Diverse sediment microbiota shape methane emission temperature sensitivity in Arctic lakes

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Diverse sediment microbiota shape methane emission temperature sensitivity in Arctic lakes

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Northern post-glacial lakes are significant, increasing sources of atmospheric carbon through ebullition (bubbling) of microbially-produced methane (CH4) from sediments. Ebulitive CH4 flux correlates strongly with temperature, reflecting that solar radiation drives emissions. However, here we show that the slope of the temperature-CH4 flux relationship differs spatially across two post-glacial lakes in Sweden. We compared these CH4 emission patterns with sediment microbial (metagenomic and amplicon), isotopic, and geochemical data. The temperature-associated increase in CH4 emissions was greater in lake middles—where methanogens were more abundant—than edges, and sediment communities were distinct between edges and middles. Microbial abundances, including those of CH4-cycling microorganisms and syntrophs, were predictive of porewater CH4 concentrations. Results suggest that deeper lake regions, which currently emit less CH4 than shallower edges, could add substantially to CH4 emissions in a warmer Arctic and that CH4 emission predictions may be improved by accounting for spatial variations in sediment microbiota.
At high latitudes, lakes and ponds are recognized as a large and understudied source of methane (CH$_4$), a radiatively important trace gas. Post-glacial lakes (formed by glaciers and receding ice sheets, leaving mineral-rich sediments) represent the largest lake area at high latitudes. Because of their areal extent, these lakes contribute to approximately two-thirds of the model-predicted natural CH$_4$ emissions above 50°N latitude. Their geochemistry and emissions are distinct from thermokarst lakes formed by permafrost thaw. With warming, permafrost thaw, and predicted increased precipitation, northern lakes are expected to receive more terrestrially derived carbon, likely increasing their carbon dioxide (CO$_2$) and CH$_4$ emissions.

Ebullition commonly accounts for >50%, sometimes >90%, of the CH$_4$ flux from post-glacial lakes, with the remainder primarily attributed to diffusion-limited hydrodynamic flux contributions. Bullition moves CH$_4$ rapidly from sediments directly to the atmosphere, typically bypassing microbial CH$_4$ oxidation in the water column. Incoming short-wave radiation and sediment temperature have been identified as strong predictors of ebullitive CH$_4$ emissions from sub-arctic post-glacial lakes on an annual basis, with higher temperatures increasing emissions during the ice-free season. However, the extent and drivers of spatial variability in this temperature response, particularly within lakes, are poorly understood.

To address this knowledge gap, we analyzed ebullitive CH$_4$ emissions over a 6-year period and collected underlying sediment cores in July 2012 from the littoral (edge) and pelagic (middle) locations of two shallow post-glacial lakes, Mellersta Harrsjön and Inre Harrsjön (Supplementary Fig. 1, Supplementary Table 1). These lakes are part of the Stordalen Mire complex, a hydrologically interconnected, discontinuous permafrost ecosystem encompassing post-glacial lakes and a mosaic palsa/wetland in approximately equal portions. The lakes contribute ~55% of the total Stordalen Mire ecosystem CH$_4$ loss, and are model sites for studying ebullitive CH$_4$ emissions via inverted funnel bubble traps at the lake surface. These ebullitive flux measurements were collected for the six summers from 2009 to 2014 every 1–3 days. Here, we analyzed 5126 ebullitive CH$_4$ emission measurements (Supplementary Table 2) from this previously published dataset for spatial patterns (edge vs. middle), and we linked these patterns to analyses of the microbiota and biogeochemistry in the underlying sediments.

**Results and discussion**

**Spatial variation in ebullitive CH$_4$ temperature sensitivity.**

Previous work has shown that annual ebullitive emissions are consistently higher from these lakes’ shallow littoral zones than their deeper pelagic cores, as expected since the shallow sediments experience higher temperatures for longer periods and also receive more substrate input from aquatic vegetation. However, assessing the temperature sensitivity of ebullition for the two lake zones in this study revealed a previously unnoticed significant difference, with ~3–5-fold higher temperature sensitivity in lake middles relative to edges (Fig. 1, Supplementary Table 3). The statistical significance of these differences was consistent across all edge-to-middle comparisons (within and between lakes) and was generally also robust to re-analyses considering three subsets of the data (removal of edge data at temperatures above those experienced by middles and removal of potential outliers at the highest and lowest temperatures experienced by lake middles, respectively), with one exception: Inre Harrsjön edges and middles were not statistically significantly different when higher temperature edge data were removed (Supplementary Table 3, Supplementary Fig. 2). Predicted future emissions from post-glacial subarctic lakes are based on current measurements of temperature responsiveness, which are dominated by ebullitive flux data from shallow lake zones because those locations currently experience a longer period of sufficient warmth for seasonal emissions than lake middles (~3 months relative to ~1 month). If, as suggested here by our spatially resolved emissions data, temperature responsiveness is substantively higher in the deeper sediments, then, as deeper regions warm and remain heated for longer before cooling off in the fall, future lake emissions would be greater than currently predicted. Thus, accurate CH$_4$ emission predictions rely on understanding the spatial heterogeneity and underlying causes of this temperature responsiveness.

Ebullition is controlled by CH$_4$ production (which is in turn driven by redox, substrates, temperature, and microbiota), consumption (driven by redox and microbiota), and the physics of bubble formation and escape (determined by sediment texture and overlying hydrostatic pressure, which is largely controlled by atmospheric conditions). Therefore, the edge-to-middle difference in temperature responsiveness of CH$_4$ ebullition could be partly due to differences in physicochemical characteristics (e.g., sediment texture, pressure, and redox), substrates (e.g., organic carbon), and/or microbiota (abundance, composition, and/or activity). Although differences in sediment texture were observed between the lake edge and middle in Mellersta Harrsjön, these differences were not consistent between lakes (Supplementary Fig. 3, Supplementary Table 4). Our previous work has shown higher and more variable ebullition rates during periods of dropping atmospheric pressure, but there were no differences in edge vs. middle locations. In terms of redox, we expect concentrations of terminal electron acceptors to be low, as the likely source would be runoff, and total sulfur and nitrogen did not correlate with ebullition rates by lake or location. In terms of measured substrates, carbon:nitrogen (C:N) ratios and bulk $\delta^{13}$C-TOC (indicative of vegetation composition) did not vary from edges to middles. Total organic carbon (TOC) varied by the lake, with similar concentrations observed between lake edge and middle in Mellersta and appreciably higher TOC in middle sediments in Inre Harrsjön. Carbon quality, as assessed by visual comparisons of organic matter composition, revealed coarse, less decomposed detritus gytta (organic-rich, peat-derived mud) in the edge sediments of both lakes, while middle sediments were characterized by fine-grained, generally more decomposed detritus gytta. Thus, higher temperature responsiveness occurred where there was lower potential substrate quality, suggesting that substrate differences do not readily explain patterns in CH$_4$ emission responses to temperature in edge vs. middle lake locations, although more detailed substrate analyses could further evaluate this in the future.

**Spatial differences in sediment microbial communities.**

Next, we sought to characterize differences in the microbiota that could contribute to the observed temperature response patterns in CH$_4$ emissions. We first used a 16S rRNA gene amplicon sequencing approach to characterize microbial community composition from the edge and middle cores from each lake (Fig. 2A, B, Supplementary Table 5). Although microbial community composition differed most significantly by depth within the sediment (Supplementary Fig. 4, Supplementary Table 6), as is typical for aquatic sediments, significant differences between lake edges and middles (Fig. 2C, PERMANOVA $p = 0.001$) revealed shared spatial patterns in microbiota and ebullitive CH$_4$ flux measurements, suggesting that microbiota could contribute to the observed temperature sensitivity in CH$_4$ emissions.

While total microbial abundances correlated most strongly with depth in the sediment and did not exhibit edge vs. middle...
differences ($p = 0.15$, Supplementary Fig. 5, Supplementary Table 7), an exploration of functional guilds revealed multiple significant edges vs. middle differences. Methanogens (defined here as populations from known methanogenic clades, Supplementary Table 5) were significantly more relatively abundant in lake middles than edges (Fig. 2D, ANOVA $p = 0.0001$). Combining relative abundances and quantitative polymerase chain reaction (qPCR) absolute abundances as a proxy for methanogen total abundances showed no significant difference between lake edges and middles ($p = 0.94$), likely due to the strong sediment-depth patterns of total cell numbers (qPCR abundances) in combination with the low relative abundances of methanogens (0.3–2.3%, consistent with other studies). Syntrophs have been shown to be upstream regulators of methane production, and they can be obligately mutualistic with methanogens, for example by producing hydrogen used in hydrogenotrophic methanogenesis. While syntrophy does not exhibit a strong phylogenetic signal, precluding its robust quantification here as a functional guild, the Syntrophaceae lineage, whose syntrophic potential was supported by reconstruction of a metagenome-assembled genome (MAG) (see below), did show significantly higher relative abundances in lake middle sediments relative to edges ($p = 0.047$). Aerobic methanotrophs, which are posited to have minimal impact on ebullitive CH$_4$ loss due to rapid bubble movement through sediment, were confined to the surface sediment layers as expected (Supplementary Table 8) and did not differ significantly in composition or relative abundance between edges and middles (ANOVA $p = 0.76$). Anaerobic methanotroph (ANME archaeal) abundances differed significantly between lake edges and middles (ANOVA $p = 0.014$) and were approximately one order of magnitude higher in edge sediments (Supplementary Tables 8 and 9). Although this could suggest that increased anaerobic methane oxidation in the edge sediments could contribute to the observed differences in temperature sensitivity, these ANME archaea comprised only 0.1% of the community on
average (range 0.0–0.6%, Supplementary Tables 5 and 8), and again, ebullition is expected to largely bypass methane oxidation. Still, future work to further constrain ANME activity at different temperatures may help to elucidate the significance of the observed patterns in ANME abundance.

Sediment incubations and modeling of CH₄ production. To more directly evaluate the CH₄ production potential of the microbial communities in these sediments at different temperatures, we performed 48 ex situ anaerobic incubations of edge and middle sediments collected in 2012 (linked directly to our microbial and biogeochemical data) and 2013 (from the same four core locations) (Supplementary Table 10). These incubations at 5 and 22 °C showed that the lake-middle sediments had significantly higher CH₄ production potentials than lake-edge sediments at both temperatures (Fig. 3), paralleling their higher methanogen and syntroph relative abundances and indicating that the lake-middle methanogens can remain metabolically active at higher temperatures, despite never yet experiencing them in situ. However, it is important to note that a difference in temperature responsiveness (i.e., different CH₄ production potentials at 5 °C, relative to 22 °C) was not observed for the edge or middle sediments (ANOVA p = 0.38 for edges, p = 0.91 for middles), as might have been expected from the in situ ebullition data (Fig. 1). One potential explanation for this is that the ex situ incubations measured relatively immediate CH₄ production when the entire sediment was maintained at a certain temperature, whereas the in situ measurements linked temperatures at the sediment/surface interface with ebullitive CH₄ flux measurements at the lake surface. Thus, the measured in situ temperatures were somewhat disconnected from the temperatures (and times) of CH₄ production, and their associated flux measurements represent CH₄ production potential integrated over the time scales of bubble generation and flow through the sediment and water column, as observed over multiple years.

Since lake surface ebullition measurements represent CH₄ production integrated across depths in the underlying sediment, we applied isotope and mass balance calculations to partition CH₄ production to its likely source depths, in order to link CH₄ production to depth-discrete microbiota. Based on stable carbon isotope values and porewater concentrations of CH₄ and dissolved inorganic carbon (DIC), we inferred total CH₄ loss (fugitive CH₄) at each depth interval (Supplementary Table 4).
Microbial metabolic predictions and ties to biogeochemistry.

To more specifically investigate links between CH4-associated microbial functional guilds and CH4 chemistry, we identified multiple known CH4-cycling clades in the 16S rRNA gene amplicon data and applied targeted metagenomic sequencing to a subset of samples to examine diagnostic genes for CH4 cycling (and to assemble genomes for metabolic pathway reconstructions, discussed further below). From the metagenomes, using hidden Markov models (see Methods), we recovered 5470 sequencing reads with high similarity to phylogenetically diverse functional genes indicative of CH4 production (mcrA) and consumption (pmoA) potential (Supplementary Fig. 6, Supplementary Table 12). The mcrA data, together with 16S rRNA gene abundances of specific lineages of known hydrogenotrophic and acetoclastic methanogens, support δ13C isotopic calculations in indicating that hydrogenotrophic methanogenesis was the dominant methanogenic pathway in these lake sediments (Supplementary Fig. 7).

Using partial least squares regressions (PLSR) and multiple linear regression (MLR) analyses, we predicted porewater CH4 concentrations from methanogen and methanotroph relative abundances, as measured via 16S rRNA gene amplicon sequencing data. Although the modeled fugitive CH4 concentrations might have provided a better depth-resolved link to CH4 production than the porewater CH4 concentrations analyzed here, we opted to use two direct measurements (microbiota and porewater CH4) in these statistical models, rather than essentially modeling a model. When using either PLSR or MLR to predict porewater CH4 concentrations, a better prediction was achieved when both depth-resolved abiotic variables (i.e., depth, TOC, DIC, δ13C-TOC, S, and TOC:TS, see Methods) and the relative abundances of predicted CH4-cycling organisms were included (PLSR: $r^2 = 0.640$, $p = 0.00001$, MLR: adjusted $r^2 = 0.752$, $p = 0.0003$), relative to including the abiotic variables alone (PLSR: $r^2 = 0.390$, $p = 0.002$, MLR: adjusted $r^2 = 0.532$, $p = 0.0004$) (Fig. 4A, B, Supplementary Table 13). These results suggest that direct measurements of microbial abundances could contribute to more accurate predictions of future CH4 emissions, consistent with previous statistical models that have linked specific microbiota to C- and/or CH4-cycling dynamics in marine ecosystems and thawing permafrost peatlands.

By expanding our PLSR analyses to consider the full microbial community, in addition to known CH4 cycles, our ability to predict CH4 concentrations improved further. This analysis considered the following groupings of 16S rRNA gene abundances as explanatory variables for the prediction of porewater CH4 concentrations: (1) each operational taxonomic unit (OTU) at >1% relative abundance in any sample (Supplementary Table 5), (2) summed lineage abundances of all bacteria and archaea (mostly at the phylum or class levels, see Supplementary Table 15 and 16), thus organization into MAGs (a population genome computationally reconstructed from shotgun metagenomic community DNA sequencing data, Supplementary Table 14). In two cases (for Aminicenantes MAG Bin 1/OTU 4 and a Methanomassiliicoccales-related Thermoplasmata, MAG Bin 16/OTU 27), a MAG was linked directly to a specific OTU in the amplicon data through a co-binned 16S rRNA gene sequence in the MAG, such that the MAG relative abundance could be inferred from the amplicon data. In all other cases, the summed abundances of amplicon OTUs in the same lineage as the MAG were used as proxies for MAG abundances. A total of 153 potential explanatory variables were considered in this PLSR analysis, 26 of which, including methanogen and methanotroph abundances, were identified as significant predictors of porewater CH4 concentrations (Fig. 4C).

Four of the top five microbial groups most predictive of porewater CH4 concentrations in the PLSR analysis were lineages for which we were able to reconstruct a MAG (Fig. 4C, Supplementary Tables 15 and 16), thus organization into MAGs helped to unravel the specific metabolic processes most predictive of carbon chemistry. In total, five MAGs were reconstructed with >85% completeness and <6% contamination (Supplementary Discussion). The best overall predictor of porewater CH4 concentrations was the Syntrophaceae class of Deltaproteobacteria, which was considered in the PLSR analysis as the summed abundance of all OTUs in this clade. Consistent with a syntrophic metabolism, including hydrogen production (e.g., in support of hydrogenotrophic methanogenesis, which is the dominant methanogenic pathway in these sediments, as described above), the Syntrophaceae MAG revealed 15 hydrogenase-associated genes, along with the capacity to ferment diverse carbon compounds (particularly carbon-sulfur compounds), with the added potential capacity for respiration (see Supplementary Discussion).

Though the Syntrophaceae lineage was overall most predictive of porewater CH4 concentrations, the most significant predictive single OTU was a member of the candidate phylum Aminice- nantes, which we also recovered as a MAG. While this lineage has been previously predicted to be fermentative, saccharolytic, and/or aerobic, our lake sediment genome revealed the metabolic potential for several C1 metabolic processes, including methanol through the assimilation of methylamines, methane-thiols, and/or dimethylsulfide, similar to previous recoveries of complete
Wood–Ljungdahl pathways for C1 metabolism via carbonyl and methyl pathways in this lineage\textsuperscript{43}. The predicted capacity for methylotrophy could explain the strong correlation between Aminicenantes relative abundance and porewater CH\textsubscript{4} concentrations.

The relative abundances of two other lineages with MAGs, the Thermoplasmata (a group of Archaea) and Phycisphaerae (a class of Planctomycetes bacteria), were also strongly predictive of both porewater CH\textsubscript{4} concentrations in the PLSR analysis and of calculated fugitive CH\textsubscript{4} in linear regressions (Supplementary Tables 11 and 16). Phylogenetic analyses showed that the Thermoplasmata Bin 19 MAG was derived from a divergent member of the Thermoplasmatales order, and it encodes the capacity for CO\textsubscript{2} production from a formate, along with peptide and amino acid degradation (as previously indicated\textsuperscript{44}) and complex carbon degradation. Our recovered Phycisphaerae

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**Fig. 4 Partial least squares regression (PLSR) statistical modeling to predict sediment CH\textsubscript{4} concentrations.** PLSR analyses tested the ability of different suites of explanatory variables to predict measured sediment CH\textsubscript{4} concentrations in the four cores from 2012 across depths ($n = 21$); in all models, all measured abiotic variables (except those related to CH\textsubscript{4} concentrations, see Methods) were included as explanatory variables, and biotic variables were added as indicated. Biotic variables included relative abundances of specific OTUs and/or summed OTU abundances grouped by taxonomy or predicted metabolism (as indicated), from 16S rRNA gene amplicon data. a Correlation coefficient ($r^2$) for PLSR models predicting sediment CH\textsubscript{4} using different combinations of explanatory variables. Each bar represents a single underlying data point, with the value of that point indicated by the bar height along the y-axis. b Linear regression of measured and model-predicted sediment CH\textsubscript{4}, considering all abiotic variables and methanogen and methanotroph abundances as explanatory variables; error band represents 95% confidence interval; each point is a sample, colored by core. c For the model with the highest $r^2$ (rightmost in panel a), all significant explanatory variables are shown ($VIP$ scores $> 1$, $n = 26$ significant explanatory variables out of 153 total variables considered). VIP scores show the relative contribution of each variable to the model, with higher VIP scores indicating a more significant contribution. Each bar represents a single underlying data point, with the value of that point indicated by the bar height along the y-axis.
population genome appears to have the capacity to metabolize a wide variety of complex carbon compounds, potentially via fermentation, consistent with previous predictions for the Planctomycetes phylum. While direct ties to CH$_4$ are not obvious in these two genomes, we speculate that their contributions to overall carbon cycling, potentially including through fermentative contributions of CO$_2$ as a substrate, may be driving these strong correlations with both CH$_4$ concentrations and modeled fugitive CH$_4$.

Interestingly, the only lineage represented by a MAG that was not a significant predictor of porewater CH$_4$ concentrations in the PLSR analysis was classified as a member of the Methanomassiliicoccales, an archaeal lineage presumed to consist exclusively of obligate H$_2$-dependent methylotrophic methanogens. We hypothesized that this lake sediment population did not have the capacity for methanogenesis, as we did not recover genes from the methanogenesis pathway in this 95% complete genome. Further analysis of this MAG, reported in a separate paper, revealed that this population is not a member of the Methanomassiliicoccales but rather is part of a new, unrelated branch of the Methanomassiliicoccales within the Thermoplasmata. All 12 MAGs that we analyzed from this new order and related lineages (including 11 MAGs from other ecosystems, mostly anoxic sediments) were found to lack >20 methanogenesis biomarker genes that are present in Methanomassiliicoccales genomes, including mcrA. Instead, the lake sediment MAG reported here is predicted to conserve energy through amino acid metabolism. While mcrA sequences that were putatively phylogenetically assigned to Methanomassiliicoccales were recovered from metagenomic reads in our GraPhM analysis here (Supplementary Table 12), these genes were presumably derived from other OTUs classified as Methanomassiliicoccales in our amplicon data, some of which may be true methanogens. These results highlight the value of reconstructing near-complete genomes for a better understanding of metabolism, along with the limitations of using 16S rRNA gene sequences to infer metabolic processes.

In conclusion, we found significant differences in the slope of the temperature vs. CH$_4$ flux relationship between sub-arctic lake edges and middles, suggesting that radiative forcing (temperature) and a concomitant increase in microbial metabolic rates are not the only primary controls on CH$_4$ emissions. These edge vs. middle differences were shared by underlying sediment microbial communities, suggesting that differences in sediment microbial community composition (i.e., not simply differences in microbial activity) contribute to spatial differences in the response of CH$_4$ emissions to increasing temperature. Specifically, we observed significant differences in microbial community composition between lake edges and middles, including significantly higher methanogen and Syntrophaceae abundances in lake middles relative to edges, and CH$_4$ emissions from lake middle sediments were significantly higher than from lake edges when incubated at the same temperatures. In addition, the relative abundances of CH$_4$-cycling organisms and their reconstructed population genomes (MAGs) were significantly better predictors of sediment CH$_4$ concentrations than abiotic variables alone. Syntrophic lineages, which can generate the hydrogen required for hydrogenotrophic methanogenesis, and lineages capable of C degradation to CO$_2$ (also potentially “upstream” of methanogenesis) were also predictive of sediment CH$_4$ concentrations. Together, these results suggest that when lake middles reach the temperatures of lake edges, they may emit even more CH$_4$ than the lake edges currently do, such that our projected future CH$_4$ emissions may be underestimated contributions from subarctic lakes, and that knowledge of microbial community composition and metabolism could improve these predictions. Future investigations that consider the combined effects of microbiota, carbon quality, and temperature on lake CH$_4$ emissions, including further exploration of sediment depth-resolved contributions to total CH$_4$ emissions across multiple locations and years, will help to provide a more comprehensive understanding of spatiotemporal controls on global CH$_4$ emissions.

**Methods**

**Field site and sample collection.** Stordalen Mire is a subarctic peatland complex located in central Swedish Lapland east of Abisko in northern Sweden (68°21′N, 19°07′E). Lakes Mel- lersta Harrsjön and Inre Harrsjön are 1.1 and 2.3 ha in area, reaching maximum depths of 7 and 5 m, respectively. These lakes are post-glacially formed, and, unlike some thermokarst lakes at high latitude, they do not have underlying permafrost or seas that emit geologic CH$_4$; the CH$_4$ is biologically derived.

Mellersta Harrsjön receives water from a small stream, while Inre Harrsjön is fed through groundwater and runoff from the surrounding mire. Ebbullitive and diffusion-limited CH$_4$ emissions from these lakes have been documented using floating funnels and chambers distributed across the lakes, and sampled frequently. Ebbullition varies spatially with higher emissions from shallow zones and in the presence of plants.

We collected quadruplicate sediment cores (four cores from two locations in each of two lakes: Mellersta Harrsjön east (68°35′38″N, 19°04′06″E) and middle (68°35′29″N, 19°04′12″E) and Inre Harrsjön east (68°35′38″N, 19°04′35″E) and middle (68°35′41″N, 19°04′56″E)) on July 10 and 18, 2012 at the Stordalen ecosystem reserve, a research site near Abisko, northern Sweden (Supplementary Table 1). Samples were taken from cores (as described below) along a depth gradient (ranging from 4 to 40 cm) for geochemical measurements and microbial DNA sequencing data.

**Geochemical data collection and analysis.** For each set of four cores, we sampled the first core for sediment C, N, and S (weight percent), percent TOC, and bulk sediment 14C and 13C of CH$_4$ and CO$_2$ on a continuous-flow Delta S isotope ratio mass spectrometer (IRMS), based on calibration with acetanilide, Atlantic cod, black spruce needles, sorghum stalks, and soil standards (UNH Stable Isotope Lab). In 2013 we also collected 15N-cod, 15N-black spruce, and 13C-sorghum ratios mass spectrometer (IRMS), based on calibration with acetanilide, Atlantic cod, black spruce needles, sorghum stalks, and soil standards (UNH Stable Isotope Lab). In 2013 we also collected sediment cores in the same locations in these lakes. We report sediment textural analyses from these cores as % sand, % silt, and % clay (Supplementary Table 4).

**Sediment porewater sampling.** Sediment porewater samples were collected from the sediment core at each replicate core (Supplementary Table 1). A 1 cm syringe core was taken at 0 cm increments from the top of the core to the bottom. The samples were then dried, ground, and split into an untreated sample for total carbon (C) and an acidified TOC sample. Details regarding sample preparation for measurement on a Perkin Elmer 2400 Series II CHNS/O Elemental Analyzer at the University of New Hampshire (UNH) were described previously. Repeatability error was established by analyzing replicate samples and calculating the standard deviation. Duplicate samples were run approximately every 10 samples. Potential outliers were also run in duplicate. Isotopic analysis was performed by combusting dried sediment samples in a Costech ECS 4010 elemental analyzer coupled to a Thermo Trace GC Ultra isotope ratio mass spectrometer (IRMS), based on calibration with acetanilide, Atlantic cod, black spruce needles, sorghum stalks, and soil standards (UNH Stable Isotope Lab). During the isotopic analysis, we sampled three cores for CH$_4$ and DIC. The third replicate core was used for the measurement of DIC. Black spruce samples were prepared by 2 cm through-drilled holes in the core and a vacuum was pulled with a 30 ml polypropylene syringe. The first ~1 ml of sediment water was discarded because of contamination with DI water. After 10 ml of sediment pore water was collected, it was injected into a 30 ml evacuated serum vial with 1 ml 30% H$_2$PO$_4$ solution. This caused forms of inorganic C in the water to form CO$_2$. A headspace sample was then evacuated and run on an infrared gas analyzer to determine the CO$_2$ concentration.

**Sediment bulk density and temperature data.** Samples for measuring ebbullition and water temperature have been described previously, and the ebbullitive CH$_4$ flux and temperature data analyzed here are a subset of those previously reported. In brief, measurements of CH$_4$ bubble flux
DNA extraction and 16S rRNA gene sequencing. A fourth replicate core was collected for DNA extraction. After coring, we pulled 2 cm sediment plugs using cut plastic syringes through pre-drilled holes cut at 4 cm increments along the core liner. Samples were immediately put in Eppendorf tubes and placed in a cooler until returned to the research station where they were stored at −80 °C until extraction.

For DNA extraction from each core depth range, 0.25 g of sediment was collected under sterile conditions and added to a MoBio PowerSoil DNA Isolation Kit (MoBio, Inc., Carlsbad, CA, USA). DNA was extracted according to the manufacturer’s instructions. PCR amplification and sequencing were performed at the Environmental Sample Preparation and Sequencing Facility at Argonne National Laboratory, in accordance with previously described protocols32–34. Briefly, 515F and 806R primers with Illumina flowcell adapter sequences were used to amplify the V4 region of bacterial and archaeal 16S rDNA genes. Each 25 µl PCR reaction contained 12 µl of PCR water (MoBio, Inc., Carlsbad, CA, USA, 10 µl of 1 x 5 PRIME Hot Master Mix (5 PRIME Inc., Bethesda, MD, USA), 1 µl each of F and R primers (5 µM concentration, 200 pM final), and 1 µl of template DNA. PCR cycling conditions were as follows: 94 °C for 5 min, 35 cycles of 94 °C for 30 s, 55 °C for 60 s, and 72 °C for 90 s, followed by 72 °C for 10 min. A PicoGreen assay (Life Technologies, Grand Island, NY, USA) was used to measure amplification concentrations. Equimolar concentrations for each barcoded sample were combined and then cleaned with the UltraClean PCR Clean-Up Kit (MoBio Inc., Carlsbad, CA, USA) and then quantified using the Qubit (Invitrogen, Carlsbad, CA, USA). DNA was then diluted to 2 nM, denatured, and then diluted to a final concentration of 4 nM with a 10% PhiX spike for sequencing on the Illumina MiSeq platform.

Quantitative PCR (qPCR). A quantitative polymerase chain reaction (qPCR) was performed to measure microbial abundances in units of 16S rRNA gene copies per g wet sediment35,36. Each reaction used 5 µl of 2x SYBR Green PCR Master Mix (Applied Biosystems, Carlsbad, CA, USA), 4 µl of template DNA, and 1 µl of primer mix. The 16S rDNA gene 1406F/1525R primer set (0.4 µM, F-GYACWCACCGCCCGT and R-AAGGAGGTGWTCCARCC) was designed to amplify the V6-V8 region of bacterial and archaeal 16S rDNA genes. The rpsL primer pair (0.2 µM, F-CTACACTGAGGTTAGCGT and R-AGCCCTGCTTACGGTCTTA) was designed to amplify bacterial 16S rRNA genes and used to quantify bacterial rpsL copies with a standard curve. The qPCR reaction contained 12 µl of 2× SYBR Green PCR Master Mix (Applied Biosystems, Carlsbad, CA, USA) and then quantified using the Qubit (Invitrogen, Carlsbad, CA, USA). DNA was then diluted to 2 nM, denatured, and then diluted to a final concentration of 4 nM with a 10% PhiX spike for sequencing on the Illumina MiSeq platform.

16S rRNA gene sequence processing and OTU table generation. Sequences were processed as previously described34. Briefly, after demultiplexing by sample, each pair of forward and reverse 16S rDNA gene reads was merged. Sequences were then quality-filtered, and singletones were removed with QIIME37 and UPARSE38. Dereplicated sequences were then clustered at 97% nucleotide identity using UCLUST v798 to generate a database containing one sequence for each OTU. Sequencing reads from the full dataset were then clustered to the database to generate an OTU table. Each OTU was assigned taxonomy via the Ribosomal Database Project taxonomic classifier39,40. Taxonomy assignments were considered as mitochondria or chloroplasts were removed. The resulting OTU table was rarefied to 3000 16S rDNA gene sequences per sample. Following this OTU table curation, 36 samples across 21 core-depth combinations were retained, of which 30 replicates were i.e., 15 pairs). For each pair of replicates, each OTU count was averaged (for 14 pairs, replicates were indistinguishable. Supplementary Fig. 1, and the OTUs were used for all downstream analyses. For the six samples without successful replicates, OTU counts from a single sample were used.

Metagenomic sequencing and bioinformatics. Based on preliminary 16S rRNA gene amplicon sequencing data from eight samples (H1M4, H1M36, H1E4, H1E8, H1M14, H1M34, H1E44, and H1M16), which generate CO2 and do not fractionate carbon, while V5 (CH4, CH4.MG) and V4 (CH4, CH4.MG2) were mapped to contigs using BWA v0.7.12-r103902, and the mean coverage of contigs was obtained using the ‘coverage’ command of CheckV v1.0.6.57. Genomes were binned using MetaBAT v0.26.38 with all five preset parameters (very sensitive, sensitive, specific, very specific, super specific), and genome completeness and contamination were estimated using CheckV59. To investigate predicted metabolic functions of interest in the metagenomic data, metagenomic reads with sequence similarity to genes diagnostic of specific metabolic functions (e.g., methane monooxygenase, pmoA, and methyl-coenzyme M reductase, mcrA, indicative of aerobic methane oxidation and methanogenesis, respectively) were identified using hidden Markov models via GraPhM v1.060.

Incubations for CH4 production rates. Anaerobic incubations of lake sediment samples were performed to assess rates of production of CH4. Four replicate sediment samples were pretreated from three depth ranges (−0.5, 1.5, 5, 10, 20 cm) from each lake and immediately sealed in a 240 ml serum vial. The headspace was immediately flushed for 5 minutes with Ultra High Purity (UHP) N2 in the field (the replacing the headspace 20 times) to establish an anaerobic headspace. The vials were stored in coolers, taken to the research station, flushed again with N2 before incubations began in the laboratory, and then stored in a refrigerator, and then stored in a refrigerator. Incubations were performed to determine sediment dry weight. We also report data from incubations in 2013 that were treated the same way with samples collected at depths consistent with changes in core sediment transitions: Inre Harrsjön edge: 2.5, 27.5, 47.5 cm; Inre Harrsjön middle: 4.5, 35, 60 cm; Mellersta Harrsjön edge: 7.5, 22.5, 37.5 cm; and Mellersta Harrsjön middle: 2.5, 27.5, 47.5 cm.

Calculations of depth-resolved fugitive CH4. Depth-resolved fugitive CH4 (total CH4 released from the sediments, including ebullitive and diffusive CH4, though ebullition is by far the dominant CH4 production pathway in these sediments, accounting for 80–88% of total emissions12) was calculated from the concentration and stable carbon isotope composition of CH4 and DIC in sediment porewater60. This approach leverages the fact that (1) microbial fermentation and respiration, (2) DIC, and (3) CO2, do not fractionate carbon, while (4) CH4, which generates CH4 and CO2 (1:1), does fractionate carbon, and (5) DIC largely remains dissolved in water, while dissolved CH4 escapes porewater by ebullition. In this framework, the measured isotopic composition of CH4 in porewater was used to calculate the fractionation factor associated with methanogenesis, assuming the standard isotopic composition of CH4 in porewater is used to determine the concentration of CH4 in porewater. We calculated the fractionation factor associated with methanogenesis, along with the measured isotopic composition of DIC in porewater, was used to determine the relative amount of DIC that came from methanogenesis vs. non-fractionating pathways (e.g., fermentation). Because any CO2 produced was assumed to stay dissolved in porewater, the relative contribution of methanogenesis to total CH4 production could be multiplied by the measured concentration of DIC to determine the concentration of CO2 and CH4 generated through methanogenesis. This generated CH4 concentration was larger than the actual measured concentration of CH4 in porewater, and the difference between the two was assigned as ‘fugitive’ CH4. Calculations assumed that the system was at a steady state.

Statistics and reproducibility. Homogeneity of regression among groups in Arhusen plots (Fig. 1 and Supplementary Fig. 2) was tested using pairwise full factorial analyses with JMP statistical software (SAS Institute Inc., Cary, NC). The significance level of the group interaction of lake zone by temperature interval was 0.05 (Supplementary Table 3).

Unless otherwise indicated, other statistical analyses were performed using Python 3.761. The rarefied OTU table was square-root transformed, and Bray-Curtis similarity matrices were generated for sample comparisons and used to make a Principal Coordinates Analysis (PCoA) plot. We used permutational
ANOVAs (PERMANOVA) to test for significant differences in microbial community composition between categorical groups of samples (e.g., between the two lakes and between the edges and middles of the lakes), and we used Mantel tests with Spearman’s rank correlations to compare microbial community composition (Bray–Curtis similarity matrices) to continuous variables (Euclidean distance matrices), including sediment depth and biogeochemical data. ANOVAs and linear regression analyses (Supplementary Tables 9 and 11) were performed with StatPlus v6.1.7.0. We performed PLSR in the R programming language via the package PLSR (function PLSR) to predict measured sediment CH4 concentrations from biotic and abiotic variables, similar to our previously described PLSR analyses. Briefly, PLSR models a causal relationship between explanatory variable(s) and the response variable being predicted (here, measured sediment CH4 concentrations). Abiotic variables included all depth-resolved abiotic measurements that were not directly related to CH4, as such measurements could be confounding variables in our analysis. The included abiotic variables were: depth, TOC, DOC, DIC, S, and TOCTS. The PLSR analysis yielded Pearson’s product–moment correlations between measured environmental and/or geochemical variables, the abundances of microbial lineages, the abundances of specific microbial populations. This allowed for a quantification of the added value of microbial abundances in predicting sediment CH4 concentrations, relative to predictions from abiotic factors alone. Variance in the projection (VIP) scores for each explanatory variable indicate the extent to which that variable was predictive of the response variable (i.e., sediment CH4 concentrations), with VIP scores ≥1 considered to be highly significant.

**Reporting summary.** Further research on file availability is described in the Nature Research Reporting Summary linked to this article.

**Data availability**

Sequencing data are available at NCBI under BioProject PRJNA667179 and also here: https://www.ncbi.nlm.nih.gov/nuccore/NC_01667179. Other raw data and relevant publications, as cited in the paper. Data underlying Figs. 1A–C (Supplementary Table 1), 2A–C (Supplementary Table 3), and 4A–C (raw data in Supplementary Tables 4–5, relevant processed data in Supplementary Tables 13 and 16).

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Author contributions
J.B.E., R.K.V., and V.I.R. designed the study, J.B.E. wrote and R.K.V. and V.I.R. substantially revised the paper. R.K.V., M.W., and N.L.F. collected the samples and J.B.E., R.K.V., M.W., J.E.I., A.O.D., M.B., and N.L.F. performed laboratory sample processing, M.W., R.K.V., and P.M.C. generated and analyzed amplicon data, R.K.V. and J.E.I. generated and analyzed geochemical data, R.K.V. and N.L.F. performed and analyzed ex situ incubations, R.B.N. generated the isotopic and mass balance calculations, R.K.V. and B.W. processed and J.B.E. and R.T. II analyzed microbial community sequencing data, J.B.E. reconstructed microbial metabolic pathways with guidance from D.H.P., C.M.S., B.J.W., and G.W.T., and J.B.E. performed the statistical modeling with guidance from S.R.S. J.B.E., R.K.V., M.W., D.H.P., R.B.N., J.E.I., C.M.S., B.J.W., R.T. II, N.L.F., A.O.D., M.B., and V.I.R. performed the data analysis. All authors contributed to project discussions, edited the paper, and approved the final version of the paper.

Competing interests
The authors declare no competing interests.

Additional information
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