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Acetoclastic archaea adaptation under increasing temperature in lake sediments and wetland soils from Alaska

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Abstract

The activity of methanogenic archaea is expected to be strongly affected under warmer temperatures, with higher greenhouse gas (GHG) emissions in the Arctic. In the sub-Arctic and Arctic freshwater sediments and wetland soils, acetoclastic methanogenesis is one of the major processes involved in methane (CH₄) release. To investigate the microbial adaptation/ tolerance at warmer temperatures and high acetate concentration, anaerobic microcosms of lake sediments and wetland soils from sub-Arctic ecosystems from Denali to Toolik regions in Alaska (USA) were set up at four temperatures (5, 10, 15 and 20 °C). In both environmental and microcosm samples, archaeal diversity was evaluated through specific archaeal 16S rRNA sequencing as well as bacterial, archaeal and methanogen abundance using quantitative PCR. Acetate amendment strongly modified the archaeal diversity highly favoring methanogens from *Methanosarcinales*, and in lower abundance, *Methanoregula* and *Bathyarchaeia* in some lake sediment. While acetoclastic groups significantly diverged among aquatic and terrestrial ecosystems, temperature did not significantly shape methanogens' diversity, showing their adaptability under warmer conditions. Faster microbial response on CH₄ production rates was observed at warmer temperatures (15 and 20 °C), bringing insights on the psychrophilic acetoclastic groups adapted to high acetate concentration with potential biotechnological application.

Keywords Methane · Acetoclastic · Archaeal community · 16S rRNA sequencing · qPCR

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INTRODUCTION

The Arctic has been one of the most susceptible regions to the effects of climate change, altering its landscape and affecting microbial activity. Constant warming has been changing several physicochemical parameters, interfering on microbial response, resulting in larger carbon input in atmosphere (Cavicchioli et al. 2019). These changes are also mentioned for Alaska state, which since the 1925s has warmed almost two times higher compared to the whole United States (Brooke et al. 2022).

High latitude wetlands and freshwater ecosystems (lakes, ponds) represent one of the largest sources of the greenhouse gas methane (CH₄), accounting for more than 40% of natural CH₄ emissions (Cui et al. 2015; Wik et al. 2016; Rosentreter et al. 2021). In these ecosystems, biogenic CH₄ is mainly derived from methanogenic archaea harboring the methyl-coenzyme M reductase (*mcr*), involved in the last step of methanogenesis process (Steinberg and Regan 2009; He et al. 2015). Methane can be produced through different pathways from H₂/CO₂, methyl groups or acetate (Conrad 1999). Acetate is formed from decomposition of organic matter (OM) and its availability is largely dependent on the physicochemical conditions, such as temperature, pH and salinity for instance (Stams et al. 2019). In low temperature freshwater sediment/ soils, as thawed permafrost, acetate is the dominant CH₄ precursor. In some peatlands, until 2/3 of the CH₄ can be originated from this pathway while H₂/ CO₂-based methanogenesis often occurs at acidic condition and mainly in deep layers (Coolen and Orsi 2015; Cui et al. 2015; Lyu et al. 2018; Kotsyurbenko et al. 2019; Brauer et al. 2020).

Acetoclastic archaea is described belonging to *Methano- sarcinales* order, encompassing few genera so far (*Methanosarcina* and *Methanosaeta*) which have been reported in freshwater sediment and soils in cold environments (Blaud et al. 2015). However due to the difficulties of isolation and characterization of new species, we aimed at evaluating the acetoclastic diversity from different ecosystems: sediment lakes and active layer (thawed portion) from two wetlands soils. Moreover, we evaluated how increasing temperature (from 5 to 20 °C) and acetate concentration could affect its composition and CH₄ production through controlled micro- cosms. Microbial incubation has been reported as useful tool and extensively employed to study diversity composition and CH₄ oxidation/production potential in polar environments (Høj et al. 2008; Blake et al. 2015; Martinez-Cruz et al. 2015).

Cold regions can harbor potential microbial metabolic pools for biotechnological processes (Parvizpour et al. 2021). There is an increasing interest in optimizing industrial process at low temperature, such as anaerobic digestion aiming to reduce heating costs (Gunnigle et al. 2015). Anaerobic digestion is a more sustainable process implying in methane generation as energy source. Since a considerable portion of the earth encompasses cold regions, adapted psychrophiles to optimize this process highly contribute for a more suitable operations in these regions (Dev et al. 2019). In this process, where polar environment can be a valuable source for such microbiota, screening for adapted microorganisms is essential and have been reported whether from Antarctic (Aguilar-Munoz et al. 2022) as Arctic or sub- Arctic (Xing et al. 2010; Petropoulos et al. 2019).

The four lakes and two wetlands belonging to different biomes (Taiga /boreal forest and tundra) are mainly situated in central Alaska, near Fairbanks, apart from Toolik (North Slope region). These environments were previously characterized regarding *in-situ* natural CH₄ emission (Walter Anthony and Anthony 2013; Greene et al. 2014; Sepulveda-Jauregui et al. 2015) and CH₄ oxidation potential and methanotroph diversity through incubations (He et al. 2015; Martinez-Cruz et al. 2015). Therefore, this work presents two main descriptions: (i) characterization of each natural environment (in situ) regarding methanogenic potential, targeting *mcrA* genes quantification, and total archaeal diversity communities, through specific 16 S rRNA; and (ii) Methanogenic incubation in acetoclastic condition under increasing temperatures where archaeal diversity was investigated as well to seek if different pattern could occur between these different ecosystems. The results gathered can contribute to a complementary characterization of these Alaskan regions, bringing insights in acetoclastic archaea capable to adapt to increasing temperature and high acetate concentration, with potentiality for industrial applications.

Material and methods

Sampling sites

Lake sediments (L) and wetland soil (W) samples were collected during summer 2016 in five different locations in Alaska State, USA: Killarney Lake (L1), Otto Lake (L2), Nutella Lake (L3), Goldstream Lake (L4), Killarney wetland adjacent to Killarney Lake (W1), and Toolik wetland (W2). Vegetation in both wetlands were different; grassland was observed in Killarney, associated to taiga / boreal forest biome, whereas peat moss (mostly *Sphagnum* spp.) was

observed in Toolik (Fig. 1).

Lake sediment samples were collected at the center of the lake at maximum depth in duplicate (A and B) at some meter of distance using an Ekman dredge and then placed in sterile plastic 1-L bottles (Nalgene®). Sample sites characteristics are summarized in Table 1. Wetland soils were collected in three points (A, B and C) and the sampling spots were divided in two subsamples, according to the depth: top (between 0–10 cm,) and bottom (10–20 cm). The average among replicates is presented aiming an overall characterization of each ecosystem (Table 2). Samples were collected in plastic, “single use” bags (Ziploc®, SC Johnson) and placed in a cool container (4 °C) before being processed, subsequently subdivided for physicochemical and molecular biology analyses. Subsamples for anaerobic incubations were transferred into a sterile plastic bag under N₂ flux and sealed.

Physicochemical analyses

Temperature, pH and conductivity were determined for each collected sample, following protocols described in Lavergne et al. (2021). Replicates of sediment/wetland soil samples were dried overnight at 105 °C to determine dry weight (DW) and then heated to 550 °C for three hours in a muffle furnace to determine organic matter content (OM). Porewater was filtered from sediments according to Jones and Willett (2006) to determine anions and cations concentrations. For that, an aliquot of 40 g of sample was suspended in 200 ml deionized water using a magnetic stirrer at room temperature for 1 h. Then, the liquid phase was filtered through a microRhizon sampler (Rhizosphere, Netherlands) and pH was measured (HI9829, Hanna). Nitrite, nitrate, and sulfate concentrations were determined through liquid chromatography (HPLC Dionex, USA) (Table 2).

Microcosms set-up

A total of 240 microcosms vials were set up and CH₄ production were determined during 90 to 370 days for lakes (L1 to L4) and 170–450 days for wetlands (W1 and W2, including subsampling points, top and bottom), depending on the CH₄ production.

Microcosms were prepared under anaerobic conditions (Pyramid GloveBox, Captair, France) in sterile glass flasks (12 ml) using four replicates per sample per temperature, sealed with rubber stoppers and aluminum crimp. As described in Lavergne et al. (2021), an aliquot of 8 g OM l⁻¹ of soil or sediment sample was added to each vial and the flasks were filled until 10 ml with mineral media (modified from Shelton and Tiedje 1984), amended with acetate (30 mM), leaving 1 ml for headspace (Online Resource 1, Table S1). For comparison purpose, the acetate concentration was set according to the protocol described in Lavergne et al. (2021), who investigated wetlands and lakes in Patagonia through incubations. The pH of mineral media was adjusted according to the pH value measured in situ. All replicates were incubated in controlled temperature chambers at 5 °C, 10 °C, 15 °C and 20 °C (± 0.4–0.8 °C) (Online Resource 1, 1). After sealing, a period of 24 h was proceeded for equilibration and then flasks were purged.

Acetate-derived methane production rates

Since microcosms contained acetate as substrate, we considered as acetate derived the methane production described throughout this work. Accumulated biogas was monitored by measuring the pressure in the microcosm headspace, with a manual manometer (Sper Scientific, USA, Portable Handheld Pressure Meter—Wide Range, model 840,065). Measurements were performed weekly or twice a week, depending on the activity of each sample. A threshold above 30 mbar was set to start quantification in GC.

Gas chromatography (GC) was used to measure accumulated CH₄ (mol) concentration in the headspace. An aliquot of 250 µl of headspace was injected using 0.5 ml sample-lock syringe (Hamilton, model 1750SL, USA) into a gas chromatographer (SRI Instruments, model 310C; injector temperature 40 °C, column temperature 150 °C, detector temperature 40 °C) equipped with a thermal conductivity detector (TCD) and Packed Column (6' X1/8" SRI Instruments, USA), using argon as carrier gas (99.9% Linde, Uruguay). Calibration curves were performed periodically in gas-tight propylene bags (FlexFoil PLUS, USA) using pure CH₄ gas (CH₄: 99.9% Linde) and nitrogen (N₂: 100%, Linde gas) and methane standard was injected at each measurement series for accurate measurements. Results were exported from software Peak Simple (version 393) and accumulated CH₄ in both gaseous and liquid phases was calculated using the ideal gas equation and Henry's law for each temperature (Online Resource 1, 1.2). Methane production rate (MPR) was calculated by linear regression, as the maximum slope of CH₄ accumulation kinetics, following Lavergne et al. (2021).

MPRs were normalized by the quantity of sample calculated as dry weight (DW) in each incubation ($\mu\text{mol CH}_4 \text{ g}^{-1} \text{ DW d}^{-1}$). We considered the latency period as the time hold to reach 10% of maximum observed MPR. At the end of the incubation (when no increased pressure was observed, and kinetic curve reached a plateau), samples were centrifuged (6000 rpm, 30 min at 4 °C). The pellet was stored at -20 °C for subsequent DNA extraction and pH measurement (Online resource 1, 1.3, Table S2). From the supernatant, the acetate consumption was checked through GC-FID (Adorno et al. 2014) (data not shown).

Q10 coefficient was calculated according to Sepulveda- Jauregui et al. (2018) to evaluate the influence of temperature on MPR. Calculation was made between 5 °C and 15 °C (Q10[5–15 °C]) and 10 °C and 20 °C (Q10[10–20 °C]). Temperature dependence of methanogenesis was also estimated using activation energy (E_a) value, applying Arrhenius equation, as described in Lavergne et al. (2021).

DNA extraction

DNA was extracted from 0.25 g of samples taken in situ (lake sediments and wetland soils) and samples taken after the methanogenic assays (centrifuged pellets obtained at the end of the methanogenic activity experiments). Power Soil Isolation kit (QIAGEN®, USA) was used following the instructor's guide. The final DNA concentration as well as DNA purity (ratio 260/280) were quantified using Nanodrop® (Thermo Scientific, USA).

Abundance of targeted *mcrA*, archaeal and bacterial 16S rRNA genes by qPCR

In order to evaluate the methanogenic abundance in each ecosystem, 16S rRNA gene (for archaea and bacteria) and the functional *mcrA* gene (gene marker for methanogenesis) were quantified by quantitative PCR (qPCR) in the in situ samples (no incubated samples). After optimization of qPCR conditions to avoid inhibition due to the sample template (data not shown), the reaction mix contained 0.4 or 1 μM of each primer, 2 μl of DNA template 10- or 100-fold diluted in ultrapure water (corresponding to 0.5–4 ng sample DNA), 10 μl of master mix (2X Takyon ROX SYBR master mix, Eurogentec, Belgium) and nuclease-free water to a final volume of 20 μl (Online Resource 1, 2, Table S3). All reactions performed in duplicate using AriaMX thermocycler (Agilent, USA) with Aria Software v.13 (Agilent, USA). Standard curves and qPCR conditions are described in Supplementary Information.

Archaeal community composition by 16S rRNA gene sequencing

Amplicon massive sequencing of the V3–V5 region of the 16S rRNA archaeal gene was performed using an archaeal-specific primer set (340F 5'-CCCTAHGGGGYGCASCA-3' and 787R 5'-GGACTACVSGGGTATCTAAT-3') (Yu et al. 2005; Pinto and Raskin 2012). A single representative sample was selected from each different incubation, considering solely top layer samples of wetland soils (W1T and W2T). Amplicons were checked on a 1% agarose gel electrophoresis and then purified using a commercial kit (ZR Zymo-clean™ Gel DNA Recovery Kit, USA). Sequencing of the 16S rRNA gene amplicon libraries was carried out by the Ion Torrent Personal Genome Machine (PGM) platform at the Biological Research Institute “Clemente Estable” (Montevideo, Uruguay).

Bioinformatics and statistical analysis

Sequence analysis was performed following the ‘quantitative insights into microbial ecology’ pipeline (QIIME2 201,910 release) (Bolyen et al. 2019). Multiplexed single-end sequencing reads (3,916,062 in total) were imported into the QIIME2. The ‘divisive amplicon denoising algorithm’ DADA2 (Callahan et al. 2016) plugin in QIIME2 was used to filter out noise and correct errors in marginal sequences, remove chimeric sequences and singletons and dereplicate the sequences, resulting in high resolution amplicon sequence variants (ASVs). ASVs represent, as closely as possible, the original biological sequence of the sequenced amplicon. Sequencing resulted in 790,220 reads ranging from 976 to 72,831 per sample representing 1,289 ASVs (sequence length of 410 pb) and were classified with a classify-sklearn trained against the most recent SILVA 16S rRNA gene reference 1381 database (Quast et al. 2013). Raw sequence data are available in the Sequence Read Archive (SRA) of NCBI under the BioProject PRJNA666194.

All the statistical analysis was performed in R v351 (R Core Team 2017) with R Studio environment (v131093). One-way ANOVA was applied to verify significant differences among MPRs and latency period (lag phase) at four temperatures, as well as the depth (top and bottom) and MPR in wetland soils. Spearman correlation tests were performed to

verify the relationship between abundance of *mcrA* with methane production rates (MPR). Pearson correlation was applied to verify the effect of temperature on MPR and latency period. T-test were applied to verify the temperature dependence of methanogenesis (Q10) and the variation of diversity (Shannon index) between in situ and each tested temperature separately. To correlate physicochemical data with MPR, a multivariate principal component analysis (PCA) was carried out ('FactoMineR' package) (Husson et al. 2013). The PCA was built with 11 variables, including MPR at 5, 10, 15 and 20 °C, environmental temperature, and pH (measured in situ), dissolved organic carbon (DOC), organic matter (OM), electric conductivity and sulfate and nitrate + nitrite concentration.

For archaeal diversity analyses, the biom file from QIIME2 was imported and analyzed through phyloseq- modified workflow (McMurdie and Holmes 2013). Alpha diversity was calculated based on the number of observed ASVs using Shannon diversity index after normalization with 976 sequences per samples (Shannon 1948; Simpson 1949). Taxon relative abundance bar charts were designed using custom R scripts and ggplot2 (v332) whereas the heatmap depicting relative abundance of ASVs (at phylum and genus level) through ampvis2 (v265) (Andersen et al. 2018).

Venn diagram (bioinformaticsbugentbe/webtools/ Venn/) was used to determine the shared ASVs considering the lowest and highest temperature in microcosms (5 and 20 °C). For Venn diagram construction we only considered 183 ASVs with a relative abundance above 1% of archaeal community. Permutational analysis of variance (PERMANOVA) was applied to analyze the influence of ecosystem and the effect of temperature incubation on the archaeal diversity distribution, (betadisper function, 'vegan' package; Oksanen et al 2013). These analyses were run in two conditions: (i) incubated + in situ samples; (ii) only incubated samples at four different temperatures. Principal coordinate analysis (PcoA) was used to evaluate the distribution of samples only in natural condition (in situ). PcoA using Bray Curtis distance metrics of non-transformed relative abundance table was computed with phyloseq and plotted using ggplot2 (Wickham 2017).

Results

Methane production rates (MPR) in lake sediments and wetland soils at different temperatures

Temperature influenced significantly both MPRs and latency periods for all microcosms samples (except for Toolik bottom which MP was not detected at 5 °C), with higher MPRs and shorter latency periods at warmer temperatures (Pearson correlation: $p < 0.05$, $p > 0.9$) (Fig. 2a and b).

In lake sediments, acetate-driven MPR varied from 3.8 ± 0.03 (Killarney Lake) to 5.6 ± 0.6 $\mu\text{moles CH}_4 \text{ g}^{-1} \text{ DW d}^{-1}$ (Otto Lake) at 20 °C. At 5 °C, MPR was about 5 times lower than at 20 °C (ANOVA $F_{1,8} = 151.8$, $p = 1.75 \cdot 10^{-6}$, $n = 8$), ranging from 0.54 ± 0.05 (Killarney Lake) to 1.15 ± 0.2 $\mu\text{moles CH}_4 \text{ g}^{-1} \text{ DW d}^{-1}$ (Nutella Lake). In wetland samples (including two depths; top and bottom), MPR increased about 4–5 times at 20 °C, compared to MPRs at 5 °C (ANOVA $F_{1,8} = 7.769$, $p = 0.031$), ranging from 4.21 ± 0.4 (Killarney—W1B) to 29.12 ± 0.7 (Toolik W2B) $\mu\text{moles CH}_4 \text{ g}^{-1} \text{ DW d}^{-1}$. In Toolik wetland, at 5 °C, we observed the highest MPR in the top layer (5.7 ± 1.8) $\mu\text{moles CH}_4 \text{ g}^{-1} \text{ DW d}^{-1}$, while CH₄ production was not detected in the bottom layer (W2 B). Latency periods pronouncedly diverged in top layer samples (W1 T \neq W2 T) (ANOVA $F_{1,8} = 6.481$, $p = 0.043$) ($n = 8$), which Killarney (W1 T) presented shorter latency period, (10 days at 20 °C to 68 at 5 °C) than samples from Toolik (W2 T) with 45 days at 20 °C to 146 at 5 °C) (Fig. 2b). 212.

Two coefficients were determined to evaluate the degree of temperature effect on methanogenesis: the Q10 and Ea (Table 3). It is worthy to note that an increase of 10 °C had a major effect in the lowest temperature range (5–15 °C) compared to the highest range (t-test, $t = 4.649$, $p = 5.61 \cdot 10^{-4}$, $n = 8$). Moreover, the overall temperature dependence of methanogenesis evaluated by the energy activation (Ea) showed similar values for lake sediment samples, mean: $79.58 \pm 17.38 \text{ kJ mol}^{-1}$, corresponding to $0.82 \pm 0.18 \text{ eV}$ and wetland soil, mean: $60 \pm 21.2 \text{ kJ mol}^{-1}$, corresponding to $0.62 \pm 0.22 \text{ eV}$. The highest values were displayed by the Otto Lake samples, reaching up to 98.7 kJ mol^{-1} (1.02 eV) (Table 3).

Physicochemical data and effects on methane production

To elucidate which physicochemical factors were related to the MPRs in sediment lakes and wetlands, principal component analysis (PCA) was performed considering 11 environmental variables. The two first dimensions of the PCA built with MPRs at the four tested temperatures (5, 10, 15 and 20 °C), in situ temperature, conductivity, pH, dissolved organic

carbon (DOC), sulfate, nitrate + nitrite concentrations and OM represented 60.21% of the total dataset variation (Table 2, Fig. 2a). OM concentration was significantly correlated to MPR in all samples at 10, 15 and 20 °C (Spearman correlation, $\rho > 0.6$, p -value < 0.05). Moreover, based on the two first dimensions of the PCA, samples were significantly clustered according to their biome, with a clear distinction between tundra (Toolik soil and Nutella Lake) and taiga (also called as boreal forest, Killarney, Goldstream) (PERMANOVA, $F = 4.18$, p -value < 0.05) (Fig. 3b).

Archaeal, bacterial 16S rRNA and *mcrA* gene abundance in sediment lake and wetland soils

To evaluate the methanogenic potential of each environment, archaeal, bacterial 16S rRNA and functional *mcrA* gene were quantified from in situ samples. The abundance of bacteria ranged from 10^9 to 10^{10} , whereas archaea ranged from 10^7 to 10^9 gene copies g^{-1} DW with highest values for the samples from Goldstream lake (L4) and Toolik wetland (W2). The abundance of functional *mcrA* gene ranged between 10^7 to 10^8 gene copies g^{-1} DW with higher values in Lake Nutella (L3), and Toolik wetland (W2, bottom samples). The proportion of *mcrA* gene regarding archaea and archaea + bacteria 16S rRNA abundances are depicted at (Online Resource 1, Fig. S1). No significant correlations were found between *mcrA* gene abundances and MPR independently of the considered temperature (Spearman correlations, $\rho < 0.8$, p -value > 0.05).

Selection of metabolically versatile *Methanosarcina* under acetate-amendment microcosms

The archaeal diversity analysis measured by Shannon index revealed the predominance of known methanogens in sediment lakes and wetland samples, in natural or incubated condition (Fig. 4).

Prior to incubation, archaeal communities from sediment lakes were dominated by acetoclastic archaea as *Methanosaeta* genus (from 45.8 to 60.9% archaeal community), followed by *Methanoregula* and *Methanobacterium* genera. Wetlands samples exhibited different pattern with dominance of *Methanobacterium* and *Methanosarcina* in Killarney (W1) and a higher diversity of genera including unclassified genera within *Bathyarchaeia* and Rice cluster II and to the recently classified *Woesearchaeales* order and *Nitrososphaeraceae* family in Toolik soil (W2) (Fig. 4). Due to selective role of acetate-amendment, archaeal diversity observed in microcosms were dominated by *Methanosarcina* (60–99% of archaeal community), at all temperatures, surpassing the previously dominant *Methanosaeta* in in situ samples, except for the samples from Goldstream Lake (L4), where the genus *Methanosaeta* prevailed at all temperatures (49–67% of archaeal community). *Methanosaeta* was also found as dominant in the samples from Otto Lake (44.7% archaeal community) and Toolik peat (52.7% archaeal community) taken from the microcosms after incubation at 15 °C. Moreover, in a small proportion, *Methanoregula* (4–6%) and *Bathyarchaeia* (~7%) were observed mainly in Goldstream Lake samples (Fig. 4).

To determine if these predominant genera were represented by the same or different species, a deeper analysis was performed using the reads to infer the amplicon sequence variants (ASVs) which revealed the genus *Methanosarcina* is represented by 97 ASVs and the genus *Methanosaeta* by 53 ASVs. Despite being affiliated to the same genus, the majority of ASVs were observed as site-specific, exceptionally some occurring in Otto Lake sample, punctually in determined temperature. For instance, ASVs 1031 classified within *Methanosarcina* were only found in Killarney wetland soils and ASVs 956 and 1046 occurring only in Toolik wetland sample. Similarly in sediment lake was observed; ASV 991 occurred solely in Killarney Lake whereas ASV 1040 in Goldstream Lake (Fig. 5a).

Venn diagram was constructed to better visualize the shared ASVs between samples in in situ soil/sediments and acetate-amended microcosms at 5 and 20 °C. In the wetland soils, only two ASVs (both classified as *Methanosarcina*) were shared between in situ and incubated samples (at 5 and 20 °C), while 10 ASVs (five classified as *Methanosaeta*, four as *Methanosarcina* and one as *Methanoregula* genera) were shared between lake sediments. A total of 10 and 16 ASVs, classified within *Methanosarcina* genus, were shared between the 5 and 20 °C for wetland soils and lakes sediments, respectively (Fig. 5b). This result suggests an important shift between the natural sample and the incubation condition due to temperature and acetate concentration, mostly in wetland soils. On the other side, the persistent acetoclastic groups (*Methanosarcina* and *Methanosaeta*) could adapt to higher temperature (5–20 °C) within a psychrotolerant range and high acetate concentration, showing high stability.

Alpha diversity analysis (Shannon index) showed a decrease after acetate-amended incubations being significantly lower at 5 °C (t-test: $t = 3.00$, $p = 0.013$), 10 (t-test: $t = 3.3903$, $p = 0.0068$) and 20 °C (t-test: $t = 3.5755$, $p = 0.0050$) compared to the in situ samples (Online resource, Fig S2). Nevertheless, no significant changes were observed among microcosms at different temperatures (ANOVA $F_3 = 0.4625$, $p = 0.7116$). Bray Curtis PCoA

based on all ASVs abundance revealed that samples significantly clustered according to the ecosystem type (PERMANOVA, $r^2 = 0.64$, $p = 0.001$, Fig. 6b and c). Generally, a clear segregation between in situ and incubated samples was observed except for the Goldstream lake sediment (L4) exhibiting a very stable archaeal composition in situ and after incubation (whatever the temperature) (Fig. 6a, Online Resource, Fig S3a). In situ lake sediments displayed similar archaeal community structure while wetland soils highly diverged between each other, reflecting the different biome they belong to (i.e., Toolik (tundra) and Killarney (taiga/ boreal forest) (Fig. 6b, Online Resource 1, Fig S3b).

Discussion

Effects of increasing temperature and other environmental factors on acetate derived CH₄ production

As expected, higher temperatures (15 and 20 °C) reduced latency period and strongly affected MPRs resulting in a 4–sixfold increase of acetate-based MPR when compared to the lowest ones (5 and 10 °C). It is well reported that temperature is a limiting factor, defining metabolic functions and our results corroborates with other works performed in Arctic environment using incubation experiments (Høj et al. 2008; Blake et al. 2015; Herndon et al. 2015; Lofton et al. 2015). Despite significant difference among latency period (lag phase), some variations observed may have occurred due to sensibility of the chromatography type (GC-TCD) used in our work.

Q10 values showed positive correlation at the lowest temperature range (5–15 °C), mainly for the samples from Otto and Goldstream Lakes (Q10 > 6), showing strong temperature sensitivity, suggesting more energy input to methanogenesis than in the other ecosystems evaluated. These values can be comparable to those found in acetoclastic incubation experiments at low temperatures (Blake et al. 2015; de Jong et al. 2018). The overall methanogenesis dependence to temperature (E_a) was on average 0.82 ± 0.18 eV in lake sediments. This value fits within the universal E_a range defined by Yvon-Durocher et al. (2014) and also corresponds to similar values recently published by Lavergne et al. (2021) using the same protocol in sub-Antarctic lake sediments.

As mentioned previously, ecosystems investigated here were studied by Sepulveda-Jauregui and collaborators (2015) through field measurements of CH₄ emission associated with biogeographical and physicochemical features. They verified lakes Nutella and Otto as non-yedoma type peatlands, emitting lower CH₄ amounts than Goldstream and Killarney classified as yedoma type peatlands. Despite diverging in natural conditions, all lake sediments showed methane production at all tested temperatures under acetoclastic condition, reaching similar rates among them, around 5 $\mu\text{moles of CH}_4 \text{ g}^{-1} \text{ DW d}^{-1}$ at 20 °C. However, considering the methanogenesis stimulation by 30 mM of acetate and the temperatures tested, the CH₄ production was lower than other reported lake sediments. From the North Slope region (Alaska), microcosm amended with 2 mM of acetate at 4 and 10 °C, presented a higher MP of 4.7 $\mu\text{moles CH}_4 \text{ g}^{-1} \text{ DW d}^{-1}$ at 10 °C (de Jong et al. 2018). In a similar study of sub-Antarctic lake sediments stimulating CH₄ production with 30 mM of acetate within the same temperature range, CH₄ production are also almost 3-times higher than rates found in our study (Lavergne et al. 2021). It is reported high concentration of acetate can inhibit methanogenesis process at low pH (Brauer et al. 2004; Kotsyurbenko et al. 2019);, but it can booster the methane production at low temperature as observed by Allan et al. (2014) using the same acetate concentration (30 mM), with higher fluxes at 4 °C rather than 22 °C. Based on Lavergne et al. (2021) which observed an increase of 127-fold in acetate incubation regarding to unamended conditions, acetate might also have stimulated MPR in these Alaskan samples. Nonetheless, there are other factors linked to methane production, such as composition of soil/ sediment (mineral/ organic) and organic matter content (Chowdhury et al. 2015).

Despite a longer latency period, wetland soil samples yielded an average 3 times higher CH₄ than lake sediments, where the highest MPR was observed from Toolik soil bottom (W2 B; 10–20 cm depth), belonging to Tundra region. These samples presented the highest OM content among all samples evaluated, with strong correlation with MPR ($\rho > 0.8$). Shaver et al. (2014) reported soils ecosystems in Toolik Lake region, stating these soils typically have high OM content overlying a poorly developed mineral soil. Thus, along with temperature, substrate availability is crucial for CH₄ cycle in natural systems. Some works performed in different locations of the Arctic report that organic rich soils may yield higher amounts of CH₄ than mineral soils; Alaska (Chowdhury et al. 2015), Siberia (Ganzert et al. 2007), Norway (Høj et al. 2008).

Methanogens abundance and methane production

mcrA gene was detected in all samples by qPCR in an abundance between 10^7 and 10^8 copies. DW^{-1} , confirming the

presence of methanogens in all the samples. While previous works have shown that *mcrA* gene could be a good molecular marker for methanogenesis (Yang et al. 2018; Zhang et al. 2019; Lavergne et al. 2021), we found no significant correlation between *mcrA* gene abundance (in situ samples) and methane production rates (MPR), which is in line with Freitag and Prosser (2009). qPCR analyses were carried out based on DNA approach, which provide us a “general” information and potential, but not the functionally active genes. Franchi et al. (2018) evaluated *mcrA* gene in anaerobic digestion process under both approaches (DNA and RNA), concluding despite high abundance, low gene expression was observed and no correlation with MPR was found as well. Despite no positive correlation, the highest abundances of *mcrA* were observed in the Toolik soils (“bot- tom”- W2 B), also presenting the highest MPR, among wet- land samples.

Methanogenic microorganisms present in Arctic lake sediments and wetland soils

In situ samples harbored *Methanosarcinales* as the most abundant, exceptionally in Toolik wetland. In Alaska, *Methanosarcinales*, *Methanomicrobiales* were found as active members in upper layer of sediment lakes from near Barrow, North Slope (Matheus Carnevali et al. 2018) and in thawed active layer from Hess Creek (Mackelprang et al. 2011). *Methanoregula* was found as the most abundant in sediment lake samples and *Methanosarcina* and *Methanobacterium* accounted for more than 90% of all classified archaeal methanogens in wetland ecosystems in the Canadian Arctic (Frank-Fahle et al. 2014; Stoeva et al. 2014). Samples from Toolik wetland were the most diverse among all studied ecosystems. *Rice cluster II* lineage have been described as dominant phylotype in thawing permafrost in Sweden (Mondav et al. 2014) and boreal peatlands in Finland (Juottonen et al. 2015). Toolik soil also harbored most *Bathyarchaeia* members (*Crenarcheota/Bathyarcheota* phylum) and almost exclusively microorganisms belonging from the recently classified *Woesearchaeales* order and *Nitrososphaeraceae*. Moreover, high abundance of not yet described genera were observed in samples from this location, suggesting this environment can harbor many unknown archaeal microorganisms so far.

Acetate addition had a selective role in microcosms, which the diversity in all samples decreased and *Methanosarcina* prevailed as the most abundant group, exceptionally in the samples from Goldstream lake, where the in situ dominant *Methanosaeta* persisted after incubation. Killarney (L1), Otto (L2) and Nutella (L3), presented similar archaeal diversity patterns; higher abundance of *Methanosaeta* in situ shifting to *Methanosarcina* predominance in microcosms, with diverse *Methanosarcina* groups (ASVs) among environments. In natural environments, low concentration of acetate and low temperatures may favor the obligate-acetoclastic *Methanosaeta*, which is more competitive than *Methanosarcina* under these conditions (Kotsyurbenko et al. 2019; Stam et al. 2019). However, despite high abundance of *Methanosaeta* in situ, the generalist feature of *Methanosarcina* to use a broad range of substrates, could provide more robustness to outcompete *Methanosaeta*. Also, *Methanosarcina* can better tolerate high acetate concentration (De Vrieze et al. 2012; Stam et al. 2019), which could have influenced their predominance in these in acetate (30 mM) amended microcosm. A plausible explanation for *Methanosaeta* prevalence in samples from Goldstream Lake (mainly ASV 885) might be associated to the absence of *Methanosarcina* not detected in the in situ samples. Also, *Methanosaeta* might be metabolically active in environmental condition, since Goldstream natural temperature was the highest among lakes (about 20 °C); once active in situ, associated to very low abundance of *Methanosarcina*, may have led it to surpass and thrive on other members. Observed in a lower proportion than *Methanosarcinales*, *Methanoregula* and *Bathyarchaeia* was detected in acetate amended microcosms from Goldstream Lake. *Bathyarchaeia* (phylum *Bathyarcheota*), previously assigned as Miscellaneous Crenarchaeota Group (MCG) is a wide group, associated with anoxic environments, like deep seafloor (Chen et al. 2020), freshwater (Fillol et al. 2015) and peatlands (Brauer et al. 2020). Their physiology is still unclear and have been reported associated with several functions as acetogenesis and methane metabolism (Evans et al. 2015; Zhou et al. 2018) and acetate consuming under sulfate-reducing condition (Na et al. 2015). *Methanoregula* is hydrogenotrophic methanogen (Kotsyurbenko et al. 2019) but is also reported in low abundance in acetate amended incubation at 30 °C (*Methanoregulaceae*/WCHA2-08) (Blake et al. 2015). Acetoclastic methanogens can compete with the acetate oxidizing bacteria, which can convert acetate to H₂ + CO₂ (Demirel and Scherer 2008). Therefore, this process might be provided *Methanoregula* presence in acetate amended microcosms, even in a small proportion. PCoA performed using all the 16S rRNA genes sequences datasets (L1, L2, L3 L4, W1 and W2), showed clustered samples according to the ecosystem and not according to the microcosm temperature, indicating methanogenic communities were influenced mostly by ecosystem type. Environment rather than temperature influencing diversity were observed in wetland samples from California, USA (He et al. 2015), in lake sediment in Germany (Glissman et al. 2004), in Chinese Zoige wetland (Tibetan plateau) (Cui et al. 2015). This results also showed a persistence of some methanogens after incubation at warmer temperatures.

Our results corroborate with other works performed through acetate incubation. *Methanosaetaceae* family was found as the most abundant in sediment lakes from Alaska with low concentration (2 mM), at 10 °C (de Jong et al. 2018). From lake sediments in Norway, Blake et al. (2015) observed this shift where *Methanosaetaceae* was the most abundant before incubation, decreasing at 5 °C and *Methanosaetaceae* and *Methanosaetaceae* compounded major part of the community at 30 °C. On the other hand, our results contrast with a study in Canadian High Arctic permafrost where *Methanococcales* and *Methanomicrobiales* were found in acetoclastic incubations (30 mM as in our study) at 4 and 22 °C (Table 4).

We observed members of *Methanosarcina* and *Methanosaeta* can outcompete other genera at all tested temperatures in favorable acetoclastic condition, showing an adaptation from 5 to 20 °C. In lower proportion, ASVs related to *Bathymarchaeia* family was detected whether in situ as acetate amended microcosms. Despite being reported inhabiting cold environments, these found genera could represent new species, since their classification is still not completed at species level, revealing new groups in these environments.

Conclusions

The present work consisted in two main objectives: firstly, the characterization of four lakes (Killarney, Otto, Nutella, Goldstream) and two wetlands soils (Killarney and Toolik) in Alaska providing physicochemical and microbial profile data regarding methanogenic archaea. Second, evaluating acetate-driven methanogenic potential from these ecosystems aiming adapted acetoclastic archaea to increasing temperature and high acetate concentration. From natural environments, our work revealed diverse composition among studied sites, with strong implications in different biogeochemical processes. Furthermore, the high abundance of Archaea classified as “uncultured” or “not classified” claims for further investigation to explore new phyla. These data will complement previous works performed in these environments in Alaska (Martinez Cruz et al. 2015; Sepulveda-Jauregui et al 2015) to better understand the complex process of CH₄ cycle in climate changes scenario. From acetoclastic microcosms, *Methanosarcina* appeared as dominant in most of samples (lake sediments and wetland soils), exception in one lake sediment, where there was the prevalence of *Methanosaeta*, with different and site-specific methanogens groups adapted to a psychrotolerant temperature range (5–20 °C). In lower proportion, members of *Bathymarchaeia* were also observed in this condition. These enriched acetoclastic groups found may contribute to further investigation regarding the application potential of psychrophilic microorganisms in the optimization of industrial process, such as anaerobic digestion and methane generation at cold temperatures.

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Declarations

Conflict of interest The authors declare no conflict of interest.

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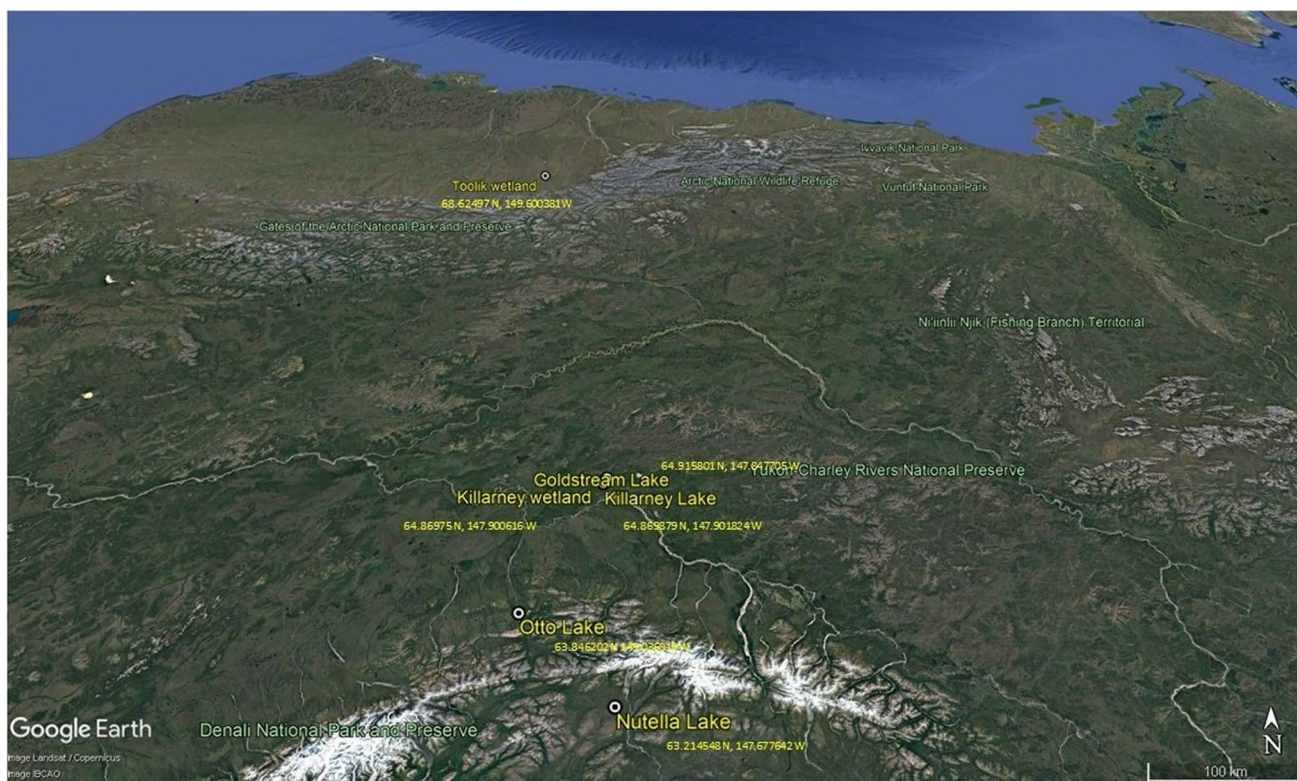


Fig. 1 Sampling spots location of four lakes and two wetland in Alaska: Lake Killarney (L1) and adjacent wetland (W1), Otto (L2), Nutella (L3), Goldstream Lake (L4), and in the north, one wetland within Toolik Lake area (W2) Source: Google Earth

Table 1 Characteristics of studied ecosystems in Alaska, US

Ecosystem	Samples	Coordinates	Location	Biome	Max Depth (m)**
Lake sediments	L1	64.869879 N, 147.901824 W	Killarney lake	Boreal forest	2.1
	L2	63.846202 N 149.036019 W	Otto lake	Tundra	3.1
	L3	63.214548 N, 147.677642 W	Nutella lake*		9.4
	L4	64.915801 N, 147.847705 W	Goldstream lake*	Boreal forest	3.3
Wetland soils	W1	64.86975 N, 147.900616 W	Killarney lake	Boreal forest	ND
	W2	68.62497 N, 149.600381 W	Toolik lake	Tundra	ND

*Informal lake names

**Source: Sepulveda-Jauregui et al. (2015); Barret et al. (2022)

ND not determined

Table 2 Physicochemical parameters measured in lake sediment and wetland soil samples

Location and samples identification	Depth (cm)	Temperature* (°C)	pH**	OM/DW (g)	DOC (mg.L ⁻¹)	Conductivity (mS/cm)	Nitrate+Nitrite (mg.L ⁻¹)	Sulfate (mg.L ⁻¹)
Killarney (L1)	0–10	10.11±0.17	7.07±0.01	0.09±0.01	21.80±0.19	1.73±0.02	0.02±0.001	2.18±0.52
Otto (L2)***	0–10	13.92	6.92	0.140	3.00	1.130	0.02	12.36
Nutella (L3)	0–10	12.42±0.14	6.14±0.17	0.17±0.11	4.84±2.99	0.47±0.29	0.02±0.009	0.07±0.01
Goldstream (L4)	0–10	19.95±0.31	6.8±0.09	0.17±0.07	12.25±0.33	1.59±0.49	0.01±0.01	4.17±3.71
Killarney (W1 T)	0–10	9.04±2.39	7.12±0.08	0.31±0.07	16.92±5.15	1.03±0.92	1.33±0.04	0.439±0.18
Killarney (W1 B)	10–20	4.15±1.20	7.23±0.01	0.090	21.05±0.76	0.32±0.36	0.99±0.14	0.370±0.09
Toolik (W2 T)	0–14	8.7±0.28	6.21±0.08	0.70±0.07	5.65±2.19	0.21±0.04	0.25±0.007	ND
Toolik (W2 B)***	14–20	3.9	6.37	0.71	7.16	0.14	0.11	ND

*Measured in situ (at the location in the moment that the sample was taken)

**Measured after extraction in water (1:4 w/w)

***Only one sample was analyzed (no replicates)

OM/DW (organic matter content per dry weight), DOC (dissolved organic carbon). The values are mean of sampling points, (field duplicates) for lakes (L1, L3, L4) and three points for wetland soils (W1, W2; T- top; B- bottom). ± indicate standard deviation between replicates

Fig. 2 Boxplot representing the influence of temperature on both CH₄ production rates (panel a) and latency periods (panel b) in acetate-amended microcosms from lakesediments and wetland soils. Type of freshwater systems was plotted by different shapes and the 6 ecosystems were represented by colors. For wetland soils, distinction was made between top (T) (disks) and bottom (B) (open circles) samples

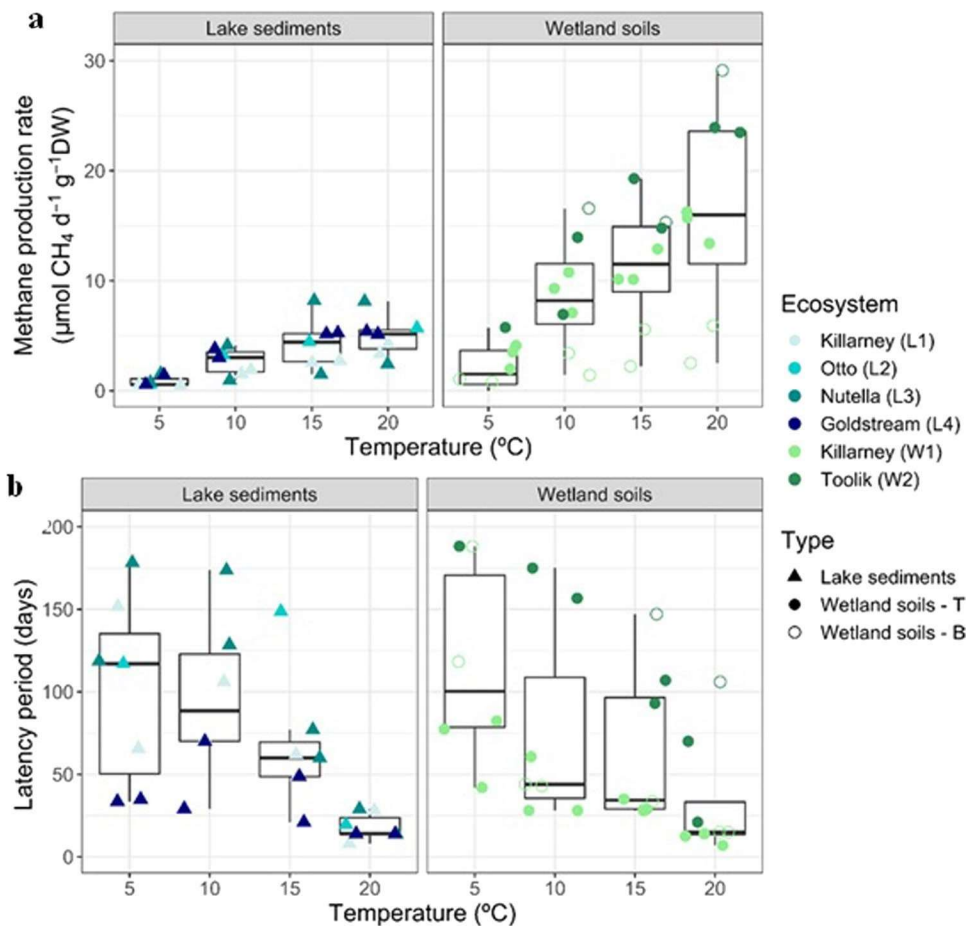


Table 3 Average Q10 coefficient and energy activation (Ea) of methane production rate, observed for lakes sediment (L1-L4) and wetland soils (W1 and W2). Depth of wetland soils is indicated by T (top) and B (bottom)

Ecosystem	Location and samples identification	Q10 (5–15 °C)	Q10 (10–20 °C)	Ea (kJ mol ⁻¹)	Ea (eV)
Lake sediments	Killarney (L1)	5.1±0.02	2.22±0.02	85.3±3.1	0.88±0.03
	Otto (L2)**	7.83	1.77	98.70	1.02
	Nutella (L3)	3.63±2.3	2.26±0.4	65.2±16.6	0.67±0.1
	Goldstream (L4)	6.44±3.8	1.56±0.2	78.3±25.9	0.81±0.2
	Killarney (W1 T)	3.74±1.3	1.7±0.2	67.8±16.5	0.70±0.1
Wetland soils	Killarney (W1 B)	4.59±3.4	1.75±0.01	65.3±33.4	0.67±0.3
	Toolik (W2 T)	3.35**	2.56±1.2	53.7±30.6	0.55±0.3
	Toolik (W2 B)*	ND	1.76	38.50	0.39

*ND Methanogenic activity was not detected at 5 °C

**Only one sample was analyzed (no replicates)

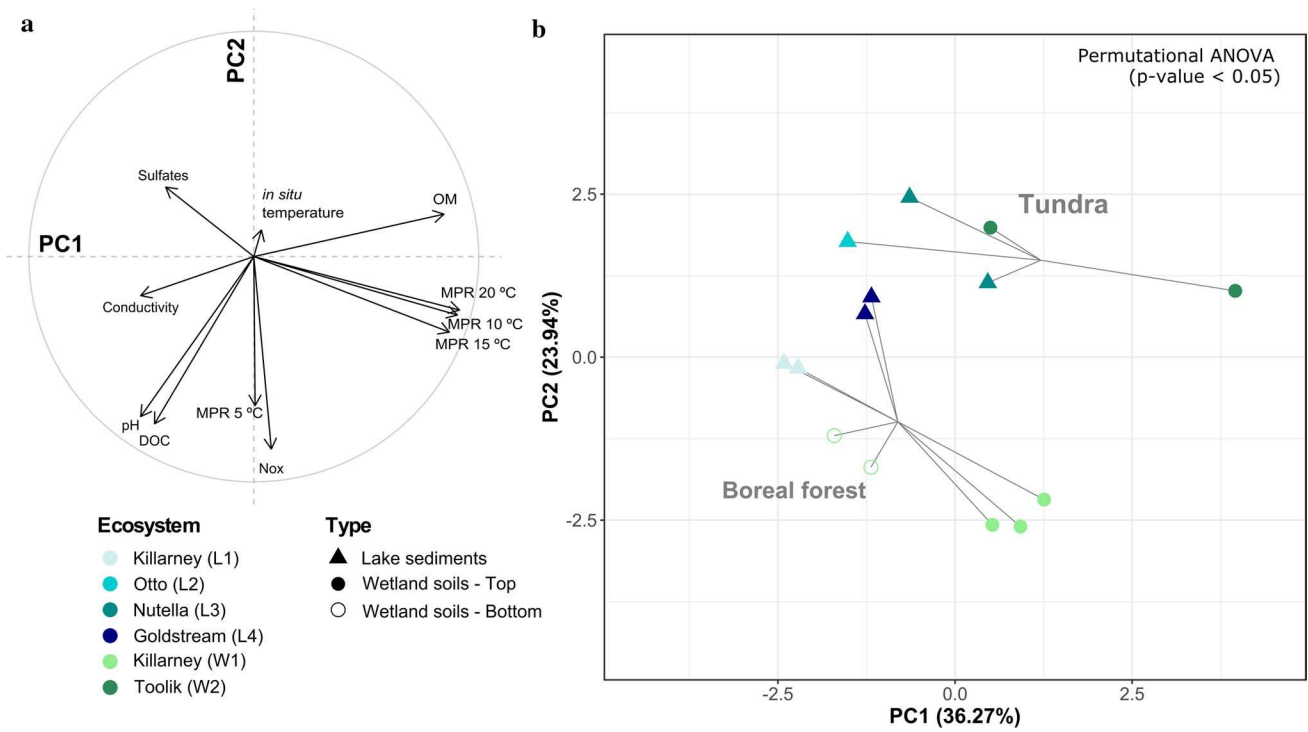


Fig. 3 Principal component analysis (PCA) performed with seven variables showing the correlation between CH₄ production rates (MPRs measured at 5, 10, 15 and 20 °C) and in situ samples physico-chemical data. Left panel **a** showed the correlation plot of variables and right panel **b** showed the site position on the ordination colored

by ecosystem, while the symbol shape represents the different ecosystem types. Significant segregation by biome (PERMANOVA, $F = 4.18$, p -value < 0.05) was plotted as spiders (95% confidence level). OM stands for organic matter and NO_x for nitrates + nitrites

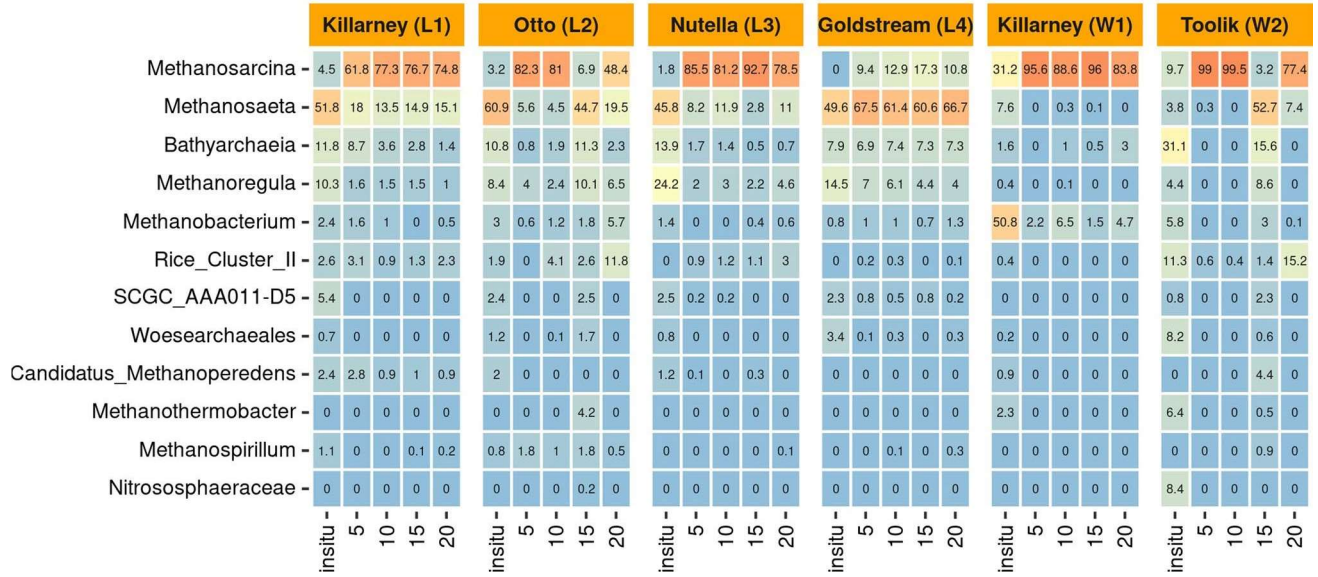


Fig. 4 Microbial composition according to archaeal 16S rRNA gene sequence analysis. Heat map showing the relative abundance at genus level in all samples: in situ and incubated at different temperatures 5, 10, 15 and 20 °C

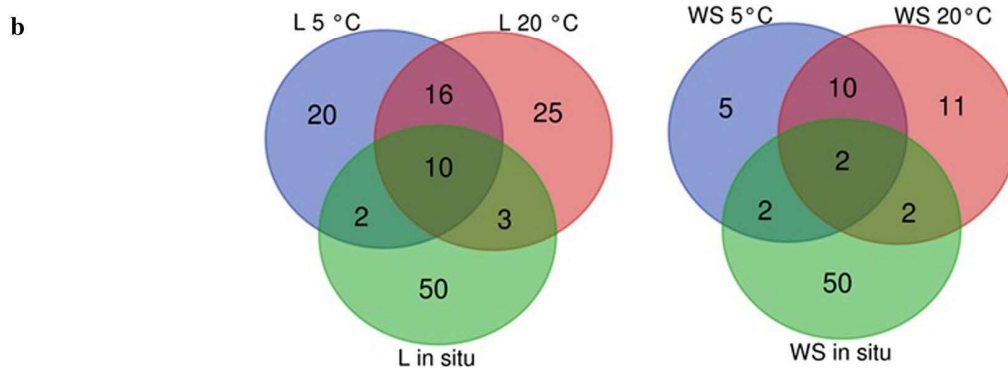
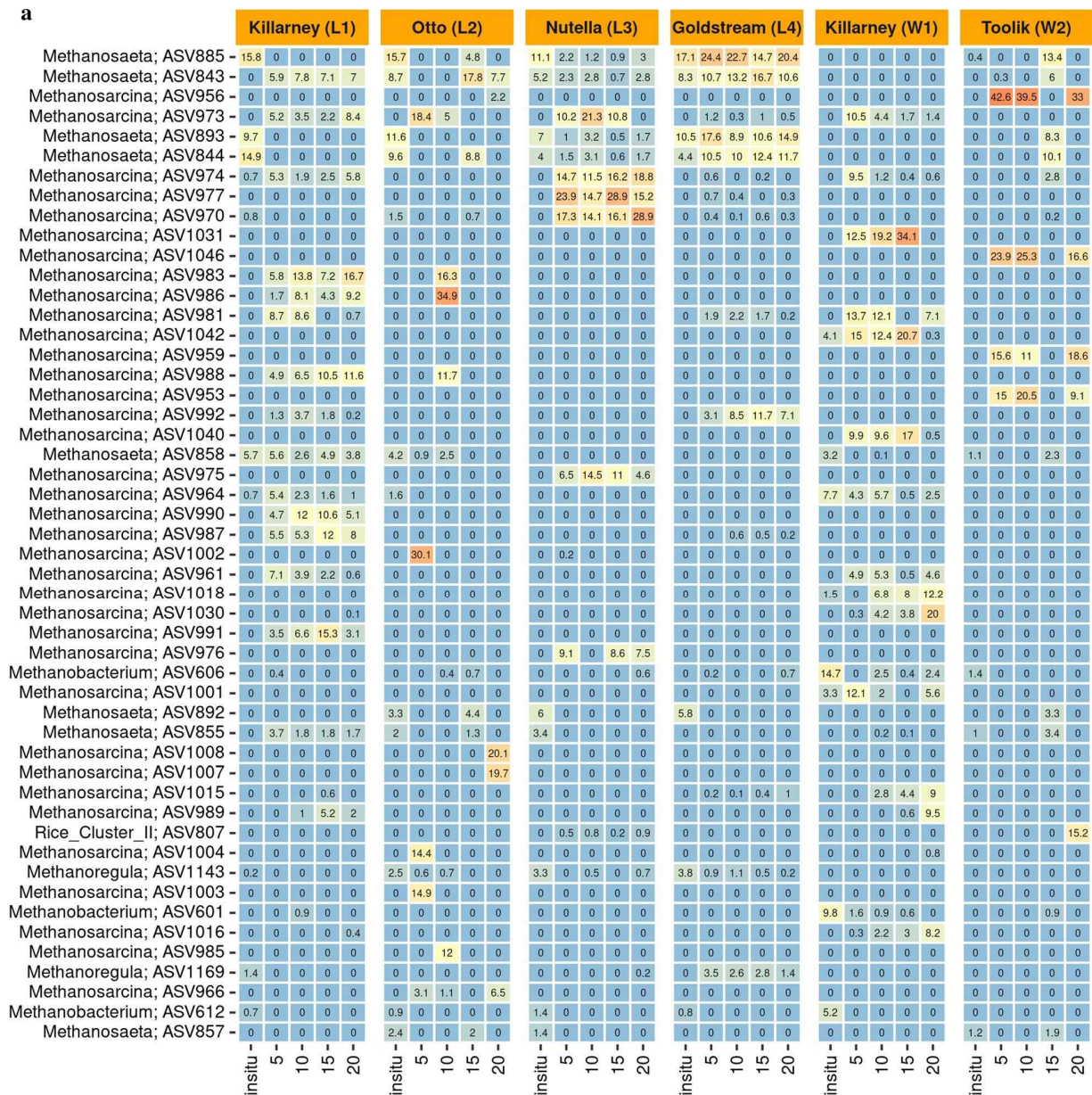


Fig. 5 a Heat map showing the ASVs relative abundance. In the heat-map a color code was used where the red means higher relative abundance and blue color means the lower relative abundance values; **b** Venn diagram showing the number of ASVs shared within the different samples including a comparison between in situ samples and samples taken after incubation at 5 and 20 °C. The samples were separated into sediment lake (L) and wetland (WS) samples

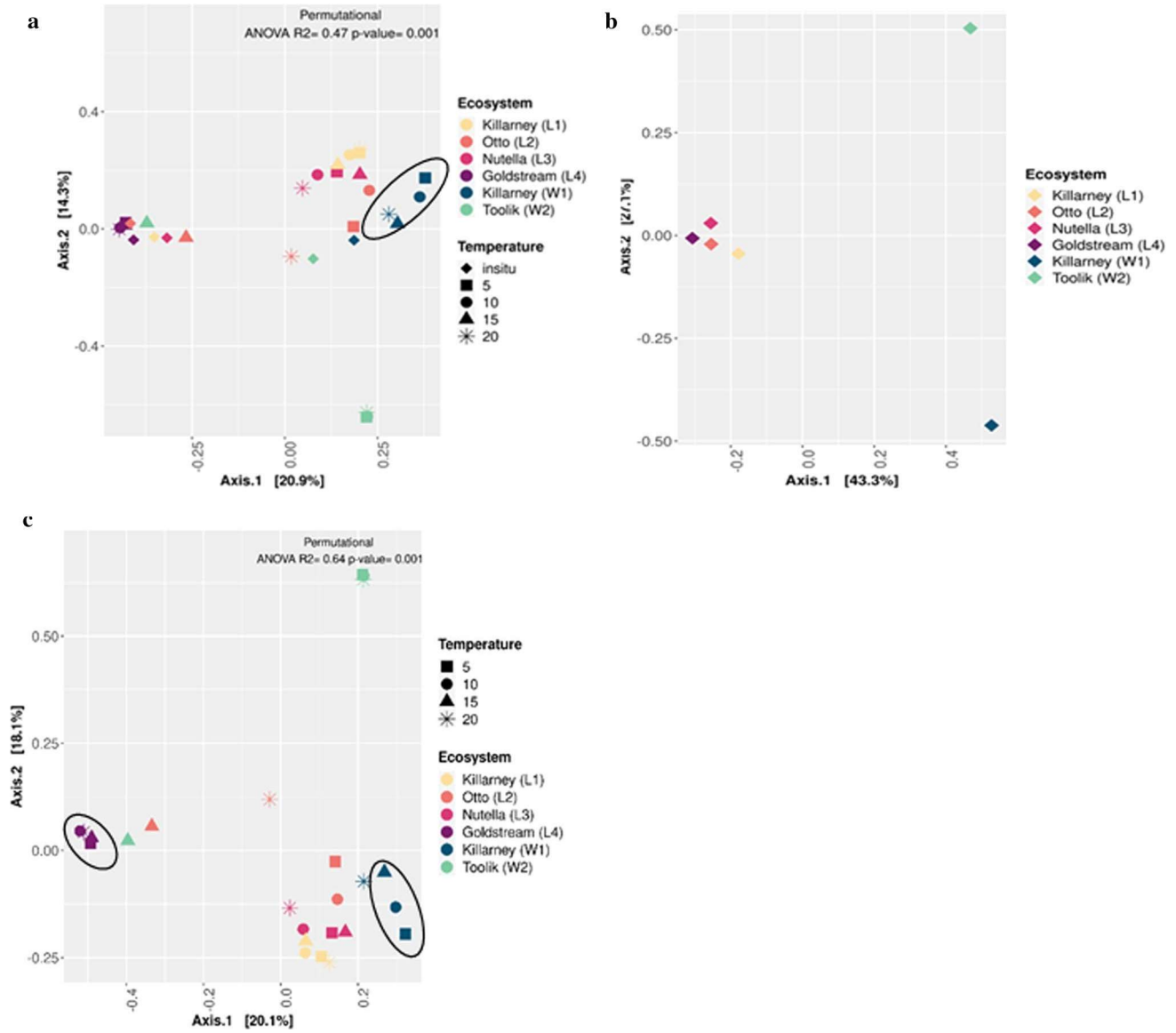


Fig. 6 Bray Curtis distance based principal coordinate analysis (PCoA) of archaeal ASVs from lake sediments and wetland soils from samples collected in situ and incubated at four different temperatures: **A** in situ and microcosms; **B** Only in situ; **C** Only microcosms at different temperatures (5, 10, 15 and 20 °C). Permutational mul-

tivariate analysis of variance (PERMANOVA) was used to identify the significant segregation between groups: A significant difference between W1, W2, L1, L2, L3, L4 (including in situ); C significant difference between W1,W2, L1, L2, L3, L4 (without in situ)

Table 4 Comparative table showing the archaeal microbial communities composition reported in Arctic and sub-Antarctic samples (in situ) and in samples taken after incubation at different temperatures in microcosms with the addition of acetate

Location	Archaea detected in the in situ samples	Archaea detected in microcosms	Temperature of incubation (°C)	Substrate amendment in the microcosms experiment	Ecosystem sampled	References
North Slope Alaska, USA	Rice Cluster II <i>Methanosaeta-ceae</i> (30%–31%)	<i>Methanosaetaceae</i> dominated after incubation at 4 and 10 °C followed by <i>Methanosarcinaceae</i>	4 and 10	Acetate 2 mM	Lake sediment	de Jong et al. (2018)
North Slope of Alaska, USA	<i>Methanosaetaceae</i>	<i>Methanosaetaceae</i> with a 1.7-fold stronger response at 10 °C	4 and 10	Acetate 14 mM	Lake sediment	Zandt et al. (2020)
Svalbard, Norway	The original sediment was dominated by the <i>Methanosaetaceae</i> group (66%)	<i>Methanosarcinaceae</i> (47%) and <i>Methanosaetaceae</i> (40%) dominate at 30 °C and <i>Methanoregulaceae</i> (50%) dominate at 5 °C	5 and 30	Acetate 10 mM	Wetland sediment	Blake et al. (2015)
Canadian High Arctic	<i>Nitrososphaerales</i>	<i>Methanococcales</i> dominates at 4 °C and <i>Methanomicrobiales</i> at 22 °C	4 and 22	Acetate 30 mM	Wetland	Allan et al. (2014)
Magallanes strait and Cape Horn, Chile	<i>Methanoregula</i> , <i>Methanobacterium</i> , <i>Methanosaeta</i>	<i>Methanosarcina</i>	5, 10, 15, 20	Acetate 30 mM	Lake sediment	Lavergne et al. (2021)
Center to north Alaska	<i>Methanosaeta</i> , <i>Methanobacterium</i> , <i>Bathyarchaeia</i>	<i>Methanosarcina</i> <i>Methanosaeta</i>	5, 10, 15, 20	Acetate 30 mM	Wetland and lake sediment	This work

