METHANE-CYCLING MICROBIAL COMMUNITIES IN PERMAFROST AFFECTED SOILS ON HERSCHEL ISLAND AND THE YUKON COAST, WESTERN CANADIAN ARCTIC

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Die Arbeiten zur vorliegenden Dissertation erfolgten hauptsächlich am Alfred-Wegener-Institut für Polar- und Meeresforschung in Potsdam. Einzelne Analysen erfolgten zusätzlich an dem Canadian National Research Council in Montréal, Kanada.

"The reward for work well done is the opportunity to do more."

Jonas Salk, American Microbiologist (1914-1995)

PREFACE

This work was funded through a doctoral scholarship from the German Environmental Foundation (DBU) to Béatrice Frank-Fahle and by the 'International Cooperation in Education and Research' program of the International Bureau of the Germany Federal Ministry of Education and Research (BMBF).

The present thesis describes the diversity, distribution and adaptation potential of methane-cycling microorganisms from the active layer of polygonal tundra found on Herschel Island and the Yukon Coast in the Canadian Western Arctic.

Field work and sampling for this study was conducted in collaboration with researchers from McGill University in Montréal, Canada during the summer expeditions Yukon Coast 2010 and 2011 on Herschel Island and on the Yukon Coast, Northwestern Canadian Arctic.

Laboratory work was mainly performed at the Alfred Wegener Institute for Polar and Marine Research, Research Unit Potsdam. Ion Torrent Sequencing analyses were performed at the National Research Council, Montréal in the research facilities of Professor Charles Greer.

The following thesis is written in English and presented as a cumulative Ph.D. thesis to the University of Potsdam (Faculty of Mathematics and Natural Sciences). It consists of a general introductory review related to the particular research field including the scientific background, the description of the study area as well as the objectives of the study. The main body of the thesis is composed of three manuscripts with first authorship. It is followed by a final synthesis including a review of the most important findings, a general conclusion, methodological remarks and future prospects.

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LIST OF ABBREVIATIONS

AMO	ammonia monooxygenase
amoA	gene encoding the α-subunit of the AMO
bp	base pairs
CH_4	methane
CO_2	carbon dioxide
EMBL	nucleotide sequence database
FAM	fluorescent dye
GenBank	international genetic database
IPCC	Intergovernmental Panel on Climate Change
kb	kilo base pairs
LIZ	internal size standard for T-RFLP
MAMO	moss-associated methane oxidation
MCR	methyl coenzyme-M reductase
mcrA	gene encoding methyl coenzyme-M reductase
MMO	methane monooxygenase
MOB	aerobic methane oxidizing bacteria
mRNA	messenger RNA
nifH	gene encoding nitrogenase reductase
NPP	net primary productivity
OTU	operational taxonomic unit
PCR	polymerase chain reaction
pMMO	particulate methane monooxygenase
pmoA	gene encoding the α-subunit of the pMMO
qPCR	quantitative (real time) PCR
rRNA	ribosomal RNA
sMMO	soluble methane monooxygenase
TOC	total organic carbon
T-RFLP	terminal restriction fragment length polymorphism

SUMMARY

Permafrost-affected ecosystems including peat wetlands are among the most obvious regions in which current microbial controls on organic matter decomposition are likely to change as a result of global warming. Wet tundra ecosystems in particular are ideal sites for increased methane production because of the waterlogged, anoxic conditions that prevail in seasonally increasing thawed layers.

The following doctoral research project focused on investigating the abundance and distribution of the methane-cycling microbial communities in four different polygons on Herschel Island and the Yukon Coast. Despite the relevance of the Canadian Western Arctic in the global methane budget, the permafrost microbial communities there have thus far remained insufficiently characterized. Through the study of methanogenic and methanotrophic microbial communities involved in the decomposition of permafrost organic matter and their potential reaction to rising environmental temperatures, the overarching goal of the ensuing thesis is to fill the current gap in understanding the fate of the organic carbon currently stored in Artic environments and its implications regarding the methane cycle in permafrost environments.

To attain this goal, a multiproxy approach including community fingerprinting analysis, cloning, quantitative PCR and next generation sequencing was used to describe the bacterial and archaeal community present in the active layer of four polygons and to scrutinize the diversity and distribution of methane-cycling microorganisms at different depths. These methods were combined with soil properties analyses in order to identify the main physico-chemical variables shaping these communities. In addition a climate warming simulation experiment was carried-out on intact active layer cores retrieved from Herschel Island in order to investigate the changes in the methane-cycling communities associated with an increase in soil temperature and to help better predict future methane-fluxes from polygonal wet tundra environments in the context of climate change.

Results showed that the microbial community found in the water-saturated and carbon-rich polygons on Herschel Island and the Yukon Coast was diverse and showed a similar distribution with depth in all four polygons sampled. Specifically, the methanogenic community identified resembled the communities found in other similar Arctic study sites and showed comparable potential methane production rates, whereas the methane oxidizing bacterial community differed from what has been found so far, being dominated by type-II rather than type-I methanotrophs. After being subjected to strong increases in soil temperature, the active-layer microbial community demonstrated the ability to quickly adapt and as a result shifts in community composition could be observed.

These results contribute to the understanding of carbon dynamics in Arctic permafrost regions and allow an assessment of the potential impact of climate change on methane-cycling microbial communities. This thesis constitutes the first in-depth study of methane-cycling communities in the Canadian Western Arctic, striving to advance our understanding of these communities in degrading permafrost environments by establishing an important new observatory in the Circum-Arctic.

ZUSAMMENFASSUNG

Permafrost beeinflusste Ökosysteme gehören zu den Regionen, in denen als Folge der globalen Erwärmung eine Veränderung des mikrobiell-kontrollierten Abbaus von organischem Material zu erwarten ist. Besonders in den Ökosystemen der feuchten Tundralandschaften kommt es zu einer verstärkten Methanpoduktion unter wassergesättigten und anoxischen Bedingungen, die durch immer tiefere saisonale Auftauschichten begünstigt werden.

Die vorliegende Doktorarbeit kontenzentrierte sich auf die Untersuchung der Abundanz und Verteilung der am Methankreislauf beteiligten mikrobiellen Gemeinschaften in vier unterschiedlichen Polygonen auf der Insel Herschel und an der Yukon Küste in Kanada. Trotz des relevanten Beitrags der kanadischen West-Arktis am globalen Methanhaushalt, sind die dortigen mikrobiellen Gemeinschaften im Permafrost bisher nur unzureichend untersucht worden.

Die zentrale Zielstellung der vorliegenden Arbeit besteht darin, die derzeitige Lücke im Verständnis der Kohlenstoffdynamik in der Arktis im Zuge von Klimaveränderungen und deren Bedeutung für den Methankreislauf in Permafrost-Ökosystemen zu schließen. Dies erfolgt durch Untersuchungen der am Abbau der organischen Substanz im Permafrost beteiligten methonogenen und methanothrophen mikrobiellen Gemeinschaften und ihrer möglichen Reaktionen auf steigende Umgebungstemperaturen.

Um dieses Ziel zu erreichen, wurde ein Multiproxy-Ansatz gewählt, der die Analyse der Gemeinschaften mittels genetischen Fingerprintmethoden, Klonierung, quantitativer PCR und moderner Hochdurchsatzsequenzierung („Next Generation Sequencing“) beinhaltet, um die in der Auftauschicht der vier untersuchten Polygone vorhandenen Bakterien- und Archaeen-Gemeinschaften zu charakterisieren sowie die Diversität und Verteilung der am Methankreislauf beteiligten Mikroorganismen in unterschiedlicher Tiefe eingehend zu analysieren. Diese Studien wurden mit physikalisch-chemischen Habitatuntersuchungen kombiniert, da diese die mikrobiellen Lebensgemeinschaften maßgeblich beeinflussen.

Zusätzlich wurde ein Laborexperiment zur Simulation der Klimaerwärmung an intakten Bodenmonolithen von der Insel Herschel durchgeführt, um die Veränderungen der am Methankreislauf beteiligten Gemeinschaften aufgrund steigender Bodentemperaturen zu untersuchen, sowie sicherere Voraussagen bezüglich der Methanfreisetzung in polygonalen Permafrostgebieten im Zusammenhang mit dem Klimawandel treffen zu können.

Die Ergebnisse zeigten, dass in den wassergesättigten und kohlenstoffreichen Polygonen der Insel Herschel und der Yukon-Küste eine diverse mikrobielle Gemeinschaft existiert, wobei die Verteilung über die Tiefe in allen vier untersuchten Polygonen ähnlich war. Insbesondere die methanogene Gemeinschaft ähnelte den Gemeinschaften, welche in ähnlichen arktischen Untersuchungsgebieten gefunden wurden und zeigte vergleichbare potenzielle Methanbildungsraten. Die Methan oxidierende bakterielle Gemeinschaft hingegen unterschied sich von bisherigen Studien und war eher von Typ-II Methanotrophen als von Typ-I Methanotrophen dominiert. Nachdem die mikrobiellen Gemeinschaften der Auftauschicht einem starken Anstieg der Bodentemperaturen ausgesetzt waren, zeigten sie die

Fähigkeit, sich schnell anzupassen und als Ergebnis dessen konnten Veränderungen in der Zusammensetzung der Gemeinschaft beobachtet werden.

Diese Ergebnisse tragen zum besseren Verständnis des Kohlenstoff-Kreislaufs in arktischen Permafrostregionen bei und ermöglichen eine Einschätzung hinsichtlich des potenziellen Einflusses des Klimawandels auf die am Methankreislauf beteiligten mikrobiellen Gemeinschaften. Mit dieser Arbeit wurde eine erste, umfassende Studie der am Methankreislauf beteiligten mikrobiellen Gemeinschaften der kanadischen West-Arktis vorgelegt, womit ein wichtiger Beitrag zum Verständnis dieser Gemeinschaften in degradierenden Permafrost-Ökosystemen geleistet wird.

I. INTRODUCTION

1.1. Scientific background

Arctic permafrost environments play a crucial role within the global carbon cycle, as northern permafrost regions contain 50% of the estimated global belowground organic carbon pool (Tarnocai *et al.*, 2009). However, with globally increasing temperatures due to climate change, these regions are recognized to be of particular significance because the permafrost is warming and degrading, releasing previously conserved organic matter in the form of greenhouse gases such as methane (CH_4) and carbon dioxide (CO_2). Because global warming is predicted to be most pronounced at northern latitudes (Figure 1) (IPCC, 2007), an increased release of these gases, especially CH_4, could have a dramatic impact on the Earth's climate system. Estimates show that up to a quarter of the carbon currently stored in permafrost soils could potentially be lost by 2100 due to global warming (Gruber 2004). In the period 1956-1990, the active layer in Russian permafrost already increased by 20 cm on average (IPCC 2007).

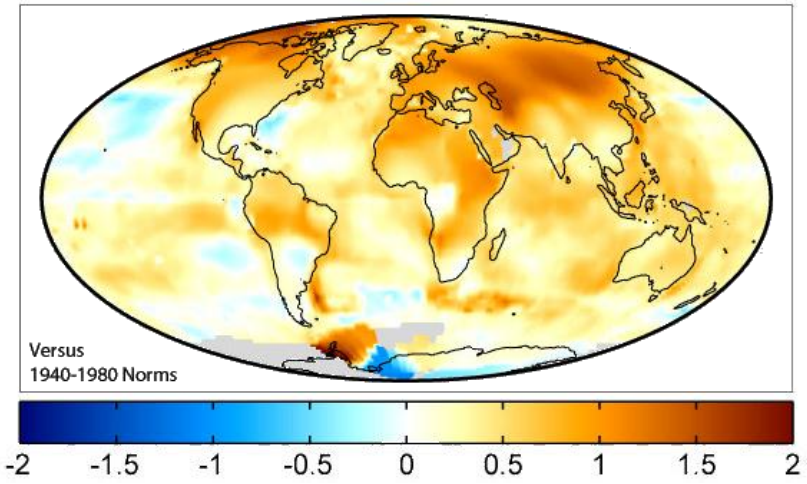

Figure 1. Difference in surface temperatures between the periods 1995 through 2004 and "normal" temperatures at the same locations, defined as the average over the interval 1940 to 1980 highlighting the immense warming of high north latitudes (modified after Robert A. Rohde, data source: Hansen et al., 2001; Reynolds et al., 2002).

Additionally, thawing permafrost will lead to increased carbon degradation and utilization by permafrost microorganisms and further affect the global carbon balance by releasing more climate relevant gases to the atmosphere. Surface temperatures are positively correlated with thawing permafrost, implying that increased temperatures will cause more permafrost to thaw. Consequently, as more organic matter is thawed, more carbon is released into the atmosphere in the form of the greenhouse gases carbon dioxide (CO_2) and methane (CH_4). As the concentration of greenhouse gases in the atmosphere increases, temperatures increase even further and the cycle continues creating a positive feedback loop (Figure 2).

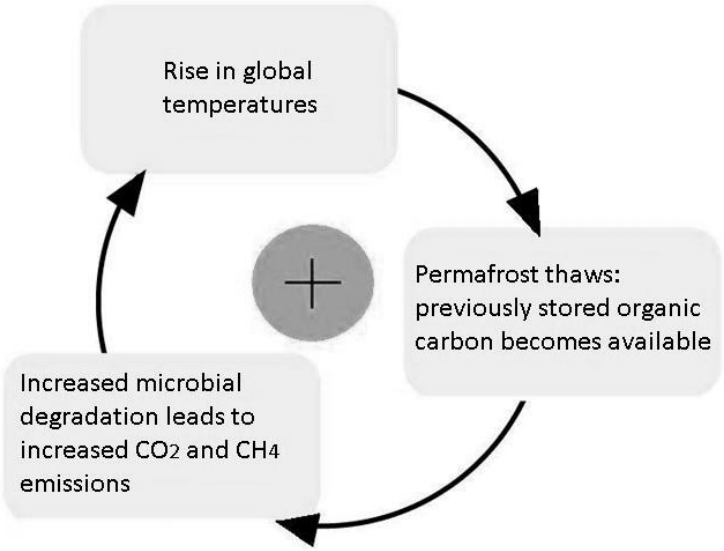

Figure 2. The positive feedback loop in degrading permafrost ecosystems (source: http://sitemaker.umich.edu/section2_group1/arctic_issues_permafrost)

Methane is chemically very active and has a 23-fold global warming potential on a per-unit-mass basis over a century compared to carbon dioxide (Zhuang et al., 2009). Approximately 18% of the current global warming can be attributed to the greenhouse effect caused by methane (Forster 2007). Between 10 and 39 Tg a^{-1} (Teragram per year) of methane are released from permafrost environments, contributing to up to 20% of global emissions (McGuire et al., 2009) making them a potent source of methane. Wet tundra ecosystems in particular are ideal sites for increased methane production because of the waterlogged, anoxic condition that prevail in seasonally increasing thawed layers (Whalen & Reeburgh, 1992). An increase in temperatures at high latitudes could potentially expose large amounts of previously conserved carbon during the next few decades (Davidson & Janssens, 2006) and lead to dramatic increases in methane and carbon dioxide production by permafrost microorganisms (Wagner, 2007). Several studies have been conducted to explore this issue in Siberia (Kobabe et al., 2004; Ganzert et al., 2007; Wagner et al., 2007; Liebner et al., 2008; Dedysh, 2009), Svalbard (Wartiainen et al., 2003; Høj et al., 2008; Graef et al., 2011) and the Canadian High Arctic (Pacheco-Oliver et al., 2002; Martineau et al., 2010; Yergeau et al., 2010) to study the characteristics and dynamics of methane-cycling communities, but despite their relevance the communities of the Canadian Western Arctic remain unexplored to date.

To better understand the fate of stored organic carbon in warming permafrost, there is a need to evaluate the abundance, function and dynamics of the microbial communities involved in its cycling and their potential reaction to the changes occurring at high latitudes. Permafrost microorganisms are highly adapted to their surrounding conditions, including extreme temperatures, freeze-thaw cycles and varying soil geochemistry, especially in the upper active layer of permafrost. Two groups of microorganisms are of particular relevance to methane cycling; methanogenic archaea and methanotrophic bacteria (refer to paragraph 4 in this chapter). Methanogenic archaea produce methane as a metabolic end-product, while

methanotrophic bacteria break down methane and utilize it as their primary energy source. Elucidating the community structure and dynamics of these microorganisms is crucial, especially regarding their adaptive abilities when confronted to rising permafrost temperatures. It is estimated that methanotrophs can oxidize up to 90% of methane produced in the soil before it can reach the atmosphere (Le Mer and Roger, 2001) making it a major sink for the methane produced by methanogens. An important concern is whether this equilibrium can hold in the context of warming temperatures, seeing that methanogens could potentially better adapt to rapid temperature fluctuations than methanotrophs (Liebner *et al.,* 2009). If this balance does not subsist and community structures are affected, this will likely lead to increased methane emissions, further impacting the Earth's climate system. The data acquired in this thesis strives to answer fundamental questions regarding the fate of the microbial methane cycle in warming permafrost environments.

1.2. Relevance of permafrost environments in the carbon cycle

Permafrost is permanently frozen ground that underlays about 25% of the exposed land area in Polar Regions (Zhang, 1999) and contains roughly one third of the world's organic carbon reserves (Tarnocai et al. 2009) (Figures 3 and 4). Permafrost soils can be divided into three zones based on temperature and depth: the "active layer" is the upper layer and is characterized by extreme temperature fluctuations between +15 and -35°C. The layer below, the upper permafrost sediments, is between 0.5 and 20m thick and has temperatures varying between 0 and -15°C. The third layer are the deeper permafrost sediments, with more stable temperatures between -5 and -10°C (French, 1996). Because of prolonged anoxic conditions and low temperatures in permafrost soils, organic matter tends to accumulate, for example through peat formation (Wagner & Liebner, 2009), and can become buried in the deeper, frozen layers through cryoturbation (Bockheim, 1998). Permafrost soils are among the most obvious environments in which current constraints on decomposition are likely to change as a result of climatic disruption, potentially exposing large amounts of previously conserved carbon during the next few decades (Davidson and Janssens, 2006). Indeed, recorded Arctic temperatures have increased to a greater extent than those of the rest of the earth (IPCC 2001). If this trend continues, it has been estimated that up to 25% of permafrost could degrade by 2100 (Anisimov, 1999) leading to a thickening of the seasonally thawed layer (active layer) releasing previously stored carbon (Nelson, 2001). In turn, intensified microbial turnover of this carbon means a dramatic increase of climatically relevant gases like methane and carbon dioxide, which represents a potential environmental hazard (Wagner *et al.*, 2005).

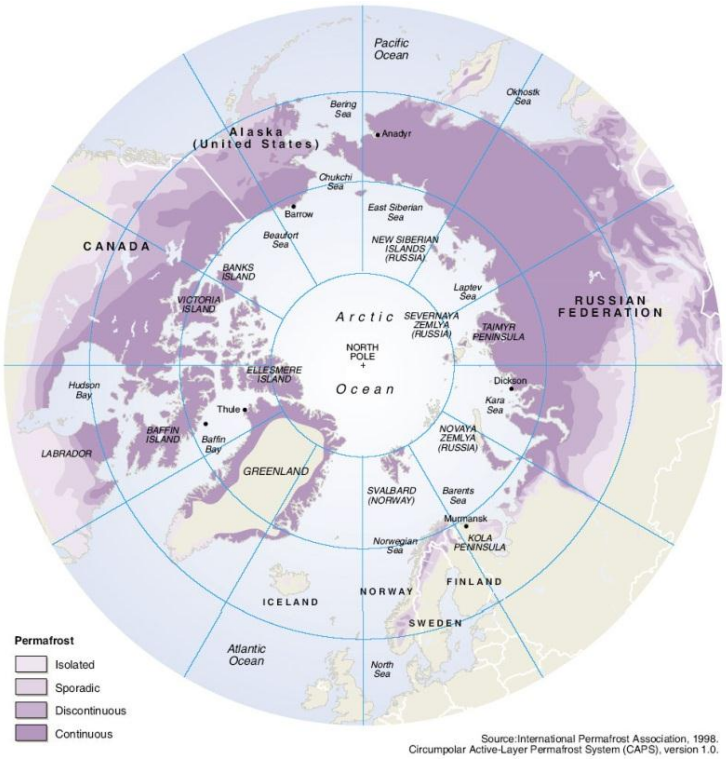

Figure 3. Latitudinal zonation of permafrost. (Source: Philippe Rekacewicz (UNEP/GRID-Arendal). Data from the International Permafrost Association, 1998. Circumpolar Active-Layer Permafrost System (CAPS), version 1.0).

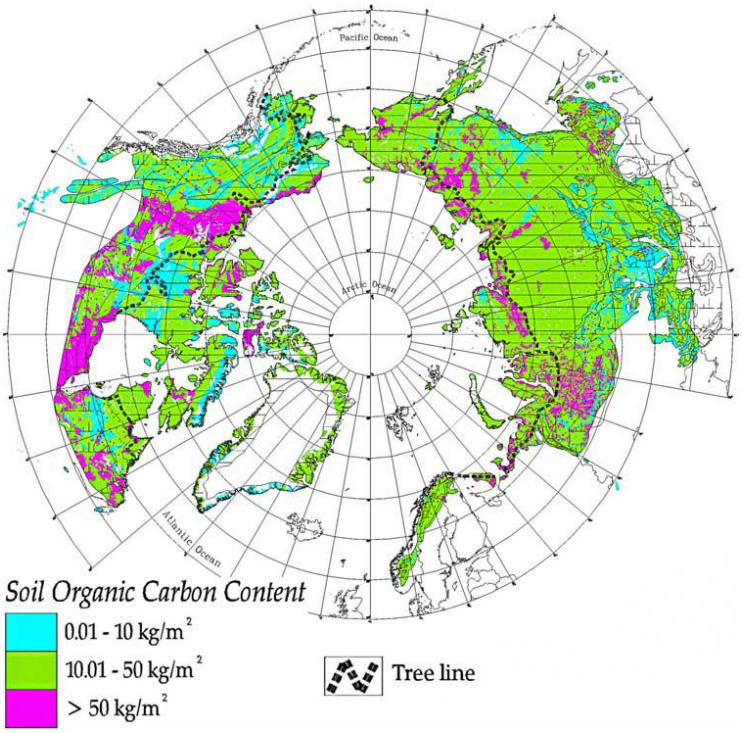

Figure 4. Distribution of soil organic carbon contents in the northern circumpolar permafrost region based on the NCSCD (Tarnocai *et al.* 2009).

1.3. Microbial processes involved in the methane cycle in permafrost

There are two main relevant microbial processes in permafrost involved in carbon cycling and methane emissions: methanogenesis, a carbon source, and methane oxidation, a carbon sink (Figure 5). Methanogenesis occurs under anoxic conditions within the predominantly wet permafrost environments, where methane is produced in the final process of hydrolysis and fermentation (Schink and Stams, 2006). The two main energy-metabolism pathways that result in methane production in low temperature environments are: the reduction of CO_2 to CH_4 and the fermentation of acetate to CH_4 and CO_2 (Conrad, 2005). Methanogenesis is driven solely by methanogenic archaea, a small group of strict anaerobes from the kingdom *Euryarchaeota* (Garcia, 2000) and can be responsible for up to 90% of total methane emissions (Ehhalt, 1978). A large part of the methane produced in permafrost environments is subsequently oxidized aerobically by methanotrophic bacteria (Hanson and Hanson, 1996). It is estimated that only 10% of the methane produced in the soil actually gets released to the atmosphere (Le Mer et Roger, 2001) making methanotrophs a major sink for the methane produced by methanogens, maintaining a relative balance (Wagner & Liebner, 2009). Moss-associated methane oxidation (MAMO) can also be an effective buffer for CH_4 emissions from permafrost-affected tundra (Liebner *et al.,* 2011) as submerged mosses are widely abundant in the polar regions. Furthermore, there are a few pathways that result in the direct release of CH_4 to the atmosphere by bypassing aerobic methane oxidation. Ebullition, for example, allows CH_4 emitted in a wetland to bypass zones of aerobic methanotrophy. Methane fluxes through plant aerenchyma can also play an important role in net CH_4 emissions from wetlands (Bridgham *et al.*, 2012). The contribution of plant-mediated CH_4 is estimated to range from ~30-100% of total CH_4 flux (Cheng *et al.*, 2006; Dorodnikov *et al.*, 2011).

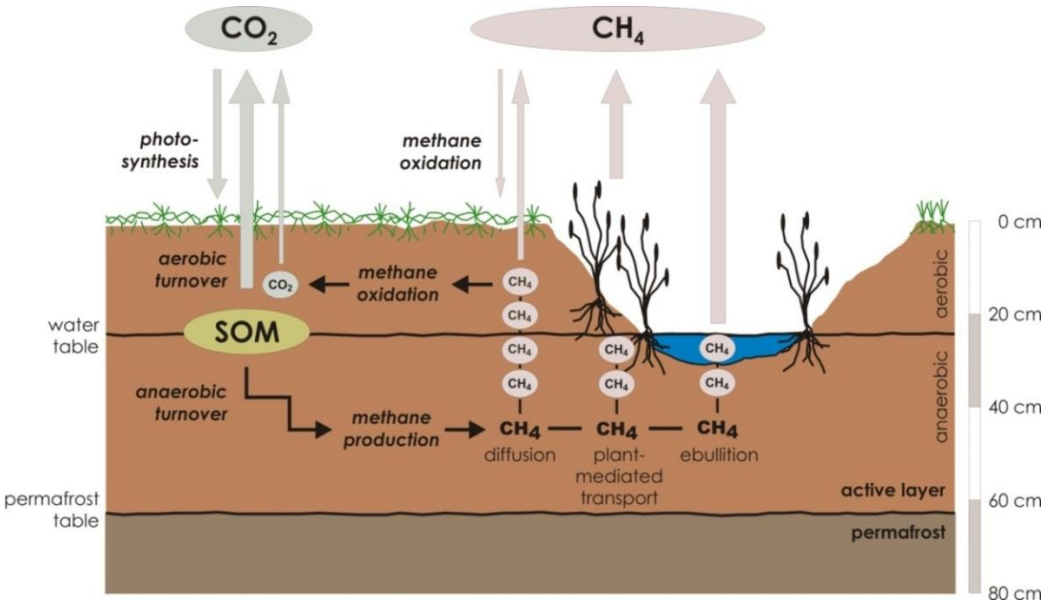

Figure 5. The carbon cycle in permafrost soils: Permafrost soils can be both a source and a sink for CO_2 and CH_4. (Wagner and Liebner, 2009)

Wagner and colleagues (2005) made the important observation that the availability of organic carbon in permafrost decreases with depth, meaning that this organic matter is less accessible to microorganisms for degradation. Liebner and colleagues (2009) posed the crucial question of whether this equilibrium will hold with the current chemical and physical changes in permafrost associated with rising temperatures. Their preliminary results indicated that while methanogens seem adapted to wider temperature fluctuations and show increased metabolic activity at higher temperatures, methanotrophs are more sensitive and show reduced activity at higher temperatures. As permafrost degrades and more organic matter becomes available, this could lead to increased methanogenesis while methanotrophy is negatively affected, clearly breaking the equilibrium.

1.4. Characterization of methane-cycling microorganisms

1.4.1. Methanogenic Archaea

Methanogenic archaea belong to the phylum *Euryarchaeota* and are ubiquitous in nature and also in extreme environments. Thanks to their unique physiology, they are able to tolerate extreme temperatures, high salinity and extreme pH-values (Wagner and Liebner, 2009). They are characteristic members of microbial communities in anoxic habitats where they are responsible for biogenic methane formation. In wet tundra soils, methanogenesis is the terminal step in the anaerobic decomposition of organic matter and is solely driven by members of *Euryarchaeota,* a small group of microorganisms which has been identified in numerous permafrost environments (Ganzert *et al.,* 2007; Steven *et al.,* 2007; Yergeau *et al.,* 2010). Active methanogenic communities make these environments a significant carbon source. Most methanogens use carbon dioxide as their electron acceptor and reduce it with hydrogen to methane. The substrates used for the methanogenic catabolic pathway can be assigned to three major groups: methylotrophic, acetoclastic and hydrogenotrophic methanogenesis. Acetoclastic methanogens include the genus *Methanosaeta* which is only able to grow on acetate. Hydrogenotrophic methanogens produce methane from hydrogen and carbon dioxide. Existing studies on methanogenesis in cold environments have discovered that methane production is limited by water availability (Høj *et al*., 2006; Christensen *et al.,* 2003) and the quality of organic matter available rather than on temperature (Wagner *et al.,* 2005, Ganzert *et al.,* 2007). Only a handful of studies exist on methanogenic populations in Arctic permafrost, (Bergman *et al*., 2000; Ganzert *et al*., 2007; Høj *et al*., 2008; Koch et al., 2009; Yergeau et al 2010). So far, only a few strains of methanogens have been isolated from Arctic environments, e.g. *Methanosarcina* species (Simankova *et al*., 2003), *Methanobacterium veterum* (Krivushin *et al.*, 2010), *Methanobacterium arcticum* (Shcherbakova *et al.*, 2011) and most recently *Methanosarcina soligelidi* (Wagner *et al.,* 2013). Nonetheless, still too little is known about the ecology and diversity of methanogens in degrading permafrost environments and their reaction to climate change.

In order to investigate the diversity of the methanogenic populations in this study, we selected a proxy for methanogenesis: the gene coding for subunit A of the methyl coenzyme-M reductase enzyme (*mcrA*). Methyl coenzyme-M is the terminal enzyme complex in the methane generation pathway, methyl coenzyme-M reductase (MCR), which catalyses the

reduction of a methyl group bound to coenzyme-M, with the accompanying release of methane (Luton *et al.*, 2002). This enzyme complex is unique and ubiquitous in methanogens (Thauer, 1998) and various studies have used it as a reliable tool for the specific detection of this group (Horz *et al.*, 2005, Lin *et al.*, 2005, Steinberg and Regan, 2008, Luke and Frenzel, 2011). The *mcrA* gene is characterized by sufficient sequence divergence to serve as a reliable diagnostic gene for the study of the methanogenic population (Luton *et al.*, 2002).

1.4.2. Methanotrophic Bacteria

Methane oxidizing bacteria (MOB) belong to the phylum *Proteobacteria: Gammaproteobacteria*, Type I and *Alphaproteobarcteria*, Type II (Hanson & Hanson, 1996) and to the phylogenetically distinct group *Verrucomicrobia* (Dunfield *et al*., 2007). MOB are present in the aerobic surface layers of the soil and in association with brown mosses such as *Sphagnum* where they play an important role in the biological oxidation of the methane produced *in-situ*. They are able to oxidize up to 90% of the methane emitted in the deeper layers before it reaches the atmosphere (Le Mer and Roger, 2001, Wagner and Liebner, 2009). The balance between methane production and oxidation is sensitive and non-linear as methanogens and methanotrophs have been observed to responds differently to temperature variations in Siberia (Ganzert *et al.*, 2007, Knoblauch *et al.*, 2008, Liebner *et al.*, 2009) and Svalbard (Høj *et al.*, 2008). Yet despite their high relevance in the global climate equation, certain polygonal tundra ecosystems in northern regions such as the Canadian Western Arctic remain poorly characterized in terms of microbial diversity and biogeochemical cycles resulting in the net release of greenhouse gases to the atmosphere. Methanotrophs can be isolated from a wide variety of environments including air, the tissues of higher organisms, soils, sediments, and freshwater and marine systems and are all obligately aerobic, gram-negative bacteria (McDonald et al., 2008). Psychrophilic and psychrotrophic MOB have been isolated from Arctic wetland soils and Siberian tundra (Wartiainen *et al.,* 2006, Omelchenko et al., 1996).

To study the diversity of methane oxidizing bacteria in polygonal tundra on Herschel Island and the Yukon coast, we selected a proxy for methane oxidation: the gene coding for subunit A of the particulate methane monooxygenase enyzyme (*pmoA*). Methane monooxygenase (MMO), found in either soluble or membrane-bound form, is responsible for the conversion of methane into methanol, which is either assimilated into biomass or oxidized to carbon dioxide (Semrau *et al.*, 1995). This gene is also characterized by sufficient sequence divergence to be used for the study of MOB (McDonald and Murrell, 1997).

1.5. Molecular ecology methods used to study methane-cycling communities

1.5.1. Culture-dependent vs. -independent methods

There are two established research approaches to investigate the microbial diversity of a given environment, culture-dependent and -independent methods (Leuko *et al*., 2007). Culture-dependent approaches to investigate the diversity of microorganisms in environmental samples are constrained by the difficulty in mimicking actual environmental growth conditions with a culture medium, resulting in the selection and enrichment of certain community representatives only. Culture-dependent techniques are generally acknowledged as being heavily biased, and the cultures obtained are unrepresentative of the distribution of microbial communities in the environment. Because cultivating microorganisms selects for some organisms while others are not culturable, these methods grossly underestimate the population diversity present in natural environments (Amann *et al*., 1995; Torsvik *et al.,* 1990). Colwell and Grimes (2000) demonstrated that only small proportions of soil microbial communities can be cultivated. Amann and colleagues (1995) estimating this number to represent less than 1% of the microorganisms actually living in the environment. Laboratory trends have therefore shifted in the past decades from culture-based methods to culture-independent methods (Øvreås and Torsvik, 1998). Culture-dependent methods are nonetheless crucial, because culturing, isolating and enriching new strains is the only way possible to describe new species based on their phenotype, providing essential information on their morphology, development, biochemical or physiological properties, metabolic products etc. Recent advances in molecular biology, however, have allowed microbial ecologists to directly access previously unexplored genetic diversity without the time consuming step of culturing. New techniques available are unaffected by the intrinsic bias introduced in culture based experiments (Babalola *et al.,* 2008), but are in turn subject to biases introduced by polymerase chain reaction (PCR), the first step in nearly all molecular biology studies (Polz and Cavanaugh, 1998; von Wintzingerode *et al.,* 1997). Advances in culture-independent molecular techniques have been applied to soil ecosystems to study microbial diversity at the molecular level.

1.5.2. Community fingerprinting methods

The application of molecular biology and genomics to environmental microbiology has enabled the proliferation of in-depth studies of the huge complexity present in natural microbial communities. The surveying of environmental biodiversity and the development of community fingerprinting methods to analyze PCR amplicons from complex environments have become routine practice in environmental microbiology studies, enabled by a range of molecular and bioinformatic techniques (Garcia-Pichel, 2008). Fingerprinting techniques generally rely on the separation and analysis of PCR products amplified from whole-community nucleic acids directly extracted from the environment (Oros-Sichler *et al.,* 2007). Different existing techniques exploit sequence variation of amplified gene fragments, such as denaturing gradient gel electrophoresis (DGGE; Muyzer *et al.,* 1993), terminal restriction fragment length polymorphism (T-RFLP; Liu *et al.,* 1997; Marsh, 1999) and automated ribosomal intergenic spacer analysis (ARISA; Fischer and Triplet, 1999).

Until recently, DGGE was one of the best molecular community fingerprinting techniques in terms of predicting the species richness and distribution (Hui *et al.,* 2008). Terminal restriction fragment length polymorphism (T-RFLP) exploit the differences in restriction enzymes digestion sites along the investigated gene (Liu *et al.,* 1997). It is a culture-independent method that was developed by Liu and colleagues (1997). In comparison to the standard 16S rRNA gene cloning approach to investigate microbial diversity, fingerprinting methods like T-RFLP hold great potential for use for rapid, high-throughput screening for differences or changes in microbial communities (Hartmann *et al*., 2005).

1.5.3. Next generation sequencing

Until recently, the most comprehensive way to analyze the biodiversity present in a sample was to build a 16S rRNA clone library and sequence as many of the resulting, unique clones as possible. However, the time cost of constructing clone libraries followed by performing capillary-based DNA sequencing is high and often limits the number of sequences investigated. Recently, there have been advances in technology that have enabled high-throughput genome sequencing to be established in research laboratories using bench-top instrumentation. These new technologies are being used to explore the vast microbial diversity in the natural environment and the untapped genetic variation that can occur in bacterial species (Hall, 2007). One of these new technologies is Ion Torrent Sequencing. This technology offers a means to more extensively sample molecular diversity in microbial populations. Hundreds of thousands of short DNA sequence reads can be generated in just under two hours. The method of Ion Torrent Sequencing was first published by Rothberg and colleagues in 2011 and is based on the detection of hydrogen ions that are released during the polymerization of DNA. A microchip on which the sample is loaded detects the release of a hydrogen ion when a nucleotide complementary to the investigated sequence is incorporated. This technology differs from other sequencing methods in that it is non-optical.

The following diagram (Figure 6) provides an overview of the combination of methods used to investigate microbial diversity and function in the polygonal tundra of Herschel Island and the Yukon Coast.

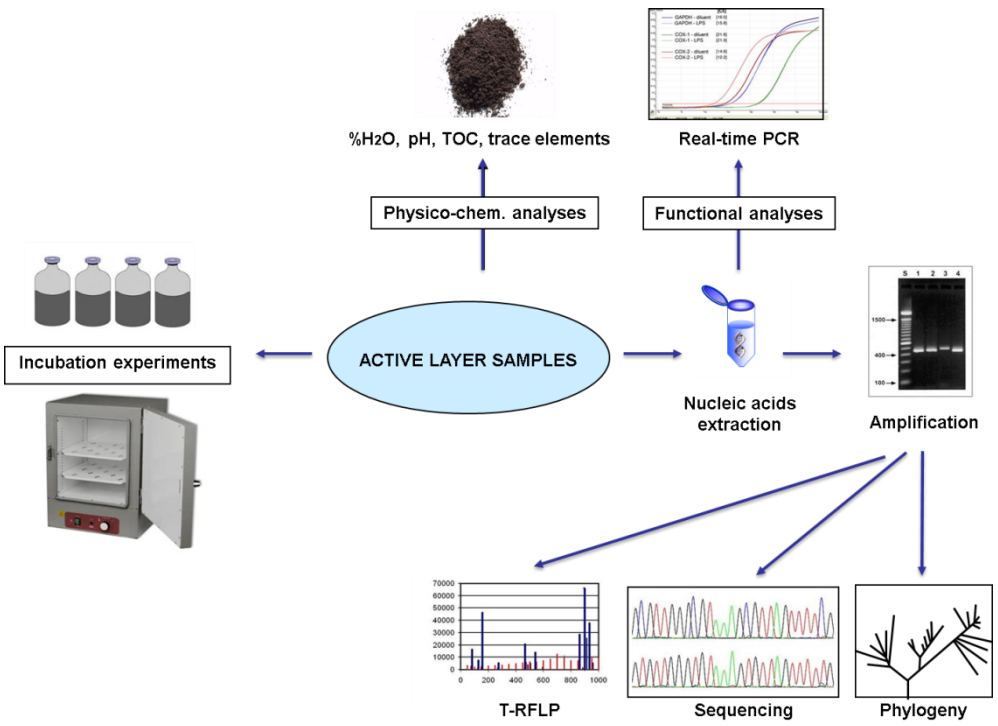

Figure 6. Overview of the methods used during this doctoral research project

1.6. Study area: Herschel Island and the Yukon Coastal Plain, Canada

Herschel Island lies in the Beaufort Sea in the northwest Canadian Arctic, 60 km from the border to Alaska within the Iwavik National Park and UNESCO World Heritage Site (Figure 7). It is situated 5 km from the mainland, its area is 112 km^2 with its highest elevation point at 182 m above sea level. The island is located in the zone of continuous permafrost and is characterized by an Artic continental climate. The Yukon Coast runs along the Beaufort Sea in the area of the Yukon Territory, which extends from the border of the U.S. state of Alaska to the border of Canada's Northwest Territory. The vegetation of the Yukon Coast and Herschel Island is described as arctic tundra, featuring the absence of trees and a dominance of dwarf shrubs, sedges and grasses, mosses, and lichens. The soils at all four sampling sites were characterized as *Hemic Glacistel* classified according to the U.S. Soil Taxonomy (Soil Survey Staff, 1998) with poor drainage and a loamy soil texture. The climate is characterized by long, cold winters and short, warm summers. The annual average temperature of the Yukon Coastal Plain is -10.2°C on the mainland and -11.3°C on Herschel Island (Burn and Zhang, 2009). A large portion of permafrost environments in the Western Canadian Arctic are characterized by patterned ground features, in particular low-centered ice-wedge polygons (Figure 8) created by cryogenic processes associated with strong seasonal freeze-thaw cycles. These low-centered polygons typically have a depressed center and elevated rim, this microrelief affecting the hydrology and organic carbon content of the soils. The polygon centres are characterized by a water level near the soil surface, which combined with the cold climatic conditions lead to an accumulation of organic matter (Shaver *et al.,* 2006).

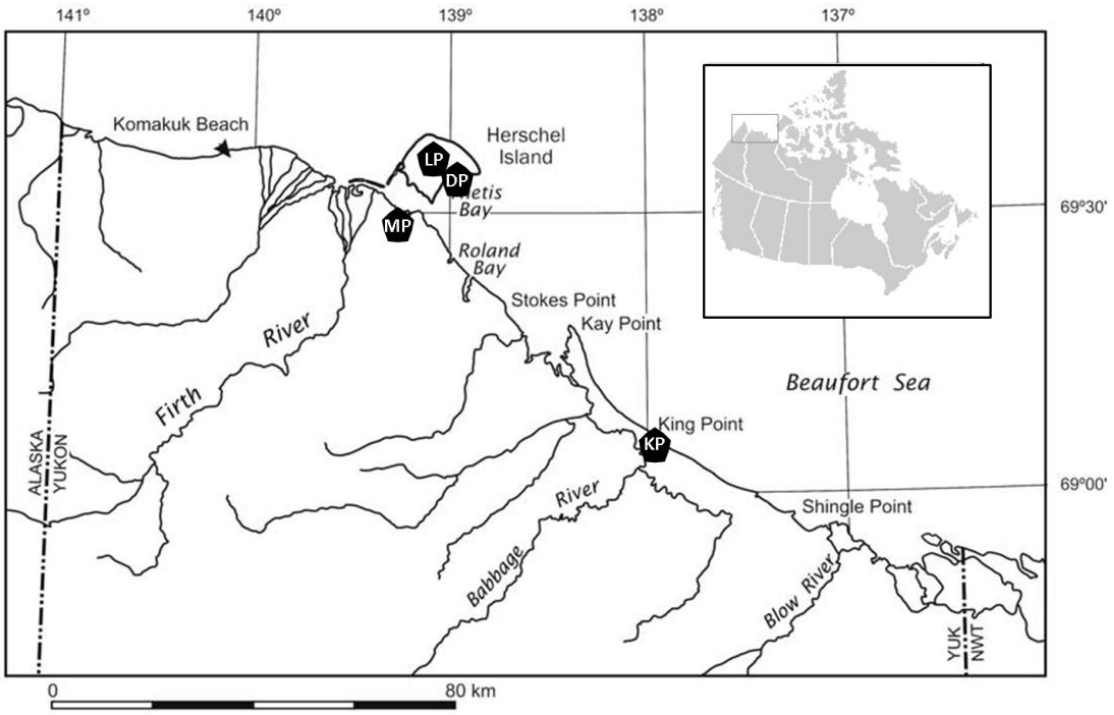

Figure 7. Map showing the geographical location of Herschel Island and the four polygon sampling sites: "Drained Lake Polygon" (DP) and "Lake Herschel Polygon" (LP) located on Herschel Island, and "Mainland Polygon" (MP) and "King Point Polygon" (KP) located on the Yukon Coast. (modified from Burn and Zhang, 2009)

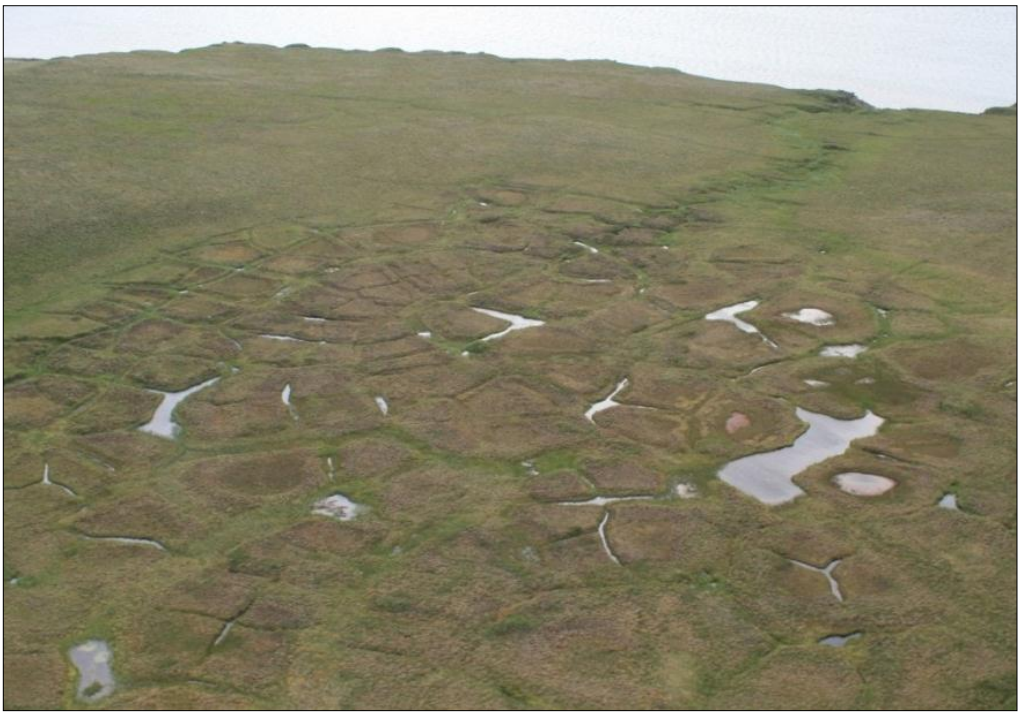

Figure 8. Aerial photography of a field of polygons on Herschel Island, Canada (B. Frank-Fahle)

The mainly anaerobic decomposition of soil organic matter in combination with the water regime and the vegetation generates high CH_4 production and emission rates from these sites (Wille *et al.,* 2008.) Today, estimates of the methane released from artic and subarctic wetlands range from 2.2% to 20% of the global methane emissions (Cao, 1998). This area of the western Canadian Arctic is being extensively studied in terms of degrading permafrost (Burn *et al*, 2009; Kokelj and Lantz, 2008). Burn and Zhang (2009) have shown that the annual average air temperatures of Herschel Island have risen by 2.5° C between the periods 1899 to 1905 and 1995 to 2007. In the same study, they have recorded an increase in permafrost thaw of 15 to 25 cm in the past twenty years. For these reasons, Herschel Island and the Yukon Coastal Plain is an ideal model system to study the fate of organic carbon stored in permafrost in response to intensified microbial degradation and the release of greenhouse gases caused by climate change.

1.7. Aims and Objectives

The main goal of this research project is to elucidate the fate of the organic carbon stored in permafrost-affected soils on Herschel Island and the Yukon Coast in the Canadian Western Arctic in the current context of climate change. This is accomplished by analyzing the abundance, dynamics and function of microbial communities involved in consuming this organic carbon, especially with respect to methane cycling. In order to attain this general goal, it is necessary to answer the following questions:

1) *Will the structure of microbial communities, particularly methanogenic and methanotrophic populations, be affected by rising permafrost temperatures?*

From existing studies and model predictions, it appears that the thawing of permafrost due to increasing temperatures in the Arctic will affect certain microbial sub-populations more than others. This study aims to use an array of culture-independent methods to clarify the diversity, abundance and dynamics of microbial communities in response to physico-chemical changes in their ecosystem caused by increasing temperatures and thawing permafrost.

2) *Are these communities flexible enough to compensate the changes occurring in their environment with regard to carbon mineralization and their function in polar ecosystems?*

Through the investigation of carbon utilization and cycling, this project aims to elucidate the role of particular microorganisms in their environment. Methane emissions are of particular interest due to their relevance in the context of global warming, and the adaptability of methanogens and methanotrophs in response to a warmer environment is crucial to maintain an equilibrium. However, preliminary evidence points towards the greater flexibility of methanogens, a major carbon source, to increasing temperatures compared to methanotrophs, a major carbon sink. The data obtained in this thesis aims to help consolidate our understanding of the carbon dynamics in melting permafrost.

1.8. Overview of manuscripts

1st Manuscript (published in 2012 in *FEMS Microbiology Ecology* **82:** 287-302)

"Methane-cycling communities in a permafrost-affected soil on Herschel Island, Western Canadian Arctic: active layer profiling of *mcrA* and *pmoA* genes."

Authors: Béatrice A. Barbier[1]*, Isabel Dziduch[1], Susanne Liebner[2#], Lars Ganzert[2§], Hugues Lantuit[1], Wayne Pollard[3] & Dirk Wagner[1#]

Aims: The aim of this study was to better understand the in situ dynamics between microbial-driven methanogenesis and methane oxidation in increasingly thawing permafrost in a low-centered, water-saturated polygon on Herschel Island, Canada.

Summary: To attain this objective, we calculated the potential methane production and oxidation rates along a vertical active layer profile. To understand abiotic factors driving methane activity, we described the physicochemical properties of the soil profile. We looked at community composition and distribution of *mcrA* and *pmoA* genes throughout the soil profile using T-RFLP analysis. We complemented the fingerprinting results by constructing clone libraries of our two genes of interest. We identified a methanotrophic community different from what was reported so far for Arctic tundra soils both in terms of community structure and potential activity. The community of methanogens was similar in composition to what we know from Arctic wet tundra and this community seems to be more stable in the circum-Arctic. We observed a clear shift from a hydrogenotrophic towards an acetoclastic community approaching the permafrost table. Such as shift though assumed to exist in tundra soils could never be shown so far.

Contribution of author and co-authors: *Béatrice Frank-Fahle* developed the structure and objectives of this study, planned and carried out field sampling and laboratory experiments, performed the data analysis and wrote the manuscript. *Isabel Dziduch* provided help with cloning and methane activity measurement. *Susanne Liebner* and *Lars Ganzert* carried out the taxonomical analysis of *pmoA* and *mcrA* sequences and contributed to the interpretation of the results and valuable discussion. *Hugues Lantuit* and *Wayne Pollard* enabled sampling and provided logistical support during the field campaign. *Dirk Wagner* contributed to structure of the manuscript, interpretation of the results and valuable discussion.

* This manuscript was published under Béatrice Frank-Fahle's maiden name (Barbier)

2nd Manuscript (submitted for publication to the *ISME Journal*)

"Microbial diversity, function and community structure in polygonal tundra from the Western Canadian Arctic using Ion Torrent sequencing and quantitative PCR."

Authors: Béatrice A. Frank-Fahle[1], Etienne Yergeau[4], Charles W. Greer[4], Hugues Lantuit[1] & Dirk Wagner[1#]

Aims : The objective of this study was to evaluate and compare the diversity and abundance of microbial communities in the active layer of four low-centered polygons on Herschel Island and the Yukon Coast, in the Canadian Western Arctic using Ion Torrent sequencing of bacterial and archaeal 16S rRNA genes and quantitative PCR of carbon- and nitrogen-cycling functional genes.

Summary: Ion Torrent sequencing of bacterial and archaeal 16S rRNA revealed the presence of all major microbial soil groups and indicated a local, vertical heterogeneity of the polygonal tundra soils with increasing depth. The overall diversity was high with Shannon indices of 5.8 for Bacteria and 4.7 for Archaea, varying only slightly between the four sampling sites. Diversity was found to be highest in the surface layers, decreasing towards the permafrost table. *Proteobacteria* and *Bacteroidetes* were the dominant members of the bacterial community at all sites. *Euryarchaeota* largely dominated the Archaeal communities, with a majority of methanogens (*Methanobacteria* and *Methanomicrobia*) found in the polygonal tundra soils. Quantitative PCR analysis of carbon and nitrogen-cycling functional genes revealed a high abundance of all genes studied, indicating that the locations studied are not nitrogen-limited, contrarily to what has been reported so far from tundra environments. This has significant implications for future carbon emissions from waterlogged tundra environments under the pronounced warming occurring in the Arctic

Contribution of author and co-authors: *Béatrice Frank-Fahle* developed the structure and objectives of this study, carried out the laboratory experiments, performed the downstream data analysis and wrote the manuscript. *Etienne Yergeau* helped with setting up Ion Torrent Sequencing analysis and provided valuable support in the downstream analysis of the sequence data and multivariate statistics, interpretation of results and discussion of the manuscript. *Charles Greer* provided the expertise, equipment and consumables for Ion Torrent Sequencing. *Hugues Lantuit* enabled sampling and provided critical comments on the manuscript. *Dirk Wagner* contributed to the interpretation of the results and valuable discussion.

3rd Manuscript (in preparation for submission to *Applied and Environmental Microbiology*)

"Dynamics and adaptation of the methane-cycling community in a permafrost-affected soil under simulated global warming."

Authors: Béatrice A. Frank-Fahle[1,*], Nadine Manke[1], Hugues Lantuit[1] & Dirk Wagner[1#]

Aims: The objective of this study was to investigate the reaction and adaptation of active-layer methane-cycling microorganisms from polygonal tundra on Herschel Island, Canada under simulated global warming.

Summary: This study presents the results of an 8 months long simulated climate warming experiment in which three intact active layer cores taken from wet polygonal tundra on Hershel Island, Canada were incubated at regularly increasing temperatures. The response of methane-cycling microbial communities in the active layer was studied using qPCR and T-RFLP. Results showed that both the methanogenic and methanotrophic communities were able to adapt rapidly to increasing temperatures and even seemed stimulated by this increase. Overall diversity did not drastically decrease over time, but a clear shift in community composition and abundance could be observed, particularly in the bacterial community towards the bottom of the active layer. Archaea appeared less affected by the temperature increase and the diversity and distribution of this community remained relatively stable over time. Overall, these results give a positive prognostic on the fate of methane-cycling microbial communities under increasing temperatures and permafrost degradation.

Contribution of author and co-authors: *Béatrice Frank-Fahle* developed the structure and objectives of this study, carried out field sampling and laboratory experiments, performed data analysis and wrote the manuscript. *Nadine Manke* contributed in setting-up and monitoring the simulation experiment, as well as laboratory and downstream analyses. *Hugues Lantuit* enabled sampling. *Dirk Wagner* developed the experimental setup and contributed to the interpretation of the results and valuable discussion.

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II. MANUSCRIPTS

MANUSCRIPT I – (Published in 2012 in *FEMS Microbiology Ecology* **82:** 287-302)

FEMS MICROBIOLOGY ECOLOGY

RESEARCH ARTICLE

Methane-cycling communities in a permafrost-affected soil on Herschel Island, Western Canadian Arctic: active layer profiling of *mcrA* and *pmoA* genes

Béatrice A. Barbier[1], Isabel Dziduch[1], Susanne Liebner[2], Lars Ganzert[2], Hugues Lantuit[1], Wayne Pollard[3] & Dirk Wagner[1]

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Editor: Max Häggblom

Keywords
methanogens; methanotrophs; methane activity; T-RFLP; diversity.

Abstract

In Arctic wet tundra, microbial controls on organic matter decomposition are likely to be altered as a result of climatic disruption. Here, we present a study on the activity, diversity and vertical distribution of methane-cycling microbial communities in the active layer of wet polygonal tundra on Herschel Island. We recorded potential methane production rates from 5 to 40 nmol h^{-1} g^{-1} wet soil at 10 °C and significantly higher methane oxidation rates reaching values of 6–10 μmol h^{-1} g^{-1} wet soil. Terminal restriction fragment length polymorphism (T-RFLP) and cloning analyses of *mcrA* and *pmoA* genes demonstrated that both communities were stratified along the active layer vertical profile. Similar to other wet Arctic tundra, the methanogenic community hosted hydrogenotrophic (*Methanobacterium*) as well as acetoclastic (*Methanosarcina* and *Methanosaeta*) members. A pronounced shift toward a dominance of acetoclastic methanogens was observed in deeper soil layers. In contrast to related circum-Arctic studies, the methane-oxidizing (methanotrophic) community on Herschel Island was dominated by members of the type II group (*Methylocystis*, *Methylosinus*, and a cluster related to *Methylocapsa*). The present study represents the first on methane-cycling communities in the Canadian Western Arctic, thus advancing our understanding of these communities in a changing Arctic.

Introduction

Arctic permafrost environments play a crucial role in the global carbon cycle. Between 10 and 39 Tg a^{-1} of methane is released from permafrost environments, contributing up to 20% of global emissions (Cao *et al.*, 1998; McGuire *et al.*, 2009) and making them the largest single natural source of methane (Christensen *et al.*, 1996). Permafrost soils are also believed to contain 50% of the global belowground organic carbon pool (Tarnocai *et al.*, 2009), a considerable reservoir for potential future release of methane.

These environments are predicted to warm more rapidly than the rest of the globe (Anisimov *et al.*, 2007) and with them, the wet tundra ecosystems which host much of the methanogenic activity because of the water-logged, anoxic conditions that prevail in seasonally deepening thawed layers (Whalen & Reeburgh, 1992),

Methane release is in fact the net result between methanogenic and methanotrophic activity. Methane can be generated *in situ* by methanogenic archaea (a group belonging to the *Euryarchaeota*) under anaerobic conditions, but it can also be oxidized by methanotrophs such as methane-oxidizing bacteria (MOB), making tundra environments act as a methane sink (Whalen *et al.*, 1990; Callaghan *et al.*, 2005; Wagner & Liebner, 2009). MOB belong to the phylum Proteobacteria and can oxidize up to 90% of the methane emitted in the deeper layers before it reaches the atmosphere (Oremland & Culbertson, 1992; Le Mer & Roger, 2001; Wagner & Liebner, 2009). The balance between methane production and oxidation is thereby fragile and nonlinear as methanogens

and methanotrophs show a different response to temperature fluctuations (Ganzert *et al.*, 2007; Høj *et al.*, 2008; Knoblauch *et al.*, 2008; Liebner *et al.*, 2009).

Changing climate conditions could dramatically alter this balance and mobilize the large carbon pools found in permafrost, potentially creating a positive feedback loop with important global implications. Several studies have been conducted to explore this issue in Siberia (Kobabe *et al.*, 2004; Ganzert *et al.*, 2007; Wagner *et al.*, 2007; Liebner *et al.*, 2008; Dedysh, 2009), Svalbard (Wartiainen *et al.*, 2003; Høj *et al.*, 2008; Graef *et al.*, 2011) and the Canadian High Arctic (Pacheco-Oliver *et al.*, 2002; Martineau *et al.*, 2010; Yergeau *et al.*, 2010) to study the characteristics and dynamics of methane-cycling communities, but the communities of the Canadian Western Arctic remain unexplored to date.

In the following paper, the vertical distribution and diversity of two functional marker genes coding for enzymes involved in the methane cycle were investigated. To look at the diversity in the methanogenic population, we selected the gene coding for subunit A of the methyl coenzyme-M reductase enzyme (*mcrA*). Methyl coenzyme-M is the terminal enzyme complex in the methane generation pathway, methyl coenzyme-M reductase (MCR), which catalyzes the reduction of a methyl group bound to coenzyme-M, with the accompanying release of methane (Luton *et al.*, 2002). This enzyme complex is unique and ubiquitous in known methanogens (Thauer, 1998), and various studies have used it as a reliable tool for the specific detection of this group (Juottonen *et al.*, 2005; Steinberg & Regan, 2008; Biderre-Petit *et al.*, 2011).

To study the diversity of MOB, we selected the gene coding for subunit A of the particulate methane monooxygenase enzyme (*pmoA*). Methane monooxygenase (MMO) is found in either soluble or membrane-bound form, except in Methylocella species where only the membrane-bound form is present (Theisen & Murrell, 2005). MMO is responsible for the conversion of methane into methanol, which is either assimilated into biomass or oxidized to carbon dioxide (Semrau *et al.*, 1995).

Both functional genes are characterized by sufficient sequence divergence to serve as a reliable diagnostic gene for the study of the two populations of interest (McDonald & Murrell, 1997; Luton *et al.*, 2002).

In this study, we aimed to better understand the *in situ* dynamics between microbial-driven methanogenesis and methane oxidation in increasingly thawing permafrost. We calculated methane production and potential oxidation rates in an active layer soil profile from polygonal tundra on Herschel Island in the Canadian Western Arctic. To understand abiotic factors driving methane activity, we described the physico-chemical properties of the soil profile. We evaluated the assortment and distribution of *mcrA* and *pmoA* signatures throughout the soil profile using T-RFLP analysis. We complemented the fingerprinting results by constructing clone libraries of our two genes of interest. The results presented give new insights into the distribution and activity of methanogenic and methanotrophic microorganisms in the active layer of a rapidly degrading permafrost environment.

Materials and methods

Site description and sample collection

Active layer samples were collected from the 'Drained Lake' low-center polygon (N 69°34′43, W 138°57′25, elevation 30 m above sea level) on Herschel Island, Western Canadian Arctic (Fig. 1) during the expedition YUKON COAST in July–August 2010. A low-center polygon is an ice-wedge polygon in which thawing of ice-rich permafrost has left the central area in a relatively depressed position (van Everdingen, 2005). The soil at this site was characterized as a hemic glacistel classified according to the U.S. Soil Taxonomy (Soil Survey Staff, 1998) with poor drainage and a loamy soil texture. Vegetation cover included roughly 35% plant litter, 40% *Carex* sp. (sedges), 15% *Salix* sp. (dwarf willow), 10% mosses with traces of *Pedicularis* sp. (wooly lousewort), and *Ledum groenlandicum* (Labrador tea). The vegetation period spans yearly from mid-June to end of September. Average air temperatures vary annually between −26.3 °C in February to 8.7 °C in July (Burn & Zhang, 2009).

The sampling site was characterized by an active layer (the layer of ground that is subject to annual thawing and freezing) consisting of a large peat horizon, with a depth of 36 cm as measured using a permafrost probe. A hole was dug to the permafrost table, one side of the hole was cleaned, and blocks of soil were taken every 5 cm with a sharp sterile knife and placed into sterile 125 mL Nalgene® screw-cap containers (Thermo Fischer Scientific Inc., Waltham, MA). The knife was wiped down and sterilized with ethanol between different samples. Soil samples were frozen immediately after sampling and stored at −20 °C upon arrival in the laboratory. All subsequent subsampling was performed under sterile and anaerobic conditions in an atmosphere-controlled glove box.

Soil physico-chemical analyses

Gravimetric moisture content of soils was determined by weighing subsamples before and after freeze-drying for 72 h.

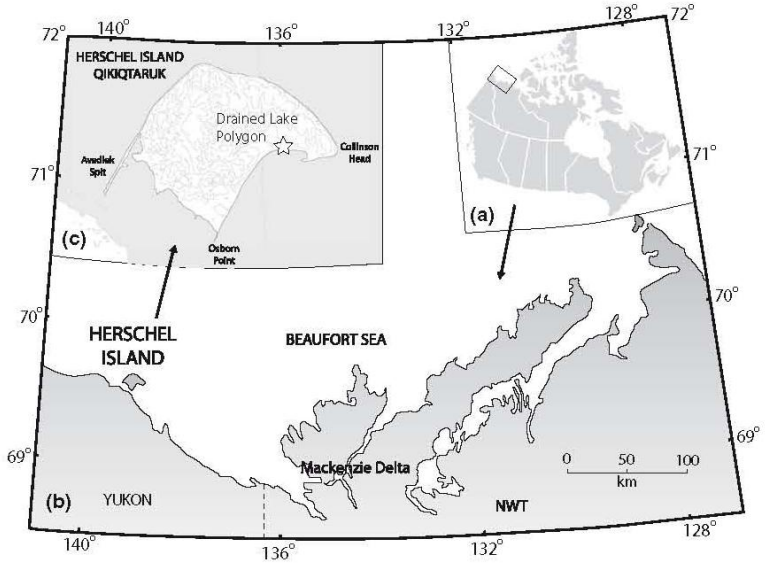

Fig. 1. Geographical location of study site (a) in Canada, (b) on the Yukon Coast and (c) Location of the Drained Lake Polygon on Herschel Island.

pH was measured using a CyberScan PC 510 Bench Meter (Eutech Instruments Pte Ltd, Singapore) following the slurry technique by mixing 1 : 2.5 mass ratio of samples and de-ionized water (Edmeades *et al.*, 1985).

Grain size was analyzed by first treating the samples with 30% H_2O_2 to digest all organic matter. After washing, the samples were freeze-dried and weighed. One per cent NH_3 solution was added to the samples and shaken for at least 24 h. Grain size was then measured at least twice for each sample with a Coulter LS 200 laser particle size analyser (Beckman Coulter, Brea, CA).

The percentage of total organic carbon (TOC) of the soils was measured in duplicate using a TOC analyzer (Elementar Vario max C, Germany). Samples prepared for analysis by freeze-drying and homogenized in an orbit mill ball-grinder (Pulverisette 5; Fritsch Ltd, Germany). The TOC content was calibrated using external standards of known elemental composition.

Water content, pH, and TOC could not be measured for the uppermost layer of the profile, as this mostly consisted of roots and plant material which were not sufficient to measure these parameters.

Methane measurements

Methanogenic activity of each soil layer was measured under simulated *in situ* conditions without substrate addition by placing 5 g of fresh soil material in 20 mL glass bottles and covered with 1 mL of sterile water under sterile, anaerobic conditions. The bottles were sealed with butyl rubber stoppers and flushed with N_2CO_2 (80 : 20% v/v). Triplicate samples were incubated in the dark at 10 °C. As a control, triplicate heat-sterilized samples were used. Samples were measured every 24 h for 1 week using an Agilent 6890 gas chromatograph (Agilent Technologies, Santa Clara, CA). Gases were separated on a Plot Q capillary column (0.53 mm diameter, 15 m length) using a gas flow of 30 mL min^{-1} with helium as carrier gas, and methane (CH_4) was measured through a flame-ionizing detector (FID). The oven and injector temperature were set at 80 °C and the detector temperature at 250 °C. All gas sample analyses were performed after calibration of the gas chromatograph with standard gases. CH_4 production rates were calculated from the linear increase in the CH_4 concentration in the headspace with time.

To study potential methane oxidation rates, fresh soil material (4 g) was placed in flat-walled culture bottles (50 mL) and distributed over the sidewall as a thin layer as described by Knoblauch *et al.* (2008). The bottles were sealed with butyl rubber stoppers and incubated horizontally. The headspace contained 2.5% v/v methane in synthetic air. Triplicate samples were incubated in the dark at 10 °C. Methane was measured repeatedly and the oxidation rates were calculated from the initial linear reduction in methane using multiple data points. Gas samples were measured in the same manner as described above. Heat-sterilized samples were used as the control.

Extraction of genomic DNA and PCR amplification

Total genomic DNA was extracted in duplicate from 0.6 g of soil using the PowerSoilTM DNA Isolation Kit (Mo Bio Laboratories, Carlsbad, CA) according to the manufacturer's protocol. Duplicates were then pooled for downstream analyses. Nucleic acids were eluted in 50 μL of elution buffer (MoBio). The concentration of the obtained genomic DNA was checked by spectrophotometry using a TrayCell (Hellma Analytics, Müllheim, Germany). DNA was then stored at −20 °C for further use in polymerase chain reaction (PCR) analyses.

PCR reactions were performed in triplicate 50 μL volumes containing between 10 and 50 ng of DNA, 0.5 μL of each 20 mM primer (forward primer labeled with the fluorescent dye carboxyfluorescein), 5 μL Q-Solution (Qiagen), 1.5 μL 10 mM dNTP mix, 5 μL 10× PCR buffer (Qiagen), 1 U of HotStar Taq DNA polymerase (Qiagen, Hilden, Germany), and PCR-grade water to 50 μL.

Primers used in the different PCR reactions are listed in Table 1. For the amplification of the archaeal *mcrA* gene, the primer pair MLf/MLr was used (Luton *et al.*, 2002). Reaction conditions were as follows: initial denaturation at 94 °C for 3 min, 35 cycles with denaturation at 94 °C for 25 s, annealing at 50 °C for 45 s, extension at 72 °C for 60 s, and a final extension at 72 °C for 5 min.

For the amplification of the methanotrophic *pmoA* gene, the primer pairs A189f/A682r and A189f/mb661r were used (Costello & Lidstrom, 1999; Holmes *et al.*, 1999) in a semi-nested PCR approach. The first PCR conditions were as follows: initial denaturation and polymerase activation at 95 °C for 5 min, 30 cycles with constant denaturation temperature at 94 °C for 45 s, decreasing annealing temperature from 62 to 52 °C for 60 s, elongation at 72 °C for 90 s, and final elongation at 72 °C for 90 s. The second PCR reaction conditions were initial denaturation and polymerase activation at 95 °C for 5 min, 22 cycles of denaturation at 94 °C for 45 s, annealing at 56 °C for 60 s, elongation at 72 °C for 90 s, and a final extension at 72 °C for 10 min.

Triplicate PCR reactions were visualized on a 1% agarose gel containing GelRed stain (Hayward, CA) and then purified using a QIAquick PCR Purification Kit (Qiagen). Purified PCR products were quantified by spectrophotometry using a TrayCell (Hellma Analytics).

Terminal restriction fragment length polymorphism (T-RFLP)

The digestion of fluorescently labeled PCR fragments using restriction enzymes was conducted in duplicate as follows. 10 U of enzyme MspI (Roche, Penzberg, Germany), 2 μL of 10× Buffer, and 500–600 ng of purified PCR product were mixed. PCR-grade water was added to 20 μL. The samples were then incubated for 3 h at 37 °C. The digestion was stopped by incubation at 80 °C for 20 min. Duplicate digests were pooled and purified using the QIAquick Purification Kit (Qiagen).

T-RFLP products (2 μL) were mixed with 0.25 μL of GeneScanTM 500 LIZ® internal size standard (Applied Biosystems, Darmstadt, Germany) and run on an ABI 3730xl DNA Analyzer (Applied Biosystems) at GATC Biotech (Konstanz, Germany). Afterward, the lengths of the fluorescently labeled terminal fragments (T-RFs) were visualized with PEAK SCANNER software (v1.0; Applied Biosystems).

T-RFLP results were analyzed statistically according to Dunbar *et al.* (2001) to yield relative abundance (%) of T-RFs. Briefly, T-RFs were aligned and clustered manually using EXCEL (Microsoft, Redmond, WA). DNA quantity between triplicate samples as well as between depth profiles was standardized in an iterative standardization procedure. For each sample, a derivative profile containing only the most conservative and reliable T-RF information was created by identifying the subset of T-RFs that appeared in all replicate profiles of a sample. Standardized, derivative profiles were then aligned. The average size of TRFs in each alignment cluster was calculated to produce a single, composite list of the T-RF sizes found among all samples. Relative signal intensity of each T-RF (%) was calculated based on the signal intensity of each individual T-RF with respect to the total signal intensity of all T-RFs in that sample. Peaks representing less that 1% of total fluorescence were eliminated from the profile to concentrate on the most representative microorganisms in each community. T-RFLP profiles were converted into

Table 1. Summary of properties of PCR primers used in this study

Primer	Target gene	Sequence (5′ 3′)	Annealing T [°C]	References
A189f	*pmoA*	GGNGACTGGGACTTCTGG	52	Holmes *et al.* (1999)
A682r	*pmoA*	GAASGCNGAGAAGAASGC	52	Holmes *et al.* (1999)
mb661r	*pmoA*	CCGGMGCAACGTCYTTACC	56	Costello & Lidstrom (1999)
MLf	*mcrA*	GGTGGTGTMGGATTCACACARTAYGCWACAGC	50	Luton *et al.* (2002)
MLr	*mcrA*	TTCATTGCRTAGTTWGGRTAGTT	50	Luton *et al.* (2002)

presence–absence data and analyzed statistically by cluster analysis based on Bray–Curtis pairwise similarities using the software PRIMER 6 (Primer-E Ltd, Lutton, UK).

Cloning and sequence analyses

Based on the obtained T-RFLP results, various profile depths with the highest representative T-RF diversity (5–10 cm and 20–25 cm for *mcrA*; 0–5 cm and 15–20 cm for *pmoA*) were chosen to establish clone libraries. Libraries for the functional genes *mcrA* and *pmoA* were created by ligating PCR products into the pGEM-T Easy vector and transformed into competent cells *Escherichia coli* JM109 using the 'pGEM-T Easy Vector Systems II' Kit (Promega, Mannheim, Germany). White colonies containing inserts were picked, suspended in 1.2 mL of nutrient broth containing ampicillin (50 μg mL^{-1}), and grown overnight at 37 °C. Colonies were screened by PCR with vector primers M13 for correct size of the insert and the amplicons were directly sequenced by GATC Biotech AG. Ninety-six clones per gene were sequenced. The sequences were edited and contigs assembled using the SEQUENCHER software (v4.7; Gene Codes, Ann Arbor, MI). Nucleotide sequences were then screened and translated into correct amino acid sequences for further phylogenetic analyses using CLC sequence viewer software (version 6.5.1). Altogether, 81 McrA and 48 PmoA deduced amino acid (aa) sequences were used.

For McrA, sequences including nearest neighbors and cultured isolates were pre-aligned using the Muscle alignment tool integrated in MEGA 5 (Tamura *et al.*, 2011). The alignment was then imported in ARB (www.arb-home.de, Ludwig *et al.*, 2004) and manually checked. A neighbor-joining tree (Saitou & Nei, 1987) was constructed in ARB with a subset of 205 McrA amino acid sequences including nearest neighbors and representative isolate sequences (163 aa).

For PmoA, the deduced amino acid sequences were imported into an ARB database containing 3708 high quality PmoA sequences and were manually aligned. A neighbor-joining tree constructed in ARB with a subset of 127 PmoA sequences including nearest neighbors and representative isolate sequences (135 aa) using a 30% base frequency filter. The distance matrix was calculated using the neighbor-joining algorithm with a Kimura correction for McrA and a PAM correction for PmoA amino acid sequences. Rarefaction analysis was performed with DOTUR (Schloss & Handelsman, 2005) based on the furthest neighbor algorithm. Operational taxonomic units (OTUs) were defined using a 14.3% cutoff value for McrA according to Hunger *et al.* (2011) and a 7% cutoff for PmoA according to Degelmann *et al.* (2010).

Nucleotide sequence accession numbers

The environmental *mcrA* and *pmoA* clone sequences recovered in this study from the active layer of a polygon on Herschel Island were have been submitted to the GenBank nucleotide sequence databases and can be found under accession numbers JQ048956-JQ049081.

Results

Characteristics of the soil

The average *in situ* day temperature at the surface of the profile was 12 °C, decreasing gradually to −0.5 °C at the permafrost table (Fig. 2a). The pH of the entire profile was slightly acidic, ranging between 5.2 and 5.6 throughout (Fig. 2b). The mineral fraction of the soil represented only roughly 30%, as calculated after concentrated acid digestion of organic matter. The mineral fraction consisted on average of 27% sand, 20% silt, and 15% clay. The soil was visibly water saturated, with gravimetric moisture contents in the profile ranging from 77% near the surface and increasing to 83% close to the permafrost table (Fig. 2c). The organic carbon content was overall very high for all profile layers, ranging from 28% in the middle layers to 23% toward the permafrost table (Fig. 2d).

Methane production and oxidation

At an incubation temperature of 10 °C and with no added substrate, no significant methane production was found in the soil surface sample (0–5 cm depth) and only a low 1.4 nmol of CH_4 per hour and per gram of wet soil (nmol h^{-1} g^{-1}) was observed in the subsequent layer (Fig. 2e). The methanogenic activity in the deeper soil layers varied from 10.3 to 38.5 nmol h^{-1} g^{-1} with the exception of one sample (20–25 cm depth) where a lower value of 4.5 nmol h^{-1} g^{-1} was observed. The maximum potential methane production rates of 38.5 nmol h^{-1} g^{-1} occurred in the middle of the soil profile at 10–15 cm depth along with 35.8 nmol h^{-1} g^{-1} above the permafrost table at 30–35 cm depth.

The potential methane oxidation rate in the same profile varied between 43.5 and 9508.1 nmol h^{-1} g^{-1} (Fig. 2f). The maximum rate of 9.51×10^3 nmol h^{-1} g^{-1} was reached at 10–15 cm depth, at the same depth where the maximum methane production rate was also observed. High rates of 6.02×10^3, 6.66×10^3, and 3.368×10^3 nmol h^{-1} g^{-1} were observed in layers between 20 cm depth and the permafrost table.

Methane concentrations in the heat-sterilized controls did not increase during the incubation.

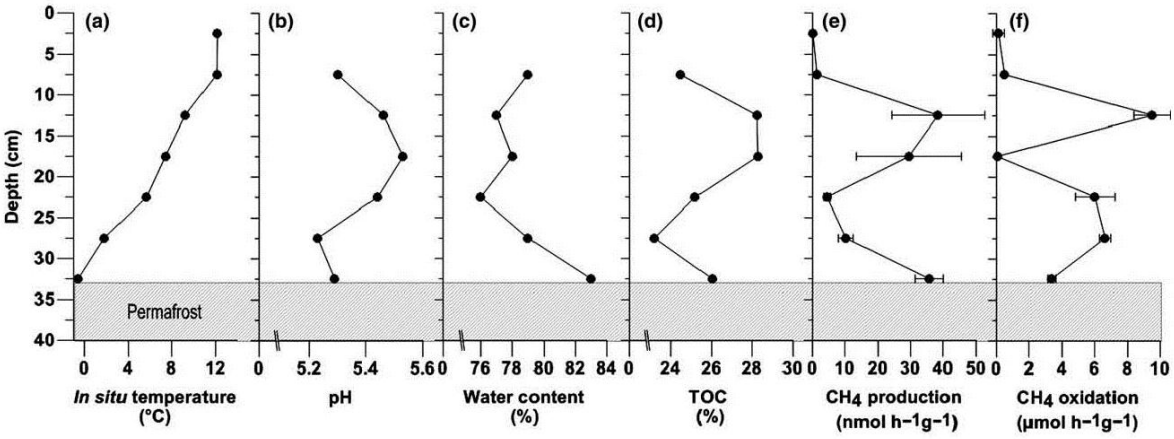

Fig. 2. Depth profile of abiotic and biotic parameters illustrating (a) active layer temperature as measured *in situ*, (b) soil pH, (c) percentage of water content, (d) percentage of total organic carbon in the soil (TOC), (e) potential methane production rate expressed in nanomol of methane per hour and gram of wet soil at 10 °C with no substrate addition, and (f) potential oxidation rate expressed in micromol of methane per hour and gram of wet soil at 10 °C in an atmosphere of 2.5% v/v methane in synthetic air. Error bars in (e) and (f) represent standard deviations.

Methanogenic and methanotrophic community structure

The community structure of methanogens and methanotrophs in the active layer profile was investigated through T-RFLP analysis of *mcrA* and *pmoA* functional genes (Fig. 3a,b). We obtained overall diverse communities, with a total of 17 T-RFs for the methanogenic archaea and 14 T-RFs for the methanotrophic bacteria.

Generally, we found that the methanogenic community became increasingly diverse with soil depth. No *mcrA* signal could be detected in the surface sample (Fig. 3a). Bray–Curtis similarity analysis of the *mcrA* T-RFLP data showed that community composition of methanogens was 80% similar between 15 and 36 cm depth. All samples taken together, excluding the surface layer, showed 60% similarity in community composition. The 5–10 cm depth sample displayed a different T-RF pattern compared with the subsequent depths, especially with respect to T-RF abundance. In this sample, the 463 bp T-RF represented 68% of total fluorescence, disappearing at the next sample depth and then reappearing in deeper layers, at a stable 10% of total T-RF abundance. A clear vertical shift in the community could be observed with predominating T-RFs in the surface layers (269, 272, 306 bp) decreasing in abundance in the deeper layers. The 269 bp T-RF could first be detected at 10–15 cm depth and represented between 35% and 55% of the community composition down to 35 cm depth. The 306 bp T-RF could first be detected at 5–10 cm depth and then gradually became more predominant in the community with increasing depth, making up 76% of the community close to the permafrost table.

The methanotrophic community based on *pmoA* showed the overall highest diversity in a depth between 10 and 30 cm of the active layer. Based on Bray–Curtis similarity analysis, the MOB community composition was heterogeneous throughout the different soil layers and samples clustered in a pairwise manner (Fig. 3b). Peaks of 245 and 246 bp clearly dominated the surface layers of the profile, representing 35% and 65%, respectively, of the total methanotrophic community between 0 and 10 cm depth. The T-RF of 117 bp first appeared at 10 cm depth and became progressively dominant in the profile with increasing depth. One T-RF of 100 bp was the only detectable peak close to the permafrost table. Overall, a shifting MOB community composition could be observed with increasing depth with T-RFs of 104, 117, 415, and 509 bp increasing in abundance while T-RFs of 245, 246 and 249 bp decreasing in abundance in the community profile.

Diversity and dominant species of *mcrA*

The clone library analysis of *mcrA* yielded a total of 85 cloned *mcrA* sequences. Four sequences resulting in < 100 amino acids were removed from further phylogenetic analyses. The diversity at the species level was low, resulting in six distinct OTU when using a cutoff value of 85.7% sequences similarity based on Hunger *et al.*, 2011 (Supporting Information, Fig. S1). Phylogenetic analyses of the clones indicated that the methanogenic community in the active layer profile was dominated by members of the genus *Methanobacterium* (1 OTU, 27 of 81 sequences), *Methanosarcina* (1 OTU, 19 sequences), *Methanosaeta* (1 OTU, 17 sequences), and *Methanocella*

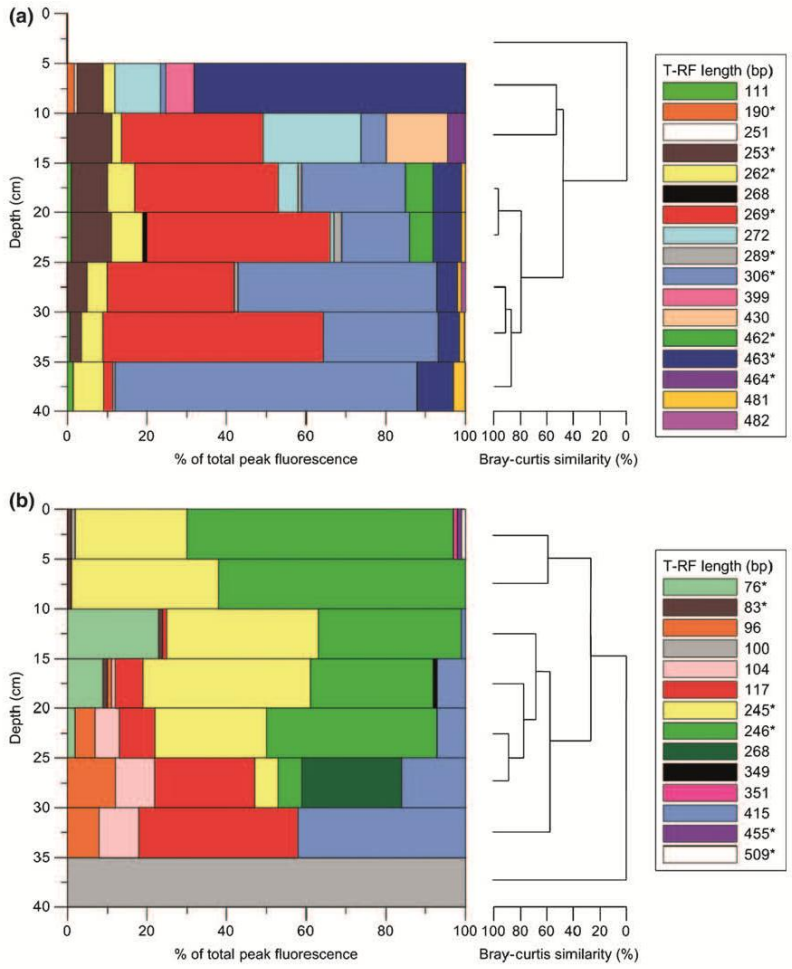

Fig. 3. Composition of methanogenic (a) and methantrophic (b) communities in an active layer profile on Herschel Island, Canada. Bars indicate the relative abundance of T-RFs of *mcrA* (a) and *pmoA* (b) functional gene amplicons. *mcrA*- and *pmoA*-based T-RFs obtained by enzymatic restriction using MspI. Numbers in the legend indicate the size of the T-RFs in base pairs (bp); an asterisk next to a T-RF size (e.g. 190*) indicates T-RFs for which a corresponding clone T-RF was found. Dendograms to the right of the histogram show similarity of T-RFLP profiles by Bray Curtis hierarchical cluster analysis.

(1 OTU, 11 sequences). To a smaller extent, sequences related to the genus *Methanosphaerula* (1 OTU, six sequences) and only one sequence could be assigned to a novel, deep-branching group with relatives found in peat (Yrjälä *et al.*, 2011), a humic bog lake (Milferstedt *et al.*, 2010), Lake Pavin (Biderre-Petit *et al.*, 2011) and wetland soil (Narihiro *et al.*, 2011) (Fig. 4).

In an attempt to identify the most dominating methanogenic species in all depths of the active layer, T-RF sizes of 50 clones for *mcrA* were determined by digesting single clones with MspI, the same enzyme used for the whole-profile T-RFLP analysis. Clones from the sample library corresponded overall to nine T-RFs obtained in the whole community profile (Table 2, Fig 3a). Out of these eight T-RFs, two dominant fragments (269 and 306 bp) found in the overall profile corresponded to *Methanosarcina*. Two fragments (462, 463 bp) corresponded to *Methanobacterium*. The same fragment (463 bp) was also found to correspond to *Methanocella*. The remaining groups were represented by single T-RFs: *Methanosarcina*/*Methanosaeta* (253 bp), *Methanocella* (262 bp), and *Methanoregula* (289 and 464 bp). The numbers of clones found representing each T-RF along with their phylogenetic affiliation as obtained after comparison with the GenBank database using the BlastN algorithm are listed in Table 2.

Diversity and dominant species of *pmoA*

The clone library analysis of *pmoA* yielded a total of 65 cloned *pmoA* sequences. Based on a 7% cutoff for the

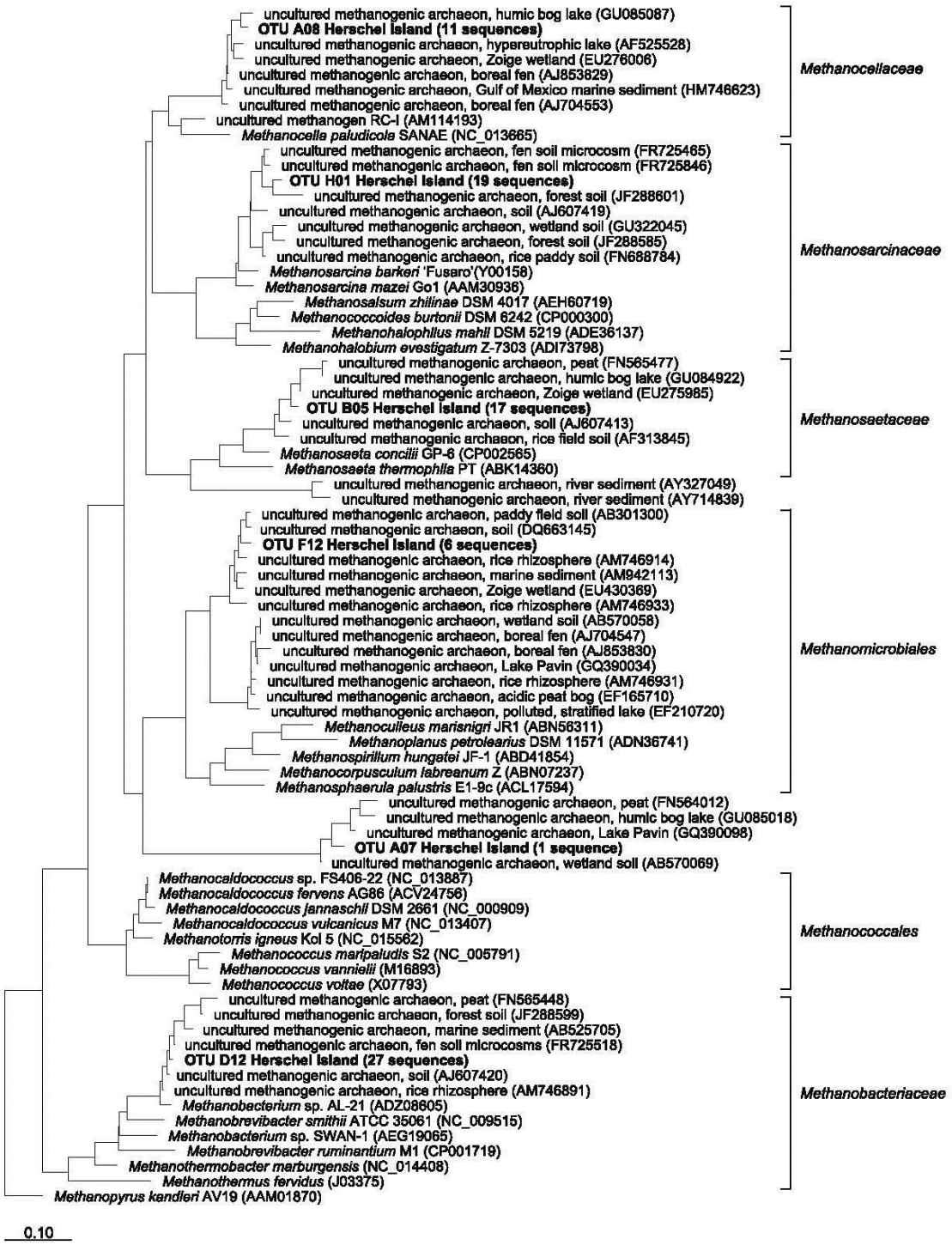

Fig. 4. Phylogenetic tree showing the relation of methanogen McrA amino acid sequences from active layer samples of Herschel Island, Canadian Western Arctic to known methanogen isolates and environmental sequences. The neighbor-joining tree was calculated from deduced amino acid sequences (159–163 aa) with *Methanopyrus kandleri* AV19 as outgroup. The 6 OTUs found in this study using a cutoff of 14.3% are in bold with the number of additional members that belong to the same OTU (in parentheses). The scale bar represents 0.10 changes per amino acid position.

Table 2. Phylogenetic assignment of active layer clones that matched the dominating soil T-RFLP peaks as obtained after comparison with the GenBank database using the BLASTN algorithm

T-RF size	Corresponding clone(s)	Accession number	Phylogenetic affiliation	Closest cultured relative	Accession number	Similarity (%)
mcrA						
190	mcrA-A07	JQ049004	*Methanomassiliicoccus*	*Methanomassiliicoccus luminyensis*	HQ896500.1	83
253	mcrA-D01	JQ049031	*Methanosarcina*	*Methanosarcina* sp. T36	AB288292.1	93
	mcrA-H03	JQ049073	*Methanosaeta*	*Methanosaeta concilii* GP-6	CP002565.1	85
262	mcrA-F10	JQ049058	*Methanocella*	*Methanocella paludicola* SANAE	AP011532.1	76
	mcrA-H04	JQ049074	*Methanocella*	*Methanocella paludicola*	AP011532.1	75
269	mcrA-E06	JQ582401	*Methanosarcina*	*Methanosarcina* sp. T36	AB288292.1	93
289	mcrA-C01	JQ049019	*Methanoregula*	Candidatus *Methanoregula boonei* 6A8	CP000780.1	83
306	mcrA-D06	JQ049035	*Methanosarcina*	*Methanosarcina* sp. T36	AB288292.1	92
	mcrA-E06	JQ582401	*Methanosarcina*	*Methanosarcina* sp. T36	AB288292.1	92
	mcrA-G01	JQ049061	*Methanosarcina*	*Methanosarcina* sp. T36	AB288292.1	92
462	mcrA-B12	JQ049018	*Methanobacterium*	*Methanobacterium* sp. AL-21	CP002551.1	90
	mcrA-C11	JQ049029	*Methanobacterium*	*Methanobacterium* sp. AL-21	CP002551.1	89
	mcrA-F09	JQ049057	*Methanobacterium*	*Methanobacterium* sp. AL-21	CP002551.1	89
	mcrA-E07	JQ049044	*Methanocella*	*Methanocella* sp. HZ254	JN081865.1	77
	mcrA-H06	JQ049076	*Methanocella*	*Methanocella* sp. HZ254	JN081865.1	76
463	mcrA-B12	JQ049018	*Methanobacterium*	*Methanobacterium* sp. AL-21	CP002551.1	90
	mcrA-C11	JQ049029	*Methanobacterium*	*Methanobacterium* sp. AL-21	CP002551.1	89
	mcrA-F09	JQ049057	*Methanobacterium*	*Methanobacterium* sp. AL-21	CP002551.1	89
	mcrA-E07	JQ049044	*Methanocella*	*Methanocella* sp. HZ254	JN081865.1	77
	mcrA-H06	JQ049076	*Methanocella*	*Methanocella* sp. HZ254	JN081865.1	76
464	mcrA-F12	JQ049060	*Methanoregula*	Candidatus *Methanoregula boonei* 6A8	CP000780.1	84
	mcrA-G12	JQ049070	*Methanoregula*	Candidatus *Methanoregula boonei* 6A8	CP000780.1	84
pmoA						
76	pmoA-C10	JQ048990	*Methylococcus*	*Methylococcus capsulatus*	AF533666.1	80
83	pmoA-E08	JQ048974	*Methylocystis*	*Methylocystis* sp. M212	JN036528.1	91
	pmoA-F04	JQ582402	*Methylocystis*	*Methylocystis* sp. M212	JN036528.1	91
245	pmoA-E08	JQ048974	*Methylocystis*	*Methylocystis* sp. M212	JN036528.1	91
	pmoA-F04	JQ582402	*Methylocystis*	*Methylocystis* sp. M212	JN036528.1	92
	pmoA-F05	JQ048976	*Methylosinus*	*Methylosinus* sp. LW2	AF150787.1	88
246	pmoA-A07	JQ582403	*Methylocystis*	*Methylocystis* sp. SS2C	AB636307.1	92
	pmoA-H01	JQ048981	*Methylocystis*	*Methylocystis* sp. M231	DQ852353.1	92
	pmoA-H04	JQ582404	*Methylocystis*	*Methylocystis parvus* strain OBBP	AF533665.1	92
	pmoA-C08	JQ582405	*Methylosinus*	*Methylosinus* sp. LW2	AF150787.1	93
455	pmoA-F02	JQ582406	*Methylocystis*	*Methylocystis* sp. SS2C	AB636307.1	92
509	pmoA-D03	JQ048967	*Methylosinus*	*Methylosinus* sp. LW2	AF150787.1	91
	pmoA-D02	JQ048966	*Methylocapsa*	*Methylocapsa acidiphila* B2	CT005238.2	79
	pmoA-A05	JQ048956	*Methylocapsa*	*Methylocapsa acidiphila* B2	CT005238.2	79

PmoA sequences (Fig. S1), 11 OTUs were defined with a clear dominance of type IIa methanotrophs (Fig. 5). A high proportion of sequences was affiliated to an uncultured *Methylocystis* cluster (3 OTUs, 17/48 sequences). Fifteen sequences (2 OTUs) clustered with uncultured *Methylosinus/Methylocystis*, eight sequences (3 OTUs) were affiliated with uncultured type Ia PmoA mainly from freshwater and mire habitats and which likely represents a new genus, seven sequences (2 OTUs) were affiliated to an uncultured cluster most closely related to *Methylocapsa* and a single sequence was most closely related to *Methylcystis* sp. 212 and related sequences from acidic and mire environments.

To identify the most dominating methanotrophic species in all depths of the active layer, T-RF sizes of 90 clones for *pmoA* were determined by digesting single clones with MspI. T-RFLP analysis of clones from the sample library corresponded to six T-RFs obtained in the whole community profile (Table 2, Fig. 3b). Out of these seven T-RFs, the two major fragments in the profile (245

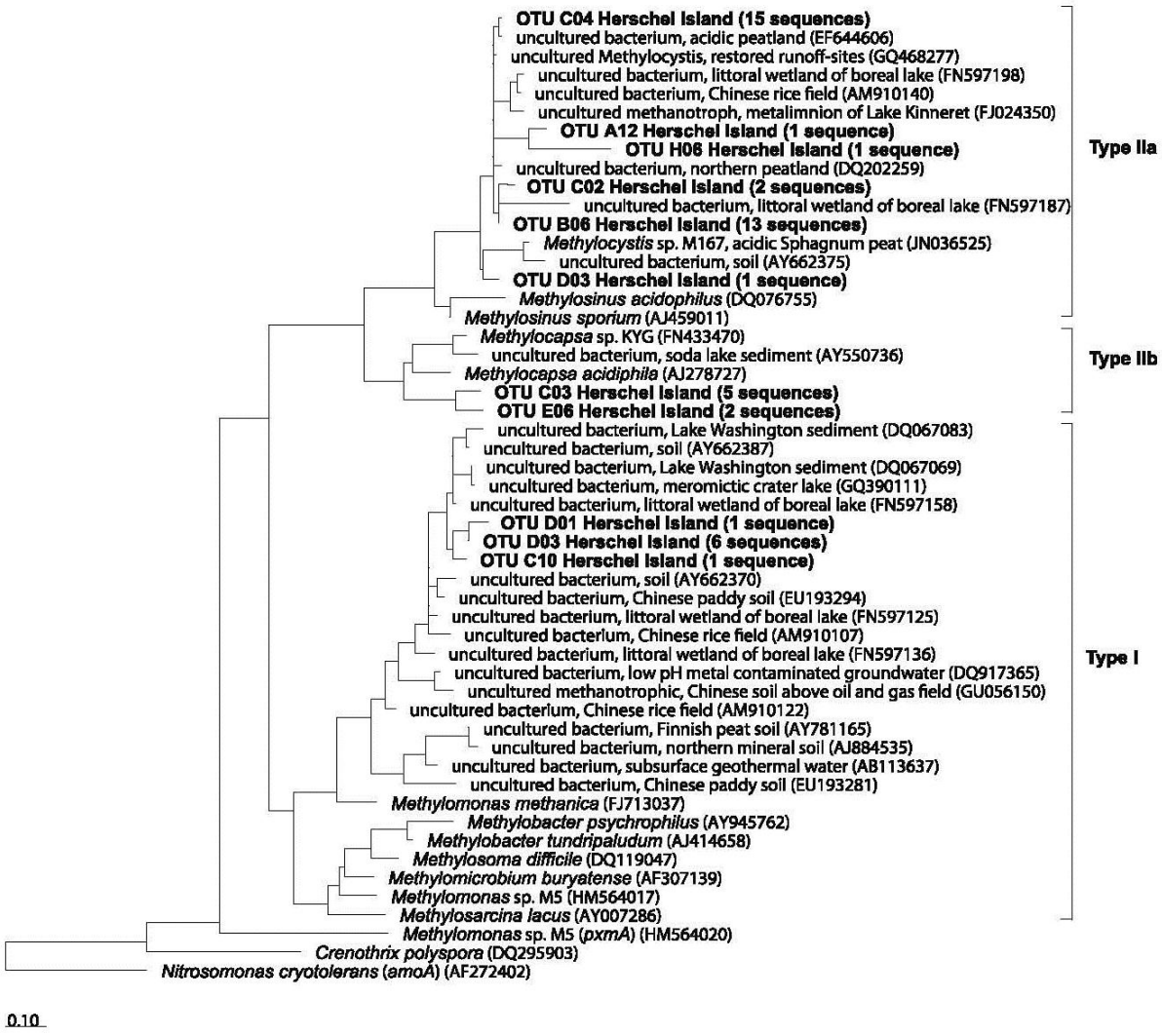

Fig. 5. Phylogenetic tree showing the relation of deduced MOB PmoA amino acid sequences from active layer samples of Herschel Island, Canadian Western Arctic to known MOB isolates and environmental sequences. The neighbor-joining tree was calculated from deduced amino acid sequences (135 aa) with *Nitrosomonas cryotolerans* as outgroup. The 11 OTUs found in this study using a cutoff of 7% are in bold with the number of additional members that belong to the same OTU (in parentheses). The scale bar represents 0.10 changes per amino acid position.

and 246 bp) corresponded to *Methylocystis* and *Methylosinus* and dominated the active layer profile between 0 and 25 cm depth. The 509 bp fragment found to correspond to *Methylocapsa* could only be detected to a small amount in the surface layer of the profile as well as between 15 and 20 cm depth. The 76 bp fragment with *Methylococcus* as the closest cultured relative was first detected in the middle of the active layer at 10 cm depth, decreasing in abundance down to 25 cm depth. The 83 bp fragment also corresponding to *Methylocystis* was detected at the same frequency in the upper half of the active layer.

The numbers of clones found representing each peak along with their phylogenetic affiliation as obtained after comparison with the GenBank database using the BlastN algorithm are listed in Table 2.

Discussion

Potential rates of methane production and methane oxidation

Although numerous studies have focused on surface methane fluxes in tundra environments in the circum-

Arctic region (e.g., Reeburgh *et al.*, 1998; Christensen *et al.*, 2003; Wille *et al.*, 2008), the microbiological communities behind these fluxes are still understudied. To understand the carbon fluxes from Arctic permafrost environments and the future development of these areas as a carbon source, it is essential to study the carbon dynamics and the microbial communities involved at different locations covering typical permafrost landscapes of the circum-Arctic. In this respect, the present study reported first results on the diversity of methane-cycling communities from a newly established environmental observatory in the Western Canadian Arctic.

It was shown that wet polygonal tundra environments on Herschel Island contained highly active methane-cycling communities. The highest potential methane production as well as oxidation rates at 10 °C in the vertical active layer profile was found in 10–20 cm soil depth, the second methane production optimum was found close to the permafrost table, while it appeared to be between 20 and 30 cm for methane oxidation. The observed potential methane production profile does not represent the typical activity pattern of methanogenesis in hydromorphic soils which would be no or little activity in the upper, oxic horizons and increasing rates in the anoxic bottom layers close to the permafrost table (Krumholz *et al.*, 1995). These methane production optima correlate with depths at which the organic carbon concentrations observed are at their highest throughout the profile, indicating a strong spatial correlation between the abundance of soil organic matter and methanogenesis as observed in previous studies in Siberia (Ganzert *et al.*, 2007). Methane oxidation rates correlated to or were just above depths at which the highest methanogenesis occurred, illustrating the close spatial location of methane production and oxidation in the studied active layer on Herschel Island. Methane production rates of 40 nmol CH_4 h^{-1} g^{-1} wet soil calculated in this study are comparable to those found *in situ* in other studies where rates of up to 39 nmol CH_4 h^{-1} g^{-1} wet soil were measured from the active layer of permafrost (Wagner *et al.*, 2003; Høj *et al.*, 2005; Metje & Frenzel, 2007). Potential methane oxidation rates in Siberian permafrost-affected soils were also calculated by Wagner *et al.* (2003) as well as in other studies (Liebner & Wagner, 2007; Knoblauch *et al.*, 2008), but the rates obtained in those studies (7–15 nmol CH_4 h^{-1} g^{-1} wet soil) were about three orders of magnitude smaller than the rate we calculated for active layer samples from Herschel Island. This could be due to differences in the activity and composition of the respective microbial communities. The activity of both processes is mainly affected by the water table position and the availability of substrates. It was shown for Siberian tundra that methane production activity decreased while oxidation rates increased concurrently with the progressing season (Wagner *et al.*, 2003). Although high methane oxidation rates can therefore be expected during the month of August, the potential methane oxidation rates calculated here are extremely high compared with what has been measured in similar arctic environments. They are rather similar to sub-Arctic rates as reported from a Finnish boreal mire (Jaatinen *et al.*, 2005) or a *Carex*-dominated fen in Alberta, Canada (Popp *et al.*, 2000). These high rates underline the importance of methanotrophic communities as the only terrestrial methane sink in Arctic wetlands (Trotsenko & Khmelenina, 2002), particularly with respect to permafrost degradation under future predicted global warming.

Structure of Herschel methane-cycling communities

Profiling *mcrA* and *pmoA* sequences along a permafrost-affected soil on Herschel Island, we extended the picture of methane-cycling communities in the Arctic and uncovered yet unseen microbial community composition in wet polygonal tundra. The MOB community found in the active layer of Herschel Island was more diverse than in other Arctic tundra environments and was dominated by type II organisms, primarily *Methylocystis*. Members of the *Methylocystis* genotype are known to thrive in methane-rich environments (Lüke *et al.*, 2010) but were not yet observed to dominate in Arctic tundra wetlands. Favorable conditions for type II methanotrophs in tundra soils were only reported for acidic *Sphagnum* peat where especially species of the genus *Methylocella* thrive (e.g., Dedysh *et al.*, 2004). However, *Methylocella* does not have a particulate methane monooxygenase and was not targeted in the present work. The MOB community on Herschel is thus unique for an Arctic tundra ecosystem and differs from what was reported until now in related studies on wet tundra of Siberia (Liebner *et al.*, 2008), Spitzbergen (Wartiainen *et al.*, 2003; Graef *et al.*, 2011) and the Canadian High Arctic (Martineau *et al.*, 2010). These studies consistently identified a dominance of type Ia methanotrophs and a generally very low diversity on the genus level (reviewed in Liebner & Wagner, 2010). In detail, we did not detect the presence of *Methylobacter tundripaludum* (Wartiainen *et al.*, 2006) or any other species of the *Methylobacter* genotype, although it was found to dominate in Arctic permafrost-affected soils of Svalbard where it was isolated (Wartiainen *et al.*, 2006; Graef *et al.*, 2011), Siberia (Liebner *et al.*, 2009) and the Canadian High Arctic (Martineau *et al.*, 2010) although a certain caution should be used when interpreting absences because of intrinsic uncertainties of the PCR reaction. Even so, these findings underline the uncommon composition of the MOB community in an Arctic environment.

In fact, the MOB community observed in this study is rather comparable to mire communities found in sub-arctic (Jaatinen *et al.*, 2005; Dedysh, 2009; Siljanen *et al.*, 2011) and temperate (Hoffmann *et al.*, 2002; Horz *et al.*, 2005; Chen *et al.*, 2008) ecosystems. Similarly, the extremely high potential methane oxidation rates observed here (discussed above) are comparable rather to what was reported in sub-arctic and temperate mires than in Arctic tundra soils (Jaatinen *et al.*, 2005; Liebner & Wagner, 2007). Given the slightly acidic pH and a mire-specific vegetation of our study site, the similarity to other mire MOB communities is not surprising. Soil temperature was also suggested to play a role in the establishment of either a type I or type II MOB community as described by Knoblauch *et al.* (2008) who observed a shift from a type I MOB community in Siberian permafrost-affected soils at low temperatures to an increase in type II MOB with increasing incubation temperature. The maritime climate and the higher annual average air temperature of −9.6 °C at our site on Herschel Island (Burn & Zhang, 2009) compared with −14.7 °C on Samoylov Island, Siberia (Wagner *et al.*, 2003), for example, could therefore have an influence in shaping the MOB toward a type II dominated community. These findings support the hypothesis that MOB communities are more sensitive to temperature variations (Liebner & Wagner, 2007), which could have implications in Arctic environments with respect to warming air temperatures and the weakening of MOB as a methane sink.

Methanogenic archaea are also known inhabitants of permafrost soils (reviewed in Wagner & Liebner, 2010). Our results indicate that hydrogenotrophic as well as acetoclastic methanogenesis occurs in active layer soils of the Western Canadian Arctic. Based on T-RFLP and sequence analyses, we found that the methanogenic archaea belonging to *Methanomicrobiales*, *Methanosarcina*, and *Methanosaeta* dominated in the active layer profile which has also been shown in other studies on archaeal diversity in Spitzbergen and Siberia (Høj *et al.*, 2005; Ganzert *et al.*, 2007). *Methanobacteria* that are hydrogenotrophic (Thauer, 1998) showed preferential colonization of the upper layer of the profile, its abundance quickly decreased with depth, while representatives of acetoclastic methanogens belonging to *Methanosarcina* and *Methanosaeta* (Thauer, 1998) were mainly found in the lower and colder soil layers. At low temperatures, there is a prevalence of the acetoclastic pathway of methanogenesis. Indeed, Conrad *et al.* (1987) showed that hydrogen-producing bacteria in paddy soils were inhibited at low temperatures, while homoacetogenesis is a dominant process in cold anoxic ecosystems (Nozhevnikova *et al.*, 1994). Hydrogenotrophic methanogenesis is hampered because of competition with acetogenic bacteria for hydrogen and carbon dioxide which produce acetate as a precursor for acetoclastic methanogens (Kotsyurbenko, 2005). Also, the availability of low molecular substances (e.g. acetate) provided by the root system of the vegetation (Chanton *et al.*, 1995; Ström *et al.*, 2003) could have an influence on the composition of the methanogenic community. Altogether, at the genus level, the community of methanogenic archaea observed here is representative of what has been found in other studies of permafrost soils (Høj *et al.*, 2005; Ganzert *et al.*, 2007; Metje & Frenzel, 2007). The low number of methanogenic OTUs in our study could be due to the low pH of the ecosystem. There are so far only a few cultured acidophilic methanogens known (Cadillo-Quiroz *et al.*, 2009; Bräuer *et al.*, 2011). This could also be due to substrate limitation even though the organic carbon concentration is high, as shown by Wagner *et al.* (2005). Indeed, methane emission rates and potential methane production in carbon-rich soils are dependent on substrate quality, which tends to decrease with the degree of decomposition (Ström *et al.*, 2003).

Based on T-RFLP fingerprints and Bray–Curtis analysis, we observed a vertical shift not only for methanogenic but also for methanotrophic communities. The surface layers clustered together and the respective community compositions of aerobic methane oxidizers were significantly different from those detected in layers closer to the permafrost table. In general, the active layer is a heterogeneous habitat in which biotic and abiotic factors, such as quantity and quality of soil organic matter, pH, soil temperature etc. vary along the soil profile (Fiedler *et al.*, 2004; Wagner *et al.*, 2005). Microbial communities close to the surface undergo large diurnal and seasonal temperature variations, influenced in the summer mainly by air temperature and solar radiation. The layers closer to the permafrost table, however, only vary by a few degrees and generally remain around 0 °C. Microorganisms close to the permafrost table are therefore more likely to be adapted to stable, cold *in situ* temperature as previously observed with methane-cycling communities in Sibera (Wagner *et al.*, 2003; Liebner *et al.*, 2009).

Conclusion

This study provides first insights into the methane-cycling microbial communities in a West Canadian permafrost soil on Herschel Island. We identified a methanotrophic community different from what was reported so far for Arctic tundra soils both in terms of community structure and potential activity. Comparative sequence analysis of uncultivated MOB revealed certain environmental distribution patterns and indicated an preference of specific genotypes to, for example, methane concentration or

salinity (Lüke *et al.*, 2010). Our results also illustrate that the composition of the MOB community in permafrost-affected soils is strongly influenced by environmental conditions such as low temperature and acidic pH. The community of methanogens was similar in composition to what we know from Arctic wet tundra and this community seems to be more stable in the circum-Arctic. We observed a clear shift from a hydrogenotrophic toward an acetoclastic community approaching the permafrost table. Such as shift, though, assumed to exist in tundra soils could never be shown so far.

Evaluating the results of this study in the scope of other studies from the Arctic, our present picture on circum-Arctic methane-cycling communities must still be considered as incomplete and biased toward the few studies conducted to date. It remains elusive whether methane-cycling communities which are specific to Arctic tundra environments truly exist.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Fig. S1. Rarefaction curves of (a) McrA sequences of methanogenic archaea and (b) PmoA sequences of methane oxidizing bacteria using a cutoff value of 14.3% and 7% respectively, from active layer samples of Herschel Island, Canadian Western Arctic.

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MANUSCRIPT II – (Submitted for publication to the *ISME Journal*)

Microbial diversity, function and community structure in polygonal tundra from the Western Canadian Arctic using Ion Torrent sequencing and quantitative PCR.

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Running title: Comparison of microbial diversity and community structure in polygonal tundra.

Keywords: active layer/ biodiversity/ environmental variables/ carbon cycle/ nitrogen cycle/ tundra.

Subject category: Environmental factors (biotic and abiotic) defining the distribution and abundance of microbial populations.

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Abstract

Permafrost-affected ecosystems including peat wetlands are among the most obvious regions in which current microbial controls on organic matter decomposition are altered as a result of climatic change. The objective of this study was to evaluate the diversity and abundance of microbial communities in the active layer of four low-centered polygons on Herschel Island and the Yukon Coast, in the Canadian Western Arctic. Ion Torrent sequencing of bacterial and archaeal 16S rRNA amplicons revealed the presence of all major microbial soil groups and indicated a local, vertical heterogeneity of the polygonal tundra soil community with increasing depth. The overall microbial diversity was high with Shannon indices of 5.8 for Bacteria and 4.7 for Archaea, varying only slightly between the four sampling sites. Diversity was found to be highest in the surface layers, decreasing towards the permafrost table. *Proteobacteria* and *Bacteroidetes* were the dominant members of the bacterial community at all sites. *Euryarchaeota* largely dominated the Archaeal community, with a majority of methanogens (*Methanobacteria* and *Methanomicrobia*) found in the polygonal tundra soils. Quantitative PCR analysis functional genes involved in carbon and nitrogen-cycling revealed a high abundance of nitrogen fixation and ammonium oxidation genes, as well as methane production and oxidation. Combined with the high amount of nitrogen and carbon measured in the soils, these results indicate a nutrient-unlimited environment for carbon degradation and the release of climate-relevant gases associated with warming, having significant implications for future carbon emissions from Arctic wetland ecosystems.

Introduction

Currently there is significant interest in the prospect of climate warming for permafrost environments as they play a crucial role within the global carbon cycle. Current controls of decomposition in such ecosystems are already changing as a result of surface warming, potentially exposing large amounts of previously conserved carbon during the next few decades (Kuhry *et al.,* 2010). Permafrost contains up to 50% of the belowground organic carbon stocks (Tarnocai *et al.*, 2009), of which a significant part could be lost within the next100 years due to globally increasing temperatures (Knoblauch *et al.*, 2013). A large portion of permafrost environments are characterized by patterned ground features, in particular low-centered ice-wedge polygons, which are created by cryogenic processes associated with strong seasonal freeze-thaw cycles. Low-centered polygons typically have a depressed center and an elevated rim, creating a microrelief which affects the hydrology and organic carbon content of the soils. A thickening of the seasonally thawed layer (active layer) of polygons and an ensuing release of previously stored organic matter can stimulate microbial decomposition of this organic carbon, resulting in a positive feedback-loop for global warming (Wagner *et al.*, 2007). Indeed, polygonal tundra is an ideal environment for increased methane production because of the waterlogged, anoxic conditions that prevail in seasonally increasing thawed layers (Whalen and Reeburgh, 1992) as illustrated in Figure 1. In wet tundra soils, methanogenesis is the terminal step in the anaerobic decomposition of organic matter and is solely driven by members of *Euryarchaeota,* a small group of microorganisms which has been identified in numerous permafrost environments (Ganzert *et*

al., 2007, Yergeau *et al.*, 2010). Active methanogenic communities make these environments a significant carbon source. Methane-oxidizing bacteria (MOB) present in the aerobic surface layers of the soil and in association with submerged mosses (Kip *et al.*, 2010, Liebner *et al.*, 2011) play an important role in the biological oxidation of the methane produced in-situ. MOB belong to the *Alpha-* and *Gamma-Proteobacteria* (Hanson and Hanson, 1996) and to the *Verrucomicrobia* (Dunfield *et al.*, 2007). They are able to oxidize up to 90% of the methane emitted in the deeper layers before it reaches the atmosphere (Le Mer and Roger, 2001, Wagner and Liebner, 2009).

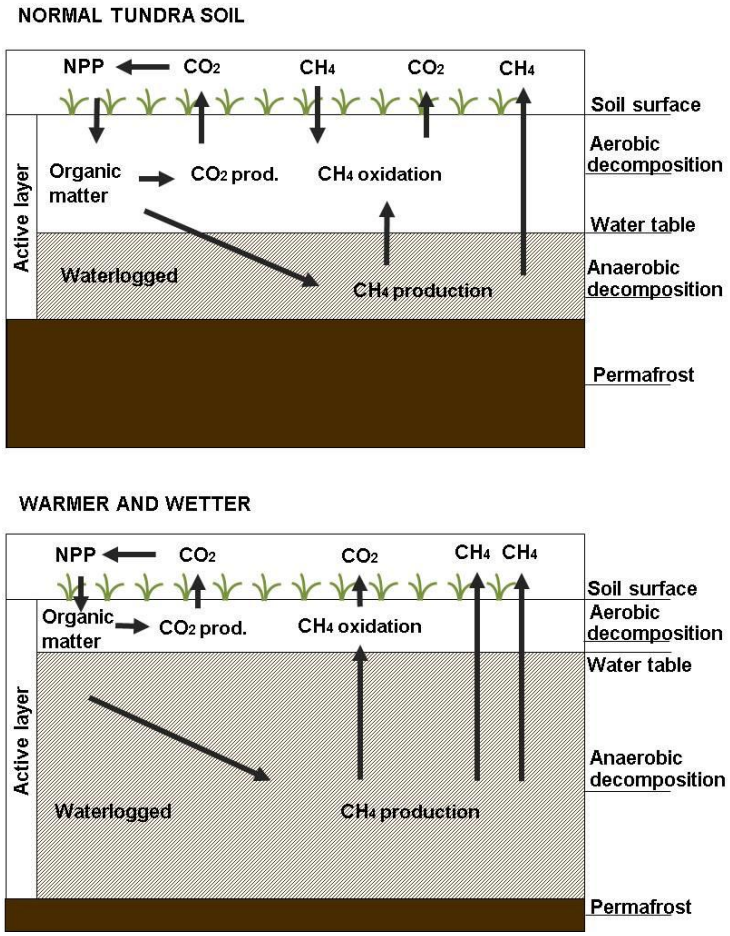


Figure 1. Model for the response of wet polygonal tundra soils under climatic warming (modified from Christensen and Cox, 1995). NPP: net primary productivity. As temperatures increase, the tundra gets warmer and wetter, the active layer thaws deeper creating favourable conditions for anaerobic decomposition and CH_4 production

The balance between methane production and oxidation in Arctic environments is sensitive and non-linear as methanogens and methanotrophs have been observed to responds differently to temperature variations in Siberia (Ganzert *et al.*, 2007, Knoblauch *et al.*, 2008, Liebner *et al.*, 2009) and Svalbard (Høj *et al.*, 2008). Furthermore, accurate predictions of the long-term rates of carbon and nitrogen cycling in arctic soils, which in turn may determine total ecosystem carbon storage (Hobbie *et al.,* 2000), plant productivity (van Wijk *et al.,* 2005) and species composition (Weintraub and Schimel, 2005), require a much greater understanding of

microbial acclimation responses (Hartley *et al.,* 2008). Yet despite their high relevance in the global climate equation (Graham *et al.*, 2012), certain polygonal tundra ecosystems in northern regions such as the Canadian Western Arctic remain poorly characterized in terms of microbial diversity and biogeochemical cycles resulting in the net release of greenhouse gases to the atmosphere.

This study builds on the findings of Barbier *et al.* (2012) who investigated a low-centered polygon on Herschel Island and found that the microbial community present differed from previous described communities in the Circum-Arctic. By extending the picture to more polygons along the Yukon Coast, we aimed to i) evaluate the bacterial and archaeal diversity in relation to active layer depth, ii) compare the microbial diversity in four seemingly similar low-centered polygons which widely occur in the Arctic based on 16S and a selection functional genes involved in carbon and nitrogen-cycling and iii) elucidate the main abiotic factors driving the microbial distribution and diversity observed.

To attain these objectives, we compared and contrasted the microbial communities present in the active layer of four polygonal tundra sites on Herschel Island and the Yukon Coast using Ion Torrent sequencing and quantitative real-time PCR. To understand abiotic factors driving this distribution, we described the physico-chemical properties of the soil profiles. Finally, we used multivariate statistics to elucidate the main biotic and abiotic factors driving the microbial community diversity and structure observed. The results presented give new insights into the distribution and function of microorganisms in a climate-sensitive environment: nitrogen and carbon rich, water saturated polygonal tundra. This study is also one of the first to validate the use of Ion Torrent sequencing for microbial ecology applications.

Materials and methods

Site description and sample collection

Active layer samples were collected from four low-centered polygons in the Western Canadian Arctic, on Herschel Island and the Yukon Coast: Drained Lake Polygon (DP, N 69°34’43, W 138°57’25), Lake Polygon (LP, N 69°36 00.6, W 139°03 56.8), King Point Polygon (KP, N 69°05 26, W 137°56 45) and Mainland Polygon (MP, N 69°28 23.7, W 139°11 06.3 (Figure 2). The soil at all four sites were characterized as Hemic Glacistel classified according to the U.S. Soil Taxonomy (Soil Survey Staff, 1998) with poor drainage and a loamy soil texture. The vegetation period on Herschel Island and the Yukon Coast spans yearly from mid-June to end of September. Average air temperatures vary annually between -26.3 °C in February to 8.7 °C in July with temperatures at the surface of the active layer ranging from -35°C in the winter to 25°C in the summer (Burn and Zhang, 2009).

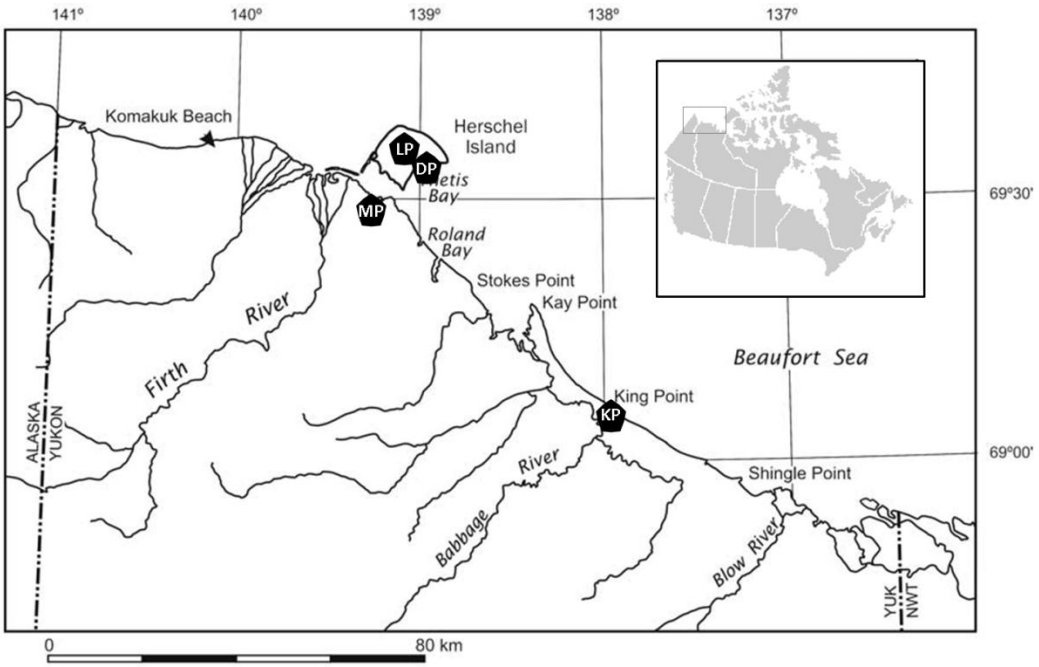


Figure 2. Geographical location of the four polygons investigated, on Herschel Island and along the Yukon Coast (modified from Burn and Zhang, 2009). DP: Drained Lake Polygon, LP: Lake Polygon, MP: Mainland Polygon and KP: King Point Polygon.

The sampling sites were characterized by an active layer (the layer of ground that is subject to annual thawing and freezing) consisting of a large peat horizon, with a depth varying between 25 cm and 36 cm as measured using a permafrost probe. Replicated sampling was conducted as follows: a large pit was dug in the center of each polygon down to the permafrost table, exposing a clean vertical profile of over a meter in width. At every 5 cm depth, soil subsamples were taken with a sharp sterile knife to cover small-scale horizontal heterogeneity. Subsamples obtained from each layer were then homogenized into sterile 125 mL Nalgene® screw-cap containers (Thermo Fischer Scientific Inc., Waltham, MA). The knife was wiped down and sterilized with ethanol between different samples. Soil samples were frozen immediately after sampling and stored at −20 °C upon arrival in the laboratory. All subsequent subsampling was performed under sterile and anaerobic conditions in an atmosphere-controlled glove box.

Soil physico-chemical analyses

Gravimetric water content of soils was determined by weighing sub-samples before and after freeze-drying until no moisture could be observed and the weight stayed constant. Conductivity and pH were measured using a CyberScan PC 510 Bench Meter (Eutech Instruments Pte Ltd, Singapore) following the slurry technique which consists in mixing 1:2.5 mass ratio of samples and de-ionized water (Edmeades *et al.*, 1985). The percentage of total organic carbon (TOC) of dried, homogenized soils was measured in duplicate using a TOC analyzer (Elementar Vario max C, Germany) after HCl (10 %) acid digestion to remove carbonates. For trace element analyses a soil slurry (5 g soil + 25 ml mili Q water) was mixed in an overhead shaker for 90 min and centrifuged at 4000 rpm for 20 min. Anions were measured by ion chromatography (Dionex-DX320), cations by inductively coupled plasma

optical emission spectrometry (ICP-OES, Perkin Elmer Optima3000XL) and HCO_3^- by titration (Metrohm Titrino 794).

DNA extraction and PCR amplification

Total genomic DNA was extracted in duplicate from 0.6 g of soil using the PowerSoil™ DNA Isolation Kit (Mo Bio Laboratories, Carlsbad, California). Duplicates were then pooled for downstream analyses. The obtained genomic DNA extracts were quantified using the PicoGreen dsDNA quantitation assay (Invitrogen, Carlsbad, CA). DNA was then stored at -20 °C for further use. 16S rRNA gene amplification was carried out using multiplex identifiers as described in Bell *et al.* (2013) for bacteria and Yergeau *et al.* (2012) for archaea. Reactions were carried out in 25 µl volumes containing 2 µl of template DNA, 0.5 µM each primer and 12.5 µl of KAPA2G Robust HotStart ReadyMix (KAPA Biosystems, Woburn, MA). Cycling conditions involved an initial 5 min denaturing step at 95°C, followed by 35 cycles of 30 s at 95°C, 30 s at 57°C, and 45 s at 72°C, and a final elongation step of 10 min at 72°C. PCR products were purified on 2.5% $w.v^{-1}$ agarose gels using the QIAquick Gel Extraction Kit (Qiagen, Valencia, CA) and quantified using the PicoGreen dsDNA quantitation assay. All 20 amplification products from the different samples were pooled in an equimolar ratio (one pool for Bacteria and one pool for Archaea) and sequenced together. A total of $3.50x10^7$ molecules were used in an emulsion PCR using the Ion OneTouch™ 200 Template Kit (Life Technologies) and the OneTouch™ instruments (Life Technologies) according to the manufacturer's protocol. The sequencing of the pooled library was done using the Personal Genome Machine™ (PGM) system and a 314 chip with the Ion Sequencing 200 kit according to manufacturer's protocol. Sequences have been deposited in the NCBI SRA database.

Quantitative PCR amplification

qPCR was performed in 20 µl volumes using the iQ SYBR green Supermix (Bio-Rad Laboratories, Hercules, CA, USA) on a Rotor-Gene 3000 apparatus (Corbett Life Science, Sydney, NSW, Australia) as previously described (Yergeau *et al.*, 2009). The primers and temperature conditions are detailed in Table 1. To check for inhibition, dilutions of the samples were made until three consecutive dilutions yielded the same results. Standards were made from 10-fold dilutions of linearized plasmids containing the gene fragment of interest that was cloned from amplified soil DNA.

Table 1. Primers and temperature profiles used for quantitative PCR analysis**.**

Functional gene	Primer Pair	Temperature °C (ann./read)	Reference
mcrA	MlF / MlR	55/83	(Luton *et al.*, 2002)
pmoA	A189F / mb661R	55/82	(Kolb *et al.* 2003)
archaeal *amoA*	amoA19F / crenamoA616r48x	50/72	(Le Roux *et al.* 2008)
bacterial *amoA*	amoA-1F / amoA2R-TC	52/81	(Nicolaisen & Ramsing 2002)
nifH	PolF / PolR	55/83	(Poly *et al.* 2001)

Bioinformatic analyses

16S rRNA gene amplicons were pre-treated using the Ribosomal Database Project (RDP) Pyrosequencing pipeline (Cole *et al.*, 2009). For bacteria, sequences having an average quality under 20, having Ns, not exactly matching the MID sequence or being shorter than 100 bp were discarded. For Archaea, sequences having an average quality under 17, having Ns, not exactly matching the MID sequence or being shorter than 75 bp were discarded. Remaining sequences were submitted to the RDP classifier (Wang *et al.*, 2007) using a 0.5 bootstrap cut-off as advised for short sequences (Claesson *et al.*, 2009). The Shannon diversity index was calculated for each depth for all four study site. For operational taxonomic unit (OTU) calculations, flowgrams from sff files were de-noised and clustered using AmpliconNoise (Quince *et al.*, 2011). Before performing AmpliconNoise calculations, datasets were normalized by randomly selecting 15,000 sequences for bacteria and 2000 sequences for Archaea. A 97% similarity cutoff was used for OTU calculation, the most stringent OTU definition allowing for intragenomic 16S rRNA variation and PCR/sequencing errors (Schloss and Handelsman, 2006; Kunin *et al.*, 2009).

Statistical analyses

Weighted-normalized Unifrac distances between each sample pair were calculated using the FastUnifrac website (Hamady *et al.*, 2010) based on the GreenGene core dataset. Most statistical analyses were performed in R (v2.13.2, The R foundation for statistical computing, Vienna, Austria). Spearman rank-order correlations (rs) were carried out using the "cor.test" function. Principal coordinate analyses (PCoA) were carried out using the "cmdcsale" function while principal component analysis (PCA) was carried out using the "rda" function of the "vegan" library. Mantel tests based on Spearman correlations were performed using the "mantel" function while permANOVA was performed using the "adonis" function of the "vegan" package. Canonical correspondence analyses (CCA) were carried out in Canoco for Windows v.4.5 (ter Braak and Šmilauer, 2002), using all the PCoA axes calculated from Unifrac distance as the "species" data and the physico-chemical variables selected by forward selection (at $P<0.05$) as the "environmental" variables.
Venny (http://bioinfogp.cnb.csic.es/tools/venny/index.html) was used to draw Venn diagrams from genus presence-absence data.

Results

Characteristics of the soil

The permafrost table was measured at 36 cm depth for DP, 25 cm for KP, 26 cm for LP and 36 cm for MP in the period from July 27th to August 2nd, 2010. All polygon sites were visibly water saturated, with gravimetric water contents ranging from 65 to 86%, with the exception of the two lower depths of KP (41-42%). The pH at polygons DP and MP was pH 5.4 on average, slightly more basic at LP (pH 5.7 to 6.8) and more acidic at KP (average pH 4.8). Total organic carbon content (TOC) was high at all sites, ranging from 20 to 40%, with the exception again of the bottom layers of KP where TOC was lower (10-11%). An overview of the physico-chemical properties of each polygon is given in Table 2.

Quantitative PCR

Overall, gene copy numbers per gram of wet soil were high for all of the functional genes investigated and in all four polygons. The only exception is the complete absence of an amplifiable *mcrA* signature in the first 15 cm depth in KP. For all genes, the copy number tended to decrease slightly (DP and LP) or remained relatively stable (KP and MP) with increasing depth (Figure 3). *NifH* was detected in the highest amount in all polygons, reaching up to 10^8 copies per gram of wet soil. Archaeal *amoA* was found to have between 10^5 to 10^6 copies.g^{-1}, while bacterial *amoA* was one or two orders of magnitude lower (10^3 to 10^4 copies.g^{-1}). *McrA* was more erratically distributed throughout the different depths and varied more between polygons, with copy numbers ranging anywhere from 10^1 to 10^7 copies.g^{-1}. Detailed gene copy number information for all functional genes can be found in Table S2 in supplementary information.

Table 2. Selected physico-chemical properties of the soils

Name of polygon GPS coordinates	Depth (cm)	Water content (%)	N (%)	C (%)	S (%)	TOC	pH	Conductivity TRef. 25°C ($\mu S.cm^{-1}$)
Drained Lake Polygon (DP)	5-10	79	1.79	26.65	0.33	24.48	5.3	68.1
	10-15	77	1.89	30.63	0.37	28.24	5.46	54.9
N 69 34 43	15-20	78	1.59	30.44	0.31	28.29	5.53	59
W 138 57 25	20-25	76	1.18	24.12	0.25	25.20	5.44	62.8
	25-30	79	1.19	26.43	0.24	23.19	5.23	74.3
	30-35	83	1.25	27.62	0.24	26.04	5.29	85.9
King Point Polygon (KP)	0-5	82	1.01	40.09	0.38	38.23	4.33	111.7
	5-10	84	1.11	38.22	0.29	37.75	4.47	91.8
N 69 05 26	10-15	86	1.11	35.91	0.27	34.31	4.96	101.7
W 137 56 45	15-20	41	0.54	13.27	0.24	11.30	5.19	28
	20-25	42	0.25	6.04	0.17	9.77	5.08	39.2
Lake Herschel Polygon (LP)	5-10	76	1.35	25.08	0.34	22.69	6.82	76.4
	10-15	76	1.33	24.92	0.4	22.66	5.73	84.7
N 69 36 00.6	15-20	69	1.14	20.62	0.31	16.56	5.83	72.5
W 139 03 56.8	20-25	77	1.76	29.77	0.35	28.09	5.9	91.3
Mainland Polygon (MP)	0-5	79	2.35	33.36	0.47	31.76	5.54	45.7
	5-10	80	2.49	39.61	0.42	38.25	5.47	40.5
N 69 28 23.7	10-15	80	2.68	42.69	0.42	39.20	5.46	38.6
W 139 11 06.3	15-20	76	1.78	31.95	0.32	29.95	5.41	45
	20-25	68	1.45	26.32	0.28	24.08	5.44	53.4
	25-30	65	1.22	23.55	0.27	21.32	5.46	49.3
	30-36	78	2	35.96	0.38	34.57	5.39	57.2

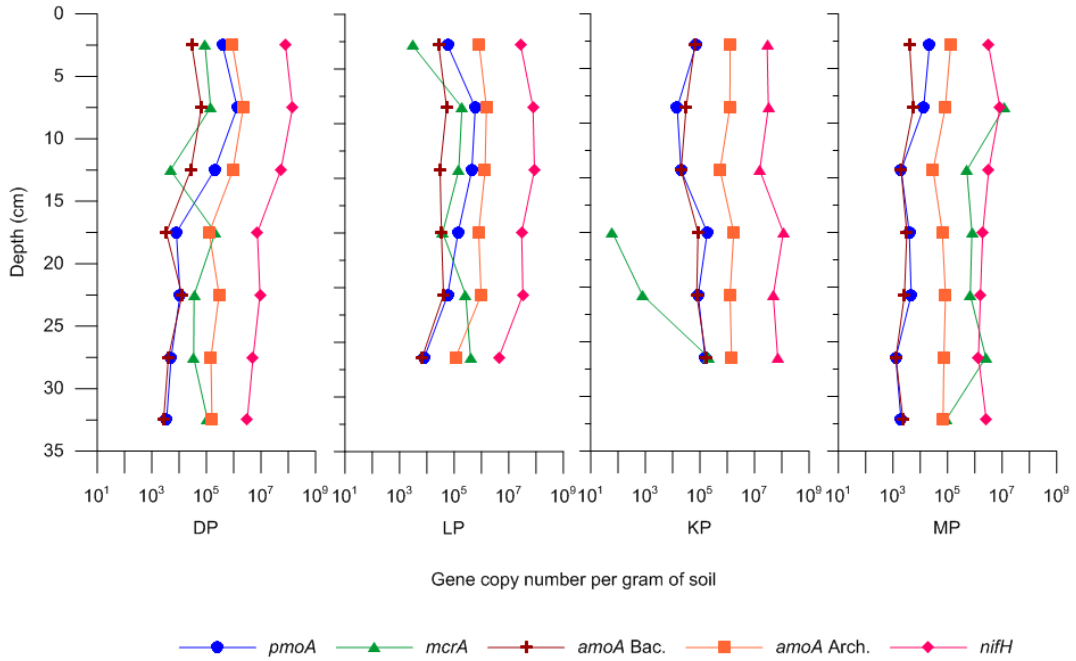


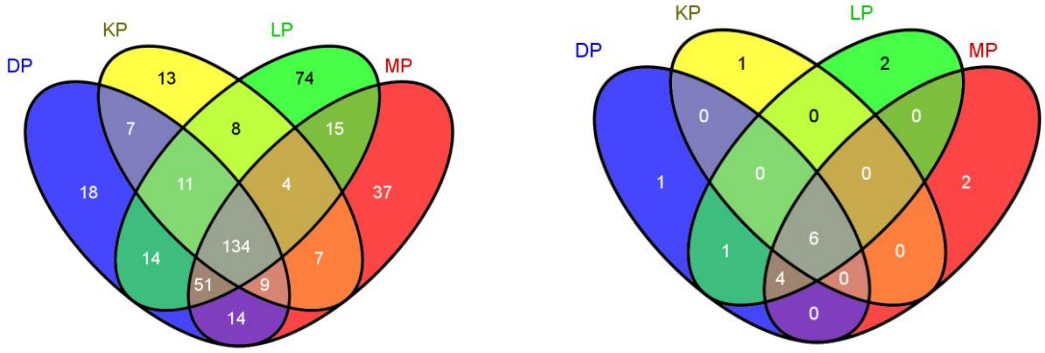
Figure 3. Real-time PCR quantification in the active layer profile of each polygon investigated for *pmoA*, *mcrA*, bacterial *amoA*, archaeal *amoA* and *nifH* genes.

Biodiversity and richness index

The bacterial community was very diverse, with an overall Shannon index (H') of 5.8. The archaeal community diversity was also high, with an overall Shannon index of 4.7. In all polygons, the bacterial diversity was highest in the surface samples, then decreasing with depth (Table 3). Archaeal diversity was highest in the upper layers of the soils, the maximum diversity found between 0-15 cm depth (Table 3). LP showed the highest diversity overall with H'6.2 for bacteria and 4.9 for archaea. Based on OTU (0.03) calculations, the highest number of OTUs was found in DP, while the highest number of archaeal OTUs was found in LP (Table S3). Out of a total of 416 bacterial genera identified in all polygons combined, 134 were shared by all four sites. DP had 18 unique genera, KP 13, LP 74 and MP 37 (Figure 4.a.). However, due to the short length of the archaeal 16S rRNA sequences and the lower number of existing archaeal genera, only 12 genera could be identified, of which 6 were shared between all polygons (Figure 4.b., grey colored field). Interestingly, 4 genera were shared between all polygons except KP.

Table 3. Shannon diversity index (H') of bacterial and archaeal 16S rRNA gene sequences with 97% similarity in the active layer of four low-centered polygons on Herschel Island and the Yukon Coast.

	DP		LP		KP		MP	
Depth (cm)	Bacteria	Archaea	Bacteria	Archaea	Bacteria	Archaea	Bacteria	Archaea
0-5	6.3	4.8	6.6	5.8	5.7	n.a.	6.2	5.1
5-10	6.2	5.0	6.4	5.7	5.7	n.a.	6.1	5.1
10-15	6.2	5.1	6.2	5.1	5.9	n.a.	5.7	4.7
15-20	6.0	4.6	5.8	4.7	5.1	5.2	5.6	4.6
20-25	5.8	4.3	6.3	4.3	5.1	4.7	5.7	4.5
25-30	5.6	4.4	n.a.	n.a.	n.a.	n.a.	5.2	4.5
30-35	5.5	4.2	n.a.	n.a.	n.a.	n.a.	5.5	4.6
Permafrost	5.5	3.9	5.8	4.1	5.1	4.5	5.6	4.7
Average	5.9	4.5	6.2	4.9	5.4	4.8	5.7	4.7



a) **b)**

Figure 4. Venn diagram illustrating the distribution of classified a) bacterial and b) archaeal genera in the four polygons.

Community composition and vertical distribution of diversity

For partial sequences of length longer than 50 bp and shorter than 250 bp, a bootstrap cutoff of 50% was shown to be sufficient to accurately classify sequences at the genus level, and to provide genus level assignments for higher percentage of sequences (Claesson *et al.*, 2009). The phylum-level compositions of bacteria at all four sites were found to be highly similar overall but varied somewhat differently with depth (Figure 5). Sequences affiliated with *Proteobacteria* accounted for 40–50% of sequences in all sites, followed by *Bacteroidetes*-affiliated sequences at 20–40% and *Actinobacteria* at 10-15%. Communities in all four polygons showed a similar evolution of community composition with depth: *Deltaproteobacteria*, *Bacteroidetes* and *Firmicutes* increasing in abundance and *Alpha-, Beta-, Gammaproteobacteria* and *Acidobacteria* decreasing with depth (Figure 5).

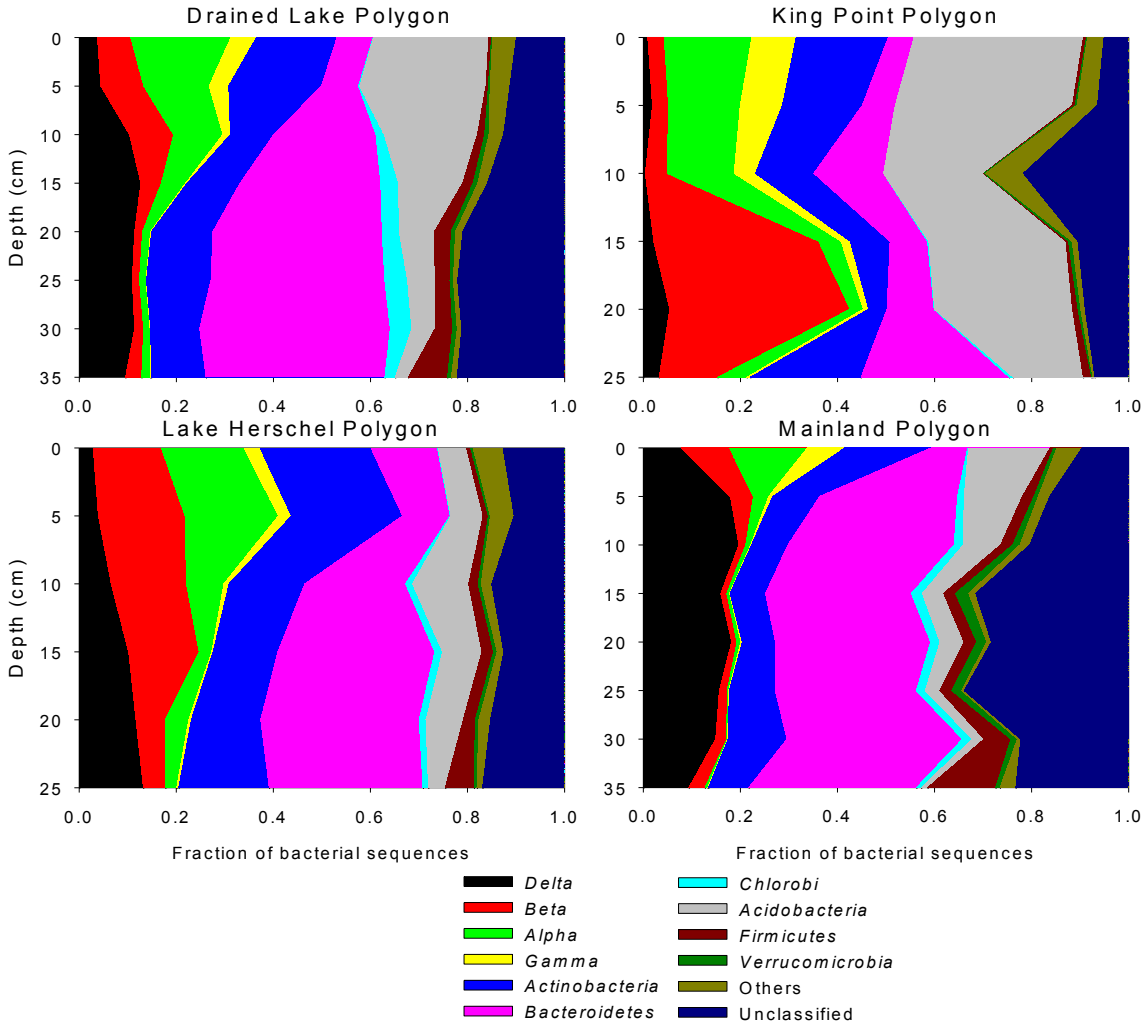


Figure 5. Distribution of bacterial phyla with depth based on 16S rRNA sequences in all four polygons. Alpha, Beta, Delta and Gamma stand for *Alphaproteobacteria*, *Betaproteobacteria*, *Deltaproteobacteria* and *Gammaproteobacteria*.

The class-level compositions of archaea were similar in DP and MP (Figure 6). No archaeal sequences could be amplified in KP from the first 15 cm of the active layer. Sequences affiliated with methanogens (*Methanobacteria* and *Methanomicrobia*) dominated in all sampling sites, representing up to 90% of all classified sequences. Within the classified sequences, *Methanobacteria* were found to clearly dominate in the surface layers of three out of the four polygons representing up to 60% of classified sequences. They decreased rapidly with depth, replaced by *Methanomicrobia* which represented more than 80% of the community in the deeper layers.

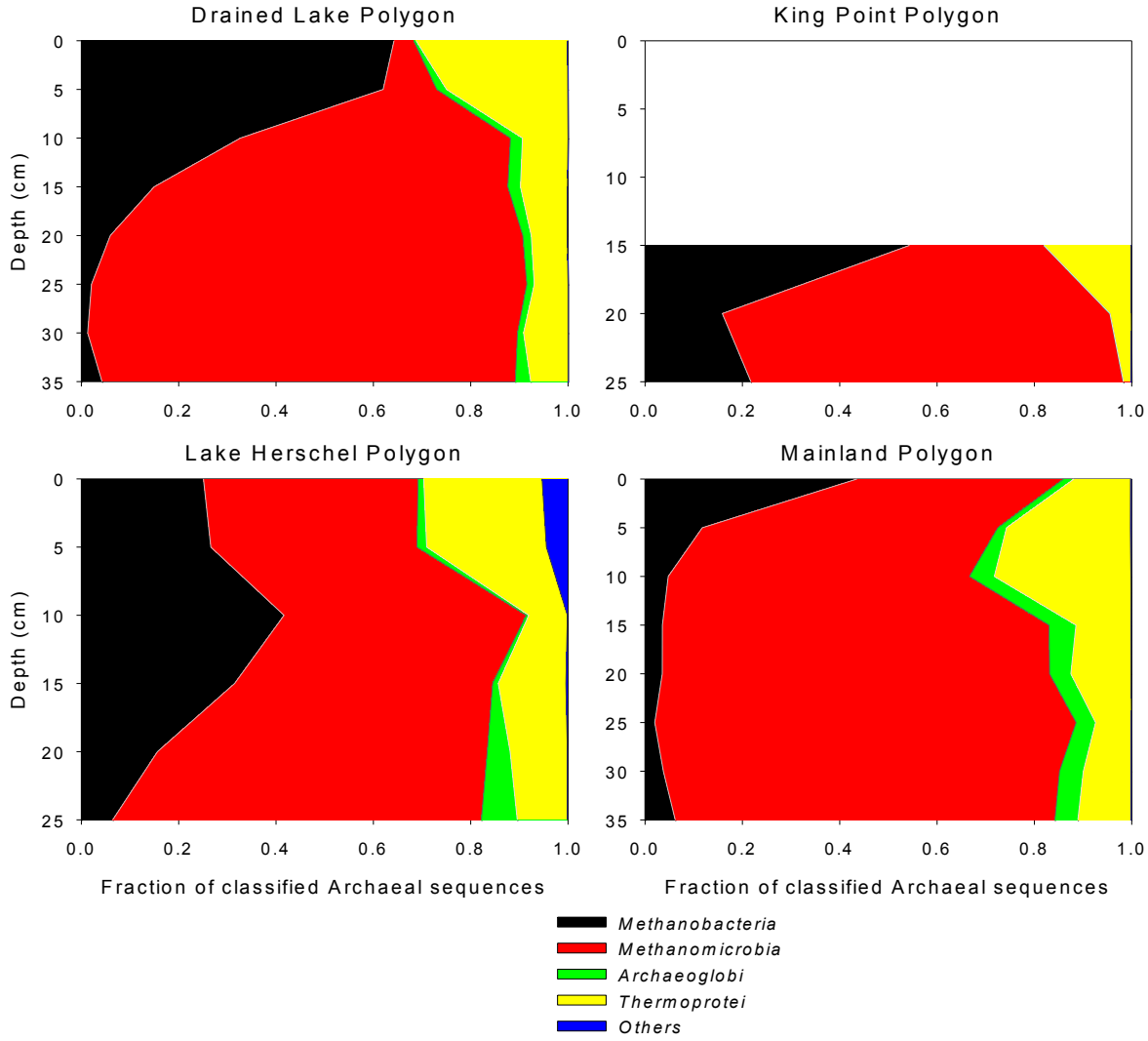


Figure 6. Distribution of archaeal classes with depth based on 16S rRNA sequences in all four polygons. No archaeal 16S rRNA sequence could be amplified from KP1-KP4.

Overall, little variation was observed for the bacterial community between all polygons, however the variation within each polygon (i.e. with increasing depth) was higher. On the contrary, the archaeal community composition was influenced by location and varied more between polygons, each having an own, polygon-specific community. For Bacteria, the KP samples were separated from all the other samples Figure 7. Another interesting trend was the separation of the majority of deep samples (15-35 cm deep) versus the majority of the shallow samples (0-10cm deep). This trend was also visible when looking at the average unifrac distance between shallow and deep samples, which was relatively high. For Archaea, again the KP samples clustered separately from all other samples. The 15 to 35 cm deep samples tightly clustered together, as visible by the relatively low unifrac distance between samples from that depth. For both archaea and bacteria, the microbial communities were more similar within a polygon then between polygons (unifrac distances), indicating some level of local adaptation.

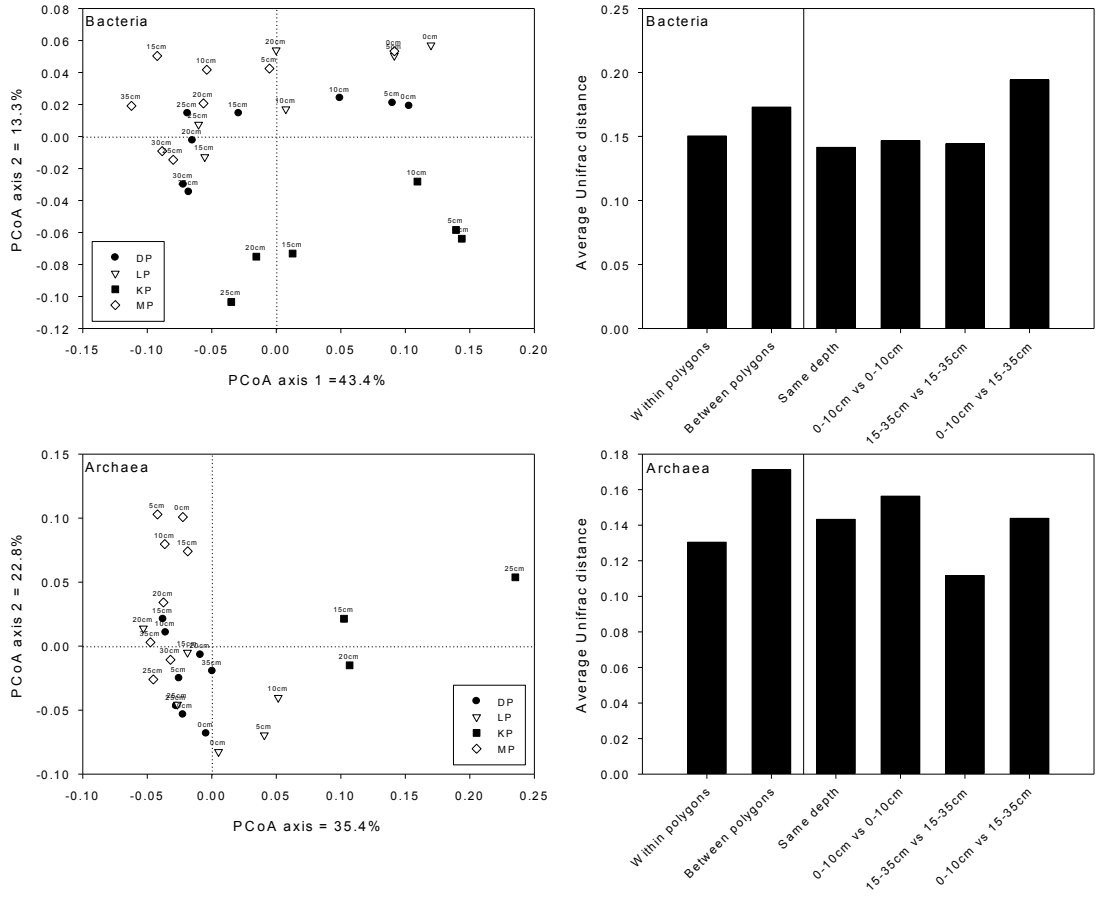


Figure 7. Unifrac analysis of 16S rRNA sequence variation within and between polygons for a) bacteria and b) archaea.

Biogeochemical analysis

To investigate potential relationships between microbial community structure and the underlying soil geochemistry, the sequence data were correlated to physico-chemical data and illustrated by heat maps (Figures 8.a and b.). Variables most consistently explanatory of both bacterial and archaeal sequence diversity patterns were depth and conductivity. Water content and nitrate had a stronger influence on the bacterial community in surface samples (Figure 8.a) while KP samples clustered separately from other samples being more influenced by C:N ratio and potassium. For archaea, samples clustered differently based on pH and a variety of trace elements such as P, K and S.

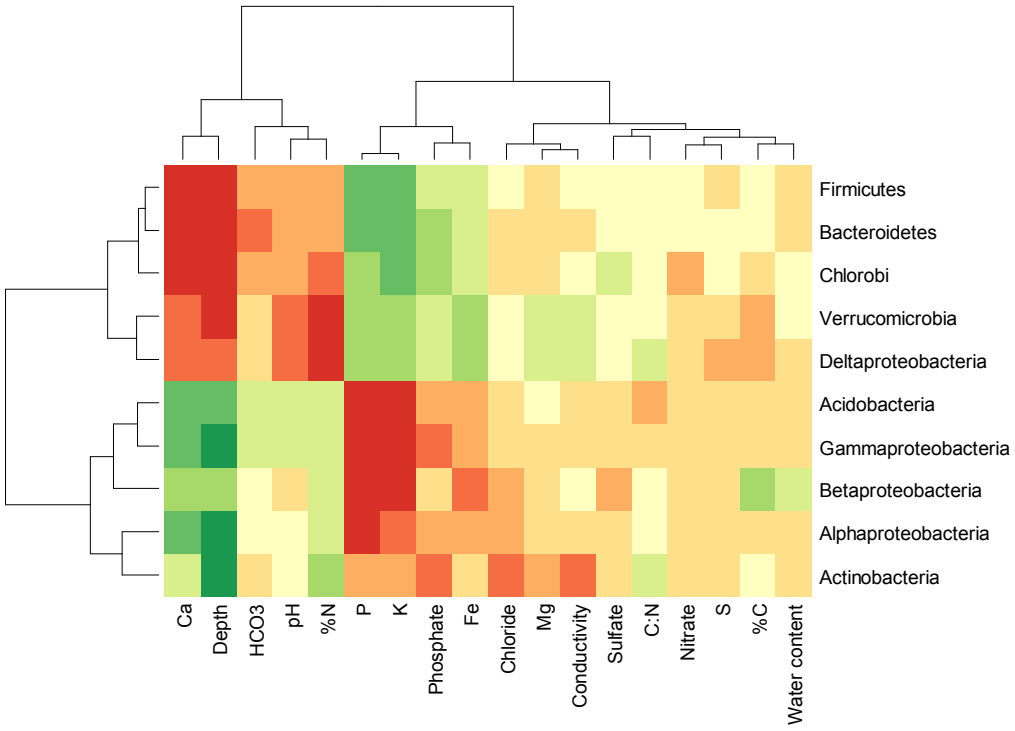


Figure 8.a. Heat maps illustrating the correlation between the taxonomical distribution of a) bacterial phyla with the physico-chemical properties of the soil. A red colour indicates a positive correlation, while a green color indicates a negative correlation. The darker the colour, the stronger the correlation.

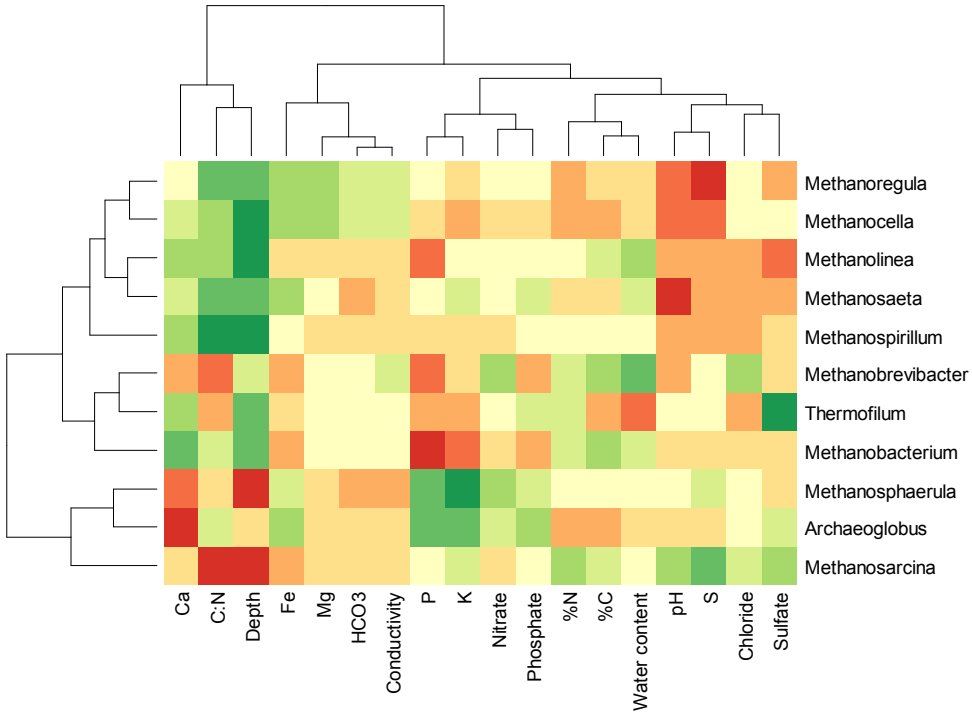


Figure 8.b. Heat maps illustrating the correlation between the taxonomical distribution of archaeal genera with the physico-chemical properties of the soil. A red colour indicates a positive correlation, while a green color indicates a negative correlation. The darker the colour, the stronger the correlation.

Discussion

Through this study, our goal was to evaluate the diversity and abundance of microbial communities in the active layer of four low-centered polygons on Herschel Island and the Yukon Coast, in the Canadian Western Arctic, and see how they compare to the microbial communities in the rest of the cirum-Arctic. We found that the polygons on Herschel Island and the Yukon Coast had similar diversity, but different community compositions. A certain level of local adaptation was illustrated by the fact that the community composition was more similar within a polygon than between two polygons,. However, within each polygon, we saw highly parallel trends, with increasing *Baceroidetes* and decreasing *Proteobacteria* in samples closer to the permafrost table. The considerable variation within the *Proteobacteria* indicates consistent forcing mechanisms related to depth. *Bacteroidetes* are known to include several psychrophilic members (Shivja *et al.*, 1992) which thrive under the stable cold conditions such as the ones found at the bottom of the active layer, close to the permafrost table. A study of polygonal tundra in the Lena Delta, Siberia also identified *Bacteroidetes* as dominant members of the soil microbial community (Liebner *et al.*, 2008). In general, the active layer is a heterogeneous habitat in which biotic and abiotic factors, such as quantity and quality of soil organic matter, pH, soil temperature etc. vary along the soil profile (Fiedler *et al.,* 2004; Wagner *et al.,* 2005, Barbier *et al.,* 2012). Microbial communities close to the surface undergo large diurnal and seasonal temperature variations. The layers closer to the permafrost table, however, only vary by a few degrees and generally remain around 0 °C. Microorganisms close to the permafrost table are therefore more likely to be adapted to stable,

cold in situ temperature as previously observed with methane-cycling communities in Siberia (Wagner *et al.,* 2003; Liebner *et al.,* 2009). Although *Proteobacteria* represented 20-40% in all four polygons in our study, there was considerable variation within this group with increasing depth; *Delta-Proteobacteria* increased while, *Alpha-*, *Beta-* and *Gamma-Proteobacteria* decreased with proximity to the permafrost table, indicating the adaptation and specialization of the various groups to changes in temperature and soil physico-chemistry. *Alphaproteobacteria,* for example, comprise largely fast-growing members which thrive in nutrient rich environments where carbon turnover is high (Thomson *et al.*, 2010). *Acidobacteria*, on the other hand, are better adapted to lower nutrient availability (Fierer *et al.,* 2007), as illustrated in our results by the occurrence of Acidobacteria towards the deeper layers at most of the study sites. Looking for the main drivers of microbial distribution in the active layer, bacterial community composition appeared mostly influenced by the decreasing temperature and oxygen gradient with increasing depth in the active layer. Indeed, depth is a known driving factor as depth (i.e. temperature) associated changes in microbial community distribution was also observed in other arctic wetlands (Galand *et al.*, 2005, Ganzert *et al.*, 2007; Liebner *et al.*, 2009).

The Archaeal community was dominated by methanogens in all four sampling locations, which is concurrent with the findings of archaeal diversity studies in Siberia and Svalbard (Ganzert *et al.*, 2007, Høj *et al.*, 2005). The Archaeal community composition, particularly methanogens, was mainly influenced by the pH of the soil, and the sampling depth which is a proxy for oxygen content and temperature. One of the sampling sites, King Point Polygon, stood out when compared to the others; as no archaeal 16S rRNA sequences could be amplified in the first half of the active layer, which was also confirmed by the absence of *mcrA* signal and the absence of any measurable methane production after incubation (data not shown). These differences can be attributed to the low pH found in the upper layers of the soil, as there are so far only a few cultured acidophilic methanogens known (Cadillo-Quiroz *et al.,* 2009; Bräuer *et al.,* 2011) and to a much higher conductivity in the top layers, most likely associated with abundant sea spray and increased salt concentration due to the proximity of the sampling site to the Beaufort Sea. Apart from the exception of KP, the high abundance of methanogenic archaea in the polygons underlines the importance of permafrost environments in view of the current climate change and the pronounced warming of the Arctic. Methanogenic archaea are recognized as widespread in permafrost-affected soils (reviewed in Wagner and Liebner, 2010).

Our results indicate that hydrogenotrophic as well as acetoclastic methanogenesis occurs in active layer soils of the Western Canadian Arctic. Within the classified sequences obtained, we found that *Methanobacteria* dominated the surface layers of all four polygons. This contrasts with other studies from archaeal diversity in northern peatlands in Siberia (Ganzert *et al.*, 2007), Finland (Juottonen *et al.*, 2005) and Svalbard (Hoj *et al.*, 2005) where no members of the family *Methanobacteriaceae* could be detected. The deeper methanogenic community switched sharply to a *Methanomicrobia* dominated community, of which most members are acetoclastic, save for the *Methanomicrobiales*. A similar switch from a hydrogenotrophic to an acetoclastic methanogenic community has been observed in the detailed study of a polygon at Drained Lake, Herschel Island (Barbier *et al.*, 2012) and in

other Arctic tundra environments (Høj *et al.*, 2005; Ganzert *et al.*, 2007; Metje and Frenzel, 2007). This supports the hypothesis that the acetoclastic pathway is favored in cold environments (Conrad *et al.*, 1987) as hydrogen-producing bacteria are inhibited at low temperatures. In cold, anoxic conditions such as those encountered close to the permafrost table, hydrogenotrophic methanogenesis is hampered because of competition for hydrogen and carbon dioxide with acetogenic bacteria (e.g. *Bacteroidetes*) which produce acetate as a precursor for acetoclastic methanogens (Kotsyurbenko, 2005). The availability of low molecular substances (e.g. acetate) provided by the root system of the vegetation (Chanton *et al.,* 1995; Ström *et al.,* 2003) could also have an influence on the composition of the methanogenic community.

Looking at an array of carbon and nitrogen functional genes also provided interesting insights into the microbial communities in the polygons. We found the abundance functional genes involved in nitrogen fixation, ammonium oxidation, methane production and oxidation to be higher by one to two orders of magnitude compared to samples from the active layer in Hess Creek, Alaska (Mackelprang *et al.*, 2011) and the Canadian High Arctic (Martineau *et al.*, 2010, Yergeau *et al.*, 2010). We detected a high abundance of nitrogen fixers and ammonia oxidizers in the active layer. In accordance with previous studies (Leininger *et al.* 2006, Yergeau *et al*., 2010), archaeal ammonia oxidation genes were detected in higher abundance than the bacterial *amoA*, in our case by almost two orders of magnitude. The dynamics of nitrogen processes may be especially important, because most tundra ecosystems are nitrogen limited and changes in nitrogen supply can affect leaf development, carbon flux and biomass production (Shaver and Kummerow, 1992; Schimel *et al.*, 1996). However, the soil nitrogen concentrations measured in the different polygons in this study was very high and the nitrogen fixation gene *nifH* was also detected in very high abundance, suggesting that nitrogen limitation on the decomposition of organic matter in the active layer and the subsequent production of CO_2 and CH_4 is unlikely. This demonstrates the presence of methanogens throughout the profile and more importantly the presence of aerobic methane oxidizers also in deeper, water saturated layers. This presence is likely supported through root exudates creating microaerobic conditions favorable for methane oxidation, as well as the possible association of MOB with mosses providing them with the necessary oxygen (Liebner *et al.*, 2011).

The limited amount of field replication is an obvious limit to the interpretation of community analysis. The ecological sensitivity of our sampling sites was an important factor in determining the amount of samples to be taken, as polygonal tundra is characterized by a complex vegetation and soil matrix that can be irreversibly damaged by excessive digging or trampling. This highlights the general difficulty of field sampling and obtaining a number of samples that is concurrently representative, statistically justified and logistically feasible. Nonetheless, polygonal tundra is characterized by homogeneous soil physical and chemical properties and surface characteristics both on a spatial and temporal scale. Water content, pH, total organic carbon content etc. were found to be homogeneous within a polygon field (Fiedler *et al*., 2004). This study is one of the first to apply the new technology of Ion Torrent Sequencing to the field of microbial ecology. This method has a great potential for future use in the field and has already been demonstrated to be a valid technology for environmental

studies (Yergeau *et al.,* 2012) allowing for cost-efficient, high-throughput sequencing which can be expected to soon rival with already established methods such as 454 Sequencing.

In summary, this study provides an important insight into the microbial functional role of Arctic polygonal tundra with respect to the carbon and nitrogen cycles, as well as a comprehensive review of the microbial diversity based on 16S rRNA sequences. The results show that despite their geographical distance, three out of the four sampling sites harbored very similar and highly diverse bacterial communities which have significant implications for future greenhouse gas emissions. Overall, we observed a vertical evolution of community composition towards the permafrost table. It is yet to be determined if these trends would apply to other similar Arctic environments, but this consistent depth effect will be key to determining the fate of the organic matter that will be released from thawing permafrost, as this process will occur in the deeper soil layers. Methanogens dominated the archaeal community, emphasizing the importance of carbon-rich polygonal tundra environments in the scope of exacerbated climate warming in Arctic regions. Specifically, carbon and nitrogen cycling genes were detected in very large amounts compared to other studies Arctic locations, pointing out the importance of the Northwest Canadian Arctic in terms of thawing permafrost and the release of climate-relevant gases into the atmosphere. We did not observe a clear separation of permafrost vs. active layer samples, rather a continuous transition from the surface all the way through the permafrost layer. This supports the idea that permafrost and active layer are highly similar to each other (Yergeau *et al.*, 2010), and that the communities that will be involved in the degradation of thawed permafrost will likely resemble the ones already in activity in the active layer.

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Supplementary information

Table S1. Trace element concentrations found at different depths in all four polygons.

Polygon	Depth (cm)	Al (µg/L)	Ba (µg/L)	Ca (µg/L)	Fe (µg/L)	K (µg/L)	Mg (µg/L)	Mn (µg/L)	Na (µg/L)	P (µg/L)	Si (µg/L)	Sr (µg/L)	Pb (µg/L)	Cr (µg/L)
Drained Lake Polygon (DP)	0-5													
	5-10	685	< 20	4.24	1460	1.99	1.8	30.6	4.84	0.55	1.07	22.7	< 20	< 20
	10-15	643	< 20	3.89	860	0.643	1.43	26	4.43	0.161	1.02	< 20	< 20	< 20
	15-20	898	< 20	4.42	1030	0.306	1.5	39.9	4.37	0.145	1.54	< 20	< 20	< 20
	20-25	972	< 20	5.33	933	0.303	1.74	52.5	4.12	< 0.10	1.3	< 20	< 20	< 20
	25-30	1510	< 20	7.35	1320	0.53	2.49	79.9	4.96	0.128	2.05	< 20	< 20	< 20
	30-35	962	< 20	8.76	1400	0.332	2.93	100	5.65	0.144	1.29	< 20	< 20	< 20
King Point Polygon (KP)	0-5	1140	< 20	1.44	1410	16.4	1.8	35.4	5.92	5.85	0.571	< 20	< 20	< 20
	5-10	1280	30.9	2.64	2040	10.9	2.17	51.1	6.19	2.34	0.718	< 20	< 20	< 20
	10-15	1660	57.6	4.48	4250	11.1	3.28	182	7.11	0.747	0.936	28.4	< 20	< 20
	15-20	585	22.3	1.13	1090	0.893	0.847	< 20	1.89	0.162	0.451	< 20	< 20	< 20
	20-25	702	35.6	1.83	2180	1.6	1.06	< 20	2.13	0.145	0.492	< 20	< 20	< 20
Lake Herschel Polygon (LP)	0-5													
	5-10	1770	21,3	7.9	2290	2,34	3.14	< 20	6.92	0.759	2.98	33.3	< 20	< 20
	10-15	935	< 20	6.53	1770	1.09	2.54	< 20	7.94	0.283	1.32	< 20	< 20	< 20
	15-20	1090	< 20	5.14	1410	0.91	2.14	< 20	6.75	0.137	1.6	< 20	< 20	< 20
	20-25	591	< 20	6.29	1050	1.11	2.64	< 20	9.14	0.156	1.02	< 20	< 20	< 20
Mainland Polygon (MP)	0-5	449	< 20	3.29	355	2.56	0.791	< 20	2.17	0.902	0.513	< 20	< 20	< 20
	5-10	315	< 20	2.97	402	1.33	0.578	< 20	1.97	0.141	0.377	< 20	< 20	< 20
	10-15	278	< 20	4.24	395	0.396	0.74	< 20	1.88	0.118	0.423	< 20	< 20	< 20
	15-20	1190	21.9	5.63	526	0.284	1.02	< 20	1.63	< 0.10	1.52	< 20	< 20	< 20
	20-25	1650	28.1	6.69	598	0.272	1.25	< 20	1.49	< 0.10	1.87	22.4	< 20	< 20
	25-30	2090	31.5	6.38	680	0.331	1.25	< 20	1.45	< 0.10	2.5	20.7	< 20	< 20
	30-36	856	25.1	7.56	506	0.274	1.38	25.7	2.05	< 0.10	1.15	23.6	< 20	< 20

Table S1. (Continued)

Polygon	Depth (cm)	V (µg/L)	Co (µg/L)	Ni (µg/L)	Cu (µg/L)	Zn (µg/L)	Fluoride (mg/L)	Chloride	Sulfate (mg/L)	Bromide (mg/L)	Nitrate (mg/L)	Phosphate (mg/L)	HCO_3^- (mg/L)
Drained Lake Polygon (DP)	0-5												
	5-10	< 20	< 20	< 20	< 20	< 20	0.05	6.43	2.14	< 0.05	0.55	0.3	7.02
	10-15	< 20	< 20	< 20	< 20	< 20	0.05	4.61	2.06	< 0.05	0.18	0.17	7.32
	15-20	< 20	< 20	< 20	< 20	< 20	0.11	4.99	1.16	< 0.05	0.15	0.11	7.63
	20-25	< 20	< 20	< 20	< 20	< 20	< 0.05	4.57	0.78	< 0.05	0.16	0.19	7.78
	25-30	< 20	< 20	< 20	< 20	< 20	< 0.05	4.73	1.04	< 0.05	0.17	0.19	10.68
	30-35	< 20	< 20	< 20	< 20	< 20	0.16	5.59	0.85	< 0.05	0.16	0.1	10.53
King Point Polygon (KP)	0-5	< 20	< 20	< 20	< 20	27,7	0.16	4.19	3.19	< 0.05	< 0.15	8.85	0
	5-10	< 20	< 20	< 20	< 20	30,3	0.05	4.14	2.35	0.09	< 0.15	2.39	3.97
	10-15	< 20	< 20	< 20	< 20	30,9	0.13	4.96	1.83	0.28	< 0.15	0.37	11.44
	15-20	< 20	< 20	< 20	< 20	< 20	< 0.05	0.77	0.88	< 0.05	< 0.15	0.25	3.05
	20-25	< 20	< 20	< 20	< 20	< 20	< 0.05	0.89	1.16	< 0.05	< 0.15	< 0.10	2.90
Lake Herschel Polygon (LP)	0-5												
	5-10	< 20	< 20	< 20	< 20	< 20	< 0.05	5.62	1.3	0.09	0.18	0.59	18.15
	10-15	< 20	< 20	< 20	< 20	< 20	0.11	6.37	4.1	< 0.05	< 0.15	0.18	10.22
	15-20	< 20	< 20	< 20	< 20	< 20	0.05	5.38	2.62	< 0.05	< 0.15	0.11	9.91
	20-25	< 20	< 20	< 20	< 20	< 20	0.09	8.02	3.03	< 0.05	< 0.15	0.16	12.36
Mainland Polygon (MP)	0-5	< 20	< 20	< 20	< 20	< 20	< 0.05	2.08	1.98	< 0.05	0.6	2.21	6.86
	5-10	< 20	< 20	< 20	< 20	< 20	< 0.05	1.72	2.12	< 0.05	0.16	0.16	5.49
	10-15	< 20	< 20	< 20	< 20	< 20	< 0.05	1.62	1.21	< 0.05	< 0.15	0.14	5.34
	15-20	< 20	< 20	< 20	< 20	< 20	0.08	1.46	1.31	< 0.05	< 0.15	< 0.10	5.64
	20-25	< 20	< 20	< 20	< 20	< 20	0.09	1	1.62	< 0.05	< 0.15	0.16	7.02
	25-30	24,8	< 20	< 20	< 20	< 20	0.12	1.07	1.72	< 0.05	< 0.15	0.17	6.25
	30-36	< 20	< 20	< 20	< 20	< 20	0.06	1.76	2.33	< 0.05	< 0.15	0.25	7.02

Table S2. qPCR results for five functional genes involved in microbial carbon and nitrogen cycling. Results presented are in gene copy number per gram of wet soil.

Polygon	Depth (cm)	*pmoA*	*amoA* Bac	*amoA* Arch	*nifH*	*mcrA*
DP1	0-5	3.93E+05	2.92E+04	9.00E+05	7.92E+07	8.96E+04
DP2	5-10	1.35E+06	6.57E+04	2.25E+06	1.44E+08	1.44E+05
DP3	10-15	1.98E+05	2.69E+04	9.34E+05	5.35E+07	4.80E+03
DP4	15-20	7.96E+03	3.42E+03	1.27E+05	7.43E+06	2.11E+05
DP5	20-25	1.09E+04	1.28E+04	2.93E+05	9.21E+06	3.54E+04
DP6	25-30	5.12E+03	4.06E+03	1.47E+05	5.14E+06	3.42E+04
DP7	30-36	3.48E+03	2.71E+03	1.61E+05	3.15E+06	1.11E+05
DP8	Permafrost	2.40E+03	1.37E+03	5.04E+04	2.32E+06	6.55E+03
LP1	0-5	5.76E+04	2.70E+04	8.24E+05	2.72E+07	2.94E+03
LP2	5-10	5.90E+05	5.26E+04	1.52E+06	7.73E+07	1.93E+05
LP3	10-15	4.44E+05	3.05E+04	1.35E+06	8.73E+07	1.47E+05
LP4	15-20	1.39E+05	3.44E+04	7.90E+05	2.97E+07	3.81E+04
LP5	20-25	5.77E+04	4.06E+04	9.75E+05	3.29E+07	2.66E+05
LP6	Permafrost	8.09E+03	6.70E+03	1.13E+05	4.33E+06	3.97E+05
KP1	0-5	7.21E+04	6.45E+04	1.28E+06	2.91E+07	0.00E+00
KP2	5-10	1.48E+04	3.11E+04	1.25E+06	3.22E+07	0.00E+00
KP3	10-15	2.03E+04	2.05E+04	5.45E+05	1.56E+07	0.00E+00
KP4	15-20	1.85E+05	8.49E+04	1.72E+06	1.13E+08	6.24E+01
KP5	20-25	8.64E+04	7.60E+04	1.27E+06	4.87E+07	7.77E+02
KP6	Permafrost	1.54E+05	1.69E+05	1.44E+06	7.05E+07	2.10E+05
MP1	0-5	2.19E+04	4.07E+03	1.30E+05	3.16E+06	0.00E+00
MP2	5-10	1.33E+04	5.67E+03	8.25E+04	8.38E+06	1.19E+07
MP3	10-15	2.00E+03	2.01E+03	2.96E+04	3.25E+06	5.12E+05
MP4	15-20	3.99E+03	3.22E+03	6.44E+04	1.95E+06	7.86E+05
MP5	20-25	4.68E+03	2.64E+03	8.42E+04	1.61E+06	6.81E+05
MP6	25-30	1.27E+03	1.29E+03	7.37E+04	1.36E+06	2.54E+06
MP7	30-35	1.99E+03	2.33E+03	6.85E+04	2.62E+06	8.80E+04
MP8	Permafrost	4.59E+03	9.80E+02	1.82E+04	8.17E+05	1.15E+04

Table S3. OTU (0.03) calculations for bacterial and archaeal 16 rRNA sequences

Polygon	Depth (cm)	OTUs (0.03) Bacteria	OTUs (0.03) Archaea
DP	0-5	8951	863
	5-10	9086	953
	10-15	7453	1027
	15-20	9155	851
	20-25	8762	864
	25-30	8258	937
	30-35	8084	900
	Permafrost	8350	701
	Average	**8512**	**887**
LP	0-5	7889	1446
	5-10	9215	1392
	10-15	8317	1111
	15-20	7856	971
	20-25	8829	767
	Permafrost	4311	697
	Average	**7736**	**1064**
KP	0-5	9084	n.a.
	5-10	8586	n.a.
	10-15	5379	n.a.
	15-20	6974	1040
	20-25	5349	837
	Permafrost	7466	924
	Average	**7140**	**934**
MP	0-5	9207	1043
	5-10	7997	1023
	10-15	7892	1007
	15-20	7171	700
	20-25	5923	933
	25-30	7326	825
	30-35	7802	821
	Permafrost	8330	599
	Average	**7706**	**869**

Figure S1. PCA analysis of the physico-chemical properties of the four polygons, all depths combined.

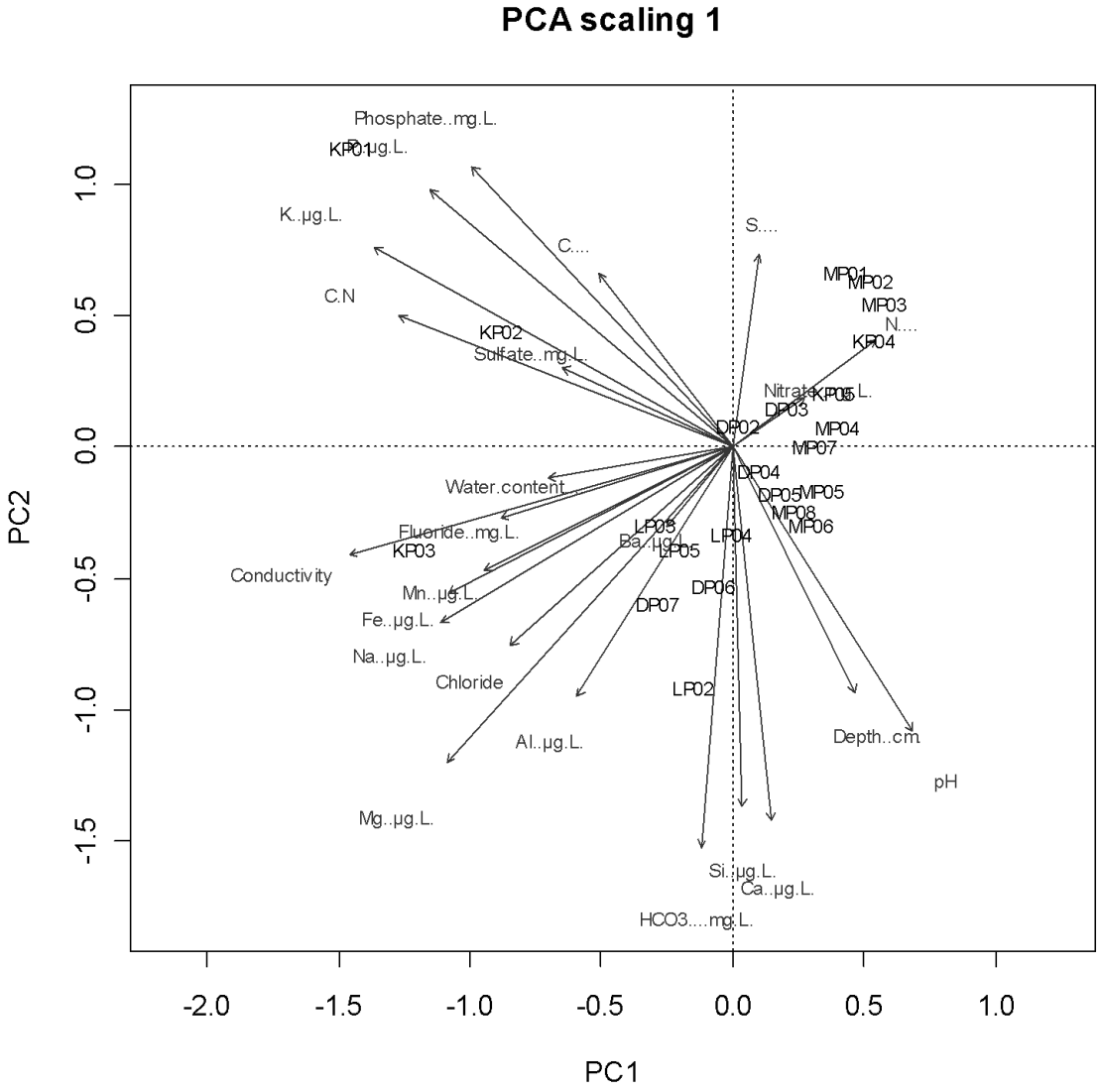
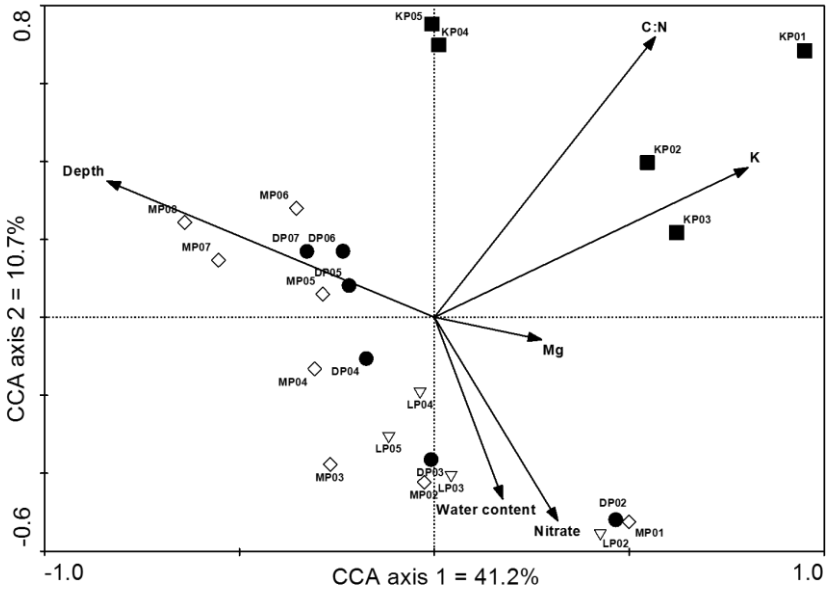
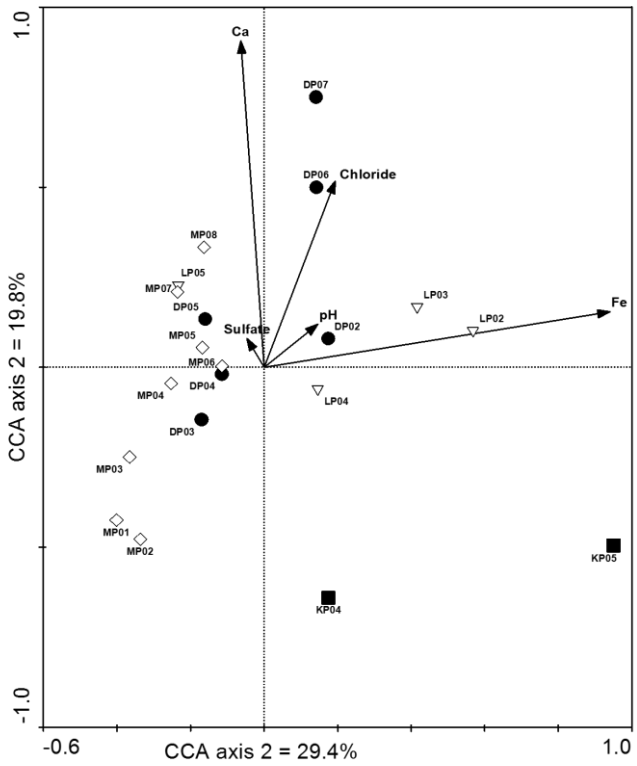


Figure S2. CCA analysis of a) bacterial and b) archaeal 16S rRNA sequences plotted with physico-chemical properties showing a strong correlation to this distribution.

a)



b)



MANUSCRIPT III – (In preparation for submission to *Applied and Environmental Microbiology*)

Dynamics and adaptation of the methane-cycling community in a permafrost-affected soil under simulated global warming.

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Running title: Adaptation of methane-cycling community under simulated global warming.

Keywords: permafrost degradation, climate change, methanogens, methanotrophs, T-RFLP

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Abstract

Permafrost-affected ecosystems including peat wetlands are among the most obvious regions in which current microbial controls on organic matter decomposition are likely to change as a result of global warming. Wet tundra ecosystems in particular are ideal sites for increased methane production because of the waterlogged, anoxic conditions that prevail in seasonally increasing thawed layers. Increased carbon degradation and utilization by permafrost microorganisms further affect the global carbon balance by releasing more climate relevant gases to the atmosphere. The objective of this study was to investigate the reaction and adaptation of active-layer methane-cycling microorganisms from polygonal tundra under simulated global warming. Here we present the results of an 8 months long simulated climate warming experiment in which three intact active layer cores taken from wet polygonal tundra on Hershel Island, Canada were incubated at regularly increasing temperatures. The response of methane-cycling microbial communities in the active layer was studied using quantitative PCR and community fingerprinting analysis (T-RFLP). Results showed that both the methanogenic and methanotrophic communities were able to adapt rapidly to increasing temperatures and even seemed stimulated by this increase. Overall microbial diversity did not decrease over time, but a clear shift in the bacterial community composition and abundance could be observed, especially towards the bottom of the active layer. The archaeal community remained somewhat more stable over time. Generally, these results give a positive prognostic on the fate of methane-cycling microbial communities under increasing temperatures and permafrost degradation, as both communities appear to be flexible enough to adapt to a rapidly changing environment.

Introduction

Arctic wet tundra ecosystems play a crucial role within the global carbon cycle because thawing permafrost leads to the release of previously frozen organic carbon, and a subsequent increased methane production due to the waterlogged, anoxic condition that prevail in the active layer of permafrost (Whalen and Reeburgh, 1992). Up to 39 Tg of methane are released from permafrost environments each year, making them the largest single natural source of methane (Christensen *et al.*, 1996) and contributing up to 20% of global emissions (Cao *et al.*, 1998, MacGuire *et al.,* 2009). Increased carbon degradation and utilization by permafrost microorganisms due to increasing soil temperatures further affect the global carbon balance by releasing more climate relevant gases to the atmosphere. The importance of permafrost soil carbon-cycling is recognized in the updated International Panel for Climate Change scenarios (IPCC, 2007). On the one hand, past extreme warming events have been linked to an increase in diversity of methanogenic archaeal (Bischoff *et al.,* 2013) and massive carbon and greenhouse gas releases from thawing permafrost (De Conto *et al.,* 2012). On the other hand, methane-oxidizing bacteria have been suggested to be more sensitive to warming and could be inhibited by stark increases in temperature (Liebner *et al.*, 2008), Temperature sensitivity of the microbial mediated decomposition of organic matter is likely to be higher in the active layer than in the permafrost because of higher substrate availability, lower potential enzyme activities

and lower microbial abundances in permafrost soils as compared with the overlaying active layer (Waldrop *et al*., 2010, Yergeau *et al*., 2010).

It is increasingly recognized that in order to predict long-term trends in ecosystem carbon fluxes and biological feedbacks, the reaction and potential for acclimation of permafrost microbial communities must be investigated (Oechel *et al.* 2000; Enquist, 2007). The potential of these communities to acclimatize to higher soil temperatures could reduce the projected carbon losses from Arctic permafrost associated with global warming (Luo *et al.* 2001). It is thus essential to understand the microbial controls on methane emissions to assess the potential for high-latitude carbon fluxes acting as a biogeochemical feedback to the climate system (Koven *et al.,* 2011).

Already, a few studies have observed important changes in microbial communities following seasonal temperature variations in tundra soils (Lipson and Schmidt 2004, Wallenstein *et al.,* 2007). Experimental warming studies in the field or in the laboratory have attempted to predict the reaction of microbial communities in a variety of peat ecosystems (Chin *et al.,* 1999; Fey and Conrad, 2000; Høj *et al.,* 2008; Metje and Frenzel, 2007; Metje and Frenzel 2005). However, most of the sites investigated were from temperate or sub-Arctic environments. Since rising temperatures are predicted to occur at a higher rate in Northern latitudes, a better understanding of microbial reaction and acclimation responses to increased warming is required to make accurate predictions of the long-term rates of carbon cycling in permafrost-affected soils.

The simulation presented in this study uses the experimental setup of permafrost microcosms developed and described by Wagner (2003). The warming simulation was performed on active-layer samples from low-centered polygons sampled on Herschel Island and the Yukon Coast. Low-centered polygons are characteristic features for polygonal tundra environments in the high Arctic and can readily be found in Alaska, the Canadian Arctic, Scandinavia and Siberia. Because of the presence of diverse methanogenic as well as methanotrophic microbial communities in polygonal tundra, these environments can act as significant methane sources or sinks, depending on the abundance, diversity and distribution of these communities. To understand the future development of Arctic permafrost environments and the potential of such areas as a carbon source or sink, the microbial communities present in three active layer cores were subjected to rapidly increasing soil temperatures over the course of eight months, mimicking an exacerbated effect of global warming.

Community fingerprinting analyses was carried out on bacterial and archaeal 16S rRNA genes to follow the reaction of the different members of the community with increasing temperatures. Functional genes involved in methane cycling were investigated by quantitative PCR to follow changes in the methanogenic and methanotrophic populations. To do so, we selected the gene coding for subunit A of the methyl coenzyme-M reductase enzyme (*mcrA*) for methanogenic archaea and the gene coding for subunit A of the particulate methane monooxygenase enzyme (*pmoA*) for methane oxidizing bacteria (MOB). Both genes were shown to exhibit sufficient sequence divergence to serve as a

reliable diagnostic gene for the study of the two populations of interest (McDonald & Murrell, 1997, Luton *et al.*, 2002).

The results presented give important new insights into the adaptation potential of Arctic microorganisms, in particular the methane-cycling community, to their rapidly degrading surroundings due to the effects of global warming.

Materials and methods

Sampling site description

Active layer core samples were collected from the "Drained Lake" low-center polygon (N 69°34'43, W 138°57'25, elevation 30 m above sea level) on Herschel Island, Western Canadian Arctic (Figure 1) during the expedition YUKON COAST in July 2011. The soil at this site was characterized as a Hemic Glacistel classified according to the U.S. Soil Taxonomy (Soil Survey Staff, 1998) with poor drainage and a loamy soil texture. Vegetation cover included roughly 35% plant litter, 40% *Carex* sp. (sedges), 15% *Salix* sp. (dwarf willow), 10% mosses with traces of *Pedicularis* sp. (wooly lousewort) and *Ledum groenlandicum* (Labrador tea) (Barbier *et al.*, 2012). Average air temperatures on Herschel Island vary annually between -26.3 °C in February to 8.7 °C in July (Burn and Zhang, 2009). The sampling site was characterized by an active layer (the layer of ground that is subject to annual thawing and freezing) consisting of a large peat horizon, with a depth of 36 cm as measured using a permafrost probe on the day of sampling (04.08.2011). Metal coring tubes with sharp edges at the bottom were pressed into the soil until they reached the permafrost horizon, then slowly retrieved upwards containing the active layer cores. Samples in the coring tubes were frozen immediately after sampling and stored at -20 °C upon arrival in the laboratory. Transfer of the cores to the permafrost microcosm columns was done under sterile conditions.

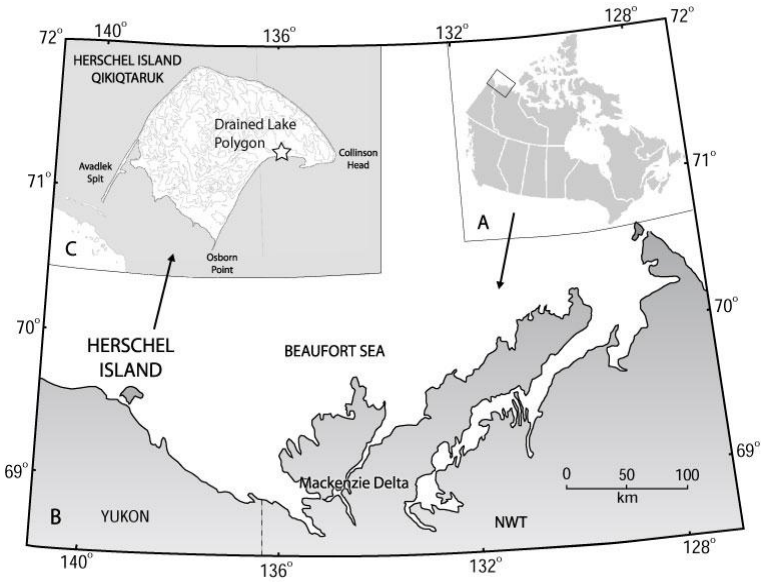

Figure 1. Geographical location of the origin of the cores A) in Canada, B) on the Yukon Coast and C) from the Drained Lake Polygon study site on Herschel Island (Barbier *et al.*, 2012).

Experimental setup

The permafrost microcosms were prepared from undisturbed soil cores including the plant cover collected at Drained Lake Polygon on Herschel Island. The permafrost microcosms were set-up following the description of Wagner (2003). The microcosms consisted of a Plexiglas tube accommodating the undisturbed soil core (Figure 2). The tubes were closed in an airtight manner by an upper and lower flange plate. The Plexiglas tubes were 50 cm long, with an inner diameter of 10 cm and a wall thickness of 0.5 cm. One side of the PVC tube walls were cut open vertically and the cuts were filled with a polyurethane elastomer to ensure the tube wall could exactly be adjusted to the undisturbed permafrost core by using pipe clamps. The top and bottom flanges consisted of two aluminum plates enclosing a cooling coil. The aluminum plates were connected by a rubber seal to the PVC tubes ensuring an airtight connection. Temperature and moisture sensors were fitted to the columns at 8 cm intervals. Two Thermo Haake cryostats (C10-K15 and WKL26; Karlsruhe, Germany) were used to cool or warm the permafrost microcosm by flowing a coolant through the coils in the flanges. Separate bottom and top cooling circuits ensured independent cooling rates and temperatures at each end of the microcosm. Continuous measurements of volumetric water content were carried out using a Campbell Scientific TDR system (Time Domain Reflectometry; Longborough, UK) consisting of a CR1000 data logger, a TDR 100 reflectometer and a SDM50x multiplexer. The TDR probes used for the simulation were 2-rod, 75 mm LP/ms laboratory probes from EASY TEST Ltd. (Lublin, Poland; Fig. 2b). Temperature measurements were made with thin film platinum RTDs (Honeywell HEL-705-U). Openings for soil sampling were made at 8, 16 and 24 cm depth and sealed with a rubber stopper. Samples were collected from the microcosms every 60 days. The starting incubation temperature was 0°C at the bottom of the cores and 10°C at the top, mimicking the permafrost table and average *in situ* conditions that were recorded at the time of sampling. Temperatures were gradually increased over the course of each month, reaching 15° C at the bottom and 35° C at the top of the soil core (Figure 3).

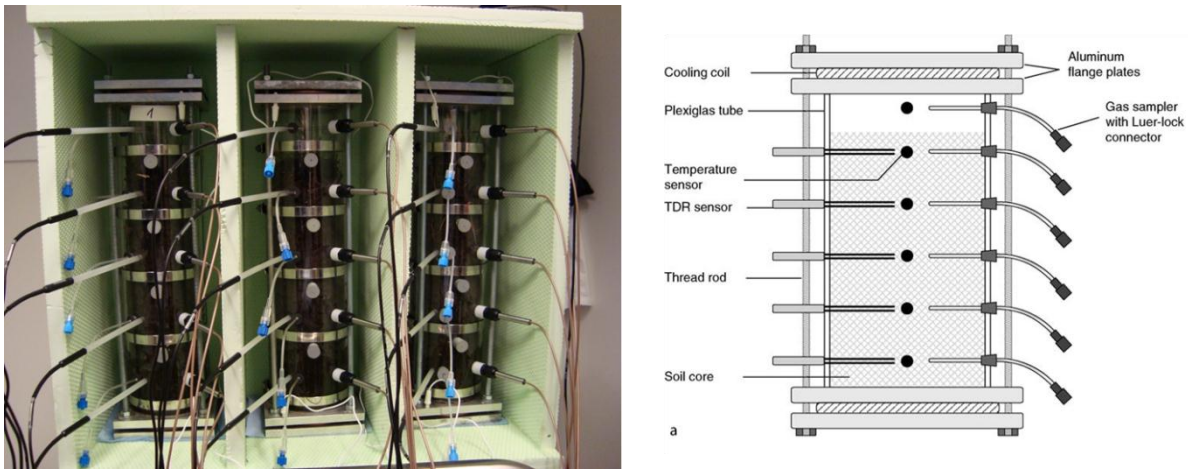

Figure 2. Photograph of the experimental setup of the columns and schematic view of the permafrost microcosm (height = 50 cm, inner diameter = 10 cm, permafrost core length = 36 cm).

Soil physico-chemical analyses.

Gravimetric moisture content of soils was previously determined by Barbier and colleagues (2012) by weighing sub-samples before and after freeze-drying for 72 h.
Conductivity and pH were measured using a CyberScan PC 510 Bench Meter (Eutech Instruments Pte Ltd., Singapore) following the slurry technique by mixing 1:2.5 mass ratio of samples and de-ionized water (Edmeades *et al.*, 1985).
Grain size was analyzed by first treating the samples with 30% H_2O_2 to digest all organic matter. After washing, the samples were freeze-dried and weighed. 1% NH_3 solution was added to the samples and shaken for at least 24 hours. Grain size was then measured at least twice for each sample with a Coulter LS 200 laser particle size analyzer (Beckman Coulter, Brea, California).
The percentage of total organic carbon (TOC) of the soils was measured using a TOC analyzer (Elementar Vario max C, Germany). Samples prepared for analysis by freeze-drying and homogenized in an orbit mill ball-grinder (Pulverisette 5, Fritsch Ltd., Germany). The TOC content was calibrated using external standards of known elemental composition.

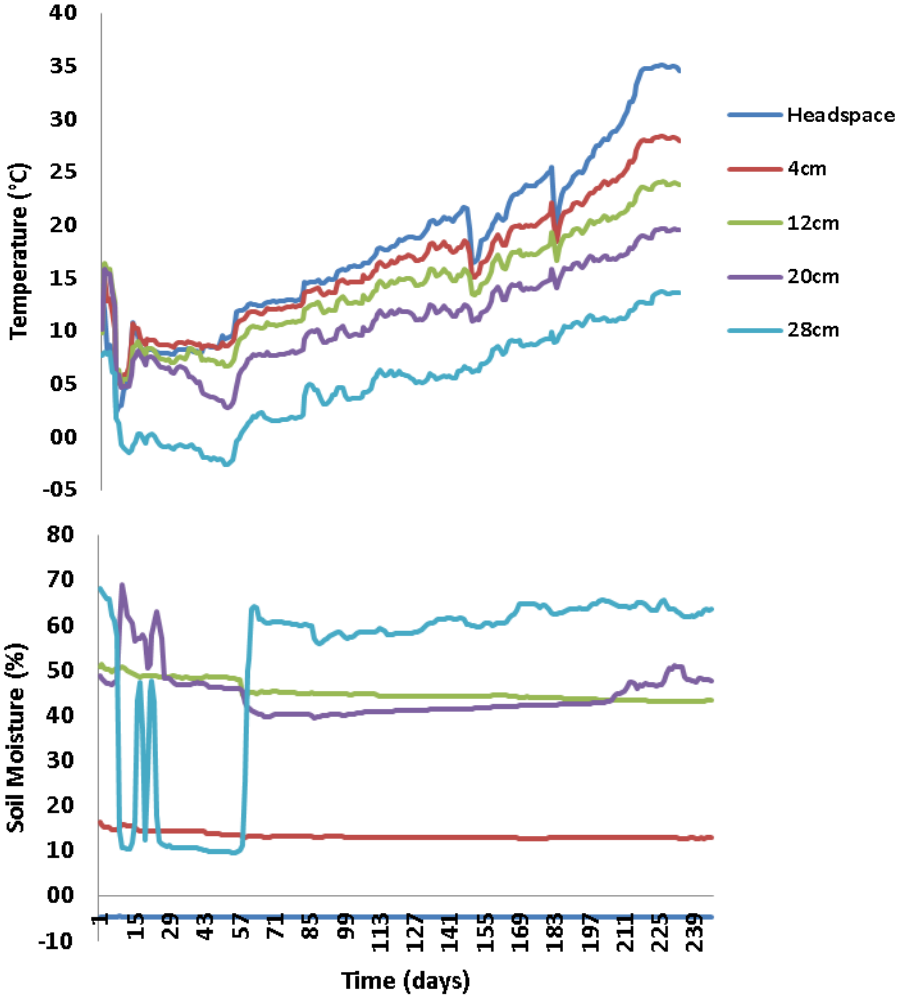

Figure 3. Average temperature (a) and soil moisture (b) as recorded by the sensors at different depths in the column over the entire course of the microcosm experiment.

Extraction of genomic DNA and PCR amplification

Total genomic DNA was extracted in triplicate from 0.3 to 0.5 g of soil using the PowerSoil™ DNA Isolation Kit (Mo Bio Laboratories, Carlsbad, California) according to the manufacturer's protocol. Triplicates were then pooled for downstream analyses. Nucleic acids were eluted in 20 to 90 µL of elution buffer (MoBio). The concentration of the obtained genomic DNA was checked by fluorescence using a Qubit fluorometer (Invotrogen, Carlsbad, U.S.A.). DNA was then stored at -20 °C for further use in polymerase chain reaction (PCR) analyses. PCR reactions were performed in triplicate 50 µL volumes containing between 10 to 50 ng of DNA, 0.5 µL of each 20 mM primer (forward primer labelled with the fluorescent dye carboxyfluorescein), 2 µL 25 mM $MgCl_2$, 2 µL 5 mM dNTP mix, 5 µL 10x PCR buffer (Qiagen), 1 U of HotStar Taq DNA polymerase (Qiagen, Hilden, Germany) and PCR-grade water to 50 µL. Primers used in the different PCR reactions are listed in Table 1. For the amplification of bacterial 16S rRNA gene, the primer pair 341F/1492R was used. Reaction conditions were as follows: initial denaturation at 95 °C for 5 min, 30 cycles with denaturation at 95 °C for 30 s, annealing at 56 °C for 45 s, extension at 72 °C for 45 s and a final extension at 72 °C for 7 min. For the amplification of the archaeal 16S rRNA gene, the primer pairs ArUn4F/915R was used. Triplicate PCR reactions were visualized on a 1% agarose gel containing GelRed stain (Hayward, California) and then purified using a QIAquick PCR Purification Kit (Qiagen). Purified PCR products were quantified using a Qubit fluorometer (Invotrogen, Carlsbad, U.S.A.).

Terminal Restriction Fragment Length Polymorphism (T-RFLP)

The digestion of fluorescently-labeled PCR fragments using restriction enzymes was conducted in duplicate as follows. 20 U of enzyme MspI (Roche, Penzberg, Germany), 4 µL of 10x Buffer and 500-600 ng of purified PCR product were mixed. PCR grade water was added to 40 µL. The samples were then incubated for 3 h at 37 ºC. The digestion was stopped by incubation at 80 ºC for 20 min. Digests were pooled and purified using the QIAquick Purification Kit (Qiagen). T-RFLP products (2 µL) were mixed with 0.25 µL of GeneScan™ 500 LIZ® internal size standard (Applied Biosystems, Darmstadt, Germany) and run on an ABI 3730xl DNA Analyzer (Applied Biosystems) at GATC Biotech (Konstanz, Germany). Afterwards, the lengths of the fluorescently labeled terminal fragments (T-RFs) were visualized with Peak Scanner software (v1.0, Applied Biosystems). T-RFLP results were analysed statistically according to Dunbar *et al.* (2001) to yield relative abundance (%) of T-RFs. Briefly, T-RFs were aligned and clustered manually using Excel (Microsoft, Redmond, Washington). DNA quantity between triplicate samples as well as between depth profiles was standardized in an iterative standardization procedure. For each sample, a derivative profile containing only the most conservative and reliable T-RF information was created by identifying the subset of T-RFs that appeared in all replicate profiles of a sample. Standardized, derivative profiles were then aligned. The average size of TRFs in each alignment cluster was calculated to produce a single, composite list of the T-RF sizes found among all samples. Relative signal intensity of each T-RF (%) was calculated based on the signal intensity of each individual

T-RF with respect to the total signal intensity of all T-RFs in that sample. Peaks representing less that 1% of total fluorescence were eliminated from the profile in order to concentrate on the most representative microorganisms in each community. T-RFLP profiles were converted into presence-absence data and analysed statistically by cluster analysis based on Bray-Curtis pairwise similarities using the software PRIMER 6 (Primer-E Ltd., Lutton, United Kingdom).

Quantitative PCR amplification

qPCR was performed in 25 µl volumes using the iQ SYBR green Supermix (Bio-Rad Laboratories, Hercules, CA, USA) on a Rotor-Gene 3000 apparatus (Corbett Life Science, Sydney, NSW, Australia). The qPCR conditions and primers are given in Table 1. Standards were made from 10-fold dilutions of linearized plasmids containing the gene fragment of interest that was cloned from amplified soil DNA. To check and compensate for inhibition, dilutions of the samples were made until three consecutive dilutions yielded the same results.

Table 1. Primers and temperature profiles used for PCR and quantitative PCR analysis.

Gene	Primer Pair	Temperature °C ann. (read.)	Reference
Archaeal 16S rRNA	ArUn4F/915R	56	(Hershberger et al., 1996, Stahl and Amann, 1991)
Bacterial 16S rRNA	341F/1492R	56	(Lane, 1991)
mcrA	MlF / MlR	55 (83)	(Luton *et al.*, 2002)
pmoA	A189F / mb661R	55 (82)	(Kolb *et al.* 2003)

Cloning and sequence analyses

Samples from day 3 after installing the system were chosen to establish clone libraries. Libraries for the bacterial and archaeal 16S rRNA genes were created by ligating PCR products into the pGEM-T Easy vector and transformed into competent cells *Escherichia coli* JM109 using the "pGEM-T Easy Vector Systems II" Kit (Promega, Mannheim, Germany). White colonies containing inserts were picked, suspended in 1.2 mL of nutrient broth containing ampicillin (50 µg mL^{-1}) and grown overnight at 37 °C. Colonies were screened by PCR with vector primers M13 for correct size of the insert and the amplicons were directly sequenced by GATC Biotech AG (Konstanz, Germany). 96 clones per gene were sequenced. The sequences were edited and contigs assembled using the Sequencher software (v4.7, Gene Codes, Ann Arbor, Michigan).

Nucleotide sequence accession numbers

The environmental bacterial and archaeal 16S rRNA clone sequences recovered in this study have been submitted to the GenBank nucleotide sequence database (awaiting accession number confirmation).

Results

Characteristics of the soil

The average *in situ* day-temperature at the surface of the profile was 12 °C, decreasing gradually to -0.5 °C at the permafrost table (Figure 3.a.). The pH of the entire profile was slightly acidic, ranging between 5.2 and 5.6 throughout (Figure 3.b.). The mineral fraction of the soil represented only roughly 30%, as calculated after concentrated acid digestion of organic matter. The mineral fraction consisted on average of 44% sand, 32% silt and 24% clay (data not shown). The soil was visibly water saturated, with gravimetric moisture contents in the profile ranging from 77% near the surface and increasing to 83% close to the permafrost table (Figure 3.c.). The organic carbon content was overall very high for all profile layers, ranging from 28% in the middle layers to 23% towards the permafrost table (Figure 3.d.).

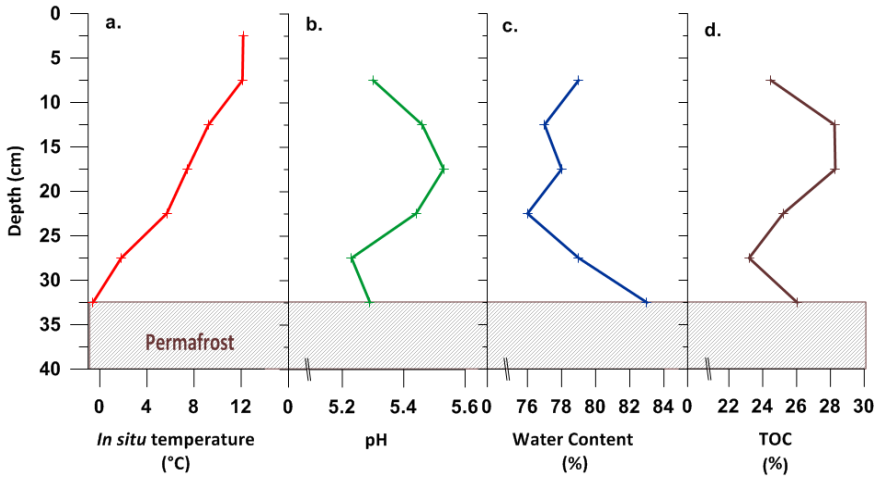

Figure 3. Depth profile of abiotic and biotic parameters illustrating (a) active layer temperature as measured *in situ*, (b) soil pH, (c) water content of the soil, (d) percentage of total organic carbon in the soil (TOC)

Abundance of methanogens and methanotrophs

The abundance of methanogens and methanotrophs in the active layer profile was investigated through qPCR analysis of *mcrA* and *pmoA* functional genes.
Generally, we found that the methanogenic community present in the deeper layers of the column steadily became more abundant over time with increasing incubation temperature, reaching 10^7 *mcrA* gene copies.g^{-1} wet soil, whereas at the top of the column *mcrA* gene copy numbers remained relatively constant throughout the entire experiment after a short decrease early on (Figure 4). Based on the quantification of *pmoA,* the methanotrophic community in the surface layer showed strong variation over time and with increasing temperature, with gene copy numbers increasing from $2.0x10^7$ to $1.6x10^8$ within 4 months and a 10 °C temperature rise. The methanotrophic community in the middle layer remained stable over time after a small initial decrease from $8x10^7$ to $2x10^7$ during the first month, while the community at the bottom of the column remained completely stable over time.

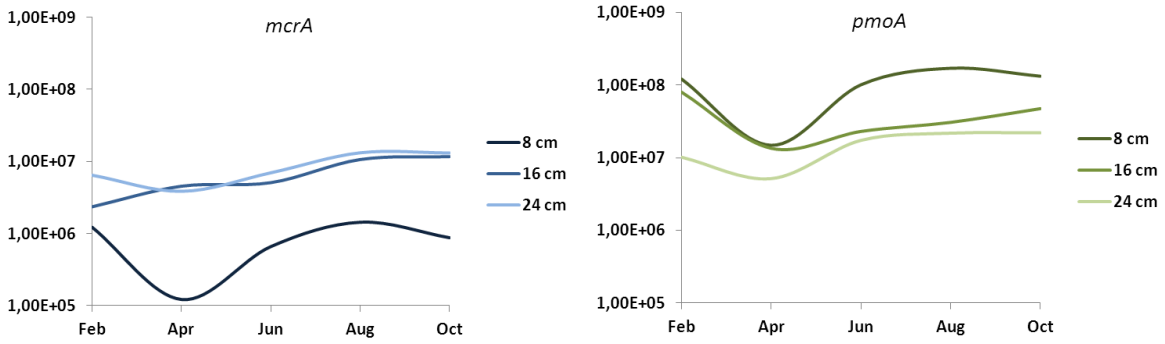

Figure 4. Quatitative PCR analysis of *mcrA* and *pmoA* genes in the permafrost microcosms at various depths over the course of the warming experiment. Results indicated are in gene copy number per gram of wet soil.

Diversity and dominant bacterial and archaeal species

The clone library analysis of bacterial 16S rRNA yielded a total of 236 cloned sequences. Sequences resulting in less than 100 amino acids were removed from further phylogenetic analyses. The bacterial community was dominated by *Proteobacteria* (32%) and *Acidobacteria* (29%), followed by *Cyanobacteria* and *Verrucomicrobia* (Figure 5.a.).A total of 8% of the sequences could not be classified to the phylum level. When looking deeper into the *Proteobacteria* sequences, half of the sequences could be identified as *Betaproteobacteria* (47%) while the Alpha- and *Gammaproteobacteria* represented 27% and 14% of the sequences, respectively (Figure 5.b.).
The clone library analysis of archaeal 16S rRNA yielded a total of 170 cloned sequences. *Euryarchaeota* dominated the archaeal sequences (40%), followed by *Crenarchaeota* (22%). A very large portion of the cloned sequences could not be identified (38%; Figure 5.c.) At the class level, half of the sequences belonged to *Methanomicrobia* (52%) and 35% to *Thermoprotei* (Figure 5.d.)

Bacterial and archaeal community fingerprints over time

At "T0" representing field conditions, the bacterial community was similar in the first and second depths, while the deepest layer (24 cm) clearly showed a different community composition (Figure 6.a.). With increasing temperature over time, a distinct shift in all layers could be observed, the most pronounced occurring close to the permafrost table. At the end of the experiment, the bacterial community at 16 and 24 cm depths had shifted significantly and showed a different composition, as illustrated by the colored bars representing different terminal restriction fragments (T-RFs). The archaeal community did not undergo as much change overall compared to the bacterial community. At T0, the archaeal community was differently distributed over the three depths sampled. During incubation and warming, the most pronounced change in community composition could be observed in the middle of the columns at 16cm depth, showing the biggest shift with time (Figure 6.b.). After a shift observed after 60 days of incubation, the community in the surface layer (8 cm) returned to a distribution comparable to the beginning.

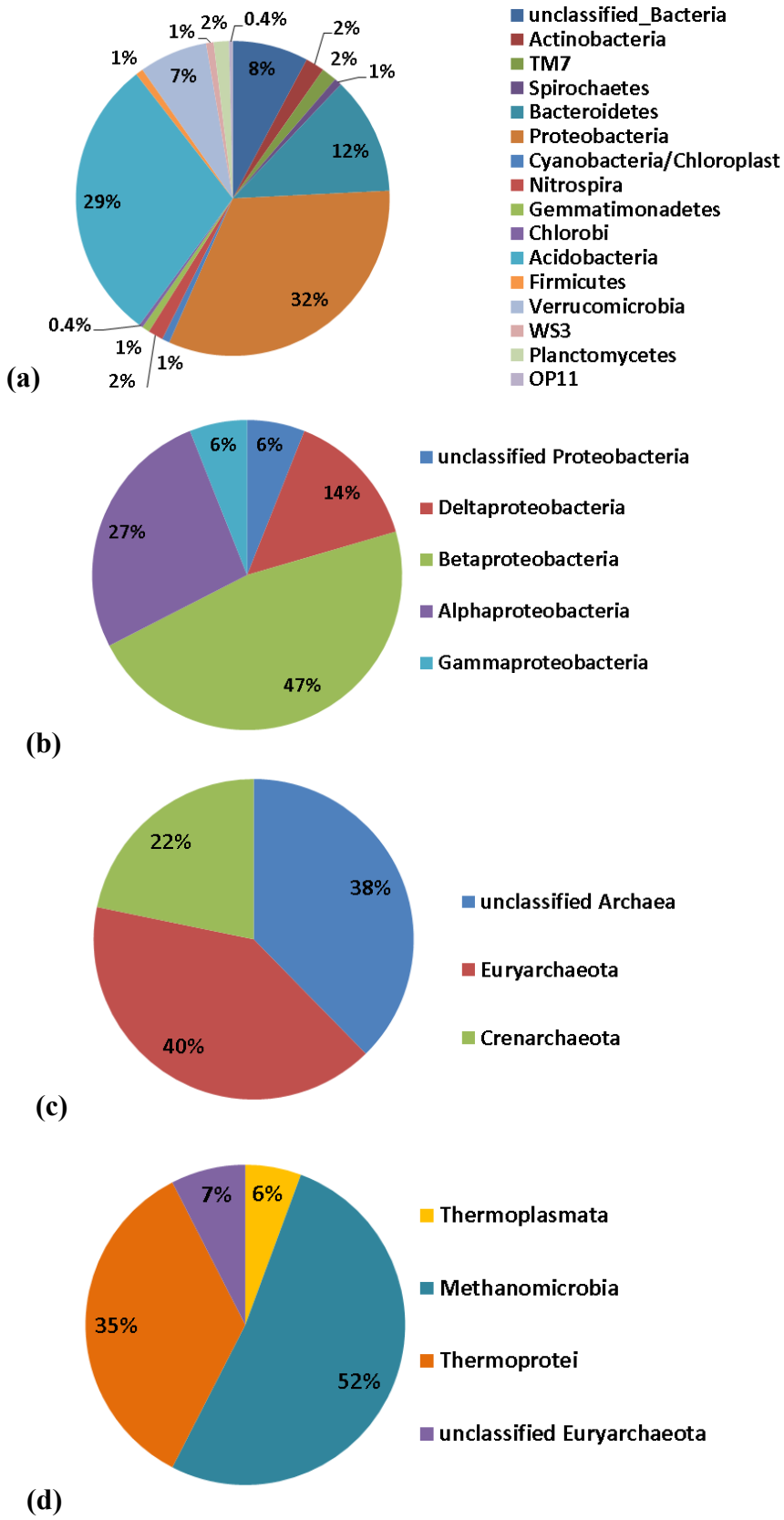

Figure 5. Taxonomical repartition of the bacterial and archaeal sequences obtained from the clone library. (a) Bacterial phyla, (b) Proteobacterial classes, (c) Archaeal phlya and (d) archaeal classes identified.

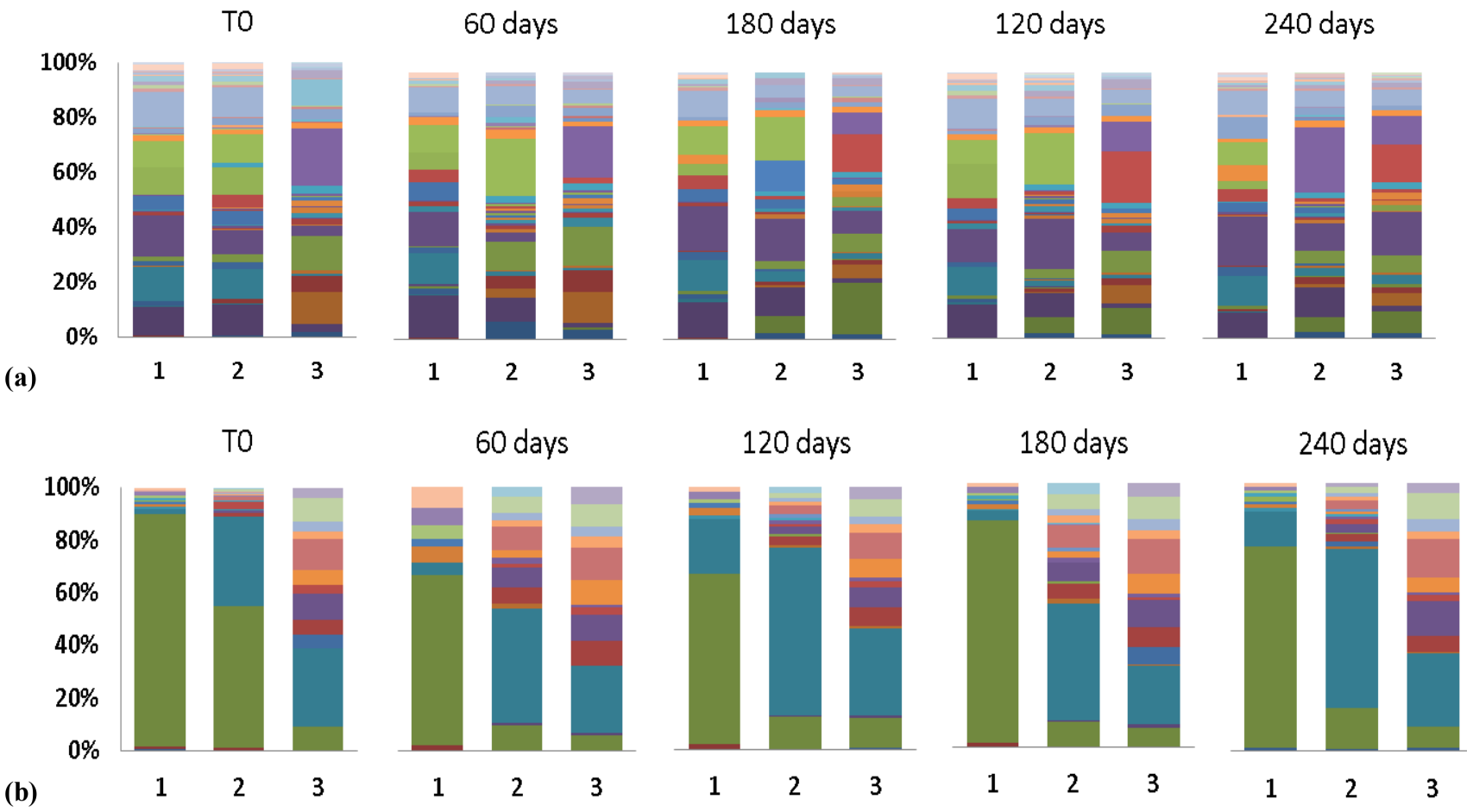

Figure 6. Bacterial (a) and archaeal (b) community fingerprints: evolution of terminal-restriction fragments representing different members of the community in three different depths over time. Numbers 1, 2 and 3 stand for column depths of 8cm, 16cm and 24cm, respectively.

Discussion

Arctic peatlands, in particular polygonal tundra, are climatic hotspots as permafrost warming and an increase in the seasonal active layer depth can lead to intensified carbon degradation and utilization by permafrost microorganisms. To understand the carbon fluxes from Arctic permafrost environments and the future development of these areas as a carbon source, we thought it advantageous to obtain direct insights into the reaction and adaptation of the active layer microbial community from a typical permafrost landscape of the Circum-Arctic.

The permafrost microcosms used in this study were originally developed to simulate the influence of the annual freeze-thaw cycles on the methane fluxes in the active layer of permafrost soils. In this study, the setup was adapted to imitate a gradual increase in soil temperature of three active layer cores. Such simulation experiments are important as they can give insights into the natural ecosystem and may yield important clues to understand the response of microbial communities to their rapidly changing environment. As described by Wagner (2003), the use of undisturbed soil cores is advantageous over smaller scale sampling as this preserves the original structure, pore system and stratification of the natural soil. In this way, a more direct correlation to *in situ* conditions and the microbial processes involved processes can be inferred from the results of the simulation. Waldrop and colleagues (2010) suggested that studies of microbial community change over natural thermokarst chronosequences are an ideal way to test the evolution of microbial degradation of organic matter and methane fluxes with increasing temperatures. In this study, we hypothesized that a gradual but strong warming of active layer cores over the course of 8 months would influence the structure and quantity of soil microbial communities, in particular the methane-cycling members of the community. We believe the length of incubation carried out in our experiment was sufficient for microbial communities to respond and adapt to the increasing temperatures, given that changes in microbial communities have been observed between seasons in tundra soils (Schadt *et al.* 2003; Lipson and Schmidt 2004; Wallenstein *et al.,* 2007), and in response to temperature changes in laboratory experiments of a similar duration (Hartley *et al.*, 2008; Pettersson and Bååth 2003).

Our results show different responses of the microbial community to warming at each depth examined, indicating an interaction between temperature and soil depth. Indeed, the experimental setup of the incubation columns had a strong effect on the community composition of bacteria, and to a lesser extent archaea, considering that over 8 months the temperature of the cores was increased from 10°C to 35°C at the surface and from 0°C to 15°C at the bottom. We observed significant shifts in bacterial composition occurring in the middle and deeper layers with increasing temperatures, while the archaeal community showed a lesser response which was mostly limited to the middle layer. Community fingerprinting analysis clearly showed a quick reaction of the communities over time to a temperature increase, visible already starting from the first month. The microbial diversity did not decrease over time, but rather shifted as some members of the community became more abundant while other decreased. The most significant change in bacterial community composition was observed in the bottom and middle layers (16-24 cm) of the cores. This is an expected response, since surface layers are naturally exposed to greater seasonal variations than the bottom of the active layer, meaning that the communities are naturally more adapted to temperature fluctuations. The bacterial community closer to the permafrost table underwent

a more pronounced shift over time, resulting in a significantly different community than what was observed under *in situ* conditions. Put in the context of degrading carbon-rich permafrost ecosystems, such a shift in composition is very likely accompanied by a shift in the function of the bacterial community and enhanced carbon loss from the environment (Hartley *et al*., 2008). In support of this hypothesis, a soil-warming study demonstrated that warming of plots during the winter produced a more active microbial community (Hartley *et al.* 2007). The thermal optimum for the activity of key carbon cycling enzymes has also been shown to increase with seasonal changes in temperature (Fenner *et al.* 2005). The archaeal community underwent less shifts in community composition, those most significant occurring in the middle of the active layer (16 cm depths). This is consistent with the results of other studies that also found that differences in temperature led to negligible changes in the composition of archaea compared with bacteria in mesotrophic peat (Kim *et al.,* 2012), in rice field soil (Fey and Conrad, 2000) in subarctic acidic peat (Metje and Frenzel, 2005, 2007). Only a few studies reported either a net decrease (Chin *et al.,* 1999) or increase (Høj *et al.,* 2008) in the diversity of archaeal communities following soil warming.

More specifically, the reaction of active-layer methane-cycling microorganisms observed in our study points towards a fast adaptation of the microbial community to rapidly increasing temperatures, as previously suggested (Hartley *et al.,* 2008). Quantitative PCR analysis showed that both the methanogenic and methanotrophic communities were able to adapt rapidly to increasing temperatures and even seemed stimulated by this increase, as indicated by the increase in gene copy number for both functional genes *pmoA* and *mcrA*. The methanogenic community increased more at the bottom of the core, while the methanotrophic community showed a stronger increase at the top. It is generally accepted that active layer warming may increase the pool size of organic carbon available for microbial degradation, as warming is known to accelerate the breakdown of recalcitrant organic matters like peats due to an increased active microbial fraction possessing sufficient energy to take part in the decomposition (Davidson and Janssens, 2006), favoring methanogenic activity by raising the supply of easily decomposable metabolic substrates from plant and organic matter decomposition (Yavitt and Seidman-Zager, 2006; Zogg *et al*., 1997). However, it is not yet understood how methanogenic conditions are related to the diversity and quantity of methanogens, and to competition of methanogens and other microbial communities in peatlands (Kim *et al.,* 2012). Acetate concentration was shown to decrease with increasing temperature in rice soil (Fey and Conrad, 2000), which could induce for example a decline in the energy available for acetoclastic methanogens relative to other microbial communities. Furthermore, enhanced vegetation growth due to increased temperatures might also potentially release more oxygen into deeper peat layers through root transport, resulting in the inhibition of some obligate anaerobes like methanogens (Fenner *et al.,* 2007) and promoting methane oxidation by methanotrophs.

These results give a positive prognostic on the fate of methane-cycling microbial communities under increasing temperatures and permafrost degradation, as even the communities closer to the permafrost table, where conditions usually remain stable and cold all year round, were able to adapt to their rapidly changing surroundings. Furthermore, previous studies have suggested that because methanotrophic communities are specialized and less diverse than methanogens, they could have more difficulty adapting to increasing

temperatures and thus be negatively affected in terms of methane oxidation (Liebner *et al.*, 2008). According to our results, though, the methanotrophic community at our study site does not seem to be inhibited by fast-increasing soil temperatures, *pmoA* gene copy numbers even increasing steadily over the course of the experiment.

An important variable to consider besides temperature is the level of the water table in the active layer. Indeed, soil moisture content was shown to have almost a stronger effect than temperature in some studies and Turetsky *et al.* (2008) hypothesized that flooding in wetlands would stimulate methane emissions beyond the effects of soil warming alone. They found that at the end of a 2-year warming experiment, the largest methane fluxes occurred in plots that had received both flooding and soil warming. This variable was not taken into account in our study, as soil moisture contents measured remained stable throughout the experiment. Nonetheless, it is clear that increased temperatures in Arctic wetlands are accompanied with changes in the local hydrology, thermal regime and vegetation (Hinzman *et al.,* 2005) all of which were demonstrated to strongly influence methane emissions (Olefeldt *et al.*, 2013). Arctic wetlands intrinsically display waterlogged conditions due to the presence of the near permafrost table acting as a water barrier and preventing drainage. A disappearance of permafrost due to higher temperatures, though, could potentially lead to better drainage of these soils, in which case the conditions would become more anaerobic and consequently unfavorable for methane production (Olfeldt *et al.*, 2013). It is therefore necessary to take into account the effects of permafrost thaw on soil moisture when assessing future methane emissions. Additional work must be done to investigate whether the experimental findings presented in this study truly illustrate the changes occurring *in situ* in Arctic wetlands, and better understand the changes in the ecological function of microbial communities in the context of global warming. It is necessary to further elucidate the susceptibility of permafrost microbial community to active layer warming and the potential effect this could have on the fate of previously conserved organic matters in Arctic wetlands.

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III. SYNTHESIS

3.1. Discussion

This study contributes to the general scientific understanding of the methane cycle in polygonal tundra environments in the current context of global warming. The analyses presented in this thesis were performed on samples from low-centred polygons found on Herschel Island and the Yukon Coast. Low-centred polygons are characteristic for polygonal tundra environments in the high Arctic and are widespread features found in Alaska, the Canadian Arctic, Scandinavia and Siberia. Arctic wet tundra ecosystems play a crucial role within the global carbon cycle because thawing permafrost leads to increased methane production due to the waterlogged, anoxic condition that prevail in the seasonally increasing thawed layer. Increased carbon degradation and utilization by permafrost microorganisms further affect the global carbon balance by releasing more climate relevant gases to the atmosphere. Because of the presence of diverse methanogenic as well as methanotrophic microbial communities in polygonal tundra, these environments can act as significant methane sources or sinks, depending on the abundance, diversity and distribution of these communities and the environmental conditions. To understand the carbon fluxes from Arctic permafrost environments and the future development of these areas as a carbon source, it is essential to study the carbon dynamics and the microbial communities involved at different locations covering typical permafrost landscapes of the circum-Arctic. However, despite the high relevance of the Canadian Western Arctic in the global methane budget, the permafrost microbial communities there had remained poorly characterized until now. In this respect, the present work reports the first results on the diversity of methane-cycling communities from a newly established environmental observatory in the Western Canadian Arctic. Microbial communities, as well as the two major groups of methane-cycling microorganisms, methanogenic archaea and methane oxidizing bacteria, were specifically investigated in the context of increasing temperature and active layer depth in polygonal tundra on Herschel Island and the Yukon Coast. Based on the methods applied and the analyses carried out in this thesis, the following conclusions could be drawn:

- *The microbial community diversity found in wet polygonal tundra on Herschel Island and the Yukon Coast is similar, even in geographically distant polygons.*

Comparing four seemingly similar polygons on Herschel Island and the Yukon Coast, the results of bacterial and archaeal community analysis showed that despite their geographical distance (up to 70 km distance between polygons), three out of the four sampling sites harbored similar and highly diverse bacterial and archaeal communities, which have significant implications for future greenhouse gas emissions. Overall, a clear vertical shift of community composition could be observed with increasing proximity to the permafrost table, illustrating the importance of temperature in shaping the microbial communities. In all polygons, the bacterial diversity was highest in the surface samples, then decreasing with depth. The top of the active layer was dominated by *Alpha*-, *Beta* and *Gammaproteobacteria* as well as *Acidobacteria*, while *Bacteroidetes* and *Firmicutes* which are known to include several psychrophilic and psychrotrophic members (Shivja *et al.*, 1992, Wartiainen *et al.*, 2006) dominated the community close to the permafrost table. At different depths, this

community composition was more similar within a polygon than between two polygons, suggesting a certain level of local adaptation. However, within each polygon, highly parallel trends in community composition could be observed, which is consistent with similar forcing mechanisms e.g. temperature extremes influencing the bacterial community distribution at all study sites. The archaeal community was dominated by methanogens in all polygons, emphasizing the importance of carbon-rich polygonal tundra environments in the scope of exacerbated climate warming in Arctic regions. The sequencing results indicated that hydrogenotrophic as well as acetoclastic methanogenesis occur in the active layer of permafrost affected soils in the Western Canadian Arctic. *Methanobacteria* were found to dominate the surface layers of all four polygons, contrasting with other studies of northern peatlands in Siberia (Ganzert *et al.*, 2007), Finland (Juottonen *et al.*, 2005) and Svalbard (Hoj *et al.*, 2005) where no members of the family *Methanobacteriaceae* could be detected in any samples. Quantitative PCR analysis revealed that carbon and nitrogen cycling genes were present in very large amounts compared to other studies in the Arctic, a result which, combined with the high availability of nitrogen in the soil system, suggests a nitrogen-unlimited system and emphasizes the importance of the Northwest Canadian Arctic in terms of thawing permafrost and the release of climate-relevant gases into the atmosphere. No clear separation of permafrost from the active layer samples could be observed in the four polygons, but rather a continuous transition of the diversity from the surface all the way through the permafrost layer. This supports the idea that permafrost and active layer are highly similar to each other (Yergeau *et al.*, 2010) and that the communities that will be involved in the future degradation of thawed permafrost will likely resemble the ones already in activity in the active layer.

- *Methanotrophic communities on Herschel Island resemble sub-Arctic mire methanotrophs rather than Arctic known communities.*

Based on the in-depth study of methane-cycling microorganisms in a representative polygon on Herschel Island, the methanotrophic community observed was found to be different from what was reported so far for Arctic tundra soils both in terms of community structure and potential methane oxidizing activity. The methane oxidizing bacteria (MOB) community composition was heterogeneous throughout the different soil layers, comparative sequence analysis revealing clear environmental distribution patterns and indicating the preference of specific genotypes to methane concentration or salinity. Moreover, the MOB community found in the active layer of Herschel Island was more diverse than in other Arctic tundra environments and was dominated by type II organisms, differing from the consistent identification of a type Ia dominated methanotrophic community that was reported until now in related studies on wet tundra soils of Siberia (Liebner *et al.,* 2008), Spitzbergen (Graef *et al.,* 2011) and the Canadian High Arctic (Martineau *et al.,* 2010). This dominance was shown to be shaped by environmental conditions such as low temperature and acidic pH. The MOB community observed in Drained Lake Polygon showed high resemblance to mire communities found in subarctic (Dedysh, 2009; Siljanen *et al.,* 2011) and temperate (Horz *et al.,* 2005; Chen *et al.,* 2008) ecosystems.

- *Methanogenic archaea found on Herschel Island are active and resemble other known Arctic communities.*

The methanogenic community observed on Herschel Island was highly active and had a similar composition when compared to the communities that have previously been described in other similar wet tundra environments, for instance in Svalbard and Siberia (Høj *et al.*, 2005, Ganzert *et al.*, 2007). Potential methane production rates calculated from microcosm incubations were very similar to those calculated from Siberian polygonal tundra (Wagner *et al.,* 2003), however the methane production profile obtained here is not representative of the typical activity pattern of methanogenesis in hydromorphic soils, in which no or little activity happens in the upper, oxic horizons whereas rates increase in the anoxic bottom layers close to the permafrost table (Wagner *et al.,* 2005). The methane production optima obtained in this study correlate with depths at which the organic carbon concentrations observed are at their highest throughout the profile, indicating a strong spatial correlation between the abundance of soil organic matter and methanogenesis, even though the anaerobic conditions in the deeper layers are theoretically more favorable for methanogenesis (Wagner *et al.,* 2003). Based on community fingerprinting and sequence analyses, it was found that *Methanomicrobiales*, *Methanosarcina* and *Methanosaeta* dominated in the active layer profile, which is concurrent with other studies on archaeal diversity in Svalbard and Siberia (Høj *et al.,* 2005; Ganzert *et al.,* 2007). A very interesting finding was the clear shift from a hydrogenotrophic towards an acetoclastic community which could be observed approaching the permafrost table. Such as shift, though theoretically hypothesized to exist in tundra soils, could never be clearly observed until now.

- *Simulated global warming through a rapid increase in the active layer temperature has a strong impact on the microbial community distribution.*

The original hypothesis that a gradual but strong warming of active layer cores over the course of 8 months would influence the structure and quantity of soil microbial communities, in particular the methane-cycling members of the community, was confirmed through the warming simulation. The microbial responses observed varied between bacteria and archaea and with depth. Significant shifts in bacterial composition were observed to occur in the middle and deeper layers with increasing temperatures, while the archaeal community showed a lesser response which was mostly limited to the middle of the active layer. Community fingerprinting analysis showed a quick reaction of the communities over time to a temperature increase, already visible during the first month. The microbial diversity did not decrease over time, but rather shifted as some members of the community became more abundant while other decreased. The most significant change in bacterial community composition was observed in the bottom half of the active layer. This is an expected response, because surface layers are naturally exposed to greater seasonal variations than the bottom of the active layer, meaning that the communities are naturally more adapted to temperature fluctuations. The layer close to the permafrost table, however, remains cold during the entire year and therefore microbial communities present there are used to more stable conditions. Put in the context of degrading carbon-rich permafrost ecosystems, such a shift in composition would likely be accompanied by a shift in the function of the microbial community, resulting in enhanced

carbon turnover and loss from the environment (Hartley *et al.,* 2008). Quantitative PCR analysis of functional genes *pmoA* and *mcrA* showed that both the methanogenic and methanotrophic communities were able to adapt rapidly to increasing temperatures and even seemed stimulated by warming. The methanogenic community increased more at the bottom of the core where anaerobic conditions are favorable for methanogenesis, while the methanotrophic community showed a stronger increase at the top, aerobic layer. Contrarily to what previous studies have suggested, the methanotrophic community at our study site does not seem to be inhibited by fast-increasing soil temperatures, *pmoA* gene copy numbers even increasing steadily over the course of the experiment. These results give a positive prognostic on the fate of methane-cycling microbial communities under warming conditions, as even the communities closer to the permafrost table which are thought to be adapted to stable, cold conditions and therefore more sensitive to warming, were able to adapt to rapidly increasing temperatures.

3.2. Conclusion

Herschel Island and the Yukon Coast harbor diverse methanotrophic communities which partly differ from other similar environments in the circum-Arctic. This illustrates that general conclusions about future methane emissions from polygonal tundra cannot be made for the entire circum-Arctic based on a single region, as the distribution, abundance and diversity of methane-cycling microbial communities varies from one region of the Arctic to the other. This demonstrates that even though the microbial communities undergo similar environmental pressures as in the rest of the circum-Arctic, i.e. extreme cold temperatures in the winter and seasonal and diurnal freeze-thaw cycles, certain microbial communities have been shaped differently, e.g. the methanotrophic community which resembles a sub-arctic rather than arctic community. This further implies that a different evolution of methane activity and methane fluxes could be expected with increasing environmental temperatures. It is crucial to take into account local heterogeneities and the fact that these communities are different, being shaped by various environmental factors. Even on the local scale, similar environments i.e. water saturated, organic carbon rich polygons can harbor different bacterial and archaeal communities. Studies have found that terrestrial tundra and methane fluxes are related to a broad range of controls (von Fischer *et al.,* 2010) such as for example the quality of soil organic matter and structure of the microbial community (Wagner *et al.,* 2005, Waldrop *et al.,* 2010). Still, the main functional groups remain the same, indicating the presence of a selective or functional adaptation pressure. This confirms that there is indeed such a thing as a biogeography of microorganisms, as proposed some decades ago by the Baas-Becking hypothesis (1934) 'everything is everywhere but the environment selects'. The environmental processes that create and sustain biogeographic patterns are responsible for spatial variation in microbial diversity.

Taken together, these results give important answers to the overarching questions posed in this thesis. Indeed, it seems that the structure of microbial communities, particularly methanogenic and methanotrophic populations, will be affected by rising permafrost temperatures. It can be hypothesized that thawing of permafrost due to increasing

temperatures in the Arctic will cause a shift in the proportion of certain groups in the microbial community, rather than cause a decrease in the overall diversity. Sites underlain by permafrost were shown to have lower methane emissions than sites with no surface permafrost (Olefeldt *et al.,* 2013), demonstrating the influence of low temperature on methane emissions. An increase in temperature can therefore be expected to lead to an increase in these emissions. Furthermore, based on the results found in the present thesis, the communities present in the active layer of polygonal tundra appear to be flexible enough to compensate the changes occurring in their environment with regard to carbon mineralization and their function in polar ecosystems. Methanotrophic communities are specialized and were observed to be less diverse than methanogens in Siberian permafrost, indicating that they could have more difficulty adapting to increasing temperatures and thus be negatively affected in terms of methane oxidation (Liebner *et al.,* 2008). Conversely, both the methanogenic and methanotrophic communities studied in this thesis showed a good potential for adaptation, even to exaggeratedly strong temperature increases. Therefore, in a scenario in which tundra environments become warmer and wetter, the balance between methane production and oxidation in Arctic wetlands can be expected to endure future climate change. An important variable to take into consideration besides increasing soil temperature is soil moisture. Arctic wetland dynamics are accompanied with changes in the local hydrology, thermal regime and vegetation (Hinzman *et al.,* 2005) all of which were shown to strongly influence CH_4 emissions by Olefeldt and colleagues (2013). They also critically remarked that such wetlands displayed waterlogged conditions due to the presence of the near permafrost table acting as a water barrier and preventing drainage. A disappearance of permafrost due to higher temperatures, though, could potentially lead to better drainage of these soils, in which case the conditions would become more anaerobic and consequently unfavorable for methane production (Olfeldt *et al.*, 2013). It is therefore necessary to take into account the effects of permafrost thaw on soil moisture when assessing future methane emissions. Further work must be done to investigate whether the experimental findings presented in this thesis truly illustrate the changes occurring *in situ* in Arctic wetlands, and further understand the changes in the ecological function of microbial communities in the context of global warming.

3.3. Remarks and further directions

An obvious limit to the extent of any study which is based on environmental samples is the amount of field replication, limiting the interpretation of results. In this case, the samples retrieved for the present PhD thesis were obtained from ecologically sensitive sites which can be readily and permanently destroyed through sampling. This was an important factor in determining the amount of samples retrieved, as wet polygonal tundra is characterized by a complex vegetation and soil matrix that can be irreversibly damaged by excessive digging or trampling. This highlights the general difficulty of field sampling and obtaining a number of samples that is concurrently representative, statistically justified and logistically feasible. Even so, polygonal tundra is characterized by homogeneous soil physical and chemical properties and surface characteristics both on a spatial and temporal scale. Water content, pH, total organic carbon content etc. were found to be homogeneous within a polygon field (Fiedler *et al*., 2004) which suggests that a limited number of samples can still be

representative. Moreover, a better sampling strategy could be applied in further studies to actually study the effect of deepening of the active layer and increasing water table accompanying climate warming in Arctic wetlands. This could be better achieved by sampling along a moisture gradient i.e. sampling polygons from the outside to the inside of a polygon field, thus including dryer and shallower polygons as well as wet and deep polygons. This would enable the comparison of the microbial communities in a variable polygonal environment, which could perhaps better demonstrate community changes with increasing depth and water saturation.

In terms of methodological limitations, community fingerprinting methods such as T-RFLP are useful for comparative analyses but they cannot be used to reliably assess the richness or diversity metrics of complex communities (Dunbar *et al.,* 2000). These methods are limited by their detection threshold and the number of peaks detected in T-RFLP can underestimate the actual richness of any community with a long-tailed rank abundance distribution (Bent *et al.,* 2007). Consequently, the sole use of fingerprinting methods cannot provide a reliable assessment of community diversity. However, these methods do hold great potential for use when rapid, high-throughput screening for differences or changes in microbial communities is more important than phylogenetic identification of specific organisms (Hartmann *et al.,* 2005). Another method used in this thesis, Ion Torrent Sequencing, is an emerging technology with great potential for future use in the field of molecular ecology. This method is still in the process of being optimized and as of yet only produces short reads, limiting the amount of taxonomical information that can be derived from the sequences obtained. Nonetheless, Ion Torrent Sequencing has been demonstrated to be a valid technology for environmental studies (Yergeau *et al.,* 2012) and as the length of reads increases, this method will become comparably efficient to pyrosequencing, for a fraction of the cost.

As a final point, the column incubation experiment to simulate exacerbated permafrost thaw and deepening of the active layer yielded very interesting results, but was limited in time to 8 months. Running a similar experiment over a longer period of time and with a more subtle increase in temperature would represent a more realistic scenario and possibly result in a different reaction and adaptation of the methane-cycling microbial communities present.

In any case, the Canadian Western Arctic, Herschel Island and the Yukon Coast in particular, are environmental “hot spots” in which the effects of global warming on permafrost environments can readily be observed. Further work must continue in this region, to better understand the reaction and adaptation of methane-cycling microbial communities in the context of climate warming and future feedbacks to the global methane cycle.

IV. REFERENCES

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