Archaea, *Bacteria*, and methane production along environmental gradients in fens and bogs

Heli Juottonen

General Microbiology
Department of Biological and Environmental Sciences
Faculty of Biosciences
and
Viikki Graduate School in Molecular Biosciences
University of Helsinki

Academic Dissertation in General Microbiology

To be presented, with the permission of the Faculty of Biosciences of the University of Helsinki, for public criticism in the auditorium 2041 at Viikki Biocenter (Viikinkaari 5, Helsinki) on June 18th, 2008, at 12 noon.

Helsinki 2008

Supervisor Docent Kim Yrjälä
General Microbiology
Department of Biological and Environmental Sciences
University of Helsinki, Finland

Reviewers Professor Pertti Martikainen
Department of Environmental Sciences
University of Kuopio, Finland

Academy Professor Kaarina Sivonen
Department of Applied Chemistry and Microbiology
University of Helsinki, Finland

Opponent Professor Vigdis Torsvik
Department of Biology
University of Bergen, Norway

ISSN 1795-7079
ISBN 978-952-10-4727-5 (paperback)
ISBN 978-952-10-4728-2 (PDF, http://ethesis.helsinki.fi)

Yliopistopaino, Helsinki 2008

Front cover picture: *Eriophorum* on a mire in Persböle, Pohja, Finland
(photo by Hannu Juottonen)

Contents

List of original publications

This thesis is based on the following articles, which are referred to in the text by their Roman numerals. In addition, some unpublished data is presented.

I Galand PE, Juottonen H, Fritze H, Yrjälä K. (2005) Methanogen communities in a drained bog: effect of ash fertilization. *Microbial Ecology* 49: 209-217.

II Juottonen H, Galand PE, Yrjälä K. (2006) Detection of methanogenic *Archaea* in peat: comparison of PCR primers targeting the *mcrA* gene. *Research in Microbiology* 157: 914-921.

III Juottonen H, Galand PE, Tuittila ES, Laine J, Fritze H, Yrjälä K. (2005) Methanogen communities and *Bacteria* along an ecohydrological gradient in a northern raised bog complex. *Environmental Microbiology* 7: 1547-1557.

IV Juottonen H, Tuittila ES, Juutinen S, Fritze H, Yrjälä K. Seasonality of 16S rDNA- and rRNA-derived archaeal communities and methanogenic potential in a boreal mire. Submitted to *The ISME Journal*.

Summary

Methanogens are anaerobic *Archaea* with unique energy metabolism resulting in production of methane (CH_4). In the atmosphere CH_4 is an effective greenhouse gas. The largest natural sources of atmospheric CH_4 are wetlands, including peat-forming mires. Methane emissions vary greatly between and within mires, depending on season and hydrological and botanical characteristics. The aim of this work was to elucidate the microbiology underlying the variation.

Methanogens and potential CH_4 production were assessed along spatial and temporal gradients of ecohydrology, season, ash fertilization, and peat depth in three Finnish boreal mires. Non-methanogenic *Archaea* and *Bacteria* were additionally addressed as potential substrate producers and competitors to methanogens. Characterization of microbial communities targeted the *mcrA* gene, essential in CH_4 production, and archaeal or bacterial 16S ribosomal RNA gene. The communities were differentiated by analysis of clone libraries, denaturing gradient gel electrophoresis (DGGE), and terminal restriction fragment length polymorphism (T-RFLP) fingerprinting.

Methanogen communities and CH_4 production changed markedly along an ecohydrological gradient from fen to bog, with changing vegetation and pH. The most acidic *Sphagnum* bog showed mainly *Methanomicrobiales*-associated, hydrogenotrophic Fen cluster methanogens, whereas the oligotrophic and mesotrophic fens with higher pH and sedge coverage had more diverse communities including acetoclastic methanogens. Season had a minor effect on the archaeal community in an acidic oligotrophic fen, but the temporal variation of CH_4 production potential was substantial. Winter potential was unexpectedly high, and active methanogens were detected in winter peat. Ash fertilization, a forestry practice for promoting tree growth, had no substantial effects on CH_4 production or methanogen communities in a drained bog, but the communities changed with peat depth. Comparison of three *mcrA* primer sets revealed that their coverage of methanogens from the drained bog was similar, but the quantitative representations of communities were primer-dependent. Bacterial and non-methanogenic archaeal groups detected in mires included *Deltaproteobacteria*, *Acidobacteria*, *Verrucomicrobia*, and *Crenarchaeota* of groups 1.1c and 1.3. Their detection forms a starting point for further studies to distinguish possible interactions with methanogens.

Overall, the results indicate that methanogen community composition reflects chemical or botanical gradients that affect CH_4 production, such as mire hydrology. Predictions of CH_4 production in the spatially heterogeneous mires could thus benefit from characterization of methanogens and their ecophysiology.

Abbreviations

aa	amino acid(s)
ANME	anaerobic methane-oxidizing *Archaea*
ANOSIM	analysis of similarity
ANOVA	analysis of variance
bp	base pair(s)
DGGE	denaturing gradient gel electrophoresis
FC	Fen cluster; a group of methanogens named after detection in a fen
FISH	fluorescence *in situ* hybridization
gdw	grams dry weight
MCR	methyl-coenzyme M reductase
mcrA	gene encoding methyl-coenzyme M reductase I α-subunit
mRNA	messenger ribonucleic acid
mrtA	gene encoding methyl-coenzyme M reductase II α-subunit
OTU	operational taxonomic unit
PCA	principal component analysis
PCR	polymerase chain reaction
ppb	parts per billion
qPCR	quantitative polymerase chain reaction
RC	Rice cluster; group of *Archaea* named after detection in rice field soil
RDA	redundancy analysis
RFLP	restriction fragment length polymorphism
rRNA	ribosomal ribonucleic acid
spp.	species
SSCP	single-strand conformation polymorphism
TGGE	temperature gradient gel electrophoresis
T-RF	terminal restriction fragment
T-RFLP	terminal restriction fragment length polymorphism

1 Introduction

1.1 Methanogenic *Archaea*

Methanogens are anaerobic prokaryotes belonging to the domain *Archaea*, the third domain of life in addition to *Eucarya* and *Bacteria* (Woese *et al.* 1990). They are metabolically unique among *Archaea* and all other organisms due to their ability to obtain energy from selected low molecular weight carbon compounds and hydrogen with stoichiometric production of methane (CH_4).

In the classification of *Archaea* into two main phyla of *Euryarchaeota* and *Crenarchaeota*, methanogens occupy the euryarchaeal branch together with non-methanogenic halophilic, thermoacidophilic, and hyperthermophilic *Archaea* (Boone and Castenholz 2001). A number of genes of the extensively studied, complex methanogenic pathway are found in non-methanogenic organisms, particularly in euryarchaeal *Archaeoglobales*, but methanogens are the only organisms employing the whole pathway with CH_4 production (Vornolt *et al.* 1995, Klenk *et al.* 1997, Chistoserdova *et al.* 1998, Thauer 1998). Methanogenesis has thus been suggested as an ancestral feature of euryarchaea which has subsequently been lost in non-methanogens (Bapteste *et al.* 2005, Gribaldo and Brochier-Armanet 2006).

Methanogens can only grow with a limited set of one- or two-carbon compounds and hydrogen. Unable to gain energy from complex compounds, methanogens are dependent on substrate supply from associated anaerobic microbial communities or geological sources. Three types of methanogenic pathways are recognized, differing in their substrates (Deppenmeier 2002):

- Hydrogenotrophic methanogens grow with hydrogen (H_2) as the electron donor and carbon dioxide (CO_2) as the electron acceptor. Some hydrogenotrophs also use formate, which is the source of both CO_2 and H_2.
- Acetoclastic methanogens cleave acetate into a methyl and a carbonyl group. Oxidation of the carbonyl group into CO_2 provides reducing potential for reduction of the methyl group into CH_4.
- Methylotrophic methanogens grow on methylated compounds such as methanol, methylamines, and methylsulphides, which act as both electron donor and acceptor or are reduced with H_2.

Some methanogens are also able to use alcohols such as ethanol and propanol as a source of H_2 for reduction of CO_2 or grow on CO (O'Brien *et al.* 1984, Zellner and Winter 1987). Taxonomically methanogens form five orders: *Methanosarcinales* (9 genera), *Methanomicrobiales* (8), *Methanobacteriales* (5), *Methanococcales* (4), and *Methanopyrales* (1 genus) (Boone and Castenholz 2001). The majority of described methanogens are able to produce CH_4 from H_2 and CO_2, and orders *Methanomicrobiales*, *Methanococcales* and *Methanopyrales* contain only hydrogenotrophic methanogens. Members of *Methanobacteriales* are also hydrogenotrophic, except the methylotrophic genus

Methanosphaera. Other methylotrophic methanogens and all acetotrophs belong to *Methanosarcinales*, including the only known obligate acetotrophs forming the family *Methanosaetaceae*. The order *Methanosarcinales* includes the most metabolically versatile methanogens; several members of the family *Methanosarcinaceae* possess all three methanogenic pathways (Garcia *et al.* 2000, Galagan *et al.* 2002).

Beyond their shared energy metabolism, methanogens are physiologically and morphologically divergent. For example, most cultured methanogens grow optimally at mesophilic temperatures (Garcia *et al.* 2000), but the temperature range of methanogenic activity reaches from psychrophilic growth of *Methanogenium frigidum* (Franzmann *et al.* 1997) and *Methanosarcina lacustris* (Simankova *et al.* 2001) at 1 °C to hyperthermophilic growth of *Methanopyrus kandleri* at 110 °C (Kurr *et al.* 1991). Several thermophilic genera are found in orders *Methanobacteriales* and *Methanococcales*. Cell forms of methanogens are highly variable even within one order and range from cocci, rods, and spirilla to sarcina and irregular plate forms (Garcia *et al.* 2000). Like cell walls of all *Archaea*, those of methanogens lack peptidoglycan and consist of pseudomurein, protein units, or a unique polymer called methanochondroitin (Kandler and König 1998).

The variety of methanogenic habitats reflects their physiological diversity and requirement of anoxic conditions. Methanogenic ecosystems include (Garcia *et al.* 2000, Chaban *et al.* 2006):

- Anaerobic environments with decomposing organic matter. These include temporarily or permanently flooded wetlands such as mires, rice fields, and salt marshes; freshwater and marine sediments, landfills, and waste digesters. In freshwater environments methanogenesis is generally acetoclastic or hydrogenotrophic but in marine sediments often methylotrophic. These environments harbour a wide range of methanogens of the orders *Methanosarcinales*, *Methanomicrobiales*, *Methanobacteriales*, and *Methanococcales* (see Chaban *et al.* 2006).
- Digestive tracts of diverse organisms including ruminants, humans, and arthropods such as termites. Anaerobic protozoa also have endosymbiotic methanogens. Because the host organism absorbs intermediates of decomposition such as acetate, methanogenesis in digestive tracts is mostly hydrogenotrophic and frequently carried out by methanogens of the order *Methanobacteriales* (Lange *et al.* 2005).
- Geothermal environments, such as hot springs, petroleum reservoirs, and seafloor hydrothermal vents, where the substrates (H_2, CO_2) originate from geological activity. Thermophilic and hyperthermophilic strains belonging to the orders *Methanobacteriales*, *Methanococcales*, and *Methanopyrales* have been isolated from these environments (e.g. Jones *et al.* 1983, Lauerer *et al.* 1986, Kurr *et al.* 1991).

1.2 Methane as a greenhouse gas

When released from methanogenic ecosystems into the atmosphere, CH_4 is a reactive and radiatively active trace gas. After water vapour and carbon dioxide (CO_2), CH_4 is the next most abundant greenhouse gas (Wuebbles and Hayhoe 2002). The global warming potential of CH_4, i.e., effectiveness as a greenhouse gas, is 25 times that of CO_2 with a 100-year time horizon (IPCC 2007). The contribution of CH_4 to the total climate warming effect of greenhouse gases is 18%. The increase in atmospheric CH_4 concentrations from 700-715 parts per billion (ppb) in 1750 to 1775 ppb in 2005 has been attributed to anthropogenic CH_4 sources (IPCC 2007).

Annual CH_4 emission is estimated to be 503-610 Tg CH_4 $year^{-1}$, and more than 70% of this is biogenic CH_4 originating from activity of methanogens (IPCC 2007). Natural biogenic sources include northern and tropical wetlands, termites, and oceans. Anthropogenic biogenic sources include rice fields, ruminants, landfills and other waste treatment facilities. Abiogenic emissions originate from fossil fuels, incomplete biomass burning, CH_4 hydrates, and geological sources (Wuebbles and Hayhoe 2002, IPCC 2007). Plants have been argued to emit 0-236 Tg CH_4 $year^{-1}$, but the magnitude and mechanism of these emissions remain unresolved (Keppler *et al.* 2006, Kirschbaum *et al.* 2006, Dueck *et al.* 2007). The primary CH_4 sinks are oxidation to CO_2 in the atmosphere and oxidation by methanotrophic bacteria in aerobic soils (Wuebbles and Hayhoe 2002, IPCC 2007).

The largest single source of CH_4 is natural wetlands, including peat-forming mires. Estimations of emissions range from 100 to 231 Tg CH_4 $year^{-1}$ (IPCC 2007). Northern wetlands, which are predominantly located >50°N, are estimated to account for 15-30% of the total CH_4 emissions from wetlands (Matthews and Fung 1987, Cao *et al.* 1996, Hein *et al.* 1997, Walter *et al.* 2001, Chen and Prinn 2006). Wetland emissions have been suggested to contribute significantly to the interannual variability of global CH_4 emissions (Mikaloff Fletcher *et al.* 2004, Bousquet *et al.* 2006). Permafrost melting due to rising temperature in Siberia and Alaska is expected to increase wetland CH_4 emissions from the northern hemisphere (Christensen *et al.* 2004, Turestsky *et al.* 2007).

1.3 Methanogenesis in mires

1.3.1 Mires

Mires are wetlands with permanently high water level, peat-forming vegetation, and accumulation of partially degraded organic matter as peat (Laine and Vasander 1996). Low rates of decomposition in anoxic peat lead to extensive carbon storage (Clymo 1984, Gorham 1991). Peatlands are important long-term carbon sinks, storing ~300 Pg of carbon, which is approximately 13% of total soil carbon (Turunen *et al.* 2002, Vasander and Kettunen 2006). Northern mires comprise only 3% of the global land area, but they cover >20% of land area of some countries in the boreal region such as Finland and Estonia (Rydin and Jeglum 2006). A considerable share of the original mire area in Finland has been drained for forestry (Paavilainen and Päivänen 1995).

Mires are acidic, nutrient poor environments with vegetation adapted to the harsh conditions. Two main types defined based on hydrology are minerotrophic fens, which receive water and nutrients from groundwater, and ombrotrophic bogs, which rely solely on atmospheric deposition. Consequently, bogs have lower nutrient and cation levels, particularly Ca, and lower pH than fens (Laine *et al.* 2000). Vegetation of northern bogs is characterized by *Sphagnum* mosses, which further acidify their surroundings (Rydin and Jeglum 2006). Fens typically have higher coverage of graminoids such as sedges (*Carex* spp.) with root systems reaching anoxic peat. As an adaptation to anoxia, *Carex* and other typical mire vascular plants have aerenchyma, intercellular spaces which form a gas conduit and allow transport of oxygen into roots (Koncalova 1990, Armstrong *et al.* 1991).

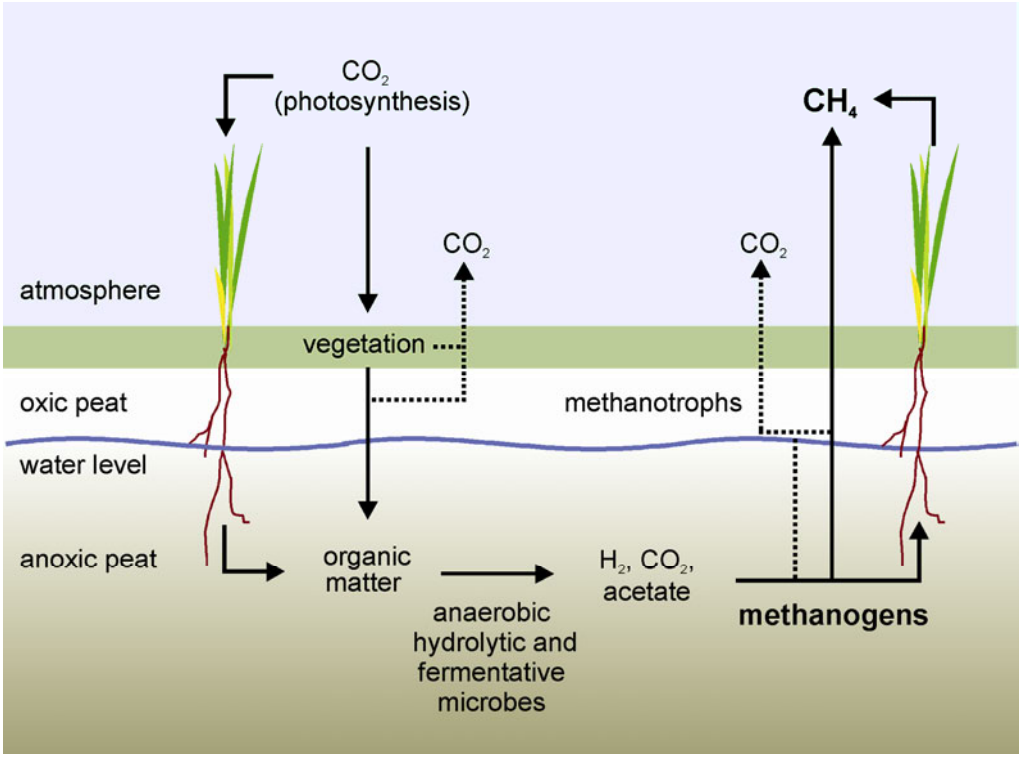

Figure 1. Schematic overview of carbon and CH_4 cycling in mires.

1.3.2 Anaerobic decomposition

The high water level in mires leads to vertical stratification with a shallow oxic layer and up to several meters of anoxic peat (Fig. 1). Above the water level, aerobic fungi and bacteria degrade organic matter to CO_2. Below the water level, oxygen level declines rapidly with depth (Lloyd *et al.* 1998). Under anoxic conditions, decomposition requires several guilds of anaerobic microbes acting in interconnected successive stages. Organic matter is converted into fermentation products, including organic acids and acetate, and finally into CH_4 and CO_2 (Fig. 2).

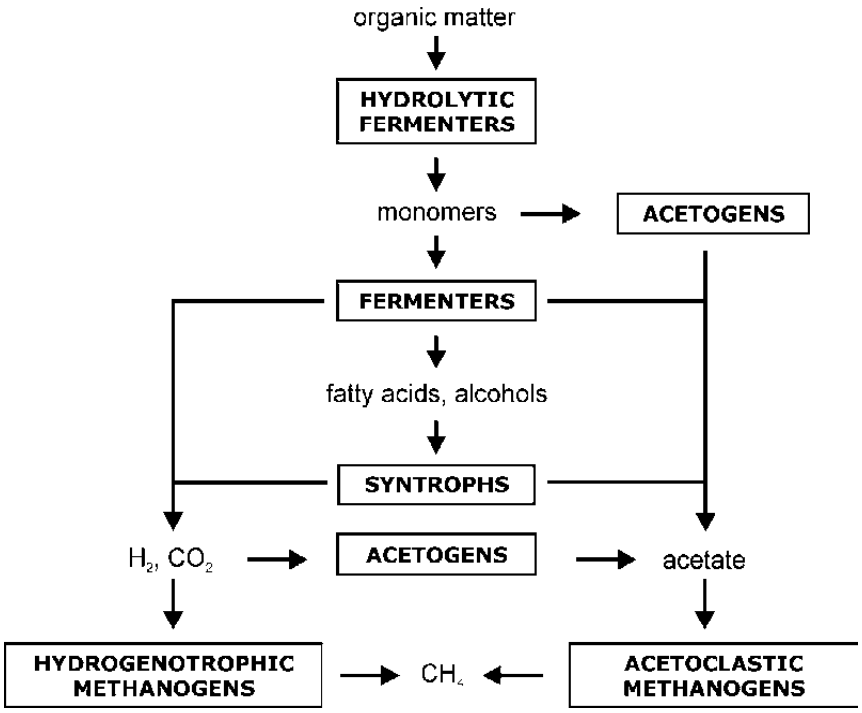

Figure 2. Schematic overview of anaerobic decomposition leading to CH_4 production. Based on Conrad (1999) and Whalen (2005).

In the first stage, hydrolytic enzymes of anaerobic bacteria and fungi break the organic polymers (e.g. cellulose, hemicellulose, starch, proteins) into monomers (sugars, amino acids). The monomers are fermented into acetate, fatty acids, alcohols, CO_2, and H_2. In peat, acetate, phenyl acetate, phenyl propionate, caproate, butyrate, and ethanol have been detected as intermediates in CH_4 production (Kotsyurbenko *et al.* 2004, Metje and Frenzel 2005, 2007). Syntrophic bacteria ferment the fatty acids and alcohols to acetate, CO_2, and H_2. Syntrophs produce H_2 and often occur in tight interaction with hydrogenotrophic methanogens; consumption of H_2 by methanogens makes the fermentation process of the syntrophs energetically feasible (Schink 1997). Acetogens produce acetate from organic monomers or from H_2 and CO_2. Acetate, CO_2, and H_2 generated at the fermentative and acetogenic steps are substrates for terminal decomposers. Because availability of oxygen and alternative electron acceptors such as sulphate (SO_4^{2-}), nitrate (NO_3^-), and ferric iron (Fe^{3+}) is generally limited in anoxic peat, the prevalent terminal process is methanogenesis.

Methane produced in the water-submerged peat layers is emitted into the atmosphere by diffusion in water, bubbling, or through the aerenchyma of vascular plants (Whalen 2005). When CH_4 passes through the oxic surface layer, depending on the thickness of the layer, more than 90% of the methane produced in anoxic peat may be oxidized into CO_2 by aerobic methanotrophic bacteria (Segers 1998, Frenzel and Karofeld 2000, Pearce and Clymo 2001). Methane transported through aerenchymatous plants largely escapes oxidation (Schimel 1995).

1.3.3 Pathways of CH_4 production

In freshwater environments, the principal precursors of CH_4 are acetate and H_2/CO_2. Acetate is considered to account for two thirds of the produced CH_4 (Whiticar *et al.* 1986, Conrad 1999). In mires, however, either hydrogenotrophic methanogenesis (Lansdown *et al.* 1992, Metje and Frenzel 2005) or acetoclastic methanogenesis may dominate (Kotsyurbenko *et al.* 2004, Metje and Frenzel 2007). Stimulation of CH_4 production by methanol in permafrost peat additionally implies potential for the methylotrophic pathway in some peat soils (Ganzert *et al.* 2007).

The pathway of methanogenesis has been observed to shift from acetoclastic in surface peat, rhizosphere, and *Carex* fens to hydrogenotrophic in more oligotrophic deeper peat and *Sphagnum*-dominated bogs (Kelly *et al.* 1992, Hornibrook *et al.* 1997, Bellisario *et al.* 1999, Popp *et al.* 1999, Chasar *et al.* 2000a, Galand *et al.* 2005). Hence, availability of fresh organic matter favours acetoclastic methanogenesis, whereas hydrogenotrophic pathway dominates in more oligotrophic and recalcitrant peat (Miyajima *et al.* 1997, Hornibrook *et al.* 2000, Ström *et al.* 2003). When acetoclastic production is substantial, the pathways appear to have seasonal shifts, with acetoclastic production being particularly important in summer when acetate levels and CH_4 production rates are high (Kelly *et al.* 1992, Avery *et al.* 1999, Chasar *et al.* 2000b).

1.3.4 Environmental factors controlling methanogenic activity

The occurrence and activity of methanogens can be assessed by measuring potential CH_4 production, i.e., microbial formation of CH_4 from endogenous or added substrates in anoxic laboratory incubations. Although sampling and preparation of peat slurries disturb the samples, potential CH_4 production estimates methanogenic activity better than CH_4 emissions or gas concentrations measured in the field. Unlike the latter methods, measurements of production potential are not affected by aerobic CH_4 oxidation or transport of old CH_4 from deeper peat. An unknown factor is anaerobic CH_4 oxidation, which occurs coupled to sulphate reduction in marine sediments (Hoehler *et al.* 1994, Orphan *et al.* 2001) and to denitrification in agriculture-influenced freshwater sediments (Raghoebarsing *et al.* 2006). Anaerobic CH_4 oxidation has only recently been reported in peat (Smemo and Yavitt 2007), and its extent and mechanism in mires is unknown. If it is prevalent, measurements of CH_4 production without considering the simultaneous anaerobic oxidation underestimate the actual methanogenic activity.

The growth and activity of methanogens occurs mainly in the anoxic portion of the peat profile and is thus regulated by water table depth. The largest CH_4 production potentials are generally measured 10-20 cm below the water level, and production declines in deeper, more decomposed peat (Williams and Crawford 1984, Sundh *et al.* 1994, Krumholz *et al.* 1995, Saarnio *et al.* 1997, Edwards *et al.* 1998). Methane production potentials and methanogens have, however, been detected in unsaturated peat and soils (Peters and Conrad 1995, Wagner and Pfeiffer 1997, Kettunen *et al.* 1999, Kobabe *et al.* 2004, Høj *et al.* 2006), suggesting methanogens survive in anoxic microenvironments or tolerate temporary

aeration. Several methanogens possess enzymes for detoxification of radical oxygen species (Galagan *et al.* 2002, Seedorf *et al.* 2004, Erkel *et al.* 2006).

In anoxic peat, the main regulator of methanogenic activity and CH_4 production is substrate supply (Svensson and Sundh 1992, Valentine *et al.* 1994, Segers 1998). Plant primary production ultimately regulates the input of organic matter, and recently fixed carbon from fresh litter or root exudates of vascular plants has been shown to support CH_4 production (Chanton *et al.* 1995, Bellisario *et al.* 1999, van den Pol-van Dasselaar and Oenema 1999, Chasar *et al.* 2000a, King and Reeburgh 2002, Ström *et al.* 2003). Fresh organic matter has higher quality, i.e., higher amount of labile carbohydrates readily available to decomposers, opposed to older material rich in recalcitrant compounds such as lignin and humic substances (Valentine *et al.* 1994, Yavitt *et al.* 2000). Water level affects substrate quality. If the level is close to surface, organic matter reaches water-saturated peat virtually undecomposed; when the level is lower, the labile compounds are degraded extensively in the thicker layer of aerobic peat, and the fraction of organic matter available for CH_4 production is more recalcitrant. However, vascular plants such as *Carex* and *Eriophorum* allocate labile carbon directly into water-saturated layer as root exudates (Joabsson *et al.* 1999). Vegetation also influences substrate quality through differences in litter chemistry and decomposability. *Sphagnum* mosses are particularly resistant to decomposition (Aerts *et al.* 1999, Kuder and Kruge 2001).

Methane production is strongly dependent on temperature and usually temperature-limited in northern mires, with maximal production at 20-35 °C (Svensson 1984, Segers 1998, Kotsyurbenko *et al.* 2004, Metje and Frenzel 2005). Incubation of acidic peat (pH 4-5) at elevated pH has lead to higher production with maximal production at pH 6-7 (Williams and Crawford 1983, Goodwin and Zeikus 1987, Dunfield *et al.* 1993, Valentine *et al.* 1994, Kotsyurbenko *et al.* 2007), suggesting that also pH limits methanogenic activity in peat. Exceptions are known where higher pH had no effect or even inhibited CH_4 production (Yavitt *et al.* 1987, Bergman *et al.* 1998, Bräuer *et al.* 2004).

If alternative electron acceptors are present, methanogens compete for substrates, particularly H_2, with other terminal decomposers. For example, sulphate reduction due to air-born sulphate deposition may decrease CH_4 production (Nedwell and Watson 1995, Dise and Verry 2001, Gauci *et al.* 2004). When the available electron acceptor is CO_2, hydrogenotrophic methanogens compete with acetogens. In some northern mires, an acetate-accumulating terminal process has been observed in connection to low CH_4 production levels (Hines *et al.* 2001, Duddleston *et al.* 2002). Although acetogenesis from H_2 and CO_2 occurs in cold soils and sediments (Schulz and Conrad 1996, Kotsyurbenko 2005), no direct evidence exists of its occurrence in peat. Acetogenesis has even been calculated to be thermodynamically unfavourable in peat (Metje and Frenzel 2007).

1.4 Detection of methanogen diversity

Biogeochemical processes, including carbon cycling, are vital for sustaining life on Earth. The ecology, physiology, and taxonomy of the microbes carrying out the processes remain, however, largely uncharacterized. As microbial activity forms the basis of these processes, unravelling how microbial diversity affects them and how biotic and abiotic factors in the ecosystem influence the microbes is essential.

Microbial communities in the environment are exceedingly complex (Torsvik *et al.* 2002, Gans *et al.* 2005). Traditional culture-dependent methods have proved inadequate to describe the vast microbial diversity; they may miss >99% of the organisms and enrich those thriving in cultures but not numerically or functionally important in the environment (Torsvik *et al.* 1990, Amann *et al.* 1995). Introduction of culture-independent, molecular methods has vastly improved the potential to describe microbial diversity (DeLong and Pace 2001). Since their introduction over 20 years ago, the methods have increased the number of recognized bacterial phyla from 12 to over 50 (Hugenholtz *et al.* 1998, Rappe and Giovannoni 2003), and recovered, for instance, a wide diversity of mesophilic *Archaea* with unknown function (Schleper *et al.* 2005).

A standard approach in molecular analysis of microbial communities starts with extraction of DNA from environmental samples, followed by PCR amplification of marker genes, differentiation of amplicons by molecular fingerprinting or cloning, and identification of the populations by DNA sequencing and phylogenetic analysis (Head *et al.* 1998). A challenge in microbial ecology has been to relate the molecular fingerprints and sequence data to ecosystem functions (Gray and Head 2001, Torsvik and Øvreås 2002). Another challenge is accurate and comprehensive description of the vast microbial diversity and its components. The high diversity and high numbers of prokaryotes pose challenges for detection of species richness, and only the most abundant species may be retrieved. Detection of community composition by PCR-based methods is highly dependent on the coverage of the primers (Baker *et al.* 2003, Forney *et al.* 2004). The major weakness of PCR-based methods is the recovery of relative abundances of taxa, because amplicon ratios may become biased during amplification (Suzuki and Giovannoni 1996, von Wintzingerode *et al.* 1997, Ishii and Fukui 2001, Lueders and Friedrich 2003).

Methanogen communities have been characterized by employing the 16S ribosomal RNA (rRNA) gene or the methyl-coenzyme M reductase gene *mcrA* as molecular markers in a wide variety of environments. These include rice field soil, wetlands, freshwater and marine sediments, hydrothermal environments, deep subsurface habitats, rumen and other digestive tracts, termites, anaerobic digesters, and landfills (reviewed by Chaban *et al.* 2006). The studies have differentiated communities by analysis of clone libraries or by community fingerprinting by denaturing gradient gel electrophoresis (DGGE), temperature gradient gel electrophoresis (TGGE), and single-strand conformation polymorphism (SSCP), which separate DNA fragments according to sequence-based melting behaviour, or by terminal restriction fragment length polymorphism (T-RFLP), which relies on differences in restriction fragment lengths between taxa (Moyer *et al.* 1994, Liu *et al.* 1997, Muyzer and Smalla 1998, Schwieger and Tebbe 1998). Additionally, fluorencence *in situ* hybridization (FISH) and membrane hybridization have been used (Raskin *et al.* 1994, Purdy *et al.* 2003).

Active methanogens have been targeted by analysis of environmental RNA (Lueders and Friedrich 2002, Koizumi *et al.* 2004, Shigematsu *et al.* 2004).

Application of molecular methods has revealed novel methanogenic or putatively methanogenic lineages. A notable example is Rice cluster I (RCI), which was first detected in rice field soil as 16S rRNA gene sequences only distantly related to *Methanomicrobiales* and *Methanosarcinales* (Grosskopf *et al.* 1998a). Subsequently, the group has been shown to be an important CH_4 producer in rice fields (Lu and Conrad 2005, Conrad *et al.* 2008), and the complete genome of a RCI methanogen has been sequenced (Erkel *et al.* 2006). The first RCI strain was recently isolated from rice field soil (Sakai *et al.* 2007).

1.4.1 Ribosomal 16S RNA gene as a molecular marker

The 16S rRNA gene encodes the small subunit of prokaryotic ribosomal RNA. As a part of the protein synthesis machinery, it has an essential function conserved across all prokaryotes, ubiquitous distribution, and lack of extensive horizontal gene transfer. It was integral for defining the three domains of life, and it has become a major tool in identification of prokaryotes. The widespread application of 16S rDNA as a molecular marker in microbial ecology has been central to discovery of numerous novel prokaryotic lineages (Hugenholtz *et al.* 1998, Rappe and Giovannoni 2003). Conserved sequence regions allow design of primers for different taxonomic levels, and interspersed variable regions and the length of the gene (~1500 bp) provide phylogenetic resolution for distinguishing taxa. Sequence similarity of <97% has been adopted to indicate that the 16S rDNA sequences represent members of different species (Stackebrandt and Goebel 1994). This threshold was later raised to 98.7-99% (Stackebrandt and Ebers 2006). The presence of multiple copies of the rRNA operon with slightly differing sequence in one organism may lead to overestimation of diversity, although mostly the sequences differ only by <1% (Acinas *et al.* 2004).

The 16S rRNA gene sequence provides the phylogenetic affiliation of the organism but tells nothing explicit of its function. Many functional microbial groups, including methanogens, are not monophyletic in 16S rRNA gene phylogeny, which hampers their detection and identification. Methanogen-specific 16S rRNA gene primers have been designed (Marchesi *et al.* 2001, Wright and Pimm 2003), but *in silico* analysis by Banning *et al*. (2005) indicated that these primer pairs amplify also non-methanogenic *Euryarchaeota* and *Crenarchaeota*. As a solution the authors developed three primer pairs, which together cover most known methanogen 16S rDNA sequence diversity. A primer set covering at least *Methanobacteriales*, *Methanosarcinales* and *Methanomicrobiales* has been developed for DGGE analysis (Watanabe *et al.* 2004). Group-specific probes and primers have also been developed for hybridization studies (Raskin *et al.* 1994) and quantitative PCR (qPCR) (Hori *et al.* 2006). A more straightforward approach for detection of methanogen 16S rDNA is application of general archaeal primers (e.g. DeLong 1992, Embley *et al.* 1992, Øvreås *et al.* 1997, Grosskopf *et al.* 1998b) and identification of methanogens by phylogenetic analysis.

1.4.2 *mcrA* as a specific marker gene for methanogens

Marker genes encoding functions specific to a functional microbial guild overcome the problem of phylogenetic dispersal. Methyl-coenzyme M reductase (MCR, EC 2.8.4.1) is an essential enzyme in CH_4 production. It catalyzes the final step of methanogenesis in which the methyl group linked to coenzyme M is reduced with formation of CH_4 (Ellermann *et al.* 1988, Deppenmeier 2002). This enzyme is present in all known methanogens, and unlike many other enzymes in the methanogenic pathway, it is absent from non-methanogenic *Archaea* and *Bacteria* (Chistoserdova *et al.* 1998, Thauer 1998, Bapteste *et al.* 2005). MCR is composed of three subunits, α, β, and γ, encoded by the operon *mcr*BDCGA (Reeve *et al.* 1997). The gene encoding the α-subunit, *mcrA*, contains conserved sequence regions, which have been related to catalytic sites of MCR (Weil *et al.* 1988, Hallam *et al.* 2003). The phylogeny of *mcrA* follows the 16S rRNA phylogeny (Springer *et al.* 1995, Lueders *et al.* 2001, Luton *et al.* 2002), allowing identification of methanogens based on *mcrA* sequences. Most methanogens possess only one copy of *mcrA*, except members of the orders *Methanobacteriales* and *Methanococcales*, which additionally have an isoenzyme MCR-II and the corresponding *mrtA* gene (Thauer 1998). Anaerobic CH_4-oxidizing *Archaea* (ANME-1 and ANME-2) harbour phylogenetically distinct *mcrA* genes (Hallam *et al.* 2003). In ANME, MCR is hypothesized to catalyze the reverse reaction of methanogenesis (Kruger *et al.* 2003, Hallam *et al.* 2004).

Several degenerate primer pairs have been designed for detection of the *mcrA* gene (Ohkuma *et al.* 1995, Springer *et al.* 1995, Hales *et al.* 1996, Luton *et al.* 2002). The primers differ in amplicon length, target site, and the level of degeneracy (Fig. 1 and Table 1 in **II**). Studies using two primer pairs have reported differences or limitations in their coverage of methanogen taxa (Lueders *et al.* 2001, Banning *et al.* 2005, Nercessian *et al.* 2005). There are also group-specific primers for quantitative PCR (Denman *et al.* 2007) or for detection of the ANME *mcrA* genes (Hallam *et al.* 2003, Nunoura *et al.* 2006). In addition to cloning and sequencing, the ME primer pair of Hales *et al*. (1996) has been applied in DGGE (Galand *et al.* 2002), the ML primers of Luton *et al*. (2002) in T-RFLP, DGGE and TGGE (Castro *et al.* 2005, Sheppard *et al.* 2005, Wilms *et al.* 2007), and the MCR pair (Springer *at al*. 1995) in T-RFLP (Lueders *et al.* 2001). The ML pair or its modification has also been used in qPCR (Radl *et al.* 2007, Goffredi *et al.* 2008).

The MCR primer pair has mainly been employed in studies of rice field soil or rice root methanogens (Chin *et al.* 1999, Ramakrishnan *et al.* 2001, Conrad *et al.* 2008), but also in floodplain wetland (Kemnitz *et al.* 2004) and hydrothermal sediment (Dhillon *et al.* 2005). The ME primers and the most recent ML primer pair have been used in a wider range of environments, ranging from wetlands and freshwater sediments (Earl *et al.* 2003, Castro *et al.* 2004, Banning *et al.* 2005, Smith *et al.* 2007) to hydrothermal, hypersaline, deep subseafloor, and CH_4 hydrate habitats (Inagaki *et al.* 2004, Newberry *et al.* 2004, Nercessian *et al.* 2005, Parkes *et al.* 2005, Smith *et al.* 2008) and rumen and animal fecal material (Tatsuoka *et al.* 2004, Ufnar *et al.* 2007). The ME pair has been used to detect *mcrA* of anaerobic methane oxidizers (Hallam *et al.* 2003, Lloyd *et al.* 2006, Lösekann *et al.* 2007).

1.5 Methanogen communities in mires

Methanogens in peat have been investigated with the 16S rRNA and *mcrA* genes as molecular markers, mainly by RFLP or sequence analysis of clone libraries or community fingerprinting, but also by probe hybridization. The studies are summarized in Table 1, together with studies of methanogenic enrichments. Members of orders *Methanosarcinales* (families *Methanosarcinaceae* and *Methanosaetaceae*), *Methanomicrobiales*, *Methanobacteriales*, and of Rice cluster I have frequently been detected in peat (Table 1). The first molecular study assessing mire methanogens detected two novel groups, R10 associated with *Methanomicrobiales* and R17 distantly related to *Methanosarcinales* (Hales *et al.* 1996). These groups have occurred in other mires as well. The R10 group has also become known as Fen cluster (FC), named based on the type of mire where the *mcrA* genes of the group were first detected as a novel lineage (Galand *et al.* 2002), or as the E2 group (Cadillo-Quiroz *et al.* 2006). The R17 group is commonly referred to as Rice cluster II (RCII) after its discovery in rice field soil (Grosskopf *et al.* 1998a).

Methanogen communities generally change with peat depth (Galand *et al.* 2002, Galand *et al.* 2003, Høj *et al.* 2005, Cadillo-Quiroz *et al.* 2006, Ganzert *et al.* 2007). Shifts related to vegetation have also been reported: communities differed between *Sphagnum*-dominated hummocks and *Eriophorum* lawns (Galand *et al.* 2003), along a successional gradient on land-uplift coast (Merilä *et al.* 2006), and between *Sphagnum*- and *Carex*-dominated Alaskan mires (Rooney-Varga *et al.* 2007). The study of Alaskan mires also suggested correlation of communities with pH and temperature. Temporal patters during the growing season have been addressed in arctic peat (Høj *et al.* 2005, 2006). In addition to peat samples, methanogen communities have been characterized in enrichment cultures (Horn *et al.* 2003, Sizova *et al.* 2003), and in incubations where the effect of temperature (Metje and Frenzel 2005, 2007, Høj *et al.* 2008) or pH (Kotsyurbenko *et al.* 2007) on community composition has been assessed.

Several isolates affiliated with *Methanobacteriales* have been obtained from peat, many of these active at low pH (Williams and Crawford 1985, Zellner *et al.* 1988, Kotsyurbenko *et al.* 2007). Recently, novel strains affiliated with *Methanomicrobiales* have been isolated from North American mires (Bräuer *et al.* 2006a, Cadillo-Quiroz *et al.* 2008). In these studies, the detection of uncultured methanogen lineages in peat has been followed by their successful isolation. "*Candidatus* Methanoregula boonei" is the first cultured member of the Fen cluster/R10/E2 group, and "*Candidatus* Methanosphaerula palustris" is the first isolate of an E1 group within *Methanomicrobiales*.

Table 1. Summary of molecular studies of methanogen communities in northern peatlands.

Marker gene (primers)	Method	Site and latitude	Detected methanogens[c]	Main findings	Reference
mcrA[a] archaeal 16S	sequencing (16S), PCR (*mcrA*)	Moorhouse blanket bog, England, 54°N	*Methanomicrobiales* (R10 group/FC), R17 group/ RCII	two novel methanogen lineages detected in peat	Hales *et al.* 1996
mcrA[a] archaeal 16S	PCR	Ellergower Moss, Scotland, 55°N	no identification	methanogens detected 9 cm below water level in a peat profile	Lloyd *et al.* 1998
archaeal 16S *mcrA*[a]	PCR, membrane hydridization	Moorhouse blanket bog, England, 54°N	*Methanosarcinaceae*, *Methanococcaceae*, *Methanobacteriaceae*	minor differences in depth distribution of groups	McDonald *et al.* 1999
mcrA[a]	sequencing	Moorhouse, Ellergower Moss, UK, 54-55°N	*Methanosarcinales*[d]	sequences differed between sites but not with depth	Nercessian *et al.* 1999
archaeal 16S *mcrA*[a]	FISH, PCR, membrane hydridization	Ellergower Moss, Scotland, 55°N	*Methanomicrobiales*, *Methanosarcinales*, *Methanococcaceae*, *Methanobacteriaceae*	methanogens detected below 14 cm in a 25-cm peat profile	Upton *et al.* 2000
mcrA[a]	clone RFLP, sequencing, DGGE	oligotrophic Salmisuo fen, Finland, 62°N	*Methanomicrobiales* (FC), RCI	communities changed with depth	Galand *et al.* 2002
archaeal 16S	clone RFLP, sequencing	Labrador Hollow conifer swamp, ombrotrophic McLean bog, NY, USA, 42°N	*Methanosarcinaceae*, *Methanosaetaceae*, RCII, *Methanomicrobiales* (FC), *Methanobacteriaceae*	same groups but different RFLP patterns in swamp and bog	Basiliko *et al.* 2003
methanogen 16S	clone RFLP, sequencing, DGGE	oligotrophic Salmisuo fen, Finland, 62°N	*Methanomicrobiales* (FC), *Methanosarcinaceae*	hummock and lawn communities differed at surface but not in deeper peat	Galand *et al.* 2003
archaeal 16S	enrichment, sequencing	*Sphagnum-Picea* bog, Germany, 50°N	*Methanomicrobiales* (FC), *Methanobacteriaceae*, *Methanosarcinaceae*	methanogens detected from serial peat dilutions and enrichments	Horn *et al.* 2003

archaeal 16S	enrichment, sequencing	ombrotrophic Bakchar Bog , Siberia, 57°N	*Methanomicrobiales* (FC), RCI, *Methanobacteriaceae*	methanogens detected in peat enrichments	Sizova *et al.* 2003
archaeal 16S	sequencing	Bakchar Bog, Siberia, 56°N, Akaiyachi Mire, Japan, 37°N, Okefenokee Swamp, USA, 30°N	*Methanosarcinaceae*, *Methanomicrobiales* (including FC), *Methanosaetaceae*	communities of subarctic, temperate, and subtropical sites differed	Utsumi *et al.* 2003
archaeal 16S	T-RFLP, sequencing	ombrotrophic Bakchar Bog, Siberia, 56°N	RCII, *Methanobacteriaceae*, *Methanosarcinaceae*, *Methanomicrobiales*	predominantly acetoclastic methanogenesis in an acidic bog (pH 4.2-4.8)	Kotsyurbenko *et al.* 2004
mcrA[b]	clone RFLP, sequencing	Lakkasuo mire complex, Finland, 61°N	FC, *Methanosaetaceae*, RCI	pathway and community differences between bog and two fens	Galand *et al.* 2005
archaeal 16S	DGGE, sequencing of bands	Solvatnet and Stuphallet, Spitsbergen, 78°N	*Methanomicrobiales*, *Methanosaetaceae*, *Methanobacteriaceae*, *Methanosarcinaceae*, RCII	differences with depth, site, and sampling time; community variation related to CO_2 emission	Høj *et al*. 2005
mcrA[b]	T-RFLP	a chronosequence of five peatlands, Siikajoki, Finland, 64°N	FC, RCI, *Methanosarcinaceae*[e]	community change in succession gradient	Merilä *et al.* 2006
archaeal 16S *mcrA*[a]	sequencing, incubations, T-RFLP	mire in eastern Finnish Lapland, 68°N	*Methanobacteriaceae*	low diversity, no substantial change in incubations at 4-45 °C, hydrogenotrophic methanogenesis	Metje and Frenzel 2005
archaeal 16S *mcrA*[a]	enrichment, sequencing	ombrotrophic McLean Bog, NY, USA, 42°N	*Methanomicrobiales* (FC)	enrichment of two similar strains, isolation of *Methanoregula boonei* (FC)	Bräuer *et al.* 2006a, 2006b
archaeal 16S	T-RFLP, sequencing	oligotrophic Chicago Bog, ombrotrophic McLean Bog, NY, USA, 42°N	*Methanomicrobiales* (FC, E1), RCII, RCI, *Methanosarcinaceae*, *Methanosaetaceae*	communities of bogs similar at surface but different in deeper peat	Cadillo-Quiroz *et al*. 2006

Marker gene (primers)	Method	Site and latitude	Detected methanogens[c]	Main findings	Reference
archaeal 16S	DGGE, sequencing of bands	Solvatnet, Stuphallet, and Sassen Valley, Spitsbergen, 78°N	*Methanosaetaceae*, *Methanobacteriaceae*	shifts in methanogen communities along a moisture gradient in upper peat layer but not deeper	Høj *et al*. 2006
archaeal 16S	clone RFLP, sequencing	continental bog, permafrost mound, and internal lawn, western Canada, 55°N	RCII, *Methanomicrobiales* (FC), *Methanosaetaceae*, *Methanosarcinaceae*, *Methanobacteriaceae*	differences between continental bog and internal lawn	Yavitt *et al.* 2006
archaeal 16S	T-RFLP, sequencing	Chicago Bog and Michigan Hollow, NY, USA, 42°N	*Methanomicrobiales* (FC, E1), RCI, RCII, *Methanosaetaceae*	different communities in acidic *Sphagnum* bog and neutral *Carex* fen	Dettling *et al.* 2007
methanogen 16S	DGGE, sequencing of bands	Laptev Sea coast, Siberia, 72-73°N	*Methanosarcinaceae*, *Methanomicrobiales*, RCII	vertical shift of communities, differences between permafrost formations	Ganzert *et al.* 2007
archaeal 16S	incubations, T-RFLP, sequencing	ombrotrophic Bakchar Bog , Siberia, 57°N	*Methanobacteriaceae*, *Methanomicrobiales*, *Methanosarcinaceae*	*Methanobacteriaceae* at low pH, three strains isolated, similar community at 15 and 25 °C	Kotsyurbenko *et al.* 2007
archaeal 16S	sequencing, incubations, T-RFLP	mire in north-western Siberia, 67°N	*Methanobacteriaceae*, *Methanosarcinaceae*	acetoclastic methanogenesis, *Methanobacteriaceae* more important in incubations with higher temperature	Metje and Frenzel 2007
archaeal 16S	DGGE, sequencing of bands and clones	12 Alaskan and 2 midlatitudes peatlands, USA, 42-69°N	*Methanobacteriaceae*, *Methanomicrobiales* (FC), *Methanosaetaceae*	archaeal community variation related to vegetation, also to pH and temperature	Rooney-Varga *et al*. 2007

archaeal 16S	T-RFLP, sequencing, enrichment and isolation	Michigan Hollow minerotrophic fen, NY, USA, 42°N	*Methanomicrobiales* (E1, FC, *Methanospirillaceae*), *Methanosaetaceae*, *Methanosarcinaceae*, *Methanobacteriaceae*, RCI, RCII	diverse community in a neutral fen, isolation of the first E1 group methanogen (*Methanosphaerula palustris*)	Cadillo-Quiroz *et al*. 2008
archaeal 16S	incubation, DGGE, sequencing	Solvatnet, Spitsbergen, 78°N	*Methanomicrobiales*, *Methanosarcinaceae*, *Methanosaetaceae*, *Methanobacteriaceae*	greater contribution of methanogens with increasing incubation temperature	Høj *et al*. 2008

[a] ME primers (Hales *et al.* 1996)
[b] ML primers (Luton *et al.* 2002)
[c] FC, Fen cluster; RCI, Rice cluster I; RCII, Rice cluster II
[d] phylogenetic affiliation uncertain because sequences are not available for analysis
[e] identification based on sequences from Lakkasuo mire complex

1.6 Non-methanogenic *Archaea* in peat

Non-methanogenic archaea in peat have been reported in methanogen studies with general archaeal 16S rRNA gene primers. Group 1.3 crenarchaea (or Rice cluster IV) have been found in several peat ecosystems (Galand *et al.* 2003, Kotsyurbenko *et al.* 2004, Høj *et al.* 2005, 2008). In a recent study, group 1.3 was the most common archaeal group in Alaskan mires (Rooney-Varga *et al.* 2007). It also occurred in arctic wet soils and peat (Høj *et al.* 2006). Other archaeal groups detected in mires include crenarchaeal Rice cluster VI and euryarchaeal groups Rice cluster V, Lake Dagow Sediment group, Marine Benthic group D, and a subaqueous cluster (Kotsyurbenko *et al.* 2004, Cadillo-Quiroz *et al.* 2008, Høj *et al.* 2008).

1.7 Bacterial communities in anoxic peat

The most studied bacteria in mires are methanotrophs (e.g. Dedysh *et al.* 1998, Jaatinen *et al.* 2005, Raghoebarsing *et al.* 2005, Chen *et al.* 2008). Factors controlling aerobic bacterial activity (Fisher *et al.* 1998, Fisk *et al.* 2003, Jaatinen *et al.* 2007) and bacteria associated with *Sphagnum* mosses have also been characterized (Opelt *et al.* 2007a, Opelt *et al.* 2007b). The bacterial communities in anoxic peat have received much less attention, despite their role in carbon cycling as substrate producers and competitors to methanogens. The few molecular studies that have characterized bacterial communities in anoxic or undefined but most likely anoxic peat have recovered mainly members of *Alphaproteobacteria*, *Acidobacteria*, *Planctomycetes*, *Verrucomicrobia*, *Deltaproteobacteria*, *Actinobacteria*, *Firmicutes*, and *Chloroflexi* (Rheims *et al.* 1996, Dedysh *et al.* 2006, Morales *et al.* 2006). Several of these groups have also been recovered in methanogenic peat enrichments (Horn *et al.* 2003, Sizova *et al.* 2003, Bräuer *et al.* 2006b), and strains of *Acidobacteria* and *Planctomycetes* have been isolated (Dedysh *et al.* 2006, Kulichevskaya *et al.* 2006). A study of 24 *Sphagnum* bogs in New England attempted to relate bacterial 16S rDNA T-RFLP fingerprints from oxic or deep anoxic (1 m) peat to a wide range of environmental variables, finding highly similar communities and a weak connection to Ca^{2+} level (Morales *et al.* 2006).

2 Aims of the study

Mires exhibit horizontal and vertical patterns of peat chemistry, vegetation, surface topography, and water level, reflected in microbial activities. Spatial and temporal variability of methane emissions has partially been related to environmental factors (Blodau 2002), but understanding of the underlying microbiology is more limited. Characterization of methanogen communities in a range of mires has revealed varied community compositions (Table 1), but when this work was initiated, studies attempting to link methanogen community composition to environmental variables were few.

The general aim of this work was to investigate methanogen communities and their activity in northern mires in relation to specific environmental gradients, and concomitantly compare methods for detecting community dynamics. *Bacteria* and non-methanogenic *Archaea*, which have received even less attention in mires, were assessed because they are potential substrate producers and competitors to methanogens. The specific objectives were to address:

- variation of methanogen communities and CH_4 production in respect to
    - ecohydrological gradient from fen to bog (**III**)
    - season in a fen where CH_4 emissions are closely monitored (**IV**)
    - ash fertilization in a drained bog (**I**)
- *Bacteria* and non-methanogenic *Archaea* in mires (**III**, **IV**)
- performance of PCR primers for the *mcrA* gene in analysis of mire methanogens (**II**)

3 Materials and methods

3.1 Sample collection

Study sites comprised three Finnish boreal mires: Lakkasuo mire complex in Orivesi and Siikaneva in Ruovesi, which are two closely situated pristine mires in southern Finland, and a drained site Pelso-Resula in Muhos, northern Finland (Table 2).

Table 2. Sampled mires.

Mire	Location	Type	Study
Lakkasuo mire complex	61°48′N, 24°19′E	ombrotrophic bog oligotrophic fen mesotrophic fen	III
Siikaneva	61°50'N, 24°12'E	oligotrophic fen	IV
Pelso-Resula	64°30′N, 26°18′E	drained bog	I, II

The sampled areas of the Lakkasuo mire complex form an ecohydrological gradient from minerotrophic fens to ombrotrophic bog. The *Sphagnum*-shrub bog has lower pH and lower levels of N, P, Ca, and Fe than the *Carex-Sphagnum* fens (Laine *et al.* 2002). Three replicate peat cores from lawn microsites at each site were collected in October 2002.

Siikaneva is an open fen, where seasonal fluctuations of CH_4 and CO_2 emissions, including winter fluxes, have been investigated (Rinne *et al.* 2007, Riutta *et al.* 2007). The samples were collected in 2005-2006 in October (end of growing season before snowfall), February (midwinter with snow cover of 35 cm), May (spring after snowmelt and temperature rise), and August (late summer after a warm, dry period). On each occasion, a peat profile was collected from three marked lawn or hollow locations.

The Pelso-Resula bog is a drained cottongrass pine bog with small Scots pines (*Pinus sylvestris*) and birches (*Betula pendula*). Ash fertilization was conducted in 1997 with 15 000 kg ha^{-1} of wood ash applied on 30×30-m plots. After five years, fertilized plots had higher pH and levels of B, Ca, and K in surface peat, enhanced tree growth, and higher abundance of *Eriophorum vaginatum* and *Rubus chamaemorus* but reduced abundance of *Sphagnum* mosses (Moilanen and Silfverberg 2004). Three replicate peat profiles were collected from fertilized plots and unfertilized control plots in May 2002.

Peat profiles were collected with a box corer (8×8×90 cm). Samples were taken as 4-cm peat slices from selected depths. The depths were measured from water table level (**I**, **II**, **III**) or peat surface (**IV**). Vegetation of the study sites is described in more detail in the articles.

3.2 Methods

The methods used in characterization of peat samples, methanogenic potential and microbial communities are described in detail in the original articles and listed in Table 3.

Table 3. Overview of chemical, molecular, and data analysis methods. Roman numerals refer to the articles I-IV.

Method	Described and used in:
Chemical analyses	
Potential CH_4 production	I, III, IV
Temperature response of CH_4 production	IV
Peat pH	I, III, IV
Nucleic acid methods	
DNA extraction	I-IV
RNA extraction	IV
Reverse transcription	IV
mcrA PCR	
ME primers (Hales *et al*. 1996)	I, II
MCR primers (Springer *et al*. 1995)	II
ML primers (Luton *et al*. 2002)	II-IV
Archaeal 16S rRNA gene PCR	IV
Bacterial 16S rRNA gene PCR	III
DGGE	I
T-RFLP	II, IV
Cloning	I-IV
RFLP screening of clone libraries	I-IV
Plasmid extraction	I, II, III
DNA sequencing	I-IV
Data analysis and statistics	
Phylogenetic analysis	I-IV
Bootstrapping	I-IV
Rarefaction analysis	I, II
Coverage values	I, III
Cluster analysis	I, III
Diversity indices	I-III
Multivariate analysis (PCA, RDA) and ANOSIM	IV
Two-way ANOVA	I, III

4 Results

4.1 Potential CH_4 production in relation to environmental gradients (I-IV)

Methane production rates from endogenous substrates at temperatures close to *in situ* conditions (10 or 14 °C) varied from 0 to ~30 nmol g^{-1} h^{-1} (Table 4). The lowest rates were measured in the drained and pristine bogs and in autumn and summer samples from Siikaneva fen. The rates were highest in the minerotrophic fens of the Lakkasuo mire complex, both less acidic than Siikaneva fen (Table 4).

Table 4. Potential CH_4 production at the depths of highest CH_4 production and pH in studied mires.

Mire	Gradient	pH	Depth from (cm)		CH_4 production (nmol $gdw^{-1}h^{-1}$)[a]	Study
			water table	peat surface		
Lakkasuo mire complex	ombrotr. bog	4.0-4.3	–20	–45	4.8 ± 6.3	III
	oligotrophic fen	4.9-5.0	0	–16	19.3 ± 14.0	
	mesotrophic fen	5.0-5.5	–10	–10	16.2 ± 14.9	
Siikaneva	autumn	3.9-4.3	–24	–20	0.4 ± 0.4	IV
	winter		– [b]	–20	11.9 ± 6.1	
	spring		–16	–20	7.4 ± 4.5	
	summer		3	–20	1.5 ± 0.6	
Pelso-Resula	control (no ash)	3.7-4.2	–20	–46	2.4 ± 4.8	I, II
	ash-fertilized	3.7-4.7	–10	–45	5.8 ± 7.4	

[a] Mean ± standard deviation, n=3. Incubation temperature for Pelso-Resula and Lakkasuo 10 °C and for Siikaneva 14 °C. Gdw, grams dry weight.
[b] not determined because peat was frozen to the depth of 10-15 cm

The production potential of Lakkasuo fens was approximately four times that of the bog (Table 4, Table 1 in **III**). In Siikaneva fen, there were seasonal differences in the production at the depths of 10 and 20 cm, with unexpectedly large potential in winter (Table 4, Fig. 3). There was no difference in the CH_4 production potential between the control and ash-fertilized sites of the drained Pelso-Resula bog (Table 4, Table 1 in **I**).

In the seasonal study on Siikaneva, the sampling depths of 10, 20, and 50 cm were kept constant from peat surface to allow sampling the same layer despite water level fluctuations. Production was generally largest at 20 cm (Fig. 3). This depth was above water table in August sampling, but even samples from the depth of 10 cm produced substantial amounts of CH_4 (Fig. 3). In Pelso-Resula and Lakkasuo, depth distribution of CH_4 production potential was examined from the water table to 40 cm below it. Production was greater 0-20 cm below the water table than in deeper peat, 30 and 40 cm below the water table (Table 1 in **I**; Table 1 in **III**).

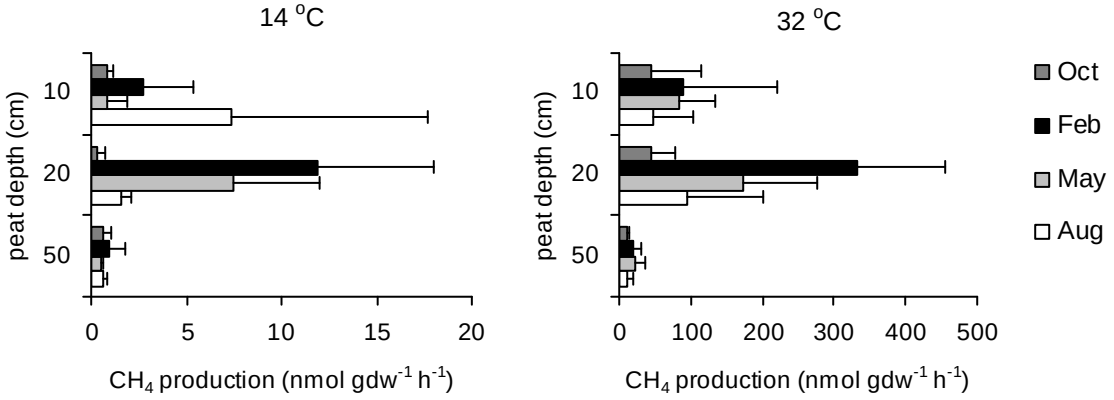

Figure 3. Depth profile of CH_4 production potential in Siikaneva fen at four times of year at 14 °C and 32 °C. Mean + standard deviation, n=3; gdw, grams dry weight. Note the different scales on x-axis.

Temperature response of CH_4 production potential in Siikaneva was determined at temperatures from 5 to 43 °C. No obvious seasonal shift in the temperature of maximal production was observed for peat from the depth of 20 cm (Fig. 4 in **IV**), and the depth distribution of CH_4 production remained similar with temperature (Fig. 3). Production was low at 5 and 14 °C, resembling the field temperature range, and substantially higher from 25 to ~35 °C with apparent optimum at ~30 °C and clear reduction above 35 °C (Fig. 4 in **IV**).

4.2 Methanogen groups (I-IV)

The methanogen groups detected as clones or T-RFLP peaks and identified by sequencing and phylogenetic analysis are summarized in Table 5, Fig. 4, and Fig. 5. *Methanomicrobiales*-associated Fen cluster (FC), Rice cluster I (RCI) or Rice cluster II (RCII), and *Methanosarcinaceae* occurred at all three mires. *Methanosaetaceae* and *Methanobacteriaceae* were only detected in the fens. *Methanosaetaceae* in Siikaneva fen were detected from RNA but not from DNA (Fig. 1 in **IV**). The year-round occurrence of FC in Siikaneva was additionally verified by PCR with specific 16S rRNA gene primers (**IV**). Lakkasuo bog and Siikaneva fen revealed similar FC and RCII 16S rRNA gene sequences with identities of 98-99%, but other groups detected in Siikaneva were absent from the bog (Table 5, Fig. 5). In Lakkasuo fens, one *mcrA* sequence type could not be assigned to any known group of methanogens (Ug in Table 5, sequence Lak19 in Fig. 4). This unidentified sequence cluster showed a very distant affiliation with *mcrA* sequences of anaerobic methane-oxidizing archaea (ANME-1) (Fig. 4).

Table 5. Methanogen groups detected in three boreal mires.

Mire	Marker gene	Gradient	Methanogen group[a]							Study
			FC	RCI	RCII	Ms	Mt	Mb[b]	Ug	
Lakkasuo mire complex	*mcrA*	ombrotrophic bog	●	●						III
		oligotrophic fen	●	●		+	●		+	
		mesotrophic fen	●	●			●	+	+	
Siikaneva	16S rRNA	autumn	●		●	●	+	+		IV
		winter	●		●	●	+	+		
		spring	●		●	●	+	+		
		summer	●		●	●	+	+		
Pelso-Resula	*mcrA*	control	●	●		+				I, II
		ash-fertilized	●	●		+				

[a] **FC** Fen cluster, **RCI** Rice cluster I, **RCII** Rice cluster II, **Ms** *Methanosarcinaceae*, **Mt** *Methanosaetaceae*, **Mb** *Methanobacteriaceae*, **Ug** unidentified *mcrA* group, ● prominent group, + minor group based on relative proportion of clones or terminal restriction fragments

[b] Detection in Siikaneva based on a shared terminal restriction fragment and RFLP pattern of *Methanobacteriaceae* and Lake Dagow Sediment euryarchaea

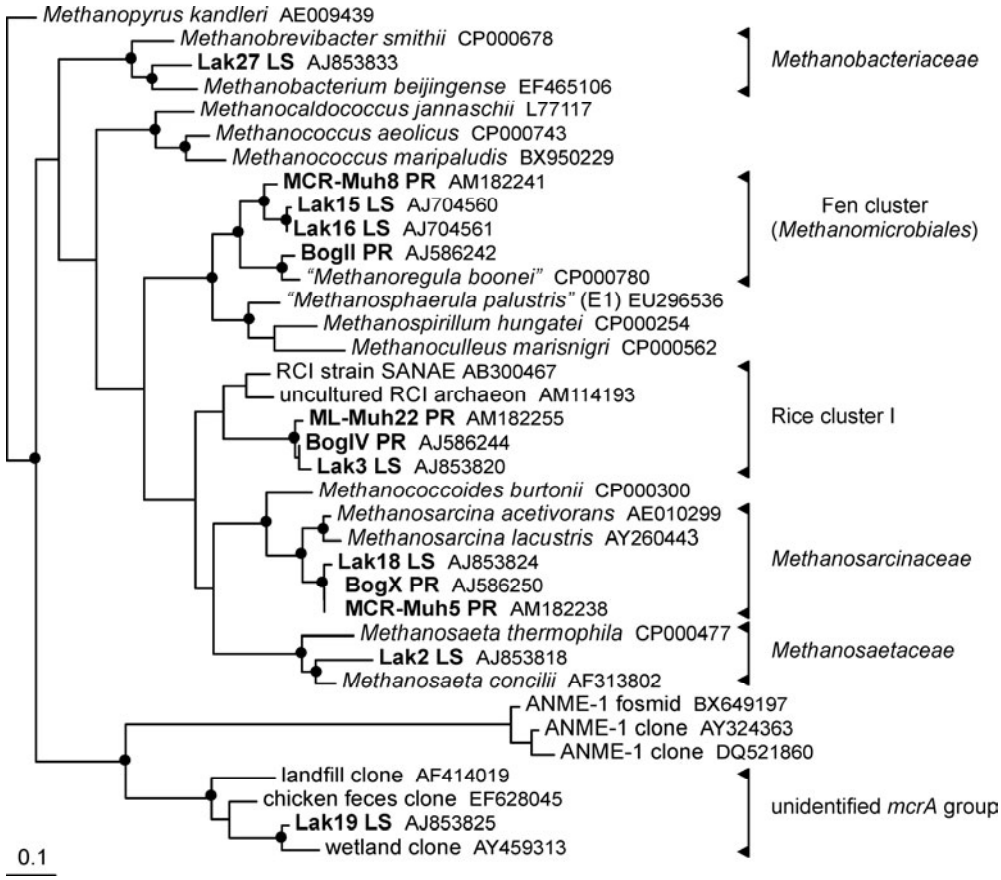

Figure 4. Maximum likelihood tree of *mcrA* sequences from the drained Pelso-Resula bog (**PR**) and Lakkasuo mire complex (**LS**). The tree was constructed from inferred amino acid sequences (130 aa) as in Study II. Scale indicates 0.1 changes per position. Filled circles mark nodes with bootstrap values >75% from 100 replicates. The sequences were selected from Studies I-III.

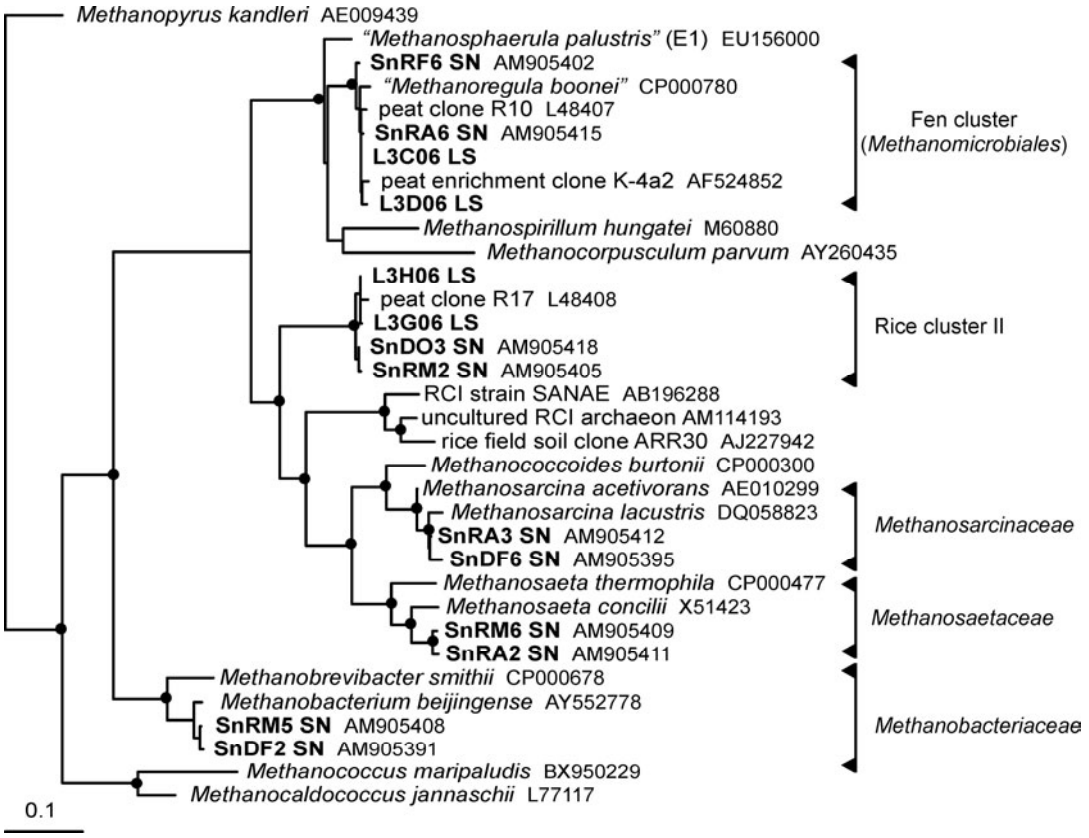

Figure 5. Maximum likelihood tree of methanogen 16S rRNA gene sequences from Siikaneva fen (**SN**) and Lakkasuo bog (**LS**). The tree was constructed from partial (~776 bp) nucleotide sequences as in Study II. Scale indicates 0.1 changes per position. Filled circles mark nodes with bootstrap values >75% from 100 replicates. The Siikaneva sequences were selected from Study IV. The Lakkasuo bog sequences originate from an unpublished clone library.

4.3 Methanogen communities in relation to environmental gradients

4.3.1 Ecohydrology (III)

Methanogens of sites forming an ecohydrological gradient from ombrotrophic bog to mesotrophic fen in the Lakkasuo mire complex were compared by RFLP and sequence analysis of *mcrA* clone libraries. The bog showed distinct communities from the fens. In the upper layer of the bog, nearly all clones belonged to Fen cluster (Fig. 6). The FC sequence types characteristic to the bog (Lak15, Lak16 in Fig. 4) were also dominant in the deeper bog layer, but rare in the oligotrophic fen and absent from the mesotrophic fen (Fig. 1 in **III**). The oligotrophic fen had 22% and the mesotrophic fen 11% of FC clones, but the sequences grouped separately from the bog sequences (Fig. 6; Fig. 1 and 3 in **III**). An archaeal 16S rDNA library was constructed and analyzed as in Study **IV** for the bog upper layer (unpublished). In the RFLP analysis, 74% of 39 clones were assigned to FC (Fig. 5), 15% to RCII, and 10% to non-methanogenic *Archaea*, hence supporting the predominance of Fen cluster in the bog.

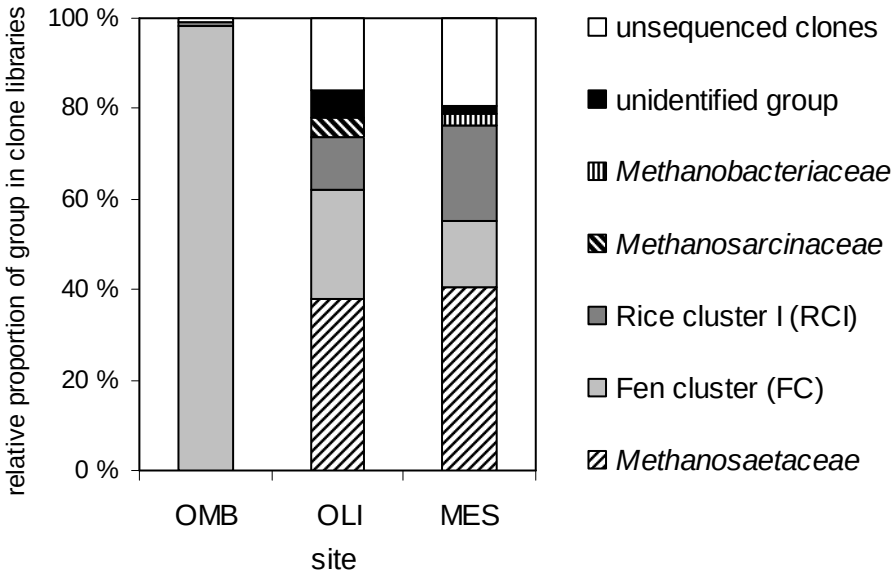

Figure 6. Distribution of methanogen groups in three Lakkasuo mire complex sites forming an ecohydrological gradient. Each column represents data from two RFLP-screened *mcrA* clone libraries from the upper peat layer (10 or 20 cm below the water table). **OMB**, ombrotrophic bog; **OLI**, oligotrophic fen; **MES**, mesotrophic fen. 'Unsequenced clones' combines minor RFLP groups from which no clones were sequenced. 'Unidentified group' refers to a sequence cluster which could not be affiliated with known methanogens (see Fig. 4).

The oligotrophic and mesotrophic fens revealed a wider range of methanogen groups, and their communities showed no substantial divergence. The largest group, constituting 38-40% of all fen clones, was *Methanosaetaceae*. This group was not detected in the upper layer of the bog and it occurred only as a rare group in the deeper bog layer (Fig. 6; Fig. 1 in **III**). RCI was detected in the fens at both depths and in the deeper bog layer, constituting 15-25% of the clones (Fig. 6). Interestingly, the unpublished bog 16S rRNA gene library revealed a small number of sequences affiliated with RCII instead (Fig. 5).

4.3.2 Season (IV)

Seasonal variation of methanogen communities in Siikaneva fen was assessed by archaeal 16S rRNA- and rDNA-based T-RFLP fingerprinting and cloning to determine whether fluctuations of temperature and CH_4 production were reflected in community composition. The analysis focused on the peat depth of 20 cm, which showed the highest methanogenic potential (Fig. 3). The major terminal restriction fragments (T-RFs) represented FC and RCII (T-RF length 393 bp), and *Methanosarcinaceae* and group 1.1c *Crenarchaeota* (186 bp). These T-RFs were detected around the year, but their relative proportions exhibited moderate temporal variation (Fig. 1 and 2 in **IV**). Redundancy analysis tentatively connected variation of DNA-derived communities to season (P=0.088). RNA-derived communities, which showed higher overall variability (Fig. 1 in **IV**), reflected differences in the CH_4 production potential (P=0.020).

One objective in the seasonal study was to determine whether methanogens are active during winter, when small CH_4 emissions have been observed at the site (Rinne *et al.* 2007, Riutta *et al.* 2007). Because detection of 16S rRNA may not conclusively indicate active archaea, winter activity of methanogens was addressed by PCR detection of *mcrA* mRNA. Successful amplification confirmed activity in winter (Fig. 5 in **IV**).

4.3.3 Peat depth (I-III)

Most of the sites showed a shift in methanogen communities between the layers with the highest CH_4 production and the deeper layers having lower capacity to produce CH_4. In the drained bog, the ME primers detected different RCI sequence types in the upper and deeper peat layer, and the ML and MCR primers supported the depth distribution (Fig. 2 in **I**, Fig. 2 in **II**). In Lakkasuo, the deeper bog layer had higher *mcrA* diversity than the FC-dominated upper layer. In the oligotrophic fen, the stratification of communities was less pronounced, and the mesotrophic fen showed no apparent stratification (Fig. 1 and 2 in **III**).

4.3.4 Ash fertilization (I, II)

Methanogen communities in the drained Pelso-Resula bog were studied by DGGE and RFLP and sequence analysis of clone libraries with *mcrA* as marker gene. Comparison of fertilized and unfertilized peat from two depths in Study **I** with the ME primers showed no major changes in the communities with ash (Fig. 2 and 3 in **I**). The most prominent sequence types were the same in unfertilized and fertilized peat, and they were affiliated with RCI (Fig. 4 in **I**). Less frequent FC sequence types forming a separate phylogenetic cluster were nearly exclusively detected in the fertilized plots (sequences T, II, and III in Fig. 2 in **I**; Fig. 4). In the four samples selected for Study **II**, where the focus was on *mcrA* primer comparison, the ML and MCR primer sets supported the detection of the main sequence types and emphasized the occurrence of the specific FC sequences (E and G in Fig. 3 in **II**) in fertilized peat.

4.4 Comparison of *mcrA* primers (II)

The ability of three *mcrA* primer sets, MCR (Springer *et al*. 1995), ME (Hales *et al*. 1996) and ML (Luton *et al*. 2002), to differentiate methanogen communities was tested with ash-fertilized and unfertilized peat sampled from two depths of the drained bog. The amplicons were compared by RFLP and sequence analysis of clone libraries. Instead of comparing the RFLP groups of individual primer sets, a sequence similarity cut off was applied to combine groups into operational taxonomic units (OTUs) to enable comparisons between the primer sets. All primer sets detected the same major OTUs affiliated with RCI and FC, but the proportions of the OTUs varied (Fig. 3 in **II**). The MCR primer set indicated presence of Fen cluster in the upper peat layer, whereas the ME and ML sets detected mainly RCI sequences (depth 1, Fig. 3 in **II**). A fifth of the sequences the MCR set recovered from upper layer peat turned out not to be *mcrA*. In the deeper layer (depth 2), the community structure depended less on the primer pair, but the ME set emphasized RCI and failed to detect *Methanosarcinaceae* when the other primers recovered the group. Each primer set also failed to detect one or more rare FC OTUs.

Several genomic sequences of *Methanosarcinales* and *Methanomicrobiales* have become available, including Fen cluster ("Candidatus *Methanoregula boonei*") and RCI genomes. The genomic full length *mcrA* sequences, previously unavailable for these groups, allow *in silico* determination of mismatches at primer binding sites. The most degenerate MCR primers showed none or single mismatches (Table 6). Primer ME1 had six mismatches to the only available *mcrA* sequence for the *Methanosarcinales* family *Methanosaetaceae*. The longest primer, MLf, had several mismatches to sequences of Rice cluster I, *Methanomicrobiales*, and *Methanosarcinales*. Hence, even the ML set, which otherwise performed best of the three primer pairs, may have shortcomings of coverage.

Table 6. Comparison of primer sequences to *mcrA* sequences from genomes of Rice cluster I, *Methanomicrobiales* and *Methanosarcinales*, and selected members of *Methanobacteriales* and *Methanococcales*.

Methanogen	Accession number	Mismatches against primer sequence[a]					
		MCRf	MCRr	ME1	ME2	MLf	MLr
Rice cluster I	AM114193	-	-	1	1	4	1
Methanocorpusculum labreanum	CP000559	1	-	-	1	4	1
Methanoculleus marisnigri	CP000562	1	-	1	2	3	2
"Methanoregula boonei"	CP000780	1	-	-	1	3	1
Methanospirillum hungatei	CP000254	1	-	-	1	3	1
Methanococcoides burtonii	CP000300	-	-	1	-	4	1
Methanosaeta thermophila	CP000477	-	-	6	2	3	2
Methanosarcina acetivorans	AE010299	-	-	-	1	3	1
Methanosarcina barkeri	CP000099	-	-	-	-	-	1
Methanosarcina mazei	AE008384	-	-	-	-	-	1
Methanobrevibacter smithii	CP000678	-	-	-	-	2	-
Methanococcus maripaludis	BX950229	-	-	-	1	2	1

[a] Full length *mcrA* sequences from the genomes were aligned with ClustalW and inspected against the primer sequences in GeneDoc software.

4.5 *Bacteria* in Lakkasuo (III)

In sequencing of bacterial 16S rDNA clones from the upper layer of the Lakkasuo bog and both fens, the main groups were *Deltaproteobacteria*, *Acidobacteria*, and *Verrucomicrobia* (Fig. 4 in **III**). Also sequences affiliated with *Planctomycetes*, other proteobacteria, *Spirochaetes*, *Bacteroidetes*, *Chloroflexi*, *Actinobacteria*, and three uncultured candidate divisions were retrieved. The number of bacterial phyla detected in the oligotrophic fen (9 phyla) and mesotrophic fen (10 phyla) exceeded the number recovered from the bog (4 phyla). Only 10 of the clones showed high sequence similarity to cultured species (*Deltaproteobacteria* or *Alphaproteobacteria*), but several resembled environmental sequences from peat or other acidic soils.

4.6 Non-methanogenic *Archaea* (III, IV)

Crenarchaeota of groups 1.3 and 1.1c were detected in Siikaneva at different seasons (Fig. 3 in **IV**). Non-methanogenic *Euryarchaeota* were less abundant in clone libraries, and they were related to *Thermoplasmatales,* Lake Dagow Sediment cluster (Glissman *et al.* 2004), or exceptionally small archaea from acid mine drainage (Baker *et al.* 2006) (Fig. 3 in **IV**). Among the bacterial sequences from Lakkasuo were also some crenarchaeal sequences, indicating that the applied 16S rRNA gene primers were not strictly specific to *Bacteria*. Nine sequences were recovered which showed 98-99% sequence similarity to group 1.3 crenarchaeal sequences from Siikaneva, a Siberian bog (Kotsyurbenko *et al.* 2004), and Finnish Salmisuo fen (Galand *et al.* 2003).

5 Discussion

5.1 Spatial and temporal patterns of methanogen communities and CH_4 production

Four aspects in boreal mires were considered in relation to methanogen communities and CH_4 production: 1) ecohydrological gradient from ombrotrophic bog to minerotrophic fens, 2) seasonal variation, 3) vertical distribution in peat profiles, and 4) effect of wood ash fertilization on mires drained for forestry.

The strongest variation of CH_4 production and methanogen community composition was associated to the shift from fen to bog in the Lakkasuo mire complex (**III**). The bog showed lower rates of CH_4 production than the fens and had distinct, low methanogen diversity dominated by the *Methanomicrobiales*-associated Fen cluster. The same pattern was detected in Lakkasuo in the following year (Galand *et al*. 2005), showing it was not transient. Similar dominance of FC has been observed in North American *Sphagnum* bogs with pH <4.3 (Cadillo-Quiroz *et al*. 2006). Low microbial activity in bogs has been related to low pH, low nutrient levels, and recalcitrant or even inhibitory nature of *Sphagnum* residue, making bog peat poor substrate for microbes (Van Breemen 1995, Verhoeven and Toth 1995, Bergman *et al*. 1999). Yet, comparisons of CH_4, CO_2, and acetate production rates in *Sphagnum*-dominated mires have suggested that the restriction of activity may concern methanogenesis in particular rather than total anaerobic microbial activity (Bridgham *et al*. 1998, Yavitt *et al*. 2005, Hines *et al*. 2008). Low pH as such could shape the communities and restrict acetoclastic production. Decrease of pH from 4.8 to 3.8 in incubations of Siberian bog peat reduced CH_4 production and shifted the pathway from acetoclastic to hydrogenotrophic (Kotsyurbenko *et al*. 2007). Methanogenic growth in bog peat could also be limited by lack of required trace elements such as Ni, Fe, and Co (Basiliko and Yavitt 2001). A third possibility is a competitive process, for example acetogenesis, which in some soils inhibits hydrogenotrophic methanogenesis especially at low temperatures (Schulz and Conrad 1996, Kotsyurbenko *et al*. 2001). The limitation of CH_4 production has been particularly severe at temperatures <15 °C (Bräuer *et al*. 2004, Kotsyurbenko *et al*. 2007, Hines *et al*. 2008). Similar pattern of conspicuously low CH_4 production potential at ≤15 °C and strong increase with temperature was also observed in Siikaneva fen, which has a low pH that is comparable to pH of bogs.

The low pH optimum of 5 of the isolated FC strain, "Candidatus *Methanoregula boonei*" (Bräuer *et al*. 2006a), indicates that the group is adapted to unusually low pH for methanogens, which could explain the prominence of FC in bogs. Another competitive advantage may be tolerance of low nutrient and cation levels. Strains were enriched from *Sphagnum* peat using acidic media mimicking the low ionic strength in bog pore water, and NaCl and KCl inhibited CH_4 production (Sizova *et al*. 2003, Bräuer *et al*. 2004, Bräuer *et al*. 2006b). Fen cluster sequences have been recovered in nearly all published studies of mires with pH <5.5 (Table 1) with few exceptions (Kotsyurbenko *et al*. 2004, Metje and Frenzel 2005). In peat with higher pH, other members of *Methanomicrobiales* such as the E1 group have been more prominent (Høj *et al*. 2005, 2006, Ganzert *et al*. 2007, Cadillo-Quiroz *et al*.

2008). The wide occurrence suggests that FC may be ubiquitous in the methanogenic layers of acidic, *Sphagnum*-dominated boreal and temperate mires.

In fens, the higher abundance of vascular plants such as sedges entails allocation of labile carbon as root exudates to the methanogenic layer. Compared to relatively homogenous bog peat, the root system creates a more heterogeneous environment. Root exudates have been shown to support CH_4 production from acetate (Ström *et al*. 2003). Accordingly, the oligotrophic and mesotrophic Lakkasuo fens had higher CH_4 production potential and more diverse methanogen communities including also acetoclastic *Methanosaetaceae*. The mesotrophic fen has also exhibited a higher fraction of acetoclastic methanogenesis than the bog (Galand *et al*. 2005). The nearby Siikaneva fen had lower pH and CH_4 production rates than the Lakkasuo fens, but the methanogen community was diverse and included acetoclastic groups (Table 5). A similar difference between *Sphagnum* bogs with FC-dominated community and a *Carex* fen with higher diversity and *Methanosaetaceae* has been observed in North America (Cadillo-Quiroz *et al*. 2006, 2008, Dettling *et al*. 2007). Archaeal communities in Alaskan mires also varied according to *Sphagnum* or *Carex* cover (Rooney-Varga *et al*. 2007). In a chronosequence of mires of Finnish land-uplift coast, methanogen communities of younger fens differed from a site in fen-bog transition stage (Merilä *et al*. 2006). The fen-bog transition is reflected in both pH and vegetation. However, different communities in *Eriophorum* lawn and poorer hummock with similar pH in an oligotrophic fen (Galand *et al*. 2003) further imply that not only pH but also the botanical composition of peat and substrate quality shape methanogen community composition.

The smaller pH shift and differences in vegetation and surface peat chemistry in the drained bog due to ash-fertilization (Moilanen and Silfverberg 2004) did not have substantial effects on methanogen communities or CH_4 production. Ash has affected archaeal and bacterial communities in forest humus (Fritze *et al.* 2000, Perkiömäki and Fritze 2002, Yrjälä *et al.* 2004), but at the Pelso-Resula bog the effect may have been restricted to surface peat, with the exception of a specific FC cluster occurring in fertilized peat. Although different *mcrA* primers gave to some extent contradicting results, the most prominent methanogen group in the drained bog was Rice cluster I. This hydrogenotrophic group also occurred in deeper peat of Lakkasuo bog (**III**) and Salmisuo fen (Galand *et al*. 2002). When draining lowers the water level, the methanogenic layer is lowered into more decomposed peat, and a higher fraction of organic matter is degraded aerobically. The low substrate availability may benefit RCI, because it has been enriched and isolated under low H_2 levels (Lu *et al.* 2005, Sakai *et al.* 2007). Another factor benefiting the group in the drained bog could be tolerance to oxygen. The genome sequence of a RCI archaeon revealed a large number of genes for oxygen detoxification (Erkel *et al*. 2006).

Season had a strong effect on CH_4 production potential, but the archaeal community composition was largely stable; temporal variation in rDNA- and rRNA-derived communities was observed as variation of relative proportions of T-RFs but not as their presence or absence. These results suggest that the population size or activity of methanogens varied substantially without a marked change in community structure. High production potential in winter has not been observed in previous studies that have included a winter sampling (Yavitt *et al.* 1987, Avery *et al.* 1999). The result was the opposite of what was expected: temperature, CH_4 emission and plant productivity would all favour high

potential activity in summer. The summer was, however, exceptionally dry, and the sampled layer was above water level, most likely diminishing methanogenic activity. A possible explanation for the high winter potential is substrate accumulation, which has been suggested as a reason for increasing potential towards autumn (Saarnio *et al*. 1997, Kettunen *et al*. 1999). When temperature declines in autumn, substrate-producing activity could exceed methanogenesis, leaving unused substrates in peat. Although high potential in the laboratory would not necessarily mean active production in the field at <2 °C, the detection of *mcrA* mRNA confirmed the presence of active methanogens in winter peat.

Seasonal pattern of CH_4 production differed in Swedish mire sites with distinct plant communities, and the difference was attributed to substrate supply (Bergman *et al.* 2000). A temporal community pattern in arctic peat was suggested to result from substrate availability (Høj *et al*. 2005). As there was some spatial variation in communities and CH_4 production even between the relatively similar sampling sites (Fig. 1 and 4 in **IV**), it would be worthwhile to compare substrate levels and seasonality of methanogens under a range of specific plant communities with a higher resolution fingerprinting method or a quantitative approach.

The archaeal 16S rRNA gene analysis of Siikaneva revealed RCII instead of RCI found in the *mcrA* studies of other mires (Table 5). This could simply be a difference in the occurrence of the groups, but because to date no *mcrA* sequences have been assigned to RCII, the possibility that *mcrA* primers do not detect RCII or that the sequences have erroneously been assigned to RCI should also be considered. As no members of RCII have been isolated, the methanogenic phenotype is currently assumed based on its phylogenetic position and occurrence in methanogenic soil enrichments (Grosskopf *et al.* 1998a, Lehmann-Richter *et al.* 1999).

5.2 Detection of methanogen communities – methodological considerations

Methanogen community analyses targeting the *mcrA* gene have the great advantage that the detected organisms are known to be CH_4 producers (or anaerobic CH_4 oxidizers). Several studies using the published primers have, however, questioned or revealed failings in the primers' species coverage or quantitative robustness (Lueders *et al.* 2001, Lueders and Friedrich 2003, Galand 2004, Banning *et al.* 2005, Nercessian *et al.* 2005). The comparison of three primer sets (**II**) showed that, in case of the drained bog, the choice of primer set had a minor effect on the recovered methanogen community composition but a major influence on the relative proportions of OTUs. Because each primer pair detected similar proportions in two different samples, the differences did not result from random PCR drift during amplification but more likely represented primer-dependent PCR selection (Wagner *et al.* 1994). The differing extent of primer-dependent variation between peat depths indicated that the properties of template such as species composition affected the outcome of amplification.

The ME primers have detected *Methanosarcinaceae* in the drained bog in Study **I** and in other environments (e.g. Lueders *et al.* 2001, Newberry *et al.* 2004), but the lack of detection in the samples of Study **II** and in Salmisuo fen (Galand 2004) suggests failings in amplification of this family. The ME set has also failed to detect *Methanosaetaceae* (Lueders *et al*. 2001, Banning *et al*. 2005), most likely due to mismatches in the forward primer

(Table 6). As these families comprise all acetoclastic methanogens, at worst the ME set could miss the entire acetoclastic population. Despite the poor performance of the MCR primers with our peat samples, they had the lowest number of mismatches to genome sequences and could therefore have the widest species coverage. The coverage is, however, achieved with high degeneracy, which may enhance quantitative bias in PCR when sequence variants with GC-rich primer binding sites are amplified preferentially over AT-rich ones (Polz and Cavanaugh 1998). Although the least degenerate ML primers showed several mismatches to *mcrA* sequences (Table 6), the primers have been shown to amplify *mcrA* from 23 methanogen strains representing all five orders (Luton *et al*. 2002). Our studies confirmed that they also detect Fen cluster and Rice cluster I. The ML set is thus currently the best choice for detection of methanogens in peat, although the effect of the abundant mismatches should be evaluated. The recent increase in availability of sequence data suitable for primer design, particularly for the orders *Methanosarcinales* and *Methanomicrobiales* which were previously poorly represented, makes modifying the existing primers or even designing entirely new *mcrA* primers a noteworthy option.

In addition to primers with good coverage, assembling a meaningful representation of methanogen communities requires a fingerprinting method with an appropriate level of resolution. Among the approaches used in this work, the two extremes in terms of resolution and the effort required per sample were the analysis of *mcrA* clones with two restriction enzymes (**III**), and the archaeal 16S rRNA gene T-RFLP (**IV**). The use of two restriction enzymes yielded a fine level of resolution, dividing most methanogen groups into several OTUs, but required time-consuming analysis of high numbers of clones. The 16S rRNA gene T-RFLP required considerably less time per sample, allowing analysis of a larger number of samples and replicates, but both main T-RFs were shared by two archaeal groups. The same combination of primers and restriction enzyme has extensively been used in T-RFLP analysis of rice field soil and even mire methanogens (Ramakrishan *et al*. 2001, Kotsyurbenko *et al*. 2004), but the T-RF of 393 bp shared between FC, RCII, and RCI (**IV**, Conrad *et al*. 2008), all groups commonly found in peat, makes the approach less ideal for differentiation of mire methanogens. Separating methanogen groups into OTUs revealed differences along the studied gradients, for example the change of RCI sequence types with depth in the drained bog (**I**), and the distinct FC OTUs occurring in Lakkasuo bog and fens (**III**). The *mcrA* gene has higher sequence divergence than the 16S rRNA gene (Springer *et al*. 1995), and therefore *mcrA* analysis should provide better prospects for differentiating smaller groups within methanogenic clusters and, for example, defining ecotypes (Palys *et al*. 1997, Cohan 2001). Although detection of only the terminal fragment lowers the resolution in T-RFLP, the higher resolution of *mcrA* combined with the swiftness of T-RFLP analysis may be the ideal compromise (Castro *et al.* 2005, Merilä *et al.* 2006).

In Study **IV**, comparison of DNA- and RNA-derived communities showed differences in T-RF proportions, and *Methanosaetaceae* were only detected from RNA. As the sole obligate acetoclastic methanogens, their detection is an indication of acetoclastic methanogenesis, and analysis of only DNA would have overlooked this group. RNA has been used in analysis of archaea and methanogens from other environments, but prior to this work not from peat. The analysis of *mcrA* mRNA was here restricted to PCR detection. Currently only one published study, addressing methanogens in a chemostat, has used *mcrA* mRNA in community analysis (Shigematsu *et al.* 2004). This approach of fingerprinting

mcrA expression would be interesting also for natural environments, regarding the resolution *mcrA* analysis offers. Combining the mRNA approach with qPCR could be particularly valuable, considering the proposal that cellular activity of methanogens may be a better predictor of CH_4 fluxes than changes of population size (Röling 2007).

5.3 *Bacteria* and non-methanogenic *Archaea* – interactions with methanogens

In methanogenic peat layers, non-methanogenic microbes, as substrate producers and competitors to methanogens, are essential for the regulation of methanogenic activity. The members of *Deltaproteobacteria* in Lakkasuo were related to syntrophic fermenters and could hypothetically function with hydrogenotrophic methanogens, although mere sequence similarity is insufficient to establish this conclusion. In a Florida wetland, hydrogenotrophic methanogens were visualized in vicinity of putative syntrophs (Chauhan *et al.* 2004). The other prominent groups, *Verrucomicrobia* and *Acidobacteria*, are abundant in soils but characterized isolates are few (Hugenholtz *et al.* 1998, Janssen 2006). Until recently, all known *Verrucomicrobia* isolates were carbohydrate degraders, but recent isolates from acidic hot springs are CH_4 oxidizers (Dunfield *et al.* 2007, Islam *et al.* 2008). The described members of *Acidobacteria* include heterotrophs and a phototroph (Liesack *et al.* 1994, Bryant *et al.* 2007). In accordance to their detection in highly acidic peat in Lakkasuo, *Acidobacteria* from soil have been shown to be more abundant and grow preferably at pH <6 (Sait *et al.* 2006). A study on bacteria in an acidic *Sphagnum-Carex* bog in Siberia later reported nearly exactly the same phyla as those found in Lakkasuo, including even some of the rarer groups such as *Planctomycetes*, *Chloroflexi*, *Bacteroidetes*, and candidate division OP3 (Sizova *et al.* 2006). The FISH analysis of the study suggested that, in contradiction to clone library data, *Planctomycetes* and *Alphaproteobacteria* were more abundant than *Acidobacteria* and *Verrucomicrobia*. Interestingly, the detected phyla did not markedly differ from those commonly observed in mineral soils (Janssen 2006).

Crenarchaea of group 1.3 occurred at Siikaneva and all three Lakkasuo mires, supporting the wide occurrence of the group not only in mineral soils (Ochsenreiter *et al.* 2003) but also in peat (Høj *et al.* 2006, Rooney-Varga *et al.* 2007). Group 1.1c crenarchaea occur in mesophilic, acidic soils (Jurgens *et al.* 1997, Yrjälä *et al.* 2004, Bomberg and Timonen 2007, Kemnitz *et al.* 2007, Hansel *et al.* 2008), but have not previously been detected in anoxic peat. In related crenarchaeal groups 1.1a and 1.1b, ammonium oxidizing organisms have been identified (Leininger *et al.* 2006, Nicol and Schleper 2006), but function of groups 1.1c and 1.3 is yet unknown. Members of group 1.3 were observed in close association with acetoclastic methanogens in anaerobic sludge (Collins *et al.* 2005), and it is tempting to speculate this group could be acetogenic.

6 Conclusions and future directions

Methanogen communities and CH_4 production potential differed strongly between sites with distinct ecohydrological status, namely fens and bogs. Fens revealed more diverse methanogen communities than bogs. The pristine and drained bog harboured hydrogenotrophs of Rice cluster I and Fen cluster. The results suggest that Fen cluster is ubiquitous in various types of acidic mires and particularly prominent in highly acidic *Sphagnum* bogs. In fens, root exudates of sedges supposedly promoted the obligate acetoclastic methanogens of the family *Methanosaetaceae* and potentially acetoclastic *Methanosarcinaceae*. The wide range of methanogens detected at Siikaneva fen with a bog-like pH (~4) demonstrated that pH alone did not define the community composition of fens and bogs. To identify the specific variables behind the influence of hydrology, further studies are needed addressing the effects of pH, other chemical properties, and vegetation on methanogen diversity. Such studies could also unravel the restrictions of methanogenesis in bogs and illuminate ecophysiology of mire methanogens.

The seasonal study demonstrated substantial temporal variation in potential CH_4 production and minor changes in archaeal DNA- and RNA-derived communities with season. However, due to low resolution, the T-RFLP analysis most likely missed some community shifts. Presumably, the seasonal temperature shifts primarily affected the size or activity of the methanogen community rather than its composition. Fingerprinting and quantifying *mcrA* mRNA would be a promising but methodologically challenging approach to clarify this issue. Temporal comparison of methanogen communities between distinct mire types or microsites could further resolve the extent of seasonal variation of methanogens. Another future objective could be determining whether the communities of bacterial substrate producers or substrate levels vary with season. The finding of high methanogenic potential and active methanogens in winter stresses the need to acknowledge microbial activity outside growing season.

The drained bog revealed a clear change of methanogen communities with peat depth, but ash fertilization had no substantial effects in the methanogenic peat layer. Comparison of three *mcrA* primer sets demonstrated that their coverage for methanogens from the drained bog was similar, but the quantitative representations of communities were primer-dependent. Particular care should therefore be taken in interpretation of *mcrA*-based abundance data, as opposed to merely assessing the presence or absence of taxa. One solution could be developing more quantitatively robust methods, for instance qPCR assays to monitor specific populations.

Detection of bacteria and non-methanogenic archaea showed that several wide groups commonly occurring in mineral soils, most with unknown function, also exist in acidic, anoxic peat. The next step would be assessing the occurrence and function of specific groups and identifying those interacting with methanogens either by supporting or inhibiting methanogenesis.

Overall, the results indicate that methanogen community composition reflects chemical or botanical gradients that affect CH_4 production, such as mire hydrology. Ecophysiological characterization of methanogens could thus benefit predictions of CH_4 production. The spatial heterogeneity of mires makes knowledge of peat chemistry indispensable for relating communities to CH_4 production capacity.

7 Acknowledgements

This work was carried out at the Department of Biological and Environmental Sciences, General Microbiology, University of Helsinki. I thank Professors Kielo Haahtela and Timo Korhonen for providing excellent working facilities. Financial support from the Academy of Finland (project 109816), the Emil Aaltonen Foundation, the Viikki Graduate School in Molecular Biosciences, the Research Foundation and Research Funds of the University of Helsinki, and the Finnish Concordia Fund is gratefully acknowledged.

My deepest gratitude goes to Docent Kim Yrjälä, the supervisor of this work, for all the opportunities and support. It has been a privilege to learn to do research under his enthusiastic guidance.

Professor Pertti Martikainen and Academy Professor Kaarina Sivonen are thanked for kindly reviewing this thesis and for the constructive comments. Kaarina Sivonen and Kielo Haahtela are also thanked for their encouragement and advice as members of the thesis follow-up group.

I sincerely thank my co-authors for their help, support, and scientific insights. I am indebted to Pierre Galand for paving the way as the “methanogen pioneer” of our lab and for all the guidance during the early years. Hannu Fritze with his clear-headed approach to research has always been ready to help which is greatly appreciated. Eeva-Stiina Tuittila, Jukka Laine, and Sari Juutinen are warmly thanked for fruitful and inspiring collaboration and for introducing a microbiologist to the world of mires.

The long- and short-term members and visitors of the MYM group have been a great help in the lab and good company. My appreciation goes especially to Timo for sharing the ups and downs of research and for the (occasionally bizarre) conversations, and to Anu for being such a great office, lab, and sampling companion. It has been a pleasure to work, teach, and have coffee with the people from both floors of YMBO. Thank you! Special thanks go to all of the staff who keep the place running smoothly.

My warmest appreciation belongs to my parents Anneli and Jaakko and brother Hannu. I am grateful for the continuous support, welcome if not always well-received distractions from work, and all the good times at home and abroad.

8 References

Acinas SG, Marcelino LA, Klepac-Ceraj V, Polz MF. (2004) Divergence and redundancy of 16S rRNA sequences in genomes with multiple rrn operons. *J Bacteriol* 186: 2629-2635.

Aerts R, Verhoeven JTA, Whigham DF. (1999) Plant-mediated controls on nutrient cycling in temperate fens and bogs. *Ecology* 80: 2170-2181.

Amann RI, Ludwig W, Schleifer KH. (1995) Phylogenetic identification and *in situ* detection of individual microbial cells without cultivation. *Microbiol Rev* 59: 143-169.

Armstrong W, Justin S, Beckett PM, Lythe S. (1991) Root adaptation to soil waterlogging. *Aquat Bot* 39: 57-73.

Avery GB, Shannon RD, White JR, Martens CS, Alperin MJ. (1999) Effect of seasonal changes in the pathways of methanogenesis on the $\delta\ ^{13}C$ values of pore water methane in a Michigan peatland. *Global Biogeochem Cycles* 13: 475-484.

Baker GC, Smith JJ, Cowan DA. (2003) Review and re-analysis of domain-specific 16S primers. *J Microbiol Methods* 55: 541-555.

Baker BJ, Tyson GW, Webb RI, Flanagan J, Hugenholtz P, Allen EE, Banfield JF. (2006) Lineages of acidophilic archaea revealed by community genomic analysis. *Science* 314: 1933-1935.

Banning N, Brock F, Fry JC, Parkes RJ, Hornibrook ER, Weightman AJ. (2005) Investigation of the methanogen population structure and activity in a brackish lake sediment. *Environ Microbiol* 7: 947-960.

Bapteste E, Brochier C, Boucher Y. (2005) Higher-level classification of the *Archaea*: evolution of methanogenesis and methanogens. *Archaea* 1: 353-363.

Basiliko N, Yavitt JB. (2001) Influence of Ni, Co, Fe, and Na additions on methane production in *Sphagnum*-dominated Northern American peatlands. *Biogeochemistry* 52: 133-153.

Basiliko N, Yavitt JB, Dees PM, Merkel SM. (2003) Methane biogeochemistry and methanogen communities in two northern peatland ecosystems, New York State. *Geomicrobiol J* 20: 563-577.

Bellisario LM, Bubier JL, Moore TR, Chanton JP. (1999) Controls on CH_4 emissions from a northern peatland. *Global Biogeochem Cycles* 13: 81-91.

Bergman I, Svensson BH, 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, Lundberg P, Nilsson M. (1999) Microbial carbon mineralisation in an acid surface peat: effects of environmental factors in laboratory incubations. *Soil Biol Biochem* 31: 1867-1877.

Bergman I, Klarqvist M, 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.

Blodau C. (2002) Carbon cycling in peatlands - A review of processes and controls. *Environ Rev* 10: 111-134.

Bomberg M, Timonen S. (2007) Distribution of cren- and euryarchaeota in Scots pine mycorrhizospheres and boreal forest humus. *Microb Ecol* 54: 406-416.

Boone DR, Castenholz RW. (2001) Bergey's Manual of Systematic Bacteriology. 2nd edn. New York, USA, Springer-Verlag, pp. 721.

Bousquet P, Ciais P, Miller JB, Dlugokencky EJ, Hauglustaine DA, Prigent C *et al.* (2006) Contribution of anthropogenic and natural sources to atmospheric methane variability. *Nature* 443: 439-443.

Bräuer SL, Yavitt JB, Zinder SH. (2004) Methanogenesis in McLean Bog, an acidic peat bog in upstate New York: stimulation by H_2/CO_2 in the presence of rifampicin, or by low concentrations of acetate. *Geomicrobiol J* 21: 433-443.

Bräuer SL, Cadillo-Quiroz H, Yashiro E, Yavitt JB, Zinder SH. (2006a) Isolation of a novel acidiphilic methanogen from an acidic peat bog. *Nature* 442: 192-194.

Bräuer SL, Yashiro E, Ueno NG, Yavitt JB, Zinder SH. (2006b) Characterization of acid-tolerant H_2/CO_2-utilizing methanogenic enrichment cultures from an acidic peat bog in New York State. *FEMS Microbiol Ecol* 57: 206-216.

Bridgham SD, Updegraff K, Pastor J. (1998) Carbon, nitrogen, and phosphorus mineralization in northern wetlands. *Ecology* 79: 1545-1561.

Bryant DA, Costas AM, Maresca JA, Chew AG, Klatt CG, Bateson MM *et al.* (2007) *Candidatus* Chloracidobacterium thermophilum: an aerobic phototrophic acidobacterium. *Science* 317: 523-526.

Cadillo-Quiroz H, Bräuer SL, Yashiro E, Sun C, Yavitt JB, Zinder SH. (2006) Vertical profiles of methanogenesis and methanogens in two contrasting acidic peatlands in central New York State, USA. *Environ Microbiol* 8: 1428-1440.

Cadillo-Quiroz H, Yashiro E, Yavitt JB, Zinder SH. (2008) Archaeal community in a minerotrophic fen and T-RFLP-directed isolation of a novel hydrogenotrophic methanogen. *Appl Environ Microbiol* 74: 2059-2068.

Cao M, Marshall S, Gregson K. (1996) Global carbon exchange and methane emissions from natural wetlands: Application of a process-based model. *J Geophys Res* 101: 14399-14414.

Castro H, Ogram A, Reddy KR. (2004) Phylogenetic characterization of methanogenic assemblages in eutrophic and oligotrophic areas of the Florida Everglades. *Appl Environ Microbiol* 70: 6559-6568.

Castro H, Newman S, Reddy KR, Ogram A. (2005) Distribution and stability of sulfate-reducing prokaryotic and hydrogenotrophic methanogenic assemblages in nutrient-impacted regions of the Florida Everglades. *Appl Environ Microbiol* 71: 2695-2704.

Chaban B, Ng SYM, Jarrell KF. (2006) Archaeal habitats - from the extreme to the ordinary. *Can J Microbiol* 52: 73-116.

Chanton JP, Bauer JE, Glaser PA, Siegel DI, Kelley CA, Tyler SC *et al.* (1995) Radiocarbon evidence for the substrates supporting methane formation within northern Minnesota peatlands. *Geochim Cosmochim Acta* 59: 3663-3668.

Chasar LS, Chanton JP, Glaser PH, Siegel DI, Rivers JS. (2000a) Radiocarbon and stable carbon isotopic evidence for transport and transformation of dissolved organic carbon, dissolved inorganic carbon, and CH_4 in a northern Minnesota peatland. *Global Biogeochem Cycles* 14: 1095-1108.

Chasar LS, Chanton JP, Glaser PH, Siegel DI. (2000b) Methane concentration and stable isotope distribution as evidence of rhizospheric processes: comparison of a fen and bog in the glacial Lake Agassiz peatland complex. *Ann Bot* 86: 655-663.

Chauhan A, Ogram A, Reddy KR. (2004) Syntrophic-methanogenic associations along a nutrient gradient in the Florida Everglades. *Appl Environ Microbiol* 70: 3475–3484.

Chen YH, Prinn RG. (2006) Estimation of atmospheric methane emissions between 1996 and 2001 using a three-dimensional global chemical transport model. *J Geophys Res* 111: doi:10.1029/2005JD006058.

Chen Y, Dumont MG, McNamara NP, Chamberlain PM, Bodrossy L, Stralis-Pavese N, Murrell JC. (2008) Diversity of the active methanotrophic community in acidic peatlands as assessed by mRNA and SIP-PLFA analyses. *Environ Microbiol* 10: 446-459.

Chin KJ, Lukow T, Conrad R. (1999) Effect of temperature on structure and function of the methanogenic archaeal community in an anoxic rice field soil. *Appl Environ Microbiol* 65: 2341-2349.

Chistoserdova L, Vorholt JA, Thauer RK, Lidstrom ME. (1998) C_1 transfer enzymes and coenzymes linking methylotrophic bacteria and methanogenic Archaea. *Science* 281: 99-102.

Christensen TR, Johansson T, Åkerman HJ, Mastepanov M, Malmer N, Friborg T *et al*. (2004) Thawing sub-arctic permafrost: Effects on vegetation and methane emissions. *Geophys Res Lett* 31: doi:10.1029/2003GL018680.

Clymo RS. (1984) The limits to peat bog growth. *Phil Trans R Soc B* 303: 605-654.

Cohan FM. (2001) Bacterial species and speciation. *Syst Biol* 50: 513-524.

Collins G, O'Connor L, Mahony T, Gieseke A, de Beer D, O'Flaherty V. (2005) Distribution, localization, and phylogeny of abundant populations of *Crenarchaeota* in anaerobic granular sludge. *Appl Environ Microbiol* 71: 7523-7527.

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.

Conrad R, Klose M, Noll M, Kemnitz D, Bodelier PLE. (2008) Soil type links microbial colonization of rice roots to methane emission. *Global Change Biol* 14: 657-669.

Dedysh SN, Panikov NS, Tiedje JM. (1998) Acidophilic methanotrophic communities from *Sphagnum* peat bogs. *Appl Environ Microbiol* 64: 922-929.

Dedysh SN, Pankratov TA, Belova SE, Kulichevskaya IS, Liesack W. (2006) Phylogenetic analysis and in situ identification of *Bacteria* community composition in an acidic *Sphagnum* peat bog. *Appl Environ Microbiol* 72: 2110-2117.

DeLong EF. (1992) Archaea in coastal marine environments. *Proc Natl Acad Sci* 89: 5685-5689.

DeLong EF, Pace NR. (2001) Environmental diversity of Bacteria and Archaea. *Syst Biol* 50: 470-478.

Denman SE, Tomkins NW, McSweeney CS. (2007) Quantitation and diversity analysis of ruminal methanogenic populations in response to the antimethanogenic compound bromochloromethane. *FEMS Microbiol Ecol* 62: 313-322.

Deppenmeier U. (2002) The unique biochemistry of methanogenesis. *Prog Nucleic Acid Res Mol Biol* 71: 223-283.

Dettling MD, Yavitt JB, Cadillo-Quiroz H, Sun C, Zinder H. (2007) Soil–methanogen interactions in two peatlands (bog, fen) in Central New York State. *Geomicrobiol J* 24: 247-259.

Dhillon A, Lever M, Lloyd KG, Albert DB, Sogin ML, Teske A. (2005) Methanogen diversity evidenced by molecular characterization of methyl coenzyme M reductase A (*mcrA*) genes in hydrothermal sediments of the Guaymas Basin. *Appl Environ Microbiol* 71: 4592-4601.

Dise NB, Verry ES. (2001) Suppression of peatland methane emission by cumulative sulfate deposition in simulated acid rain. *Biogeochemistry* 53: 143-160.

Duddleston KN, Kinney MA, Kiene RP, Hines ME. (2002) Anaerobic microbial biogeochemistry in a northern bog: Acetate as a dominant metabolic end product. *Global Biogeochem Cycles* 16: doi:10.1029/2001GB001402.

Dueck TA, de Visser R, Poorter H, Persijn S, Gorissen A, de Visser W *et al*. (2007) No evidence for substantial aerobic methane emission by terrestrial plants: a ^{13}C-labelling approach. *New Phytol* 175: 29-35.

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

Dunfield PF, Yuryev A, Senin P, Smirnova AV, Stott MB, Hou S *et al*. (2007) Methane oxidation by an extremely acidophilic bacterium of the phylum *Verrucomicrobia*. *Nature* 450: 879-882.

Earl J, Hall G, Pickup RW, Ritchie DA, Edwards C. (2003) Analysis of methanogen diversity in a hypereutrophic lake using PCR-RFLP analysis of *mcr* sequences. *Microb Ecol* 46: 270-278.

Edwards C, Hales BA, Hall GH, McDonald IR, Murrell JC, Pickup R *et al.* (1998) Microbiological processes in the terrestrial carbon cycle: methane cycling in peat. *Atmos Environ* 32: 3247-3255.

Ellermann J, Hedderich R, Bocher R, Thauer RK. (1988) The final step in methane formation. Investigations with highly purified methyl-CoM reductase (component C) from *Methanobacterium thermoautotrophicum* (strain Marburg). *Eur J Biochem* 172: 669-677.

Embley TM, Finlay BJ, Thomas RH, Dyal PL. (1992) The use of rRNA sequences and fluorescent probes to investigate the phylogenetic positions of the anaerobic ciliate *Metopus palaeformis* and its archaeobacterial endosymbiont. *J Gen Microbiol* 138: 1479-1487.

Erkel C, Kube M, Reinhardt R, Liesack W. (2006) Genome of Rice Cluster I *Archaea*–the key methane producers in the rice rhizosphere. *Science* 313: 370-372.

Fisher MM, Graham JM, Graham LE. (1998) Bacterial abundance and activity across sites within two northern Wisconsin *Sphagnum* bogs. *Microb Ecol* 36: 259-269.

Fisk MC, Ruether KF, Yavitt JB. (2003) Microbial activity and functional composition among northern peatland ecosystems. *Soil Biol Biochem* 35: 591-602.

Forney LJ, Zhou X, Brown CJ. (2004) Molecular microbial ecology: land of the one-eyed king. *Curr Opin Microbiol* 7: 210-220.

Franzmann PD, Liu Y, Balkwill DL, Aldrich HC, Conway de Macario E, Boone DR. (1997) *Methanogenium frigidum* sp. nov., a psychrophilic, H_2-using methanogen from Ace Lake, Antarctica. *Int J Syst Bacteriol* 47: 1068-1072.

Frenzel P, Karofeld E. (2000) CH_4 emission from a hollow-ridge complex in a raised bog: The role of CH_4 production and oxidation. *Biogeochemistry* 51: 91-112.

Fritze H, Perkiömäki J, Saarela U, Katainen R, Tikka P, Yrjälä K *et al.* (2000) Effect of Cd-containing wood ash on the microflora of coniferous forest humus. *FEMS Microbiol Ecol* 32: 43-51.

Galagan JE, Nusbaum C, Roy A, Endrizzi MG, Macdonald P, FitzHugh W *et al.* (2002) The genome of *M. acetivorans* reveals extensive metabolic and physiological diversity. *Genome Res* 12: 532-542.

Galand PE. (2004) Methanogenic *Archaea* in boreal peatlands. Dissertationes Biocentri Viikki Universitatis Helsingiensis 15/2004. PhD thesis, University of Helsinki.

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

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

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

Gans J, Wolinsky M, Dunbar J. (2005) Computational improvements reveal great bacterial diversity and high metal toxicity in soil. *Science* 309: 1387-1390.

Ganzert L, Jurgens G, Münster U, Wagner D. (2007) Methanogenic communities in permafrost-affected soils of the Laptev Sea coast, Siberian Arctic, characterized by 16S rRNA gene fingerprints. *FEMS Microbiol Ecol* 59: 476-488.

Garcia JL, Patel BKC, Ollivier B. (2000) Taxonomic, phylogenetic and ecological diversity of methanogenic *Archaea*. *Anaerobe* 6: 205-226.

Gauci V, Matthews E, Dise N, Walter B, Koch D, Granberg G, Vile M. (2004) Sulfur pollution suppression of the wetland methane source in the 20th and 21st centuries. *Proc Natl Acad Sci* 101: 12583-12587.

Glissman K, Chin KJ, Casper P, Conrad R. (2004) Methanogenic pathway and archaeal community structure in the sediment of eutrophic Lake Dagow: Effect of temperature. *Microb Ecol* 48: 389-399.

Goffredi SK, Wilpiszeski R, Lee R, Orphan VJ. (2008) Temporal evolution of methane cycling and phylogenetic diversity of archaea in sediments from a deep-sea whale-fall in Monterey Canyon, California. *ISME J* 2: 204-220.

Goodwin S, Zeikus JG. (1987) Ecophysiological adaptations of anaerobic bacteria to low pH: Analysis of anaerobic digestion in acidic bog sediments. *Appl Environ Microbiol* 53: 57.

Gorham E. (1991) Northern peatlands: Role in the carbon cycle and probable responses to climate warming. *Ecol Appl* 1: 182-195.

Gray ND, Head IM. (2001) Linking genetic identity and function in communities of uncultured bacteria. *Environ Microbiol* 3: 481-492.

Gribaldo S, Brochier-Armanet C. (2006) The origin and evolution of Archaea: a state of the art. *Phil Trans R Soc B* 361: 1007-1022.

Grosskopf R, Stubner S, Liesack W. (1998a) Novel euryarchaeotal lineages detected on rice roots and in the anoxic bulk soil of flooded rice microcosms. *Appl Environ Microbiol* 64: 4983-4989.

Grosskopf R, Janssen PH, Liesack W. (1998b) Diversity and structure of the methanogenic community in anoxic rice paddy soil microcosms as examined by cultivation and direct 16S rRNA gene sequence retrieval. *Appl Environ Microbiol* 64: 960-969.

Hales BA, Edwards C, Ritchie DA, Hall G, Pickup RW, Saunders JR. (1996) Isolation and identification of methanogen-specific DNA from blanket bog peat by PCR amplification and sequence analysis. *Appl Environ Microbiol* 62: 668-675.

Hallam SJ, Girguis PR, Preston CM, Richardson PM, DeLong EF. (2003) Identification of methyl coenzyme M reductase A (*mcrA*) genes associated with methane-oxidizing archaea. *Appl Environ Microbiol* 69: 5483-5491.

Hallam SJ, Putnam N, Preston CM, Detter JC, Rokhsar D, Richardson PM, DeLong EF. (2004) Reverse methanogenesis: Testing the hypothesis with environmental genomics. *Science* 305: 1457-1462.

Hansel CM, Fendorf S, Jardine PM, Francis CA. (2008) Changes in bacterial and archaeal community structure and functional diversity along a geochemically variable soil profile. *Appl Environ Microbiol* 74: 1620-1633.

Head IM, Saunders JR, Pickup RW. (1998) Microbial evolution, diversity, and ecology: A decade of ribosomal RNA analysis of uncultivated microorganisms. *Microb Ecol* 35: 1-21.

Hein R, Crutzen PJ, Heimann M. (1997) An inverse modeling approach to investigate the global atmospheric methane cycle. *Global Biogeochem Cycles* 11: 43-76.

Hines ME, Duddleston KN, Kiene RP. (2001) Carbon flow to acetate and C_1 compounds in northern wetlands. *Geophys Res Lett* 28: 4251-4254.

Hines ME, Duddleston KN, Rooney-Varga J, Fields D, Chanton JP. (2008) Uncoupling of acetate degradation from methane formation in Alaskan wetlands: Connections to vegetation distribution. *Global Biogeochem Cycles* doi:10.1029/2006GB002903 (in press).

Hoehler TM, Alperin MJ, Albert DB, Martens CS. (1994) Field and laboratory studies of methane oxidation in an anoxic marine sediment: Evidence for a methanogen-sulfate reducer consortium. *Global Biogeochem Cycles* 8: 451-463.

Høj L, Olsen RA, Torsvik VL. (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 LE, Olsen RA, Torsvik VL. (2006) Effects of water regime on archaeal community composition in Arctic soils. *Environ Microbiol* 8: 984-996.

Høj L, Olsen RA, Torsvik VL. (2008) Effects of temperature on the diversity and community structure of known methanogenic groups and other archaea in high Arctic peat. *ISME J* 2: 37-48.

Hori T, Haruta S, Ueno Y, Ishii M, Igarashi Y. (2006) Dynamic transition of a methanogenic population in response to the concentration of volatile fatty acids in a thermophilic anaerobic digester. *Appl Environ Microbiol* 72: 1623-1630.

Horn MA, Matthies C, Kusel K, Schramm A, Drake HL. (2003) Hydrogenotrophic methanogenesis by moderately acid-tolerant methanogens of a methane-emitting acidic peat. *Appl Environ Microbiol* 69: 74-83.

Hornibrook ERC, Longstaffe FJ, Fyfe WS. (1997) Spatial distribution of microbial methane production pathways in temperate zone wetland soils: Stable carbon and hydrogen isotope evidence. *Geochim Cosmochim Acta* 61: 745-753.

Hornibrook ERC, Longstaffe FJ, Fyfe WS. (2000) Evolution of stable carbon isotope compositions for methane and carbon dioxide in freshwater wetlands and other anaerobic environments. *Geochim Cosmochim Acta* 64: 1013-1027.

Hugenholtz P, Goebel BM, Pace NR. (1998) Impact of culture-independent studies on the emerging phylogenetic view of bacterial diversity. *J Bacteriol* 180: 6793.

Inagaki F, Tsunogai U, Suzuki M, Kosaka A, Machiyama H, Takai K *et al.* (2004) Characterization of C_1-metabolizing prokaryotic communities in methane seep habitats at the Kuroshima Knoll, southern Ryukyu Arc, by analyzing *pmoA*, *mmoX*, *mxaF*, *mcrA*, and 16S rRNA genes. *Appl Environ Microbiol* 70: 7445-7455.

IPCC. (2007) Climate change 2007: The physical science basis. Contribution of working group I to the fourth assessment report of the intergovernmental panel on climate change. Cambridge, UK and New York, USA, Cambridge University Press, pp. 996.

Ishii K, Fukui M. (2001) Optimization of annealing temperature to reduce bias caused by a primer mismatch in multitemplate PCR. *Appl Environ Microbiol* 67: 3753-3755.

Islam T, Jensen S, Reigstad LJ, Larsen O, Birkeland NK. (2008) Methane oxidation at 55 °C and pH 2 by a thermoacidophilic bacterium belonging to the *Verrucomicrobia* phylum. *Proc Natl Acad Sci* 105: 300-304.

Jaatinen K, Tuittila ES, Laine J, Yrjälä K, Fritze H. (2005) Methane-oxidizing bacteria in a Finnish raised mire complex: Effects of site fertility and drainage. *Microb Ecol* 50: 429-439.

Jaatinen K, Fritze H, Laine J, Laiho R. (2007) Effects of short- and long-term water-level drawdown on the populations and activity of aerobic decomposers in a boreal peatland. *Global Change Biol* 13: 491-510.

Janssen PH. (2006) Identifying the dominant soil bacterial taxa in libraries of 16S rRNA and 16S rRNA genes. *Appl Environ Microbiol* 72: 1719-1728.

Joabsson A, Christensen TR, Wallen B. (1999) Vascular plant controls on methane emissions from northern peatforming wetlands. *Trends Ecol Evol* 14: 385-388.

Jones WJ, Leigh JA, Mayer F, Woese CR, Wolfe RS. (1983) *Methanococcus jannaschii* sp. nov., an extremely thermophilic methanogen from a submarine hydrothermal vent. *Arch Microbiol* 136: 254-261.

Jurgens G, Lindström K, Saano A. (1997) Novel group within the kingdom *Crenarchaeota* from boreal forest soil. *Appl Environ Microbiol* 63: 803-805.

Kandler O, König H. (1998) Cell wall polymers in Archaea (Archaebacteria). *Cell Mol Life Sci* 54: 305-308.

Kelly CA, Dise NB, Martens CS. (1992) Temporal variations in the stable carbon isotopic composition of methane emitted from Minnesota peatlands. *Global Biogeochem Cycles* 6: 263-269.

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

Kemnitz D, Kolb S, Conrad R. (2007) High abundance of *Crenarchaeota* in a temperate acidic forest soil. *FEMS Microbiol Ecol* 60: 442-448.

Keppler F, Hamilton JT, Brass M, Rockmann T. (2006) Methane emissions from terrestrial plants under aerobic conditions. *Nature* 439: 187-191.

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

King JY, Reeburgh WS. (2002) A pulse-labeling experiment to determine the contribution of recent plant photosynthates to net methane emission in arctic wet sedge tundra. *Soil Biol Biochem* 34: 173-180.

Kirschbaum MUF, Bruhn D, Etheridge DM, Evans JR, Farquhar GD, Gifford RM *et al.* (2006) A comment on the quantitative significance of aerobic methane release by plants. *Funct Plant Biol* 33: 521-530.

Klenk HP, Clayton RA, Tomb JF, White O, Nelson KE, Ketchum KA *et al.* (1997) The complete genome sequence of the hyperthermophilic, sulphate-reducing archaeon *Archaeoglobus fulgidus*. *Nature* 390: 364-370.

Kobabe S, Wagner D, Pfeiffer EM. (2004) Characterisation of microbial community composition of a Siberian tundra soil by fluorescence in situ hybridisation. *FEMS Microbiol Ecol* 50: 13-23.

Koizumi Y, Takii S, Fukui M. (2004) Depth-related change in archaeal community structure in a freshwater lake sediment as determined with denaturing gradient gel electrophoresis of amplified 16S rRNA genes and reversely transcribed rRNA fragments. *FEMS Microbiol Ecol* 48: 285-292.

Koncalova H. (1990) Anatomical adaptations to waterlogging in roots of wetland graminoids: Limitations and drawbacks. *Aquat Bot* 38: 127-134.

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

Kotsyurbenko OR, Glagolev MV, Nozhevnikova AN, Conrad R. (2001) Competition between homoacetogenic bacteria and methanogenic archaea for hydrogen at low temperature. *FEMS Microbiol Ecol* 38: 153-159.

Kotsyurbenko OR, Chin KJ, Glagolev MV, Stubner S, Simankova MV, Nozhevnikova AN, Conrad R. (2004) Acetoclastic and hydrogenotrophic methane production and methanogenic populations in an acidic West-Siberian peat bog. *Environ Microbiol* 6: 1159-1173.

Kotsyurbenko OR, Friedrich MW, Simankova MV, Nozhevnikova AN, Golyshin PN, Timmis KN, Conrad R. (2007) Shift from acetoclastic to H_2-dependent methanogenesis in a west Siberian peat bog at low pH values and isolation of an acidophilic *Methanobacterium* strain. *Appl Environ Microbiol* 73: 2344-2348.

Kruger M, Meyerdierks A, Glockner FO, Amann R, Widdel F, Kube M *et al.* (2003) A conspicuous nickel protein in microbial mats that oxidize methane anaerobically. *Nature* 426: 878-881.

Krumholz LR, Hollenback JL, Roskes SJ, Ringelberg DB. (1995) Methanogenesis and methanotrophy within a *Sphagnum* peatland. *FEMS Microbiol Ecol* 18: 215-224.

Kuder T, Kruge MA. (2001) Carbon dynamics in peat bogs: Insights from substrate macromolecular chemistry. *Global Biogeochem Cycles* 15: 721-728.

Kulichevskaya IS, Pankratov TA, Dedysh SN. (2006) Detection of representatives of the *Planctomycetes* in *Sphagnum* peat bogs by molecular and cultivation approaches. *Microbiology* 75: 329-335.

Kurr M, Huber R, König H, Jannasch HW, Fricke H, Trincone A *et al.* (1991) *Methanopyrus kandleri*, gen. nov. and sp. nov. represents a novel group of hyperthermophilic methanogens, growing at 110 °C. *Arch Microbiol* 156: 239-247.

Laine J, Vasander H. (1996) Ecology and vegetation gradients of peatlands. In: Vasander H (ed.) Peatlands in Finland. Helsinki, Finnish Peatland Society, pp. 10-19.

Laine J, Minkkinen K, Laiho R, Tuittila E-S, Vasander H. (2000) Suokasvit–turpeen tekijät (Mire vegetation and peat formation). Publications from the Department of Forest Ecology, University of Helsinki 24: 1-55.

Laine J, Komulainen V, Laiho R, Minkkinen K, Rasinmäki A, Sallantaus T *et al.* (2002) Lakkasuo–opas suon ekosysteemiin (Lakkasuo–guide to a mire ecosystem). Publications from the Department of Forest Ecology, University of Helsinki 26: 1-120.

Lange M, Westermann P, Ahring BK. (2005) Archaea in protozoa and metazoa. *Appl Microbiol Biotechnol* 66: 465-474.

Lansdown JM, Quay PD, King SL. (1992) CH_4 production via CO_2 reduction in a temperate bog: a source of ^{13}C-depleted CH_4. *Geochim Cosmochim Acta* 56: 3493-3503.

Lauerer G, Kristjansson JK, Langworthy TA, König H, Stetter KO. (1986) *Methanothermus sociabilis* sp. nov., a second species within the *Methanothermaceae* growing at 97 °C. *Syst Appl Microbiol* 8: 100-105.

Lehmann-Richter S, Grosskopf R, Liesack W, Frenzel P, Conrad R. (1999) Methanogenic archaea and CO_2-dependent methanogenesis on washed rice roots. *Environ Microbiol* 1: 159-166.

Leininger S, Urich T, Schloter M, Schwark L, Qi J, Nicol GW *et al.* (2006) Archaea predominate among ammonia-oxidizing prokaryotes in soils. *Nature* 442: 806-809.

Liesack W, Bak F, Kreft JU, Stackebrandt E. (1994) *Holophaga foetida* gen. nov., sp. nov., a new, homoacetogenic bacterium degrading methoxylated aromatic compounds. *Arch Microbiol* 162: 85-90.

Liu WT, Marsh TL, Cheng H, Forney LJ. (1997) Characterization of microbial diversity by determining terminal restriction fragment length polymorphisms of genes encoding 16S rRNA. *Appl Environ Microbiol* 63: 4516-4522.

Lloyd D, Thomas KL, Hayes A, Hill B, Hales BA, Edwards C *et al.* (1998) Micro-ecology of peat: Minimally invasive analysis using confocal laser scanning microscopy, membrane inlet mass spectrometry and PCR amplification of methanogen-specific gene sequences. *FEMS Microbiol Ecol* 25: 179-188.

Lloyd KG, Lapham L, Teske A. (2006) An anaerobic methane-oxidizing community of ANME-1b archaea in hypersaline Gulf of Mexico sediments. *Appl Environ Microbiol* 72: 7218-7230.

Lösekann T, Knittel K, Nadalig T, Fuchs B, Niemann H, Boetius A, Amann R. (2007) Diversity and abundance of aerobic and anaerobic methane oxidizers at the Haakon Mosby mud volcano, Barents Sea. *Appl Environ Microbiol* 73: 3348-3362.

Lu Y, Conrad R. (2005) *In situ* stable isotope probing of methanogenic archaea in the rice rhizosphere. *Science* 309: 1088-1090.

Lu Y, Lueders T, Friedrich MW, Conrad R. (2005) Detecting active methanogenic populations on rice roots using stable isotope probing. *Environ Microbiol* 7: 326-336.

Lueders T, Friedrich MW. (2002) Effects of amendment with ferrihydrite and gypsum on the structure and activity of methanogenic populations in rice field soil. *Appl Environ Microbiol* 68: 2484-2494.

Lueders T, Friedrich MW. (2003) Evaluation of PCR amplification bias by terminal restriction fragment length polymorphism analysis of small-subunit rRNA and *mcrA* genes by using defined template mixtures of methanogenic pure cultures and soil DNA extracts. *Appl Environ Microbiol* 69: 320-326.

Lueders T, Chin KJ, Conrad R, Friedrich M. (2001) Molecular analyses of methyl-coenzyme M reductase alpha-subunit (*mcrA*) genes in rice field soil and enrichment cultures reveal the methanogenic phenotype of a novel archaeal lineage. *Environ Microbiol* 3: 194-204.

Luton PE, Wayne JM, Sharp RJ, Riley PW. (2002) The *mcrA* gene as an alternative to 16S rRNA in the phylogenetic analysis of methanogen populations in landfill. *Microbiology* 148: 3521-3530.

Marchesi JR, Weightman AJ, Cragg BA, Parkes RJ, Fry JC. (2001) Methanogen and bacterial diversity and distribution in deep gas hydrate sediments from the Cascadia Margin as revealed by 16S rRNA molecular analysis. *FEMS Microbiol Ecol* 34: 221-228.

Matthews E, Fung I. (1987) Methane emission from natural wetlands: global distribution, area, and environmental characteristics of sources. *Global Biogeochem Cycles* 1: 61-86.

McDonald IR, Upton M, Hall G, Pickup RW, Edwards C, Saunders JR *et al*. (1999) Molecular ecological analysis of methanogens and methanotrophs in blanket bog peat. *Microb Ecol* 38: 225-233.

Merilä P, Galand PE, Fritze H, Tuittila E, Kukko-oja K, Laine J, Yrjälä K. (2006) Methanogen communities along a primary succession transect of mire ecosystems. *FEMS Microbiol Ecol* 55: 221-229.

Metje M, 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.

Metje M, Frenzel P. (2007) Methanogenesis and methanogenic pathways in a peat from subarctic permafrost. *Environ Microbiol* 9: 954-964.

Mikaloff Fletcher SE, Tans PP, Bruhwiler LM, Miller JB, Heimann M. (2004) CH_4 sources estimated from atmospheric observations of CH_4 and its $^{13}C/^{12}C$ isotopic ratios: 1. Inverse modeling of source processes. *Global Biogeochem Cycles* 18: doi:10.1029/2004GB002223.

Miyajima T, Wada E, Hanba YT, Vijarnsorn P. (1997) Anaerobic mineralization of indigenous organic matters and methanogenesis in tropical wetland soils. *Geochim Cosmochim Acta* 61: 3739-3751.

Moilanen M, Silfverberg K. (2004) Short-term effects of wood ash fertilization on two drained mires in Central Finland. In: Päivänen J (ed.) Wise Use of Peatlands. Proceedings of the 12th International Peat Congress. International Peat Society, Jyväskylä, pp. 464-471.

Morales SE, Mouser PJ, Ward N, Hudman SP, Gotelli NJ, Ross DS, Lewis TA. (2006) Comparison of bacterial communities in New England *Sphagnum* bogs using terminal restriction fragment length polymorphism (T-RFLP). *Microb Ecol* 52: 34-44.

Moyer CL, Dobbs FC, Karl DM. (1994) Estimation of diversity and community structure through restriction fragment length polymorphism distribution analysis of bacterial 16S rRNA genes from a microbial mat at an active, hydrothermal vent system, Loihi Seamount, Hawaii. *Appl Environ Microbiol* 60: 871-879.

Muyzer G, Smalla K. (1998) Application of denaturing gradient gel electrophoresis (DGGE) and temperature gradient gel electrophoresis (TGGE) in microbial ecology. *Antonie Leeuwenhoek* 73: 127-141.

Nedwell DB, Watson A. (1995) CH_4 production, oxidation and emission in a UK ombrotrophic peat bog: Influence of SO_4^{2-} from acid rain. *Soil Biol Biochem* 27: 893-903.

Nercessian D, Upton M, Lloyd D, Edwards C. (1999) Phylogenetic analysis of peat bog methanogen populations. *FEMS Microbiol Lett* 173: 425-429.

Nercessian O, Bienvenu N, Moreira D, Prieur D, Jeanthon C. (2005) Diversity of functional genes of methanogens, methanotrophs and sulfate reducers in deep-sea hydrothermal environments. *Environ Microbiol* 7: 118-132.

Newberry CJ, Webster G, Cragg BA, Parkes RJ, Weightman AJ, Fry JC. (2004) Diversity of prokaryotes and methanogenesis in deep subsurface sediments from the Nankai Trough, Ocean Drilling Program Leg 190. *Environ Microbiol* 6: 274-287.

Nicol GW, Schleper C. (2006) Ammonia-oxidising *Crenarchaeota*: Important players in the nitrogen cycle? *Trends Microbiol* 14: 207-212.

Nunoura T, Oida H, Toki T, Ashi J, Takai K, Horikoshi K. (2006) Quantification of *mcrA* by quantitative fluorescent PCR in sediments from methane seep of the Nankai Trough. *FEMS Microbiol Ecol* 57: 149-157.

O'Brien J, Wolkin R, Moench T, Zeikus J. (1984) Association of hydrogen metabolism with unitrophic or mixotrophic growth of *Methanosarcina barkeri* on carbon monoxide. *J Bacteriol* 158: 373-375.

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

Ohkuma M, Noda S, Horikoshi K, Kudo T. (1995) Phylogeny of symbiotic methanogens in the gut of the termite *Reticulitermes speratus. FEMS Microbiol Lett* 134: 45-50.

Opelt K, Berg C, Schönmann S, Eberl L, Berg G. (2007a) High specificity but contrasting biodiversity of *Sphagnum*-associated bacterial and plant communities in bog ecosystems independent of the geographical region. *ISME J* 1: 502-516.

Opelt K, Chobot V, Hadacek F, Schonmann S, Eberl L, Berg G. (2007b) Investigations of the structure and function of bacterial communities associated with *Sphagnum* mosses. *Environ Microbiol* 9: 2795-2809.

Orphan VJ, House CH, Hinrichs KU, McKeegan KD, DeLong EF. (2001) Methane-consuming archaea revealed by directly coupled isotopic and phylogenetic analysis. *Science* 293: 484-487.

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

Paavilainen E, Päivänen J. (1995) Peatland Forestry–Ecology and Principles. Berlin, Springer-Verlag, pp. 248.

Palys T, Nakamura LK, Cohan FM. (1997) Discovery and classification of ecological diversity in the bacterial world: the role of DNA sequence data. *Int J Syst Bacteriol* 47: 1145-1156.

Parkes RJ, Webster G, Cragg BA, Weightman AJ, Newberry CJ, Ferdelman TG *et al.* (2005) Deep sub-seafloor prokaryotes stimulated at interfaces over geological time. *Nature* 436: 390-394.

Pearce DME, Clymo RS. (2001) Methane oxidation in a peatland core. *Global Biogeochem Cycles* 15: 709-720.

Perkiömäki J, Fritze H. (2002) Short and long-term effects of wood ash on the boreal forest humus microbial community. *Soil Biol Biochem* 34: 1343-1353.

Peters V, Conrad R. (1995) Methanogenic and other strictly anaerobic bacteria in desert soil and other oxic soils. *Appl Environ Microbiol* 61: 1673-1676.

Polz MF, Cavanaugh CM. (1998) Bias in template-to-product ratios in multitemplate PCR. *Appl Environ Microbiol* 64: 3724-3730.

Popp TJ, Chanton JP, Whiting GJ, Grant N. (1999) Methane stable isotope distribution at a *Carex* dominated fen in North Central Alberta. *Global Biogeochem Cycles* 13: 1063-1078.

Purdy KJ, Munson MA, Cresswell-Maynard T, Nedwell DB, Embley TM. (2003) Use of 16S rRNA-targeted oligonucleotide probes to investigate function and phylogeny of sulphate-reducing bacteria and methanogenic archaea in a UK estuary. *FEMS Microbiol Ecol* 44: 361-371.

Radl V, Gattinger A, Chronakova A, Nemcova A, Cuhel J, Simek M *et al.* (2007) Effects of cattle husbandry on abundance and activity of methanogenic archaea in upland soils. *ISME J* 1: 443-452.

Raghoebarsing AA, Smolders AJP, Schmid MC, Rijpstra WIC, Wolters-Arts M, Derksen J *et al.* (2005) Methanotrophic symbionts provide carbon for photosynthesis in peat bogs. *Nature* 436: 1153-1156.

Raghoebarsing AA, Pol A, van de Pas-Schoonen KT, Smolders AJ, Ettwig KF, Rijpstra WIC *et al.* (2006) A microbial consortium couples anaerobic methane oxidation to denitrification. *Nature* 440: 918-921.

Ramakrishnan B, Lueders T, Dunfield PF, Conrad R, Friedrich MW. (2001) Archaeal community structures in rice soils from different geographical regions before and after initiation of methane production. *FEMS Microbiol Ecol* 37: 175-186.

Rappe MS, Giovannoni SJ. (2003) The uncultured microbial majority. *Annu Rev Microbiol* 57: 369-394.

Raskin L, Stromley JM, Rittmann BE, Stahl DA. (1994) Group-specific 16S rRNA hybridization probes to describe natural communities of methanogens. *Appl Environ Microbiol* 60: 1232-1240.

Reeve JN, Nölling J, Morgan RM, Smith DR. (1997) Methanogenesis: Genes, genomes, and who's on first*? J Bacteriol* 179: 5975-5986.

Rheims H, Rainey FA, Stackebrandt E. (1996) A molecular approach to search for diversity among bacteria in the environment. *J Ind Microbiol Biotechnol* 17: 159-169.

Rinne J, Riutta T, Pihlatie M, Aurela M, Haapanala S, Tuovinen JP *et al.* (2007) Annual cycle of methane emission from a boreal fen measured by the eddy covariance technique. *Tellus B* 59: 449-457.

Riutta T, Laine J, Aurela M, Rinne J, Vesala T, Laurila T *et al.* (2007) Spatial variation in plant community functions regulates carbon gas dynamics in a boreal fen ecosystem. *Tellus B* 59: 838-852.

Röling WFM. (2007) Do microbial numbers count? Quantifying the regulation of biogeochemical fluxes by population size and cellular activity. *FEMS Microbiol Ecol* 62: 202-210.

Rooney-Varga JN, Giewat MW, Duddleston KN, Chanton JP, Hines ME. (2007) Links between archaeal community structure, vegetation type and methanogenic pathway in Alaskan peatlands. *FEMS Microbiol Ecol* 60: 240-251.

Rydin H, Jeglum JK. (2006) The Biology of Peatlands. New York, USA, Oxford University Press, pp. 343.

Saarnio S, Alm J, Silvola J, Lohila A, Nykänen H, Martikainen PJ. (1997) Seasonal variation in CH_4 emissions and production and oxidation potentials at microsites on an oligotrophic pine fen. *Oecologia* 110: 414-422.

Sait M, Davis KER, Janssen PH. (2006) Effect of pH on isolation and distribution of members of subdivision 1 of the phylum *Acidobacteria* occurring in soil. *Appl Environ Microbiol* 72: 1852-1857.

Sakai S, Imachi H, Sekiguchi Y, Ohashi A, Harada H, Kamagata Y. (2007) Isolation of key methanogens for global methane emission from rice paddy fields: A novel isolate affiliated with the clone cluster rice cluster I. *Appl Environ Microbiol* 73: 4326-4331.

Schimel JP. (1995) Plant transport and methane production as controls on methane flux from arctic wet meadow tundra. *Biogeochemistry* 28: 183-200.

Schink B. (1997) Energetics of syntrophic cooperation in methanogenic degradation. *Microbiol Mol Biol Rev* 61: 262-280.

Schleper C, Jurgens G, Jonuscheit M. (2005) Genomic studies of uncultivated archaea. *Nat Rev Microbiol* 3: 479-488.

Schulz S, Conrad R. (1996) Influence of temperature on pathways to methane production in the permanently cold profundal sediment of Lake Constance. *FEMS Microbiol Ecol* 20: 1-14.

Schwieger F, Tebbe CC. (1998) A new approach to utilize PCR-single-strand-conformation polymorphism for 16S rRNA gene-based microbial community analysis. *Appl Environ Microbiol* 64: 4870-4876.

Seedorf H, Dreisbach A, Hedderich R, Shima S, Thauer RK. (2004) $F_{420}H_2$ oxidase (FprA) from *Methanobrevibacter arboriphilus*, a coenzyme F_{420}-dependent enzyme involved in O_2 detoxification. *Arch Microbiol* 182: 126-137.

Segers R. (1998) Methane production and methane consumption: A review of processes underlying wetland methane fluxes. *Biogeochemistry* 41: 23-51.

Sheppard SK, McCarthy AJ, Loughnane JP, Gray ND, Head IM, Lloyd D. (2005) The impact of sludge amendment on methanogen community structure in an upland soil. *Appl Soil Ecol* 28: 147-162.

Shigematsu T, Tang Y, Kobayashi T, Kawaguchi H, Morimura S, Kida K. (2004) Effect of dilution rate on metabolic pathway shift between aceticlastic and nonaceticlastic methanogenesis in chemostat cultivation. *Appl Environ Microbiol* 70: 4048-4052.

Simankova MV, Parshina SN, Tourova TP, Kolganova TV, Zehnder AJB, Nozhevnikova AN. (2001) *Methanosarcina lacustris* sp. nov., a new psychrotolerant methanogenic archaeon from anoxic lake sediments. *Syst Appl Microbiol* 24: 362-367.

Sizova MV, Panikov NS, Tourova TP, Flanagan PW. (2003) Isolation and characterization of oligotrophic acido-tolerant methanogenic consortia from a *Sphagnum* peat bog. *FEMS Microbiol Ecol* 45: 301-315.

Smemo KA, Yavitt JB. (2007) Evidence for anaerobic CH_4 oxidation in freshwater peatlands. *Geomicrobiol J* 24: 583-597.

Smith JM, Castro H, Ogram A. (2007) Structure and function of methanogens along a short-term restoration chronosequence in the Florida Everglades. *Appl Environ Microbiol* 73: 4135-4141.

Smith JM, Green SJ, Kelley CA, Prufert-Bebout L, Bebout BM. (2008) Shifts in methanogen community structure and function associated with long-term manipulation of sulfate and salinity in a hypersaline microbial mat. *Environ Microbiol* 10: 386-394.

Springer E, Sachs MS, Woese CR, Boone DR. (1995) Partial gene sequences for the A subunit of methyl-coenzyme M reductase (*mcr*I) as a phylogenetic tool for the family *Methanosarcinaceae*. *Int J Syst Bacteriol* 45: 554-559.

Stackebrandt E, Goebel BM. (1994) Taxonomic note: a place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *Int J Syst Evol Microbiol* 44: 846-849.

Stackebrandt E, Ebers J. (2006) Taxonomic parameters revisited: tarnished gold standards. *Microbiol Today* 33: 152-155.

Ström L, Ekberg A, Mastepanov M, Christensen TR. (2003) The effect of vascular plants on carbon turnover and methane emissions from a tundra wetland. *Global Change Biol* 9: 1185-1192.

Sundh I, Nilsson M, Granberg G, Svensson BH. (1994) Depth distribution of microbial production and oxidation of methane in northern boreal peatlands. *Microb Ecol* 27: 253-265.

Suzuki MT, Giovannoni SJ. (1996) Bias caused by template annealing in the amplification of mixtures of 16S rRNA genes by PCR. *Appl Environ Microbiol* 62: 625-630.

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

Svensson BH, Sundh I. (1992) Factors affecting methane production in peat soils. *Suo* 43: 183-190.

Tatsuoka N, Mohammed N, Mitsumori M, Hara K, Kurihara M, Itabashi H. (2004) Phylogenetic analysis of methyl coenzyme-M reductase detected from the bovine rumen. *Lett Appl Microbiol* 39: 257-260.

Thauer RK. (1998) Biochemistry of methanogenesis: a tribute to Marjory Stephenson. *Microbiology* 144: 2377-2406.

Torsvik V, Øvreås L. (2002) Microbial diversity and function in soil: from genes to ecosystems. *Curr Opin Microbiol* 5: 240-245.

Torsvik V, Goksøyr J, Daae FL. (1990) High diversity in DNA of soil bacteria. *Appl Environ Microbiol* 56: 782-787.

Torsvik V, Øvreås L, Thingstad TF. (2002) Prokaryotic diversity–magnitude, dynamics, and controlling factors. *Science* 296: 1064-1066.

Turestsky MR, Wieder RK, Vitt DH, Evans RJ, Scott KD. (2007) The disappearance of relict permafrost in boreal north America: Effects on peatland carbon storage and fluxes. *Global Change Biol* 13: 1922-1934.

Turunen J, Tomppo E, Tolonen K, Reinikainen A. (2002) Estimating carbon accumulation rates of undrained mires in Finland – application to boreal and subarctic regions. *Holocene* 12: 69-80.

Ufnar JA, Ufnar DF, Wang SY, Ellender RD. (2007) Development of a swine-specific fecal pollution marker based on host differences in methanogen *mcrA* genes. *Appl Environ Microbiol* 73: 5209-5217.

Upton M, Hill B, Edwards C, Saunders J, Ritchie DA, Lloyd D. (2000) Combined molecular ecological and confocal laser scanning microscopic analysis of peat bog methanogen populations. *FEMS Microbiol Lett* 193: 275-281.

Utsumi M, Belova SE, King GM, Uchiyama H. (2003) Phylogenetic comparison of methanogen diversity in different wetland soils. *J Gen Appl Microbiol* 49: 75-83.

von Wintzingerode F, Göbel UB, Stackebrandt E. (1997) Determination of microbial diversity in environmental samples: pitfalls of PCR-based rRNA analysis. *FEMS Microbiol Rev* 21: 213-229.

Valentine DW, Holland EA, Schimel DS. (1994) Ecosystem and physiological controls over methane production in northern wetlands. *J Geophys Res* 99: 1563-1571.

van Breemen N. (1995) How *Sphagnum* bogs down other plants. *Trends Ecol Evol* 10: 270-275.

van den Pol-van Dasselaar A, Oenema O. (1999) Methane production and carbon mineralisation of size and density fractions of peat soils. *Soil Biol Biochem* 31: 877-886.

Vasander H, Kettunen A. (2006) Carbon in boreal peatlands. In: Wieder K, Vitt DH (ed.) Boreal Peatland Ecosystems. Berlin, Springer-Verlag, pp. 165–194.

Verhoeven JTA, Toth E. (1995) Decomposition of *Carex* and *Sphagnum* litter in fens: Effect of litter quality and inhibition by living tissue homogenates. *Soil Biol Biochem* 27: 271-275.

Vornolt J, Kunow J, Stetter KO, Thauer RK. (1995) Enzymes and coenzymes of the carbon monoxide dehydrogenase pathway for autotrophic CO_2 fixation in *Archaeoglobus lithotrophicus* and the lack of carbon monoxide dehydrogenase in the heterotrophic *A. profundus*. *Arch Microbiol* 163: 112-118.

Wagner A, Blackstone N, Cartwright P, Dick M, Misof B, Snow P *et al*. (1994) Surveys of gene families using polymerase chain reaction: PCR selection and PCR drift. *Syst Biol* 43: 250-261.

Wagner D, Pfeiffer EM. (1997) Two temperature optima of methane production in a typical soil of the Elbe river marshland. *FEMS Microbiol Ecol* 22: 145-153.

Walter B, Heimann M, Matthews E. (2001) Modeling modern methane emissions from natural wetlands. I. Model description and results. *J Geophys Res* 106: 34189-34206.

Watanabe T, Asakawa S, Nakamura A, Nagaoka K, Kimura M. (2004) DGGE method for analyzing 16S rDNA of methanogenic archaeal community in paddy field soil. *FEMS Microbiol Lett* 232: 153-163.

Weil CF, Cram DS, Sherf BA, Reeve JN. (1988) Structure and comparative analysis of the genes encoding component C of methyl coenzyme M reductase in the extremely thermophilic archaebacterium *Methanothermus fervidus*. *J Bacteriol* 170: 4718-4726.

Whalen SC. (2005) Biogeochemistry of methane exchange between natural wetlands and the atmosphere. *Environ Eng Sci* 22: 73-94.

Whiticar MJ, Faber E, Schoell M. (1986) Biogenic methane formation in marine and freshwater environments: CO_2 reduction vs. acetate fermentation—Isotope evidence. *Geochim Cosmochim Acta* 50: 693-709.

Williams RT, Crawford RL. (1983) Microbial diversity of Minnesota peatlands. *Microb Ecol* 9: 201-214.

Williams RT, Crawford RL. (1984) Methane production in Minnesota peatlands. *Appl Environ Microbiol* 47: 1266-1271.

Williams RT, Crawford RL. (1985) Methanogenic bacteria, including an acid-tolerant strain, from peatlands. *Appl Environ Microbiol* 50: 1542-1544.

Wilms R, Sass H, Köpke B, Cypionka H, Engelen B. (2007) Methane and sulfate profiles within the subsurface of a tidal flat are reflected by the distribution of sulfate-reducing bacteria and methanogenic archaea. *FEMS Microbiol Ecol* 59: 611-621.

Woese CR, Kandler O, Wheelis ML. (1990) Towards a natural system of organisms: Proposal for the domains Archaea, Bacteria, and Eucarya. *Proc Natl Acad Sci* 87: 4576-4579.

Wright AG, Pimm C. (2003) Improved strategy for presumptive identification of methanogens using 16S riboprinting. *J Microbiol Methods* 55: 337-349.

Wuebbles DJ, Hayhoe K. (2002) Atmospheric methane and global change. *Earth Sci Rev* 57: 177-210.

Yavitt JB, Lang GE, Wieder RK. (1987) Control of carbon mineralization to CH_4 and CO_2 in anaerobic, *Sphagnum*-derived peat from Big Run Bog, West Virginia. *Biogeochemistry* 4: 141-157.

Yavitt JB, Williams CJ, Wieder RK. (2000) Controls on microbial production of methane and carbon dioxide in three *Sphagnum*-dominated peatland ecosystems as revealed by a reciprocal field peat transplant experiment. *Geomicrobiol J* 17: 61-88.

Yavitt JB, Williams CJ, Wieder RK. (2005) Soil chemistry versus environmental controls on production of CH_4 and CO_2 in northern peatlands. *Eur J Soil Sci* 56: 169-178.

Yavitt JB, Basiliko N, Turetsky MR, Hay AG. (2006) Methanogenesis and methanogen diversity in three peatland types of the discontinuous permafrost zone, boreal western continental Canada. *Geomicrobiol J* 23: 641-651.

Yrjälä K, Katainen R, Jurgens G, Saarela U, Saano A, Romantschuk M, Fritze H. (2004) Wood ash fertilization alters the forest humus *Archaea* community. *Soil Biol Biochem* 36: 199-201.

Zellner G, Winter J. (1987) Secondary alcohols as hydrogen donors for CO_2-reduction by methanogens. *FEMS Microbiol Lett* 44: 323-328.

Zellner G, Bleicher K, Braun E, Kneifel H, Tindall BJ, Macario EC, Winter J. (1988) Characterization of a new mesophilic, secondary alcohol-utilizing methanogen, *Methanobacterium palustre* spec. nov. from a peat bog. *Arch Microbiol* 151: 1-9.