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

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Running title: \textit{mcrA} and \textit{pmoA} profiling in a permafrost-affected soil

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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 to 10 µmol h\(^{-1}\) g\(^{-1}\) wet soil. Terminal restriction fragment length polymorphism (T-RFLP) and cloning analyses of \textit{mcrA} and \textit{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 (\textit{Methanobacterium}) as well as acetoclastic (\textit{Methanosarcina} and \textit{Methanosaeta}) members. A pronounced shift towards 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 (\textit{Methylcystis}, \textit{Methylosinus}, and a cluster related to \textit{Methylcapsa}). The present study represents the first on methane-cycling communities in the Canadian Western Arctic, thus advancing our understanding on 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 are released from permafrost environments, contributing up to 20% of global emissions (Cao et al., 1998, MacGuire 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 due to the waterlogged, 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 \textit{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 non linear 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, Hoj 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 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 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 methane oxidizing bacteria, 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 & Murell, 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, Massachusetts). 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 done under sterile and anaerobic conditions in an atmosphere-controlled glove box.

Soil physico-chemical analyses

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

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 analysed by first treating the samples with 30% H2O2 to digest all organic matter. After washing, the samples were freeze-dried and weighed. 1% NH3 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 analyser (Beckman Coulter, Brea, California).

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 1mL of sterile water under sterile, anaerobic conditions. The bottles were sealed with butyl rubber stoppers and flushed with N₂CO₂ (80:20% v/v). Triplicate samples were incubated in the dark at 10°C. As a control, triplicate heat-sterilised samples were used. Samples were measured every 24 h for one week using an Agilent 6890 gas chromatograph (Agilent Technologies, Santa Clara, California). 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 (CH₄) 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 done after calibration of the gas chromatograph with standard gases. CH₄ production rates were calculated from the linear increase of the CH₄ 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-sterilised 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 PowerSoil™ DNA Isolation Kit (Mo Bio Laboratories, Carlsbad, California) 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 to 50 ng of DNA, 0.5 µL of each 20 mM primer (forward primer labelled with the fluorescent dye carboxyfluorescein), 5 µL Q-Solution (Qiagen), 1.5 µL 10 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 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 reaction conditions were as follow: 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 °C to 52 °C for 60 s, elongation at 72 °C for 90 s; 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, California) and then purified using a QIAquick PCR Purification Kit (Qiagen). Purified PCR products were quantified by spectrophotometry using a TrayCell (Hellma Analytics, Müllheim, Germany).

**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 10x 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 ºC. The digestion was stopped by incubation at 80 ºC 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 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 than 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).

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 (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 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. 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% towards 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 CH₄ 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 x10³ 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 x10³, 6.66 x10³ and 3.368 x10³ nmol h⁻¹ g⁻¹ were observed in layers between 20 cm depth and the permafrost table.
Methane concentrations in the heat-sterilised controls did not increase during the incubation.

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 (Figs 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 cm 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 to 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 base pairs (bp), 272 bp, 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 35-55% 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 cm 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 bp and 246 bp clearly dominated the surface layers of the profile, representing 35% and 65% respectively of the total methanotrophic community between 0 cm 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 operational taxonomic units (OTU) when using a cutoff value of 85.7% sequences similarity based on Hunger et al., 2011 (Supplementary Material). 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* (1 OTU, 11 sequences). To a smaller extent, sequences related to the genus *Methanosphaerula* (1 OTU, 6 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 \textit{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 9 T-RFs obtained in the whole community profile (Table 2.A., Fig 3.A). Out of these 8 T-RFs, 2 dominant fragments (269 bp and 306 bp) found in the overall profile corresponded to \textit{Methanosarcina}. Two fragments (462 bp, 463 bp) corresponded to \textit{Methanobacterium}. The same fragment (463 bp) was also found to correspond to \textit{Methanocella}. The remaining groups were represented by single T-RFs; \textit{Methanosarcina}/\textit{Methanosaeta} (253 bp), \textit{Methanocella} (262 bp), and \textit{Methanoregula} (289 bp and 464 bp). The numbers of clones found representing each T-RF along with their phylogenetic affiliation as obtained after comparison to the GenBank database using the BlastN algorithm are listed in Table 2.A.

**Diversity and dominant species of \textit{pmoA}**

The clone library analysis of \textit{pmoA} yielded a total of 65 cloned \textit{pmoA} sequences. Based on a 7% cutoff for the PmoA sequences (Supplementary Material), 11 OTUs were defined with a clear dominance of type IIA methanotrophs (Fig. 5). A high proportion of sequences was affiliated to an uncultured \textit{Methylocystis} cluster (3 OTUs, 17/48 sequences). 15 sequences (2 OTUs) clustered with uncultured \textit{Methylosinus/Methylocystis}, 8 sequences (3 OTUs) were affiliated with uncultured type Ia PmoA mainly from freshwater and mire habitats and which likely represents a new genus, 7 sequences (2 OTUs) were affiliated to an uncultured cluster most closely related to \textit{Methylocapsa} and a single sequence was most closely related to \textit{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 \textit{pmoA} were determined by digesting single clones with
MspI. T-RFLP analysis of clones from the sample library corresponded to 6 T-RFs obtained in the whole community profile (Table 2.B., Fig. 3.B). Out of these 7 T-RFs, the two major fragments in the profile (245 bp 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 cm 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 to the GenBank database using the BlastN algorithm are listed in Table 2b.

### 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. Christensen *et al.*, 2000, Reeburgh *et al.*, 2008, 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 were 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 CH₄ 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 CH₄ 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 and colleagues (2003) as well as in other studies (Liebner & Wagner, 2007, Knoblauch et al., 2008), but the rates obtained in those studies (7-15 nmol CH₄ 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 to 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 (Luke 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* (Wartianen 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 (Wartianen 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 and colleagues (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 to -14.7°C on Samoylov Island, Siberia (Wagner et al., 2003) for example could therefore have an influence in shaping the MOB towards 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 which 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 paddie soils were inhibited at low temperatures, while homoacetogenesis is a dominant process in cold anoxic ecosystems (Nozhevnikova et al., 1994). Hydrogenotrophic methanogenesis is hampered due to 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, Metje & Frenzel, 2007, Ganzert et al., 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 and colleagues (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 Siberia (Wagner et al., 2003, Liebner et al., 2007).

**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 (Luke 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 towards an acetoclastic community approaching the permafrost table. Such a 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 towards the few studies conducted to date. It remains elusive whether methane-cycling communities which are specific to Arctic tundra environments truly exist.

Acknowledgements

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Literature


Wagner D & Pfeiffer EM (1997) Two temperature optima of methane production in a

arctic wetlands on the islands of svalbard (Norway) - denaturing gradient gel
electrophoresis analysis of soil DNA and enrichment cultures. *Can J Microbiol* **49**:
602-612.

Wartiainen I, Hestnes AG, Mcdonald IR & Svenning MM (2006) *Methylobacter tundripaludum* sp. nov., a methane-oxidizing bacterium from arctic wetland soil on

water table level and peat temperatures in boreal peatlands. *Ecol Model* **192**: 441-
456.

Whalen SC & Reeburgh WS (1992) Interannual variations in tundra methane emission:
a 4-year time series at fixed sites. *Global Biogeochem Cy* **6**: 139-159.

Whalen SC, Reeburgh WS & Sandbeck KA (1990) Rapid methane oxidation in a

from Siberian arctic polygonal tundra: eddy covariance measurements and

Arctic permafrost revealed by metagenomic sequencing, qPCR and microarray
analyses. *ISME J* **4**: 1206-1214.

Yrjälä K, Tuomivirta TT, Juottonen H, Putkinen A, Tuittila ES, Penttil T, Laine J,
Peltoniemi K & Fritze H (2011) CH4 production and oxidation processes in a boreal
fen ecosystem after long-term water table drawdown. *Glob Chang Biol* **17**: 1311-
1320.
### Table 1. Summary of properties of PCR primers used in this study.

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

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Figure legends

Figure 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.

Figure 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⁻¹ methane in synthetic air. Error bars in (e) and (f) represent standard deviations.

Figure 3. Composition of methanogenic (A) and methanotrophic (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.
Figure 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.

Figure 5. Phylogenetic tree showing the relation of deduced methane oxidizing bacteria PmoA amino acid sequences from active layer samples of Herschel Island, Canadian Western Arctic to known methane oxidizing bacteria 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.