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Molecular and biogeochemical evidence for methane cycling beneath the western margin of the Greenland Ice Sheet

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Abstract:

Microbial processes that mineralize organic carbon and enhance solute production at the bed of polar ice sheets could be of a magnitude sufficient to affect global elemental cycles. To investigate the biogeochemistry of a polar subglacial microbial ecosystem, water discharged during the summer of 2012 from Russell Glacier at the western margin of the Greenland Ice Sheet near Kangerlussuaq was sampled and analyzed. The molecular data implied that the most abundant and active component of the subglacial microbial community at these marginal locations were bacteria within the order *Methylococcales* (59 to 100% of reverse transcribed [RT]-rRNA sequences). mRNA transcripts of the particulate methane monooxygenase (*pmoA*) from these taxa were also detected, confirming that methanotrophic bacteria were functional members of the subglacial ecosystem. Dissolved methane ranged between 2.7 to 83 μM in the subglacial waters analyzed, and its concentration was inversely correlated with dissolved oxygen while positively with electrical conductivity. Subglacial microbial methane production was supported by $\delta^{13}C$-CH_4 values between -64‰ and -62‰ together with the recovery of RT-rRNA sequences that classified within the *Methanosarcinales* and *Methanomicrobiales*. Under aerobic conditions, >98.5% of the methane was consumed during a 33 day incubation at ~4°C. An estimated methane oxidation rate of 0.31 ±0.01 μM day^{-1} provided direct evidence of the potential for methanotrophy in the subglacial environment. Our results support the occurrence of active methane cycling beneath this region of the Greenland Ice Sheet, where microbial communities poised in the oxygenated drainage channels could serve as significant sinks for subglacially generated methane.

Introduction

Environments below inland portions of the Greenland Ice Sheet are dark, cold, and poorly ventilated, with basal melt in regions of elevated heat flux providing the main source of liquid water at the bed (Bell et al, 2008). In the absence of direct exchange with the atmosphere, aerobic respiration and chemical oxygen consumption create anoxia, providing conditions favorable for obligately anaerobic processes such as methanogenesis (Skidmore et al, 2005; 2010; Tranter et al, 2005; Wadham et al, 2008). Whether or not the basal zones of ice sheets are sources of atmospheric methane will depend on the *in situ* rates of methane production and consumption (i.e. methanotrophy) and how effectively gases are advected to the margin. While it is hypothesized that substantial subglacial methane reservoirs exist (Wadham et al, 2012), the release of which could potentially influence atmospheric methane concentrations during deglaciation (Wadham et al, 2008; Wadham et al, 2012), there have been few data available on microbial carbon transformations beneath ice sheets.

Measurements of methane excesses in basal ice (Souchez et al, 1995; Miteva et al, 2009) and frozen water (Christner et al, 2012) have suggested that methanogenesis occurs beneath the Greenland Ice Sheet. It is also possible that the erosion of pre-glacial permafrost through glaciological processes could provide an additional source of legacy methane under the ice (Miteva et al, 2009). Laboratory and *in situ* measurements of methane production, and molecular analyses have confirmed that methanogenic archaea are active members of the microbial communities in these biomes (Bárcena et al, 2010; Boyd et al, 2010; Stibal et al, 2012a; b). However, data to assess carbon dynamics in these ecosystems have been challenging to obtain, and our understanding about the structure and diversity of microbial communities inhabiting the basal environments of polar ice sheets is still very limited.

Here we present molecular and biogeochemical data to investigate the role of microbes in subglacial carbon transformations in West Greenland. Near the ice sheet margin, surface melt enters the subglacial hydrological system through crevasses and moulins, transporting water, atmospheric gases, nutrients, and allochthonous carbon to the base (Tranter et al, 2005). Microbiological and physico-chemical characteristics of this aqueous environment were examined by collecting and analyzing subglacial outflows from the western margin of the Greenland Ice Sheet near Kangerlussuaq during the melt season in 2012. Our results show the dominance of active methanotrophic bacteria as well as phylotypes from a diverse assortment of obligately anaerobic bacteria and methanogenic archaea, confirming that a range of oxic to anoxic conditions are present in the subglacial environment at the marginal zone (Yde et al, 2010; Stibal et al. 2012a, b). The implications of unearthing a methanotrophic component in the subglacial ecosystem, and how effluxes and cycling of methane occur beneath the Greenland Ice Sheet, are discussed.

Materials and Methods

Study site description

Sampling at the western margin of the Greenland Ice Sheet was conducted at Russell Glacier (67°7’6” N, 50°10’5” W), a land terminating outlet glaciers located approximately 30 km east of Kangerlussuaq (Fig. 1). Following a recession for about five decades, most of the margin of Russell Glacier advanced recently between 30-70 m from 1984 to 1999 (Knight et al. 2000). The local climate of the area is characteristic for an arctic dessert (Hobbs 1927) with mean annual and summer air temperatures of -5.1°C and 9.8°C (June to August), respectively, between 1948 and 2003 (Aelby and Fritz 2009). Annual precipitation averaged around 158 mm yr^{-1}

during the same time (Aelby and Fritz 2009). The geology of the area consists of Archaean granitic, tonalitic gneisses, and the Kangâmiut dykes that are composed of metamorphosed to amphibolite facies of the southern Nagssugtoqidian orogen (Mayborn & Lesher 2006).

Sampling

Sampling was conducted between 21 May and 11 September 2012 at Russell Glacier (designated SUB-RG; Fig. 1A) to describe the biogeochemistry of a subglacial outflow. For direct comparison of microbiological and chemical parameter, water samples from a supraglacial stream (designated SUPRA-RG; Fig. 1A) and a proglacial moraine stream (67°9'24" N, 50°3'54" W; designated MS; Fig 1), respectively, were collected. During a short field campaign in 2013, additional water samples were collected from SUB-RG on 31 August to investigate methane oxidation rates in subglacial discharge.

Water samples for the nucleic acid extractions were collected two to three times a week from SUB-RG (n=38) and once a week from SUPRA-RG when water was flowing (n=12). Each sample was approximately 50 L of water, maintained at ~4°C after collection, and processed within 4-6 hours. Particulates larger than 0.2 μm in the water were concentrated by filtration through 142 mm Supor-200 0.2 μm pore size membrane filters (Pall) (~10L per filter). Separate filters were collected for the RNA and DNA extractions. All samples were maintained at -20°C while at the Kangerlussuaq International Science Support (KISS) facility (≤ 65 days), and subsequently, were transferred to -80°C for permanent storage when received at Louisiana State University or the University of Washington.

Molecular biological analysis

The nucleic acid extraction procedure included lysozyme digestion in combination with a bead beating step, followed by phenol and chloroform extraction and isopropanol precipitation. For RNA isolation, genomic DNA was eliminated from the extracts with DNase (TURBO DNA-free kit, Ambion) according to the manufacturer's guidelines and total RNA was purified using the MEGAclear™ kit (Ambion). Complementary DNA was reverse transcribed from RNA by priming with random hexamers and SuperScript II reverse transcriptase (Invitrogen) according to the manufacturer's specifications. The Illumina MiSeq system (Covance Genomics Laboratory, Seattle, WA) was used to sequence amplicons of the hypervariable V4 region of the 16S rRNA gene using the primers described by Bates et al, (2011; Supplemental Table S1).

The sequences obtained were analyzed using the Mothur platform v.1.29.1 (Schloss et al, 2009). After quality screening, the dataset was divided into rare (i.e. <1%) and abundant (i.e. >1%) sequences. An operational taxonomic unit (OTU) was defined as all sequences that had ≥97% identity. OTUs were sorted based on being shared or unique to the SUB-RG and SUPRA-RG environment. Metabolically active OTUs were inferred by analyzing the ratio of the relative abundance of sequences obtained in the 16S RT-rRNA and rRNA gene data sets.

The 16S rRNA from methanogenic archaea and mRNA of particulate methane monooxygenase subunit A (*pmoA*) were amplified by RT-PCR of total RNA from the SUB-RG and SUPRA-RG samples (Supplemental Table S1). Clone libraries were generated and sequenced (n=48 clones per library) on an ABI 3130XL Genetic analyzer (Genomics Facility, Louisiana State University). OTUs for the PmoA amino acid sequences were defined on a ≥93% similarity level (Degelmann et al, 2010). The V4 sequence data was deposited in the NCBI SRA database under the accession number SRP029752, the 16S rRNA sequences from methanogenic taxa as KF649970 - KF649974, and *pmoA* gene sequences as KF649975 - KF649979.

Methane concentration and isotopic analysis

Water samples were collected on eight occasions from SUB-RG, and about once a week from M-S (n=6)between 8 August 2012 and 9 September 2012. Duplicate 120 mL serum vials without headspace were collected, one of which was fixed immediately by the addition of 0.5 mL of 12N HCl. The water samples were stored at ~4°C and analyzed four to five months after collection on an SRI 8610C gas chromatograph. $\delta^{13}C$-CH_4 isotopic analyses was performed by the Stable Isotope Laboratory at the University of Arkansas.

Methane oxidation rates were investigated from water samples collected on 31 August 2013 at SUB-RG. For a direct comparison to ambient methane concentrations, water samples (n=3) were also collected from a nearby atmosphere equilibrated river. Outflow samples (n=27) were collected in 160 mL serum vials without headspace. Triplicate subglacial and river samples were fixed on site with 0.5 mL of 100% HCl immediately after collection. The remaining 24 SUB-RG samples were incubated at 4°C for 24, 48, 72, and 96 hours, with the final time point acquired upon return to the laboratory at Louisiana State University 33 days post collection. At each time point triplicate samples were fixed with 0.5 mL of 100% HCl. 'Laboratory' and 'field' controls were included in the experimental design to account for methane losses during transport and storage over time. Likewise, these controls were prepared in triplicate. Serum vials were filled with deionized water and sealed without headspace. Subsequently, 5 mL of deionized water were displaced with 10 cm^3 of an 11,000 ppm CH_4 standard (Sigma Aldrich). Samples were mixed for 5 min to equilibrated head space gases with water. Head space volume was then replaced by the injection of 5 ml of an equally equilibrated sample. The final control samples were fixed with 0.5 mL of 100% HCl. While the lab-controls remained at Louisiana State

University, field controls were transported to Kangerlussuaq, Greenland, and returned with the collected samples. All samples were stored at 4°C until analyses on the SRI 8610C gas chromatograph.

Detailed methodology

The Supplemental Materials contain explicit details on the (i) sample collection and *in situ* measurements; (ii) nucleic acid extraction; (iii) amplification of 16S rRNA genes, reverse transcribed 16S rRNA molecules, and mRNA of functional genes; (iv) sequencing and analysis; and (v) determination of dissolved methane concentrations and $\delta^{13}C$-CH_4 isotopes.

Results

Physicochemical characteristics of the supra- and subglacial waters

The water samples from the subglacial outflow at SUB-RG encompassed the early melt water that emerged from the glacier, high discharge related to increasing surface melt during summer, and the late season when surface melt water input to the basal environment diminished and the subglacial outflow decreased (Fig. 1A2-4). At the beginning of July, a slight deviation in the subglacial drainage was observed, and the outflow location migrated ~50 m to the side.

The pH of the subglacial water at SUB-RG ranged from 5.1 to 7.8 during the 2012 field season, with an average pH of 6.8 ± 0.5 s. d. (Supplemental Fig. S1). Electrical conductivity (EC) was highest at the beginning and at end of the melt season (46.8 and 64.0 $\mu S\ cm^{-1}$, respectively) and decreased to values generally below 10 $\mu S\ cm^{-1}$ from the beginning of June to the end of August. The SUB-RG discharge water temperature ranged between 0.0 to 0.1°C and contained an average dissolved oxygen (DO) concentration of 13.87 ± 1.50 $mg\ L^{-1}$. The most significant change in the DO occurred between 3 and 9 September when it decreased from 13.97 to 10.14 mg L^{-1} (Supplemental Fig. S1). There were also occasions in August and September when lower DO values were observed, and collectively the DO data were significantly negatively correlated with the EC data (Pearson correlation; $r=-0.939$, $p<0.001$). After a snowfall and subsequent melt on 9 September, extensive melting lead to an abrupt increase in the DO concentration and decrease in EC on 11 September (Supplemental Fig. S1). In contrast, the DO and EC in the SUPRA-RG varied little throughout the melt season and ranged from 14.12 to 14.38 mg $L^{-1}$and 1.6 to 1.7 µS cm^{-1}, respectively. The pH of SUPRA-RG remained fluctuated between values of 5.4 to 6.9.

Dissolved methane measurements

Dissolved methane concentrations were low in water samples collected from the moraine stream MS and ranged between 5 and 18 nM. In contrast, dissolved methane in the subglacial outflow from SUB-RG between 2.7 to 83 µM and were, on average, approximately 3000--fold higher when compared to MS (Fig 2). The high dissolved methane concentrations at SUB-RG were positively correlated with EC (Pearson correlation; $r=0.985$, $p<0.001$) and negatively correlated with DO ($r=-0.990$, $p<0.001$; Fig. 2). The $\delta^{13}C$-CH_4 values in the samples from SUB-RG (n=7) ranged between -64‰ to -62‰. Unfixed water samples contained 1.7 to 22 nM of methane after incubation at ~4°C for four to five months; this is an average of 0.08 ± 0.08% of the concentration observed in samples fixed immediately after collection, indicating methane consumption (Fig. 2). A similar decrease in dissolved methane concentrations was observed when SUB-RG was revisited in August 2013. Unfixed water samples contained 1.2 ± 0.73% of the initial methane after incubation at 4°C for 33 days. Methane oxidation followed a biphasic decay (Fig. 3) with 54% of methane already consumed during the first four days of incubation. An oxidation rate of 0.31 ± 0.01 μM CH_4 day^{-1} was estimated from the first-order decrease (rate constant=0.138; Fig. 3). Methane concentration in fixed laboratory and field controls were not statistically different from the initial inoculum (9.1 ± 0.5 μM) after 85 days of storage at 4°C (one way ANOVA; p=0.528), indicating that methane loss was not an artifact of bottle leakage

Identification of the autochthonous subglacial microbial community

Based on seasonal changes in EC, DO, and select analytes (i.e. total Fe and SO_4^{2+}; Supplemental Table 2) in the subglacial discharge, nucleic acids from 19 SUB-RG and five SUPRA-RG samples were selected for community structure analysis via 16S RT-rRNA and 16S rRNA gene sequencing. Amplicon sequences of the hypervariable V4 region of the 16S rRNA and 16S rRNA gene were examined using Mothur, and 1,693,724 bacterial sequences passed all quality screening criteria. Subsequently, the analysis focused on the dominant fraction of sequences in each barcoded sample (i.e. >1%), which comprised a total of 891,785 bacterial sequence reads. To identify the source of origin, OTUs classified within the subglacial and supraglacial microbial communities were sorted based on being either shared or unique to the SUB-RG and SUPRA-RG samples (Supplemental Fig S2, S3). Of all sequences in the 16S RT-rRNA and 16S rRNA gene libraries, 76% and 61% were shared and 3% and 15%, respectively, were unique to the SUPRA-RG samples.

There were 200,650 sequences unique to the SUB-RG samples, accounting for 21% and 24% of the sequences in the 16S RT-rRNA and gene libraries, respectively, and comprising a total of 43 OTUs. Of the 12 OTUs classified in the 16S RT-rRNA dataset; only six of these were also present in the 16S rRNA gene library (Supplemental Fig. S4 A, B). The remaining 31 OTUs were only present in the 16S rRNA gene library (Supplemental Fig. S4 C). The majority of the sequences unique to the subglacial discharge were phylogenetically related to a single OTU of the methanotrophic order *Methylococcales* (OTU1). From May to September 2012, OTU1 represented 59-100% of the SUB-RG 16S RT-rRNA and 0-66% of the 16S rRNA gene libraries (Fig. 4). The ratio of sequences for each phylotype in the 16S RT-rRNA and rRNA gene libraries was used as an indicator for metabolic activity and was the largest for OTU1 (4:1).

Phylogenetic analysis indicated that OTU1 had 97% identity to the 16S rRNA gene of *Methylobacter tundripaludum* and *Methylobacter psychrophilus* (Fig. 5A). All RT-PCR amplicons of *pmoA* mRNA from SUB-RG (n=5; 9 and 27 August; 5, 7, and 9 September) indicated the presence of the approximately 500 bp size product and subsequent clone libraries from 9 September confirmed that bacteria affiliated with the *Methylococcales* were metabolically active in the subglacial samples (Fig. 5B); however, *pmoA* transcripts were not detectable in SUPRA-RG (n=2; 8 and 29 August). The most abundant phylotype in the PmoA library (74% of the clones) had an amino acid sequence 99% similar to *M. tundripaludum* (PmoA_OTU1; Fig. 5B), which agreed well with the most abundant taxon in the 16S rRNA data (Fig. 5A). Two additional PmoA OTUs (PmoA_OTU3, OTU5) were identified from the library that were most closely related to *M. psychrophilus*. OTU2 and OTU4 had close PmoA amino acid sequence similarities with *Methylovulum miyakonense*.

The remaining eleven 16S RT-rRNA OTUs unique to subglacial samples were represented by seven bacterial taxonomic orders. OTUs classifying within the orders *Sphingobacterales* (≤ 17%) and *Burkholderiales* (≤ 13%) were found in approximately half of the SUB-RG RT-rRNA sequence libraries (Fig. 4, B). The *Verrucomicrobiales* (≤ 13%) appeared consistently in the RT-rRNA libraries towards the end of August and the beginning of

September, whereas *Vibrionales* were only present on 9 September. OTUs related to the *Actinomycetales*, *Rhodospirales,* and *Rhizobiales* were detected on 28 July in the SUB-RG 16S RT-rRNA libraries (Fig. 4, B). Sequences obtained from the *Rhizobiales* contained a single OTU that classified within the methanotrophic genus *Methylocella* (Fig. 5A).

SUB-RG 16S rRNA gene libraries were significantly different compared to the indigenous SUB-RG 16S RT-rRNA dataset (Mothur-based AMOVA, $p<0.001$). Of the 31 OTUs exclusively found in the 16S rRNA gene libraries, OTUs related to strict anaerobes within the orders *Anaerolineales* (≤ 51%) and *Holophagales* (≤ 19%), as well as *Coriobacteriales*, *Syntrophobacterales*, and *Clostridiales* were abundant (Fig. 4A). Further, OTUs predominately related to bacterial genera known for facultative anaerobic or fermentative metabolisms were identified (Fig. 4, Supplemental Fig. S2).

Although none of the amplified sequences obtained from the V4 region of the 16S rRNA gene were identified as archaeal, the use of methanogen specific 16S rRNA gene primers and RT-PCR was successful in amplifying sequences related to species within the *Methanosarcinales* and *Methanomicrobiales* in the SUB-RG and SUPRA-RG samples (Fig. 6), but identical OTUs were not shared between the two environments. Three OTUs were classified in the SUPRA-RG samples within the order *Methanosarcinales,* with the most abundant OTU (SUPRA-RG_Methanogen_OTU1; most closely related to *Methanosarcina mazei*) accounting for 95% of the clone library. The two SUB-RG methanogen OTUs, SUB-RG_Methanogen_OTU1 (88% of the clones) and SUB-RG_Methanogen_OTU2 (12% of the clones) were most closely related to *Methanococcoides alaskense* and *Methanoregula boonei*, respectively (Fig. 6).

Discussion:

This study coincided with the most widespread surface melt event observed in the past 120 years, and liquid water was present on 98.6% of the ice sheet surface during 12 July 2012 (Nghiem et al, 2012). This exceptional high melting also increased bulk runoff from the ice sheet margin by 113% compared to 2009 in the study site hydrological catchment area (Tedstone et al, 2013). Given the ubiquity of ephemeral supraglacial aquatic ecosystems (e.g. McMillan et al, 2007; Stibal et al, 2010; 2012c; Cameron et al, 2012; Irvine-Fynn et al, 2012; Telling et al, 2012), microorganisms, dissolved gases, and nutrients in the runoff inevitably enter the englacial drainage system. These surface contributions alter hydrological flowpath, affect access to atmospheric oxygen and the redox potential of environments at the glacier bed, and as such have important implications for understanding biogeochemical reactions that occur beneath ice sheets, as well as, interpreting data obtained from subglacial outflows at the margin.

A priori it was reasonable to assume that the bulk composition of microbial assemblages present within the subglacial discharge would represent an amalgam of species that originated from ecosystems on the surface and beneath the ice. For example, phototrophic organisms would not be expected to be active members of a sub-ice ecosystem, and the recovery of 16S rRNA sequences from cyanobacteria (Fig. S2) suggested these species originated from habitats on the ice surface. To address this issue, we conducted a comparative analysis of the supra- and subglacial data to deduce the source of the phylotypes observed (Fig. 3, Supplemental Fig. S2). In addition to cyanobacteria, all OTUs classifying within the *Methylophilales* and *Deinococcales* were inferred to be of supraglacial origin as members of these orders were either unique to SUPRA-RG samples or shared between both environments (Supplemental Fig. S2). One potential caveat of this approach is that generalist species with ecological roles in both the surface and sub-ice ecosystem were neglected. As such, our data should be viewed as a conservative appraisal of the endogenous subglacial microbial community composition.

In agreement with previous molecular surveys of Russell Glacier (Yde et al, 2010; Stibal et al, 2012a) and other subglacial environments (Christner et al, 2001; Skidmore et al, 2005; Lanoil et al, 2009; Hamilton et al, 2013), the phylotypes unique to the subglacial outflow samples were dominated by bacteria in the phyla *Bacteroidetes*, *Actinobacteria*, *Firmicutes, Acidobacteria*, and *Proteobacteria*, the latter of which was the predominant phylum in the 16S RT-rRNA dataset. A single phylotype with 97% 16S rRNA sequence identity to the Type I methanotrophs *Methylobacter tundripaludum* and *M. psychrophilus* (OTU1) was the most abundant OTU in the subglacial discharge samples, representing up to 66% and 100% of the 16S rRNA gene and RT-rRNA sequences, respectively (Fig. 4). Phylogenetically similar taxa have also been found to be dominant in arctic soils (e.g. Berestovskaya et al, 2002; Liebner et al, 2009; Martineau et al, 2010) and low temperature lakes (Borrel et al, 2011 and references within), supporting the view that this group of methanotrophs plays an important role in arctic environments. Additionally, two less abundant phylotypes with high sequence identity to Alpha- (Type II) and Gammaproteobacterial (Type I) methanotrophs were found to be unique to the subglacial samples (Fig. 5). It is interesting to speculate on the environmental factors which may affect the distribution of Type I and Type II methanotrophs in the subglacial environment. Berestovskaya et al, (2002) showed that the Type II methanotroph *Methylocella palustris* (related to OTU3; Fig. 5A) was more abundant than the Type I methanotrophs *M. psychrophilus* (OTU1; Fig. 5A) at low pH (4 to 6). Furthermore, Type II methanotrophs appear to be favored at temperatures >15°C (Mohanty et al, 2007) and can outcompete Type I methanotrophs when oxygen becomes limited and the methane concentration is high (Amaral et al, 1995).

Bioavailability of nitrogen and copper has also been found to affect the distribution of methanothrophs (Hanson and Hanson 1996). It is noteworthy that Type II methanotrophs were abundant in the RG-SUPRA (Broemsen 2013). Similar to findings on methanotrophic communities within glacial forefield sediments (Bárcena et al, 2010), Type II methanotrophs may dominate glacial environments where they exclusively rely on atmospheric methane sources. Given that most of the supraglacial runoff is routed to the base of the ice sheet, the minimal detection of Type II methanotrophs in subglacial waters indicates that the physicochemical conditions (i.e. near saturated DO concentrations, near circumnutral pH on a seasonal average, temperatures close to the freezing point) in the subglacial aquatic ecosystem during the period of study (Supplemental Fig. S1) was likely to be more favorable for Type I methanotrophs.

RT-rRNA data were mapped onto 16S rRNA gene data set and the ratio of 16S RT-rRNA: rRNA gene sequences form each OTU was calculated to highlight relative ribosomal expression in the community (Blazewicz et al, 2013). The ratio of 16S RT-rRNA: rRNA gene sequences for OTU1 was the largest (4:1) observed for any OTU, suggesting this phylotype was both abundant and likely metabolically active in the subglacial environment (Supplemental Fig. S4A). Further evidence for the *in situ* activity of methanotrophs was provided through the detection of *pmoA* mRNA sequences that were closely related to the PmoA amino acid sequence of *M. tundripaludum* and *M. psychrophilus* (OTU1: 99% and OTU2-5: 90-96% sequence similarity, respectively). Direct confirmation of methanotrophic activity was provided by the methane consumption in water samples during incubation at ~4°C, and the estimated *in vitro* methane oxidation rate of 0.31 ± 0.01 µM day^{-1} indicates the potential of methanotrophs as a biological sink for methane in this subglacial ecosystem. This methane oxidation rate in the subglacial microcosm experiments lies at the higher end of reported rates and was between 2.5 to 46 times higher compared with those reported for temperate aquatic systems (Kelley 2003, Carini et al, 2005) but lower when compared to rates from arctic lakes (Lofton et al. 2014).

A subglacial channelized drainage system is expected to be the major water flow near the ice sheet margin during summer melt. With high velocities, residence time in these effective drainage systems will be short, and enhanced microbial activity is more likely confined to basal sediments and the flanking marginal zones (Tranter et al, 2005). Periodic changes in water pressure in the main channels may flush this marginal zones (Hubbard et al, 1995), eluting sediments and microbes back into the channelized system with falling discharge. Importantly, these channel margin habitats provide conditions favorable for microorganisms capable of aerobic respiration as well as those that generate energy via fermentation or anaerobic respiration (Tranter et al, 2005). Therefore, the abundance of phylotypes related to obligately aerobic species (Fig. 5) together with methanogens and other known anaerobes in the orders *Anaerolineales* (≤ 51%) and *Holophagales* (≤ 19%) (Fig. 4A) was consistent with a range of redox conditions existing in the environment beneath the ice sheet. Previous studies have provided evidence for methanogenesis in subglacial sediments from alpine valley glaciers (Boyd et al, 2010) and beneath polar ice sheets (Bárcena et al, 2010; Christner et al, 2012). Specifically, Stibal et al, (2012b) reported methane production rates in subglacial sediments from Russell Glacier yielding approximately 10 pmol methane cell^{-1} day^{-1} at 1°C. The dissolved methane concentrations we documented in subglacial outflow waters ranged between 2.7 to 83 µM and were similar in magnitude to values reported in freshwater wetlands (Devol et al, 1988) and temperate swamps (Amaral & Knowles, 1994). The δ^{13}C-CH$_4$ data (range of -62‰ to -64‰) were slightly heavier than values observed in recently deglaciated lakes near Kangerlussuaq (-

63‰ to -67; Walter Anthony et al, 2012) and were consistent with expectations for methane produced from a mixture of methyl type-fermentation and CO_2 reduction (Whiticar 1999). For comparison, the $\delta^{13}C\text{-}CH_4$ values from the basal ice of GRIP (-81 to -84‰; Souchez et al, 2006) and GISP2 (-76‰; Miteva et al, 2009) were isotopically lighter and characteristic of methane produced by CO_2 reduction (Whiticar 1999). It is important to note that we lack an endmember source and alteration of the methane isotopic signature would have occurred *in situ* as a result of methane oxidation. Hence, a strict interpretation of the isotopic data should be avoided.

Significant quantities of organic carbon from vegetation, soils, and lacustrine and marine sediments are thought to have been overridden by ice masses and sequestered globally (Wadham et al, 2008; 2012). If mineralized to methane, modeled estimates suggest an equivalent of 1.31 to 7.28×10^{14} m^3 of methane gas could be stored beneath the East Antarctic Ice Sheet alone (Wadham et al, 2012). Methane reservoirs of this magnitude could have significant climatic implications; however, the net flux of methane from these regions will ultimately depend on the sources and sinks for methane beneath the ice sheet. The recovery of RT-rRNA molecules related to species of the *Methanosarcinales* and *Methanomicrobiales* (Fig. 6) support that viable methanogens were present beneath this portion of the ice sheet. The phylotypes observed formed clades with clones previously observed in sediments from the Russell Glacier (Stibal et al, 2012b) and alpine glaciers (Boyd et al, 2010), suggesting that specific lineages of methanogens may be common to subglacial environments. The nearest neighbors to these OTUs have the capacity to produce methane from CO_2 reduction (Bräuer et al, 2011) and methylated compounds (Singh et al, 2005). While methanogens would not be metabolically active in the oxygenated subglacial waters we sampled (Fig. S1), methanogenesis would be favorable in anoxic regions beneath the ice sheet. As such, methane produced in anoxic regions within the subglacial aquifer (i.e. sediment layers and discharge channel margins, and the distributed drainage system) could diffuse or be advected to the oxygenated, channelized drainage system at the ice sheet margin (Hewitt 2011), providing conditions favorable for aerobic methanotrophy. Our data support the working hypothesis that anoxic-oxic transitions in the subglacial environments affect the nature of microbial communities and carbon cycling at the ice sheet bed (Yde et al, 2010; Stibal et al, 2012a, b). Whether or not the prominence of methane oxidation in the marginal zones is seasonally limited to periods of increased inputs of surface runoff (e.g. Chandler et al, 2013) or occurs throughout the year (e.g. anaerobic methanotrophy; Strous 2010) merits further study.

The dissolved methane concentrations we observed in subglacial waters (Fig. 2) indicate that this region of the ice sheet was a source of atmospheric methane. We used cumulative discharge measured between 7 May and 27 August 2012 from Leverett Glacier (2.2 km^3; Tedstone et al, 2013) to provide seasonal methane fluxes. This glacier is the southern lobe of the Russell-Leveritt Glacier catchment, draining approximately half (~1200 km^2; Lawson et al, 2013) of the entire hydrological catchment area (~2400 km^2; Fitzpatrick et al, 2013). When the average methane concentration over one month of sampling in the subglacial outflow (n=8) is scaled to the Leverett Glacier discharge as the ideal proxy for the Russell Glacier watershed, the dissolved methane flux during the four months of summer melt season from Russell Glacier is estimated to be 990 Mg CH_4. Given the turbulence of the subglacial outflow and a rate of methane oxidation with a relatively long half-life in the order of four days (Fig. 3), dissolved methane was likely to be rapidly exchanged with the atmosphere by diffusive flux rather than being oxidized in the pro-glacial stream. Considering that the Russell-Leverett Glacier is representative of many land-terminating Greenland outlet glaciers (Lawson et al, 2013) and that methane contributions from other polar freshwater and soil environments are approximately only two orders of magnitude higher (90-200 Gg CH_4 a^{-1}; Bridgham et al, 2013), one could speculate that the Greenland Ice sheet as a whole may contribute significantly to global annual methane emissions.

Conclusion

Our molecular and biogeochemical data indicate that methanotrophic bacteria were both abundant and active members of the microbial communities found in regions beneath the western margin of the Greenland Ice Sheet. These data have expanded our understanding of the subglacial carbon cycle and point towards the existence of a complex subglacial ecosystem at the oxygenated marginal zones driven by organic carbon mineralization, methanogenesis, and methanotrophy. Stibal et al. (2012b) and Wadham et al. (2012) predict that the degradation of organic carbon beneath the world's ice sheets would lead to a substantial subglacial methane reservoir that could have significant climatic implications if released to the atmosphere during deglaciation. In the absence of empirical data, such modeling efforts have been unable to account for the potential of aerobic methanotrophy as a sink for methane in ventilated regions near the ice sheet margin. The subglacial waters we analyzed provide the first evidence for subglacial methanotrophy, supported by the dominance of methanotrophs in the subglacial discharge (Fig. 4), gene transcripts (Fig 5B), and biological methane oxidation measurements (Fig.3), indicating that the microbial, subglacial community could be an essential controlling factor on the net release of subglacial methane. Our findings make clear the need for further research to constrain methane reservoirs beneath ice masses and assess how microbial activities in subglacial ecosystems influence methane cycling and its flux to the atmosphere.

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Supplementary Information accompanies this paper on The ISME Journal website (http://www.nature.com/ismej)

Conflict of interest statement:

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Titles and legends to figures:

Figure 1: Sampling locatations on the western margin of the Greenland Ice Sheet. The sites were located just east of Kangerlussuaq. (A) Subglacial outflow (SUB-RG) and supraglacial stream (SUPRA-RG) at Russell Glacier. (A1) Early season, (A2) mid-season, and (A3) late season discharge at SUB-RG. (B) Moraine stream (MS).

Figure 2: A: Dissolved methane concentrations in subglacial discharge (●) and after four to five months of incubation at 4°C (▼). B: Correlations between dissolved methane, electrical conductivity (EC; ■), and dissolved oxygen (DO; ●) in subglacial discharge at Russell Glacier (SUB-RG) , and the moraine river (MS). Note that different scales are used on the x-axes.

Figure 3: Biological methane oxidation in subglacial discharge incubated at 4°C for 33 days. Error bars represent standard deviations from triplicate measurements. Note the log-scale on the y-axis.

Figure 4: Seasonal distribution of the microbial assemblage unique to the subglacial outflow at Russell Glacier (SUB-RG). The relative abundance of (A) 16S rRNA gene and (B) 16S RT-rRNA sequences. The missing sample on 25 June in the 16S rRNA gene dataset was the result of a technical error during sequencing.

Figure 5: Phylogenetic analysis of (A) 16S RT-rRNA sequences related to Type I and Type II methanotrophs and (B) PmoA amino acid sequences from subglacial samples at Russell Glacier (SUB-RG). Filled circles (●) and bold lettering indicate the clones obtained. The 16S RT-rRNA and PmoA amino acid neighbor joining trees were rooted with the 16S rRNA gene of

Clostridium frigoriphilum and AmoA sequences of the family *Nitrosomonadaceae*, respectively. Evolutionary distance was computed using the Jukes-Cantor method. Gaps and missing data were eliminated from the alignments. Bootstrap values (1000 replications) >50% are shown. The scale bar represents 3 and 10% divergence of nucleic acid sequence and amino acid sequence, respectively. The numbers in brackets following the OTU indicate the number of sequences represented for each OTU. Environmental clades of methanotrophs were included in the tree [upland soil clade (USC), glacial forefield soils, Greenland, and Jasper Ridge clade (JR1)] for reference. Genbank accession numbers are shown in parentheses.

Figure 6: Phylogenetic analysis of methanogen 16S RT-rRNA clone libraries from supraglacial (SUPRA-RG) and subglacial (SUB-RG) water samples at Russell Glacier using the neighbor joining method. Evolutionary distances were computed using the Jukes-Cantor method. Evolutionary analyses were conducted in MEGA v5.2.2. The tree is rooted with the 16S rRNA gene sequence of *Clostridium frigoriphilum* and bootstrap values (1000 replications) >50% are shown. The scale bar represents 5% divergence of nucleic acid sequence. The sequences obtained are shown in bold: (♦) SUPRA-RG OTUs, (●) SUB-RG OTUs; numbers in parentheses indicate the number of clones grouped with a ≥97% identity level. Selected 16S rRNA sequences of clone sequences obtained from other subglacial systems and type species were obtained from public databases. Genbank accession numbers are shown in parentheses.

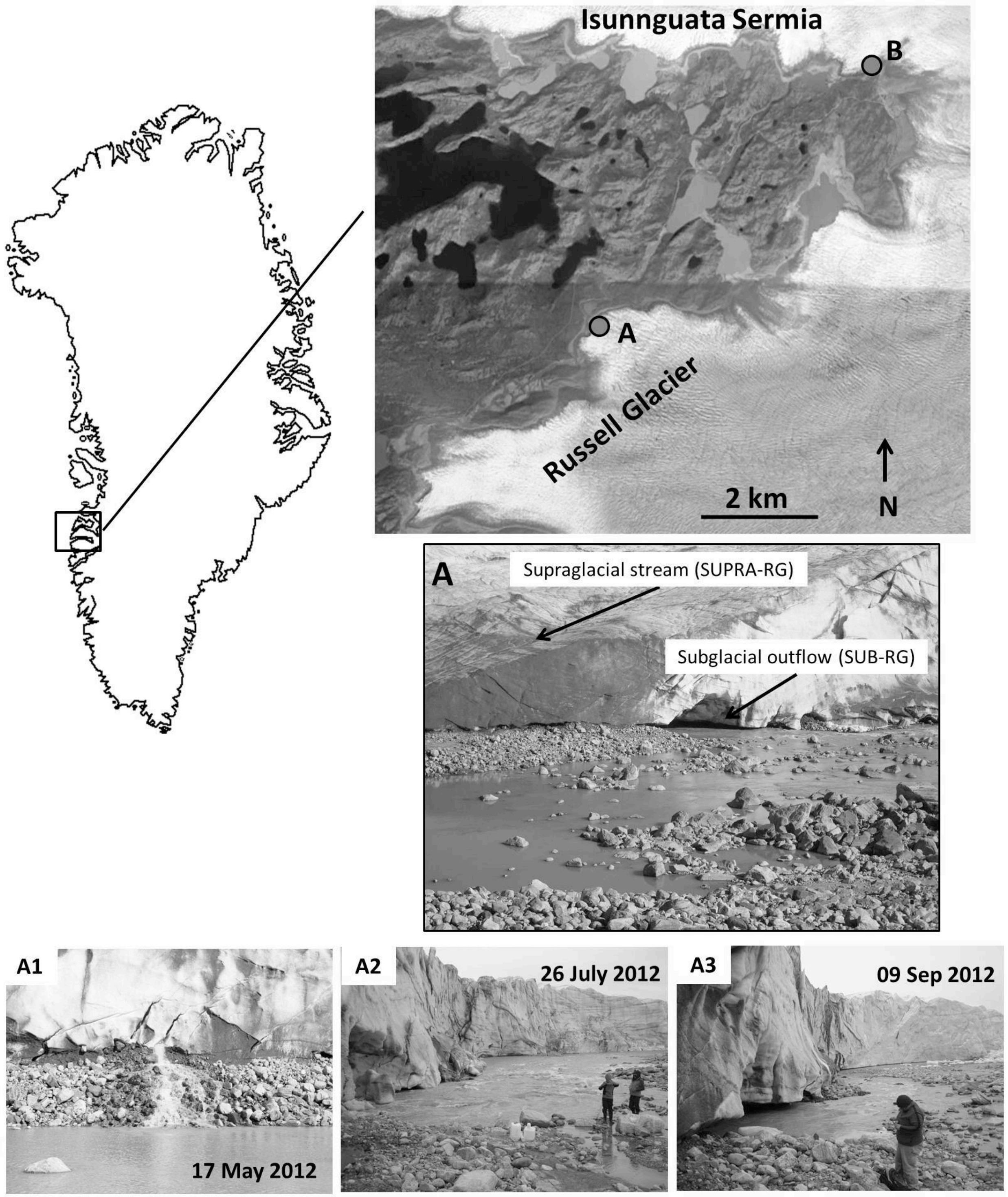
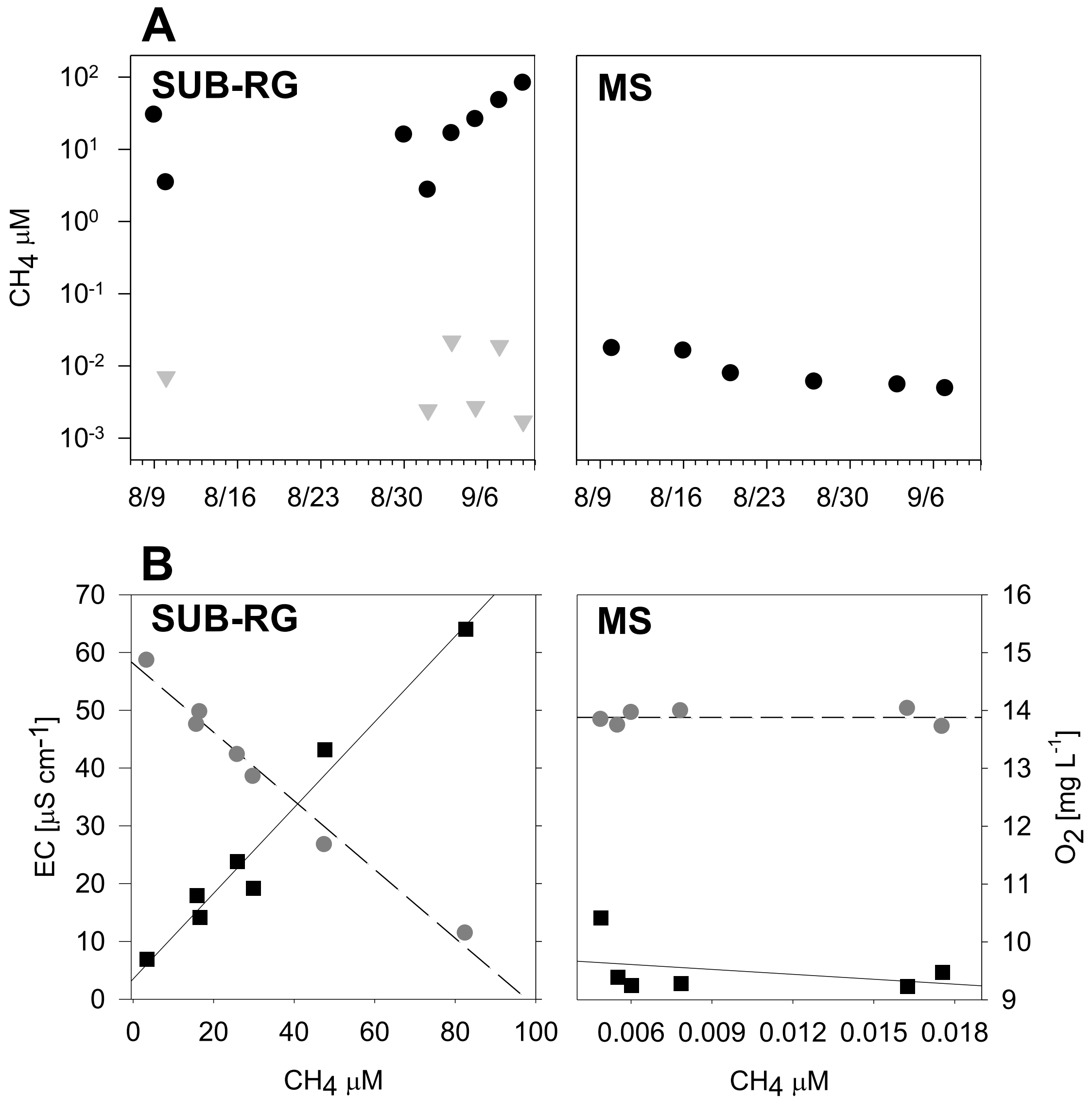

Isunnguata Sermia
B
A
Russell Glacier
2 km
N
A
Supraglacial stream (SUPRA-RG)
Subglacial outflow (SUB-RG)
A1
17 May 2012
A2
26 July 2012
A3
09 Sep 2012

Figure 1:


A
SUB-RG
MS
10^2
10^1
10^0
10^{-1}
10^{-2}
10^{-3}
CH_4 µM
8/9
8/16
8/23
8/30
9/6
B
SUB-RG
MS
70
60
50
40
30
20
10
0
EC [µS cm^{-1}]
0
20
40
60
80
100
CH_4 µM
0.006
0.009
0.012
0.015
0.018
CH_4 µM
16
15
14
13
12
11
10
9
O_2 [mg L^{-1}]

Figur2:

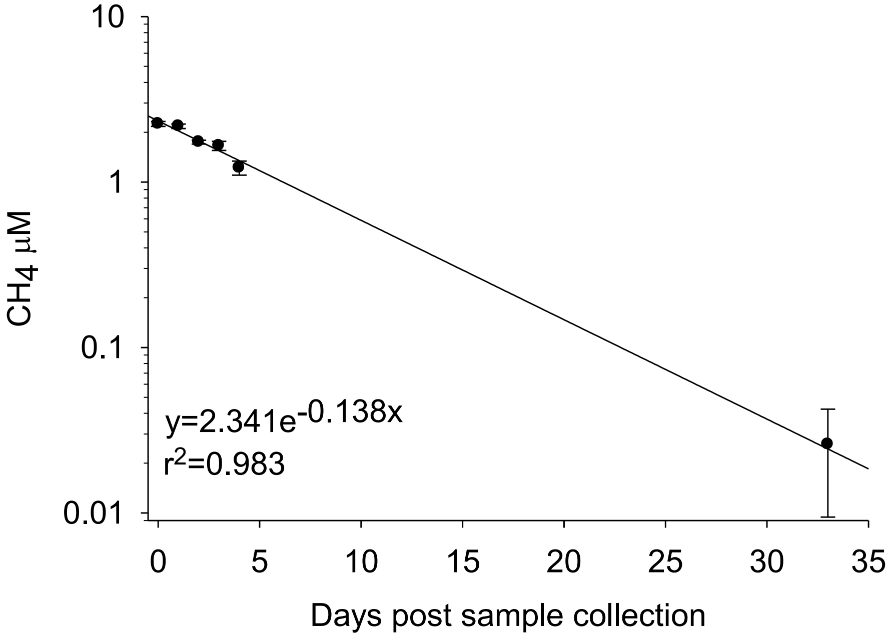

10
1
0.1
0.01
CH4 μM
y=2.341e-0.138x
r2=0.983
0
5
10
15
20
25
30
35
Days post sample collection

Figure 3:

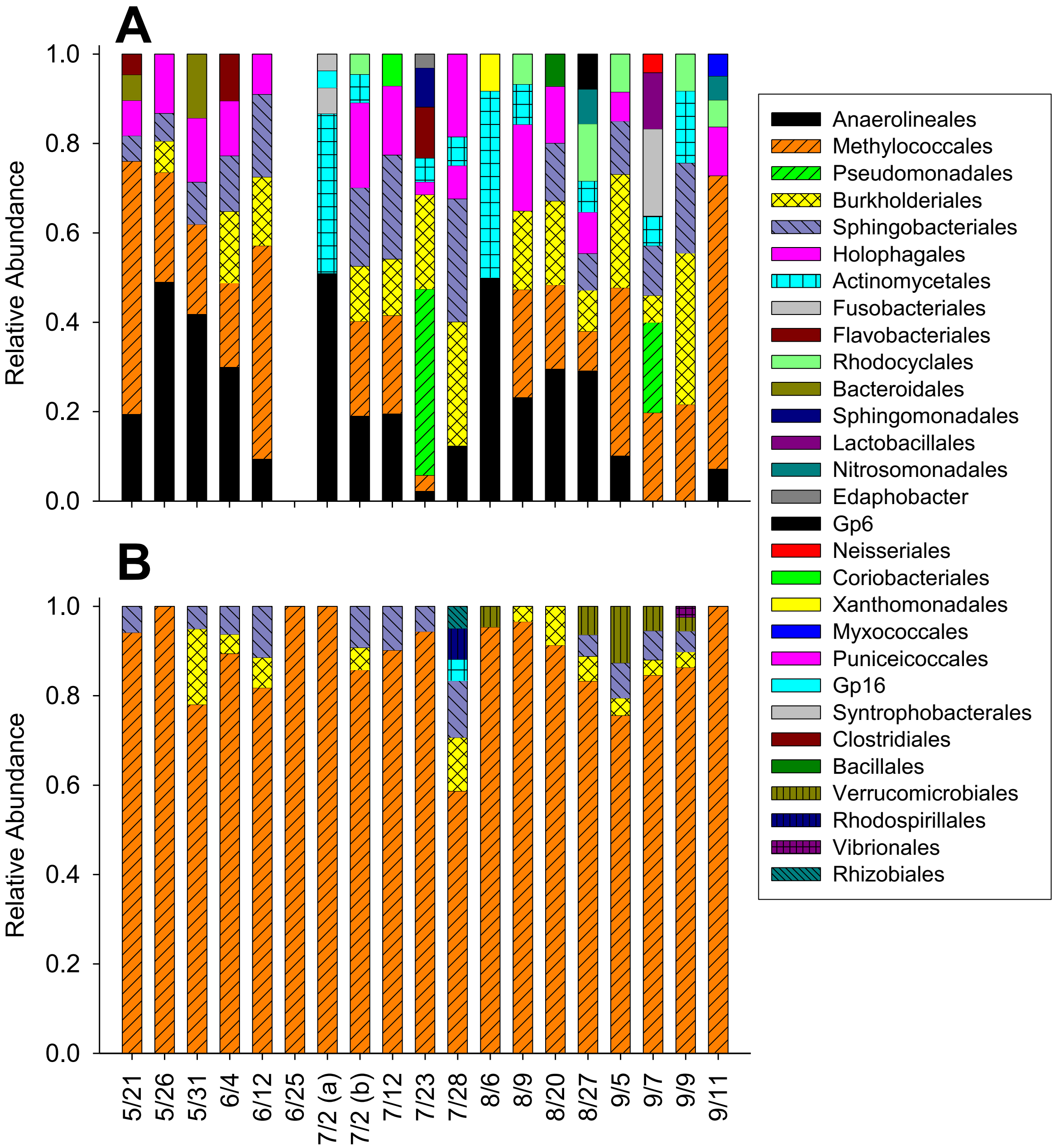

A
B
Relative Abundance
1.0
0.8
0.6
0.4
0.2
0.0
5/21
5/26
5/31
6/4
6/12
6/25
7/2 (a)
7/2 (b)
7/12
7/23
7/28
8/6
8/9
8/20
8/27
9/5
9/7
9/9
9/11
Anaerolineales
Methylococcales
Pseudomonadales
Burkholderiales
Sphingobacteriales
Holophagales
Actinomycetales
Fusobacteriales
Flavobacteriales
Rhodocyclales
Bacteroidales
Sphingomonadales
Lactobacillales
Nitrosomonadales
Edaphobacter
Gp6
Neisseriales
Coriobacteriales
Xanthomonadales
Myxococcales
Puniceicoccales
Gp16
Syntrophobacterales
Clostridiales
Bacillales
Verrucomicrobiales
Rhodospirillales
Vibrionales
Rhizobiales

Figure 4:

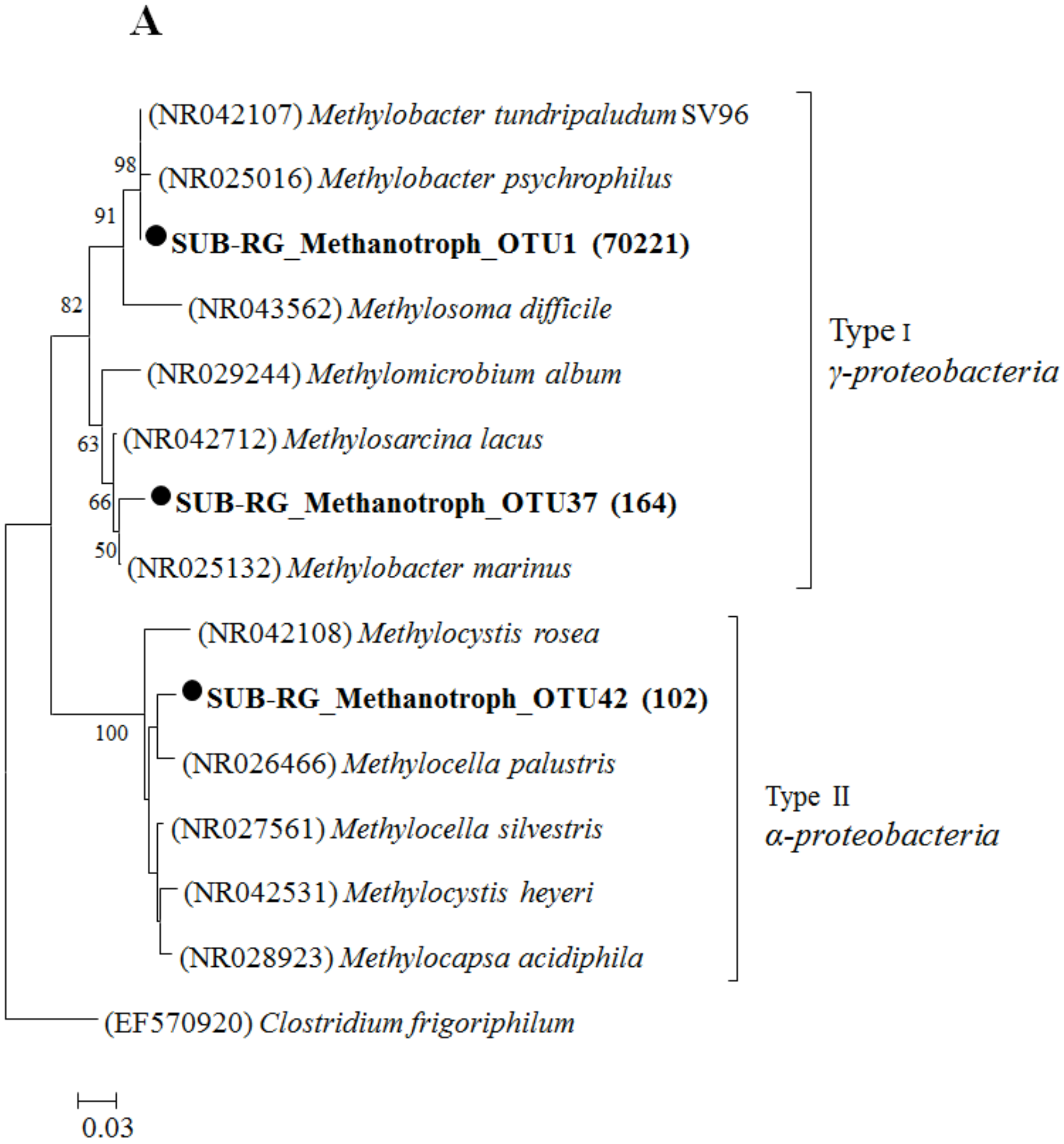
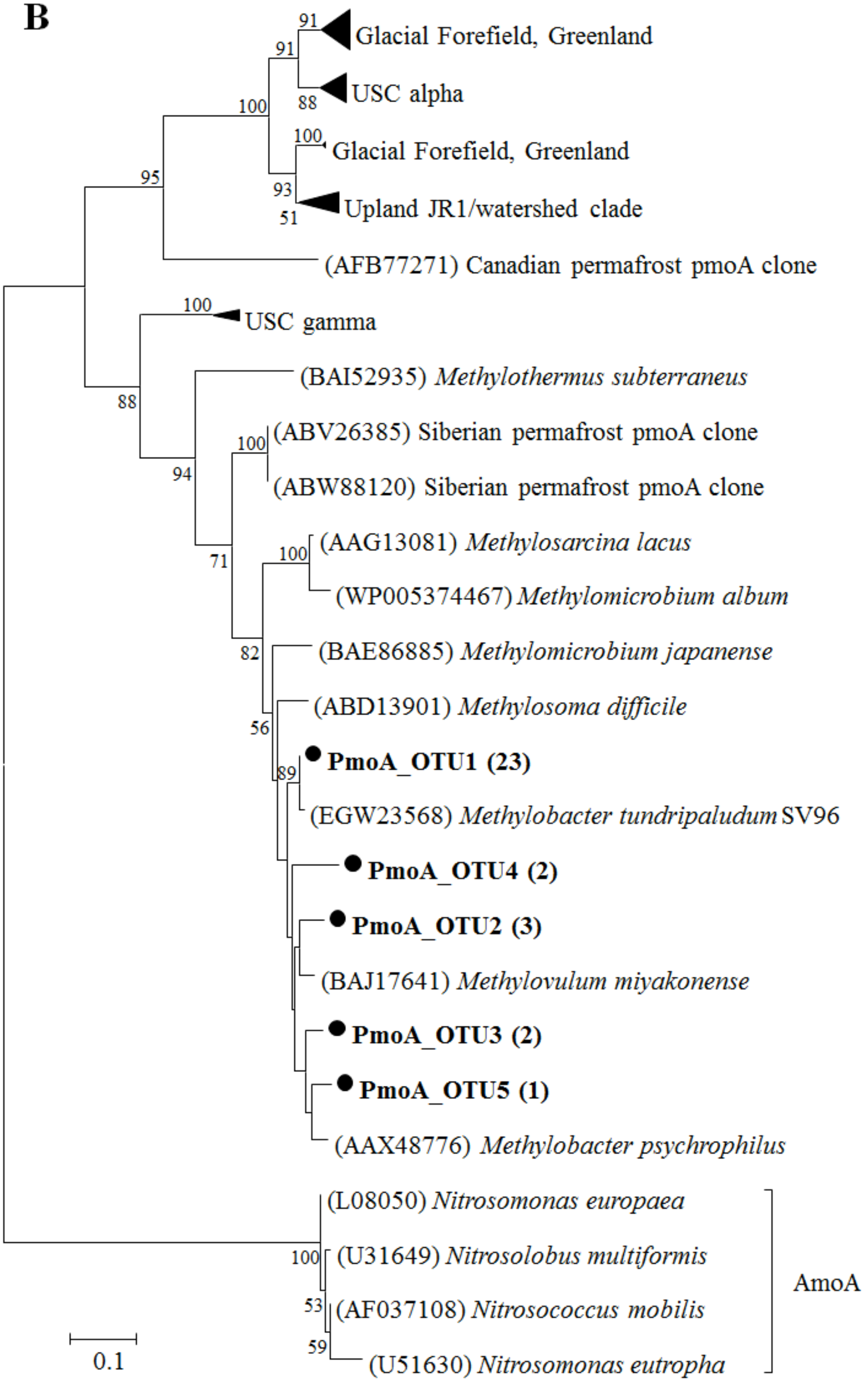
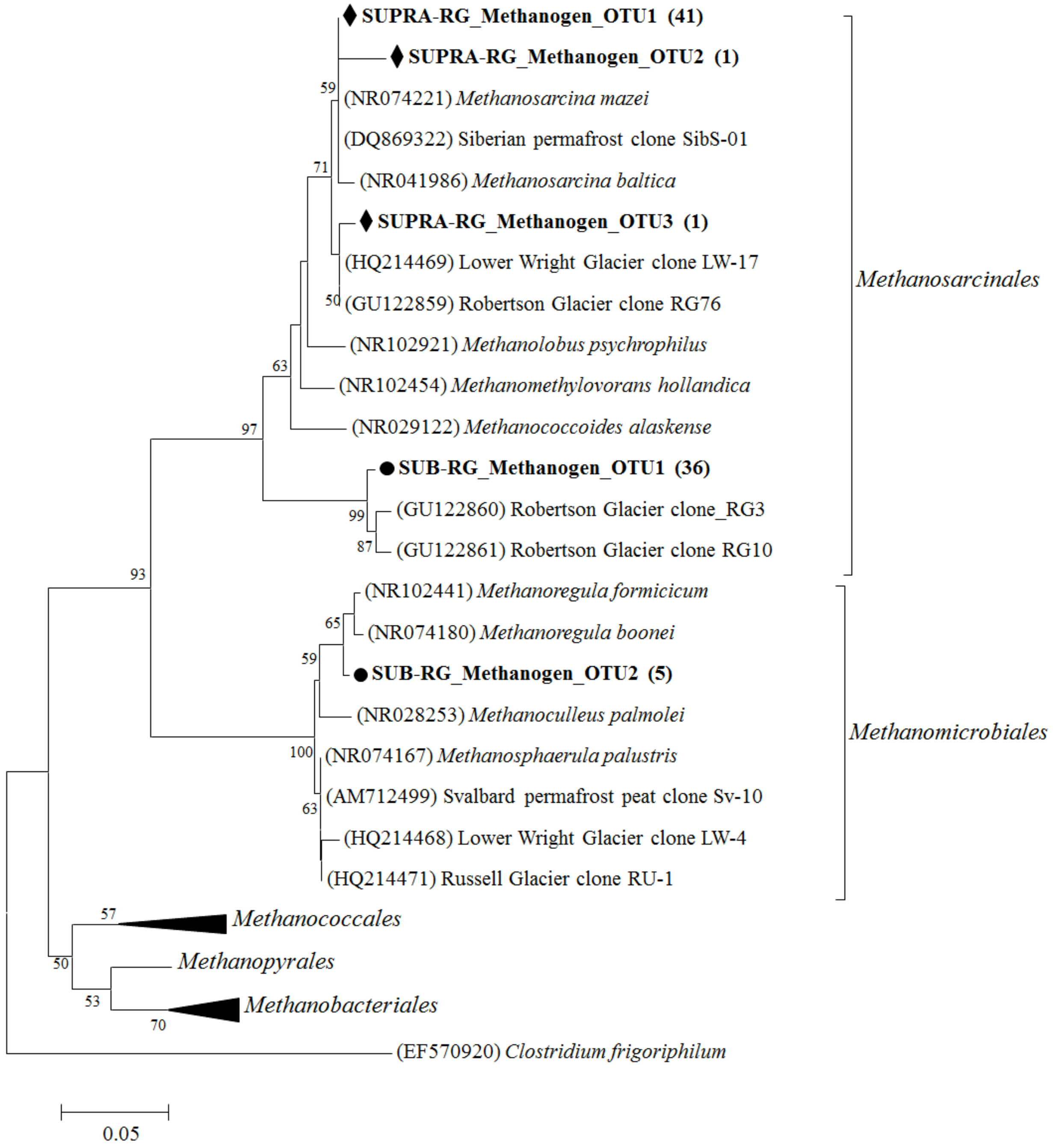

A
(NR042107) Methylobacter tundripaludum SV96
98
(NR025016) Methylobacter psychrophilus
91
SUB-RG_Methanotroph_OTU1 (70221)
82
(NR043562) Methylosoma difficile
(NR029244) Methylomicrobium album
63
(NR042712) Methylosarcina lacus
66
SUB-RG_Methanotroph_OTU37 (164)
50
(NR025132) Methylobacter marinus
Type I
γ-proteobacteria
(NR042108) Methylocystis rosea
SUB-RG_Methanotroph_OTU42 (102)
100
(NR026466) Methylocella palustris
(NR027561) Methylocella silvestris
(NR042531) Methylocystis heyeri
(NR028923) Methylocapsa acidiphila
Type II
α-proteobacteria
(EF570920) Clostridium frigoriphilum
0.03

Figure 5A:



B
91
91
Glacial Forefield, Greenland
100
88
USC alpha
100
Glacial Forefield, Greenland
95
93
51
Upland JR1/watershed clade
(AFB77271) Canadian permafrost pmoA clone
100
USC gamma
(BAI52935) Methylothermus subterraneus
88
100
(ABV26385) Siberian permafrost pmoA clone
94
(ABW88120) Siberian permafrost pmoA clone
100
(AAG13081) Methylosarcina lacus
71
(WP005374467) Methylomicrobium album
82
(BAE86885) Methylomicrobium japanense
56
(ABD13901) Methylosoma difficile
● PmoA_OTU1 (23)
89
(EGW23568) Methylobacter tundripaludum SV96
● PmoA_OTU4 (2)
● PmoA_OTU2 (3)
(BAJ17641) Methylovulum miyakonense
● PmoA_OTU3 (2)
● PmoA_OTU5 (1)
(AAX48776) Methylobacter psychrophilus
(L08050) Nitrosomonas europaea
100
(U31649) Nitrosolobus multiformis
53
(AF037108) Nitrosococcus mobilis
59
(U51630) Nitrosomonas eutropha
AmoA
0.1

Figure 5B:



♦ SUPRA-RG_Methanogen_OTU1 (41)
♦ SUPRA-RG_Methanogen_OTU2 (1)
59
(NR074221) Methanosarcina mazei
(DQ869322) Siberian permafrost clone SibS-01
71
(NR041986) Methanosarcina baltica
♦ SUPRA-RG_Methanogen_OTU3 (1)
(HQ214469) Lower Wright Glacier clone LW-17
50
(GU122859) Robertson Glacier clone RG76
(NR102921) Methanolobus psychrophilus
63
(NR102454) Methanomethylovorans hollandica
97
(NR029122) Methanococcoides alaskense
● SUB-RG_Methanogen_OTU1 (36)
99
(GU122860) Robertson Glacier clone_RG3
87
(GU122861) Robertson Glacier clone RG10
Methanosarcinales
93
(NR102441) Methanoregula formicicum
65
(NR074180) Methanoregula boonei
59
● SUB-RG_Methanogen_OTU2 (5)
(NR028253) Methanoculleus palmolei
100
(NR074167) Methanosphaerula palustris
(AM712499) Svalbard permafrost peat clone Sv-10
63
(HQ214468) Lower Wright Glacier clone LW-4
(HQ214471) Russell Glacier clone RU-1
Methanomicrobiales
57
Methanococcales
50
Methanopyrales
53
Methanobacteriales
70
(EF570920) Clostridium frigoriphilum
0.05

Figure 6: