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1 **Methane-cycling communities in a permafrost-affected soil on Herschel Island,**

2 **Western Canadian Arctic: active layer profiling of *mcrA* and *pmoA* genes.**

3

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14 Running title: *mcrA* and *pmoA* profiling in a permafrost-affected soil

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21

22 **Abstract:**

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

40

41

42 **Introduction**

43 Arctic permafrost environments play a crucial role in the global carbon cycle. Between
44 10 and 39 Tg a^{-1} of methane are released from permafrost environments, contributing up
45 to 20% of global emissions (Cao *et al.*, 1998, MacGuire *et al.,* 2009) and making them
46 the largest single natural source of methane (Christensen *et al.*, 1996). Permafrost soils
47 are also believed to contain 50% of the global belowground organic carbon pool
48 (Tarnocai *et al.*, 2009), a considerable reservoir for potential future release of methane.
49 These environments are predicted to warm more rapidly than the rest of the globe
50 (Anisimov *et al.,* 2007) and with them, the wet tundra ecosystems which host much of
51 the methanogenic activity due to the waterlogged, anoxic conditions that prevail in
52 seasonally deepening thawed layers (Whalen & Reeburgh, 1992),
53 Methane release is in fact the net result between methanogenic and methanotrophic
54 activity. Methane can be generated in-situ by methanogenic archaea (a group belonging
55 to the *Euryarchaeota*) under anaerobic conditions, but it can also be oxidized by
56 methanotrophs such as methane oxidizing bacteria (MOB), making tundra environments
57 act as a methane sink (Whalen *et al.*, 1990, Callaghan *et al.*, 2005, Wagner & Liebner,
58 2009). MOB belong to the phylum Proteobacteria and can oxidize up to 90% of the
59 methane emitted in the deeper layers before it reaches the atmosphere (Oremland &
60 Culbertson, 1992, Le Mer & Roger, 2001, Wagner & Liebner, 2009). The balance
61 between methane production and oxidation is thereby fragile and non linear as
62 methanogens and methanotrophs show a different response to temperature fluctuations
63 (Ganzert *et al.*, 2007, Høj *et al.*, 2008, Knoblauch *et al.*, 2008, Liebner *et al.*, 2009).
64 Changing climate conditions could dramatically alter this balance and mobilize the large
65 carbon pools found in permafrost, potentially creating a positive feedback loop with
66 important global implications. Several studies have been conducted to explore this issue
67 in Siberia (Kobabe *et al.*, 2004, Ganzert *et al.*, 2007, Wagner *et al.*, 2007, Liebner *et al.*,

68 2008, Dedysh 2009), Svalbard (Wartiainen *et al.*, 2003, Høj *et al.*, 2008, Graef *et al.*,
69 2011) and the Canadian High Arctic (Pacheco-Oliver *et al.*, 2002, Martineau *et al.*,
70 2010, Yergeau *et al.*, 2010) to study the characteristics and dynamics of methane-
71 cycling communities, but the communities of the Canadian Western Arctic remain
72 unexplored to date.
73 In the following paper the vertical distribution and diversity of two functional marker
74 genes coding for enzymes involved in the methane cycle were investigated. To look at
75 the diversity in the methanogenic population, we selected the gene coding for subunit A
76 of the methyl coenzyme-M reductase enzyme (*mcrA*). Methyl coenzyme-M is the
77 terminal enzyme complex in the methane generation pathway, methyl coenzyme-M
78 reductase (MCR), which catalyses the reduction of a methyl group bound to coenzyme-
79 M, with the accompanying release of methane (Luton *et al.*, 2002). This enzyme
80 complex is unique and ubiquitous in known methanogens (Thauer, 1998) and various
81 studies have used it as a reliable tool for the specific detection of this group (Juottonen
82 *et al.*, 2005, Steinberg & Regan, 2008, Biderre-Petit *et al.*, 2011).
83 To study the diversity of methane oxidizing bacteria, we selected the gene coding for
84 subunit A of the particulate methane monooxygenase enzyme (*pmoA*). Methane
85 monooxygenase (MMO) is found in either soluble or membrane-bound form, except in
86 Methylocella species where only the membrane-bound form is present (Theisen &
87 Murell, 2005). MMO is responsible for the conversion of methane into methanol, which
88 is either assimilated into biomass or oxidized to carbon dioxide (Semrau *et al.*, 1995)..
89 Both functional genes are characterized by sufficient sequence divergence to serve as a
90 reliable diagnostic gene for the study of the two populations of interest (McDonald &
91 Murrell, 1997, Luton *et al.*, 2002).
92 In this study we aimed to better understand the *in situ* dynamics between microbial-
93 driven methanogenesis and methane oxidation in increasingly thawing permafrost. We

94 calculated methane production and potential oxidation rates in an active layer soil
95 profile from polygonal tundra on Herschel Island in the Canadian Western Arctic. To
96 understand abiotic factors driving methane activity, we described the physico-chemical
97 properties of the soil profile. We evaluated the assortment and distribution of *mcrA* and
98 *pmoA* signatures throughout the soil profile using T-RFLP analysis. We complemented
99 the fingerprinting results by constructing clone libraries of our two genes of interest.
100 The results presented give new insights into the distribution and activity of
101 methanogenic and methanotrophic microorganisms in the active layer of a rapidly
102 degrading permafrost environment.
103
104 **Materials and methods**
105 **Site description and sample collection**
106 Active layer samples were collected from the "Drained Lake" low-center polygon (N
107 69°34'43, W 138°57'25, elevation 30 m above sea level) on Herschel Island, Western
108 Canadian Arctic (Fig. 1) during the expedition YUKON COAST in July-August 2010.
109 A low-center polygon is an ice-wedge polygon in which thawing of ice-rich permafrost
110 has left the central area in a relatively depressed position (van Everdingen, 2005). The
111 soil at this site was characterized as a hemic glacistel classified according to the U.S.
112 Soil Taxonomy (Soil Survey Staff, 1998) with poor drainage and a loamy soil texture.
113 Vegetation cover included roughly 35% plant litter, 40% *Carex* sp. (sedges), 15% *Salix*
114 sp. (dwarf willow), 10% mosses with traces of *Pedicularis* sp. (wooly lousewort) and
115 *Ledum groenlandicum* (Labrador tea). The vegetation period spans yearly from mid-
116 June to end of September. Average air temperatures vary annually between -26.3 °C in
117 February to 8.7 °C in July (Burn & Zhang, 2009).
118 The sampling site was characterized by an active layer (the layer of ground that is
119 subject to annual thawing and freezing) consisting of a large peat horizon, with a depth

120 of 36 cm as measured using a permafrost probe. A hole was dug to the permafrost table,
121 one side of the hole was cleaned and blocks of soil were taken every 5 cm with a sharp
122 sterile knife and placed into sterile 125 mL Nalgene® screw-cap containers (Thermo
123 Fischer Scientific Inc., Waltham, Massachusetts). The knife was wiped down and
124 sterilized with ethanol between different samples. Soil samples were frozen
125 immediately after sampling and stored at -20 °C upon arrival in the laboratory. All
126 subsequent subsampling was done under sterile and anaerobic conditions in an
127 atmosphere-controlled glove box.
128
129 **Soil physico-chemical analyses**
130 Gravimetric moisture content of soils was determined by weighing sub-samples before
131 and after freeze-drying for 72 h.
132 pH was measured using a CyberScan PC 510 Bench Meter (Eutech Instruments Pte
133 Ltd., Singapore) following the slurry technique by mixing 1:2.5 mass ratio of samples
134 and de-ionized water (Edmeades *et al.*, 1985).
135 Grain size was analysed by first treating the samples with 30% H_2O_2 to digest all
136 organic matter. After washing, the samples were freeze-dried and weighed. 1% NH_3
137 solution was added to the samples and shaken for at least 24 hours. Grain size was then
138 measured at least twice for each sample with a Coulter LS 200 laser particle size
139 analyser (Beckman Coulter, Brea, California).
140 The percentage of total organic carbon (TOC) of the soils was measured in duplicate
141 using a TOC analyzer (Elementar Vario max C, Germany). Samples prepared for
142 analysis by freeze-drying and homogenized in an orbit mill ball-grinder (Pulverisette 5,
143 Fritsch Ltd., Germany). The TOC content was calibrated using external standards of
144 known elemental composition.

145 Water content, pH and TOC could not be measured for the uppermost layer of the
146 profile, as this mostly consisted of roots and plant material which were not sufficient to
147 measure these parameters.
148
149 **Methane measurements**
150 Methanogenic activity of each soil layer was measured under simulated in-situ
151 conditions without substrate addition by placing 5 g of fresh soil material in 20 mL
152 glass bottles and covered with 1mL of sterile water under sterile, anaerobic conditions.
153 The bottles were sealed with butyl rubber stoppers and flushed with N_2CO_2 (80:20% v
154 v^{-1}). Triplicate samples were incubated in the dark at 10°C. As a control, triplicate heat-
155 sterilised samples were used. Samples were measured every 24 h for one week using an
156 Agilent 6890 gas chromatograph (Agilent Technologies, Santa Clara, California). Gases
157 were separated on a Plot Q capillary column (0.53 mm diameter, 15 m length) using a
158 gas flow of 30 mL min^{-1} with helium as carrier gas and methane (CH_4) was measured
159 through a flame ionizing detector (FID). The oven and injector temperature were set at
160 80 °C and the detector temperature at 250 °C. All gas sample analyses were done after
161 calibration of the gas chromatograph with standard gases. CH_4 production rates were
162 calculated from the linear increase of the CH_4 concentration in the headspace with time.
163 To study potential methane oxidation rates, fresh soil material (4 g) was placed in flat-
164 walled culture bottles (50 mL) and distributed over the sidewall as a thin layer as
165 described by Knoblauch *et al.* (2008). The bottles were sealed with butyl rubber
166 stoppers and incubated horizontally. The headspace contained 2.5% v v^{-1} methane in
167 synthetic air. Triplicate samples were incubated in the dark at 10 °C. Methane was
168 measured repeatedly and the oxidation rates were calculated from the initial linear
169 reduction in methane using multiple data points. Gas samples were measured in the
170 same manner as described above. Heat-sterilised samples were used as the control.

171

172 **Extraction of genomic DNA and PCR amplification**

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

180 PCR reactions were performed in triplicate 50 µL volumes containing between 10 to 50
181 ng of DNA, 0.5 µL of each 20 mM primer (forward primer labelled with the fluorescent
182 dye carboxyfluorescein), 5 µL Q-Solution (Qiagen), 1.5 µL 10 mM dNTP mix, 5 µL
183 10x PCR buffer (Qiagen), 1 U of HotStar Taq DNA polymerase (Qiagen, Hilden,
184 Germany) and PCR-grade water to 50 µL.

185 Primers used in the different PCR reactions are listed in Table 1. For the amplification
186 of the archaeal *mcrA* gene, the primer pair MLf / MLr was used (Luton *et al.*, 2002).
187 Reaction conditions were as follows: initial denaturation at 94 °C for 3 min, 35 cycles
188 with denaturation at 94 °C for 25 s, annealing at 50 °C for 45 s, extension at 72 °C for
189 60 s and a final extension at 72 °C for 5 min.

190 For the amplification of the methanotrophic *pmoA* gene, the primer pairs A189f / A682r
191 and A189f / mb661r were used (Costello & Lidstrom, 1999, Holmes *et al.*, 1999) in a
192 semi-nested PCR approach. The first PCR reaction conditions were as follow: initial
193 denaturation and polymerase activation at 95 °C for 5 min, 30 cycles with constant
194 denaturation temperature at 94 °C for 45 s, decreasing annealing temperature from 62
195 °C to 52 °C for 60 s, elongation at 72 °C for 90 s; final elongation at 72 °C for 90 s. The
196 second PCR reaction conditions were initial denaturation and polymerase activation at

197 95 °C for 5 min, 22 cycles of denaturation at 94°C for 45 s, annealing at 56°C for 60 s,
198 elongation at 72 °C for 90 s, and a final extension at 72 °C for 10 min.
199 Triplicate PCR reactions were visualized on a 1% agarose gel containing GelRed stain
200 (Hayward, California) and then purified using a QIAquick PCR Purification Kit
201 (Qiagen). Purified PCR products were quantified by spectrophotometry using a
202 TrayCell (Hellma Analytics, Müllheim, Germany).
203

204 **Terminal Restriction Fragment Length Polymorphism (T-RFLP)**

205 The digestion of fluorescently-labeled PCR fragments using restriction enzymes was
206 conducted in duplicate as follows. 10 U of enzyme MspI (Roche, Penzberg, Germany),
207 2 µL of 10x Buffer and 500-600 ng of purified PCR product were mixed. PCR grade
208 water was added to 20 µL. The samples were then incubated for 3 h at 37 ºC. The
209 digestion was stopped by incubation at 80 ºC for 20 min. Duplicate digests were pooled
210 and purified using the QIAquick Purification Kit (Qiagen).
211 T-RFLP products (2 µL) were mixed with 0.25 µL of GeneScan™ 500 LIZ® internal
212 size standard (Applied Biosystems, Darmstadt, Germany) and run on an ABI 3730xl
213 DNA Analyzer (Applied Biosystems) at GATC Biotech (Konstanz, Germany).
214 Afterwards, the lengths of the fluorescently labeled terminal fragments (T-RFs) were
215 visualized with Peak Scanner software (v1.0, Applied Biosystems).
216 T-RFLP results were analysed statistically according to Dunbar *et al.* (2001) to yield
217 relative abundance (%) of T-RFs. Briefly, T-RFs were aligned and clustered manually
218 using Excel (Microsoft, Redmond, Washington). DNA quantity between triplicate
219 samples as well as between depth profiles was standardized in an iterative
220 standardization procedure. For each sample, a derivative profile containing only the
221 most conservative and reliable T-RF information was created by identifying the subset
222 of T-RFs that appeared in all replicate profiles of a sample. Standardized, derivative

223 profiles were then aligned. The average size of TRFs in each alignment cluster was
224 calculated to produce a single, composite list of the T-RF sizes found among all
225 samples. Relative signal intensity of each T-RF (%) was calculated based on the signal
226 intensity of each individual T-RF with respect to the total signal intensity of all T-RFs
227 in that sample. Peaks representing less that 1% of total fluorescence were eliminated
228 from the profile in order to concentrate on the most representative microorganisms in
229 each community. T-RFLP profiles were converted into presence-absence data and
230 analysed statistically by cluster analysis based on Bray-Curtis pairwise similarities using
231 the software PRIMER 6 (Primer-E Ltd., Lutton, United Kingdom).
232

233 **Cloning and sequence analyses**

234 Based on the obtained T-RFLP results, various profile depths with the highest
235 representative T-RF diversity (5-10 cm and 20-25 cm for *mcrA*; 0-5 cm and 15-20 cm
236 for *pmoA*) were chosen to establish clone libraries. Libraries for the functional genes
237 *mcrA* and *pmoA* were created by ligating PCR products into the pGEM-T Easy vector
238 and transformed into competent cells *Escherichia coli* JM109 using the "pGEM-T Easy
239 Vector Systems II" Kit (Promega, Mannheim, Germany). White colonies containing
240 inserts were picked, suspended in 1.2 mL of nutrient broth containing ampicillin (50 µg
241 mL^{-1}) and grown overnight at 37 °C. Colonies were screened by PCR with vector
242 primers M13 for correct size of the insert and the amplicons were directly sequenced by
243 GATC Biotech AG (Konstanz, Germany). 96 clones per gene were sequenced. The
244 sequences were edited and contigs assembled using the Sequencher software (v4.7,
245 Gene Codes, Ann Arbor, Michigan). Nucleotide sequences were then screened and
246 translated into correct amino acid sequences for further phylogenetic analyses using
247 CLC sequence viewer software (version 6.5.1). Altogether, 81 McrA and 48 PmoA
248 deduced amino acid (aa) sequences were used.

249 For McrA, sequences including nearest neighbors and cultured isolates were pre-aligned
250 using the Muscle alignment tool integrated in MEGA 5 (Tamura *et al.*, 2011). The
251 alignment was then imported in ARB (www.arb-home.de, Ludwig *et al.*, 2004) and
252 manually checked. A neighbor joining tree (Saitou & Nei, 1987) was constructed in
253 ARB with a subset of 205 McrA amino acid sequences including nearest neighbors and
254 representative isolate sequences (163 aa).
255 For PmoA, the deduced amino acid sequences were imported into an ARB database
256 containing 3708 high quality PmoA sequences and were manually aligned. A neighbor
257 joining tree constructed in ARB with a subset of 127 PmoA sequences including nearest
258 neighbors and representative isolate sequences (135 aa) using a 30% base frequency
259 filter. The distance matrix was calculated using the neighbor joining algorithm with a
260 Kimura correction for McrA and a PAM correction for PmoA amino acid sequences.
261 Rarefaction analysis was performed with DOTUR (Schloss & Handelsman, 2005) based
262 on the furthest neighbor algorithm. OTUs were defined using a 14.3% cutoff value for
263 McrA according to Hunger *et al.* (2011) and a 7% cutoff for PmoA according to
264 Degelmann *et al.* (2010).
265
266 **Nucleotide sequence accession numbers**
267 The environmental *mcrA* and *pmoA* clone sequences recovered in this study from the
268 active layer of a polygon on Herschel Island were have been submitted to the GenBank
269 nucleotide sequence databases and can be found under accession numbers JQ048956-
270 JQ049081.
271
272 **Results**
273 **Characteristics of the soil**

274 The average *in situ* day-temperature at the surface of the profile was 12 °C, decreasing
275 gradually to -0.5 °C at the permafrost table (Fig. 2a). The pH of the entire profile was
276 slightly acidic, ranging between 5.2 and 5.6 throughout (Fig. 2b). The mineral fraction
277 of the soil represented only roughly 30%, as calculated after concentrated acid digestion
278 of organic matter. The mineral fraction consisted on average of 27% sand, 20% silt and
279 15% clay. The soil was visibly water saturated, with gravimetric moisture contents in
280 the profile ranging from 77% near the surface and increasing to 83% close to the
281 permafrost table (Fig. 2c). The organic carbon content was overall very high for all
282 profile layers, ranging from 28% in the middle layers to 23% towards the permafrost
283 table (Fig. 2d).
284

285 **Methane production and oxidation**

286 At an incubation temperature of 10°C and with no added substrate, no significant
287 methane production was found in the soil surface sample (0-5 cm depth) and only a low
288 1.4 nmol of CH_4 per hour and per gram of wet soil (nmol $h^{-1}g^{-1}$) was observed in the
289 subsequent layer (Fig. 2e). The methanogenic activity in the deeper soil layers varied
290 from 10.3 to 38.5 nmol $h^{-1}g^{-1}$ with the exception of one sample (20-25 cm depth) where
291 a lower value of 4.5 nmol $h^{-1}g^{-1}$ was observed. The maximum potential methane
292 production rates of 38.5 nmol $h^{-1}g^{-1}$ occurred in the middle of the soil profile at 10-15
293 cm depth along with 35.8 nmol $h^{-1}g^{-1}$ above the permafrost table at 30-35 cm depth.
294 The potential methane oxidation rate in the same profile varied between 43.5 and
295 9508.1 nmol $h^{-1}g^{-1}$ (Fig. 2f). The maximum rate of 9.51 $x10^3$ nmol $h^{-1}g^{-1}$ was reached at
296 10-15 cm depth, at the same depth where the maximum methane production rate was
297 also observed. High rates of 6.02 $x10^3$, 6.66 $x10^3$ and 3.368 $x10^3$ nmol $h^{-1}g^{-1}$ were
298 observed in layers between 20 cm depth and the permafrost table.

299 Methane concentrations in the heat-sterilised controls did not increase during the
300 incubation.
301
302 **Methanogenic and methanotrophic community structure**
303 The community structure of methanogens and methanotrophs in the active layer profile
304 was investigated through T-RFLP analysis of *mcrA* and *pmoA* functional genes (Figs
305 3.a,b). We obtained overall diverse communities, with a total of 17 T-RFs for the
306 methanogenic archaea and 14 T-RFs for the methanotrophic bacteria.
307 Generally, we found that the methanogenic community became increasingly diverse
308 with soil depth. No *mcrA* signal could be detected in the surface sample (Fig. 3a). Bray-
309 Curtis similarity analysis of the *mcrA* T-RFLP data showed that community
310 composition of methanogens was 80% similar between 15 cm and 36 cm depth. All
311 samples taken together, excluding the surface layer, showed 60% similarity in
312 community composition. The 5-10 cm depth sample displayed a different T-RF pattern
313 compared to the subsequent depths, especially with respect to T-RF abundance. In this
314 sample, the 463 bp T-RF represented 68% of total fluorescence, disappearing at the next
315 sample depth and then reappearing in deeper layers, at a stable 10% of total T-RF
316 abundance. A clear vertical shift in the community could be observed with
317 predominating T-RFs in the surface layers (269 base pairs (bp), 272 bp, 306 bp)
318 decreasing in abundance in the deeper layers. The 269 bp T-RF could first be detected at
319 10-15 cm depth and represented between 35-55% of the community composition down
320 to 35 cm depth. The 306 bp T-RF could first be detected at 5-10 cm depth and then
321 gradually became more predominant in the community with increasing depth, making
322 up 76% of the community close to the permafrost table.
323 The methanotrophic community based on *pmoA* showed the overall highest diversity in
324 a depth between 10 cm and 30 cm of the active layer. Based on Bray-Curtis similarity

325 analysis the MOB community composition was heterogeneous throughout the different
326 soil layers and samples clustered in a pairwise manner (Fig. 3b). Peaks of 245 bp and
327 246 bp clearly dominated the surface layers of the profile, representing 35% and 65%
328 respectively of the total methanotrophic community between 0 cm and 10 cm depth.
329 The T-RF of 117 bp first appeared at 10cm depth and became progressively dominant in
330 the profile with increasing depth. One T-RF of 100 bp was the only detectable peak
331 close to the permafrost table. Overall, a shifting MOB community composition could be
332 observed with increasing depth with T-RFs of 104, 117, 415 and 509 bp increasing in
333 abundance while T-RFs of 245, 246 and 249 bp decreasing in abundance in the
334 community profile.
335

336 **Diversity and dominant species of *mcrA***

337 The clone library analysis of *mcrA* yielded a total of 85 cloned *mcrA* sequences. Four
338 sequences resulting in < 100 amino acids were removed from further phylogenetic
339 analyses. The diversity at the species level was low, resulting in six distinct operational
340 taxonomic units (OTU) when using a cutoff value of 85.7% sequences similarity based
341 on Hunger *et al.*, 2011 (Supplementary Material). Phylogenetic analyses of the clones
342 indicated that the methanogenic community in the active layer profile was dominated by
343 members of the genus *Methanobacterium* (1 OTU, 27 of 81 sequences),
344 *Methanosarcina* (1 OTU, 19 sequences), *Methanosaeta* (1 OTU, 17 sequences) and
345 *Methanocella* (1 OTU, 11 sequences). To a smaller extent, sequences related to the
346 genus *Methanosphaerula* (1 OTU, 6 sequences) and only one sequence could be
347 assigned to a novel, deep-branching group with relatives found in peat (Yrjälä *et al.*,
348 2011), a humic bog lake (Milferstedt *et al.*, 2010), Lake Pavin (Biderre-Petit *et al.*,
349 2011) and wetland soil (Narihiro *et al.*, 2011) (Fig. 4).

350 In an attempt to identify the most dominating methanogenic species in all depths of the
351 active layer, T-RF sizes of 50 clones for *mcrA* were determined by digesting single
352 clones with MspI, the same enzyme used for the whole-profile T-RFLP analysis. Clones
353 from the sample library corresponded overall to 9 T-RFs obtained in the whole
354 community profile (Table 2.A., Fig 3.A). Out of these 8 T-RFs, 2 dominant fragments
355 (269 bp and 306 bp) found in the overall profile corresponded to *Methanosarcina*. Two
356 fragments (462 bp, 463 bp) corresponded to *Methanobacterium*. The same fragment
357 (463 bp) was also found to correspond to *Methanocella*. The remaining groups were
358 represented by single T-RFs; *Methanosarcina/ Methanosaeta* (253 bp), *Methanocella*
359 (262 bp), and *Methanoregula* (289 bp and 464 bp). The numbers of clones found
360 representing each T-RF along with their phylogenetic affiliation as obtained after
361 comparison to the GenBank database using the BlastN algorithm are listed in Table 2.A.
362

363 **Diversity and dominant species of *pmoA***

364 The clone library analysis of *pmoA* yielded a total of 65 cloned *pmoA* sequences. Based
365 on a 7% cutoff for the PmoA sequences (Supplementary Material), 11 OTUs were
366 defined with a clear dominance of type IIa methanotrophs (Fig. 5). A high proportion of
367 sequences was affiliated to an uncultured *Methylocystis* cluster (3 OTUs, 17/48
368 sequences). 15 sequences (2 OTUs) clustered with uncultured
369 *Methylosinus/Methylocystis*, 8 sequences (3 OTUs) were affiliated with uncultured type
370 Ia PmoA mainly from freshwater and mire habitats and which likely represents a new
371 genus, 7 sequences (2 OTUs) were affiliated to an uncultured cluster most closely
372 related to *Methylocapsa* and a single sequence was most closely related to *Methylcystis*
373 sp. 212 and related sequences from acidic and mire environments.
374 To identify the most dominating methanotrophic species in all depths of the active layer,
375 T-RF sizes of 90 clones for *pmoA* were determined by digesting single clones with

376 MspI. T-RFLP analysis of clones from the sample library corresponded to 6 T-RFs
377 obtained in the whole community profile (Table 2.B., Fig. 3.B). Out of these 7 T-RFs,
378 the two major fragments in the profile (245 bp and 246 bp) corresponded to
379 *Methylocystis* and *Methylosinus* and dominated the active layer profile between 0 and
380 25 cm depth. The 509 bp fragment found to correspond to *Methylocapsa* could only be
381 detected to a small amount in the surface layer of the profile as well as between 15 cm
382 and 20 cm depth. The 76 bp fragment with *Methylococcus* as the closest cultured
383 relative was first detected in the middle of the active layer at 10 cm depth, decreasing in
384 abundance down to 25 cm depth. The 83 bp fragment also corresponding to
385 *Methylocystis* was detected at the same frequency in the upper half of the active layer.
386 The numbers of clones found representing each peak along with their phylogenetic
387 affiliation as obtained after comparison to the GenBank database using the BlastN
388 algorithm are listed in Table 2b.
389

390 **Discussion**

391

392 ***Potential rates of methane production and methane oxidation***

393 Although numerous studies have focused on surface methane fluxes in tundra
394 environments in the circum-Arctic region (e.g. Christensen *et al.*, 2000, Reeburgh *et al.*,
395 2008, Wille *et al.*, 2008), the microbiological communities behind these fluxes are still
396 understudied. To understand the carbon fluxes from Arctic permafrost environments
397 and the future development of these areas as a carbon source, it is essential to study the
398 carbon dynamics and the microbial communities involved at different locations
399 covering typical permafrost landscapes of the circum-Arctic. In this respect, the present
400 study reported first results on the diversity of methane-cycling communities from a
401 newly established environmental observatory in the Western Canadian Arctic.

402 It was shown that wet polygonal tundra environments on Herschel Island contained
403 highly active methane-cycling communities. The highest potential methane production
404 as well as oxidation rates at 10 °C in the vertical active layer profile were found in 10-
405 20 cm soil depth, the second methane production optimum was found close to the
406 permafrost table, while it appeared to be between 20 and 30 cm for methane oxidation.
407 The observed potential methane production profile does not represent the typical
408 activity pattern of methanogenesis in hydromorphic soils which would be no or little
409 activity in the upper, oxic horizons and increasing rates in the anoxic bottom layers
410 close to the permafrost table (Krumholz *et al.*, 1995). These methane production optima
411 correlate with depths at which the organic carbon concentrations observed are at their
412 highest throughout the profile, indicating a strong spatial correlation between the
413 abundance of soil organic matter and methanogenesis as observed in previous studies in
414 Siberia (Ganzert *et al.*, 2007). Methane oxidation rates correlated to or were just above
415 depths at which the highest methanogenesis occurred, illustrating the close spatial
416 location of methane production and oxidation in the studied active layer on Herschel
417 Island. Methane production rates of 40 nmol CH_4 h^{-1} g^{-1} wet soil calculated in this study
418 are comparable to those found *in situ* in other studies where rates of up to 39 nmol CH_4
419 h^{-1} g^{-1} wet soil were measured from the active layer of permafrost (Wagner *et al.*, 2003,
420 Høj *et al.*, 2005, Metje & Frenzel, 2007). Potential methane oxidation rates in Siberian
421 permafrost-affected soils were also calculated by Wagner and colleagues (2003) as well
422 as in other studies (Liebner & Wagner, 2007, Knoblauch *et al.*, 2008), but the rates
423 obtained in those studies (7-15 nmol CH_4 h^{-1} g^{-1} wet soil) were about three orders of
424 magnitude smaller than the rate we calculated for active layer samples from Herschel
425 Island. This could be due to differences in the activity and composition of the respective
426 microbial communities. The activity of both processes is mainly affected by the water
427 table position and the availability of substrates. It was shown for Siberian tundra that

428 methane production activity decreased while oxidation rates increased concurrently with
429 the progressing season (Wagner *et al.*, 2003). Although high methane oxidation rates
430 can therefore be expected during the month of August, the potential methane oxidation
431 rates calculated here are extremely high compared to what has been measured in similar
432 arctic environments. They are rather similar to sub-Arctic rates as reported from a
433 Finnish boreal mire (Jaatinen *et al.*, 2005) or a *Carex*-dominated fen in Alberta, Canada
434 (Popp *et al.*, 2000). These high rates underline the importance of methanotrophic
435 communities as the only terrestrial methane sink in Arctic wetlands (Trotsenko &
436 Khmelenina, 2002), particularly with respect to permafrost degradation under future
437 predicted global warming.
438
439 ***Structure of Herschel methane-cycling communities***
440 Profiling *mcrA* and *pmoA* sequences along a permafrost-affected soil on Herschel
441 Island, we extended the picture of methane-cycling communities in the Arctic and
442 uncovered yet unseen microbial community composition in wet polygonal tundra. The
443 MOB community found in the active layer of Herschel Island was more diverse than in
444 other Arctic tundra environments and was dominated by type II organisms, primarily
445 *Methylocystis*. Members of the *Methylocystis* genotype are known to thrive in methane
446 rich environments (Luke *et al.*, 2010) but were not yet observed to dominate in Arctic
447 tundra wetlands. Favorable conditions for type II methanotrophs in tundra soils were
448 only reported for acidic *Sphagnum* peat where especially species of the genus
449 *Methylocella* thrive (e.g. Dedysh *et al.*, 2004). However *Methylocella* does not have a
450 particulate methane monooxygenase and was not targeted in the present work. The
451 MOB community on Herschel is thus unique for an Arctic tundra ecosystem and differs
452 from what was reported until now in related studies on wet tundra of Siberia (Liebner *et*
453 *al.*, 2008), Spitzbergen (Wartiainen *et al.*, 2003, Graef *et al.*, 2011) and the Canadian

454 High Arctic (Martineau *et al.*, 2010). These studies consistently identified a dominance
455 of type Ia methanotrophs and a generally very low diversity on the genus level
456 (reviewed in Liebner & Wagner, 2010). In detail, we did not detect the presence of
457 *Methylobacter tundripaludum* (Wartianen *et al.*, 2006) or any other species of the
458 *Methylobacter* genotype, although it was found to dominate in Arctic permafrost-
459 affected soils of Svalbard where it was isolated (Wartianen *et al.*, 2006, Graef *et al.*,
460 2011), Siberia (Liebner *et al.*, 2009) and the Canadian High Arctic (Martineau *et al.*,
461 2010) although a certain caution should be used when interpreting absences because of
462 intrinsic uncertainties of the PCR reaction. Even so, these findings underline the
463 uncommon composition of the MOB community in an Arctic environment. In fact, the
464 MOB community observed in this study is rather comparable to mire communities
465 found in sub-arctic (Jaatinen *et al.*, 2005, Dedysh, 2009, Siljanen *et al.*, 2011) and
466 temperate (Hoffmann *et al.*, 2002, Horz *et al.*, 2005, Chen *et al.*, 2008) ecosystems.
467 Similarly, the extremely high potential methane oxidation rates observed here
468 (discussed above) are comparable rather to what was reported in sub-arctic and
469 temperate mires than in Arctic tundra soils (Jaatinen *et al.*, 2005, Liebner & Wagner,
470 2007). Given the slightly acidic pH and a mire-specific vegetation of our study site, the
471 similarity to other mire MOB communities is not surprising. Soil temperature was also
472 suggested to play a role in the establishment of either a type I or type II MOB
473 community as described by Knoblauch and colleagues (2008) who observed a shift from
474 a type I MOB community in Siberian permafrost-affected soils at low temperatures to
475 an increase in type II MOB with increasing incubation temperature. The maritime
476 climate and the higher annual average air temperature of -9.6°C at our site on Herschel
477 Island (Burn & Zhang, 2009) compared to -14.7°C on Samoylov Island, Siberia
478 (Wagner *et al.*, 2003) for example could therefore have an influence in shaping the
479 MOB towards a type II dominated community. These findings support the hypothesis

480 that MOB communities are more sensitive to temperature variations (Liebner & 481 Wagner, 2007), which could have implications in Arctic environments with respect to 482 warming air temperatures and the weakening of MOB as a methane sink.

483 Methanogenic archaea are also known inhabitants of permafrost soils (reviewed in 484 Wagner & Liebner, 2010). Our results indicate that hydrogenotrophic as well as 485 acetoclastic methanogenesis occurs in active layer soils of the Western Canadian Arctic. 486 Based on T-RFLP and sequence analyses, we found that the methanogenic archaea 487 belonging to *Methanomicrobiales, Methanosarcina* and *Methanosaeta* dominated in the 488 active layer profile which has also been shown in other studies on archaeal diversity in 489 Spitzbergen and Siberia (Høj *et al.*, 2005, Ganzert *et al.*, 2007). *Methanobacteria* which 490 are hydrogenotrophic (Thauer, 1998) showed preferential colonization of the upper 491 layer of the profile, its abundance quickly decreased with depth while representatives of 492 acetoclastic methanogens belonging to *Methanosarcina* and *Methanosaeta* (Thauer, 493 1998) were mainly found in the lower and colder soil layers. At low temperatures there 494 is a prevalence of the acetoclastic pathway of methanogenesis. Indeed, Conrad *et al.* 495 (1987) showed that hydrogen-producing bacteria in paddie soils were inhibited at low 496 temperatures, while homoacetogenesis is a dominant process in cold anoxic ecosystems 497 (Nozhevnikova *et al.*, 1994). Hydrogenotrophic methanogenesis is hampered due to 498 competition with acetogenic bacteria for hydrogen and carbon dioxide which produce 499 acetate as a precursor for acetoclastic methanogens (Kotsyurbenko, 2005). Also, the 500 availability of low molecular substances (e.g. acetate) provided by the root system of 501 the vegetation (Chanton *et al.*, 1995; Ström *et al.*, 2003) could have an influence on the 502 composition of the methanogenic community. Altogether, at the genus level the 503 community of methanogenic archaea observed here is representative of what has been 504 found in other studies of permafrost soils (Høj *et al.*, 2005, Metje & Frenzel, 2007, 505 Ganzert *et al.*, 2007). The low number of methanogenic OTUs in our study could be due

506 to the low pH of the ecosystem. There are so far only a few cultured acidophilic
507 methanogens known (Cadillo-Quiroz *et al.*, 2009, Bräuer *et al.*, 2011). This could also
508 be due to substrate limitation even though the organic carbon concentration is high, as
509 shown by Wagner and colleagues (2005). Indeed, methane emission rates and potential
510 methane production in carbon rich soils are dependent on substrate quality, which tends
511 to decrease with the degree of decomposition (Ström *et al.*, 2003).
512 Based on T-RFLP fingerprints and Bray-Curtis analysis, we observed a vertical shift not
513 only for methanogenic but also for methanotrophic communities. The surface layers
514 clustered together and the respective community compositions of aerobic methane
515 oxidizers were significantly different from those detected in layers closer to the
516 permafrost table. In general, the active layer is a heterogeneous habitat in which biotic
517 and abiotic factors, such as quantity and quality of soil organic matter, pH, soil
518 temperature etc. vary along the soil profile (Fiedler *et al.*, 2004, Wagner *et al.*, 2005).
519 Microbial communities close to the surface undergo large diurnal and seasonal
520 temperature variations, influenced in the summer mainly by air temperature and solar
521 radiation. The layers closer to the permafrost table, however, only vary by a few degrees
522 and generally remain around 0 °C. Microorganisms close to the permafrost table are
523 therefore more likely to be adapted to stable, cold *in situ* temperature as previously
524 observed with methane-cycling communities in Sibera (Wagner *et al.*, 2003, Liebner *et*
525 *al.*, 2007).
526
527 **Conclusion**
528
529 This study provides first insights into the methane-cycling microbial communities in a
530 West Canadian permafrost soil on Herschel Island. We identified a methanotrophic
531 community different from what was reported so far for Arctic tundra soils both in terms

532 of community structure and potential activity. Comparative sequence analysis of
533 uncultivated MOB revealed certain environmental distribution patterns and indicated an
534 preference of specific genotypes to, for example, methane concentration or salinity
535 (Luke *et al.*, 2010). Our results also illustrate that the composition of the MOB
536 community in permafrost affected-soils is strongly influenced by environmental
537 conditions such as low temperature and acidic pH. The community of methanogens was
538 similar in composition to what we know from Arctic wet tundra and this community
539 seems to be more stable in the circum-Arctic. We observed a clear shift from a
540 hydrogenotrophic towards an acetoclastic community approaching the permafrost table.
541 Such as shift though assumed to exist in tundra soils could never be shown so far.
542 Evaluating the results of this study in the scope of other studies from the Arctic, our
543 present picture on circum-Arctic methane-cycling communities must still be considered
544 as incomplete and biased towards the few studies conducted to date. It remains elusive
545 whether methane-cycling communities which are specific to Arctic tundra environments
546 truly exist.
547

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549

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Literature

569 Anisimov OA (2007) Potential feedback of thawing permafrost to the global climate
570 system through methane emission. *Environ Res Lett* **2:** 045016 (7pp)
571 Anisimov OA, Vaughan DG, Callaghan TV, Furgal C, Marchant H, Prowse TD,
572 Vilhjálmsson H & Walsh JE (2007) Polar regions (Arctic and Antarctic). Climate
573 Change 2007: Impacts, Adaptation and Vulnerability. *Contribution of Working*
574 *Group II to the Fourth Assessment Report of the Intergovernmental Panel on*
575 *Climate Change* (Parry ML, Canziani OF, Palutikof JP, van der Linden PJ &
576 Hanson CE, eds), pp. 653-685. Cambridge University Press, Cambridge, UK.
577 Biderre-Petit C, Jézéquel D, Dugat-Bony E, Lopes F, Kuever J, Borrel G, Viollier E,
578 Fonty G & Peyret P (2011) Identification of microbial communities involved in the
579 methane cycle of a freshwater meromictic lake. *FEMS Microbiol Ecol* **77:** 533-545.
580 Bräuer SL, Cadillo-Quiroz H, Ward RJ, Yavitt JB & Zinder SH (2011) *Methanoregula*
581 *boonei* gen. nov., sp. nov., an acidiphilic methanogen isolated from an acidic peat
582 bog. *Int J Syst Evol Microbiol* **61:** 45-52.
583 Burn CR & Zhang Y (2009) Permafrost and climate change at Herschel Island
584 (Qikiqtaruq), Yukon Territory, Canada. *J Geophys Res* **114:** F02001 16pp.
585 doi:10.1029/2008JF001087
586 Cadillo-Quiroz H, Yavitt JB & Zinder S H (2009) *Methanosphaerula palustris* gen.
587 nov., sp. nov., a hydrogenotrophic methanogen isolated from a minerotrophic fen
588 peatland. *Int J Syst Evol Microbiol* **59:** 928–935.
589 Callaghan TV, Björn LO, Chapin FS III, Chernov Y, Christensen TR, Huntley B, Ims
590 R, Johansson M, Riedlinger DJ, Jonasson S, Matveyeva N, Oechel WC, Panikov N
591 & Shaver GR (2005) Arctic tundra and polar desert ecosystems. *Arctic Climate*

592 *Impact Assessment* (C. Symon, L. Arris & B. Heal, eds), pp. 243–352. Cambridge
593 University Press, Cambridge.
594 Cao M, Gregson K & Marshall S (1998) Global methane emission from wetlands and
595 its sensitivity to climate change. *Atmos Environ* **32:** 3293-3299.
596 Chanton JP, Bauer JE, Glaser PA, Siegel DI, Kelley CA, Tyler SC, Romanowicz EH &
597 Lazrus A (1995) Radiocarbon evidence for the substrates supporting methane
598 formation within northern Minnesota peatlands. *Geochim Cosmochim Ac* **59:** 3663-
599 3668.
600 Chen Y, Dumont MG, Cébron A & Murrell JC (2007) Identification of active
601 methanotrophs in landfill cover soil through detection of expression of 16S rRNA
602 and functional genes. *Environ Microbiol* **9:** 2855-2869
603 Chen Y, Dumont MG, McNamara NP, Chamberlain PM, Bodrossy L, Stralis-Pavese N
604 & Murrell JC (2008) Diversity of the active methanotrophic community in acidic
605 peatlands as assessed by mRNA and SIP-PLFA analyses. *Environ Microbiol* **10:**
606 446-459.
607 Christensen N, Bartuska A, Brown J *et al.* (1996) The report of the Ecological Society
608 of America Committee on the scientific basis for ecosystem management. *Ecol*
609 *Appl* **6:** 665-691.
610 Christensen TR, Ekberg A, Ström L *et al.* (2003) Factors controlling large scale
611 variations in methane emissions from wetlands. *Geophys Res Lett* **30:** 1414.
612 Conrad R, Schütz H & Babbel M (1987) Temperature limitation of hydrogen turnover
613 and methanogenesis in anoxic paddy soil. *FEMS Microbiol Ecol* **45:** 281-289.
614 Costello AM & Lidstrom ME (1999) Molecular Characterization of Functional and
615 Phylogenetic Genes from Natural Populations of Methanotrophs in Lake
616 Sediments. *Appl Environ Microb* **65:** 5066-5074.

617 Davidson EA & Janssens IA (2006) Temperature sensitivity of soil carbon
618 decomposition and feedbacks to climate change. *Nature* **440:** 165.
619 Dedysh SN, Berestovskaya YY, Vasylieva LV, Belova SE, Khmelenina VN, Suzina
620 NE, Trotsenko YA, Liesack W & Zavarzin GA (2004) *Methylocella*
621 *tundrae* sp. nov., a novel methanotrophic bacterium from acidic peatlands of
622 tundra, *Int J Syst Evol Microbiol* **54:** 151-156.
623 Dedysh S (2009) Exploring methanotroph diversity in acidic northern wetlands:
624 Molecular and cultivation-based studies. *Microbiology* **78:** 655-669.
625 Degelmann DM, Borken W, Drake HL & Kolb S (2010) Different atmospheric
626 methane-oxidizing communities in European beech and Norway spruce soils. *Appl*
627 *Environ Microbiol* **76:** 3228-3235.
628 Dunbar J, Ticknor LO & Kuske CR (2001) Phylogenetic Specificity and reproducibility
629 and new method for analysis of terminal restriction fragment profiles of 16S rRNA
630 genes from bacterial communities. *Appl Environ Microb* **67:** 190-197.
631 Fiedler S, Wagner D, Kutzbach L & Pfeiffer E-M (2004) Element redistribution along
632 hydraulic and redox gradients of low-centered polygons, Lena Delta, Northern
633 Siberia. *Soil Sci Soc Am J* **68:** 1002-1011.
634 Forster P, Ramaswamy V, Artaxo P *et al.* (2007) Changes in atmospheric constituents
635 and in radiative forcing. *Climate Change 2007: the Physical Science Basis.*
636 *Contribution of Working Group I to the Fourth Assessment Report of the*
637 *Intergovernmental Panel on Climate Change* (Solomon S, Qin D, Manning M,
638 Chen Z, Marquis M, Averyt KB & Tignor M, eds), pp. 129-234. Cambridge
639 University Press, Cambridge.
640 Ganzert L, Jurgens G, Münster U & Wagner D (2007) Methanogenic communities in
641 permafrost-affected soils of the Laptev Sea Coast, Siberian Arctic, characterized by
642 16S rRNA gene fingerprints. *FEMS Microbiol Ecol* **59:** 476–488.

643 Graef C, Hestnes AG, Svenning MM & Frenzel P (2011) The active methanotrophic
644 community in a wetland from the High Arctic. *Environ Microbiol Rep* **3:** 466-472.
645 Grosse G, Harden J, Turetsky M, McGuire AD, Camill P, Tarnocai C, Frolking S,
646 Schuur EAG, Jorgenson T, Marchenko S, Romanovsky V, Wickland KP, French N,
647 Waldrop M, Bourgeau-Chavez L & Streigl RG (2011) Vulnerability of high-
648 latitude soil organic carbon in North America to disturbance. *J Geophys Res-Biogeo*
649 **116:** 23 pp.
650 Gruber S, Hoelzle M & Haeberli W (2004) Permafrost thaw and destabilization of
651 Alpine rock walls in the hot summer of 2003. *Geophys Res Lett* **31:** L13504
652 Hoffmann T, Horz H-P, Kemnitz D & Conrad R (2002) Diversity of the particulate
653 methane monooxygenase gene in methanotrophic samples from different rice field
654 soils in china and the philippines. *Syst Appl Microbiol* **25:** 267-274.
655 Høj L, Olsen RA & Torsvik VL (2005) Archaeal communities in High Arctic wetlands
656 at Spitsbergen, Norway (78°N) as characterized by 16S rRNA gene fingerprinting.
657 *FEMS Microbiol Ecol* **53:** 89-101.
658 Høj L, Rusten M, Haugen LE, Olsen RA & Torsvik VL (2006) Effects of water regime
659 on archaeal community composition in Arctic soils. *Environ Microbiol* **8:** 984-996.
660 Høj L, Olsen RA & Torsvik VL (2008) Effects of temperature on the diversity and
661 community structure of known methanogenic groups and other archaea in high
662 Arctic peat. *ISME J* **2:** 37-48.
663 Holmes AJ, Roslev P, McDonald IR, Iversen N, Henriksen K & Murrell JC (1999)
664 Characterization of methanotrophic bacterial populations in soils showing
665 atmospheric methane uptake. *Appl Environ Microbiol* **65:** 3312-3318.
666 Horz H-P, Rich V, Avrahami S & Bohannan BJM (2005) Methane-oxidizing bacteria in
667 a california upland grassland soil: diversity and response to simulated global
668 change. *Appl Environ Microbiol* **71:** 2642-2652.

669 Hunger S, Schmidt O, Hilgarth M, Horn MA, Kolb S, Conrad R & Drake HL (2011)
670 Competing formate- and carbon dioxideutilizing prokaryotes in an anoxic methane-
671 emitting fen soil. *Appl Environ Microbiol* **77:** 3773-3785.
672 Hutchens, E., Radajewski, S., Dumont, M.G., McDonald, I.R., and Murrell, J.C. (2004)
673 Analysis of methanotrophic bacteria in Movile Cave by stable isotope probing.
674 *Environ Microbiol* **6:** 111–120
675 Jaatinen K, Tuittila ES, Laine J, Yrjälä K & Fritze H (2005) Methane-oxidizing bacteria
676 in a finnish raised mire complex: effects of site fertility and drainage. *Microb Ecol*
677 **50:** 429-439.
678 Joabsson A & Christensen TR (2001) Methane emissions from wetlands and their
679 relationship with vascular plants: an Arctic example. *Glob Change Biol*, **7:** 919–
680 932.
681 Juottonen H, Galand PE, Tuittila ES, Laine J, Fritze H & Yrjälä K (2005) Methanogen
682 communities and *Bacteria* along an ecohydrological gradient in a northern raised
683 bog complex. *Environ Microbiol* **7:** 1547-1557.
684 Kalyuzhnaya MG, Makutina VA, Rusakova TG, Nikitin DV, Khmelenina VN, Dmitriev
685 VV & Trotsenko YA (2002) Methanotrophic communities in the soils of the
686 Russian northern taiga and subarctic tundra. *Microbiology* **72:** 264-271.
687 Knoblauch C, Zimmermann U, Blumenberg M, Michaelis W & Pfeiffer EM (2008)
688 Methane turnover and temperature response of methane-oxidizing bacteria in
689 permafrost-affected soils of northeast Siberia. *Soil Biol Biochem* **40:** 3004-3013.
690 Kobabe S, Wagner D & Pfeiffer E-M (2004) Characterisation of microbial community
691 composition of a Siberian tundra soil by fluorescence in situ hybridisation. *FEMS*
692 *Microbiol Ecol* **50:** 13–23.

693 Kotsyurbenko OR (2005) Trophic interactions in the methanogenic microbial
694 community of lowtemperature terrestrial ecosystems. *FEMS Microbiol Ecol* **53:** 3-
695 13.
696 Krause S, Lüke C & Frenzel P (2009) Spatial heterogeneity of methanotrophs: a
697 geostatistical analysis of *pmoA*-based T-RFLP patterns in a paddy soil. *Environ*
698 *Microbiol Rep* **1:** 393-397.
699 Krumholz LR, Hollenback JL, Roskes SJ & Ringelberg DB (1995) Methanogenesis and
700 methanotrohpy within a Sphagnum peatland. *FEMS Microbiol Ecol* **18:** 215-224.
701 Laanbroek HJ (2010) Methane emission from natural wetlands: interplay between
702 emergent macrophytes and soil microbial processes. A mini-review. *Ann Bot-*
703 *London* **105:** 141-153.
704 Le Mer J & Roger P (2001) Production, oxidation, emission and consumption of
705 methane by soils: A review. *Eur J Soil Biol* **37:** 25-50.
706 Liebner S, Harder J & Wagner D (2008) Bacterial diversity and community structure in
707 polygonal tundra soils from Samoylov Island, Lena Delta, Siberia. *Int Microbiol*
708 **11:** 195–202.
709 Liebner S, Rublack K, Stuehrmann T & Wagner D (2009) Diversity of aerobic
710 methanotrophic bacteria in a permafrost active layer soil of the Lena Delta, Siberia.
711 *Microb Ecol* **57:** 25-35.
712 Liebner S & Wagner D (2007) Abundance, distribution and potential activity of
713 methane oxidising bacteria in permafrost soils from the Lena Delta, Siberia.
714 *Environ Microbiol* **9:** 107-117
715 Liebner S & Wagner D (2010) Permafrost – Current and future challenges to study
716 methanotrophy in permafrost affected tundra and wetlands. *Handbook of*
717 *Hydrocarbon and Lipid Microbiology* (Timmis KN, ed), pp 2173-2179. Springer-
718 Verlag, Berlin Heidelberg, Germany.

719 Lin J-L, Joye SB, Scholten JCM, Schafer H, McDonald IR & Murrell JC (2005)
720 Analysis of methane monooxygenase genes in Mono Lake suggests that increased
721 methane oxidation activity may correlate with a change in methanotroph
722 community structure. *Appl Environ Microbiol* **71:** 6458-6462.
723 Ludwig W, Strunk O, Westram R *et al.* (2004) ARB: a software environment for
724 sequence data. *Nucleic Acids Res* **32:** 1363-1371.
725 Luke C & Frenzel P (2011) The potential of *pmoA* amplicon pyrosequencing for
726 methanotroph diversity studies. *Appl Environ Microbiol* 05355-05311.
727 Lüke C, Krause S, Cavigiolo S, Greppi D, Lupotto E & Frenzel P (2010) The
728 biogeography of wetland rice methanotrophs. *Environ Microbiol* **12:** 862-872.
729 Luton PE, Wayne JM, Sharp RJ & Riley PW (2002) The *mcrA* gene as an alternative to
730 16S rRNA in the phylogenetic analysis of methanogen populations in landfill.
731 *Microbiology* **148:** 3521-3530.
732 Martineau C, Whyte LG & Greer CW (2010) Stable isotope probing analysis of the
733 diversity and activity of methanotrophic bacteria in soils from the Canadian High
734 Arctic. *Appl Environ Microbiol* **76:** 5773-5784.
735 McDonald IR & Murrell JC (1997) The particulate methane monooxygenase gene
736 *pmoA* and its use as a functional gene probe for methanotrophs. *Fems Microbiol*
737 *Lett* **156:** 205-210.
738 McDonald IR, Bodrossy L, Chen Y & Murrell JC (2008) Molecular Ecology
739 Techniques for the Study of Aerobic Methanotrophs. *Appl Environ Microbiol* **74:**
740 1305-1315.
741 McGuire AD, Anderson LG, Christensen TR, Dallimore S, Guo L, Hayes DJ, Heimann
742 M, Lorenson TD, Macdonald RW &. Roulet N (2009) Sensitivity of the carbon
743 cycle in the Arctic to climate change. *Ecol Monogr* **79:** 523-555.

744 Metje M & Frenzel P (2007) Methanogenesis and methanogenic pathways in a peat
745 from subarctic permafrost. *Environ Microbiol* **9:** 954-964
746 Milferstedt K, Youngblut ND & Whitaker RJ (2010) Spatial structure and persistence of
747 methanogen populations in humic bog lakes. *ISME J* **4:** 764-776.
748 Natali SM, Schuur EAG, Trucco C, Pries CEH, Crummer KG & Lopez AFB (2011)
749 Effects of experimental warming of air, soil and permafrost on carbon balance in
750 Alaskan tundra. *Glob Change Biol* **17:** 1394-1407.
751 Narihiro T, Hori T, NagataO, Hoshino T, Yumoto I & Kamagata Y (2011) The impact
752 of aridification and vegetation type on changes in the community structure of
753 methane-cycling microorganisms in Japanese wetland soils. *Biosci Biotechnol*
754 *Biochem* **75:** 1727-1734.
755 Nelson FE, Anisimov OA & Shiklomanov NI (2001) Subsidence risk from thawing
756 permafrost. *Nature* **410:** 889-890.
757 Nozhevnikova AN, Kotsyurbenko OR & Simankova MV (1994) Acetogenesis at low
758 temperature. *Acetogenesis* (Drake HL, ed), pp. 416-431. Chapman & Hall, London,
759 UK.
760 Oremland RS & Culbertson CW (1992) Importance of methane-oxidizing bacteria in
761 the methane budget as revealed by the use of a specific inhibitor. *Nature* **356:** 421-
762 423.
763 Pacheco-Oliver M, McDonald IR, Groleau D, Murrell JC & Miguez CB (2002)
764 Detection of methanotrophs with highly divergent *pmoA* genes from Arctic soils.
765 *Fems Microbiol Lett* **209:** 313-319.
766 Pester M, Friedrich MW, Schink B & Brune A (2004) *pmoA*-Based analysis of
767 methanotrophs in a littoral lake sediment reveals a diverse and stable community in
768 a dynamic environment. *Appl Environ Microbiol* **70:** 3138-3142.

769 Popp TJ, Chanton JP, Whiting GJ & Grant N (2000) Evaluation of methane oxidation in
770 the rhizosphere of *Carex* dominated fen in north central Alberta, Canada.
771 *Biogeochemistry* **51:** 259-281.
772 Reeburgh WS, King, JY, Regli S K, Kling GW, Auerbach N A & Walker DA (1998) A
773 CH_4 emission estimate for the Kuparuk River basin, Alaska. *J Geophys Res* **103:**
774 29005-29014.
775 Saitou N & Nei N (1987). The neighbour-joining method - a new method for
776 constructing phylogenetic trees. *Mol Biol Evol* **4:** 406-425.
777 Schloss PD & Handelsman J (2005) Introducing DOTUR, a computer program for
778 defining operational taxonomic units and estimating species richness. *Appl Environ*
779 *Microbiol* **71:** 1501-1506.
780 Semrau J, Chistoserdov A, Lebron J *et al.* (1995) Particulate methane monooxygenase
781 genes in methanotrophs. *J Bacteriol* **177:** 3071-3079.
782 Siljanen HMP, Saari A, Krause S *et al.* (2011) Hydrology is reflected in the functioning
783 and community composition of methanotrophs in the littoral wetland of a boreal
784 lake. *FEMS Microbiol Ecol* **75:** 430-445.
785 Steinberg LM & Regan JM (2008) Phylogenetic comparison of the methanogenic
786 communities from an acidic, oligotrophic fen and an anaerobic digester treating
787 municipal wastewater sludge. *Appl Environ Microbiol* **74:** 6663-6671.
788 Ström L, Ekberg A, Mastepanov M & Christensen TR (2003) The effect of vascular
789 plants on carbon turnover and methane emissions from a tundra wetland. *Global*
790 *Change Biol* **9:** 1185-1192.
791 Tamura K, Peterson D, Peterson N, Stecher G, Nei M & Kumar S (2011) MEGA5:
792 Molecular Evolutionary Genetics Analysis using Maximum Likelihood,
793 Evolutionary Distance, and Maximum Parsimony Methods. *Mol Biol Evol* **28:**
794 2731-2739.

795 Tarnocai C, Canadell JG, Schuur EAG, Kuhry P, Mazhitova G & Zimov S (2009) Soil
796 organic carbon pools in the northern circumpolar permafrost region. *Global*
797 *Biogeochem Cy* **23:** 1-11
798 Thauer RK (1998) Biochemistry of methanogenesis: a tribute to Marjory Stephenson
799 *Microbiology* **144:** 2377-2406.
800 Theisen AR & Murrell JC (2005) Facultative methanotrophs revisited. *J Bacteriol* **187:**
801 4303-4305.
802 Trotsenko Y & Khmelenina V (2002) Biology of extremophilic and extremotolerant
803 methanotrophs. *Arch Microbiol* **177:** 123.
804 van Everdingen R O (ed) (2005) Multi-Language Glossary of Permafrost
805 and Related Ground-Ice Terms, *International Permafrost Association*,
806 University of Calgary, Calgary, Canada. (Available at http://nsidc.org/
807 fgdc/glossary)
808 Wagner D, Kobabe S, Pfeiffer EM & Hubberten HW (2003) Microbial controls on
809 methane fluxes from a polygonal tundra of the Lena Delta, Siberia. *Permafrost*
810 *Periglac* **14:** 173-185.
811 Wagner D, Gattinger A, Embacher A, Pfeiffer EM, Schloter M & Lipski A (2007)
812 Methanogenic activity and biomass in Holocene permafrost deposits of the Lena
813 Delta, Siberian Arctic and its implication for the global methane budge. *Glob*
814 *Change Biol* **13:** 1089-1099.
815 Wagner D & Liebner S (2009) Global warming and carbon dynamics in permafrost
816 soils: methane production and oxidation. *Permafrost Soils, Vol. 16* (Margesin R,
817 ed), pp. 219-236. Springer, Berlin, Heidelberg.
818 Wagner D & Liebner S (2010) Methanogenesis in arctic permafrost habitats. *Handbook*
819 *of Hydrocarbon and Lipid Microbiology* (Timmis KN, ed), pp. 666-663. Springer-
820 Verlag, Berlin Heidelberg, Germany.

821 Wagner D & Pfeiffer EM (1997) Two temperature optima of methane production in a
822 typical soil of the Elbe river marshland. *FEMS Microbiol Ecol* **22:** 145-153.
823 Wartiainen I, Hestnes AG & Svenning MM (2003) Methanotrophic diversity in high
824 arctic wetlands on the islands of svalbard (Norway) - denaturing gradient gel
825 electrophoresis analysis of soil DNA and enrichment cultures. *Can J Microbiol* **49:**
826 602-612.
827 Wartiainen I, Hestnes AG, Mcdonald IR & Svenning MM (2006) *Methylobacter*
828 *tundripaludum* sp. nov., a methane-oxidizing bacterium from arctic wetland soil on
829 the Svalbard islands, Norway (78°N). *Int J Syst Evol Microbiol* **56:** 109–113.
830 Weiss R, Shurpali NJ, Sallantaus T, Laiho R, Laine J & Alm J (2005) Simulation of
831 water table level and peat temperatures in boreal peatlands. *Ecol Model* **192:** 441-
832 456.
833 Whalen SC & Reeburgh WS (1992) Interannual variations in tundra methane emission:
834 a 4-year time series at fixed sites. *Global Biogeochem Cy* **6:** 139-159.
835 Whalen SC, Reeburgh WS & Sandbeck KA (1990) Rapid methane oxidation in a
836 landfill cover soil. *Appl Environ Microbiol* **56:** 3405-3411.
837 Wille C, Kutzbach L, Sachs T, Wagner D & Pfeiffer E-M (2008) Methane emission
838 from Siberian arctic polygonal tundra: eddy covariance measurements and
839 modeling. *Glob Change Biol* **14:** 1395-1408.
840 Yergeau E, Hogues H, Whyte LG & Greer CW (2010) The functional potential of high
841 Arctic permafrost revealed by metagenomic sequencing, qPCR and microarray
842 analyses. *ISME J* **4:** 1206-1214.
843 Yrjälä K, Tuomivirta TT, Juottonen H, Putkinen A,Tuittila ES, Penttil T, Laine J,
844 Peltoniemi K & Fritze H (2011) CH_4 production and oxidation processes in a boreal
845 fen ecosystem after long-term water table drawdown. *Glob Chang Biol* **17:** 1311-
846 1320.

847 Zhuang Q, Melack JM, Zimov S, Walter KM, Butenhoff CL & Khalil MAK (2009)
848 Global methane emissions from wetlands, rice paddies, and lakes. *Trans Am*
849 *Geophys Union (Eos)* **90:** 37-38.
850

851 **Tables & Figures**

852

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

854

Primer	Target gene	Sequence (5'-3')	Annealing T [°C]	Reference
A189f	*pmoA*	GGNGACTGGGACTTCTGG	52	Holmes *et al.*
A682r	*pmoA*	GAASGCNGAGAAGAASGC	52	Holmes *et al.*
mb661r	*pmoA*	CCGGMGCAACGTCYTTACC	56	Costello & Lidstrom
MLf	*mcrA*	GGTGGTGTMGGATTCACACART AYGCWACAGC	50	Luton *et al.*
MLr	*mcrA*	TTCATTGCRTAGTTWGGRTAGTT	50	Luton *et al.*

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856

857 Table 2. Phylogenetic assignment of active layer clones that matched the dominating soil T-RFLP peaks as obtained after comparison to the

858 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

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861

862 **Figure legends**

863 Figure 1. Geographical location of study site (A) in Canada, (B) on the Yukon Coast
864 and (C) Location of the Drained Lake Polygon on Herschel Island.

865

866 Figure 2. Depth profile of abiotic and biotic parameters illustrating (a) active layer
867 temperature as measured *in situ,* (b) soil pH, (c) percentage of water content, (d)
868 percentage of total organic carbon in the soil (TOC), (e) potential methane production
869 rate expressed in nanomol of methane per hour and gram of wet soil at 10 °C with no
870 substrate addition and (f) potential oxidation rate expressed in micromol of methane per
871 hour and gram of wet soil at 10 °C in an atmosphere of 2.5% v v^{-1} methane in synthetic
872 air. Error bars in (e) and (f) represent standard deviations.

873

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

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883

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

891

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