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# Lake Sediments From Littoral and Profundal Zones are Heterogeneous but Equivalent Sources of Methane Produced by Distinct Methanogenic Communities-A Case Study From Lake Remoray

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# JGR Biogeosciences

## RESEARCH ARTICLE

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### Key Points:

- Methane concentration and production potential in lake sediments were spatially heterogeneous and similar in littoral and profundal zones
- Spatial patterns of methane concentration and production were associated with variations in nature and quality of sediment organic matter
- Weighted correlation network analysis revealed five distinct sediment methanogen communities spatially distributed across the lake

### Supporting Information:

Supporting Information may be found in the online version of this article.

### Correspondence to:

V. Tardy,  
[vpm.tardy@gmail.com](mailto:vpm.tardy@gmail.com)

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### Author Contributions:

**Conceptualization:** David Etienne, Laurent Millet, Emilie Lyautey  
**Data curation:** Vincent Tardy  
**Funding acquisition:** David Etienne, Laurent Millet, Emilie Lyautey  
**Investigation:** Vincent Tardy  
**Methodology:** David Etienne, Laurent Millet, Emilie Lyautey  
**Resources:** David Etienne, Emilie Lyautey  
**Supervision:** Emilie Lyautey  
**Visualization:** Vincent Tardy  
**Writing – original draft:** Vincent Tardy, Emilie Lyautey  
**Writing – review & editing:** Vincent Tardy, Emilie Lyautey

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## Lake Sediments From Littoral and Profundal Zones are Heterogeneous but Equivalent Sources of Methane Produced by Distinct Methanogenic Communities—A Case Study From Lake Remoray

Vincent Tardy<sup>1,2</sup> , David Etienne<sup>1,2</sup> , Laurent Millet<sup>3</sup>, and Emilie Lyautey<sup>1,2</sup>

<sup>1</sup>Pôle R&D 'ECLA, Le Bourget-du-Lac, France, <sup>2</sup>University Savoie Mont Blanc, INRAE, CARTELE, Thonon-les-Bains, France, <sup>3</sup>Chrono-Environnement, Université de Franche-Comté / CNRS, Besançon, France

**Abstract** Lake ecosystems contribute significantly to atmospheric methane and are likely to become even bigger methane emitters with the global spread of hypoxia/anoxia in freshwater ecosystems. Here we characterized the spatial heterogeneity of methane production potential, methane concentration, archaeal and bacterial communities across Lake Remoray sediment during the summer period when hypoxic conditions settle in the deepest part of the water column. It was hypothesized that methane concentration and production would be higher in the deeper part of the lake, our results showed that some littoral areas exhibited similar or higher values than the deepest area. The full 16S rRNA gene sequencing dataset counted 41 OTUs affiliated with methanogenic species in abundances that depended more on sampling-site location than on the water depth gradient. The methanogenic co-occurrence network revealed the existence of five distinct sub-communities, suggesting the presence of different methanogenic niches across Lake Remoray. The variation in abundance of the two larger methanogenic sub-communities was significantly related to methanogenesis potential and sediment methane concentration across-lake but further studies investigating their real activities would provide additional insights. In a globally changing environment (temperature, eutrophication, ...) a better understanding of the functional specificities and characteristics of the potential of methane cycle actors would allow us to better predict their future implications for greenhouse gas production and mitigation.

**Plain Language Summary** Many lakes worldwide produce methane, a greenhouse gas with a global warming potential 72 times higher than CO<sub>2</sub>. However, global estimations of methane emission from lake ecosystems would be improved by including spatially resolved datasets. Particularly to extend the knowledge about methane production both in the deeper areas of these ecosystems as well as in the shallower and littoral areas. In this study, we measured methane concentrations and production in sediment at different locations from one lake, located in France. Using a DNA approach, we also analyzed the microorganisms inhabiting sediment to detect species able to produce methane. We observed that sediments in littoral zones harbored distinct methanogen species and exhibited as high methane concentrations than in the deeper anoxic areas. This suggests that in the sediments of littoral zones methane production is also an active process carried out by distinct microbial actors.

## 1. Introduction

Methane ranks just behind carbon dioxide as a major greenhouse gas, with a 12-year lifetime in the atmosphere, a global warming potential 72 times higher than CO<sub>2</sub> over a 20-year timescale (25 times higher over 100 years), and a radiative forcing of +0.48 W m<sup>-2</sup> (IPCC, 2007). Methane concentration in the atmosphere is currently about 1,850 ppb (Nisbet et al., 2019), which is an increase of more than 150% since the pre-industrial era and rising fast at the unprecedented rate of >5 ppb yr<sup>-1</sup> in the 2004–2017 period (Nisbet et al., 2019).

Atmospheric methane is mostly anthropogenic: fossil fuel extraction and agricultural practices account for about 60% of methane emissions (Saunois et al., 2020). Other sources are natural: wetlands ecosystems are the biggest natural source of atmospheric methane, followed by lake ecosystems which account for 6%–16% of non-anthropogenic emissions (Bastviken et al., 2004). Depending on the methodologies applied, lakes are estimated to be responsible for up to 50 (Johnson et al., 2022) to 150 Tg (Saunois et al., 2020) of C-CH<sub>4</sub> emitted to the atmosphere per year. Recent models project that lake emissions will increase under the combined effects of

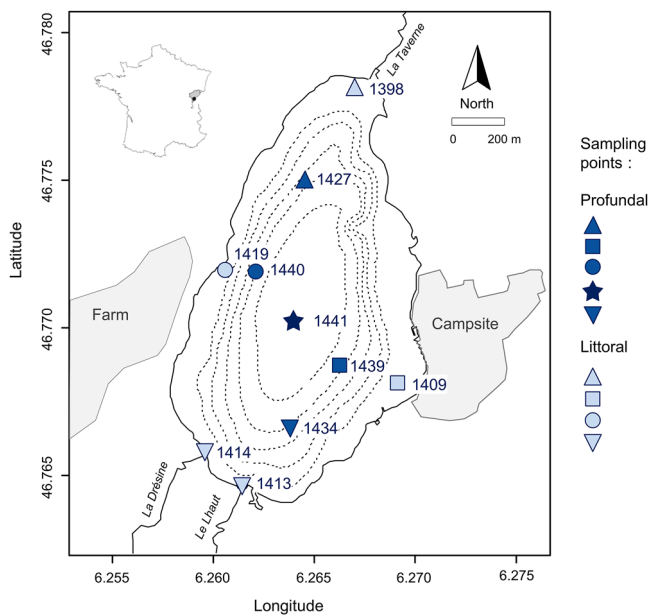
rising temperature (Cavicchioli et al., 2019; Fuchs et al., 2016) and global-scale eutrophication of water bodies (Beaulieu et al., 2019; D'Ambrosio & Harrison, 2021; Sepulveda-Jauregui et al., 2018). The isotopic signature of atmospheric methane is also changing, as  $\delta^{13}\text{C}-\text{CH}_4$  has fallen from  $-47.06\text{‰}$  to  $-47.36\text{‰}$  in less than 10 years, likely reflecting changes in both sources and sinks of atmospheric methane (Nisbet et al., 2019).

Microbial actors of the methane cycle are the methanogenic archaea, which are involved under anoxic conditions in the final stage of reducing organic matter (OM) into methane (Evans et al., 2019), and methanotrophic organisms (archaea and bacteria). Methanotrophs oxidize methane into carbon dioxide under both anaerobic and aerobic conditions (Bhattarai et al., 2019; Kang et al., 2019) with efficiencies ranging from 30% to 99% in lake ecosystems (Bastviken et al., 2008). In lakes, methanogenesis mainly takes place in the sediment and in the deeper water column layers under temporary or permanent anoxic conditions (Bastviken et al., 2008; Lehours et al., 2016; Liu et al., 2017; Peeters et al., 2019) although methanogenesis in oxic conditions has been reported (i.e., the methane paradox, Bižić et al. [2020]; Grossart et al. [2011]; Wang et al. [2021]). Methane oxidation is carried out aerobically by methanotrophic bacteria or anaerobically in the presence of sulfates or nitrates as terminal electron acceptors by methanotrophic Archaea (Borrel et al., 2011). If not oxidized, methane can be emitted to the atmosphere through four well-described pathways: bubbling, diffusion, during lake overturn when methane trapped in the hypolimnion is released, and via flows related to aquatic plants and roots that facilitate sediment–water column gas exchanges (Bastviken et al., 2004).

Among the abiotic factors likely to affect the methane cycle, those that vary spatially and seasonally and with stratification of the water column will define the location of the oxic/anoxic interface and influence methanogenic and methanotrophic community diversity and activity rates (Chaudhary et al., 2013; Chowdhury & Dick, 2013; Froelich et al., 1979; Thomas et al., 2018). Methane oxidation dynamics are influenced by methane (Semrau et al., 2010), oxygen (Bastviken et al., 2002; Bosse et al., 1993; Duc et al., 2010), or nitrogen concentrations (Bosse et al., 1993), and by methanotroph grazing by benthic invertebrates (Murase & Frenzel, 2008). Methanogenesis depends more on redox potential (Lehours et al., 2005), quality and quantity of available OM (Bastviken et al., 2008), and temperature (Duc et al., 2010). In particular, it has been shown that methane production in lake sediments is correlated with the C/N ratio of OM: methane production rates are higher at  $\text{C/N} < 10$  (Duc et al., 2010). This suggests that methanogenic and methanotrophic communities are likely to be sensitive to variations in redox potential but also to qualitative and quantitative variations in OM inputs. At the lake scale, seasonally-driven water column stratification maximizes the spatial occurrence of favorable redox methanogenesis conditions in the hypolimnion (Deemer & Harrison, 2019), and thermal stratification is expected to last for longer periods in future global climate change scenarios (Butcher et al., 2015; Jenny et al., 2020; Sahoo et al., 2016).

When assessing the methane cycle microbial actors, recent studies observed the absence of relationships between the abundances of methanogens and/or methanotrophs and the recorded activity rates of fluxes (Emerson et al., 2021; Pierangeli et al., 2021), making these descriptors weak predictors of the methane cycle activity rates. Opposite, bacterial community composition was shown to significantly influence methanogenic community activity, particularly through the strong relationships occurring between fermentative bacteria and methanogenic archaea (Bertolet et al., 2019; Emerson et al., 2021; Lavergne et al., 2021; Pierangeli et al., 2021), with fermentative bacteria supplying substrates to methanogenic archaea, thus supporting trophic cooperation. At horizontal scales, methanogenic communities are generally different between low and high water depth areas, with site-related changes in the relative abundances of the major genera (Berberich et al., 2020).

Here we aimed to assess the spatial heterogeneity of methane production potential, concentration, and diffusion in the first 10 cm of Lake Remoray sediments (France). Lake Remoray is characterized by eutrophication due to OM, nutrient and contaminant inputs from the watershed, and by long periods of hypoxia in the deep water column (Belle et al., 2016). In July 2017, sediment cores were collected from 10 sampling sites across the 95-ha lake. Environmental descriptors were related to physicochemistry of the sediment (OM content). Abundances of archeal and bacterial communities involved in methane production and oxidation were assessed by targeting *mcrA* (methyl coenzyme M reductase) and *pmoA* (particulate methane monooxygenase) genes as functional markers. Diversity, structure, and composition of sediment archeal and bacterial communities were assessed using an Illumina sequencing approach targeting 16S ribosomal RNA (rRNA) genes. The whole sequencing dataset served to characterize the methanogenic communities by co-occurrence network analysis.



**Figure 1.** Map of Lake Remoray showing the sediment-core sampling points used in the year-2017 campaign. Lake bathymetry is represented in dashed black lines showing 5-m-depth contours.

We expected that (a) methane concentration, production, and diffusion in sediment would be higher in the deeper zones due to extensive recent OM accumulation and to the longer time period of anoxia in overlying water column layers, and that (b) spatial variations of methane concentration, production, and diffusion in sediment would be related to the diversity and to their metabolic genetic potentials (i.e., DNA-based abundance of the functional communities) of sediment methanogenic communities.

## 2. Materials and Methods

### 2.1. Study Site and Core Collection

Lake Remoray (46°46'12" N; 6°15'49" E; Figure 1) is located in the Jura Mountains (eastern France). The water depth of this moderately-sized lake (95 ha) reaches 27 m. The lake is currently meso-eutrophic, with an accumulation of organic matter in the central and deepest part of the lake (Belle et al., 2017). It is fed by two tributaries (the Drésine and the Lhaut), has one outlet (the Taverne) (Figure 1), and is bordered by a farm and a campsite. The lake is surrounded mainly by a mix of *Juncus* and *Phragmites* species and species affiliated with the Cyperaceae family. The catchment basin of Lake Remoray consists of 46.7% forest (mainly coniferous forest), 42.5% agricultural land, 5.5% peat bog, 2.6% water area, and 2.7% urban area (Belle et al., 2016).

In 2017 (4 and 5 July), short (<1m) 90-mm-diameter sediment cores were collected at 10 sampling sites located all over the lake using a UWITEC gravity corer (Figure 1). The 10 sites were selected out of the 44 sites analyzed previously (Tardy et al., 2021), to include the deepest site [#1441] along with littoral-zone (<5 m) and profundal-zone (about 20 m deep) sites representing the four characteristic areas of the lake: tributaries [#1413, #1414, and #1434, close from the two lake tributaries, the Drésine and the Lhaut], outlet [#1398 and #1427], campsite [#1409 and #1439], and farm [#1419 and #1440] (Figure 1). At sampling time, water temperature at the water–sediment interface was  $5 \pm 0.06^\circ\text{C}$  for profundal-zone and  $21.7 \pm 0.05^\circ\text{C}$  for littoral zone, and thermal stratification was established at 5 m depth. Two cores were collected at each site, and the first 10 cm of sediment from the sediment water interface in each core were sub-sampled directly in the field. For the first core, sediment samples were collected through a pre-pierced liner using precut autoclaved syringes at determined sampling depths ( $n = 10$ , every centimetre for the first 10 cm), and used for biogeochemical measurements (sediment methane concentrations and sediment to water diffusive flux calculations) and microbiological analyses (molecular-based analyses). For the second sediment core, sediment samples were extruded using a core extruder and 2-cm sediment sections were collected using a core cutter ( $n = 5$ , every 2 cm for the 10 first centimeters) to serve for biogeochemical measurements (potential activity rates) and sedimentological analysis.

### 2.2. Sediment Characteristics

Sediment particle size distribution was determined by laser diffraction on dry sediments (Mastersizer S, Malvern, United Kingdom), and particles were grouped by median grain size. Sediment water content was estimated by drying sediment at 60°C for 48 hr. Organic matter (OM) content was measured by loss-on-ignition (Heiri et al., 2001). Organic carbon (OC) content and porosity were calculated from water and OM contents using formulas provided elsewhere (Avnimelech et al., 2001).

### 2.3. Sediment Methane Concentrations and Diffusion

Sediment methane concentrations and diffusion were estimated according to a method modified by Thomas et al. (2018). Sediment samples (0.5 ml) were collected from the sediment core every centimetre through pre-pierced holes and were placed into glass vials with NaOH (4 ml of 2.5% NaOH in 20-ml vials), covered with a butyl stopper, and sealed with an aluminum crimp. Dissolved sediment methane concentrations were measured

in the headspace by gas chromatography using an Agilent 490 MicroGC thermal conductivity detector equipped with a PoraPLOT Q column with helium as vector gas.

Diffusive methane ( $\text{CH}_4$ ) fluxes from the sediments ( $J_s$ ) were calculated based on Fick's first law using the top 10 cm of measured sediment methane concentrations. The equation from Maerki et al. (2004) was used to correct for porosity and tortuosity. The methane diffusion coefficient,  $D$ , was interpolated by sampling site water temperature at the water–sediment interface according to Lerman (1979).  $J_s$  was calculated according to Sollberger et al. (2014) as follows:

$$J_s = -\frac{D}{F} \times \frac{\delta\text{CH}_4}{\delta z}$$

where  $F$  is the formation factor of the sediment and  $z$  is the depth.

#### 2.4. Sediment Methane Production Potential

Sediment methane production potentials were measured using a protocol modified from Fuchs et al. (2016) by incubating lake sediment under controlled conditions. Immediately following field sampling, wet sediment from each targeted 2-cm section of extruded sediment was placed in a flask, filled with sediment to the top to exclude air capture, sealed, and stored at 4°C until potential activity measurements. For methane production potential, 5–10 g of wet sediment was transferred to 150 ml flasks added with 10 ml of demineralized water. The flasks were hermetically sealed with rubber caps, inerted with helium, and incubated at 20°C under constant moderate agitation during the course of the experiment. Headspace gases were extracted through a needle punched into each flask septa, and were sampled directly to the gas chromatograph using a 50 ms injection time. Sediment methane concentrations were measured by gas chromatography ( $n = 1$ ) at  $t_0 = 1$  hr (allowing 1 hr of medium equilibration) and at 5 hr using the Agilent 490 MicroGC thermal conductivity detector. For each flask, dry weights of incubated sediments were determined after drying at 105°C for 48 hr. Methane production was expressed as ng of C- $\text{CH}_4$  g<sup>-1</sup> sediment dry weight [DW] h<sup>-1</sup> based on the increase in methane concentrations.

#### 2.5. Molecular Analyses

The molecular analysis workflows used here were already been published elsewhere (Lyautey et al., 2021; Tardy et al., 2021; Thomas et al., 2018). Samples for nucleic acid-based analyses were collected using pre-cut auto-claved syringes, transferred to cryovials, immediately stored on liquid nitrogen in the field, taken back to the lab, and held at -20°C until nucleic acid extraction was completed. Total DNA was extracted from 0.5 g of wet sediment using a NucleoSpin Soil Kit following the manufacturer's instructions, and using SL1 lysis buffer and additive Enhancer SX buffer (Macherey-Nagel, Hoerd, France). The extracted DNA was quantified fluorometrically after staining with QuantiFluor dsDNA Dye (QuantiFluor dsDNA System, Promega, Charbonnières-les-Bains, France) using a Plate Chameleon™ fluorometer (Hidex, Turku, Finland; excitation: 485 nm, emission: 590 nm).

The abundances of total and functional organisms were assessed by quantitative PCR targeting the 16S rRNA, *mcrA*, and *pmoA* genes. Real-time PCR was carried out in triplicate on DNA extracts for each sample. For 16S rRNA gene analysis, reactions were done in a final volume of 25 μl containing 1 × Brilliant II SYBR® Green QPCR Master Mix (Agilent), 0.3 mg ml<sup>-1</sup> bovine serum albumin (Sigma-Aldrich), 0.3 μM of each primer (EUB-341F: 5'-CCT ACG GGA GGC AGC AG-3' and EUB-515R: 5'-ATT ACC GCG GCT GCT GG-3'; López-Gutiérrez et al., 2004), and 0.5 μl DNA extract. Thermal cycling conditions were an initial cycle of 95°C for 10 min, 45 cycles of 95°C for 15 s, 60°C for 30 s, and 72°C for 30 s, and then a melt-curve step (55°C–95°C). A plasmid containing a single copy of 16S rRNA genes amplified from *Escherichia coli* (Bacteria) was diluted from 10<sup>8</sup> to 10<sup>1</sup> copies per assay and used in triplicate to produce the standard curve. Amplification efficiency was 105%. Methanogen and methanotroph abundance was evaluated by quantitative PCR on *mcrA* and *pmoA* genes using the protocol described in Fuchs et al. (2016) but with a final qPCR mix adjusted to 25 μl.

PCR amplification for high-throughput 16S rRNA sequencing was carried out with the universal primer pair 515F (5'-GTGYCAGCMGCCGCGTA-3') and 909R (5'-CCCCGYCAATTCMTTTRAGT-3') targeting the hypervariable V4-V5 region of the 16S rRNA gene (Y. Wang & Qian, 2009) according to the protocol from Tardy et al. (2021). Sequencing was realized by Fasteris (Geneva, Switzerland) on an Illumina HiSeq 2500 system with



2 × 250 bp. Analysis yielded 6.7 Gb of sequences with an average error rate of 0.822% and an average Q30 of 90.3%.

## 2.6. Bioinformatics Analysis

Bioinformatics analysis was carried out according to Tardy et al. (2021). Adapter sequences were removed using Trimmomatic (Bolger et al., 2014) and barcode-sorted using a Fasteris internal script (Perl script). Sequences were then processed using the FROGS (Find Rapidly OTUs with Galaxy Solution) Galaxy-supported pipeline (Escudié et al., 2018). Paired-end reads were joined using FLASH (Magoc & Salzberg, 2011) and quality-checked using FastQC. Sequences with non-mismatching primers were kept and then filtered by size (between 350 and 500 bp), and those containing N bases were discarded. The 16S rRNA gene sequences were then denoised and clustered using the Swarm method (Mahé et al., 2014) with a maximum 3-bases difference. Clusters having less than 0.005% abundance and with occurrence in less than two samples of the total library were deleted. Chimeras were removed using the vchime tool of the vsearch package (Rognes et al., 2016). Affiliation was done using the Silva SSU database v.123 (Quast et al., 2013) via BLAST (Altschul et al., 1990) with multiple affiliations allowed and manual curation. All analyses were done on the Galaxy instance of the INRA MIGALE bioinformatics platform (<http://migale.jouy.inra.fr>). The raw datasets are available in the European Bioinformatics Institute (EBI) database system (in the European Nucleotide Archive) under project accession number PRJEB 43875.

## 2.7. Statistical Analysis

Sediment methane production potential, methane concentration, methane diffusion, microbial density, *mcrA*/16S rRNA genes ratio, and *pmoA*/16S rRNA genes ratio were compared between the littoral and profundal zones of Lake Remoray using a nonparametric Kruskal–Wallis test for each stratum of sediment core samples. Statistical results for each comparison were presented in Table S1.

Based on the taxonomic classification, methanogenic sequences were extracted from the sequencing dataset. We used all the orders of methanogen known in literature and which are represented by Methanobacteriales, Methanomassiliicoccales, Methanomicrobiales, Methanosarcinales, Methanomethyliales, and Methanofastidiosales (Evans et al., 2019). Spatial variations in archeal and bacterial and methanogenic community structures across Lake Remoray were characterized using UniFrac distance (Lozupone & Knight, 2005). Nonmetric multidimensional scaling (NMDS) was used to graphically depict spatial variations between the archeal and bacterial, and methanogenic communities. The significance of the observed clustering of samples on the ordination plot was assessed by analysis of variance using distance matrices (ADONIS test, 999 permutations).

We used the ‘DESeq2’ R package (Love et al., 2014) to calculate the significant changes in methanogenic composition (log<sub>2</sub> fold change in the relative abundance of each OTU) between the littoral and profundal zones of Lake Remoray. Differences in methanogenic OTU abundances between in-lake depth zones were considered significant if their adjusted *p* < 0.01.

The methanogenic community co-occurrence network was built using the ‘igraph’ R package. Pearson correlations between all pairs of 41 OTUs (OTUs found in at least 15 samples) were calculated, and the *p*-values were adjusted using Benjamini–Hochberg (BH) method (Benjamini & Hochberg, 1995). Only significant correlations (*p* < 0.05, post-BH correction) with *R* > 0.5 were graphed. Network modules (subcommunities in the network) were identified using the ‘WGCNA’ R package (Langfelder & Horvath, 2008) on the adjacency matrix of Pearson correlation values between each pair of OTUs with a thresholding power of 6. The adjacency matrix was transformed into a topological overlap matrix to minimize possible errors, and the OTUs were clustered in modules with a minimum number of three OTUs per module. Modules having <0.3 dissimilarities in eigengenes were subsequently merged, and Pearson correlations between merged modules and the methane functional parameter dataset (methane production, methane, OM concentration, and *mcrA*/16S rRNA genes ratio) were calculated.

All statistical analyses were performed using the free R software (version 3.5.3). Data and R codes are available at the Zenodo repository (<https://doi.org/10.5281/zenodo.6518228>).

**Table 1**  
*Sediment Texture (Percentage of Clay, Silt, and Sand) and Percentage of Organic Matter (OM) and Organic Carbon (OC) for Each Sediment Core*

	GPS code	Clay (%)	Silt (%)	Sand (%)	OM (%)	OC (%)
Littoral zone	1398	1.5 ± 1.8	68.3 ± 2.8	30.2 ± 2.2	12.6 ± 2.1	7.4 ± 1.3
	1409	1.3 ± 1.2	66.6 ± 5.8	32.1 ± 4.7	8.8 ± 1.4	5.2 ± 0.8
	1413	0.2 ± 0.5	18.8 ± 15.8	80.9 ± 16.3	10.0 ± 2.9	5.9 ± 1.7
	1414	nd	Nd	nd	24.5 ± 8.4	14.4 ± 5.0
	1419	0.3 ± 0.7	71.6 ± 3.6	28.1 ± 4.0	9.2 ± 0.8	5.4 ± 0.5
Profundal zone	1427	3.9 ± 3.5	73.5 ± 10.4	22.6 ± 8.6	11.8 ± 0.3	7.0 ± 0.2
	1434	0.6 ± 0.3	82.2 ± 4.9	17.2 ± 4.7	15.6 ± 1.3	9.2 ± 0.8
	1439	1.6 ± 0.8	71.6 ± 10.9	26.8 ± 10.2	12.2 ± 0.1	7.2 ± 0.1
	1440	2.2 ± 2.6	78.4 ± 8.0	19.4 ± 5.6	12.1 ± 0.4	7.1 ± 0.2
	1441	3.5 ± 2.3	70.8 ± 9.1	25.7 ± 7.9	16.2 ± 1.4	9.6 ± 0.8

*Note.* Value represent the average and standard deviation of the parameter measured every 2 cm along the top 10 cm of sediment cores.

### 3. Results

#### 3.1. Sediments Characteristics

Sediment texture was relatively similar between the different sampling sites of Lake Remoray, with about 73% silt, 25% sand, and 2% clay (Table 1). Only sampling site #1413 close to the Lhaut tributary had a different sediment texture, with 19% silt, 81% sand, and less than 1% clay.

Organic matter (OM) and organic carbon (OC) content in the first 10 cm of sediment cores varied from 6.3% to 38% and from 3.7% to 22%, respectively, depending on sampling-site location (Table 1). The highest OM and OC contents were found at sampling site #1414 close to lake tributaries, with in average of the first 10 cm of the sediment core containing an average of 24% OM and 14% OC. The deepest sampling site (#1441) was characterized by 16% OM content and 9.6% OC content.

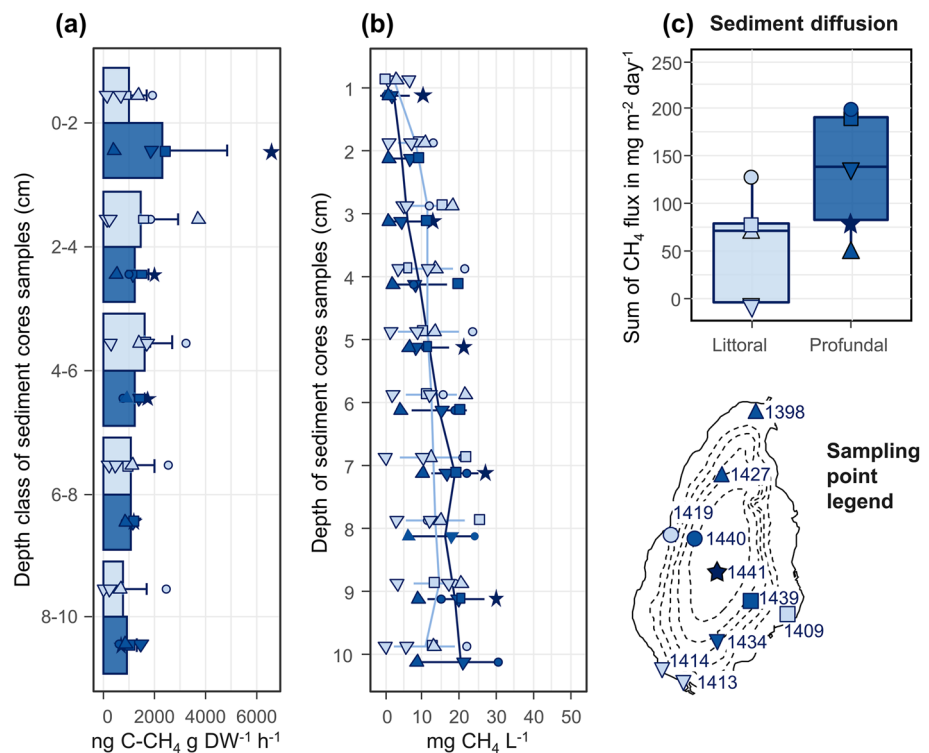
#### 3.2. Methane Production Potential, Concentration, and Sediment Diffusion Across Lake Remoray

Sediment methane production potential, concentration, and diffusion were not related to the water depth at the sampling site (littoral vs. profundal zone) and were not significantly different between stratum depths along sediment cores (Kruskal-Wallis test in all sediment strata; Table S1) (Figure 2).

In the profundal zone, sediment methane production potential was maximal in the first 2 cm of sediment cores (Figure 2a). The highest methane potential production was recorded at the deepest sampling site (#1441), with 6,563 ng C-CH<sub>4</sub> g DW<sup>-1</sup> h<sup>-1</sup>. Sediment methane production potential decreased gradually along the strata of sediment cores at all profundal-zone sampling sites. Average methane production potential in the littoral zone varied from 789 to 1,674 C-CH<sub>4</sub> g DW<sup>-1</sup> h<sup>-1</sup> along the sediment core strata. The highest littoral-zone methane production potential was recorded at sampling site #1419, with 1,833–3,263 C-CH<sub>4</sub> g DW<sup>-1</sup> h<sup>-1</sup> over the first 10 cm of the sediment core. The lowest methane production potential was recorded at sampling sites #1413 and #1414, with an average of 179 and 705 C-CH<sub>4</sub> g DW<sup>-1</sup> h<sup>-1</sup>, respectively, over the first 10 cm of the sediment core.

Sediment methane concentrations increased with sediment core depth (Figure 2b), ranging from 2 to 8.2 mg CH<sub>4</sub> L<sup>-1</sup> for littoral-zone cores and 2.6–11 mg CH<sub>4</sub> L<sup>-1</sup> for profundal-zone cores. The deepest sampling site (#1441) had the highest methane concentrations along the sediment core. Sampling site #1413, in the tributaries area, had the lowest methane concentrations in the sediment core.

Calculations of methane diffusion along the first 10 cm of the sediment cores found significantly higher average diffusive methane fluxes in the profundal zone (Kruskal–Wallis test,  $p = 0.04$ ) (Figure 2c). The highest methane fluxes were recorded at profundal-zone sites #1439 and #1440 (192 and 200 mg CH<sub>4</sub> m<sup>-2</sup> day<sup>-1</sup>, respectively). The lowest fluxes were recorded at littoral-zone sampling sites #1413 and #1414 (−4 and −7 mg CH<sub>4</sub> m<sup>-2</sup> day<sup>-1</sup>).



**Figure 2.** Average values for methane production potential (a), methane concentration (b), and sediment methane diffusion (c) along the first 10 cm of sediment cores for littoral-zone (light blue) and profundal-zone (blue) areas of Lake Remoray. Symbols represent the different sampling points across Lake Remoray (see Figure 1). Sediment methane diffusion (c) represents the sum of methane fluxes calculated between each stratum for each sampling point.

### 3.3. Genetic Potential of Methane Cycle Functioning Across Lake Remoray

In every sampling-depth zone, bacterial densities assessed as 16S rRNA gene copies  $g^{-1}$  DW (Figure 3a) decreased from top to bottom of the sediment cores. No significant differences were observed between littoral and profundal zones (confirmed by Kruskal–Wallis tests for all sediment strata; Table S1) and the variation of bacterial densities depended more on sampling-site location across Lake Remoray.

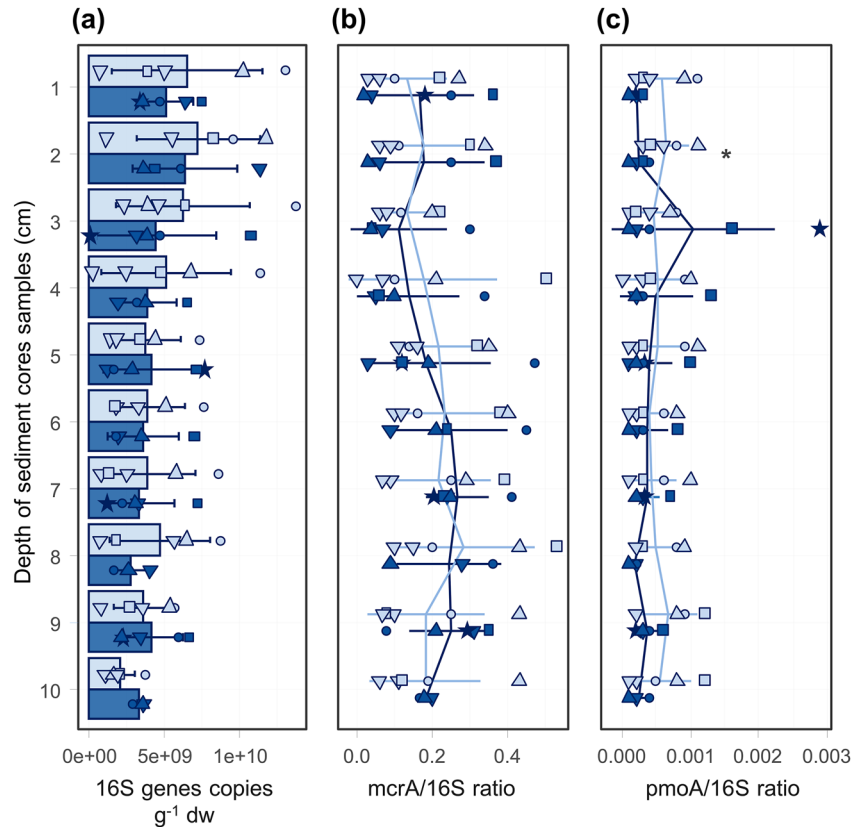
Gene-based potential methanogenesis (*mcrA*/16S rRNA genes ratio) and methanotrophy (*pmoA*/16S rRNA genes ratio) (Figures 3b and 3c) varied depending on sampling-site location across Lake Remoray. There were no significant differences between littoral and profundal zones, except *pmoA*/16S rRNA genes ratio which was significantly higher in the 2-cm stratum of littoral-zone sediment cores. The microbial community had 700-fold higher methanogenesis potential than methanotrophy potential. The highest methanogenesis potential was found for profundal-zone site #1440 (average *mcrA*/16 ratio of 0.31 over the whole sediment core) and for littoral-zone sampling site #1398 (average *mcrA*/16 ratio of 0.34 over the whole sediment core). The lowest methanogenesis potential was found for littoral-zone sampling sites #1413 and #1414 (average *mcrA*/16S rRNA genes ratio of 0.09 and 0.08, respectively, over the whole sediment core).

### 3.4. Archeal and Bacterial, and Methanogenic Community Structure Across Lake Remoray

The high-throughput 16S rRNA gene sequencing approach applied here generated 3,788,355 quality sequences and recovered 1566 OTUs affiliated to both the Archaea and Bacteria domains.

NMDS analysis of the full archeal and bacterial sequence dataset highlighted a distinct difference in sediment community structure between littoral and profundal zones (Figure 4a) (ADONIS test,  $R^2 = 0.13$ ,  $p = 0.001$ ). This difference was associated with different taxonomic compositions: Proteobacteria, Bacteroidetes, Planctomycetes, Acidobacteria, and Chloroflexi were more abundant in the profundal zone whereas Cyanobacteria, Spirochetes,





**Figure 3.** Microbial density (a), genetic potential of methanogens (b) and methanotrophs (b) along the sediment cores for littoral zone (light blue) and profundal-zone (blue) area of Lake Remoray. Barplots and vertical lines represent the averages of sampling points from littoral and profundal zones for each sampling stratum of sediment cores, with standard deviation ( $n = 5$ ). Symbols represent the different sampling points across Lake Remoray (see Figure 1). Asterisks indicate significant differences between littoral-zone and profundal-zone sediment.

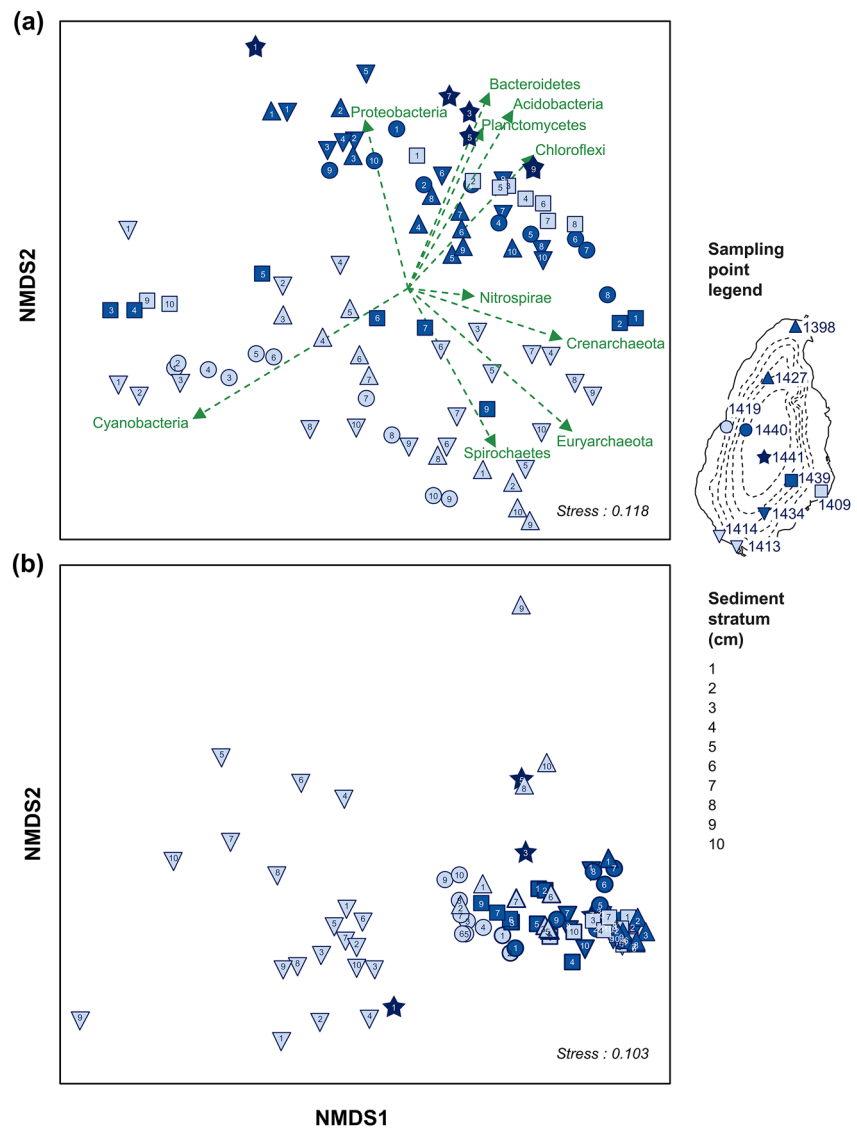
and Euryarchaeota were more abundant in the littoral zone of Lake Remoray. There was no effect of sampling depth along the sediment cores on microbial structure (ADONIS tests;  $R^2 = 0.10$ ,  $p = 0.911$ ).

Based on the taxonomic classification in the 16S rRNA gene sequences dataset, we identified 41 OTUs affiliated with the methanogenic group, representing 6.11% of the total sequences. NMDS analysis of the methanogenic-sequences dataset revealed two distinct groups on the ordination plot (Figure 4b). The first group was represented by sediment cores collected in the tributaries area, and the second group clustered the sediment cores from all other sampling sites across the lake. ADONIS test revealed a significantly distinct methanogenic community structure between littoral and profundal zones ( $R^2 = 0.22$ ,  $p = 0.001$ ), mainly driven by methanogen sequences from the tributaries area. For all sampling site locations, no significant difference in methanogen community structure was observed in the first 10 cm of sediment cores ( $R^2 = 0.03$ ,  $p = 0.999$ ).

DESeq2 analysis on the 41 methanogenic OTUs identified methanogenic genera accounting for the differences between littoral and profundal zones (Figure 5). Profundal-zone sediments had a higher relative abundance of OTUs affiliated to the Methanomicrobiales order (81% of methanogenic sequences) compared to the littoral zone (65% of methanogenic sequences). Sediments in the littoral zone had a two-time higher relative abundance of OTUs affiliated with *Candidatus methanoperedens*, *C. methanomethylicus*, and the Methanosaeta genus.

### 3.5. Linking Methanogenic Communities and CH<sub>4</sub>-Cycling Parameters Across Lake Remoray

The methanogenic community co-occurrence network was built based on pairwise correlations between the 41 OTUs affiliated with methanogens. The co-occurrence pattern within the methanogenic community was determined by only keeping significant Pearson's correlations. The resulting network (Figures 6a and 6b) was

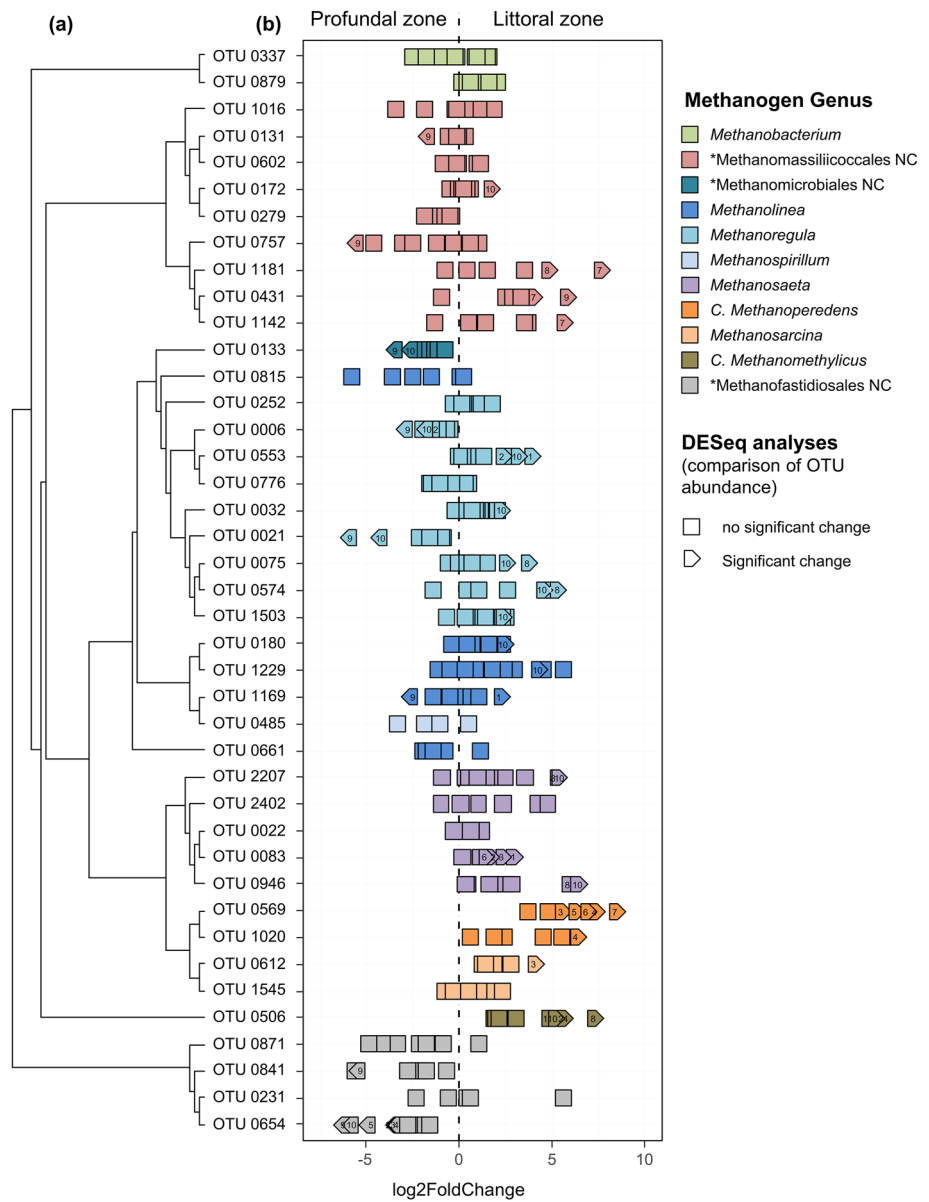


**Figure 4.** Non-metric multidimensional scaling (NMDS) ordination plot derived from weighted pairwise Unifrac distances for (a) bacterial and archaeal and (b) methanogen communities for all strata of sediment cores sampled across Lake Remoray. Symbols represent sampling points, and numbers inside represent the sediment stratum. Stress values for the two ordination plots were  $<0.2$ , which indicates that these data were well represented by the two-dimensional scaling. Vectors in the bi-plot overlay represent significant correlations ( $p < 0.05$ ) between phylum abundance and genetic structure. The angle and length of the vector indicate the direction and strength of the variable.

composed of 131 edges (all edges were positive correlations) representing 15% of possible within-network connections (network density). The topological network parameters were: average degree = 6.4, diameter = 3.3, modularity = 0.48, transitivity = 0.73, average path length = 2.46.

Covariation similarity analysis between methanogenic OTUs showed 5 distinct modules within a methanogenic network and containing more than three OTUs (Figure 6b). Analysis of the proportion of methanogenic OTUs composing the network modules of Lake Remoray (Figure 6c) showed that the methanogenic OTUs of modules 1 and 2 were dominant in sediments close to the lake tributaries. The methanogens of module 5 dominated the profundal zones while the OTUs of module 3 were more abundant in littoral zones. The methanogens composing module 4 were found specifically in profundal zones, especially in the deepest sediment of Lake Remoray.

Analysis of module–functional parameter relationships based on the 100 samples collected from the 10 sample cores (Figure 6d) revealed that sub-communities of modules 1 and 2 were only significantly correlated to OM

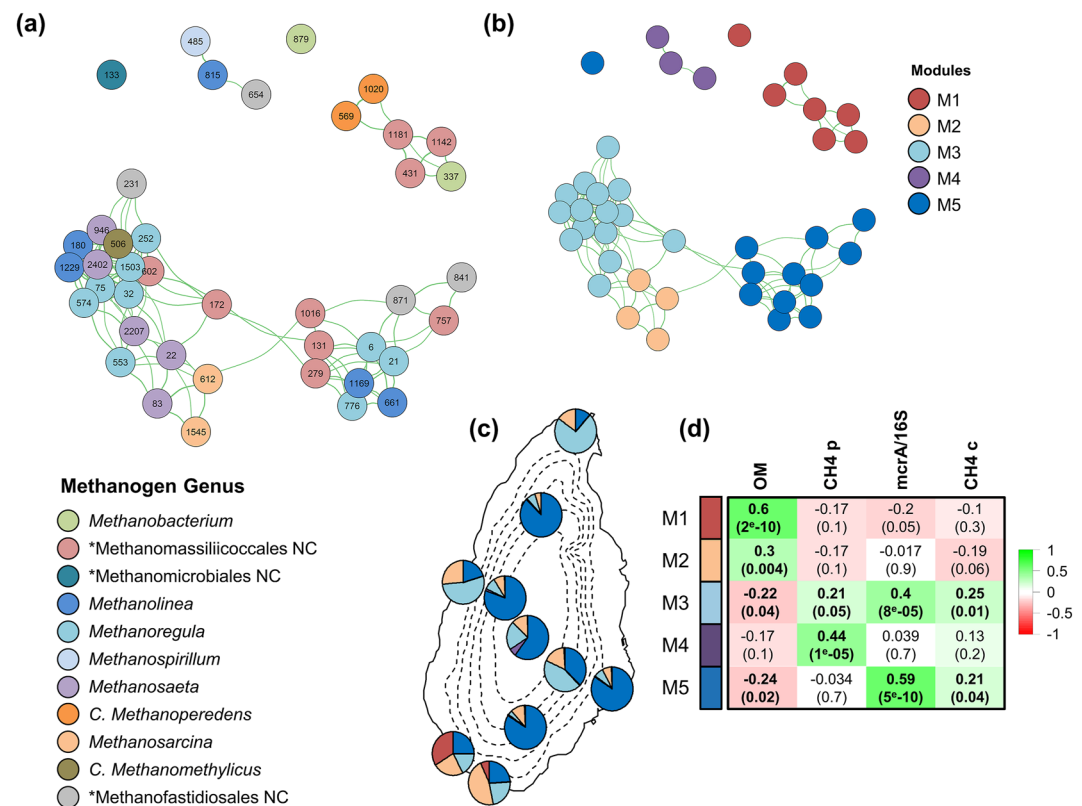


**Figure 5.** Phylogenetic tree of 41 methanogen OTUs extracted from the 16S rRNA gene sequencing dataset (a) and log<sub>2</sub> fold changes in relative abundance of methanogen OTUs between sediment cores sampled in littoral-zone and profundal-zone areas of Lake Remoray (b). Each symbol represents the comparison between littoral-zone and profundal-zone areas for a given stratum and OTU. Numbers in white represent the stratum depths (in cm) where significant change was observed. OTUs not classified at genus level (NC) were assigned to order level.

concentration, whereas modules 3 and 4 (representing the largest methanogenic sub-communities) were negatively correlated to OM and significantly positively correlated to sediment methane concentration and functional methanogenesis potential. Sediment methane production in Lake Remoray was significantly correlated to the abundance of module-3 and module-4 sub-communities.

#### 4. Discussion

Lake Remoray presents long periods of hypoxia in the deepest part of the water column (Belle et al., 2016) due to an excess of autochthonous material deposition driven by primary overproduction and, to a lesser extent, to organic material inputs carried by its two tributaries (Tardy et al., 2021). Methanogenic species affiliated with



**Figure 6.** Co-occurrence networks of methanogen communities in the first 10 cm of Lake Remoray sediment cores. Nodes (circles) represent the methanogen OTU colored by (a) taxonomic affiliation at genus level and (b) by modules. The edges (lines) represent significant Pearson's correlations at  $R > 0.5$ . Green and red lines indicate positive and negative relationships between OTUs. The map of Lake Remoray (c) shows the proportion of modules within methanogen communities. The correlation plot (d) represents module–functional parameter relationships according to Pearson's correlations between eigengenes of modules and CH<sub>4</sub>-cycling parameters (OM, Organic Matter concentration; CH<sub>4</sub>p, methane production; mcrA/16S rRNA genes ratio, the genetic potential of methanogenesis; CH<sub>4</sub>c, methane concentration). Numbers in bold indicate significant correlations with  $p$  values in brackets.

the Methanomicrobia class were shown to be widely distributed in the surficial sediment layers of lake Remoray's deepest parts (Tardy et al., 2021), allowing to expect methane production in the sediment and methane diffusion to the water column, at least during periods of hypoxia. The aim of this study was to characterize the spatial variation of sediment methane production across Lake Remoray sediment during the summer period when dissolved oxygen levels decrease sharply with depth in the water column. Due to seasonally anoxic periods in profundal sediment and differences in OM content and quality, our main hypotheses were that it would select for specific and more abundant methanogenic communities within the sediment microbial assemblages, and that sediment methane production potential would increase from the littoral zone to the deepest zone of Lake Remoray.

#### 4.1. Spatial Heterogeneity of Sediment Methane Production and Concentration Across Lake Remoray

Both methane production potential and methane concentration in sediment were more related to sampling-site location than to the water depth of the collected sediment cores (Holgerson & Raymond, 2016; Juutinen et al., 2003; Thebrath et al., 1993). Similar methane production rates were recorded in the sediment collected in the littoral zone (close to the farm and outlet) and in sediments collected in the deeper zones, with the exception of littoral-zone sampling sites located in the tributary plumes that had the lowest values for methane production potential.

The literature shows that methane production rates depend on the amount, nature, and composition of OM inputs (Berberich et al., 2020; Bertolet et al., 2020; Duc et al., 2010; Grasset et al., 2018). In a controlled experiment, West et al. (2012) found higher methane production rates following algal biomass input than terrestrial carbon

addition whereas methanogenesis potential was similar between conditions. Duc et al. (2010) studied sediments of eight boreal and temperate northern lakes and found higher methane production rates in sediments characterized by a C:N ratio below 10, whatever the different incubation temperatures tested. In Lake Remoray, sediment had a higher C:N ratio (average C:N ratio = 12) in the tributary area than in any other location of the lake (average C:N ratio = 8) (Tardy et al., 2021).

Sediment methane production potential measured in Lake Remoray spanned a higher range than rates measured in Lake Onego (0–456 ng C-CH<sub>4</sub> g DW<sup>-1</sup> h<sup>-1</sup>; Thomas et al. [2018]), Lake Dago (1,201–4,203 ng C-CH<sub>4</sub> g DW<sup>-1</sup> h<sup>-1</sup>; Chan et al. [2005]) and Swedish lakes (24–1,996 ng C-CH<sub>4</sub> g DW<sup>-1</sup> h<sup>-1</sup>; Duc et al. [2010]). However, these values should be interpreted with care, (a) as potential production rates were estimated using different laboratory incubation temperatures (20°C here, 4°C in Thomas et al. [2018], 10°C in Chan et al. [2005]; and 4°C, 10°C, 20°C, and 30°C in Duc et al. [2010]) and (b) because the studied ecosystems varied based on different environmental settings (such as lake depth and area and geographical zones). Even though the incubation times used here were short (5 hr), methane production is still temperature-dependent (Fuchs et al., 2016; Schulz et al., 1997; Yvon-Durocher et al., 2014) and this could have influenced the values obtained. However, the incubation temperature used here was chosen to reflect the water temperature recorded right above the sediment interface at the sampling site in the shallower areas (21.7 ± 0.05°C) of the lake.

The highest sediment potential methane production rates were in the top 6 cm of sediment cores. However, the sediment methane concentrations measured in situ in the sediment core layers increased with distance from the water–sediment interface at every sampling location. This could be related to (a) sub-favorable in situ conditions for methanogenesis, likely related to oxygen concentration or redox potential, and/or to (b) methane loss from sediment to water column though either oxidation, diffusive emission, and ebullition, or any combinations of these. Conceptual models based on methane concentration versus depth curves could help to estimate the balance between oxidation and emission (Bernard, 1979), but they are only qualitative and remain difficult to interpret. The analysis of the carbon isotopic signature of methane would have helped to quantify the contribution of methane oxidation (Bastviken et al., 2002) to diffusion mitigation. Methane oxidation can occur under both aerobic and anaerobic conditions and is a metabolic pathway supported by both archeal and bacterial microbial species (Lehours et al., 2016). Here, aerobic methanotrophs community abundance, assessed by quantifying the relative abundance of the *pmoA* gene, represented only 0.01% to 0.03% of the sediment bacterial community. This abundance was estimated using a DNA-based approach, so care should be taken as an unknown fraction of the community might be inactive. Although only sparsely represented, aerobic methanotrophs have very efficient enzymatic activities and can oxidize significant amounts of methane even at low populations densities (Mayr, Zimmermann, Dey, et al., 2020; Mayr, Zimmermann, Guggenheim, et al., 2020; Reis et al., 2020). However, aerobic methane oxidation is more likely to occur in the water column, as oxygen diffusion in the sediment is expected to be low, aerobic methane oxidation may not be the only pathway involved in Lake Remoray sediment, especially in the deeper area of the lake and/or deeper layers of the sediment column, even though methanotrophs are also active in anoxic conditions (Oswald et al., 2016). Anaerobic oxidation of methane is coupled with nitrate, sulfate, or iron reduction (Lehours et al., 2016). The occurrence of the anaerobic methanotrophs *Ca. Methanoperedens*-related sequences suggest that the anaerobic oxidation of methane using nitrate as terminal electron acceptor via the reverse methanogenesis pathway (Haroon et al., 2013) could take place in Lake Remoray, as previously described in other lakes (Deutzmann et al., 2014; Thomas et al., 2018). Further studies, based both on pore-water chemistry and the carbon isotopic signature of methane, are needed to better estimate how different oxidation pathways contribute to methane emission mitigation.

In Lake Remoray, methane diffusion from the sediment to the water column varied according to sampling depth, ranging from 50 to 200 mg CH<sub>4</sub> m<sup>-2</sup> d<sup>-1</sup> in profundal and littoral sampling points close to the farm, outlet, and campsite. These methane diffusion figures are higher than previously reported for Lake Geneva (up to 37 mg CH<sub>4</sub> m<sup>-2</sup> d<sup>-1</sup>; Sollberger et al. [2014]), Lake Stechlin (up to 39 mg CH<sub>4</sub> m<sup>-2</sup> d<sup>-1</sup>; Li et al. [2021]) and Lake Onego (up to 95 mg CH<sub>4</sub> m<sup>-2</sup> d<sup>-1</sup>; Thomas et al. [2018]) but consistent with values reported for Lake Soppensee (up to 253 mg CH<sub>4</sub> m<sup>-2</sup> d<sup>-1</sup>; Vachon et al. [2019]). This confirms the large contribution of the sediment of Lake Remoray to the methane cycle. However, sediment methane concentration and diffusion were measured during the summer oxygen depletion period and would probably be even higher in late fall when stronger hypoxia/anoxia prevails in the hypolimnion. Our work only focused on the diffusive component of the methane transfer from the sediment to the water column, and ebullition fluxes, which could also lead to the transfer of the important amount of methane to the water column and the atmosphere, were not addressed (Praetzel et al., 2021).



Given that Lake Remoray annually experiences long anoxia-periods in the deeper layers of the water column, we expected a higher contribution of methane production in the sediment in the profundal than in the littoral areas. Previous studies have reported higher methane production in littoral zones relative to profundal-zone sediment (Holgerson & Raymond, 2016; Juutinen et al., 2003; Li et al., 2018, 2021; Murase et al., 2003; Thebrath et al., 1993). Altogether, this highlights the importance of taking into account the spatial resolution of microbial activities within the sediment at the lake scale.

#### 4.2. Spatial Variation of Sediment Archeal and Bacterial, and Methanogenic Communities

As observed in other freshwater lake ecosystems, water depth significantly affected sediment archeal and bacterial community composition (Ruuskanen et al., 2018; Wu et al., 2019; J. Zhang et al., 2015; L. Zhang et al., 2019). Littoral-zone sediment was dominated by Cyanobacteria species (29.7% of total sequences) while profundal-zone sediment was dominated by Proteobacteria, Bacteroidetes, and Chloroflexi (25.4%, 21.2%, and 10.5% of total sequences, respectively). There was no significant effect of in-core stratum depth on archeal and bacterial structure and composition. This finding contrasts with numerous studies showing a vertical structuration of bacterial and archeal communities with the sediment depth in lake ecosystems (Billard et al., 2015; Ding et al., 2015; Han et al., 2020; Rissanen et al., 2019; Wurzbacher et al., 2017; Xiong et al., 2015). These works, however, focused on larger depth ranges, from 20 to 100 cm, likely integrating more diverse and changing environmental parameters. Archeal and bacterial composition changes were associated with different patterns of variation in chemical properties along the sediment depth, such as OM quality, oxygen concentration, or redox potential. The lack of vertical structuration of sediment archeal and bacterial communities found here suggests that chemical properties in the first 10 cm of the sediment are relatively homogeneous.

Based on the taxonomic affiliation in the 16S rRNA gene sequences dataset, we identified 41 OTUs affiliated with archaea involved in the methanogenesis function. The relative abundance of methanogenic sequences varied from 1% to 17% according to location and in-core sediment depth, and as previously underlined for methanotrophs, this abundance is likely to include both active and inactive microorganisms. A large proportion of sequences was affiliated with *Methanoregula* (67% of total methanogenic sequences – 38% in profundal-zone and 29% in littoral-zone). *Methanoregula* is a hydrogenotrophic genus able to reduce CO<sub>2</sub> with hydrogen as an electron donor, and it has already been reported as predominant in many freshwater lakes (Berberich et al., 2020; Biderre-Petit et al., 2019; Borrel et al., 2011; Wen et al., 2017). The second most abundant methanogen was *Methanosarcina* (15% of total methanogenic sequences – 5% in profundal-zone and 10% in littoral-zone), an acetotrophic genus able to produce methane with acetate as the final electron donor (Ferry, 2010). Based on the DNA abundance ratio of these two methanogenic populations in sediment, it suggests that methanogenesis in Lake Remoray results mainly from the hydrogenotrophic pathway and to a lesser extent the acetoclastic pathway, but this would require to be confirmed by activity measurements. A previous study suggested that the hydrogenotrophic pathway outcompetes the acetoclastic pathway in lake sediment if OM is recalcitrant (Liu et al., 2017). However, since the abundance of microbial communities can not directly be related to their respective activities, further transcriptomic analyses could provide additional information on the link between community structure, abundance, and activity.

*Methanosarcina* was the more abundant methanogen in the littoral zone whereas species affiliated with *Methanomicrobiales* and *Methanofastidiosales* were the more abundant methanogen in profundal sediments. Littoral sediments located in the tributary area had a specific methanogenic composition. In this part of the lake, the methanogenic community was largely associated with OTUs affiliated with *Methanobacterium* and *Methanosarcina*, highlighting the significant horizontal structuration of the methanogenic community at the whole-lake scale. A few studies have characterized the horizontal distribution of sediment methanogenic communities within a single lake ecosystem. Only Berberich et al. (2020) in Lake Harsha showed a large variation in methanogenic community composition and structure between riverine, lacustrine, and transitional areas of the lake, as well as a higher proportion of *Methanosarcina* species in the riverine zone.

To go further in the characterization of the methanogenic communities, a co-occurrence network was built from the 41 OTUs detected in all sediment samples. The resulting methanogenic network highlighted exclusively positive relationships between the different OTUs, suggesting the absence of competition and the predominance of facilitative interactions within the sediment methanogenic community of Lake Remoray. The other interesting metric from the methanogenic network is modularity, which was positive and high enough ( $Q = 0.48$ ) to point

to the possible presence of community structures (or ‘modules’) within the network (Newman, 2006). In biotic co-occurrence networks, the presence of sub communities is usually related to the heterogeneity of habitat and to the diversification of ecological niches in the studied ecosystem (Barberán et al., 2012; Ren et al., 2020). Our analysis of Lake Remoray sediment found five distinct methanogenic sub communities (modules) in which interactions are more frequent than with the rest of the community. Two of these sub communities were particularly large (modules 3 and 5; Figure 6) and both grouped the interactions between OTUs affiliated to *Methanoregula*, *Methanolinea*, *Methanosaeta*, and *Methanomassiliicoccales* species. Three other smaller sub communities (module 1, 2, and 4) were identified with specific taxonomic compositions. Consistently with methanogenic community structure and composition, the abundance of sub communities varied according to the sampling location in Lake Remoray. Two specific methanogenic sub communities were found exclusively in the sediment of the deeper area (module 4) and sediments close to tributaries (module 1), thus further confirming the existence of diverse methanogenic niches across Lake Remoray sediment and again highlighting the need to integrate lake-scale characterization of methanogenic communities when assessing lake methane emissions.

#### 4.3. Linking Spatial Variation of Methanogenic Communities to Methane Production Rate in Lake Remoray

Analysis of module structure in the co-occurrence network can also provide substantial information on the functional and ecological properties of various ecosystems (Barberán et al., 2012). For instance, numerous studies have used module structure to explain the influences of environmental parameters on microbial community structure in lake ecosystems (Cao et al., 2018; Capo et al., 2017; Ren et al., 2017; Xu et al., 2018; Zhao et al., 2016). Functional relationships between modules showed that the amount of OM in the sediments was a key factor of variation in the abundance of methanogenic sub communities, influencing four modules of the network. The quantity and quality of sediment OM are now well recognized as significant drivers of variation in sediment methanogenic communities, shaping abundance, genetic structure, and taxonomic composition (Berberich et al., 2020; Bertolet et al., 2019; Billard et al., 2015). In Lake Remoray, higher sediment OM content positively influenced modules 1 and 2, which are more abundant in sediments close to tributaries. As described in Section 4.1, sediment OM content and C:N ratio is higher in this part of the lake, mainly due to allochthonous OM deposition by tributaries. Conversely, modules 3 and 5 were negatively influenced by sediment OM content and their large abundances were associated with autochthonous OM depositions characterized by a lower C:N ratio and related to the lake's high primary production. Taken together, these results suggest that the origin (allochthonous vs. autochthonous) and quantity of OM deposition in Lake Remoray sediment have led to a diversification of ecological niches for the methanogenic community.

The two larger modules (modules 3 and 5) were positively associated with methanogenesis potential and sediment methane concentration, suggesting that the sediment methane production of Lake Remoray is mainly carried out by the biotic interactions between methanogenic taxa composing these two sub communities. However, only one of these modules was positively associated with methane production rate. On the other hand, the smaller methanogenic sub community in the network (module 3) represented by the interactions between three methanogenic taxa (*Methanolinea*, *Methanofastidiosales*, and *Methanospirillum*) was strongly associated with methane production rate. This suggests that in the deeper part of Lake Remoray, sediment methane production intensity depends mainly on the activity of a handful of methanogenic species. However, this interpretation warrants caution, as production rates were estimated as a methane potential measured under controlled in-lab conditions, and would require further analyses such as transcriptomic or proteomic to have a more accurate assessment. The absence of any strong relationship between modules 3 and 5 and the methane production rate could be due to the experimental approach used. The relationship between variations in the abundance of methanogenic communities and methane production rate in lake sediment is difficult to explore (Bertolet et al., 2019). Indeed, methane production rate does not rely solely on the presence, abundance, and activity of methanogenic species, and several environmental parameters also have to be factored in, such as OM composition, temperature, ion content (i.e.,  $\text{SO}_4^{2-}$ ,  $\text{Fe}^{3+}$ ,  $\text{NO}_2^-$ ,  $\text{NO}_3^-$ ), and pH (Bertolet et al., 2019; Borrel et al., 2011; Evans et al., 2019; Fuchs et al., 2016).

## 5. Conclusion

Methane production and methanogenesis potential in sediments were expected to be higher in profundal areas of the lake where annual hypoxia occurs during the late summer–winter period, but we found that some littoral zones contribute as much or more to sediment methane diffusion to the water column. Spatial patterns of sediment methane concentration and production potential were associated with variations in the nature and quality of sediment organic matter and the presence of specific methanogenic sub communities. Further studies on methanogenic and methanotrophic communities, eventually using recent metagenomics approaches could help to better assess the functional importance of these community differences at the lake scale. Measurements of ebullition, eventually methane production in oxic areas, and diffusion of methane to the atmosphere are also needed to create a more complete picture of the methane cycle in this lake. Further studies are also needed to address the sediment-to-water column continuum and improve our predictions of methane emissions from lakes. Combining molecular and geochemistry approaches with powerful isotopic approaches could valuably integrate both the spatial and temporal scales.

## Conflict of Interest

The authors declare no conflicts of interest relevant to this study.

## Data Availability Statement

Data supporting the analyses and conclusions are available at the Zenodo repository (<https://doi.org/10.5281/zenodo.6518228>) and at the cited references.

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