currently no evidence that endosymbiotic methanogens are present in Arctic wetlands. There are however reports that there is a large diversity and high numbers of terrestrial protozoa both at Antarctic and Arctic sites (Schmidt, 1999; Smith, 1972). Endosymbiotic methanogens would not be exposed to competition from bacteria using alternative electron acceptors, and would have a steady supply of hydrogen from the hydrogenosomes, despite low temperatures. Therefore, the possibility that such a life strategy could be important in Arctic wetlands deserves further study.

In total, ten sequences clustered with groups that to date contain no cultured isolates (Fig. 3, blue print). One sequence (RCII) clustered within Rice Cluster II (Grosskopf *et al.*, 1998a). This cluster has also previously been detected in northern wetlands (Kotsyurbenko *et al.*, 2004; Basiliko *et al.*, 2003; Edwards *et al.*, 1998). No cultured members of this cluster exist, but its branching within the phylogenetic radiation of the *Methanosarcinales* and *Methanomicrobiales* indicates that it is a novel methanogenic group (Grosskopf *et al.*, 1998a). Two sequences clustered within a clade named Sediment 1 (Robertson *et al.*, 2005; Hugenholtz, 2002; Jurgens, 2002), which includes sequences in Rice Cluster V (Grosskopf *et al.*, 1998a) (**paper I**). This clade has also been detected in other northern wetlands (Kotsyurbenko *et al.*, 2004; Galand *et al.*, 2003). Since the clade Sediment 1 currently contains no cultured members, the physiological traits of the corresponding organisms are unknown. It should be noted that sequences in this cluster have a relatively large heterogeneity, and hence the hitherto detected sequences may represent only a small fraction of a large unknown phylogenetic diversity (**paper I**). Six sequences formed a cluster that was remotely related to sequences in Marine Group II (DeLong, 1992) and Marine Benthic Group D (Vetriani *et al.*, 1999; Munson *et al.*, 1997) and also to fen-cluster III (Galand *et al.*, 2003) (**paper II**). Hence, the new cluster belongs to the branch with the clusters Group II, Vadin, SAGMA S/T and Group III in the recent review by Schleper and coworkers (2005) (Schleper *et al.*, 2005). Again, the physiology of the corresponding organisms is currently unknown. One sequence was affiliated with Group 1.3b of uncultivated *Crenarchaeota* (Ochsenreiter *et al.*, 2003). Results from an initial survey of archaeal communities in Svalbard wet soils and sediments showed

that this sequence was present in a wide range of samples (Fig. 3 in **paper II**). The corresponding band was relatively strong in most DGGE-profiles when present, indicating that the corresponding organisms were numerically abundant in wet Arctic soils and sediments (**paper II**). This cluster has also previously been detected in northern wetlands (Kotsyurbenko *et al.*, 2004; Galand *et al.*, 2003; Utsumi *et al.*, 2003).

4.2 Culturability of methanogenic archaea from Spitsbergen soils

Enrichment cultures were made with peat from Solvatnet and Stuphallet. The aim was to evaluate whether methanogenic archaea that were detected directly in peat could be cultivated in the laboratory, and whether additional methanogenic archaea could be detected by cultivation (**paper I**). Enrichment cultures were made with the carbon sources carbonate, formate, acetate, methanol, and trimethylamine (TMA), with H₂/CO₂ (80:20 v/v) or N₂/CO₂ (80:20 v/v) as the headspace gas. DGGE-profiling indicated that most methanogenic archaea that were detected directly in peat community DNA could grow in enrichment cultures at 10°C. Only two novel sequences were recovered from analyses of the enrichment cultures, and both of these sequences had only one mismatch with sequences recovered from peat community DNA analyses (Fig. 3, green print). However, the *Methanosarcina* strain detected in culture could not be detected in peat community DNA, and *vice versa*. This *Methanosarcina* strain became the dominant methanogen after several transfers. The results indicate that culture-based methods may provide additional information on the archaeal community composition present *in situ*. In addition, the data show that culture-based studies can introduce bias relative to the *in situ* situation, and comparison with the *in situ* communities is necessary in order to make ecologically relevant conclusions based on such studies (**paper I**).

It is difficult to isolate methanogenic archaea from cold terrestrial systems due to their slow growth rates and high sensitivity to oxygen. Nevertheless, we attempted to isolate methanogenic archaea from the enrichment cultures using the agar dilution method as described by Widdel and Pfennig (1984) and by Ljungdahl and Wiegel (1986). Colonies picked from agar dilution tubes were used to inoculate liquid medium. In this way, highly enriched cultures, but no pure cultures were obtained.

One of the cultures resulted in only one band in the archaeal DGGE profile, and the resulting sequence was identical to Msar2 recovered from the original enrichment cultures. Microscopy showed that single cells with a strong F₄₂₀ autofluorescence dominated this culture. The only published methanogenic isolate from Arctic tundra is a novel ecotype of *Methanosarcina mazei* isolated from weakly acidic (pH 6.1) tundra wetland soil in Polar Ural, Russia (Simankova *et al.*, 2003; Nozhevnikova *et al.*, 2001). The isolation process of this strain lasted five years (Nozhevnikova *et al.*, 2001), demonstrating the difficulties associated with isolation of methanogenic archaea from cold, terrestrial habitats. The ecological relevance of this isolate is unknown (Simankova *et al.*, 2003).

4.3 Effects of soil water regime on archaeal community composition

Many process-related studies have shown that methane emissions are correlated with soil moisture or water table levels (Bubier, 1995; Bubier *et al.*, 1993), and it has been suggested that this parameter can act as an on-off switch for methane emissions on the ecosystem scale (Christensen *et al.*, 2003). However, it was not known whether this effect was caused by changes in the overall activity of methanogenic and methanotrophic populations only, or whether differences in the microbial community structures were involved (**paper II**). The variation in archaeal community composition associated with water regime in Spitsbergen soils and sediments was predominant over variation between different types of continuously wet environments such as peatlands, riparian soils and lake sediments (**paper II**). Methanogenic archaea and Group 1.3b of *Crenarchaeota* were detected in continuously wet environments, while sequences affiliated with a novel cluster of *Euryarchaeota* were only detected in soils that dried out during the summer season. It should be noted that a nested PCR protocol with high sensitivity was used, and the results show that methanogenic archaea were not abundant in the tested mesic soils.

These results are consistent with data from river floodplains (Kemnitz *et al.*, 2004), where methanogenic archaea were detected only in soils from frequently and permanently flooded sites. In contrast, good survival of methanogenic archaea during dry periods have been demonstrated for rice field soils, where onset of

methanogenesis after flooding is limited by substrate availability rather than by biomass of methanogenic archaea (Mayer and Conrad, 1990). Our data suggest that during shorter periods with water logging such as during spring-melt, methane production from Arctic soils could currently be limited by low biomass of methanogenic archaea (**paper II**). We suggest that the reason why populations of methanogenic archaea are not sustainable in the tested soils with intermediate to good draining is the low growth rate of methanogens during spring melt and early summer when these soils are wet. In addition, the formation of syntrophic aggregates may be necessary at low temperatures to facilitate diffusional transfer of H₂, and this could also take a long time at low temperatures (Kotsyurbenko, 2005). The situation may change if climate change leads to increased soil temperatures (**papers II and III**). Since the adaptation to low temperature seems to vary significantly between different wetlands (**paper III**, see section 4.5), it is possible that also the water regime conditions that can support methanogenic populations may differ between soil types.

Studies of a natural soil moisture gradient (**paper II**) indicated that members of *Methanomicrobiales* were relatively tolerant to soil aeration. The result suggest that the detected members of *Methanomicrobiales* were more tolerant to less reduced conditions, or alternatively that they were relatively better competitors for substrate under such conditions. A relatively high dominance of *Methanomicrobiales*-affiliated sequences in drier soils was previously demonstrated for a boreal Finnish fen, where sequences affiliated with *Methanomicrobiales* were dominant in upper layers of hummocks (Galand *et al.*, 2003).

4.4 Distribution of methanogenic populations in space and time

Knowledge of the *in situ* distribution of methanogenic populations in space and time is important for ecological interpretation of results from controlled systems, for modelling and upscaling efforts, and for making predictions of community responses to climate change. The papers included in this thesis include analyses of differences between sites (**papers I and II**), differences with soil depth (**papers I and II**), and seasonal differences (**papers I, II**).

4.4.1 Variation between sites

In this section the observed variation in archaeal community composition between sites is discussed. Site-specific differences in seasonal trends and in trends with depth are discussed below.

The archaeal community composition differed between individual wet sites (**papers I and II**). This is consistent with several recent studies of methanogenic communities in northern wetlands, which have demonstrated site-specific differences in the archaeal communities (Merilä *et al.*, 2006; Galand *et al.*, 2005b; Juottonen *et al.*, 2005; Kotsyurbenko *et al.*, 2004; Basiliko *et al.*, 2003; Galand *et al.*, 2003). Most notably, we observed differences in the detection of methanogenic archaea with potential or acetoclastic methanogenesis, i.e. *Methanosaeta* and *Methanosarcina*, between sites.

A few recent studies have addressed the relative contribution of the major methanogenic pathways and the archaeal community structure in the same peat samples (Galand *et al.*, 2005b; Metje and Frenzel, 2005; Kotsyurbenko *et al.*, 2004). In all cases the distribution of methanogenic populations was found to be consistent with trends in the relative contribution of acetoclastic methanogenesis (see also section 3.6). We detected methanogenic archaea with potential for acetoclastic methanogenesis in wetland and lake sediments at the Brøgger Peninsula (Solvatnet and Stuphallet), and also in the lake sediment from the Sassen Valley. In accordance with the literature, this suggests that the contribution of acetoclastic methanogenesis could be relatively high at these sites. In contrast, the methanogenic archaea detected in peat from the Sassen Valley, and in soils from the Advent Valley, including fluvial deposits and soils collected along a natural soil moisture gradient (**paper II**), all belonged to groups that are only capable of hydrogenotrophic methanogenesis. This suggests that acetoclastic methanogenesis may be of less importance at those sites (< 67%).

Along the soil moisture gradient the soil pH was relatively low (4.0 –5.1 in CaCl₂). Hence, the inability to detect methanogenic archaea capable of acetoclastic methanogenesis at these sites, is consistent with the notion that soils with low pH

favour hydrogenotrophic methanogenesis (Kotsyurbenko, 2005; Brauer *et al.*, 2004; Horn *et al.*, 2003). However, some recent studies have shown that acetoclastic methanogenesis may be important in some acidic peat bogs (Kotsyurbenko *et al.*, 2004), so the effect of low pH on the relative contribution of hydrogenotrophic methanogenesis could vary between systems. The inability to detect methanogenic archaea with potential for acetoclastic methanogenesis in peat from the Sassen Valley and in mineral soils from the Advent Valley is currently not easily explained, but it could potentially be related to the availability of labile organic carbon (Hornibrook *et al.*, 2000; Schoell, 1988). Further studies are needed to elucidate the mechanisms behind variations in the relative contribution of hydrogenotrophic methanogenesis in northern wetlands.

4.4.2 Variation with soil depth

Depth-related differences in the archaeal community composition were observed only at sites with significant trends in the soil physicochemical conditions with depth. Soil depth affected the archaeal community composition at Solvatnet (**paper I**) and at the driest (A) and the intermediate (B) positions along the soil moisture gradient (**paper II**). At these sites the soil cores had visible layering, and clear trends with depth in the physicochemical conditions were demonstrated. In contrast, at Stuphallet and at the wettest position (C) along the gradient, there was no visible layering of the soil core, and there were no clear trends with depth in the archaeal community composition (**papers I and II**). This is consistent with a recent study by Merilä and coworkers (2006), where depth-related differences were detected only for the bog site where the peat layers differed more from each other (Merilä *et al.*, 2006). We suggest that the depth-related variation in archaeal community composition is determined primarily by depth-profiles of physicochemical parameters such as the soil aeration, the redox potential and the availability of methanogenic substrates.

In general, microbial communities in upper soil layers are more exposed to seasonal changes and site-related differences than communities in lower layers where the physicochemical conditions are more homogenous. In accordance with this, the archaeal community composition varied more in upper than in lower soil along the natural soil moisture gradient, (**paper II**). This was correlated with the soil aeration, which varied along the transect for the upper soil layer while the lower soil layers

were water saturated at all positions (see section 4.3). This is consistent with results from a Finnish boreal fen, where the spatial heterogeneity was larger in upper soil layers as compared to at larger depths (Galand *et al.*, 2003).

4.4.3 Seasonal variability

Seasonal trends in the archaeal community composition were addressed for field samples from the peatlands at Solvatnet and Stuphallet (**paper I**) and for field samples taken along a natural soil moisture gradient (**paper II**).

Clear seasonal trends were seen for the site Solvatnet, where changes in populations of *Methanosarcina*, *Methanobacteriaceae* and *Methanosaeta* were observed (**paper I**). Canonical correspondence analysis (CCA) showed that the detection of a DGGE-band affiliated with *Methanosarcina* (Msar1) was correlated with the measured CO₂-emissions, suggesting that the relative dominance of this population was related to the general microbial activity and hence the availability of substrates (**paper I**). The seasonal and depth-related trends in the detection of this band was consistent with previously reported trends in dissolved organic carbon (Wagner *et al.*, 2003), acetate, and the contribution of acetoclastic methanogenesis (Avery *et al.*, 1999) (**paper I**). A slurry experiment with peat from Solvatnet suggested that detection of the *Methanosarcina* population was correlated with changes in substrate availability (**paper III**). However, the experiment indicated that the detected population utilized other substrates than acetate, at least in some slurries (**paper III**). Further studies are needed to elucidate the substrate use of this population.

DGGE profiles of Solvatnet field samples showed that the two bands affiliated with *Methanobacteriaceae* (Mb) and *Methanosaeta* (Msae) were of similar strength in the upper and lower soil layers in July both years, while later in the summer season the relative intensity of *Methanobacteriaceae* increased in upper soil layers, and the relative intensity of *Methanosaeta* increased in lower soil layers (**paper I**). In the slurry experiment the relative intensity of the DGGE-band Msae increased with time. This was consistent with increasing consumption of acetate, but could also be related to that the fact that the slow-growing *Methanosaeta* needed time to become detectable (**paper III**). The band affiliated with *Methanobacteriaceae* however, was relatively

strong in the DGGE profiles of all the slurries, so this experiment provided no new information on how the dynamics of this population is controlled (**paper III**).

At Stuphallet there was a high variability in the DGGE-profiles, and the eight studied samples produced no identical profiles (**paper I**). The archaeal community at Stuphallet was therefore possibly less stable than at the other sites, and hence more sampling dates should have been applied to detect any trends. In contrast to the immediate onset of methane production in slurries with Solvatnet soil (**paper III**), slurries with soil from Stuphallet showed an initial lag phase for methane accumulation that was seen also on a logarithmic scale (Rolf Arnt Olsen, unpublished data). The results are therefore consistent with a study of riparian soils, which demonstrated that archaeal groups were more dynamic during incubation of soils where methane production is preceded by a lag phase (Kemnitz *et al.*, 2004). Moreover, studies have shown that methanogenic bioreactors with a high percentage of *Methanomicrobiales*- and *Methanosaeta*-affiliated 16S rRNA genes have lower microbial community structure replicability as compared to reactors with a high percentage of *Methanosarcina*-affiliated 16S rRNA genes, despite being more functionally stable (Fernandez *et al.*, 2000). These studies therefore support the notion that the observed high variability in the archaeal community at Stuphallet was real, and suggest that the dominating degradation processes may vary between Solvatnet and Stuphallet (Fernandez *et al.*, 2000).

At points along the soil moisture gradient no clear seasonal trends were observed (**paper II**). This is consistent with a recently published field study from rice paddy soil, where the community composition was stable throughout the season despite significant changes in both total methanogenesis and the contribution from acetoclastic methanogenesis (Krüger *et al.*, 2005).

4.5 Effects of temperature on archaeal community structure and methane production

Several studies have addressed the effects of temperature on methane production and methane oxidation in northern wetland soils (Kotsyurbenko *et al.*, 2004; Wagner *et al.*, 2003; Dunfield *et al.*, 1993; Svensson, 1984). However, until recently (Metje and

Frenzel, 2005) there was no information on the effects of soil temperature on the archaeal community structure in peat from northern wetlands. We therefore performed a controlled laboratory experiment where the effects of temperature on archaeal community structure, methane production, and fatty acid accumulation were addressed (**paper III**). During the first part of the experiment no volatile fatty acids (VFA) were detected, showing that the process under study was degradation of biopolymers rather than labile organic carbon (**paper III**). It should be noted that the situation in the field would differ from this, since freeze-thaw cycles during spring thaw and actively growing vegetation would affect the substrate supply. However, the DGGE-fingerprints of archaeal communities in the slurries were similar or identical to DGGE-fingerprints of communities in field samples (**paper I**), and hence it can be assumed that the observed temperature effects are ecologically relevant (**paper III**).

The slurry experiment demonstrated that the overall process of methanogenic degradation of biopolymers in Solvatnet peat at temperatures $\geq 10^{\circ}\text{C}$ was poorly adapted to low temperatures (**paper III**). The temperature response (Q_{10} value) for the linear phase of methane accumulation was 14.7 for the interval 10-20 $^{\circ}\text{C}$, and the corresponding Ratkowsky plot (Ratkowsky *et al.*, 1983) gave an apparent minimum temperature for the overall process of 6.5 $^{\circ}\text{C}$. The methane accumulation at 1 $^{\circ}\text{C}$ and 5 $^{\circ}\text{C}$ corresponded to less than 0.5% of the methane accumulation at 25 $^{\circ}\text{C}$. At 10 $^{\circ}\text{C}$ the temporal development in the slurry appeared to be delayed as compared to the higher temperatures, and at the end of the experiment the CO_2/CH_4 ratio and the fatty acid accumulation pattern was similar to the conditions in the beginning of the experiment in slurries incubated at the higher temperatures (**paper III**).

FISH analysis of microbial communities in the Solvatnet peat slurries in week 4 showed that different archaeal communities were active at temperatures below and above 10 $^{\circ}\text{C}$. Changes were seen in the cell morphologies, in the abundance of active archaeal cells, and in the ability to detect groups of methanogenic archaea using specific probes (MSMX860 and MB310, see below) (**paper III**). At 1 $^{\circ}\text{C}$ and 5 $^{\circ}\text{C}$, the archaeal probe ARCH915 detected a high fraction (11-12%) of the total prokaryotic community, while no cells were detected with the group specific methanogen probes. Such high archaeal fractions have previously been detected by FISH in Antarctic waters, and this suggests that archaeal communities may be more abundant in low

temperature environments. No archaeal populations were detected only at low temperatures by DGGE, however. This suggested that archaeal populations that were abundant at low temperatures were not detected by the primer sets used for the DGGE-analysis (**paper III**). Highly structured sphere shaped cell aggregates were detected with archaeal probes, including MSMX860 and MB310, at temperatures $\geq 10^{\circ}\text{C}$ (**paper III**). The organized structure suggests that the aggregates could consist of cells living in syntrophic associations. The inability to detect cells with the probes MSMX860 and MB310 at lower temperatures was most likely related to their low activity level. In week 4, the methane production was not yet visible on the linear scale at 1°C and 5°C .

In combination with the process-related data, the DGGE-profiles suggested that the dynamics of most populations of methanogenic archaea was primarily controlled by the availability of substrates for methanogenesis (**paper III**). The temporal development of the archaeal community appeared to be delayed at the low temperatures, in accordance with the effect of temperature on the process-related parameters. The results suggested that the growth and activity of most populations of methanogenic archaea were restricted by substrate availability rather than by direct temperature effects. This is consistent with field data, where significant methane emissions were recorded at days where the soil temperatures were below 5°C (**paper I**). Based on these observations we present the hypothesis that field emissions of methane from Solvatnet are probably primarily due to a continuous supply of easily degradable substrates rather than to the degradation of complex biopolymers. This is consistent with a recent study by Yavitt and Seidmann-Zager (2006), which suggested that methanogenic conditions in northern peat soils rely on a constant supply of easily degradable metabolic substrates. It is also consistent with previous studies that have shown that methane production is primarily substrate dependent (Wagner *et al.*, 2003), and that methane production and archaeal populations in peat soils are specifically associated with fresh organic material (Wachinger *et al.*, 2000).

No methanogenic populations were detected only in the DGGE profiles from slurries incubated at high temperatures. This indicates that populations present *in situ* could compete relatively well in the slurries even at temperatures well above current soil temperatures in the Arctic. In addition, the specific methane production (per cell

activity) for the general population of methanogenic archaea was highest at 25°C (**paper III**). This shows that the temperature optimum for activity was relatively high compared to the soil temperatures in the field. These results are consistent with previous functional studies with uncharacterized soil slurries which have demonstrated temperature optima in the range 20°C – 28°C (Dunfield *et al.*, 1993; Svensson, 1984). However, preliminary studies of enrichment cultures with peat from the Advent Valley (wettest point of gradient (C), **paper II**) showed that the addition of substrates (H₂/CO₂, acetate) caused growth of populations not detectable in the inoculum peat, especially at higher incubation temperatures (15°C and 20°C) (Marte Rusten, personal communication). Hence, if climate change causes changes also in the substrate availability through for instance changes in vegetation, this can cause alterations in the predominant populations of methanogenic archaea.

The effect of temperature on the temporal development of methane accumulation phases (Fey *et al.*, 2004; van Hulzen *et al.*, 1999, **paper III**), the accumulation pattern of volatile fatty acids (**paper III**, Fey *et al.*, 2004) and the development of the archaeal community (**paper III**), suggests that more attention should be given to the field conditions present in early spring. For instance, it is uncertain whether the conditions present at the start of the Solvatnet slurry experiment (**paper III**), when none of the measured fatty acids could be detected, deviates substantially from the situation present in the field in early spring after the soil has been exposed to freeze-thaw cycles. Better knowledge of the situation in field samples in early spring could aid in predicting the effect of a temperature increase during the ecologically relevant time frame of an Arctic summer season. In addition, further studies should focus on the availability of methanogenic substrates throughout the summer season.

The poor temperature adaptation revealed for methanogenic degradation in the wetland at Solvatnet (**paper III**) was in contrast to a recent report from a northern acidic mire where methane production at 4°C corresponded to 10% of the methane production at 25°C and the theoretical minimum temperature was estimated to -5°C (Metje and Frenzel, 2005). The variation in temperature response between these two sites is consistent with previous functional studies which have demonstrated relatively large variations in the temperature response (Q₁₀) between different peat types (Bergman *et al.*, 2000; Bergman *et al.*, 1998). Bergman *et al.* (2000) showed that

within an acidic mixed mire (pH 3.5-4.5) there were differences between plant communities in the seasonal average temperature response (Q_{10}) for methane production. These differences were primarily caused by differences in organic matter quality, which depended on the presence of vascular plants and the time period when the organic material was exposed to oxic conditions before reaching the anaerobic zone. The temperature response also varied over the season. The authors argued that this might be explained by seasonal variation in the supply of substrates and in some cases also by changes in the active anaerobic microbial community (Bergman *et al.*, 2000). It is currently uncertain whether the variation related to the microbial community was correlated with changes in degradation pathways and archaeal community composition, or whether the effect was exclusively a function of differences in abundance of microbial populations and their activity.

5. CONCLUSIONS

The range of archaeal groups detected in Spitsbergen soils was consistent with previous studies of wet soils and sediments, both from temperate and northern regions. The recovered sequences were affiliated with *Methanomicrobiales*, *Methanobacteriaceae*, *Methanosarcina*, *Methanosaeta*, Rice Cluster II, Sediment 1, a new euryarchaeotal cluster and Group 1.3b of *Crenarchaeota*. In addition, FISH analysis of soil slurries revealed a large archaeal community at low temperatures (1°C and 5°C) which is currently unknown.

Studies of enrichment cultures with peat from Solvatnet and Stuphallet demonstrated that most populations detected by PCR-DGGE in field samples could grow in enrichment cultures at 10°C. Two populations were detected only in enrichment cultures, one was affiliated with *Methanomicrobiales* and the other with *Methanosarcina*. Interestingly, the studies showed that another *Methanosarcina* strain was favoured in culture than the one detected in field samples. This strain was relatively fast-growing and became the dominant methanogen in later enrichment steps. This demonstrates that culture based studies should include monitoring of the methanogenic populations to evaluate whether the results are ecologically relevant. Attempts to isolate methanogenic archaea were not successful, but highly enriched cultures of the *Methanosarcina* strain were obtained.

Soil water regime was found to be critical for the presence of abundant methanogenic populations in Spitsbergen soils. The variability associated with soil water regime was predominant over variability between different wet sites and seasonal variations. This partly explains earlier observations that soil hydrology is an important controlling factor for seasonal methane emissions (Christensen *et al.*, 2003; Bubier, 1995; Bubier *et al.*, 1993). The results emphasize that knowledge on the effect of a climate change on the distribution of wet soils is of utmost importance for predicting the effects of climate change on methane emissions from northern wetlands.

Differences in the archaeal community composition between wet sites were demonstrated. Especially, differences in the occurrence of methanogens capable of acetoclastic methanogenesis (*Methanosaeta*, *Methanosarcina*) were conspicuous. Seasonal changes in the archaeal community composition were detected at some sites, while other sites had more stable archaeal communities. Depth related changes were observed only at sites with significant changes in the physicochemical conditions between the tested soil depths.

The overall process of methanogenic degradation of biopolymers in Solvatnet peat operating at temperatures $\geq 10^{\circ}\text{C}$ 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 $^{\circ}\text{C}$, and the corresponding Ratkowsky plot gave an apparent minimum temperature of 6.5 $^{\circ}\text{C}$. The methane accumulating at 1 $^{\circ}\text{C}$ and 5 $^{\circ}\text{C}$ corresponded to less than 0.5% of the methane accumulating at 25 $^{\circ}\text{C}$. Temperature affected the temporal development in the slurries as seen for the transition between methane accumulation phases, the CO_2/CH_4 ratio, the accumulation of volatile fatty acids and the development of the archaeal communities. At 5 $^{\circ}\text{C}$ and 10 $^{\circ}\text{C}$ the accumulation of isobutyrate was conspicuous. FISH analysis revealed a large (10-12% of the total count), unidentified, active archaeal community at low temperatures (1 $^{\circ}\text{C}$ and 5 $^{\circ}\text{C}$). DGGE-profiling showed that populations of methanogenic archaea could grow at all tested temperatures, and indicated that their dynamics was primarily controlled by substrate availability rather than by direct temperature effects. The results showed that at Solvatnet, field emissions of methane at low temperatures are probably due to a continuous supply of easily degradable substrates rather than the degradation of complex organic polymers. The results suggested that more attention should be given to the supply of methanogenic substrates and to the field conditions present in early spring. The differences seen between the temperature effects on the methane production in Solvatnet peat and acidic mire from northern Finland (Metje and Frenzel, 2005) demonstrates that the temperature response may vary between sites. Such differences can in part be related to differences in the dominant degradation pathways and the archaeal community structure.

6. FUTURE PERSPECTIVES

Based on available data, a few sites that vary in pH and in dominating vegetation types should be selected for further studies. The future investigations should follow three main paths:

- 1) Studies targeting the dynamics and activity of individual populations in combination with detailed analyses of the microbial processes. RNA-based analyses and possibly stable isotope probing should be used for identification of active populations, in combination with T-RFLP, DGGE and real-time PCR for tracking the dynamics of individual populations. The studies should also include the use of inhibitors and radioactive tracers, and measurements of metabolic intermediates. The overall aim of these studies should be to thoroughly understand how the microbial community involved in methane production at a few selected sites is controlled.
- 2) Studies aiming to determine whether archaeal communities of different composition also differ in their methane production potentials and in their response to changes in different environmental parameters such as addition of substrate, drying, changes in temperature, and freeze-thaw cycles. The studies should include samples collected at different times of the year, including early spring.
- 3) If it is confirmed that soils with different archaeal community structures differ in their methane production potentials and response to environmental changes, efforts should be made to classify archaeal community types which respond differently to environmental changes. If possible, these archaeal community types should be linked to more easily measured variables such as vegetation type, primary production, water table levels and seasonal soil temperatures. If the black box containing the microbial community can be closed again in this way, then the information on the archaeal community can be implemented in upscaling and modelling efforts

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