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# Spatial Patterns of Soil Development, Methane Oxidation, and Methanotrophic Diversity along a Receding Glacier Forefield, Southeast Greenland

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## Abstract

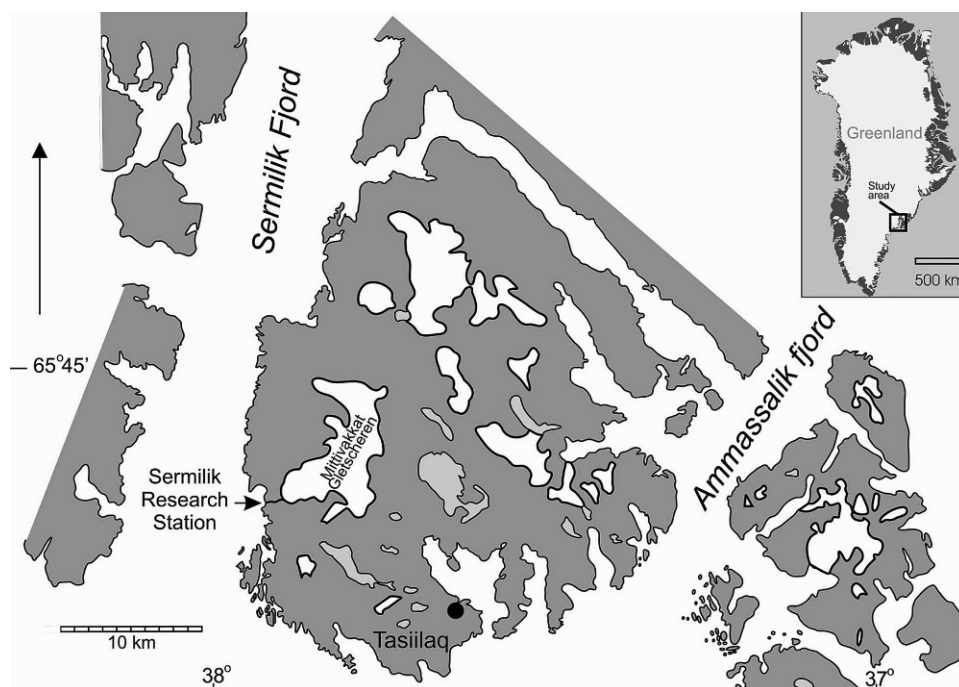
Increasing global annual temperature leads to massive loss of ice cover worldwide. Consequently, glaciers retreat and ice-covered areas become exposed. We report on a study from the Mittivakkat Gletscher forefield in Southeast Greenland with special focus on methanotrophy in relation to exposure time to the atmosphere. The Mittivakkat Gletscher has receded since the end of the Little Ice Age (LIA; about AD 1850) and has left behind a series of deposits of decreasing age concurrently with its recession. Soil samples from this chronosequence were examined in order to elucidate main soil variables, as well as the activity and community structure of methanotrophs, a group of microorganisms involved in regulation of atmospheric methane. Soil variables revealed poor soil development, and incubation experiments showed methane consumption rates of  $2.14 \text{ nmol CH}_4 \text{ day}^{-1} \text{ g}_{\text{soil}}^{-1}$  at  $22^\circ\text{C}$  and  $1.24 \text{ nmol CH}_4 \text{ day}^{-1} \text{ g}_{\text{soil}}^{-1}$  at  $10^\circ\text{C}$  in the LIA terminal moraine. Methane consumption was not detected in younger samples, despite the presence of high-affinity methanotrophs in all samples. This was indicated by successful amplification of partial *pmoA* genes, which code for a subunit of a key enzyme involved in methane oxidation. In addition, the results of the diversity study show that the diversity of the methanotrophic community at the younger, recently deglaciated site P5 is poorer than the diversity of the community retrieved from the LIA moraine. We put forward the hypothesis that aerobic methanotrophs were at very low abundance and diversity during glaciation probably due to anoxia at the ice-sediment interface and that colonization after deglaciation is not completed yet. More detailed studies are required to explain the causes of discrepancy between activity and presence of high-affinity methanotrophs and its relation to the transit from ice-covered probably anoxic to ice-free oxic conditions.

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## Introduction

A consequence of glacier recession on land is the exposure of deglaciated forefields to environmental factors and changing conditions, such as exposure to air, rainfall, and more pronounced temperature changes. This may affect several potential climatic feedback mechanisms: (1) the change in albedo (solar energy reflection), due to a darker surface which causes a positive climatic feedback, as it would conserve heat in the system; (2) altered biogeochemical weathering reactions with changed atmospheric  $\text{CO}_2$  and  $\text{O}_2$  sequestration rates; (3) deposition of organic matter as a consequence of plant colonization and soil formation; and (4) changes in microbial community compositions and activity, which could result in altered respiration rates that affect the production or consumption of greenhouse gases such as  $\text{CO}_2$ ,  $\text{CH}_4$  and  $\text{N}_2\text{O}$ . Despite the growing interest in recent years in studying microbial communities in recently deglaciated forelands (Sigler et al., 2002; Sigler and Zeyer, 2002; Tschirko et al., 2003; Bekku et al., 2004; Kaštovská et al., 2005; Kandeler et al., 2006; Nemergut et al., 2006; Bardgett et al., 2007; Hämmerli et al., 2007; Yoshitake et al., 2007; Schmidt et al., 2008; Duc et al., 2009; Lazzaro et al., 2009; Strauss et al., 2009), little is known about production and consumption rates of greenhouse gases in this type of environment. This is especially true for microbial communities involved in the production and consump-

tion of one of the major greenhouse gases, methane. Although the concentration of methane in the atmosphere ( $\sim 1.8 \text{ ppm}$ ) is much lower than the concentration of  $\text{CO}_2$  ( $\sim 380 \text{ ppm}$ ) (IPCC, 2007), its impact on heat storage by atmospheric gases is significant due its 25 times greater capacity to absorb infrared radiation compared to  $\text{CO}_2$ . The major source of methane in the Arctic is the anaerobic degradation of organic matter stored in tundra wetlands (Bloom et al., 2010). The authors report that methane emission from arctic wetlands increased by 30% from 2003 to 2007, due to a temperature rise that increased the turnover rates of biochemical reactions and transformed permafrost into wetlands. In addition, there is substantial evidence in deglaciated forelands for widespread anoxic conditions, which is a precondition for methanogenesis and ultimately methane release (Wadham et al., 2007). Also, recently deglaciated forelands may release  $\text{CH}_4$  that was stored underneath glaciers in the past. These pulses may have an episodic impact on atmospheric  $\text{CH}_4$  concentrations (Wadham et al., 2008). In the context of an increasing release of methane as a consequence of permafrost melting and deglaciation, glacial forefields that increase in area as a consequence of glacial melting and recession may become significant sites for methane consumption. Methane consuming bacteria may find more favorable conditions as glaciers recede, in particular due to facilitated access to oxygen, and may consequently counterbalance some of the stimulated methane emission.



**FIGURE 1. Map of Ammassalik Island, Southeast Greenland.**

In addition to the microbial communities and their activity *per se*, the numerous controlling factors that may affect microbial community composition and activity based on their influence on physical (diffusivity, temperature regime, water content, occurrence of permafrost, time since deglaciation), chemical (pH, organic matter, total C, and N content), biological (colonization rates of lichens, mosses, and vascular plants) and sedimentological (mineralogy, texture) processes have only partly been resolved.

Interestingly, glacier forefields represent unique natural chronosequences with the oldest till deposits furthest away from the present glacier terminus. The low proglacial terrain between moraines may work as a sink for glaciofluvial sediments, but during glacier recession sediment supply becomes reduced and the location of meltwater portals may shift rapidly, both of which may lead to stream incision into proglacial outwash sediments (e.g. Benn et al., 2003). Ultimately, this process leads to formation of a set of degradational terraces where the highest terraces are the oldest. These settings, as they are found in the Mittivakkat Gletscher valley in Southeast Greenland, provide excellent opportunities to study the microbial community distribution in an environment where the geomorphological history is relatively easy to reconstruct.

The aim of this study is to examine the diversity of methanotrophic microbial communities and their activity in a deglaciated foreland, where they are established in deposits of different maturity stages. Investigating this group of microorganisms provides a better understanding of the pioneer processes occurring in these environments, their magnitude and development, and the way microbial adaptations occur during changing environmental conditions. In addition, methanotrophs represent a highly specialized group of bacteria using methane as a carbon and energy source, and they are found in widely distributed ecosystems (Dedysh et al., 1998; Trotsenko and Khmelenina, 2005). On a global perspective, methanotrophs represent a net sink of CH<sub>4</sub> able to consume approximately 20 to 60 Tg of CH<sub>4</sub> per year (King, 1997; Holmes et al., 1999) and thus play a very important role in the regulation of concentrations of methane, one

of the major greenhouse gases in the atmosphere. Hitherto, studies in Greenland and other arctic regions have focused on methane emission due to permafrost melting (Wagner et al., 2005; Liebner and Wagner, 2007; Mastepanov et al., 2008; Kirpotin et al., 2009). To the best of our knowledge this is the first study that examines methane consumption in a recently deglaciated forefield in Greenland. We hypothesize that deglaciated forefields may act as sinks for greenhouse gases like methane and consequently counterbalance some of the effects that result from an increased emission from melting permafrost regions.

### Study Area

Since the end of the Little Ice Age (LIA) (about AD 1850), local glaciers surrounding the Greenland Ice Sheet have experienced significant recession, whereas the part of the ice sheet margin that terminates on land has remained stationary (Kelly and Lowell, 2009).

The Mittivakkat Gletscher (65°42'N, 31°48'W), an ice cap (31 km<sup>2</sup>) on Ammassalik Island in Southeast Greenland (Fig. 1) exemplifies this observation in an excellent manner and was therefore selected as a natural laboratory where the abiotic (glacial-driven weathering) and biotic (evolution of microbial and other biological communities) consequences of glacial recession could be monitored as changes occurred.

The low arctic climate in the area of study is characterized by a mean annual precipitation of 984 mm (AD 1961–1990) and a mean annual air temperature (MAAT) of −1.7 °C (1961–1990), but in the last decade the MAAT has increased to about 0.0 °C (Cappelen, 2009a). The mean monthly air temperature (1961–1990) ranges from −8.1 °C in March to 6.4 °C in July (Cappelen, 2009b). There are no indications of contemporary permafrost at low altitudes. Vegetation cover is sparse and dominated by a dwarf shrub heath habitat with dwarf birch, greyleaf willow, and fellfield communities based on cushion plants (Jakobsen et al., 2008) as well as the abundant presence of lichens. The geology of Ammassalik Island mainly consists of Archean granite gneiss (Bridgwater, 1976).



TABLE 1

Sample site characteristics and estimated age range of the chronosequence.

Sample no.	Distance from reference (m)	Sediment types	Age (AD)
P1	Outermost moraine ridge (0)	Till	1850–1900
P2	180	Glaciofluvial	↓ 1920s
P3	250	Glaciofluvial	
P4	370	Glaciofluvial	
P5	500	Till	1920s

### GLACIER RECESSION HISTORY

The study site is located in a proglacial valley in front of the main outlet glacier descending from the ice cap. Five sampling sites were selected that were representative along a chronosequence transect where glacier recession has occurred. Based on the receding trend shown by the glacier since the LIA, the following deglaciation history can be deduced (Table 1, Fig. 2): (1) P1: outermost moraine–till. During the culmination of the LIA (AD 1650–1850), the outermost moraine was formed. It consists of two aligned segments with both angular and rounded boulders. It is unknown when the glacier started to recede from the outermost moraine, but it most likely occurred between 1850 and 1900. The remaining four sample sites were all deposited after the formation of the outermost moraine. (2) P2: glaciofluvial deposit. Glacier recession resulted in the formation of a degradational glaciofluvial terrace, which is lower than the outwash in front of the outermost moraine. (3) P3: glaciofluvial deposit. Further glacier recession resulted in incision of the river forming a terrace lower than the terrace related to P2. (4) P4: glaciofluvial deposit. Additional glacier recession formed a lower terrace, which is covered by a bryophyte mat and consists of relatively few boulders. (5) P5: till. The last sample corresponds to an inner moraine system, mainly consisting of large boulders, which most likely represents a short period of stillstand or a minor advance event, which occurred prior to the abrupt MAAT increase of 1.5 °C during the 1920s. The meltwater portal at the inner moraine is associated with a glaciofluvial terrace that is lower than the P4 terrace. The first photograph of the Mittivakkat Gletscher shows that in 1933 the glacier terminus had retreated additionally 50–60 m from the inner moraine (Fristrup, 1970). This geomorphological evolution with successive lowering of glaciofluvial terraces during glacier recession and formation of boulder-rich moraines during short periods of glacier stillstand or minor re-advance continues all the way to the present glacier terminus. The approximate distances

between the outermost moraine (P1) and the other sampling sites are 180 m to P2, 250 m to P3, 370 m to P4, and 500 m to P5 samples (the samples' nomenclature corresponds to the distance from the outermost moraine).

## Materials and Methods

### SAMPLE COLLECTION

Sediment samples were collected during a fieldwork campaign in August 2008. During sampling, the top 3–5 cm were removed since it consisted of gravel and plant material, which are not relevant for the analysis of the fine material (<2 mm). About 1 kg of the upper soil (5–10 cm depth) was collected from an area covering approximately 0.5 m<sup>2</sup> at each of the five sampling sites (P1–P5), and stored in darkness at 4 °C in soil sampling–adequate plastic bags (LDPE model, Norlip A/S, Denmark) until further analysis. Plant roots and stones were removed from each sample by handpicking prior to further analysis. Subsamples from each site were used for pH measurements, particle size analysis, and soil organic carbon and total nitrogen measurements. Four subsamples from each site were used for high-affinity methane oxidation analysis.

### SOIL ANALYSIS

#### Sample Pretreatment

Soil samples that were used for physical and chemical analyses were dried at 60 °C for 48 hours, until they reached constant weight. Samples were then sieved through a 2 mm sieve, and approximately 50 g of each sample was retained for grinding in a Disc Mill RS 200 equipped with a wolfram-carbide mortar (Retsch GmbH, Haan, Germany), which is a necessary pretreatment before measuring total organic carbon and total nitrogen. The weight of hygroscopic water was determined from the samples by drying an approximately 15 g sample at 110 °C for 16 hours (Nørnberg and Dalsgaard, 2005). Before further calculations, the sample weights were corrected for hygroscopic water content.

As a preparation for particle size analyses, complete oxidation of organic matter in the samples is required. For this purpose the samples were kept in 6% peroxide (H<sub>2</sub>O<sub>2</sub>) solution for six days while heated in sand baths. By adding 6% H<sub>2</sub>O<sub>2</sub> to a subsample under a microscope it was determined that complete oxidation had occurred. Then, the fraction of each sample less than 2 mm was sieved again with 250-μm and 63-μm sieves, and finally the fraction under 63 μm was stored in a 0.002M sodium pyrophosphate solution to avoid flocculation.

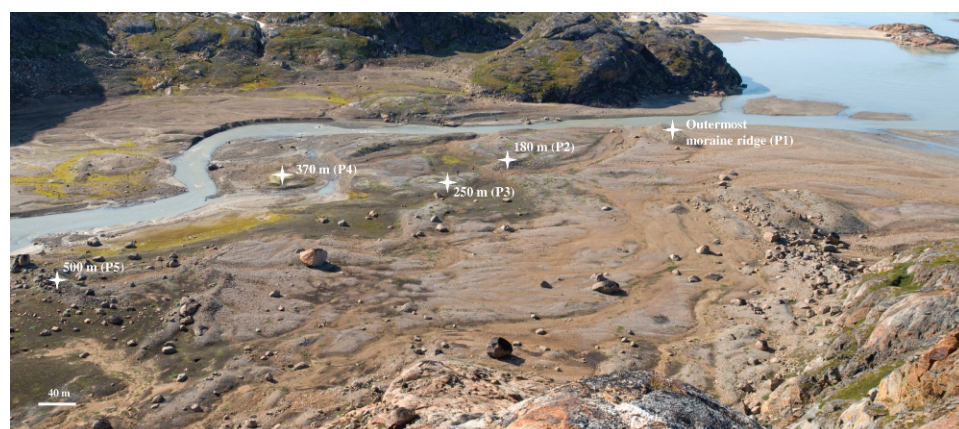


FIGURE 2. Overview of the sampling locations in the proglacial Mittivakkat Valley looking southwest. The present glacier front is located 1 km east from the innermost site (P5). Photo: Ebbe N. Bak.

**TABLE 2**  
**Main soil parameters.**

	P1 (Outermost moraine ridge)	P2 (180 m)	P3 (250 m)	P4 (370 m)	P5 (500 m)
<b>pH</b>					
<b>dH<sub>2</sub>O</b>	5.65	5.16	5.67	5.58	5.54
<b>KCl</b>	4.34	4.18	4.31	4.33	4.25
<b>Sand (%)</b>	80.7	77.2	85.2	80.9	89.5
<b>Silt (%)</b>	15.7	18.0	10.1	13.7	8.5
<b>Clay (%)</b>	3.6	4.8	4.7	5.4	2.0
<b>C (%)</b>	0.199 (0.001)*	0.188 (0.004)*	0.166 (0.002)*	0.128 (0.007)*	0.153 (0.002)*
<b>N (%)</b>	0.017 (0.000)*	0.013 (0.000)*	0.012 (0.004)*	0.010 (0.002)*	0.009 (0.001)*
<b>C:N</b>	11.9	14.4	14.1	12.5	17.3

\* For % C and % N, values are means based ( $\pm$ SE, calculated as  $SD/\sqrt{n}$ ,  $n = 2$ ).

### *Analysis of Soil Variables*

For particle size determination, the three fractions obtained from each pretreated subsample were measured on a particle size analyzer based on a HELOS laser diffraction sensor equipped with the QUIXEL dispersion unit (Sympatec GmbH, Clausthal-Zellerfeld, Germany). Results were then visualized and processed with the WINDOX 5 software package (Sympatec GmbH, Clausthal-Zellerfeld, Germany).

Soil pH was analyzed with a pHM 64 Research pH meter (Radiometer Copenhagen, Brønshøj, Denmark) on two subsamples from each site; one measured in distilled water (w/w) and the other in 1M KCl (w/w), to give a better description of the actual and potential acidity of the material (Table 2). The estimated error with this procedure is 2% of the measured pH value.

Total organic carbon was analyzed by a dry combustion procedure, followed by a gravimetric determination of the developed amount of CO<sub>2</sub> (Nørnberg and Dalsgaard, 2005). Measurements were based on duplicates from each sample site, and standards (110 °C dried sugar samples, from which theoretical values are known) were analyzed at the beginning and end of the procedure to ensure the precision of the analysis.

Total organic nitrogen was analyzed by the Kjeldahl method, using a Kjeltec™ 2300 Auto Distillation Unit (FOSS, Hillerød, Denmark). The analysis is based on the release of nitrogen by wet combustion in concentrated H<sub>2</sub>SO<sub>4</sub> in three steps: digestion, distillation, and titration (Bradstreet, 1954), and, as with carbon, duplicates of each sample were measured.

### *INCUBATION EXPERIMENTS WITH SOIL SAMPLES*

In order to study the potential high-affinity methane oxidation rates of the soil samples, incubation experiments at two different temperatures and atmospheric methane concentrations were performed. For this purpose four 3.5 g soil subsamples (wet weight) from each site were introduced into a 120 mL serum flask and then sealed with a butyl-rubber stopper covered by an aluminum clamp to ensure gas-tight conditions during the experiment. The samples were incubated at room temperature (~22 °C) and at 10 °C (in the temperature range measured during the sampling campaign in August 2008), and headspace methane concentrations were followed over time. The methane concentration of the headspace gas mixture equaled atmospheric methane concentrations (~1.8 ppm), since the purpose of the incubations was, as mentioned before, to determine the potential of high-affinity methane oxidation (i.e., at low methane mixing ratios, corresponding to atmospheric levels).

A time series of gas samples was analyzed from each incubation process up to 70 and 98 hours. Analysis of the CH<sub>4</sub> concentration was performed by gas chromatography on a SRI 310C GC (SRI, Torrance, California, U.S.A.) with a 3' × 1/8" silica gel packed column (column i.d. 8600-PK1A), using helium as the carrier gas with a flow of 6 psi. For methane analysis, the oven temperature was 40 °C and the temperature in the FID detector 155 °C. Gas samples (500 µL) were injected into the GC system and analyzed by simultaneous integration of the peaks in the chromatograph with the Peak Simple 2000 software (SRI, Torrance, California, U.S.A.). Standards in the range of the expected headspace CH<sub>4</sub> concentrations (from 0.2 ppm up to 2.3 ppm, as lowest and highest concentrations, respectively, including a series of 8 standards with intervals of ~0.2 ppm) were injected in the system before and after the measurements to verify their quality and perform calibration.

### *MOLECULAR ANALYSIS*

#### *Soil DNA Extraction*

Approximately 0.5 g of sample was used for DNA extraction with the Fast DNA Spin Kit for Soil (Bio101, La Jolla, California, U.S.A.). The sample material was transferred to a lysing tube containing a mixture of ceramic and silica particles and subjected to vigorous shaking in a beadbeater. Then kit reagents were used to give a complete sample homogenization and protein solubilization, and finally DNA was purified following the GENE-CLEAN procedure (Bio101, La Jolla, California, U.S.A.) obtaining PCR-ready genomic DNA.

#### *Cloning and Sequencing of pmoA Gene PCR Products*

Prior to the microbial diversity study of soil samples from the chronosequence, two pairs of primer sets targeting the *pmoA* gene were used in order to assess the presence of methanotrophs with *pmoA* genes. The main goal of the comparison was to determine which primer set would result in the most efficient *pmoA* amplification. The two primer sets that were used were the pair A189F-mb661R (Costello and Lidstrom, 1999) and the pair A189F-A650R (Bourne et al., 2001). Based on the study of Bourne et al. (2001), the A189F-mb661R PCR primer set tends to amplify a broader range of *pmoA* sequences compared to the primer set A189F-A650R. However, the former primer set apparently does not amplify sequences that code for high-affinity *pmoA* genes, which were successfully amplified with the A189F-A650R primer set. We tested both primer sets using DNA extracts from our five

sampling sites. Amplification of *pmoA* genes with the A189F-mb661R primer set was only successful with DNA extract from the oldest outermost moraine while the A189F-A650R primer set resulted in PCR products in soil samples from all sites, suggesting that a better retrieval of high-affinity methanotrophs was achieved with these primers. Since we were interested in the community that could consume atmospheric methane and since we also wanted to address the methanotrophic communities at more recently deglaciated sites we decided to use exclusively the A189F-A650R primer set.

PCR products obtained with the *pmoA*-specific primer set A189F-A650R (Bourne et al., 2001) for the outermost moraine sample (P1) and the 500 m sample (P5), corresponding to the oldest and youngest investigated deposits, respectively, were used for cloning with the pGEM<sup>®</sup>-T vector system (Promega, Madison, Wisconsin, U.S.A.) in various steps: ligation of the vector with the PCR product, and posterior transformation of the derived vector containing the insert of interest into competent cells of *E. coli* strain JM 109. Subsequently, screening of cells containing the plasmid with the insert was performed. PCR-screening with vector-specific primers was carried out in order to check the size of the inserts. Finally, after growth overnight of cultures of the clones, plasmids were purified with the GenElute<sup>™</sup> Plasmid Miniprep kit (Sigma-Aldrich, St. Louis, Missouri, U.S.A.). Plasmid DNA was sequenced by the MacroGen sequencing service (Seoul, Korea) on both strands using the primers M13f and M13r, which are targeting the flanking region of the insert.

#### Sequence Analysis

Sequences of the targeted region of the *pmoA* gene were compared with GenBank sequences using the NCBI BLAST software package (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>), in order to find regions of similarity with other *pmoA* sequences in the database. Raw sequences were analyzed using the Bioedit software tool (Ilbis Therapeutics, Carlsbad, California, U.S.A.). Vector and primer sequences were removed. The nucleotide sequences were aligned against *pmoA* sequences obtained from the NCBI Nucleotide Sequence Database using the ARB program package (Ludwig et al., 2004). A maximum likelihood tree was constructed with Phylml (DNA) using the default settings of the ARB package. Sequences were deposited into the EMBL<sup>®</sup> database (<https://www.ebi.ac.uk/embl/>) with the following Accession Numbers: clone library P1 (FN651642–FN651658); clone library P5 (FN651807–FN651824).

## Results

### SOIL ANALYSIS

The main soil variables considered in this study were: pH, particle size distribution, soil organic carbon, and total nitrogen as well as the C:N ratio (Table 2). The pH values measured in distilled water indicated the actual acidity of the soil, while the potential acidity value is indicated by the KCl measurement. The pH for KCl is approximately one unit lower than the distilled water value, which is an indication of a low base saturation in the samples.

Particle size distribution in each sampled location refers to clay particles as the fraction smaller than 2 µm, silt as the fraction between 2 µm to 20 µm, and sand particles as the fraction between 20 µm to 2000 µm. The overall particle size distribution pattern corresponds to a loamy sand soil texture type (Berlinger et al., 2001).

Values of soil organic carbon showed a general decrease from the outermost moraine towards the inner part of the valley (Table 2). The 370 m (P4) sample showed the lowest content in soil organic carbon. Differences in nitrogen content were not significant; however, there was a slight decrease from the outer to the inner part of the sampling transect.

### INCUBATION EXPERIMENTS

Methane was consumed at atmospheric levels in the outermost moraine ridge, while the other sampled locations did not show a significant change in CH<sub>4</sub> headspace concentration within the time of incubation. The CH<sub>4</sub> consumption observed in the outermost moraine ridge sample occurred for both incubation temperatures, 22 °C and 10 °C (Figs. 3A and 3B, respectively) and it is following first-order kinetics. A first-order oxidation rate constant (k) was calculated for the outermost moraine sample for both incubation temperatures based on the slope of the linear part of the semilogarithmic plots, and k values were then multiplied by the initial CH<sub>4</sub> concentrations for both experiments in order to obtain initial consumption rates.

Rates of atmospheric methane consumption on the oldest material (outermost moraine ridge) were different between the two incubation experiments, being 2.14 nmol CH<sub>4</sub> day<sup>-1</sup> g<sub>soil</sub><sup>-1</sup> at 22 °C, and 1.24 nmol CH<sub>4</sub> day<sup>-1</sup> g<sub>soil</sub><sup>-1</sup> for the 10 °C incubation.

In the 22 °C incubation experiment, CH<sub>4</sub> concentration continued decreasing regardless of the low headspace concentrations left after three days of incubation, and at the end of the experiment the headspace concentration reached less than 0.5 ppmv.

### DIVERSITY ANALYSIS

#### Analysis of *pmoA* Clone Libraries from Sites P1 and P5

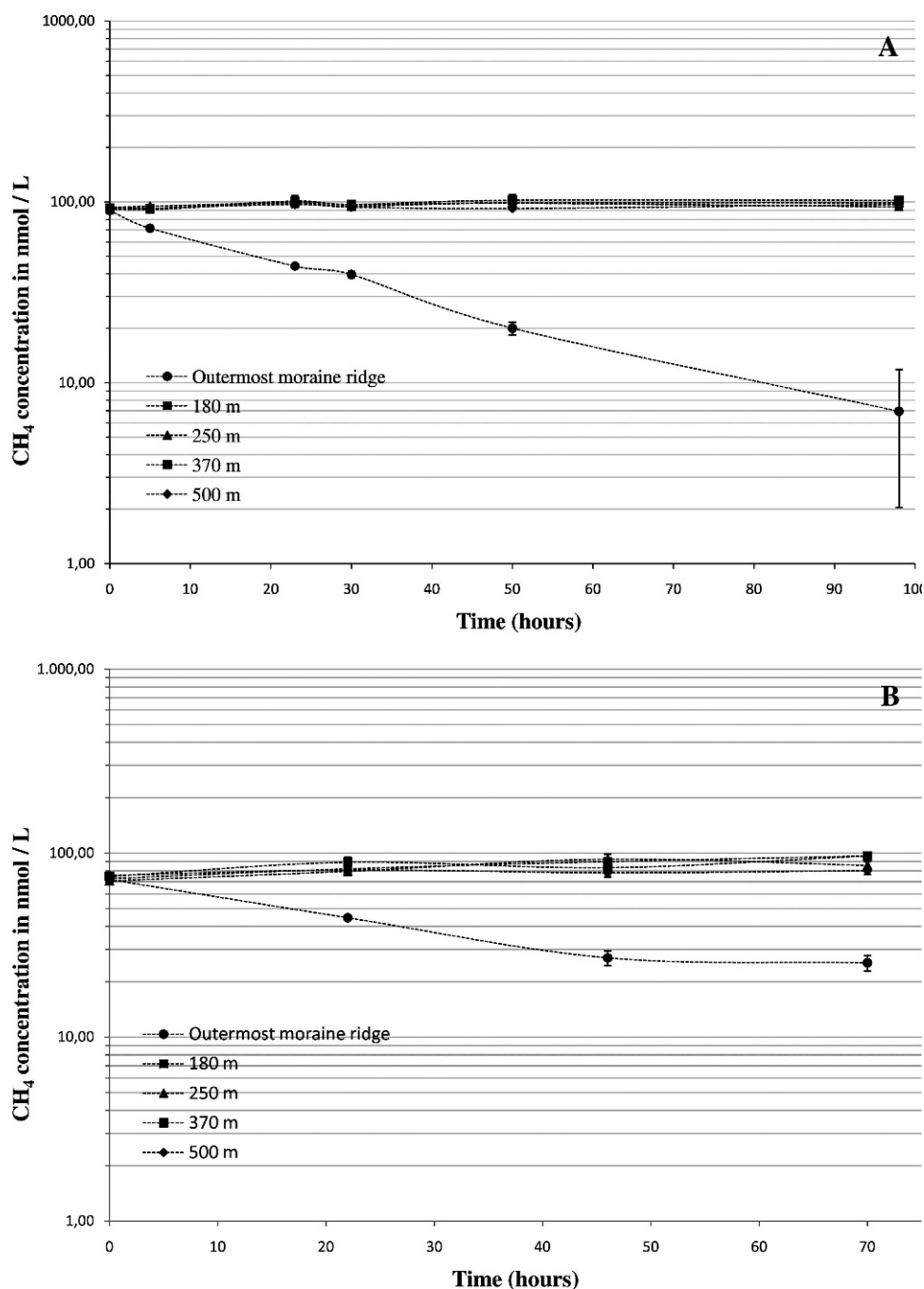
A total of 61 clones were analyzed: 34 clones were obtained from the outermost moraine samples (indexed as P1) and 27 clones from the innermost sample (indexed as P5) (Fig. 4). All clones grouped with type II methanotrophs. The largest fraction of the clones (38 out of 61) formed one distinct cluster of closely related sequences with a maximum within cluster sequences dissimilarity of <3% (Cluster 4). The cluster contained 26 clones from site P5 and 12 clones from site P1. Sequences within this cluster shared <90% sequence similarity with their closest clone relatives deposited in the database. Of the remaining clones, one (G1-251\_p5) was situated on a distinct branch sharing <85% sequence similarity with sequences in the NCBI database, while the remaining clones (all from site P1) form 3 distinct clusters with a within-cluster similarity of >95% and a between cluster similarity of <90%. All clones shared <70% sequence similarity with cultured type II methanotrophs such as *Methylocystis* sp. and *Methylocapsa acidiphila*.

## Discussion

### SOIL DEVELOPMENT

Initial stages of soil formation in the Mittivakkat valley has previously been examined by Jakobsen (1990), who observed a gradual change in soil-forming processes with age. Soil horizons had developed to a depth of 6 cm at the LIA moraine and only reached depths of few centimeters in younger soil profiles. Also, Jakobsen (1990) observed translocation of inorganic Si and Al compounds from the top soil horizons into depths greater than 30 cm. The chronosequence in our study focuses on soil profiles



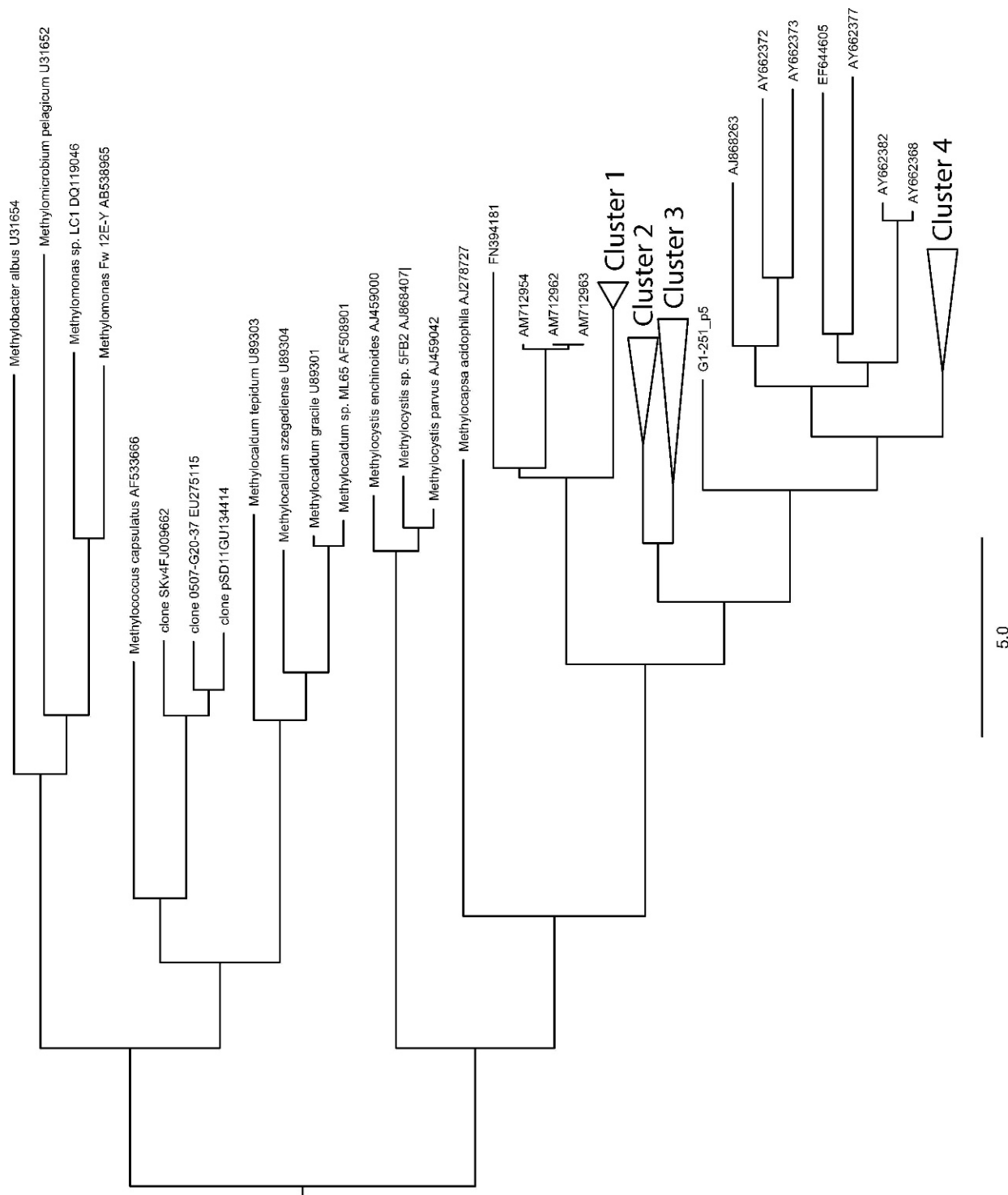


**FIGURE 3.** (A) Semilogarithmic plot of CH<sub>4</sub> headspace concentrations over 98 hours. Incubation temperature 22 °C. Plotted values are means, error bars indicate  $\pm$ SE ( $n = 4$ ). (B) Semilogarithmic plot of CH<sub>4</sub> headspace concentrations over 70 hours. Incubation temperature 10 °C. Plotted values are means, error bars indicate  $\pm$ SE ( $n = 4$ ).

with ages between 1850 and 1920, so the initial podzolization processes observed by Jakobsen (1990) are likely to be present.

The characteristics of the parent material may affect soil properties at various degrees. Soil pH can be strongly influenced by the parent material, e.g. highly calcareous parent material in the Robson Glacier forefield are reflected in high pH values ranging from 7.5 to 8 (Sondheim and Standish, 1983). The Mittivakkat proglacial valley is not affected by calcareous substrates in the parent material, which is reflected in the low pH measured in the chronosequence. Matthews (1992) stated that a decline in pH with time appears to be an almost universal feature for glacier forefield chronosequences, but this is not the case for the Mittivakkat Gletscher forefield, since the outermost moraine ridge presents a very similar pH value to that found at the innermost moraine. This feature could be explained by the fact that very distinct glacial chronosequences are being compared.

Along the Mittivakkat chronosequence, vegetation is very sparse and spread in patches, and in all moraine deposits the upper sediment considered in this study is based on a mineral soil with very poor development contrary to the trends found in chronosequences reported by other authors (Crocker and Major, 1955; Messer, 1988; Matthews, 1992). Other exceptions to this universal trend have recently been reported (Breen and Lévesque, 2008; Strauss et al., 2009), revealing the variability among different glacier forefields. Soil pH decrease with age in a glacial forefield is an expected characteristic observed in forefields where soils reach a relatively mature structure and where vegetation is increasingly present with soil age affecting the pH. For this pattern to be observed, a clear change in vegetation cover, vegetation type, and hence soil development is required, otherwise pH from mineral soils in early stages after glacial retreat will not show a decrease (Crocker and Major, 1955).



**FIGURE 4.** Phylogenetic tree based on partial *pmoA* sequences (A189F–A650R) showing the relation between sequences retrieved from the oldest (P1) and the youngest (P5) moraine within the Mittivakkat Gletscher forefield. The tree was constructed using the maximum likelihood algorithm of the ARB software package. The scale bar represents a 5% sequence divergence.



The texture of the sediment at the five locations showed similar trends, with distributions that were characteristic for a loamy sand deposit. Despite the general similarity of particle size distributions between sites, the innermost moraine (P5) presented almost 10% more sand than the two outermost sites (P1 and P2). This difference in sand content when comparing the two moraines P1 and P5 could indicate that the outermost moraine primarily consists of redeposited glaciofluvial sediments, whereas the innermost moraine mainly comprises sediments from valley-side mass wasting processes. Clay content can be relatively low in glacial forefields, as found in other studied areas (Strauss et al., 2009). The percentage of clay in the 370 m (P4) site as well as in the other glaciofluvial-like deposits (P2 and P3) was slightly higher compared to that found in the moraine sites P1 and P5. This is most likely a consequence of the different genetic processes influencing the deposits; P2, P3, and P4 originated from predominantly fluvial activity, and P1 and P5 were affected by glacial intervention, which will result in finer and coarser textures, respectively. In the case of P4 the clay content could also have been enhanced by eolian deposition due to the dense moss layer covering the site.

Soil organic carbon and total nitrogen levels were low as a result of the relative immaturity of the soils. As a general pattern, Matthews (1992) suggested that in glacier forefields an increase in soil organic carbon with sediment age should be expected, based on an increase in litter residues in more mature terrain. Nitrogen content would also show a similar trend, with rapid increases in early stages of soil development. This is in accordance with the Mittivakkat Gletscher chronosequence, where increases in soil organic carbon content and total nitrogen are observed from the inner moraine system represented by P5 towards the outermost moraine ridge (P1). Soil organic carbon showed the lowest value at the 370 m (P4) site, despite the dense bryophyte mat and thereby the higher amount of litter present compared to the rest of the samples. As suggested by Jonasson et al. (1998), microbial communities in arctic environments cause efficient nutrient immobilization and thereby low availability of these nutrients for the plants. At the same time, high microbial decomposition of soil organic matter would lead to a loss of carbon to the atmosphere, which may not be completely compensated by increased plant carbon fixation, thus contributing to the decreased content in soil organic carbon observed at this site.

#### POTENTIAL METHANE OXIDATION FROM A GLACIER FOREFIELD

The presence of methanotrophic bacteria in various cold environments has been reported by several authors (Dedysh et al., 1998; Trotsenko and Khmelenina, 2005; Wagner et al., 2005; Knoblauch et al., 2008), demonstrating that methanotrophs are able to colonize harsh cryospheric and acidic habitats (Dedysh et al., 1998; Trotsenko and Khmelenina, 2001). In addition, investigations of potential methane oxidation have been performed with tundra and permafrost soils comprising both *in situ* and *in vitro* approaches (Whalen and Reeburgh, 1990; Roslev and Iversen, 1999; Berestovskaya et al., 2001; Liebner and Wagner, 2007). Despite the variety of studies of methanotrophy and methanotrophic bacteria in cold environments, this study is, to our knowledge, the first to address methanotrophy in an arctic glacier forefield. Our results reveal by the retrieval of *pmoA* PCR products that high-affinity methanotrophs are present in soil samples from all the sites that we investigated

along the deglaciation chronosequence of Mittivakkat Gletscher valley. To our surprise, only in a soil sample of the LIA terminal moraine (P1) was the identification of a methanotrophic community accompanied by the ability of the community to oxidize methane at atmospheric concentrations. The discrepancy between the presence of methanotrophs and the absence of activity could be a consequence of the PCR procedure that ideally could identify the presence of a single methanotrophic bacterial cell in the sample (Stepanauskas and Sieracki, 2007), while the activity measurements lack the same degree of sensitivity and thus would not be able to detect and quantify the activity of small populations. We thus hypothesize that the area that has been deglaciated since the LIA harbors methanotrophic bacterial communities but that the population size of these communities is apparently not sufficiently large to account for measurable atmospheric methane oxidation rates. This hypothesis needs to be tested by the application of quantitative molecular methods such as FISH or q-PCR that shed light on the size of the methanotrophic population. It must be emphasized that the molecular methods used in this study do not discern between living and dead organisms and/or metabolically active or non-active microorganisms, and conclusions must therefore be drawn from the two approaches (incubations and clone libraries) independently. A further study based on Stable Isotope Probing (SIP) would be highly suitable to address the question of how time since deglaciation affects metabolically active communities.

*In vitro* experiments showed that regardless of the low pH of the soil, atmospheric methane consumption could be observed over time at room temperature and at 10 °C for the outermost moraine ridge sample (P1), when incubated in the laboratory. Rates ( $2.14 \text{ nmol CH}_4 \text{ g}_{\text{soil}}^{-1} \text{ day}^{-1}$  and  $1.24 \text{ nmol CH}_4 \text{ g}_{\text{soil}}^{-1} \text{ day}^{-1}$  at 22 °C and 10 °C, respectively) were in a range comparable to that observed in other incubation experiments with initial atmospheric headspace concentrations (Table 3). Nevertheless, the rates measured in this study are substantially lower than those observed in other studies of cold environments with higher initial headspace concentrations. Soil slurries from peat areas in Canada showed potential methane oxidation rates ranging from 15 to  $110 \text{ nmol CH}_4 \text{ g}_{\text{peat}}^{-1} \text{ day}^{-1}$  when incubated at different temperatures ranging from 0–35 °C (Dunfield et al., 1992), whereas non-flooded forest soil from the Canadian taiga yielded rates varying from 28 to  $66 \text{ nmol CH}_4 \text{ g}_{\text{soil}}^{-1} \text{ day}^{-1}$  (Jugnia et al., 2006). However, in both experiments the headspace volume was enriched in methane, high above atmospheric levels, increasing the substrate availability and thereby the potential of oxidizing methane. In contrast, the experiments conducted in our study did not include enrichments, since it was our aim to observe the ability of the indigenous methanotrophs to oxidize methane at atmospheric concentrations, thereby simulating *in situ* conditions in terms of substrate availability. On the other hand, *in situ* measurements of the potential of methane oxidation presented an approximately fifty-fold increase in tundra soils, when comparing static chambers with initial  $\text{CH}_4$  concentrations of 1.8 and 500 ppm, respectively (Whalen and Reeburgh, 1990), indicating that *in situ* methane consumption rates based on initial atmospheric concentrations of  $\text{CH}_4$  are expected to be low, presumably due to substrate limitation. The *in vitro* studies reported here will be followed up on by more detailed *in situ* measurements in order to investigate methane fluxes in the moraine system at the forefield and in deposits in closer proximity to the glacier front, thus extending our insight into methanotrophy along the chronosequence of the receding Mittivakkat Gletscher.

**TABLE 3**  
**Methane oxidation rates in different incubation experiments.**

Sample site	Sample characteristics	native pH	CH <sub>4</sub> oxidation rate	Initial CH <sub>4</sub> concentration	Reference
Zackenbergl, Greenland	Heathland	4.3	0.98 nmol g <sup>-1</sup> day <sup>-1</sup>	Atmospheric (~1.8 ppm)	Roslev and Iversen (1999)
Pantanal, Brazil	Rain forest	4.9	0.50 nmol g <sup>-1</sup> day <sup>-1</sup>	Atmospheric (~1.8 ppm)	Roslev and Iversen (1999)
Canadian subarctic	Non-flooded forest soil	—	28–66 nmol g <sup>-1</sup> day <sup>-1</sup>	Enriched headspace	Jugnia et al. (2006)
Hudson Bay lowland, Canada	Peat slurry	—	15–110 nmol g <sup>-1</sup> day <sup>-1</sup>	Enriched headspace	Dunfield et al. (1992)
Mata Atlântica, Brazil	Forested ferrasol	3.6	7.2 10 <sup>-4</sup> nmol g <sup>-1</sup> day <sup>-1</sup>	Atmospheric (~1.8 ppm)	Dörr et al. (2010)
Ammassalik Island, Greenland	Moraine	4.3	2.14 nmol g <sup>-1</sup> day <sup>-1</sup>	Atmospheric (~1.8 ppm)	This study

#### COMPARISON OF THE METHANOTROPHIC DIVERSITY BETWEEN THE LIA AND AN INNER MORaine

Although potential high-affinity methane oxidation was only observed in the outermost moraine ridge, under the same incubation procedures a *pmoA*-positive response using PCR-based *pmoA* gene amplification was found for all deposits, confirming the presence of methanotrophs in all samples. The diversity of the high-affinity methanotrophic community was addressed by cloning and sequencing of *pmoA* genes retrieved from site P1 and P5, respectively. These were representing the different intrinsic histories with respect to ice coverage and exposure. Despite the fact that the clone libraries were relatively small, containing 34 clones from site P1 and 27 clones from site P5, it is evident that the overall diversity among the sequenced *pmoA* gens is limited since 38 out of 61 sequences grouped within the same cluster (cluster 4). The overall distance within this cluster was <3% and considering that the tree was constructed from nucleotide and not from amino acid sequences it could be assumed that the sequences within that cluster were almost identical when it comes to the functional protein. Although the diversity in general is limited, there is a tendency to a larger diversity at the P1 site than at site P5. While 26 out of 27 site P5 *pmoA* sequences are found within the same cluster, only 12 of 34 P1 *pmoA* sequences group together and were found in the same cluster as the P5 sequences. The other P1 *pmoA* sequences group on distinct branches and form 3 additional clusters with no representation of site P5 clones. Thus the clone library results support the view that high-affinity methanotrophic communities had a very restricted diversity during glaciation and that the invasion of new strains is relatively slow. This conclusion is also supported by our observation that no PCR product was obtained when the A189F-mb661R PCR primer set was used. This primer set covers a broader range of *pmoA* genes, but is less efficient in amplifying high-affinity methanotrophic bacteria (Bourne et al., 2001). Thus, the absence of a PCR product when using this primer set was applied provides an independent indication of limited methanotrophic diversity.

All *pmoA* sequences retrieved from sampling sites P1 and P5 were affiliated with sequences clustering among the so-called type II methanotrophs, which belong to the  $\alpha$ -subdivision of the *Proteobacteria*, thus confirming the specificity of the A189F-A650R primer set. Strains belonging to this type are also characterized by their ability to fix nitrogen and to grow at relatively low pH (Graham et al., 1993). Both characteristics would be advantageous in the environment from which the sequences were retrieved. The concentration of fixed nitrogen in the system is very low (Table 2) and would favor microorganisms with the ability to fix nitrogen. At all sites the pH of the soil is acidic (Table 2) with values ranging from pH 5.16 (site P2) to pH 5.67 (site P3).

Interestingly, none of the *pmoA* sequences was closely affiliated to *pmoA* sequences from glacial or other cold/arctic

environments but rather to high-affinity methanotrophs from subarctic (Jaatinen et al., 2004), temperate (Dunfield et al., 1999; Henkel et al., 2000; Bourne et al., 2001; Singh et al., 2009), or even subtropical regions (Dörr et al., 2010). However, the relatively large phylogenetic distance (<90% similarity with closest relative) of the clusters indicates that Mittivakkat Valley methanotrophs belong to distinct lineages of microorganisms that may have specific adaptations to the cold climate at the site of sampling. More intensive cloning efforts as well as physiological studies of methanotrophic isolates from these sites are needed to address this topic in more depth.

The sampling procedure applied in this study was designed with focus on including those study sites which were most relevant for the early LIA deglaciation history of the forefield based on a series of geomorphological units. The subjective selection of discrete points for analyzing chronosequences may, in some cases, be considered insufficient for specific measurements due to spatial bias. To minimize potential biases, the studies by Breen and Lévesque (2008) and Strauss et al. (2009) used sampling approaches with several replicates at different study sites. For more detailed studies of soil variables and their changes along chronosequences it would be recommendable to establish transects where several samples with similar age and distance from the glacier front could be collected, thereby minimizing the risk of spatial bias. However, as the primary objective of this pioneering study was to determine whether deglaciated forefields act as sinks for atmospheric methane, the sampling strategy with using subsamples at each individual sampling site in the chronosequence is considered sufficient, since the selected geomorphological units are assumed to be homogeneous systems in terms of time of exposure, drainage, and parent material. It is thus unlikely that small spatial variations within the units could lead to significantly different conclusions.

#### Conclusions and Perspectives

This study is the first investigation addressing the activity and diversity of methanotrophic bacteria from the Mittivakkat Gletscher forefield in Southeast Greenland. Our data indicate that methanotrophic populations are present in all samples from the area, but an *ex situ* activity was exclusively recorded in material from the oldest outermost moraine. The diversity of the high-affinity methanotrophic community is relatively limited but the methanotrophs from distinct lineages may represent differential adaptations to the specific abiotic environmental conditions of the region, which has such low annual temperatures, low pH, and low nitrogen content. The large phylogenetic distance (>30% difference) to cultured methanotrophs would support an isolation effort in order to learn more about the physiology of these microbes. Here in particular, their methane consuming potential at low temperatures and at acidic pH would be of interest, as the

results may lead to future biotechnological applications of these microorganisms.

The Mittivakkat Gletscher forefield is an ideal natural laboratory to investigate biotic and abiotic processes induced by climate change in arctic regions. The region is particularly suited to study the combined evolution of biological and geological features in previously ice-covered regions, studies that are only in their infancy in Greenland. The study site has many advantages such as very limited direct influence of human activity, close proximity to the Sermilik field station (which provides an excellent base even for long-term investigation), and a long monitoring record of meteorological, hydrological, and glaciological data.

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