

This is a self-archived version of an original article. This version may differ from the original in pagination and typographic details.

Author(s): Rissanen, Antti; Peura, Sari; Mpamah, Promise; Taipale, Sami; Tirola, Marja; Biasi, Christina; Mäki, Anita; Nykänen, Hannu

Title: Vertical stratification of bacteria and archaea in sediments of a small boreal humic lake

Year: 2019

Version: Published version

Copyright: © FEMS 2019.

Rights: CC BY 4.0

Rights url: <https://creativecommons.org/licenses/by/4.0/>

Please cite the original version:

Rissanen, A., Peura, S., Mpamah, P., Taipale, S., Tirola, M., Biasi, C., Mäki, A., & Nykänen, H. (2019). Vertical stratification of bacteria and archaea in sediments of a small boreal humic lake. *FEMS Microbiology Letters*, 366(5), Article fnz044. <https://doi.org/10.1093/femsle/fnz044>

RESEARCH LETTER – Environmental Microbiology

Vertical stratification of bacteria and archaea in sediments of a small boreal humic lake

Antti J. Rissanen^{1,2,*†}, Sari Peura^{2,‡}, Promise A. Mpamah², Sami Taipale², Marja Tirola², Christina Biasi³, Anita Mäki² and Hannu Nykänen³

¹Tampere University, Faculty of Engineering and Natural Sciences, Korkeakoulunkatu 10, FI-33720, Tampere, Finland, ²University of Jyväskylä, Department of Biological and Environmental Science, PO Box 35, FI-40014, Jyväskylä, Finland and ³University of Eastern Finland, Department of Environmental and Biological Sciences, PO Box 1627, FI-70211, Kuopio, Finland

*Corresponding author: Tampere University, Faculty of Engineering and Natural Sciences, Korkeakoulunkatu 10, 33720 Tampere, Finland. Tel: +358 40 1981145; Fax: +358 3 3641392; E-mail: antti.rissanen@tuni.fi

†Present Address: Swedish University of Agricultural Sciences, Science for Life Laboratory, Department of Forest Mycology and Plant Pathology, Box 7026,750 07, Uppsala, Sweden.

One sentence summary: DNA sequencing and biomass analysis show strong vertical stratification of microbial communities in sediments of a small humic boreal lake.

†Antti J. Rissanen, <http://orcid.org/0000-0002-5678-3361>

Editor: Nathan Basiliko

ABSTRACT

Although sediments of small boreal humic lakes are important carbon stores and greenhouse gas sources, the composition and structuring mechanisms of their microbial communities have remained understudied. We analyzed the vertical profiles of microbial biomass indicators (PLFAs, DNA and RNA) and the bacterial and archaeal community composition (sequencing of 16S rRNA gene amplicons and qPCR of *mcrA*) in sediment cores collected from a typical small boreal lake. While microbial biomass decreased with sediment depth, viable microbes (RNA and PLFA) were present all through the profiles. The vertical stratification patterns of the bacterial and archaeal communities resembled those in marine sediments with well-characterized groups (e.g. *Methanomicrobia*, *Proteobacteria*, *Cyanobacteria*, *Bacteroidetes*) dominating in the surface sediment and being replaced by poorly-known groups (e.g. *Bathyarchaeota*, *Aminicenantes* and *Caldiserica*) in the deeper layers. The results also suggested that, similar to marine systems, the deep bacterial and archaeal communities were predominantly assembled by selective survival of taxa able to persist in the low energy conditions. Methanotrophs were rare, further corroborating the role of these methanogen-rich sediments as important methane emitters. Based on their taxonomy, the deep-dwelling groups were putatively organo-heterotrophic, organo-autotrophic and/or acetogenic and thus may contribute to changes in the lake sediment carbon storage.

Keywords: lake; sediment; bacteria; archaea; 16S rRNA; biomass

Received: 14 May 2018; Accepted: 23 February 2019

© FEMS 2019. This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted reuse, distribution, and reproduction in any medium, provided the original work is properly cited.

INTRODUCTION

A large number of small humic forest lakes are typical for the boreal region (Downing et al. 2006). They are globally significant carbon stores and hot spots of processes producing greenhouse gases, especially methane (Molot and Dillon 1996; Bastviken et al. 2004; Kortelainen et al. 2004; Kankaala et al. 2007; Wik et al. 2016). As microbes are important mediators of biogeochemical processes, data on the composition and structuring mechanisms of the bacterial and archaeal communities in these lakes is crucial in the study of global carbon cycling and in mitigation and prediction of climate change.

Lake sediment microbial communities are generally understudied relative to their marine counterparts (Petro et al. 2017). The studies have also mostly focused on lakes in the temperate area, and information on sediment microbial communities of small boreal lakes is scarce (Koizumi, Kojima and Fukui 2003; Ye et al. 2009; Borrel et al. 2012; Wurzbacher et al. 2017). Sediments of marine systems and lakes are characterized by increasing energy limitation, i.e. increasing contribution of recalcitrant organic matter and decreasing availability of electron acceptors, with depth, which can also cause variation in their microbial communities (Petro et al. 2017; Wurzbacher et al. 2017). However, the vertical stratification patterns of the biomass (abundance) and composition of microbial communities in the sediments of small boreal lakes was not explored in previous studies (Steger et al. 2011; Youngblut, Dell'Aringa and Whitaker 2014; Rissanen et al. 2017). In marine sediments, the abundance of microbes decrease downwards alongside with increasing energy limitation (Petro et al. 2017; Starnawski et al. 2017). Marine sediments are also characterized by a steep vertical stratification in the microbial community composition with well-characterized taxonomic groups (groups for which functional data and many cultivated representatives exist) being replaced by poorly-known groups (groups for which negligible/scant functional data and no or only a few cultivated representatives exist) when moving deeper in the sediment (Petro et al. 2017; Starnawski et al. 2017). Instead, the few existing lake sediment studies from the temperate area indicate that the stratification patterns of the abundance and composition of microbial communities differ between lakes (Koizumi, Kojima and Fukui 2003; Ye et al. 2009; Borrel et al. 2012; Wurzbacher et al. 2017). Similar to marine sediments, the composition of bacterial and archaeal communities changed considerably and the abundance of microbes (concentration of DNA and cells) decreased with sediment depth in Lake Stechlin (Wurzbacher et al. 2017). In contrast, despite drastic vertical change in the composition of archaea, the abundance of bacteria and archaea (16S rRNA gene copy number) did not change with depth in Lake Pavin (Borrel et al. 2012), and the abundance of bacteria even increased but their composition did not change with depth in Lake Taihu (Ye et al. 2009). However, the vertical stratification patterns of the sediment communities of small humic-rich boreal lakes cannot be predicted based on these results, as they differ considerably from the temperate lakes in the physicochemical conditions that structure the microbial communities, for example in the quality and quantity of organic matter (Xiong et al. 2015). For instance, variations in dissolved organic carbon (DOC) concentrations indicate that both the quantity of organic matter and the contribution of non-labile allochthonous organic matter is larger in small humic-rich boreal lakes (up to 25 mg C L⁻¹) than in the previously studied temperate lakes (up to 1.8–7 mg C L⁻¹) (Mothes, Koschel and Proft 1985; Viollier et al. 1995; Sugiyama et al. 2005; Ye et al. 2015). Because methane produced in the sediments

accumulate in high amounts in the lower parts of the water column (Houser et al. 2003; Kankaala et al. 2007; Peura et al. 2012), it can be expected that methanogens are especially abundant and methanotrophs rare in the sediments of small boreal lakes. However, further studies are needed to confirm this as well as to explore, whether and how the bacterial and archaeal community changes in the low-energy conditions below the zone dominated by methanogens.

The mechanisms controlling the vertical variations in microbial community composition in sediments of lakes are also unclear. Evidence from marine sediments suggests that the transition from the surface to deep subsurface biosphere is due to filtering (i.e. selection) of populations from the surface that leave only a subset of taxa to populate the deeper energy limited zones (Petro et al. 2017). This mechanism was shown by a low number of operational taxonomic units (OTUs) that are present at all depths (i.e. persisting OTUs) but make up a significant proportion of the total sediment communities (Petro et al. 2017). It may well explain the vertical distribution of bacterial and archaeal communities also in lakes, but has not been previously investigated.

We wanted to fill the knowledge gap on sediment microbial communities of small humic-rich boreal lakes. Therefore, we characterized the vertical variation in bacterial and archaeal communities in sediments (26 cm deep cores) of a small boreal humic forest lake (Lake Alinen Mustajärvi) via DNA/RNA quantification, next-generation sequencing of 16S rRNA gene amplicons, phospholipid fatty acid (PLFA)—analysis and quantitative-PCR (qPCR) of methyl coenzyme M reductase (*mcrA*) gene (a biomarker gene of methanogenic archaea). Furthermore, the vertical variation in the sediment organic matter quality was assessed via analyzing the content and stable isotopic ratios of carbon and nitrogen of the bulk sediment. Our main aim was to resolve the vertical change in the bacterial and archaeal community composition and in the microbial biomass alongside with the increasing energy limitation (i.e. increasing organic matter recalcitrance) with depth in the study lake sediments. We also specifically aimed to verify our expectation that methanogens are abundant and methanotrophs rare in the sediments of the study lake. Furthermore, we aimed to elucidate whether the vertical change in bacterial and archaeal communities in the sediments of the study lake are predominantly controlled by the same mechanism as in their marine counterparts, i.e. by selective survival of taxa able to persist under the energy limitation.

MATERIALS AND METHODS

Study lake

Lake Alinen Mustajärvi (A = 0.7 ha, max. depth = 6.5 m, V = 31 000 m³ and DOC = ~10–20 mg C L⁻¹) is a small, humic, headwater lake located in southern Finland (61° 12'N; 25° 06'E). The catchment area is <0.5 km² and consists of >90% coniferous forest and <10% peatland. The lake is ice covered each year from late November to late April. Alinen Mustajärvi is spring meromictic. Thus, the water column mixes completely only during autumn. The lake water column is stratified with respect to temperature and oxygen during both summer and winter. Most parts of the year, the sediment surface is exposed to anoxic and cold hypolimnion water with relatively stable temperature (4–6°C) (Nykänen et al. 2014).

Sampling

Sampling was done through holes drilled in ice at the deepest point of the lake on Apr 4th 2012. Water column profiles of temperature and oxygen concentrations were measured *in situ* using a portable field meter (YSI model 58, Yellow Springs Instruments, Yellow Springs, Ohio, USA). The sediment surface was exposed to anoxic and cold water (4.5°C) during the time of sampling representing typical conditions of the sediments for most of the year. Sediment samples were collected using a slicing (height of the slicing ring = 1 cm) Limnos Sediment Sampler (Limnos.pl, Komorów, Poland) (length = 94 cm, Ø = 9.4 cm) connected to a gravity corer. Altogether, two cores (thus, $n = 2$, length 26 cm from the water-sediment interface), were collected from 3 meters apart from each other. For the 0–20 cm zone (0 cm = water-sediment interface), the cores were divided into 1 cm thick layer-specific samples (i.e. layers: 0–1 cm, 1–2 cm, ... 18–19 cm, 19–20 cm), except that layer 15–16 cm was not sampled. For the 20–26 cm zone, the cores were divided into 2 cm thick samples (i.e. 20–22 cm, 22–24 cm and 24–26 cm). Immediately after collection, the samples were homogenized and subsamples were collected from each sample for DNA/RNA extraction and for determination of dry weight as well as content and isotopic composition of C and N of bulk sediment. The sediments were stored frozen (at outdoor temperature, -19°C) until transported to the laboratory. Samples were merged into pools corresponding to 2 to 6 cm thick depth layers (20 samples, 10 layers per core) for phospholipid fatty acid (PLFA) analysis (layers: 0–2 cm, 2–4 cm, 4–6 cm, 6–8 cm, 8–10 cm, 10–12 cm, 12–15 cm, 16–18 cm, 18–20 cm and 20–26 cm). All the samples were then stored frozen (at -20°C) before further analyses which took place within 1–2 months from sampling. Sediment age of the study lake is not known. However, sediment age has been determined for a closely located (within 5 km distance in the same forest area) Lake Valkeakotinen, which represents a similar type of small, shallow (max. depth 6.7 m), humic, spring-meromictic headwater lake. Based on these analyses, a 26 cm long sediment core represent approximately 200 years of sediment accumulation (Wickstrom and Tolonen 1987; Pajunen 2004).

Analysis of chemical sediment qualities

Subsamples of frozen sediment were freeze-dried for determination of the dry weight of each layer. Freeze-dried sediment was also homogenized and weighted into small tin cups for analysis of content and stable isotope ratios of C and N using a Thermo Finnigan Flash EA1112 elemental analyser connected to a Thermo Finnigan DELTAplus Advantage continuous-flow stable isotope ratio mass spectrometer (Thermo Fisher Scientific, Waltham, Massachusetts, USA). Isotopic composition of C and N is expressed in terms of δ value, which is parts per thousand differences from a standard (Vienna Pee Dee Belemnite for C and atmospheric N_2 for N): $\delta^{13}\text{C}$ or $\delta^{15}\text{N} = [(R_{\text{sample}}/R_{\text{standard}}) - 1] * 10^3$, where R is the $^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$ —ratio.

PLFA extraction and analysis

Total lipids of the freeze-dried samples were extracted and fractionated into neutral, glyco-, and phospholipids, and the phospholipid fraction was analyzed as explained before (Bligh and Dyer 1959; Mpamah et al. 2017). The amount (μg fatty acids g^{-1} dw) of BrFA (sum of i14:0, i15:0, a15:0, i16:0, i17:0, a17:0 and i18:0) was used as a biomarker of living bacterial biomass. BrFA were

chosen for this purpose as they are specifically of bacterial origin, generally related to gram-positive prokaryotes, sulphate-reducing bacteria and other anaerobic bacteria in sediments (Zhukova 2005).

Nucleic acid—based analyses

DNA and RNA were simultaneously extracted from frozen sediment samples (190–340 mg) using a previously published protocol based on bead-beating and phenol–chloroform extraction (Griffiths et al. 2000). Nucleic acid yields of extractions were determined with Qubit 2.0 Fluorometer and Qubit™ dsDNA HS Assay Kit for DNA and Qubit™ RNA HS Assay Kit for RNA (Thermo Fisher Scientific). DNA extractions were stored at -20°C before sequencing and qPCR analyses.

The abundance of methanogenic archaea was studied via qPCR of the *mcrA* gene as described in detail in Supplemental Methods (Supplemental Methods, Supporting Information). Furthermore, the community structure of bacteria and archaea was studied by next-generation sequencing of bacterial and archaeal 16S rRNA gene amplicons. Primers, PCR, preparation of NGS libraries, and the sequencing (Ion Torrent™ Personal Genome Machine) are described in detail in Supplemental Methods. Sequencing analysis of archaeal 16S rRNA genes was done from 43 samples representing almost all the collected depth-layers from the two sample cores. However, for sequencing analysis of bacterial 16S rRNA genes and for qPCR analysis, equal amount of DNA from each sample were pooled into samples corresponding to the same depth layers as in PLFA analysis (20 samples, 10 layers per core, see above).

Bioinformatic analyses

Mothur was used in subsequent sequence analyses (Schloss et al. 2009). Barcodes and primer sequences, as well as low-quality sequences (containing sequencing errors in primer or barcode sequences, ambiguous nucleotides and homopolymers longer than eight nucleotides) were removed. Thereafter, alignment, chimera-removal, pre-clustering (to reduce PCR-amplification and sequencing errors), taxonomic classification (Silva v128 database), division of sequences into OTUs (at 97% similarity level), singleton removal and subsampling of each sample to the size of the smallest sample were conducted as previously described (Rissanen et al. 2018). Sequences classified as chloroplast, mitochondria and eukaryota were removed from all libraries. Furthermore, bacterial sequences were removed from archaeal libraries and vice versa. Finally, there were 1239 and 11 837 archaeal and bacterial sequences per sample with an average length of 270 and 308 bp, respectively. Sequence variation was adequately covered in these libraries as shown by Good's coverage that varied 0.91–0.97 and 0.88–0.95 for archaeal and bacterial libraries, respectively.

Bacterial and archaeal OTUs were classified into 1) well-characterized groups (groups with functional data and cultivated representatives) and 2) poorly-known groups (groups with negligible/scant functional data and either no or only a few cultured representatives) according to Table 1. We also specifically studied the contribution of methanogens and methanotrophs in the archaeal and bacterial communities. Furthermore, to assess whether the deep bacterial and archaeal communities predominantly assemble by selective survival of different taxa, we analyzed the number of surface layer OTUs surviving depth-wise from layer to layer, and the relative abundance of persisting

Table 1. Well-characterized and poorly-known taxa detected in the study lake sediments using 16S rRNA gene sequencing.

| Group | Archaea | Bacteria |
|--------------------|---|---|
| Well-characterized | Classes: <i>Methanobacteria</i> , <i>Methanomicrobia</i> | Phyla: <i>Acidobacteria</i> , <i>Actinobacteria</i> , <i>Armatimonadetes</i> , <i>Bacteroidetes</i> , <i>Chlamydiae</i> , <i>Cyanobacteria</i> , <i>Deinococcus-Thermus</i> , <i>Fibrobacteres</i> , <i>Firmicutes</i> , <i>Fusobacteria</i> , <i>Gemmatimonadetes</i> , <i>Lentisphaerae</i> , <i>Nitrospirae</i> , <i>Planctomycetes</i> , <i>Proteobacteria</i> , <i>Spirochaetae</i> , <i>Verrucomicrobia</i> |
| | Poorly-known ^a | |
| | unclassified <i>Archaea</i> | unclassified <i>Bacteria</i> |
| | Classes: <i>Thermoplasmata</i> | Phyla: <i>AC1</i> , <i>Acetothermia</i> , <i>Aminicenantes</i> , <i>Atribacteria</i> |
| | Phyla: <i>Aenigmarchaeota</i> , <i>Altiarchaeales</i> , <i>Bathyarchaeota</i> , <i>Diapherotrites</i> , <i>Hadesarchaea</i> , <i>Lokiarchaeota</i> , <i>Miscellaneous Euryarchaeotic</i> <i>Group (MEG)</i> , <i>Parvarchaeota</i> , <i>Thaumarchaeota</i> , <i>Woesearchaeota</i> , <i>YNPFFA</i> | <i>Berkelbacteria</i> , <i>BRC1</i> <i>Caldiserica</i> , <i>Chlorobi</i> , <i>Chloroflexi</i> , <i>Cloacimonetes</i> , <i>Elusimicrobia</i> , <i>FCPU426</i> , <i>Gracilibacteria</i> , <i>Hydrogenedentes</i> , <i>Ignavibacteriae</i> , <i>Latescibacteria</i> , <i>LCP-89</i> , <i>Microgenomates</i> , <i>Omnitrophica</i> , <i>Parcubacteria</i> , <i>Peregrinibacteria</i> , <i>RBG-1</i> , <i>Saccharibacteria</i> , <i>SR1</i> , <i>TA06</i> , <i>TM6</i> , <i>WS1</i> , <i>WS2</i> , <i>WS6</i> |

^aThe detected 16S rRNA gene sequences assigned to *Chlorobi* were not from the known phototrophic genera nor were *Chloroflexi*—sequences from known organohalide respiring genera or *Thermoplasmata*—sequences from known methanogenic genera. This supports the classification of these taxa into the group of poorly-known microbes.

OTUs (i.e. OTUs present in each layer) (Petro et al. 2017; Starnawski et al. 2017). For the analyses, the values of the replicate samples were averaged for each layer. Furthermore, archaea were studied with similar layering as bacteria by averaging the values of sample layers of archaeal samples (e.g. 0–1 cm and 1–2 cm layers) to represent layering of bacterial samples (e.g. 0–2 cm layer). For comparison, we conducted a similar analysis of assembly mechanisms of bacterial and archaeal communities for sediments of Lake Stechlin via reanalysis of a recently published dataset (Additional File 11 in Wurzbacher et al. 2017).

Accession numbers

16S rRNA gene sequences were deposited in NCBI's short read archive under accession number SRP120305.

RESULTS AND DISCUSSION

There were vertical variations in the bulk sediment C%, N%, C:N—ratio, $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$, which can be caused by variations in organic matter input and in diagenetic processes (Fig. S1, Supporting Information). We lack information on the history of the lake to assess the relative importance of these factors. However, the generally higher C:N and $\delta^{15}\text{N}$ and lower $\delta^{13}\text{C}$ in deep than in surface layers can be expected to reflect decomposition and, thus, increased energy limitation due to increased organic matter recalcitrance with depth. This is caused by preferential microbial degradation of labile, high-nitrogen organic compounds with high $\delta^{13}\text{C}$ leaving the residual organic matter ^{15}N -enriched and ^{13}C -depleted (Fig. S1, Supporting Information) (Lehmann et al. 2002). Of recalcitrant organic compounds, especially lignin, which is an important component of organic matter leaching to freshwater systems in the boreal area, could lead to low $\delta^{13}\text{C}$ in sediments (Benner et al. 1987; Amon et al. 2012).

Based on the content of DNA, RNA and branched fatty acids (BrFA) of the sediment, the microbial biomass and abundance was highest at the top 4 cm below which it generally decreased until the deepest layer of the sample cores (Fig. 1A and B). Decrease in biomass and abundance was caused by downward increasing energy limitation agreeing with results from marine and some lake systems (Haglund et al. 2003; Petro et al. 2017; Starnawski et al. 2017; Wurzbacher et al. 2017). However, the presence of biomarkers of viable microbes (i.e. RNA and fatty acids, Blagodatskaya and Kuzyakov 2013) down to the deepest layers of the sediment core indicate that the conditions in the deep layers supported microbial life (Fig. 1A and B). This agrees with Haglund et al. (2003) showing active cells down to 25 cm depth in sediments of Lake Erken. Interestingly, the depth-wise decrease was more profound with nucleic acids than with BrFAs (Fig. 1A and B). Similarly, DNA content decreased much more than cell numbers with sediment depth in Lake Stechlin (Wurzbacher et al. 2017). As nucleic acids originate from both eukaryotes and prokaryotes and BrFAs only from bacteria, the difference in the vertical distribution of nucleic acids and BrFAs may be partially explained by steeper decrease in eukaryotic biomass than in bacterial biomass (Wurzbacher et al. 2017). However, the generally larger vertical reduction in DNA than in cell numbers (Lake Stechlin) and bacterial BrFAs (our study) can be also due to vertical reduction in cell-size and cell-specific DNA contents. Decrease in cell and genome size could be a consequence of adaptation to nutrient limited conditions in the deep layers (Giovannoni, Thrash and Temperton 2014). In fact, as described below, the relative abundance of taxa featuring small genome size increased in the deeper sediment.

Sediment bacterial and archaeal communities were vertically stratified with some poorly-known taxonomic groups increasing deeper in the sediment (Figs 2–4), which agrees with results from temperate lakes (Borrel et al. 2012; Wurzbacher et al. 2017) and from marine systems (Petro et al. 2017; Starnawski

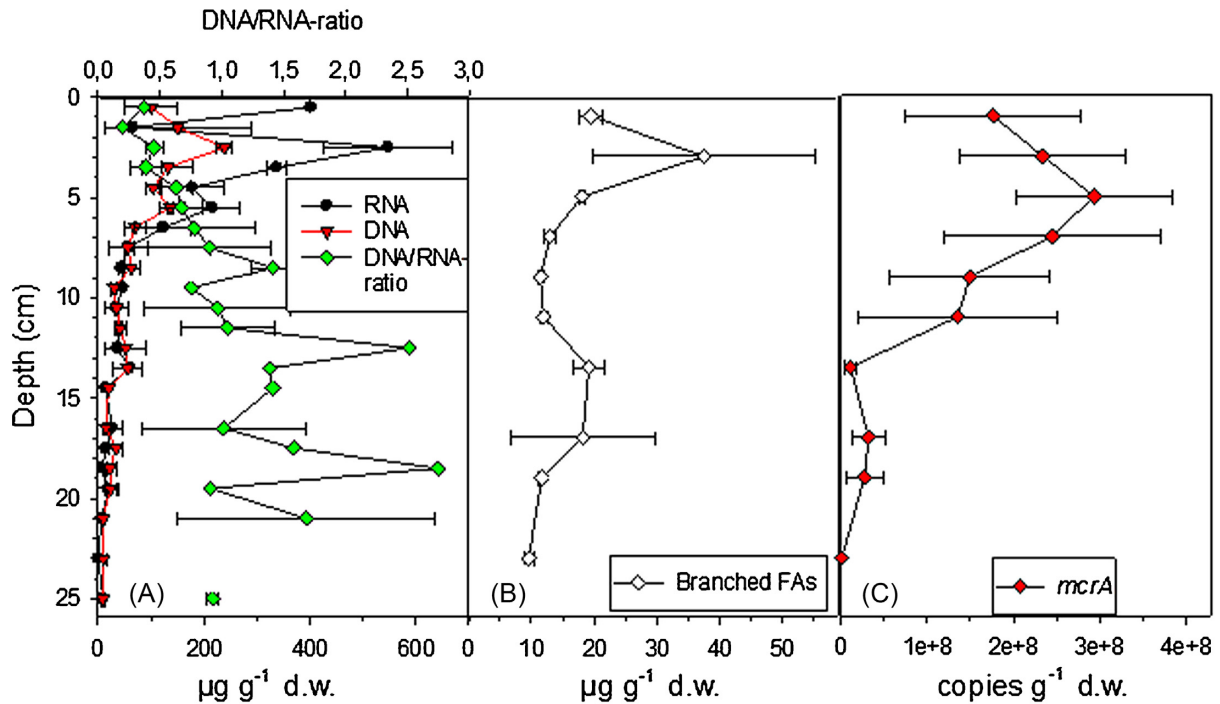


Figure 1. Vertical variation in the (A) amount of RNA and DNA as well as in the DNA:RNA—ratio, (B) amount of BrFA (sum of i14:0, i15:0, a15:0, i16:0, i17:0, a17:0 and i18:0) in PLFA fraction and (C) *mcrA* gene copy numbers in the sediments of the study lake. Results represent mean of two replicate cores and their average deviation. Depth of each data point is the average depth of the particular study layer. DNA:RNA—ratio could not be calculated for the layer 22–24 cm because RNA was below detection limit (in A).

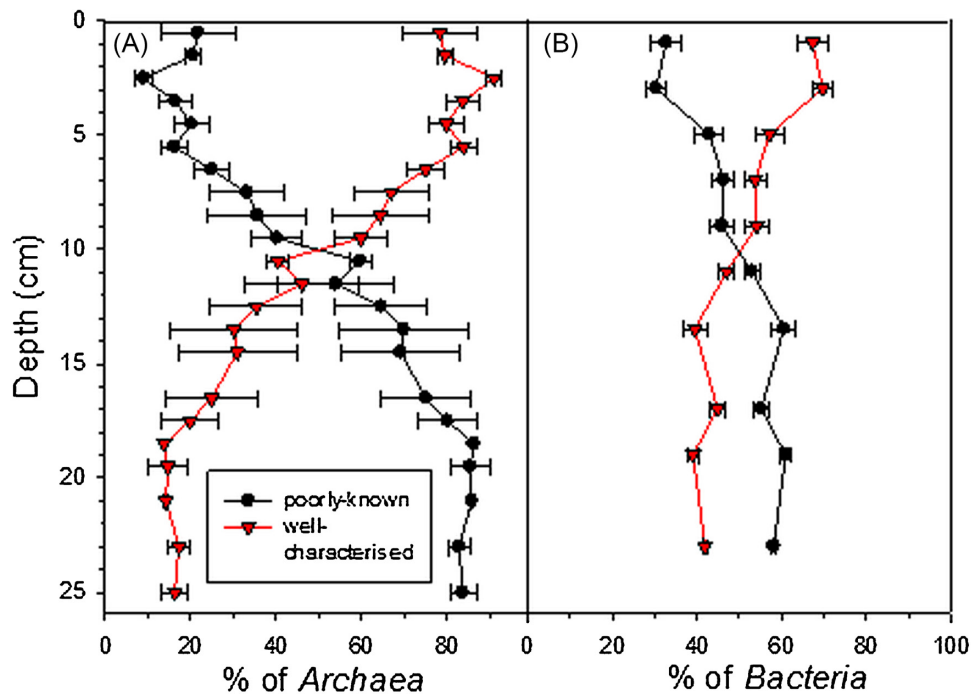


Figure 2. Vertical variation in the sum of the relative abundances of poorly-known and well-characterized (A) archaeal and (B) bacterial taxa, including also unclassified Archaea (in A) and Bacteria (in B), in the sediments of the study lake based on 16S rRNA gene sequencing. Results represent mean of two replicate cores and their average deviation. Depth of each data point is the average depth of the particular study layer. See Table 1 for the list of taxa in the study lake sediments.

et al. 2017). This suggests that it is a widespread pattern in many types of organic sediment ecosystems. However, the possible effect of relic DNA on the diversity estimates has to be acknowledged (Carini et al. 2016). Indeed, the depth-wise increase in DNA:RNA—ratio could reflect increasing amount of relic DNA (Fig. 1A). However, increase in DNA:RNA—ratio can be also due to microbes being less active and producing less RNA in deep than in surface layers. Similar to Wurzbacher et al. (2017), we suggest that extractable relic DNA in our study lake was very short-lived. Furthermore, relic DNA is expected to mostly affect the results on the rare biosphere (Wurzbacher et al. 2017), which we did not target in our study.

As expected, methanogens belonging to the phyla *Euryarchaeota* dominated archaeal communities in the upper sediment layers (Fig. 3A). The vertical variation in *mcrA* abundance further confirmed that methanogens were very important in the top 14 cm layer (Fig. 1C). The largest poorly-known archaeal group was *Bathyarchaeota*, which dominated the lower layers (Fig. 3B). Other dominant poorly-known archaeal groups (i.e. consisting > 1% of the archaeal 16S rRNA gene sequences in the dataset) were unclassified *Archaea*, *Thaumarchaeota* and *Thermoplasmata*, which also had generally higher relative abundance in the deep than in the surface layers, as well as *Woeisearchaeota*, which had its highest relative abundance in the middle of the sediment core (Fig. 3B). The dominant groups of well-characterized bacteria (i.e. consisting > 1% of bacterial 16S rRNA gene sequences in the dataset) consisted of *Proteobacteria*, *Cyanobacteria*, *Bacteroidetes* and *Acidobacteria* which had generally higher relative abundance in the surface layers than in the deep layers, as well as of *Firmicutes* and *Spirochaetae*, which showed contrasting depth patterns (Fig. 4A). Of the dominant groups of poorly-known bacteria, *Caldiserica*, *Aminicenantes*, *Chlorobi* and unclassified *Bacteria* had a higher relative abundance in the deep layers than in the surface layers, while other groups either decreased towards bottom, i.e. *TM6* and *Omnitrophica*, or showed an elliptical distribution pattern, i.e. *Chloroflexi*, *Parcubacteria*, *AC1* and *Ignavibacteriae* (Fig. 4B and C). Comparison of the results with previous literature also suggest that genome sizes of the bacterial and archaeal cells decreased with sediment depth. Published genome sizes of the largest deep sediment phyla, *Caldiserica*, 1.6 Mb (*Caldicericum exile*, KEGG genome database), *Aminicenantes*, 2.5 Mb (metagenome assembled genome in Robbins et al. 2016) and *Bathyarchaeota*, ~0.6–1.9 Mb (Castelle et al. 2018) are within the lower end of range reported for the largest surface layer phyla, *Proteobacteria*, ~0.2–10 Mb, *Cyanobacteria*, ~1.6–9 Mb, *Bacteroidetes*, ~0.3–6 Mb and *Euryarchaeota*, ~1.6–5.9 Mb (Castelle et al. 2018).

Putative acetoclastic (acetate—consuming) methanogens (*Methanosaeta*) dominated over hydrogenotrophic (H_2 and CO_2 —consuming) methanogens (*Methanomicrobiales*) with a much higher ratio (up to 9:1) than the theoretically predicted 2:1 for methane production in acetoclastic:hydrogenotrophic pathways in complete methanogenic degradation of organic matter (Fig. 3A) (Conrad 1999). This could be due to low temperature (4–6°C for most of the year) and pH (5–6) in the lake bottom increasing the relative contribution of acetoclastic pathway via increase in the bacterial acetate production (Phelps and Zeikus 1984; Glissmann et al. 2004; Nykänen et al. 2014). *Methanosaeta*—methanogens may have also partially sustained their metabolism through CO_2 —reduction linked to direct interspecies electron transfer (Rotaru et al. 2014). The downward changes in the relative contribution of acetoclastic and hydrogenotrophic methanogens was a further indication of increasing organic matter recalcitrance in deeper depths

(Fig. 3A) (Conrad, Claus and Casper 2009). Furthermore, half of *Proteobacteria* consisted of fermentative *Deltaproteobacteria* (*Syntrophobacterales* and *Syntrophorhabdaceae*). In addition, fermentative function has been proposed for *Firmicutes*, *Bacteroidetes*, *Parcubacteria*, *TM6*, *Chloroflexi* and *Ignavibacteriae* (McInerney et al. 2008; Iino et al. 2010; Wrighton et al., 2012, 2014; Hug et al. 2013; Kantor et al. 2013; Podosokorskaya et al. 2013; Kallistova, Goel and Nozhevnikova 2014; Wasmund et al. 2014). Thus, a significant proportion of sediment bacteria potentially provided substrates for methanogenesis.

As expected, methanotrophs did not make a significant contribution to the sediment bacterial and archaeal community (Fig. S2). In fact, anaerobic methane oxidizing bacteria [i.e. NC10-phyla (Ettwig et al. 2010)] or archaea [i.e. ANME—archaea (Knittel and Boetius 2009)] or aerobic verrucosomic microbial methanotrophs were not detected in the sediments. Archaeal genus *Methanosarcina*, whose member, *M. acetivorans*, can potentially mediate anaerobic methane oxidation, was also very rare, only up to 0.25% of archaeal 16S rRNA genes (Fig. S2B, Supporting Information) (Yan et al. 2018). In addition, aerobic methanotrophs in the order *Methylococcales* and in the family *Methylocystaceae* consisted only up to 0.6% and 1.7% of bacterial 16S rRNA gene sequences, respectively (Fig. S2A, Supporting Information). These results differ from those of the water column of the same study lake, where *Methylococcales* were up to 16% of bacteria (Rissanen et al. 2018). They also disagree with those from sediments of larger boreal lakes, where aerobic methanotrophs had a higher relative abundance (i.e. *Methylococcales* were up to 8% and *Methylocystaceae* up to 4% of bacteria) and which harbored also anaerobic methane oxidizing bacteria and archaea (Rissanen et al. 2017). However, *Methylococcales* in the water column of the study lake resided close to the oxic-anoxic interface (Rissanen et al. 2018). Furthermore, the sediments of the larger dimictic boreal lakes usually have an oxic surface all the year around, whereas the water layers above the sediment surface of small spring-meromictic boreal lakes are mostly completely anoxic, except during autumn-mixing. Thus, the lower and temporally more variable availability of oxygen explained the low relative abundance of aerobic methanotrophs in the sediments of the study lake. Furthermore, profiles of sulfate and hydrogen sulfide indicate active sulfate reduction in the anoxic water columns of small boreal humic lakes suggesting that the electron acceptors used in anaerobic methane oxidation are exhausted for most of the year (Schiff et al. 2017; Rissanen et al. 2018). This very likely prevented the establishment of populations of anaerobic methane oxidizing bacteria and archaea in the sediments of the study lake. Anaerobic methanotrophs were also absent in the water column of the study lake (Rissanen et al. 2018). In contrast, aerobic methanotrophs sustained their life also during the long anoxic periods, yet the type of their anaerobic metabolism is mostly uncharacterized (Roslev and King 1994; Bar-Or et al. 2017; Martinez-Cruz et al. 2017).

The OTUs, that were present in all depth layers (persisting OTUs), had only very minor contribution to the total richness (5%–9% and 5%–17% of the number of bacterial and archaeal OTUs), yet constituted a large fraction of the communities (41%–67% and 73%–90% of bacterial and archaeal sequences, respectively) (Fig. 5). This agrees with results from marine sediments (Petro et al. 2017; Starnawski et al. 2017). The persisting OTUs represented the same taxonomic groups as the dominant groups of poorly-known and well-characterized bacteria and archaea (e.g. *Cyanobacteria*, *Caldiserica*, *Methanosaeta* and *Bathyarchaeota*), and, thus, played a significant role in structuring the major vertical

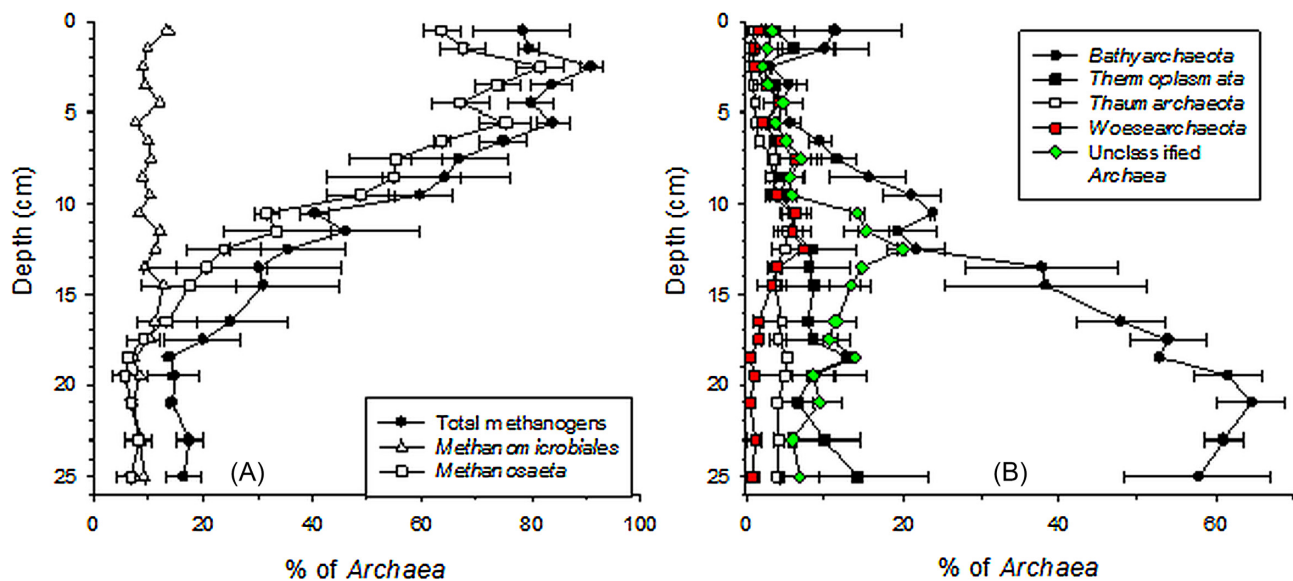


Figure 3. Vertical variation in the relative abundance of dominant (i.e. consisting > 1% of the archaeal 16S rRNA gene sequences in the dataset) (A) well-characterized archaeal (i.e. euryarchaeotal methanogens), and (B) poorly-known archaeal taxa as well as unclassified *Archaea* in the sediments of the study lake based on 16S rRNA gene sequencing. Results represent mean of two replicate cores and their average deviation. Depth of each data point is the average depth of the particular study layer.

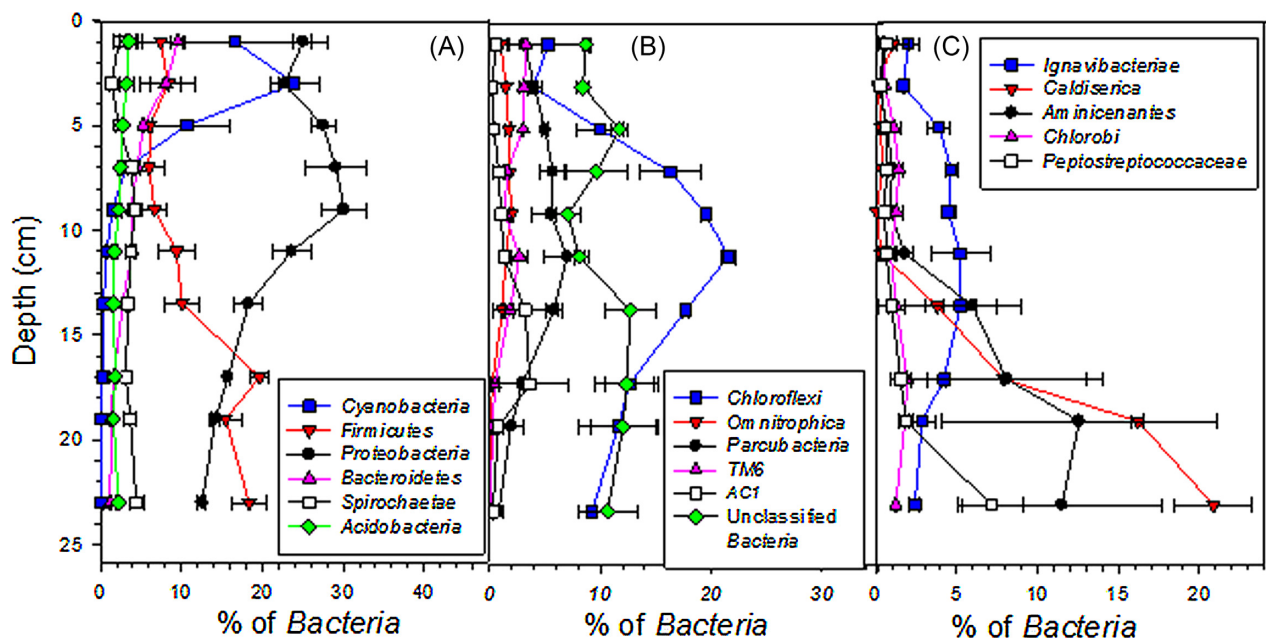


Figure 4. Vertical variation in the relative abundance of (A) dominant (i.e. consisting > 1% of the bacterial 16S rRNA gene sequences in the dataset) well-characterized bacterial phyla, (B) and (C) dominant poorly-known bacterial phyla and unclassified *Bacteria*, as well as, (C) a putative protein degrading family *Peptostreptococcaceae* (*Firmicutes*), in the sediments of the study lake based on 16S rRNA gene sequencing. Results represent mean of two replicate cores and their average deviation. Depth of each data point is the average depth of the particular study layer.

patterns in the microbial community (Figs S3, Supporting Information; Figs 3 and 4). In accordance, our reanalysis of the dataset from Wurzbacher et al. (2017) showed that, in Lake Stechlin, the persisting OTUs had only minor contribution to the total richness (2%–9% and 4%–11% of bacterial and archaeal OTUs) but still constituted a significant fraction of the communities (29%–41% and 35%–71% of bacterial and archaeal sequences) (Fig. S4A and B, Supporting Information). Altogether, this suggests that the bacterial and archaeal communities in the deep sediments

of the lakes are predominantly assembled by the same mechanism as in their marine counterparts, i.e. by selective survival of taxa able to persist under the energy limitation. The community assembly mechanism could not be inferred for other previously studied lake sediments because they were done using low-resolution methods (DGGE or TTGE) (Koizumi, Kojima and Fukui 2003; Ye et al. 2009; Borrel et al. 2012).

The dominant deep sediment phyla, *Caldiseica*, *Aminicenantes* and *Bathyarchaeota* are typical members of freshwater systems, including lakes (Borrel et al. 2012; Faraq et al. 2014;

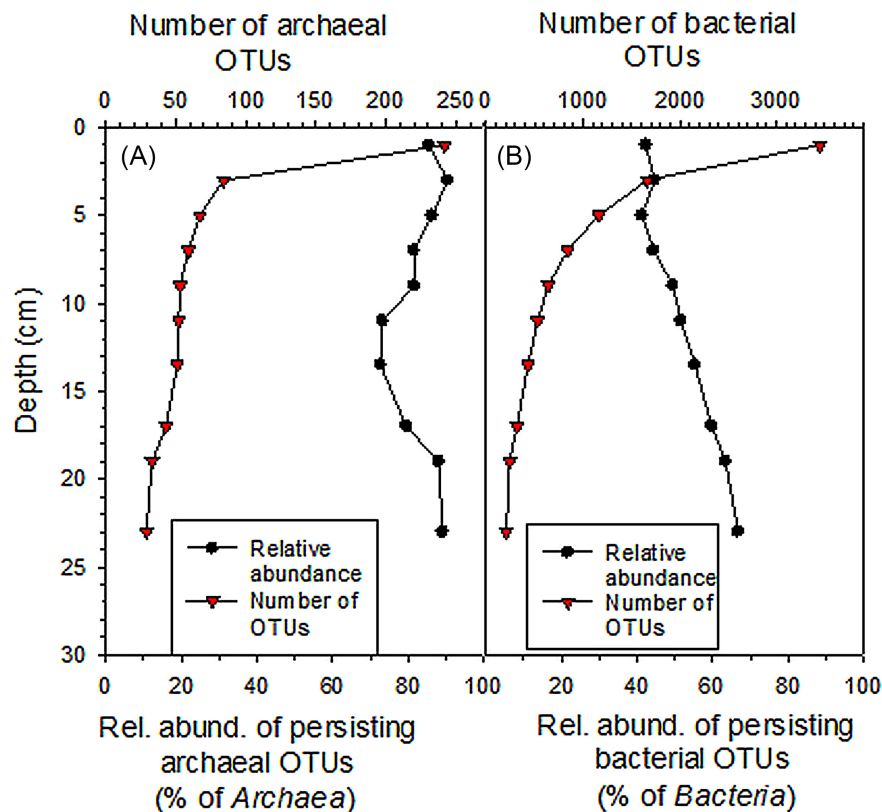


Figure 5. Vertical distribution in the number of surface layer OTUs surviving depth-wise from layer to layer, and in the relative abundance of persisting OTUs (i.e. OTUs present in each layer from top to bottom) in the sediments of the study lake for (A) Archaea and (B) Bacteria, based on 16S rRNA gene sequencing. Thus, number of OTUs at the top-most layer represent number of all the OTUs at the surface, whereas number of OTUs at the bottom-most layer represent number of persisting OTUs (i.e. OTUs present in each layer from top to bottom). Depth of each data point is the average depth of the particular study layer.

Wurzbacher et al. 2017). The relative abundance of these phyla also increased with depth in Lake Stechlin (Fig. S4C, Supporting Information) (Wurzbacher et al. 2017), and an archaea-specific study showed a similar pattern for *Bathyarchaeota* in Lake Pavin (Borrel et al. 2012). Furthermore, *Bathyarchaeota* is a key microbial phylum in deep marine sediments (Starnawski et al. 2017). Thus, members of these phyla are adapted to living in low-energy conditions. Blastn-searches confirmed that the *Bathyarchaeota* in the study lake were not closely related to the putative methanogenic members of this phylum (Altschul et al. 1990; Evans et al. 2015). Instead, previous metagenomic, single-cell genomic and culture-dependent analyses suggest that members of *Bathyarchaeota*, *Caldiserica* and *Aminicenantes* are organo-heterotrophs, yet, organo-autotrophic and acetogenic lifestyle has been also proposed for some members of *Bathyarchaeota* (Mori et al. 2009; Lloyd et al. 2013; Rinke et al. 2013; He et al. 2016; Lazar et al. 2016; Yu et al. 2018). As recently shown by Yu et al. (2018) for marine sediment *Bathyarchaeota* and suggested by our stable isotopic data (see above, Fig. S1A, Supporting Information), lignin might be an important energy source for the *Bathyarchaeota* in the sediments of the study lake and generally in sediments of lakes having forested catchments. However, protein-degrading function has been also specifically proposed for both *Bathyarchaeota* and *Aminicenantes* (Mori et al. 2009; Lloyd et al. 2013; Rinke et al. 2013; Lazar et al. 2016). In support of this, a well-characterized protein-mineralizing family, *Pep-tostreptococcaceae* (Slobodkin 2014), showed a concurrent downward increase in its relative abundance in the study lake (Fig. 4C). Yet, the same did not take place in Lake Stechlin (Wurzbacher

et al. 2017) (Fig. S4C, Supporting Information). It is still tempting to speculate that the capability for protein degradation, for example via degrading dead microbial biomass or stable organometallic complexes that contain proteins and other labile organic matter (Lalonde et al. 2012), would be an advantageous trait in low energy conditions. Furthermore, besides energy metabolism based on fermentation and acetogenesis, as suggested for *Bathyarchaeota* (He et al. 2016; Lazar et al. 2016), anaerobic respiration linked to reduction of poorly reactive Fe(III) minerals may also become a thermodynamically favorable process in electron acceptor—poor conditions below the methanogenesis zone in lake sediments (Bar-Or et al. 2017). Consequently, sulfur-respiration could be also promoted in the deep sediments via abiotic sulfide oxidation linked to iron reduction. In support of that, the only cultured representative of *Caldiserica*, *Caldisericum exile*, do not ferment but grows by anaerobic respiration reducing thiosulfate, sulfite and elemental sulfur (Mori et al. 2009).

CONCLUSION

Based on results from the boreal study lake and the previously studied temperate lakes, it can be concluded that lake sediment bacterial and archaeal communities generally follow a similar stratification pattern as communities in marine systems with the relative importance of poorly-known groups increasing with depth. The results also suggest that, similar to marine systems, the bacterial and archaeal communities in deep sediment layers of lakes are predominantly assembled by selective

survival of taxa able to persist in the low energy conditions. The dominance of fermentative bacteria and methanogenic archaea and the rarity of methanotrophs in the study lake adds to the growing body of evidence on the role of the sediments of small boreal humic lakes as important methane emitters. As the poorly-known, deep-dwelling groups potentially contribute to changes in the lake sediment carbon store, future studies utilizing culture-independent and -dependent study methods are needed to resolve their activities in lake sediments and under different physicochemical conditions.

SUPPLEMENTARY DATA

Supplementary data are available at [FEMSLE](https://femsle.onlinelibrary.wiley.com/doi/10.1111/femsle.12345) online.

ACKNOWLEDGEMENTS

We thank staff at the Lammi Biological Station for supporting the field work. We also thank the two anonymous reviewers and the editor for their constructive comments.

FUNDING

This work was supported by The Academy of Finland [Grants 136455 and 140964 to HN, and 286642 to AJR], European Research Council [ERC consolidator grant 615146 (Seventh Framework Programme) to MT] and Maj & Tor Nessling Foundation [CB].

Conflict of interest. None declared.

REFERENCES

- Altschul SF, Gish W, Miller W et al. Basic local alignment search tool. *J Mol Biol* 1990;**215**:403–10.
- Amon RMW, Rinehart AJ, Duan S et al. Dissolved organic matter sources in large Arctic rivers. *Geochim Cosmochim Acta* 2012;**94**:217–37.
- Bar-Or I, Elvert M, Eckert W et al. Iron-coupled anaerobic oxidation of methane performed by a mixed bacterial-archaeal community based on poorly reactive minerals. *Environ Sci Technol* 2017;**51**:12293–301.
- Bastviken D, Cole J, Pace M et al. Methane emissions from lakes: Dependence of lake characteristics, two regional assessments, and a global estimate. *Global Biogeochem Cycles* 2004;**18**:GB4009.
- Benner R, Fogel ML, Sprague EK et al. Depletion of ¹³C in lignin and its implications for stable carbon isotope studies. *Nature* 1987;**329**:708–10.
- Blagodatskaya E, Kuzyakov Y. Active microorganisms in soil: Critical review of estimation criteria and approaches. *Soil Biol Biochem* 2013;**67**:192–211.
- Bligh EG, Dyer WJ. A rapid method of total lipid extraction and purification. *Can J Biochem Physiol* 1959;**37**:911–7.
- Borrel G, Lehours A-C, Crouzet O et al. Stratification of *Archaea* in the deep sediments of a freshwater meromictic lake: Vertical shift from methanogenic to uncultured archaeal lineages. *PLoS One* 2012;**7**:e43346.
- Carini P, Marsden PJ, Leff JW et al. Relic DNA is abundant in soil and obscures estimates of soil microbial diversity. *Nat Microbiol* 2016;**2**:16242.
- Castelle CJ, Brown CT, Anantharaman K et al. Biosynthetic capacity, metabolic variety and unusual biology in the CPR and DPANN radiations. *Nat Rev Microbiol* 2018;**16**:629–45.
- Conrad R, Claus P, Casper P. Characterization of stable isotope fractionation during methane production in the sediment of a eutrophic lake, Lake Dagow, Germany. *Limnol Oceanogr* 2009;**54**:457–71.
- Conrad R. Contribution of hydrogen to methane production and control of hydrogen concentrations in methanogenic soils and sediments. *FEMS Microbiol Ecol* 1999;**28**:193–202.
- Downing JA, Prairie YT, Cole JJ et al. The global abundance and size distribution of lakes, ponds, and impoundments. *Limnol Oceanogr* 2006;**51**:2388–97.
- Ettwig KF, Butler MK, Le Paslier D et al. Nitrite-driven anaerobic methane oxidation by oxygenic bacteria. *Nature* 2010;**464**:543–8.
- Evans PN, Parks DH, Chadwick GL et al. Methane metabolism in the archaeal phylum Bathyarchaeota revealed by genome-centric metagenomics. *Science* 2015;**350**:434–8.
- Faraq IF, Davis JP, Youssef NH et al. Global patterns of abundance, diversity and community structure of the *Aminicenantes* (Candidate Phylum OP8). *PLoS One* 2014;**9**:e92139.
- Giovannoni SJ, Thrash JC, Temperton B. Implications of streamlining theory for microbial ecology. *ISME J* 2014;**8**:1553–65.
- Glissmann K, Chin KJ, Casper P et al. Methanogenic pathway and archaeal community structure in the sediment of eutrophic Lake Dagow: Effect of temperature. *Microb Ecol* 2004;**48**:389–99.
- Griffiths RI, Whiteley AS, O'Donnell AG et al. Rapid method for coextraction of DNA and RNA from natural environments for analysis of ribosomal DNA- and rRNA-based microbial community composition. *Appl Environ Microbiol* 2000;**66**:5488–91.
- Haglund AL, Lantz P, Törnblom E et al. Depth distribution of active bacteria and bacterial activity in lake sediment. *FEMS Microbiol Ecol* 2003;**46**:31–8.
- He Y, Li M, Perumal V et al. Genomic and enzymatic evidence for acetogenesis among multiple lineages of the archaeal phylum *Bathyarchaeota* widespread in marine sediments. *Nat Microbiol* 2016;**1**:16035.
- Houser JN, Bade DL, Cole JJ et al. The dual influences of dissolved organic carbon on hypolimnetic metabolism: organic substrate and photosynthetic reduction. *Biogeochemistry* 2003;**64**:247–69.
- Hug LA, Castelle CJ, Wrighton KC et al. Community genomic analyses constrain the distribution of metabolic traits across the *Chloroflexi* phylum and indicate roles in sediment carbon cycling. *Microbiome* 2013;**1**:22.
- Iino T, Mori K, Uchino Y et al. *Ignavibacterium album* gen. nov., sp. nov., a moderately thermophilic anaerobic bacterium isolated from microbial mats at a terrestrial hot spring and proposal of *Ignavibacteria* classis nov., for a novel lineage at the periphery of green sulfur bacteria. *Int J Syst Evol Microbiol* 2010;**60**:1376–82.
- Kallistova AY, Goel G, Nozhevnikova AN. Microbial diversity of methanogenic communities in the systems for anaerobic treatment of organic waste. *Microbiology* 2014;**83**:462–83.
- Kankaala P, Taipale S, Nykänen H et al. Oxidation, efflux, and isotopic fractionation of methane during autumnal turnover in a polyhumic, boreal lake. *J Geophys Res Biogeosciences* 2007;**112**:G02033.
- Kantor RS, Wrighton KC, Handley KM et al. Small genomes and sparse metabolisms of sediment-associated bacteria from four candidate phyla. *MBio* 2013;**4**:e00708–13.
- Knittel K, Boetius A. Anaerobic oxidation of methane: progress with an unknown process. *Annu Rev Microbiol* 2009;**63**:311–34.

- Koizumi Y, Kojima H, Fukui M. Characterization of depth-related microbial community structure in lake sediment by denaturing gradient gel electrophoresis of amplified 16S rDNA and reversely transcribed 16S rRNA fragments. *FEMS Microb Ecol* 2003;**46**:147–57.
- Kortelainen P, Pajunen H, Rantakari M et al. A large carbon pool and small sink in boreal Holocene lake sediments. *Global Change Biology* 2004;**10**:1648–53.
- Lalonde K, Mucci A, Ouellet A et al. Preservation of organic matter in sediments promoted by iron. *Nature* 2012;**483**:198–200.
- Lazar CS, Baker BJ, Seitz K et al. Genomic evidence for distinct carbon substrate preferences and ecological niches of *Bathyarchaeota* in estuarine sediments. *Environ Microbiol* 2016;**18**:1200–11.
- Lehmann MF, Bernasconi SM, Barbieri A et al. Preservation of organic matter and alteration of its carbon and nitrogen isotope composition during simulated and in situ early sedimentary diagenesis. *Geochim Cosmochim Acta* 2002;**66**:3573–84.
- Lloyd KG, Schreiber L, Petersen DG et al. Predominant archaea in marine sediments degrade detrital proteins. *Nature* 2013;**496**:215–8.
- Martinez-Cruz K, Leewis M-C, Herriott IC et al. Anaerobic oxidation of methane by aerobic methanotrophs in sub-Arctic lake sediments. *Sci Total Environ* 2017;**607**:23–31.
- McInerney MJ, Struchtemeyer CG, Sieber J et al. Physiology, ecology, phylogeny, and genomics of microorganisms capable of syntrophic metabolism. *Annals of the New York Academy of Sciences*. 2008;**1125**:58–72.
- Molot LA, Dillon PJ. Storage of terrestrial carbon in boreal lake sediments and evasion to the atmosphere. *Global Biogeochem Cycles* 1996;**10**:483–92.
- Mori K, Yamaguchi K, Sakiyama Y et al. *Caldisericum exile* gen. nov., sp. nov., an anaerobic, thermophilic, filamentous bacterium of a novel bacterial phylum, *Caldiserica* phyl. nov., originally called the candidate phylum OP5, and description of *Caldiserica a caeae* fam. nov., *Caldisericales* ord. no. *Int J Syst Evol Microbiol* 2009;**59**:2894–8.
- Mothes G, Koschel R, Proft G. The chemical environment. In: Casper SJ (ed). *Lake Stechlin, a temperate oligotrophic lake*. Dordrecht: Dr. W. Junk Publishers, 1985, 87–128.
- Mpamah PA, Taipale S, Rissanen AJ et al. The impact of long-term water level draw-down on microbial biomass: a comparative study from two peatland sites with different nutrient status. *Eur J Soil Biol* 2017;**80**:59–68.
- Nykänen H, Peura S, Kankaala P et al. Recycling and fluxes of carbon gases in a stratified boreal lake following experimental carbon addition. *Biogeosci Discuss* 2014;**11**:16447–95.
- Pajunen H. *Järvisedimentit Kuiva-Aineen Ja Hiilen Varastona*. Espoo, Finland: Geological Survey of Finland, Report of Investigation 160, 2004. (in Finnish).
- Petro C, Starnawski P, Schramm A et al. Microbial community assembly in marine sediments. *Aquat Microb Ecol* 2017;**79**:177–95.
- Peura S, Eiler A, Bertilsson S et al. Distinct and diverse anaerobic bacterial communities in boreal lakes dominated by candidate division OD1. *ISME J* 2012;**6**:1640–52.
- Phelps TJ, Zeikus JG. Influence of pH on terminal carbon metabolism in anoxic sediments from a mildly acidic lake. *Appl Environ Microbiol* 1984;**48**:1088–95.
- Podosokorskaya OA, Kadnikov VV, Gavrillov SN et al. Characterization of *Melioribacter roseus* gen. nov., sp. nov., a novel facultatively anaerobic thermophilic cellulolytic bacterium from the class *Ignavibacteria*, and a proposal of a novel bacterial phylum *Ignavibacteriae*. *Environ Microbiol* 2013;**15**:1759–71.
- Rinke C, Schwientek P, Sczyrba A et al. Insights into the phylogeny and coding potential of microbial dark matter. *Nature* 2013;**499**:431–7.
- Rissanen AJ, Karvinen A, Nykänen H et al. Effects of alternative electron acceptors on the activity and community structure of methane-producing and consuming microbes in the sediments of two shallow boreal lakes. *FEMS Microbiol Ecol* 2017;**93**:fix078.
- Rissanen AJ, Saarenheimo J, Tiirola M et al. Gammaproteobacterial methanotrophs dominate methanotrophy in aerobic and anaerobic layers of boreal lake waters. *Aquat Microb Ecol* 2018;**81**:257–76.
- Robbins SJ, Evans PN, Parks DH et al. Genome-centric analysis of microbial populations enriched by hydraulic fracture fluid additives in a coal bed methane production well. *Front Microb* 2016;**7**:731.
- Roslev P, King GM. Survival and recovery of methanotrophic bacteria starved under oxic and anoxic conditions. *Appl Environ Microbiol* 1994;**60**:2602–8.
- Rotaru A-E, Shrestha PM, Liu F et al. A new model for electron flow during anaerobic digestion: direct interspecies electron transfer to *Methanosaeta* for the reduction of carbon dioxide to methane. *Energy Environ Sci* 2014;**7**:408–15.
- Schiff SL, Tsuji JM, Wu L et al. Millions of boreal shield lakes can be used to probe Archean Ocean biogeochemistry. *Sci Rep* 2017;**7**:46708.
- Schloss PD, Westcott SL, Ryabin T et al. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Appl Environ Microbiol* 2009;**75**:7537–41.
- Slobodkin A. The Family *Peptostreptococcaceae*. In: Rosenberg E, DeLong EF, Lory S et al. (eds). *The Prokaryotes: Firmicutes and Tenericutes*. Berlin, Heidelberg: Springer Berlin Heidelberg, 2014, 291–302.
- Starnawski P, Bataillon T, Ettema TJG et al. Microbial community assembly and evolution in seafloor sediment. *PNAS* 2017;**114**:2940–5.
- Steger K, Premke K, Gudaszc C et al. Microbial biomass and community composition in boreal lake sediments. *Limnol Oceanogr* 2011;**56**:725–33.
- Sugiyama Y, Anegawa A, Inokuchi H et al. Distribution of dissolved organic carbon and dissolved fulvic acid in mesotrophic Lake Biwa, Japan. *Limnology* 2005;**6**:161–8.
- Viollier E, Jézéquel D, Michard G et al. Geochemical study of a crater lake (Pavin Lake, France): Trace-element behaviour in the monimolimnion. *Chem Geol* 1995;**125**:61–72.
- Wasmund K, Schreiber L, Lloyd KG et al. Genome sequencing of a single cell of the widely distributed marine subsurface *Dehalococcoidia*, phylum *Chloroflexi*. *ISME J* 2014;**8**:383–97.
- Wickstrom K, Tolonen K. The history of airborne polycyclic aromatic hydrocarbons (PAH) and perylene as recorded in dated lake sediments. *Water Air Soil Pollut* 1987;**32**:155–75.
- Wik M, Varner RK, Anthony KW et al. Climate-sensitive northern lakes and ponds are critical components of methane release. *Nat Geosci* 2016;**9**:99–105.
- Wrighton KC, Castelle CJ, Wilkins MJ et al. Metabolic interdependencies between phylogenetically novel fermenters and respiratory organisms in an unconfined aquifer. *ISME J* 2014;**8**:1452–63.
- Wrighton KC, Thomas BC, Sharon I et al. Fermentation, hydrogen, and sulfur metabolism in multiple uncultivated bacterial phyla. *Science* 2012;**337**:1661–5.

- Wurzbacher C, Fuchs A, Attermeyer K et al. Shifts among *Eukaryota*, *Bacteria*, and *Archaea* define the vertical organization of a lake sediment. *Microbiome* 2017;5:41.
- Xiong W, Xie P, Wang S et al. Sources of organic matter affect depth-related microbial community composition in sediments of Lake Erhai, Southwest China. *J Limnol* 2015;74:310–23.
- Yan Z, Joshi P, Gorski CA et al. A biochemical framework for anaerobic oxidation of methane driven by Fe(III)-dependent respiration. *Nat Commun* 2018;9:1642.
- Ye LL, Wu XD, Liu B et al. Dynamics of dissolved organic carbon in eutrophic Lake Taihu and its tributaries and their implications for bacterial abundance during autumn and winter. *J Freshw Ecol* 2015;30:129–42.
- Ye W, Liu X, Lin S et al. The vertical distribution of bacterial and archaeal communities in the water and sediment of Lake Taihu. *FEMS Microb Ecol* 2009;70:263–76.
- Youngblut ND, Dell'Aringa M, Whitaker RJ. Differentiation between sediment and hypolimnion methanogen communities in humic lakes. *Environ Microbiol* 2014;16:1411–23.
- Yu T, Wu W, Liang W et al. Growth of sedimentary *Bathyarchaeota* on lignin as an energy source. *PNAS* 2018;115:6022–7.
- Zhukova NV. Variation in microbial biomass and community structure in sediments of Peter the Great Bay (Sea of Japan/East Sea), as estimated from fatty acid biomarkers. *Ocean Sci J* 2005;40:34–42.