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Anaerobic oxidation of methane and associated microbiome in anoxic water of Northwestern Siberian lakes

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HIGHLIGHTS
• Anaerobic oxidation of CH$_4$ (AOM) was a major sink in the water of 4 Siberian lakes.
• AOM mitigated 60–100% of the produced CH$_4$.
• All four lakes shared the same predominant methanotrophs in AOM hotspots.
• AOM was attributed to Methylobacter and other Methylomonadaceae.
• Methanotrophs co-occurred with denitrifiers and iron-cycling partners.

GRAPHICAL ABSTRACT

Abstract

Arctic lakes emit methane (CH$_4$) to the atmosphere. The magnitude of this flux could increase with permafrost thaw but might also be mitigated by microbial CH$_4$ oxidation. Methane oxidation in oxic water has been extensively studied, while the contribution of anaerobic oxidation of methane (AOM) to CH$_4$ mitigation is not fully understood. We have investigated four Northern Siberian stratified lakes in an area of discontinuous permafrost nearby Igarka, Russia. Analyses of CH$_4$ concentrations in the water column demonstrated that 60 to 100% of upward diffusing CH$_4$ was oxidized in the anoxic layers of the four lakes. A combination of pmoA and mcrA gene qPCR and 16S rRNA gene metabarcoding showed that the same taxa, all within Methylomonadaceae and including the predominant genus Methylobacter as well as Crenothrix, could be the major methane-oxidizing bacteria (MOB) in the anoxic water of the four lakes. Correlation between Methylomonadaceae and OTUs within Methylotenera, Geothrix and Geobacter genera indicated that AOM might occur in an interaction between MOB, denitrifiers and iron-cycling partners. We conclude that MOB within Methylomonadaceae could have a crucial impact on CH$_4$ cycling in these Siberian Arctic lakes by mitigating the majority of produced CH$_4$ before it leaves the anoxic zone. This finding emphasizes the importance of AOM by Methylomonadaceae and extends our knowledge about CH$_4$ cycle in lakes, a crucial component of the global CH$_4$ cycle.

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1. Introduction

High-latitude lakes, mostly located in permafrost-dominated areas (Smith et al., 2007), are major sources of methane (CH₄) to the atmosphere. It has been estimated that lakes located above 50° of northern latitude are responsible for 18.8 Tg y⁻¹, i.e. 26% of total lake CH₄ emissions that are estimated to 71 Tg y⁻¹ (Saunois et al., 2019). Lake CH₄ emissions represent about 19% of non-anthropogenic global CH₄ sources in bottom-up estimations. According to the International Panel on Climate Change (IPCC, 2013), the global mean surface temperatures are projected to increase between 1.7 °C and 4.8 °C by 2100 and the climate is changing twice as fast in the Arctic (Cohen et al., 2014). Under the global warming scenario of +2 °C, the reduction of permafrost area by 40% due to thawing (Chadburn et al., 2017) will disturb the arctic biome hydrology through lake and pond formation, expansion, and drainage (Yonk et al., 2015). Permafrost region comprises 50% of the global soil organic carbon stock (Hugelius et al., 2014; Strauss et al., 2017) and therefore future thawing is likely to shift the balance of the global carbon cycle by making organic carbon available to microbial decomposition and conversion into CH₄ (McGuire et al., 2009; Wik et al., 2016). From the global mean radiative forcing of 2.83 W m⁻² (van Grinsven et al., 2020; Milucka et al., 2015; Oswald et al., 2016b; Rissanel et al., 2018) Out of four lakes, a niche-partitioning analysis conducted on methane-oxidizing bacteria concluded that some strains within Crocophilax and Methylothermobacter (Methylococcales) prefer oxygen-deficient conditions (Mayr et al., 2019). MOB could survive for a certain time under anoxic conditions without being active, between intermittent periods of O₂ availability (Blees et al., 2014). The microaerobic MOB Methylospira mobilis (Methylcoccales) has been recently isolated from northern wetland and shows adaptation to micro-oxic habitats. Its survival capacity to a wide range of O₂ concentrations has been attributed to low- and high-affinity oxidases (Oshkin et al., 2019). The recently proposed mechanisms underlying the hypoxia stress response in Methylobacter involves a complex interconnection between nitric oxide reductase, quorum sensing, the secondary metabolite tundraene, and methanol dehydrogenase functions (Yu et al., 2020). Until now, MOB activity has been evidenced in hypoxic and microaerobic pure cultures, but not in strictly anaerobic ones, to our knowledge. However, the metabolic basis and origin of oxygen for AOM by aerobic MOB within interacting microbiomes, has not been described. Furthermore, the contribution of this puzzling phenomenon to the CH₄ budgets in lakes remains to be estimated.

A Northern Siberian lake in which the methane oxidation rate in the anoxic water was high enough to fully mitigate the CH₄ flux from the bottom was recently reported (Thalasso et al., 2020). Here, we show that AOM substantially mitigated CH₄ in the anoxic waters of three neighbouring stratified lakes. By combining CH₄ diffusion-reaction modelling with in-depth analysis of the microbial key players, we provide the first evidence that Methylomonadaceae could act as a major CH₄ sink in anoxic lake water.

2. Material and methods

2.1. Field sites

Four stratified glacial lakes under thermokarstic influence were sampled in August 2016 (L1, L2, L3, L4) around Igarka, Russia (67.465°N, 86.578°E), on the eastern bank of the Yenisei river. The lakes are located in a discontinuous permafrost area (Streletsky et al., 2015) characterized by a patchwork of boreal forest, lakes and peatlands, including palsa complexes. Annual mean temperature is 8.3 °C, the annual precipitation is 495 mm and the elevation is around 55 m AMSL. The lake areas ranged from 0.7 to 6.5 ha, and the lake maximum depths ranged from 6 to 12 m.

2.2. In situ physico-chemical characterization

Before sampling, the water column was characterized at the center of the lakes at two replicate locations a few meters apart (A, B). Since L2 is formed by two distinct basins (Thalasso et al., 2020), a location from the second basin was added, approximately 200 m to the west and called location D. Dissolved oxygen (DO), with a detection limit of 10 μg L⁻¹, temperature, pH, conductivity and redox potential were measured with a multiparametric probe (HI 9828, Hanna Instrument, Woonsocket, RI, US).
Dissolved CH₄ concentrations along the water column were determined using a membrane-integrated cavity output spectrometry (MICOS) method (Gonzalez-Valencia et al., 2014). This method, described in more detail in the Supplementary material, is based on a gas-liquid equilibration module. Briefly, a continuous flow of water, pumped from the desired depth of the water column, is forced to equilibrate with a continuous flow of CH₄- and CO₂-free nitrogen, which is then measured with an ultraportable greenhouse gas analyzer (UGGA; Model 30P, Los Gatos Research, San Jose, CA, USA). After proper calibration, this method allowed for the continuous measurement of dissolved CH₄ at high-resolution, with a frequency of 1 s⁻¹. Thus, a weighted probe containing a water filter and connected to a water pump and the MICOS device was continuously lowered at constant speed, through the water column, which allowed for about 50 dissolved CH₄ concentration data points per meter of water column. The lower detection limit of the method under the present configuration was 5 nmol L⁻¹. Vertical CH₄ fluxes through the water column and the net methane production rate (NMPR) within the water column were derived from the estimation of turbulent diffusion of CH₄ across the concentration gradient according to the method established previously (Kankaala et al., 2006), also described in the Supplementary material.

As it will be shown in the results and discussion section, we observed in all lakes that the epilimnion and the hypolimnion were segregated by a layer of water column where dissolved CH₄ concentration was minimum. This layer, that acted as a buffer zone between hypo- and epilimnion, was called minimum methane zone (MMZ). From that observation, we characterized the CH₄ mass balance with four parameters. First, the total CH₄ oxidation in the hypolimnion was established by integration of the NMPR over the entire hypolimnion depth, expressed per unit of lake area, and identified as rMMZ hereafter. Second, the vertical CH₄ exchange rate between the epilimnion and the MMZ was established from the maximal flux determined at the interface between both layers and identified as r_EPI hereafter. Third, CH₄ and CO₂ fluxes from the lakes to the atmosphere were determined using the static chamber method at the surface of the lakes in a recirculation mode coupled to the UGGA (Gerardo-Nieto et al., 2017). These fluxes were included into the CH₄ mass balance and identified as r_ATM hereafter. Fourth, a mass balance over the epilimnion showed that r_MMZ and r_ATM, both being CH₄ output, must be compensated by an equivalent input. This input was also considered in the CH₄ mass balance, as the sum of r_MMZ and r_ATM, and identified as r_EPI hereafter. Details of the methods used to determine fluxes, r_ATM, r_MMZ, r_EPI, and r_ATM are provided in the Supplementary material.

### 2.3. Sampling and physicochemical analysis

Water samples (one from the oxic epilimnion and one from the anoxic hypolimnion) were collected at replicate points A and B in all lakes with a water sampler (2.2 L Van Dorn Bottle). Water samples were collected in the same way from eight different depths at point D in L2 (1, 2, 4, 5, 6, 8, 9, 10 m-depth). Superficial sediments were sampled using a mud-sampler. Water and sediment samples were kept at 4 °C for no >24 h prior to further processing.

The stable isotopes of dissolved methane (δ¹³C-CH₄ and δD-CH₄) and dissolved inorganic carbon (δ¹³C-DIC), dissolved organic and inorganic carbon concentrations, optical properties of dissolved organic matter, suspended solids, trace elements, major anion and cation concentrations, were analyzed in the water samples. The corresponding material and methods are described in Supplementary material.

### 2.4. Prokaryotic community analysis

#### 2.4.1. Sample preparation and DNA extraction

After prefiltration at 80 μm (nylon net filters, Merck Millipore, Cork Ireland), water samples from each site were filtered at 0.22 μm (nitrocellulose GSWP membrane filters, Merck Millipore, Cork, Ireland) until filter clogging, i.e. after 275 to 1480 mL of flow through, depending on lake and depth. The 0.22-μm filter was frozen at −20 °C until DNA extraction. DNA was extracted from the water filters and the sediment with the PowerWater and PowerSoil DNA isolation kits, respectively (MoBio Laboratories, Inc., Carlsbad, CA, USA). The DNA extracts were stored at −20 °C.

#### 2.4.2. Quantitative PCR

The abundances of bacterial 16S rRNA gene (total Bacteria), archaeal 16S rRNA gene (total Archaea), pmoA gene (encoding the beta subunit of particulate methane monoxygenase, phylogenetic marker for MOB) and mcrA gene (encoding the alpha subunit of methyl coenzyme M reductase, phylogenetic marker for methanogens and ANMEs) were estimated by quantitative PCR (qPCR). All measurements were performed in 20 μL duplicates with Taqly SYBR master mix (Eurogentec, Liège, Belgium) and 0.4 ng of template DNA, using a CFX96 thermocycler (Bio-Rad Laboratories, Hercules, CA, US). Primer sequences and concentrations, thermocycling conditions, and standard curve preparation are detailed in Supplementary Table S1.

#### 2.4.3. 16S rRNA gene sequencing

The V4-V5 region of archaeal and bacterial 16S rRNA genes was amplified from the DNA extracts using 515F and 928R primers (GTGYYCAGCMGCCCGCTA and CCCCCGCAATTCCMTTTRAGT) (Wang and Qian, 2009) and MTP Taq DNA polymerase (Sigma-Aldrich, Lyon, France). The thermocycling procedure was the following: 2 min denaturation at 94 °C, 30 cycles of 60 s at 94 °C for denaturation, 40 s at 65 °C for annealing and 30 s at 72 °C for elongation, followed by 10 min at 72 °C for final elongation. PCR products were sequenced using Illumina Miseq paired-end sequencing (2 × 250-bp). The nucleotide sequences have been deposited to the European Nucleotide Archive under BioProject code PRJEB36731, using the nomenclature detailed in Supplementary Table S2.

#### 2.4.4. Bioinformatics and statistical analyses

After demultiplexing, the reads were merged using Flash (Magoc and Salzberg, 2011), with a minimum overlap between the forward and reverse sequences of 10 base pairs and maximum mismatch of 1% in the overlapping region (Lluch et al., 2015). The sequence dataset was then processed using the FROGS pipeline (Escudie et al., 2017). Briefly, sequences were denoised and Operational Taxonomic Units (OTUs) were identified using Swarm (Mahé et al., 2014). Chimera sequences were then removed using vsearch. OTUs that were below 0.005% of the total abundance across the sample set were removed, as previously recommended (Bokulich et al., 2013). A taxonomic affiliation was assigned to each OTU using Blast (Altschul et al., 1990) against SILVA 132 (Pintail 80) rRNA database (Quast et al., 2013).

The OTU sequences of methanogenic Archaea and MOB were extracted. For methanogens, OTUs belonging to the following lineages were considered: Methanomicrobia, Methanococcoides, Methanobacteriia, Methanopyri and Verslauwearchaeia classes, and Methanosillaciicoccales and Methanofastidiosoideas orders. For aerobic MOB, OTUs belonging to the following lineages were considered: Methylococcaceae, Methylophilaceae, Methylothermaceae and Methylocaldiphilaceae families, Methylobacterium, Methylophilus, Methylocella, Methylocystis, Methyloferula, Methylosinus and Methylovirgula genera.

Two phylogenetic trees were constructed, one for aerobic MOB (including 15 MOB OTUs from this study), and one for methanogens/ANME (including 14 OTUs from this study). In these phylogenetic trees we included 45 and 43 reference sequences from the SILVA 132 database (Quast et al., 2013), representative of methanogens/ANME and MOB lineages, respectively. In addition, we included the two best environmental matches to our OTUs in BLAST. The selected OTUs and the reference sequences were aligned using SeaView 4 (Gouy et al., ...
2010) and maximum-likelihood phylogenetic trees were reconstructed using PhyML version 3 (Guindon and Gascuel, 2003).

All statistical analyses were conducted in R 3.4.3, using the vegan (Oksanen et al., 2013) and Phylsoeq (McMurdo and Holmes, 2013) packages. The OTU abundance matrix was normalized by rarefying to the lowest number of reads per sample (9105). Alpha diversity was assessed through the calculation of observed richness and Simpson index. The depth effect on alpha diversity indexes was evaluated by ANOVA (SpTank et al., 2010) and maximum-likelihood phylogenetic trees were reconstructed using PhyML version 3 (Guindon and Gascuel, 2003).

The analysis of CH₄ depth profiles using a diffusion-reaction model (Kankaala et al., 2006) allowed to determine the NMPR, which once integrated over the entire hypolimnion and expressed per unit of lake area, gave an AOM rate ranging from 1.29 to 307 mg CH₄ m⁻² h⁻¹, identified as r_AOM in Fig. 2 (see below). These AOM rates were higher in lakes showing higher maximum CH₄ concentrations (L3, L4). Interestingly, the rate of methane oxidation measured by van Grinsven et al. (2020) in laboratory incubations was also maximal in the anoxic water of lake Lacamas (27.6 mg m⁻² h⁻¹) and falls into our range. The CH₄ oxidation observed in the hypolimnion raises the important question of the oxic or anoxic nature of the process, although several arguments support AOM. First, the hypolimnetic CH₄ oxidation was found well below the oxic and the maximum CH₄ oxidation activity was always observed several meters below the oxic (2.9 to 5.2 m below), except in L3, where the maximum CH₄ oxidation took place 0.5 m below the oxic. Thus, where CH₄ oxidation was observed, the DO availability was, at the most, inferior to 10 µg L⁻¹, i.e., detection limit of the probe, and probably much lower. Second, assuming a DO transfer from the upper layer of the water column, the rates of CH₄ oxidation from 1.29 to 307 mg CH₄

A general upward decrease of dissolved CH₄ concentration was observed in the hypolimnion of all lakes, with contrasted patterns between lakes, but all supporting evidences of AOM. First, a minimum methane zone (MMZ) was observed in all lakes; i.e., zone of the water column where dissolved CH₄ concentration was lower than in the epilimnion and the hypolimnion. The latter implies that a downward CH₄ flux at the bottom of the epilimnion and an upward CH₄ flux in the hypolimnion were observed, MMZ acting as a diffusional barrier, illustrated in Fig. 2. The existence of a diffusional barrier in stratified lakes has been previously suggested (DelSontro et al., 2018b; Peeters et al., 1996; Thalasso et al., 2020), segregating the CH₄ cycling in the oxic epilimnion from the hypolimnion dominantly anoxic. The segregation between the CH₄ cycling observed in the epilimnion and the hypolimnion was also evidenced by the stable isotopic signature, with significantly higher stable isotopic signature of CH₄ in the epilimnion (δ¹³C-CH₄ = -47.8 ± 7.3, p < 0.0001, Kruskal-Wallis test) and lower fractionation factor (α = 1.04 ± 0.01, p < 0.0001) compared to the bottom of the lakes (Supplementary Table S3). These higher values of δ¹³C-CH₄ and lower α in the epilimnion might reflect a higher contribution of acetoclastic production of CH₄. Besides, the coupled increase of δ¹³C-CH₄ and δ¹⁴N-CH₄ (Supplementary Table S3) from the hypolimnion to the epilimnion also suggests a contribution of CH₄ oxidation to the epilimnion isotopic signature. The expected concentration profiles, assuming a simple diffusive flux from the bottom of the lakes to the MMZ, were clearly above the observed concentration profiles, in all lakes (Fig. 2A). Thus, a net CH₄ oxidation in the hypolimnion must be considered in order to explain the observed profiles. The existence of a diffusional barrier and AOM is particularly clear in L2, where CH₄ concentrations decreased to undetectable levels between 4 and 6.5 m depth, i.e. well below the oxicline (3 m). This interesting case of complete CH₄ mitigation in the anoxic layer is similar to that described in Lago di Cadagno (Milucka et al., 2015), Lake Zug (Oswald et al., 2016b), and Lake Lugano (Blees et al., 2014), all being large meromictic alpine lakes in Switzerland.

3. Results and discussion

3.1. Evidence of CH₄ oxidation in anoxic water

The depth profiles of DO showed that the four lakes were oxygen-stratified at the time of sampling, with a clear oxic and fully anoxic water underneath (Fig. 1). The oxicline was at 2.0, 2.5, 4.0 and 1.5 m depth in L1, L2, L3, and L4, respectively. The lakes were also thermally stratified, with temperatures ranging from 3.4 to 3.9 °C in the anoxic hypolimnion and from 15.6 to 18.3 °C in the oxic epilimnion (see Supplementary Table S3). In the four lakes, the maximal dissolved CH₄ concentrations ranged between 1.11 mg L⁻¹ (in L1) and 18.86 mg L⁻¹ (in L3), just above the sediment and decreased to approximately 0.01 mg L⁻¹ in the epilimnion (Fig. 1). The δ¹³C signature of CH₄ (δ¹³C-CH₄ = -79.2 ± 5.4‰) and apparent fractionation factor (α = 1.08 ± 0.01, Supplementary Table S3) revealed that CH₄ at the bottom of the four lakes originated mainly from hydrogenotrophic methanogenesis (Hornbrook et al., 2000; Whiticar and Faber, 1986). This was supported by the presence of methanogen communities in sediments and bottom water, which revealed that OTUs classified as acetoclastic methanogens (all in Methanoseta genus in the present dataset) accounted for only 10 ± 8% of the methanogen communities in the sediment (Supplementary Fig. S1).

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respectively) and at additional point D in L2 (grey). For microbial ecology, respectively. In lake L2, an additional pro
by independent measurements of field duplicates at some meters of distance (A, B) at the center of the lake, corresponding to the deepest zone of the lakes (light and dark red, respectively). In lake L2, an additional pro
field duplicates (A, B) at the center of the lake, corresponding to the deepest zone of the lakes (light and dark red, respectively). At additional point D in L2 (grey). For microbial ecology, field duplicates (A, B) of water samples were collected in the four lakes, at two depths (oxic and anoxic water), as indicated by the circles. In L2, additional water samples were collected with higher resolution at eight depths along pro...
The prokaryotic communities in oxic waters were significantly correlated with higher temperature and redox conditions (p < 0.001), and were characterized by higher abundance of photosynthetic Cyanobacteria (Fig. 3B; p < 0.009). The community structure in anoxic water correlated significantly with higher contents of dissolved CH4, Fe and Mn (Fig. 3A; p < 0.05).

### 3.3. ANME and Methylomirabilales are not major CH4-oxidizers

In the depth profile of point D in L2, qPCR assays revealed a maximum abundance of archaea at the bottom of the lake (9.6 m), with archaecal 16S rRNA gene copy number reaching 2.5 ± 0.1% of the total prokaryotic 16S rRNA gene copy number (Fig. 4A). The mcrA gene also reached its maximum abundance at 9.6 m, with the copy numbers representing 0.57 ± 0.13% of the total prokaryotic community. However, within the maximal CH4-oxidation zone (i.e. between 9 and 6.5 m), the mcrA gene was at the detection limit (<0.04% of the community), suggesting that neither ANME nor methanogens (through trace methane oxidation, Timmers et al., 2017) were involved in AOM in this lake.

ANME form lineages within the Methanomicrobia class. The phylogenetic comparison of reference ANME sequences and Methanomicrobia OTUs from the 16S rRNA gene sequencing confirmed that none of the OTUs detected in lakes 1, 2, 3 and 4 was closely related to ANME (Supplementary Fig. S2). The bacterial order Methylomirabilales, which includes the anaerobic methane oxidizer Candidatus Methylomirabilis, was not identified in the 16S rRNA gene libraries from any of the four lakes.

The qPCR and 16S rRNA gene sequencing approaches are both based on amplification reactions. PCR biases should be examined to avoid misinterpretations. The primer set targeting the mcrA gene for qPCR (mlas and mcrA-rev) fails to target ANME-2d sequences, as shown in Supplementary Table S4 and as previously reported (Vaksmaa et al., 2017). Nonetheless, this primer set has 100% coverage for the mcrA genes of other ANME lineages. The universal primer set used for 16S rRNA gene sequencing (515F and 928R) covered >98.5% of anaerobic methane oxidizers lineages: ANME-1a/1b, ANME-2a/2b, ANME-2c, ANME-2d and Ca. Methylomirabilis (Supplementary Table S4). Although the primers coverage was not 100% and was estimated on a limited number of taxonomically-afﬁliated sequences available in the databases, these analyses suggest it is unlikely that our combination of qPCR and sequencing approaches would miss major anaerobic methane oxidizers in this study.
Fig. 3. Microbial community structure. (A) Principal Component Analysis (PCA) of the filtered and normalized OTU abundance table of microbial communities along the water column in the four Siberian lakes (L1 to L4, indicated by the symbol shape) at different relative sampling depths (as indicated by the color gradient). Ellipses represent significant depth-based clustering (p-value < 0.002 by non-parametric MANOVA), after excluding L3, showing community stratification between oxic water, MMZ (methane minimum zone) and AOM zone (anaerobic oxidation of methane). Significant correlations between ordination and environmental variables are represented (envfit function, p-value < 0.05) (SUVA: specific ultraviolet absorbance; Cond: conductivity; FI: fluorescence index; DIC: dissolved inorganic carbon; T: temperature). (B) Identification of the 42 most discriminant OTUs explaining the community distribution on the ordination (envfit function, p-value < 0.012) labeled with their OTU number. Arrow color represents the OTU taxonnic affiliation at the phylum, family or genus levels.
Taken together, these results show that AOM in the anoxic waters of lakes L1, L2, L3 and L4 could not be attributed to ANMEs, methanogens, or members of Methylomirabilales.

3.4. Predominance of methane-oxidizing bacteria (MOB) in anoxic waters

MOB were more abundant in the AOM zones than in the oxic waters of all lakes (Table 1). Genes encoding the functional marker of MOB (pmoA) amounted to between 1.1 and 17.4% of the 16S rRNA gene counts in the AOM zones of all four lakes, which was 19 to 189 times higher than in the corresponding oxic waters (Table 1 and Fig. 4). In the 16S rRNA gene libraries, MOB OTUs represented from 3.6 to 21.8% of total prokaryotes in the AOM zone, which is 7 to 93 times higher than in the corresponding oxic waters of the four lakes (Table 1). Interestingly, in L2, the maximum MOB abundance (pmoA: 2.5% of total prokaryotes, and MOB OTUs: 6.5% of the community) was observed at 8 m depth, identified as the AOM hotspot (Fig. 2B).

The 16S rRNA gene sequencing revealed that MOB communities were not only stratified in terms of abundance but also in terms of composition (Fig. 4B). MOB OTUs in oxic water were dominated by a unique Methylomonadaceae sequence (OTU_1717), affiliated to Methyloparacoccus, reaching 38–93% of the MOB in this layer. It is recognized that γ-MOB are often favored by low temperature (Sundh et al., 2005) and outcompete alphaproteobacterial MOB at low CH4 and high DO (Amaral and Knowles, 1995; Chowdhury and Dick, 2013; Henckel et al., 2000), excluding MOB that oxidize atmospheric methane (Tveit et al., 2019).

In the MMZ of L2, the MOB community was small (<0.3% of the total prokaryotes, Fig. 4). Alphaproteobacterial MOB from Beijerinckiaaceae dominated this small fraction (Fig. 4B) and all five Beijerinckiaaceae members (OTUs 4110, 6215, 6978, 7183 and 8644) were affiliated to Methylobacterium. Facultative methanotrophy capacity has been reported for Methylobacterium organophilum and M. populi (Patel et al., 1982; VanAken et al., 2004), but these observations were later contradicted (Semrau and Dispirito, 2011). Thus, although it is uncertain whether Methylobacterium spp. can oxidize CH4, the observation of few obligate MOB in the MMZ was consistent with the undetectable levels of CH4 in that water layer.

In the AOM zone of all lakes, where MOB were the most abundant, sequences affiliated to Methylomonadaceae (γ-proteobacteria) accounted for >91% of the MOB sequences. The prevalence of aerobic γ-MOB in sediments (He et al., 2012; Martinez-Cruz et al., 2017; Rissanen et al., 2017) or at the oxic/anoxic interface of the water column (Table 2) was reported previously. The ability of Methylomonadaceae to oxidize CH4 in anoxic conditions was evidenced by incubation activity measurements coupled to DNA- or PLFA-stable isotope probing in sediment samples (Martinez-Cruz et al., 2017), or to fluorescence in situ hybridization and/or nanometer-scale secondary ion mass spectrometry in water (Oswald et al., 2015). However, in the water column, most studies reported maximal CH4 oxidation activity (measured in incubations under in-situ O2 concentration) at the oxycline or just above the oxycline (Table 2), concurring with maximal MOB abundance. Only two previous studies evidenced a maximum abundance of γ-MOB exclusively in anoxic water, together with maximal AOM activity.
In L2, the values refer to profiles with higher resolution (depths 1 and 2 in the oxic zone; depths 8, 9 and 10 in the AOM zone).

<table>
<thead>
<tr>
<th>Lake</th>
<th>Method of AOM activity determination</th>
<th>Depth of maximal CH₄ oxidation activity</th>
<th>Significance of AOM in CH₄ budget</th>
<th>Predominant MOB in the water column at max. abundance depth</th>
<th>MOB identification and quantification methods</th>
<th>Reference</th>
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<tr>
<td>L1</td>
<td>Oxic zone</td>
<td>Oxic</td>
<td>γ-MOB</td>
<td>CARD-FISH</td>
<td>16S rRNA gene sequencing</td>
<td>Oswald et al. (2016a)</td>
</tr>
<tr>
<td></td>
<td>Anoxic zone</td>
<td>Anoxic</td>
<td>Methylobacter</td>
<td>CARDS-FISH</td>
<td>16S rRNA and pmoA gene sequencing</td>
<td>Kallistova et al. (2018)</td>
</tr>
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<td>Incubations</td>
<td>Oxic/anoxic</td>
<td>Oxic</td>
<td>CARD-FISH</td>
<td>16S rRNA and pmoA gene sequencing</td>
<td>Oswald et al. (2016b)</td>
</tr>
<tr>
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<td>Oxic</td>
<td>γ-MOB</td>
<td>CARD-FISH</td>
<td>16S rRNA and pmoA gene sequencing</td>
<td>Mayr et al. (2019)</td>
</tr>
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<td>Pavin</td>
<td>Reactive transport modelling</td>
<td>Oxic/Oxycline</td>
<td>Methylomonadaceae</td>
<td>CARD-FISH and transcript sequencing</td>
<td>CARD-FISH and transcript sequencing</td>
<td>Biderre-Petit et al. (2011); Lopes et al. (2011)</td>
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<tr>
<td>Rotsee</td>
<td>Incubations</td>
<td>Oxic</td>
<td>γ-MOB</td>
<td>CARD-FISH</td>
<td>16S rRNA and pmoA gene sequencing</td>
<td>Oswald et al. (2015)</td>
</tr>
<tr>
<td>Brownie, Canyon</td>
<td>CH₄ stable carbon isotope δ¹³C CH₄</td>
<td>Oxic</td>
<td>Methylococcales, mainly Methylobacter</td>
<td>CARD-FISH</td>
<td>16S rRNA and pmoA gene sequencing</td>
<td>Mayr et al. (2019)</td>
</tr>
<tr>
<td>Lacamas</td>
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<td>Anoxic</td>
<td>Methylobacter</td>
<td>CARD-FISH</td>
<td>16S rRNA and pmoA gene sequencing</td>
<td>van Grinsven et al. (2020)</td>
</tr>
<tr>
<td>Alines-Mustajärvi</td>
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<td>Anoxic</td>
<td>Methylobacter</td>
<td>CARD-FISH</td>
<td>16S rRNA and pmoA gene sequencing</td>
<td>Rissanen et al. (2018)</td>
</tr>
<tr>
<td>Mekkojärvi</td>
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<td>Methylobacter</td>
<td>CARD-FISH</td>
<td>16S rRNA and pmoA gene sequencing</td>
<td>This study</td>
</tr>
<tr>
<td>L1 to L4</td>
<td>Diffusion-reaction modelling</td>
<td>Anoxic</td>
<td>Methylomonadaceae</td>
<td>CARD-FISH</td>
<td>16S rRNA and pmoA gene sequencing</td>
<td>This study</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Anoxic</td>
<td>Methylomonadaceae</td>
<td>CARD-FISH</td>
<td>16S rRNA and pmoA gene sequencing</td>
<td>This study</td>
</tr>
</tbody>
</table>

a Only when a comparison is possible between oxic/anoxic depths; i.e. when methane oxidation was assessed at O₂ concentrations that are representative of in situ conditions for each depth. The slash (/) indicates that equivalent activities were found at different depths.

b The slash (/) indicates that equivalent abundances were found at different depths.

c Potential AOM measured using CH₄ incubations in situ (or δ¹³C CH₄ incubations in the case of Kallistova et al., 2018), or without spiked substrate in the case of van Grinsven et al. (2020).

d CARD-FISH combined with nanoSIMS, targeting the following known CH₄-oxidizing groups: Gammaproteobacteria MOB (Mgama484 and -705 probes), AOM-associated archaea (AAA, AAAA-FW-641 and -834 probes), ANMEs (ANME-1-350, -2-538 probes).

e Similar in situ results were reported from three consecutive sampling years, however the complete profiles of MO activities and MOB abundances were only measured in 2013 samples: therefore, only the 2013 data are presented in the present Table.

f Only the complete campaigns (including both isotopy and microbial community analysis) are included in the present table.
in relative abundances of OTUs 112 and 349 contributed to the segregation of communities from AOM hotspots on the abundance pattern of differential OTUs (Fig. 6) and on the PCA ordination (p-value $b 0.012$; Fig. 3B), suggesting that CH$_4$ cycling shapes a large proportion of the microbial communities in these lakes.

OTU 112 was the overall most abundant MOB, representing 1.4 to 16.2% of the total number of sequences in the hypolimnia (Fig. 6). OTU 112 shared 99.5% sequence similarity with Methylobacter tundripaludum SV96 (NR_042107, closest cultured relative), which was isolated from Arctic wetland soil (Wartiainen et al., 2006) and identified as the most active MOB in anoxic arctic peat (Tveit et al., 2014). The OTU 112 sequence was identical (100% similarity) to sequences retrieved from sediments of large deep lakes: Lake Constance in Germany (EF101325), Lake Baikal in Siberia (GU911445.1) and Lake Onego in Russia (MH205693; Fig. 5B). AOM was observed in the sediments of these same lakes in other studies (Deutzmann et al., 2014; Pimenov et al., 2014; Thomas et al., 2018). Despite the different processes occurring in water and soil or sediment ecosystems, the main MOB OTUs found in the water column in this study have close relatives in wetland soil and sediment habitats. Furthermore, in the water column of several lakes, the genus Methylobacter was also the most abundant MOB at the oxic-anoxic transition (Table 2). Previously it was shown that phytophylotypes within Methylobacter can occupy different niches according to oxygen availability (Biderre-Petit et al., 2011).

While OTU 112 was most abundant, overall, OTU 349 outnumbered it in the anoxic water of L3 (up to 6.8% of the community). Interestingly, in lake L2, OTU 349 reached its maximum abundance at 8 m depth (3.7% of the community), just at the AOM hotspot (Figs. 2B, 6). OTU 349 had no close cultured relative. Its closest environmental sequence was derived from Lake Mizugaki water (AB754129, 99.7% similarity). OTU 349 shared 99.5% similarity with several environmental sequences from sediments of Lake Onego (MH205708), Fe-rich microbial mats (LN870874), ice covered Qalluuraq lake in Alaska (JN626674) and hypolimnion water from permafrost thaw pond in Canada (JN656780). Again, AOM activities were detected in most of these environments (He et al., 2012; Thomas et al., 2018).

OTU 1925, classified as Crenothrix sp., was present in the anoxic water of the four lakes, but at lower abundances than OTUs 112 and 349. It contributed to the segregation of communities from AOM hotspots on the abundance pattern of differential OTUs (Fig. 6) and on the PCA ordination (p-value $b 0.012$; Fig. 3B), suggesting that CH$_4$ cycling shapes a large proportion of the microbial communities in these lakes.
and 349 (<1.4% of the community). *Crenothrix* spp. are as-yet-uncultured multicellular and filamentous γ-MOB. Some *Crenothrix* were found to prefer oxygen-deficient niches (Mayr et al., 2019). Interestingly, Oswald et al. (2017, 2016b) demonstrated that γ-MOB in Lake Zug were active under oxic, sub-oxic and anoxic conditions. Despite their lower 16S rRNA gene abundance compared to other Methylomonadaceae, *Crenothrix* members were the major contributors to methane oxidation under oxygen-deficient conditions, as revealed by stable isotope labeling combined with nanoSIMS (Oswald et al., 2017).

Under hypoxia, AOM can be coupled to denitrification, within a single MOB organism, as experimentally confirmed in *Methylomonas denitrificans* (Kits et al., 2015b) and *Methylomicrobium album* strain BG8 (Kits et al., 2015a). Respiratory nitrate and nitrite reductases are encoded in over one third of Methylococcales genomes, including *Methylobacter* and *Crenothrix* species (Smith et al., 2018). Methane-dependent growth under nitrate-reducing conditions was experimentally demonstrated for *Crenothrix*, but evidence for in situ nitrate reduction is lacking (Oswald et al., 2017). In our study, it can be hypothesized that N-oxides could be used as electron acceptors by the three

Fig. 6. Left: Heatmap showing the relative abundance (log-transformed) of 81 OTUs identified by DESeq2 as differentially abundant (p < 0.001) between the AOM, MMZ and oxic zones, in the four Siberian lakes (L1 to L4). Right: Heatmap showing the corresponding logarithmic fold change (log2FC) estimated for each OTU between two depth zones. Within each lake, the sampling point (field replicate) is indicated by the letter A, B or D, and the relative sampling depth (normalized by maximal depth) is indicated by the last numeric digit in the sample names. Samples and OTUs are arranged by hierarchical clustering on the basis of their differential abundance patterns, using Bray-Curtis distance. For each OTU, the taxonomic affiliation is provided at the genus level, or at the highest available level (f: family; o: order; c: class; p: phylum).
predominant Methylomonadaceae MOB in the anoxic waters. Soluble nitrate concentration in the anoxic waters was below quantification limit (0.5 mg L\(^{-1}\) of N-NO\(_3^-\)) and nitrite concentration never exceeded 0.02 mg L\(^{-1}\) of N-NO\(_2^-\) (Supplementary Table S3). Since N-oxides are extremely reactive intermediary species with high turnover (Zhu-barker et al., 2014), these low concentrations do not conclusively exclude nitrate or nitrite as electron acceptors.

3.6. Potential reductase partnerships with Methylomonadaceae for performing AOM

The most significant discriminant OTUs (i.e. explaining community segregation along depth) were identified on the PCA (Fig. 3B) and the differentially abundant OTUs between AOM, MMZ and oxic zones were identified by DESeq2 (Fig. 6). Both approaches gave highly congruent results. Their taxonomy as well as their closest relatives are provided in Supplementary Table S5. Among these OTUs, the ones displaying significant correlation with the abundance of the main MOB OTUs ( Spearman correlation coefficient > 0.75) were presented in Table 3. Such correlations give insights into co-occurrence and co-variation of OTUs, but do not prove the existence of ecological interactions (Freilich et al., 2018; Peterson et al., 2020).

A high correlation was observed between the relative abundance of the main Methylomonadaceae and the discriminant Methyliphilaceae OTU 104 (Table 3, Supplementary Table S5), with 100% identity to the facultative methylotroph Methylotenera versatilis (Kalyuzhnaya et al., 2012). The co-occurrence of γ-MOB and Methyliphilaceae has been reported (He et al., 2015, 2012; Hernandez et al., 2015; Martinez-Cruz et al., 2017). OTU 104 could potentially serve as a partner for AOM by accepting electrons for N-oxide reduction, since the denitrification capacity of Methylotenera has been evidenced (Kalyuzhnaya et al., 2012; Oshkin et al., 2015).

In our ecosystems, several clues point at the coupling between CH\(_4\)- and Fe-cycles. In the AOM zone, the discriminant OTUs 3, 24 and 131 (Fig. 3, Supplementary Table S5, Table 3) co-occurring with the main Methylomonadaceae, were related to Rhodofex ferreducticans (100% similarity), Geothrix fermentans (99.7%) and Geobacter sp. (99.2%), respectively, all recognized as Fe-reducers (Coates et al., 1999; Finneran et al., 2003; Holmes et al., 2007). The addition of Fe-oxides has been documented to effectively stimulate CH\(_4\) oxidation by γ-MOB (Osvald et al., 2016b, 2016a), suggesting that Fe-reduction could shuttle the electrons produced by CH\(_4\) oxidation. Total Fe concentrations were higher in the AOM zones (up to 3194 μg L\(^{-1}\); Supplementary Table S3) and significantly correlated with the community structure (p < 0.03; Fig. 3A). The presumably important role of Fe in the anoxic water of the four lakes is supported by the significant correlation of the main Methylomonadaceae (OTUs 112 and 349) with OTUs 564 and 3251, identified as Candidatus Omnitrophus, which requires Fe for magnetosome biosynthesis (Kolinko et al., 2016). However, we acknowledge that measurements of Fe oxidation state would be needed to ascertain the availability of Fe-oxides.

Another possible way of coupling CH\(_4\)- and Fe-cycles relies on the regeneration of Fe-oxides that could serve subsequently as electron acceptors for AOM. OTUs 723, 313, 334 and 373 significantly co-occurred with the two main Methylomonadaceae (Table 3, Fig. 6). They were respectively related to Gallionella capsiferriformans, Sediiminibacterium, Sideroxydans lithrophilicus and Prokibacteraeaceae, recognized as Fe-oxidizing bacteria (Emerson, 2018; Fabisch et al., 2016, 2013; lino et al., 2015; Li et al., 2015; Wang et al., 2012) (Supplementary Table S5). Oxidized compounds could also be regenerated through an interplay of biotic and abiotic cryptic reactions (Melton et al., 2014; Zhu-barker et al., 2014; Postma, 1985). Organic matter can also serve to fuel AOM, either directly as terminal electron acceptors, or indirectly to regenerate more common oxidants (Bai et al., 2019; Reed et al., 2017). Here, organic acids could be produced by fermentative bacteria such as OTUs 1220, 1263, 1387 (Supplementary Table S5, Table 3, Fig. 6).

Even if it is now recognized that Methylomonadaceae are not restricted to oxic environments, monooxygenases responsible for CH\(_4\) oxidation catalysis do need oxygen. In the hypolimnion of the four lakes, oxygen might be present at concentrations below the detection limit (10 μg L\(^{-1}\)). On one hand, oxygen might be produced by oxygenic photosynthesis in apparently anoxic water. This hypothesis is supported by studies performed in the high-altitude meromictic Lago di Cadagno (Milucka et al., 2015) and in monomictic lake Rotsee (Osvald et al., 2015), which concluded that methane oxidation at similar depths (respectively 12 and 9 m) was light-dependent. In our study, sequences affiliated to oxygenic photosynthetic organisms accounted for 0.4 to 3.6% of the total sequences in hypolimnia samples, excluding L3 where they reached 26.9% (Fig. 6), distributed between Cyanobacteria (19 to 86%) and chloroplastic sequences. None of these photosynthetic OTUs exhibited an abundance pattern positively correlated with the main MOB OTUs. Oxygenic photosynthesis might partially explain the observed methane oxidation in the four lakes, but we showed in paragraph 3.1 that photosynthetic activity would not be sufficient to aerobically oxidize the whole amount of CH\(_4\). On the other hand, in the dark, biological intracellular dioxygen production has been documented through chlorite or nitric oxide dismutation (Ettwig et al., 2012) but, as previously mentioned, our dataset did not contain bacteria known to rely on nitric oxide dismutation for AOM, such as Methylomonas-like bacteria. Nevertheless, a wide phylogenetic diversity of NO dismutase (NOD) genes and homologues was recently found in various environments (Reimann et al., 2015; Zhu et al., 2017), suggesting that intracellular oxygen supplying is more widespread than previously thought.

4. Conclusion

In this study, the characterization of lake microbial communities in combination with high-resolution biogeochemical analyses were carried out to shed light on the water methane profiles observed in four Northern Siberian lakes. Three predominant γ-MOB, affiliated to Methylobacter, Crenothrix and unclassified Methylomonadaceae, were identified as potential key players in lake methane cycling, being highly abundant in the zones of maximum methane oxidation in the anoxic waters of the four lakes. The oxygen supply for fueling monooxygenase activity in Methylomonadaceae members residing in anoxic waters is still elusive, but hypolimnetic bacteria that co-occurred with the γ-MOB included putative Fe-oxidizing and Fe-reducing bacteria and denitrifying Methyliphilaceae. This suggests that AOM could result from a tightly interacting microbiome, possibly through cryptic biogeochemical cycling involving aerobic MOB, denitrifiers and iron cycling microorganisms. More research is needed to ascertain that the microorganisms identified in this DNA-based study were really active in the four lakes and to unravel the metabolic interactions, e.g. through metatranscriptomics and/or stable isotope probing approaches. It was previously suggested that Methylomonadaceae might have a role in AOM in thermally stratified high-latitude or high-altitude lakes. Our study corroborates this idea and further indicates that Methylomonadaceae-driven AOM could be widespread among stratified lakes and has an important ecological function for the regulation of atmospheric GHG emissions.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2020.139588.

Data availability

The nucleotide sequences have been deposited to the European Nucleotide Archive under BioProject code PRJEB36731, using the nomenclature detailed in Supplementary Table S2. Access to the sequences and metadata is facilitated through the georeferenced mARs database (https://ipt.biodiversity.aq/resource?r=methanobase&v=1.4).
Table 3
Correlations between the relative abundance of the main MOB (OTUs 112 and 349) and the relative abundance of discriminant OTUs (identified on PCA and by differential analysis) in the AOM zone of the four Siberian lakes. The range of relative abundances in the four lakes and Spearman correlation coefficients are provided in the table. Only OTUs displaying correlation coefficients higher than 0.75 with at least one of the two MOBs were shown. All p-values were <0.002. The taxonomy of the discriminant OTUs was confirmed by Blast and their potential function inferred from literature (Supplementary Table S3).

<table>
<thead>
<tr>
<th>Discriminant OTU number</th>
<th>Taxonomic identification</th>
<th>Putative function</th>
<th>Relative abundance (%)</th>
<th>Spearman correlation coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>OTU 104*</td>
<td>Methylotenera</td>
<td>Methylothroph, denitrifier</td>
<td>0.0-12.3</td>
<td>0.85</td>
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<td>OTU 24*</td>
<td>Geothrix</td>
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<td>0.78</td>
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<td>Fe-reducer</td>
<td>0.6-7.0</td>
<td>0.85</td>
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<tr>
<td>OTU 313*</td>
<td>Sediminibacterium</td>
<td>Fe-oxidizer</td>
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<td>0.75</td>
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<tr>
<td>OTU 334</td>
<td>Sideroxydans</td>
<td>Fe-oxidizer</td>
<td>0.8-10.0</td>
<td>0.75</td>
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<tr>
<td>OTU 373*</td>
<td>Prolixibacteraceae</td>
<td>Fe-oxidizer</td>
<td>0.0-1.0</td>
<td>0.73</td>
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<td>OTU 723*</td>
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<td>OTU 564*</td>
<td>Ca. Ommotrophus</td>
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<td>0.0-1.0</td>
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<td>OTU 3251</td>
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<td>0.0-5.7</td>
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<td>OTU 341</td>
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<td>0.0-1.2</td>
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<td>OTU 628*</td>
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<td>OTU 252</td>
<td>Syntrophus</td>
<td>Syntrophic VFA and aromatic compound oxidizer</td>
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<td>0.0-1.5</td>
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<tr>
<td>OTU 1387</td>
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<td>0.0-0.9</td>
<td>0.86</td>
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</tbody>
</table>

*The OTUs marked by a star were identified by both correlation with PCA ordination (emfit) and differential abundance pattern (DESeq2); the others were only identified by differential abundance pattern (DESeq2), with the respective significance thresholds selected for each method.

CRediT authorship contribution statement

Léa Cabrol: Conceptualization, Investigation, Methodology, Supervision, Writing - original draft. Frédéric Thalasso: Conceptualization, Investigation, Formal analysis, Writing - review & editing. Laure Gandois: Methodology, Investigation, Writing - review & editing. Armando Sepulveda-Jauregui: Methodology, Investigation, Writing - review & editing. Karla Martinez-Cruz: Methodology, Investigation, Writing - review & editing. Roman Teisserenc: Resources. Nikita Tananaev: Resources. Alexander Tveit: Writing - review & editing. Mette M. Svenning: Writing - review & editing. Maiaen Barret: Conceptualization, Investigation, Methodology, Supervision, Writing - original draft.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References


