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Article in FEMS Microbiology Ecology · September 2021

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## RESEARCH ARTICLE

# Spatial distribution of sediment archaeal and bacterial communities relates to the source of organic matter and hypoxia – a biogeographical study on Lake Remoray (France)

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**One sentence summary:** This biogeographical study gives an extensive picture on the origin of organic matter in the sediment compartment and the related diversity of sediment microbial communities in a lake with deoxygenated deep water layers.

Editor: Tillmann Lueders

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## ABSTRACT

Bottom waters hypoxia spreads in many lakes worldwide causing severe consequences on whole lakes trophic network. Here, we aimed at understanding the origin of organic matter stored in the sediment compartment and the related diversity of sediment microbial communities in a lake with deoxygenated deep water layers. We used a geostatistical approach to map and compare both the variation of organic matter and microbial communities in sediment. Spatialisation of C/N ratio and  $\delta^{13}\text{C}$  signature of sediment organic matter suggested that Lake Remoray was characterized by an algal overproduction which could be related to an excess of nutrient due to the close lake-watershed connectivity. Three spatial patterns were observed for sediment microbial communities after the hypoxic event, each characterized by specific genetic structure, microbial diversity and composition. The relative abundance variation of dominant microbial groups across Lake Remoray such as Cyanobacteria, Gammaproteobacteria, Deltaproteobacteria and Chloroflexi provided us important information on the lake areas where hypoxia occurs. The presence of methanogenic species in the deeper part of the lake suggests important methane production during hypoxia period. Taken together, our results provide an extensive picture of microbial communities' distribution related to quantity and quality of organic matter in a seasonally hypoxic lake.

**Keywords:** hypoxia; biogeography; lake sediment; organic matter; eutrophication; microbial diversity

## INTRODUCTION

Human activities have become one of the main drivers of lake ecosystem evolutions during the last two centuries, inducing profound changes on the biodiversity and the biogeochemical

cycles functioning (Tranvik et al. 2009; Anderson, Bennion and Lotter 2014). A major and dramatic change observed for lake ecosystems is the recent global spread of oxygen depletion observed seasonally or permanently in lake bottom-water

Received: 16 April 2021; Accepted: 31 August 2021

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(Jenny et al. 2016a,b). This phenomenon is mainly caused by the eutrophication of water bodies, induced by an increase of nutrient inputs due to human activities in the watershed. This stimulates biomass production in the euphotic area, followed by its sedimentation and decomposition through microbial respiration (Jenny et al. 2016b; Robinson 2019). Global warming tends to enhance this phenomenon by increasing aerobic microbial decomposition and/or strengthening thermal stratification (Golosov et al. 2012). As a result, dissolved oxygen concentration decreases along the water column leading to a bottom-water hypoxia when oxygen concentrations are below 2 mg O<sub>2</sub> L<sup>-1</sup> (Biddanda et al. 2018), or anoxia, when dissolved oxygen concentration is null (Friedrich et al. 2014). Hypoxia leads to severe consequences on the biology of aquatic organisms (fish and invertebrates) and can lead, in some case, to so-called 'dead zones' where benthic fauna life's is not possible anymore (Diaz and Rosenberg 2008; Weinke and Biddanda 2018; Galic, Hawkins and Forbes 2019). Another adverse effect of hypoxia/anoxia is the shift toward microbial fermentation and anaerobic respiration, both influencing methane production and the release of this powerful greenhouse gas in the water column (Grasset et al. 2018) and in the atmosphere if the methanotrophic mitigation of CH<sub>4</sub> emissions is not efficient or impaired (Bastviken et al. 2008).

In lake ecosystems, sediments are complex habitats densely colonized by a huge diversity of microorganisms (mainly bacteria and archaea), playing major roles in aquatic food webs, in nutrient cycling, and in organic matter transformation (Martins et al. 2011; Thevenon et al. 2011). In the deepest lake areas, under the combined influence of higher water column productivity, lake morphometry, and thermal stratification, sediments are more likely to present hypoxic and anoxic conditions supporting the occurrence of microbial consortia able to process organic matter through fermentation and anaerobic respiration (Schwefel et al. 2018).

Spatial and temporal variation of sediment bacterial and archaeal diversity have been extensively studied over the last decade in freshwater ecosystems (Szabó et al. 2011; Zhang et al. 2014, 2019; Chen et al. 2015; Xiong et al. 2015; Huang et al. 2017; Wu et al. 2017; Ruuskanen et al. 2018). Several environmental parameters such as water depth, organic matter quantity and quality, nutrient content, and contaminants have been reported to shape their abundance, structure and diversity (Haller et al. 2011; Zhang et al. 2014; Xiong et al. 2015). However, despite the growing interest on consequences of hypoxia on aquatic food web of lakes (Galic, Hawkins and Forbes 2019), little is known about the spatial distribution of microbial communities in freshwater lake sediments exhibiting hypoxia periods. This question is of major importance since these communities are an essential component of lake trophic network functioning. The few studies dealing with freshwater hypoxia/anoxia mainly focused on microbial communities inhabiting lake water column (Wilhelm et al. 2006, 2014; Li, Xing and Wu 2012; Diao et al. 2017). Their results showed microbial composition shifts along the depth gradient of the water column in response to seasonal hypoxia. In marine ecosystems, some studies examined the horizontal and/or vertical microbial communities' variation in hypoxic sediments (Mahmoudi et al. 2015; Jessen et al. 2017; Mori et al. 2018). The authors notably observed a large change of sediment bacterial structure and composition in the hypoxic zone, with bacterial taxa replacement from Gammaproteobacteria to Deltaproteobacteria. In lake ecosystems, composition shifts were also considered in the sediment column, along vertical sampling designs, and demonstrated that the microbial community distribution was under the influence of electron acceptor availability

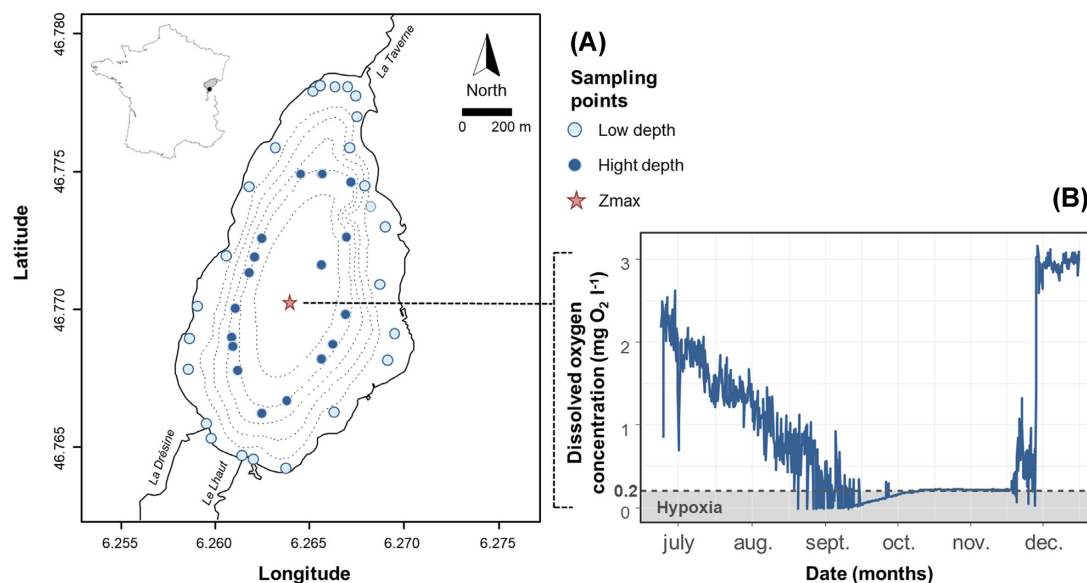
(Wurzbacher et al. 2017; Rissanen et al. 2019; Han et al. 2020). In this context, the aim of this study was to characterize the horizontal distribution of sediment microbial communities (Archaea and Bacteria) across a hypoxic lake.

Lake Remoray is located in the Jura Mountains (eastern France) and presents a trophic dysfunction, due to high organic matter accumulation in the benthic compartment, thus causing long hypoxia periods in the deepest part of the water column with the expansion of a 'dead-zone' (Belle et al. 2016). To date, the origin of sedimentary organic matter and the processes associated with its accumulation are still poorly understood, and the spatial variation of sediment microbial communities in lakes with hypoxia events has not been explored so far. In this context, the study aims to characterize (i) the origin and the quantity of organic matter inputs in the Lake sediment compartment, and (ii) the spatial variation of lake sediment microbial communities under the combined influence of long annual hypoxic events and organic matter characteristics in the sediment compartment. For this, sediment cores from 44 sampling points were collected across the 95 ha of Lake Remoray right after the seasonal hypoxia period that concerned the deepest parts of the lake. For each sampling point, quantitative and qualitative characterizations of organic matter were performed on the first centimeter of the collected sediment cores, using different parameters: organic matter content and C/N ratio allowed to assess OM quantity and quality, while the use of two parameters (total carotenoids and 3 $\beta$ -Cholestan-3 $\beta$ -ol TMS concentrations) allowed to discriminate between autochthonous and allochthonous sources of OM. In parallel, we analyzed the diversity, structure and composition of sediment microbial communities (bacteria and archaea) using an Illumina sequencing approach targeting 16S ribosomal RNA (rRNA) genes. In this study, we used a geostatistical approach to map and compare both the variation of organic matter parameters, microbial genetic structure and the major archaeal and bacterial taxa across Lake Remoray. Additionally, we performed variance partitioning analysis to quantify the contribution of quantitative and qualitative organic matter parameters in the variation of genetic structure and the major taxa of sediment microbial community. In this study, we hypothesized that (i) the organic matter accumulation in sediment mainly come from the overproduction of the phytoplanktonic biomass in the water column, (ii) the presence and the spatial distribution of some microbial taxa are related to the hypoxic conditions occurring in the deepest part of Lake Remoray, and (iii), that the nature and quantity of organic matter inputs from the watershed are significant drivers in the distribution of sediment microbial communities.

## MATERIALS AND METHODS

### Study site and sample collection

Lake Remoray (46°46'12" N; 6°15'49" E; Fig. 1A) is located in the Jura Mountains (eastern France). The water depth of this moderately sized lake (95 ha) reaches 27 m, the lake is presently mesoeutrophic with accumulation of organic matter in the central and deepest part of the lake (Belle et al. 2017). The lake is fed by two tributaries (the Drésine and the Lhaut) and bordered by a farm and a campsite (Fig. 1A). The watershed basin of Lake Remoray consists of 46.7% forest (mainly coniferous forest), 42.5% agricultural parcels, 5.5% peat bog, 2.6% water area, and 2.7% urban area (Belle et al. 2016).



**Figure 1.** Map of Lake Remoray with sediment samples location in 2016 (A). Bathymetry is represented with dashed white line every 5 m and Zmax corresponds to the deepest site of the lake. Dissolved oxygen concentration measured at location Zmax over 7-months period (July to December 2016), (B). Grey dashed line represents the threshold dissolved oxygen value to consider hypoxia, according to Diaz and Rosenberg (2008).

In 2016 (December 2<sup>nd</sup>), 44 short sediment cores (90-mm diameter) were collected all over the lake sediment surface using a UWITEC gravity corer after a 4-months hypoxia period occurred in the deepest part of the lake (Fig. 1A and B), and concerned the water column from 15 to 27 m deep at the end of the period. Only the first centimeter of sediment was sampled for each core, using a core extruder and a core cutter. Subsamples were stored at 4°C upon return to the lab (within 6 hrs) except for subsamples dedicated to nucleic acid and pigment analyses that were immediately stored in a dry shipper filled with liquid nitrogen (-196°C) upon return to the lab, followed by a storage at -20°C until nucleic acid and pigment extractions.

### Organic matter characterization of Lake Remoray sediments

For each sediment sample, total carbon (TC), organic carbon (OC) content and nitrogen content (TN) were analyzed using a vario MAX CNS analyzer (Elementar) at Chrono-Environnement Laboratory. For organic carbon analyses (OC), the carbonates were removed following the addition of 3.7% HCl until the effervescence stops. Carbon isotopic composition ( $\delta^{13}\text{C}$ ) was analyzed using an Isotope Ratio Mass Spectrometer interfaced with an Elemental Analyzer (EA-IRMS) at INRA Nancy (Champenoux). Prior to isotopic analysis, the sediment samples were heat dried at 60°C during 24 h and carbonates were removed by the addition of 1 M HCl solution until effervescence ceased.

Concentration of photosynthetic pigment was analyzed to estimate the organic matter input from primary producers (Guilizzoni et al. 2011). Photosynthetic pigments were extracted from fresh sediments overnight with a solution of acetone and water (90:10) in a closed tube under nitrogen atmosphere, and then centrifuged at 3000 × rpm for 10 min. The extract obtained was used to quantify the total carotenoids by spectrophotometry, following the recommendations of Guilizzoni et al. (2011). The lipid analysis of 3 $\beta$ -Cholestan-3 $\beta$ -ol TMS in sediment used as fecal biomarker of herbivorous (Zocatelli et al. 2017) was performed to characterize the nature of indigenous organic matter inputs following the method of Pearson et al. (2007).

### Sequencing of 16S rRNA gene

Total sediment DNA was extracted from 0.5 g of wet sediment using a NucleoSpin Soil Kit (Macherey-Nagel EURL) following the manufacturer's instructions, and using SL1 lysis buffer and additive Enhancer SX buffer. The extracted DNA was quantified fluorometrically after staining with QuantiFluor dsDNA Dye (QuantiFluor dsDNA System, Promega) using a Plate Chameleon<sup>TM</sup> fluorometer (Hidex; excitation: 485 nm, emission: 590 nm).

PCR amplification for high throughput 16S rRNA sequencing was carried out with the universal primer pair 515F (5'-GTGYCAGCMGCCGCGGTA-3') and 909R (5'-CCCCGYCAATTCMTT TRAGT-3') targeting the V4-V5 hypervariable region of the 16S rRNA gene (Wang and Qian 2009). Indexes were integrated to both primers following the dual-indexing procedure described by Kozich et al. (2013). Triplicates PCR amplification for each sample were realized with a total amount of ~5 ng of DNA per reaction. Amplicon products were quantified using Picogreen assay (Life Technologies, Carlsbad, USA) and pooled equimolarly. The final pool was purified with CleanPCR beads (CleanNA). Sequencing was realized by Fasteris (Geneva, Switzerland) on an Illumina HiSeq with 2 × 250 bp. The analysis yielded 6.7 Gb of sequences with average error rate of 0.822%, and average Q30 of 90.3%.

### Bioinformatic analysis

Adapters were removed using trimmomatic (Bolger, Lohse and Usadel 2014) and reads were sorted according to their barcodes using a Fasteris internal script (Perl script). Sequences were then processed using the FROGS (Find Rapidly OTUs with Galaxy Solution) Galaxy-supported pipeline (Escudie et al. 2018). Paired-ends reads were joined using FLASH (Magoc and Salzberg 2011) and quality check was performed using FastQC. Sequences with primers having no mismatch were kept. They were 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 3 bases maximum difference, deletion of clusters having less than 0.005% abundance and cluster occurrence in



minimum 2 samples of the total library. Chimeras were removed using vchime of vsearch package (Rognes et al. 2016). Taxonomic classification was done using the Silva SSU database 123 (Quast et al. 2013) through BLAST (Altschul et al. 1990) with allowed multiple affiliation 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 Short Read Archive) under project accession number PRJEB 43 876.

## Statistical analysis

All statistical analyses were performed with R free software (<http://www.r-project.org/>).

### Library size homogenization

Prior to analysis, sequencing data was rarefied to the smallest sample in order to remove bias comparison of diversity and communities similarity indices between sites (Weiss et al. 2017; Willis 2019). All analyses were also conducted on proportional data set, as recommended by McMurdie and Holmes (2014), and similar trends were observed (data not shown).

### Ordination method

Differences in microbial community structure between locations were characterized using weighted Unifrac distance (Lozupone and Knight 2005). Non-Metric Multidimensional Scaling (NMDS) was then used to graphically depict differences between sediment microbial communities.

### Mapping using geostatistics

A geostatistical method was used to map organic matter parameters, microbial community structure (i.e. coordinates of the two axes of the NMDS analysis run on the weighted UniFrac distance matrix) and the relative abundance of major microbial phyla. Only bacterial and archaeal phyla representing more than 0.5% of total sequences were mapped. Since these variables did not follow a normal distribution, a transformation was applied before modeling the spatial correlations. Log transformation was applied for both organic matter parameters and NMDS scores, and the arcsine square root transformation was applied for the relative abundance of bacterial and archaeal phyla. In conventional geostatistical analysis, an estimate of a variogram model is computed based on the observations, which describe the spatial variation of the property of interest. This model is then used to predict the property at unsampled locations using kriging (Webster and Oliver 2007). For this, we used a similar procedure than previously described to map bacterial communities in soil (Constancias et al. 2015). Briefly, a common method for variogram estimation is first to calculate the empirical variogram by the method of moment, and then to fit a model to the empirical variogram by (weighted) nonlinear least squares. We tried to fit several models (spherical, matern, gaussian and exponential models) and retained the one that minimized the objective function (Minasny and McBratney 2005). The validity of the best fitted geostatistical model was then assessed in terms of the standardized squared prediction errors (SSPE) using the results of a leave one out cross validation. If the fitted model was a valid representation of the spatial variation of the microbial property, then these errors would have a  $\chi^2$  distribution with a mean of 1 and median of 0.455 (Lark 2002). The mean and median values of the SSPE were also calculated for

1000 simulations of the fitted model to determine the 95% confidence limits and to obtain a map of the kriging standard error. The geostatistical analysis gstat package was used for variogram analysis and kriging.

### Variance partitioning

The relative contributions of depth gradient and organic matter parameters in shaping the patterns of sediment microbial diversity and bacterial and archaeal taxa relative abundance were estimated by variance partitioning. For bacterial and archaeal taxa, only phyla representing more than 0.1% and the class and order representing more than 1% of total sequence were analyzed. The explanatory variables were selected to reduce the autocorrelation in the models and to obtain the most parsimonious models. Overall, 5 environmental parameters of the nine measured were selected for the analysis. These were Depth and Total Carbon (TC), C/N ratio (C/N), 3 $\beta$ -Cholestan-3 $\beta$ -ol TMS (Choles) and total carotenoids (Car.) as organic matter parameters. All selected environmental variables were transformed by applying Box-Cox transformation prior to the analyses. Diversity index and relative abundance of microbial taxa were standardized to guarantee an approximated Gaussian and homoscedastic residual distribution of the model. To determine the environmental parameters significantly shaping bacterial and archaeal phyla and microbial diversity, a stepwise selection procedure was first applied to significant explanatory variables by maximizing the adjusted  $r^2$  while minimizing the Akaike Information Criterion (AIC) (Ramette 2007). Statistical significance was assessed by 1000 permutations of the reduced model.

### Constrained ordination method

The selected explanatory variables were subsequently used to build a constrained ordination plot using the best-fitted model in a distance-based redundancy analysis (db-RDA) (Legendre and Anderson 1999) in vegan. ANOVA permutation tests were used to assess the significance of the individual axes and the overall models.

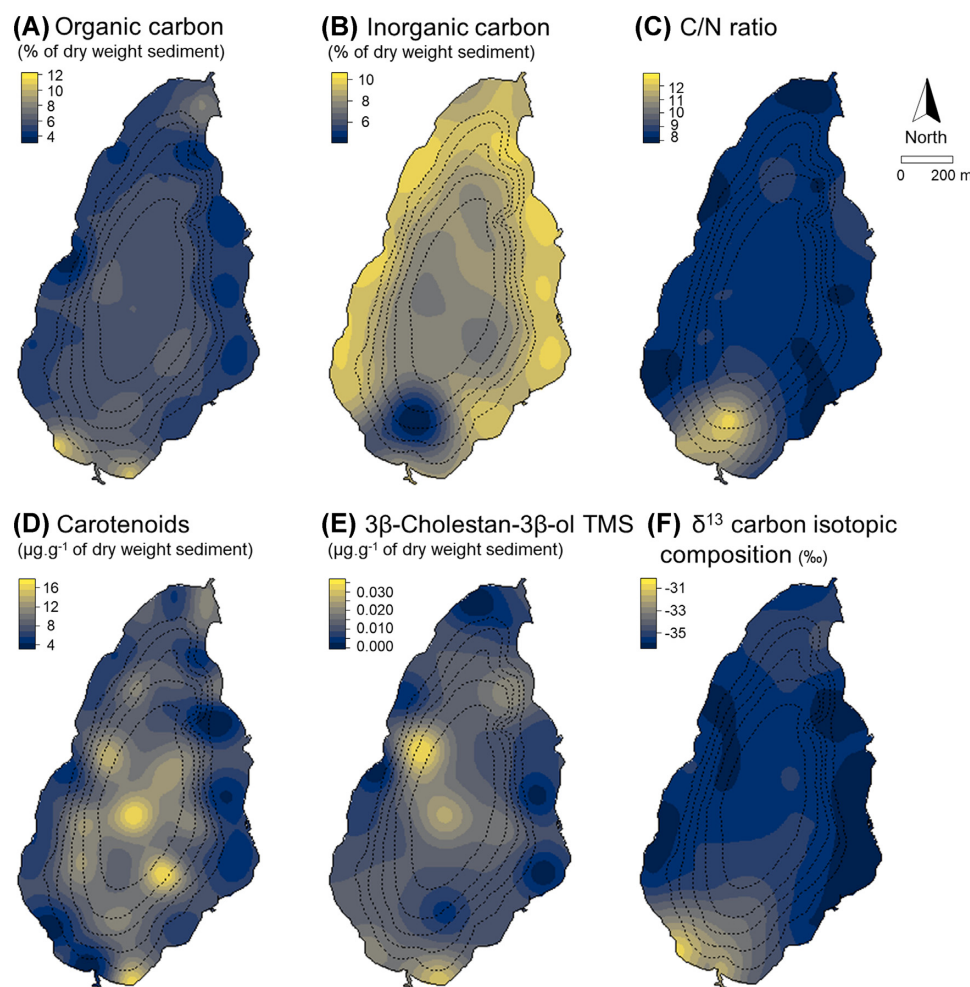
## RESULTS

### Spatial characterization of qualitative and quantitative parameters of organic matter across Lake Remoray sediment

Mapping of organic matter parameters across Lake Remoray revealed high organic carbon concentrations in the southern area of the lake close to the tributaries, representing 10–12% of dry weight sediment (Fig. 2A). In the high depth areas of the lake, organic carbon content was slightly higher than in littoral areas, ranging from 5.4 to 6.9% of dry weight sediments. Conversely, inorganic carbon concentration related to carbonate precipitation was more important in littoral areas representing 8–9% of dry weight sediments (Fig. 2B).

The C/N ratio and  $\delta^{13}\text{C}$  of sediment were relatively homogeneous across the lake averaging  $8.8 \pm 1.1$  and  $-35 \pm 1.5\text{‰}$ , respectively (Fig. 2C). The area close to the tributaries was the only to exhibit higher values of C/N ratio and  $\delta^{13}\text{C}$  reaching up to 12.5 and  $-29.41\text{‰}$ , respectively.

Spatial analysis of pigments (total carotenoids) in sediment revealed higher concentrations in the deeper area of Lake Remoray with maximum value for the deepest sampling point of Lake ( $17.7 \mu\text{g g}^{-1}$  of dry weight sediment), (Fig. 2D). Another concentration hotspot of pigment was observed south east of the lake. The fecal marker 3 $\beta$ -Cholestan-3 $\beta$ -ol, mainly produced by



**Figure 2.** Mapping of parameters characterizing the quantity and quality of sediment organic matter across Lake Remoray. The colour scale to the left of each map indicates the extrapolated parameter values. The quality parameters and the model types are detailed in Table S1 (Supporting Information).

herbivores, was found in high concentration in the south area of the lake and in the deepest area of the lake close to the farming area (Fig. 2E).

### Spatial distribution of microbial diversity across Lake Remoray

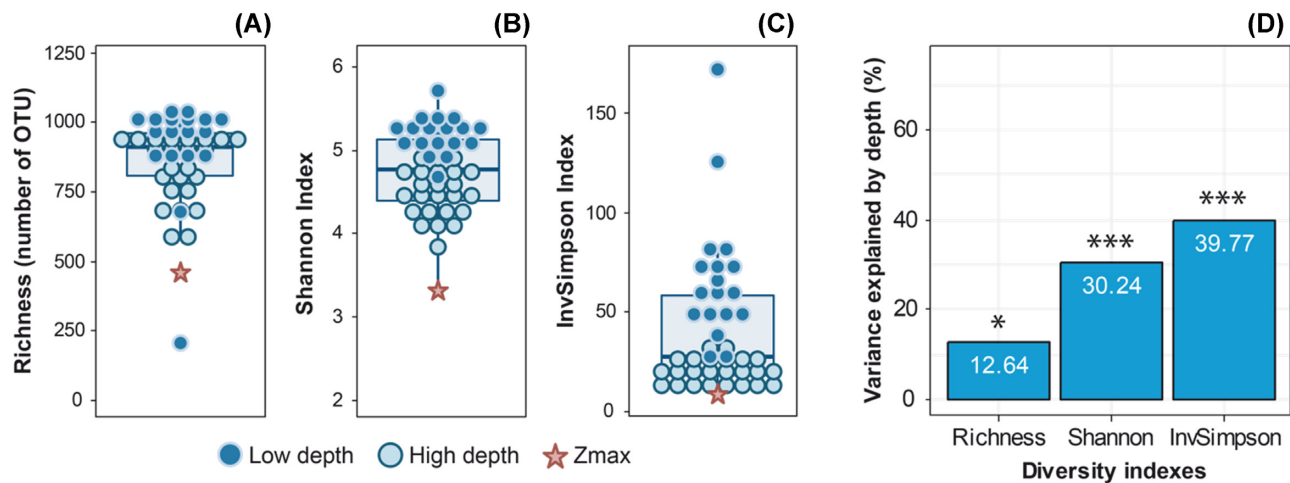
Illumina sequencing of the 44 samples yielded a total of 3323941 high-quality 16S rRNA gene sequences and described 1 566 different OTUs. Among them, 36239 quality sequences per sample were sub-sampled to allow efficient comparison of the data sets and avoid biased community comparisons.

Analysis of richness, Shannon and InvSimpson indexes (Fig. 3A–C) revealed differences of diversity levels between sampling areas in Lake Remoray sediment. Sediments in the low depth area exhibited lower microbial diversity level than high depth sediments, except for Zmax point (maximum lake depth) which harboured the lowest level of microbial diversity. Distribution of microbial diversity level according to Depth was confirmed by variance partitioning analysis (Fig. 3D). Among the five explanatory variables tested in our model, only Depth contributed significantly in the variation of diversity levels (Fig. 3D), accounting for up to 39.77% of the InvSimpson index variation.

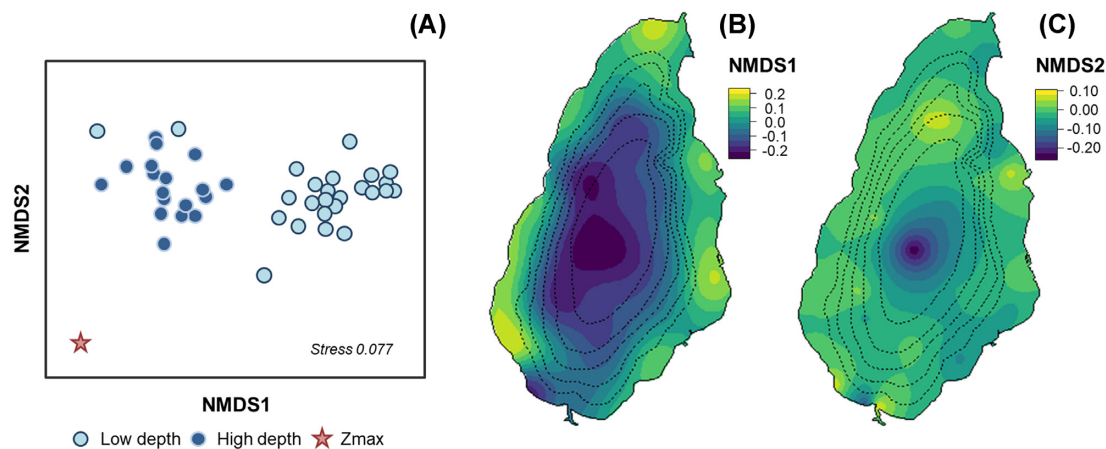
### Spatial variation of microbial genetic structure across Lake Remoray

The NMDS ordination of weighted UniFrac distance revealed two distinct groups based on microbial community structures between Lake Remoray sediments collected from the low and the high depth areas (Fig. 4A). Mapping NMDS1 coordinates confirmed the distribution of sediment microbial communities along the lake depth gradient (Fig. 4B) with an effective range of 224 m (Interpolation distance of geostatistical model). Different structure was also observed for the sediment microbial community sampled close to the lake tributaries. The NMDS2 map exhibited strong variations in community structure for the sediment microbial community thriving in the deepest area of the lake (Zmax point), (Fig. 4C). The robustness of these interpolated maps was supported by the cross validation statistics (Table S1, supporting information).

Db-RDA analysis on weighted UniFrac distance with our selected environmental variables showed a pattern of sample clustering similar to the NMDS ordination plot (Fig. 5A). The model significantly explained 52.7% of sediment microbial community variation across Lake Remoray (Fisher test,  $P < 0.01$ ). According to variation partitioning, Depth was the best driver of this variability accounting for 33.86% of the total variance



**Figure 3.** Variation of Richness (A), Shannon index (B) and InvSimpson index (C) according to sampling area; low depth, high depth and Zmax (maximal lake depth). Significant contribution of depth parameter in the variation of diversity indexes (D). Significance of variables was determined by 1000 permutations with \*\*\* $P < 0.001$ ; \* $P < 0.05$ .



**Figure 4.** Non-metric multidimensional scaling (NMDS) ordination plot derived from the weighted pairwise Unifrac distances of bacterial communities (A). Zmax point corresponds to the deepest site of the lake. Stress value was  $< 0.2$  which indicates that this data was well-represented by the two dimensional representation. Mapping of microbial community structure based on the score of axes 1 (B) and 2 (C) of the NMDS analysis. For each map, the colour scale bar indicates the prediction of scores of NMDS axis. The quality parameters and the model types are detailed in Table S1, Supporting Information.

(Fig. 5A and B). It was followed by the C/N ratio (14.95%) and TC (3.79%).

### Mapping major archaeal and bacterial phyla across Lake Remoray

Maps of the 11 most representative phyla (representing more than 0.5% of total sequences) were drawn by applying a geo-statistical approach based on their relative abundance (Fig. 6). Due to the large relative abundance of the Proteobacteria phyla, the Alphaproteobacteria, Deltaproteobacteria and Gammaproteobacteria class were used. No mapping was performed for Actinobacteria since spatial autocorrelation range was smaller than the average distance between sampling points (43 m and 100 m, respectively).

Phyla exhibited heterogeneous spatial distributions with geographical patch sizes ranging from 187 m to 637 m. Three major spatial distribution patterns were observed and structured according to the depth gradient of Lake Remoray (Fig. 6). Cyanobacteria and Alphaproteobacteria were dominant in the low depth area while the Bacteroidetes, Acidobacteria,

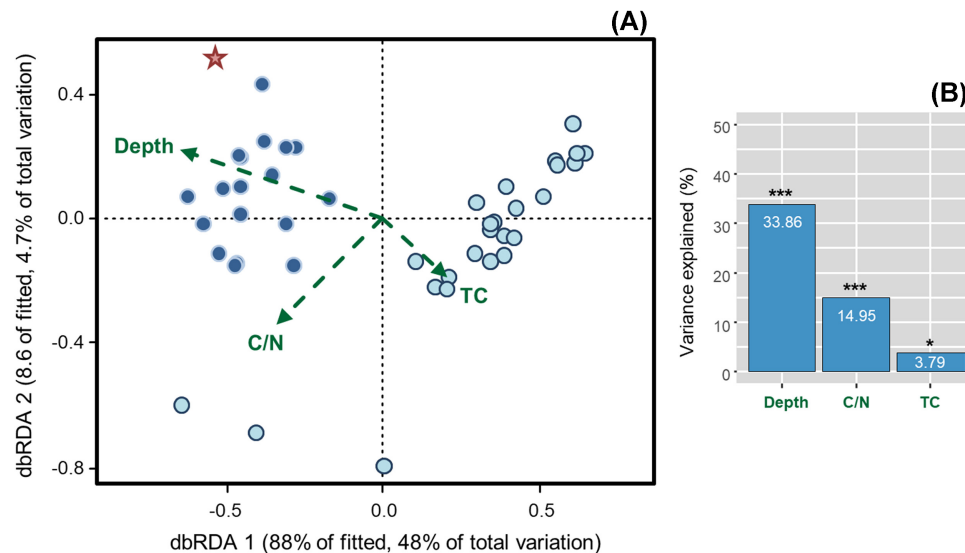
Deltaproteobacteria, and Verrucomicrobia were more abundant in the high depth area of Lake Remoray. In the deepest area of the lake, a Chloroflexi hotspot was observed and represents 23% of the total community. Other phyla such as Gammaproteobacteria, Euryarchaeota and Planctomycetes exhibited spottiest distributions across Lake Remoray.

### Drivers of major microbial taxa distribution in Lake Remoray

Multiple regression model with selected variables allowed to explain the variation of 19 bacterial and archaeal phyla (Fig. 7), ranging from 9.8% for Thermotogae to 71% for Cyanobacteria phyla. Actinobacteria and Euryarchaeota were the only taxa whose variations were not explained by the model parameters.

Consistently with our previous results, water depth is the best driver of microbial phyla distribution across the lake, explaining the relative abundance variation of 13 phyla, with up to 63% of variation explained for the Deltaproteobacteria class. Relative abundances of Cyanobacteria and Alphaproteobacteria significantly decreased with lake depth. With depth increase,





**Figure 5.** Distance-based Redundancy Analysis (dbRDA) ordination plot of microbial community structure (based on weighted pairwise Unifrac distances) fitted to environmental variables (A) and variance explained by significant model variables (B). Only significant explanatory variables are shown with \*\*\* $P < 0.001$ ; \*\* $P < 0.01$ ; \* $P < 0.05$ .

the relative abundance of 9 taxa, including Bacteroidetes and Deltaproteobacteria, increased. The second best driver of microbial phyla distribution was the C/N ratio of sediment organic matter, accounting for 5.9%–23% of microbial taxa relative abundance variation. This driver positively influenced 6 phyla and negatively influenced 3 phyla.

The presence of carotenoids in the sediment compartment (car.), illustrating sediment organic matter of phytoplanktonic origin, was the third parameter explaining the distribution of five microbial phyla. It influenced positively the variation of Chloroflexi (21% of explained variance), Gemmatimonadetes and Thermotogae (15.4 and 9.8% of explained variance, respectively).

Total carbon (TC) used in our model also significantly accounted for microbial distribution across Lake Remoray, explaining between 4.8 and 16.9% of phyla relative abundance variation (Fig. 7). It influenced positively the variation of Crenarcheota and Cyanobacteria and negatively, the variation of Bacteroidetes and Gemmatimonadetes.

Presence of  $\beta$ -Cholestan- $\beta$ -ol fecal contamination marker in sediment organic matter was the less important driver in spatial variation of sediment microbial communities. It positively influenced the abundance of Kiritimatiellaeota and Deltaproteobacteria, explaining 5.7%–3.5% of spatial variation.

## DISCUSSION

As observed for many lakes worldwide, seasonal hypoxia was reported in the deepest part of the French Lake Remoray by Belle and colleagues (2016). Using a paleolimnological approach, the trophic dysfunction of this lake was dated back to the 1970s, but the origin and causes are still unknown (Belle et al. 2015). In this study, we aimed at understanding how organic matter distribution and origin in the sediment compartment and the annual limitation of oxygen diffusion in the sediment top layers in the deepest part of the lake could control the diversity of sediment microbial communities, a major component of lake trophic network functioning. For this, we provided an extensive picture of organic matter origin and microbial communities in

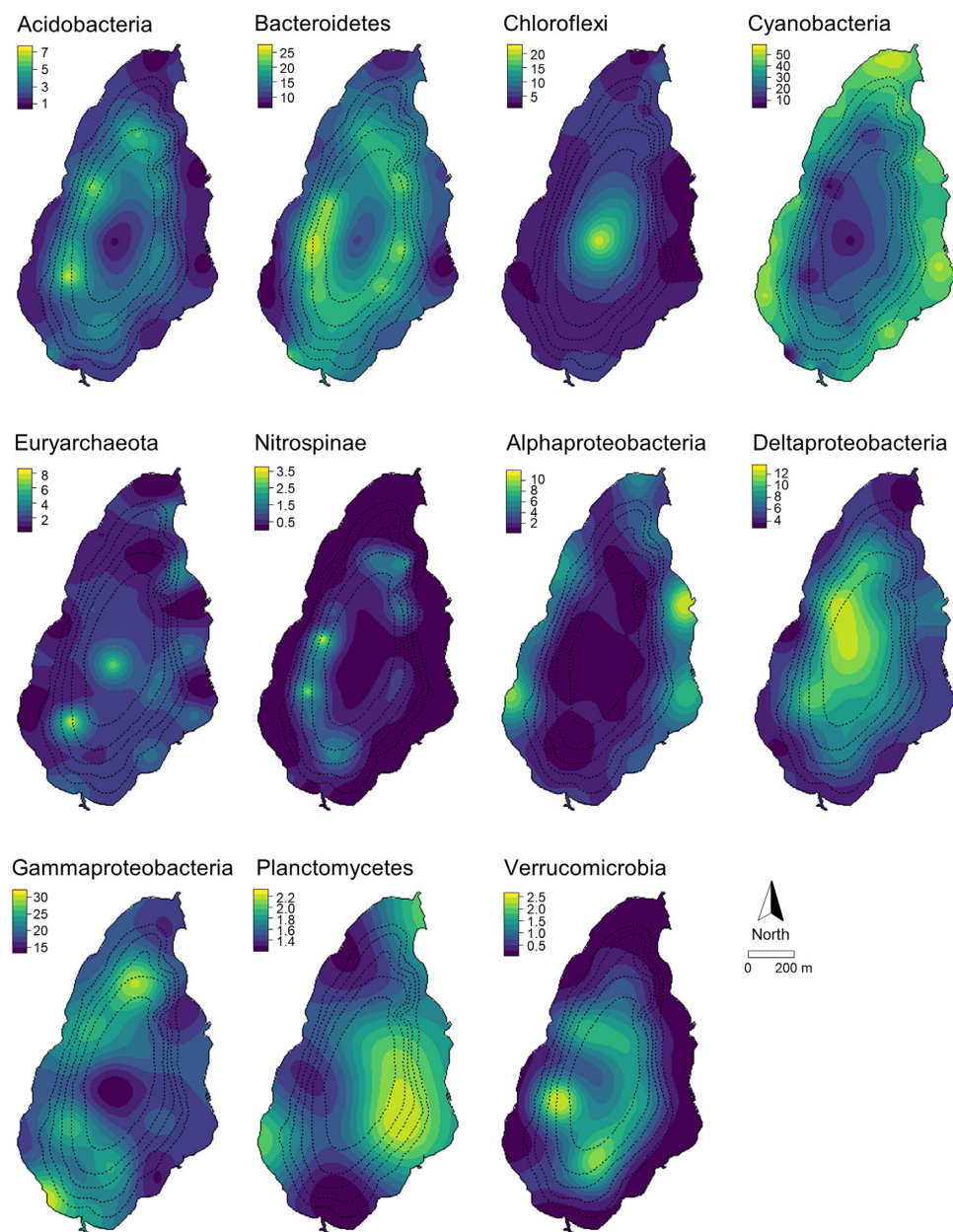
the first centimeter of Lake Remoray sediments after the 2016 hypoxia period.

## Origin of organic matter input in Lake Remoray sediment

Spatial characterization of both C/N ratio and  $\delta^{13}\text{C}$  signature of sediment organic matter (Fig. 2) showed weak values for the whole lake locations indicating that organic matter inputs are mainly of aquatic origin (Meyers and Lallier-Vergès 1999). Furthermore, the high concentration of pigments in sediments of deeper areas indicates an overproduction of the phytoplanktonic biomass in the water column. Organic matter accumulation in lake Remoray sediments results thus from a poor transfer of the phytoplanktonic organic matter in the trophic network. This degradation of trophic transfer efficiency could be explained by (i) the eutrophication of water body leading to an algal overproduction exceeding the transfer capacities of the trophic network (Salk et al. 2016; Karpowicz et al. 2020) or community changes toward less edible species, (ii) the presence of toxic contaminants inhibiting both these transfers and organic matter mineralization (Rodríguez et al. 2018; Nilsen et al. 2019), or (iii) the combination of former two mechanisms. Further investigation on sediment contamination is needed to confirm these hypotheses.

In the southern area of Lake Remoray, next to the two tributary outlets, the sediment was characterized by higher organic carbon concentrations, C/N ratios and  $\delta^{13}\text{C}$  compared to other lake locations. This single pattern indicates a slightly larger proportion of allochthonous organic matter in sediments (Meyers and Lallier-Vergès 1999) and is explained by the outlets of two tributaries crossing a wetland upstream. In this part of the lake, fecal sterol analyses also revealed a fecal contamination sourced from herbivore animals. Another fecal contamination area specific to herbivores was observed in the North-West area of the lake, located in the vicinity of a cattle farm and of grazing pasture areas. Altogether, this suggests that the two tributaries as well as runoff from the watershed could be important point and diffuse sources of nutrients (dissolved N and P).





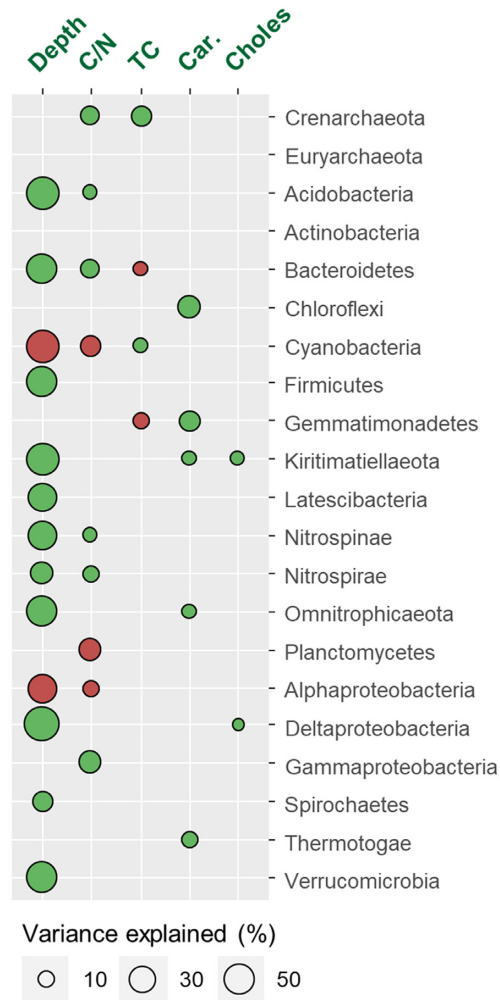
**Figure 6.** Mapping of the most dominant bacterial and archaeal phyla abundance across Lake Remoray. Proteobacteria phylum is represented by the most dominant classes. The colour scale on the top left side of each map indicates the extrapolated relative abundance values. The quality parameters and the model types are detailed in Table S1, Supporting Information.

Based on this data, Lake Remoray functioning is characterized by a strong lake-watershed connectivity representing an important source of nutrients and particulate organic matter causing lake eutrophication. As a consequence, this eutrophication stimulates the internal production of algal organic matter, likely causing, as for many lakes worldwide (Jenny *et al.* 2016b; Friedrich *et al.* 2014) long hypoxia periods in the water column. This also results in a heterogeneous distribution of organic matter in the sediment compartment, at the lake scale, both quantitatively and qualitatively.

#### Spatial distribution of sediment microbial communities after lake hypoxia period

Seasonal hypoxia leads to profound changes in the benthic fauna composition with major consequences on lakes biogeo-

chemical processes (Middelburg and Levin 2009; Friedrich *et al.* 2014; Weinke and Biddanda 2018). The top layers of lake sediment are generally oxygenated for a few millimeters when the interface water column is oxygenated, and can contain DNA from past and from present microbial community as well as from microorganisms inhabiting the benthic compartment or transferred from the overlying water column by sedimentation (Ellegaard *et al.* 2020). Consequently, the characterization of sediment microbial communities using DNA-based approaches allowed accessing both to microbial communities that developed during the hypoxia period and to active microbial communities at sampling time, right after the water column turnover and the subsequent reoxygenation of the top sediment layers. Three distinct patterns of sediment microbial communities were observed, each characterized by different levels of diversity, genetic structure and microbial composition (Figs 3, 4 and 6).



**Figure 7.** Variance partitioning results of most dominant bacterial and archaeal phyla according to model parameters. Proteobacteria phylum is represented by the most dominant classes. The height of the shape indicates the percentage of variance explained by parameters. Missing values indicate that no variable of the related group was retained in the model. The colours depict the direction of the standardized partial regression coefficients (green, positive effect; red, negative effect).

The first pattern was observed for sediments located in the lower depth area (0–2 m depth), with microbial communities strongly dominated by Cyanobacteria (from 20 to 50% of total sequences). The major cyanobacterial taxa detected were related to *Cyanobium* (57% of total cyanobacterial sequences) and *Microcystis* genera (14% of total cyanobacterial sequences). Both genera clusters photoautotrophic cyanobacterial organisms whose dominance in the shallower areas of the lake is also likely related to light availability throughout the water column. Cyanobacterial groups are frequently encountered in lake systems and global proliferations of cyanobacteria have even been reported in diverse aquatic habitats since the beginning of the Anthropocene period (Kosten et al. 2012; Taranu et al. 2015). Both global climate change and anthropic eutrophication are the main causes of cyanobacteria expansion and proliferation (Paerl and Huisman 2008). Cyanobacteria species generally outcompete other microbial taxa when nutrient concentrations and/or temperature increase (Kosten et al. 2012; Beaulieu, Pick and Gregory-Eaves 2013; Sukenik, Quesada and Salmaso 2015) and can lead to fast algal bloom. Cyanobacterial proliferation

may be also involved into the mechanisms causing lake hypoxia (Scavia et al. 2014; Weinke and Biddanda 2018). Cyanobacteria have detrimental effects on zooplankton due to their poor nutritional quality and the production of toxins (Sukenik, Quesada and Salmaso 2015; Karpowicz et al. 2020) reducing the efficiency of their organic matter transfer in food webs. As a result, organic matter accumulation in Lake Remoray sediments could be related to a decrease of transfer efficiency in the food web caused by cyanobacteria species, and their large dominance in microbial communities could be explained by an excess of nutrient supply from the lake-watershed connectivity.

The second pattern corresponds to microbial communities inhabiting deep areas of the lake (18–27 m depth). In this area, sediment microbial communities were dominated by Bacteroidetes, Deltaproteobacteria, Acidobacteria and Verrucomicrobia bacterial groups commonly found in freshwater lake sediments (Zhang et al. 2014; Ruuskanen et al. 2018). These bacterial groups exhibit large phylogenetic and metabolic diversity and are often associated with turnover of nutrients and organic matter in freshwater sediments (Newton et al. 2011; Cardman et al. 2014). Previous works have also reported an increase of both Verrucomicrobia and Bacteroidetes group occurrence in sediment during periods following Cyanobacterial blooms (Eiler and Bertilsson 2004; Louati et al. 2015). In agreement with this observation, the large proportion of these two bacterial groups in the high depth area of the lake could be related to Cyanobacterial dominance occurring in the water body during the summer period.

Interestingly, the abundant class of Gammaproteobacteria displayed a spatial pattern sharing low and high depth sediment areas and overlapping with the spatial pattern of Deltaproteobacteria. In the literature, previous works have already observed a shift between Gammaproteobacteria to Deltaproteobacteria in marine and freshwater sediments and was associated with oxygen gradient (Mahmoudi et al. 2015; Jessen et al. 2017; Mori et al. 2018). Gammaproteobacteria dominated surface sediment under well-oxygenated conditions while Deltaproteobacteria prevailed in sediment under hypoxic conditions (Mahmoudi et al. 2015). This finding suggests that the spatial area of Deltaproteobacteria in Lake Remoray sediment could be representative of the past hypoxic zone of Lake Remoray during the summer and fall periods.

The third microbial pattern corresponds to the deepest area of the lake (30 m depth) where microbial communities were characterized by the lowest level of diversity, and a very distinct genetic structure dominated by Chloroflexi, Thermotogae and Euryarchaeota species. Among these phyla, many sequences were affiliated to Dehalococcoidia, Thermotogae and Methanomicrobia classes representing 22%, 21% and 7% of total sequences, respectively. These taxa are described as strict anaerobic organisms inhabiting organic-rich sediment (Bhandari and Gupta 2014; Biderre-Petit et al. 2016; Pala et al. 2018) and their presence is consistent with the long anoxic conditions occurring in the deepest area of Lake Remoray. Despite the natural mixing of bottom waters before the sampling campaign and the subsequent increase of dissolved oxygen, the large predominance of anaerobic microbial communities suggests that the reoxygenation did not drastically affect microbial communities. Anoxic conditions could however have remained in most of the first centimeter of sediment analyzed here. The return of aerobic conditions at sediment-water interface for short terms might prevent the resilience of aerobic benthic communities with long-term consequences on biogeochemical cycles functioning, such as organic matter mineralization and nutrients turnover

(Middelburg and Levin 2009). Permanent anoxic conditions in sediment particularly promote the anaerobic respiration of organic matter, resulting in the formation of various reduced substances such as ammonium, iron, manganese, hydrogen sulphide and methane (Middelburg and Levin 2009).

The presence of methanogenic communities, mainly represented by the Methanomicrobia class has to be underlined. In the deeper areas of the lake, the production and diffusion of methane through the water column could represent an important microbial process for an ecosystem with long hypoxia/anoxia periods (Gelesh et al. 2016). Given the large contribution of methane emission to global climate change (Dean et al. 2018; Reay et al. 2018), further investigations need to be addressed to evaluate the potential methane production from Lake Remoray sediment compartment at various spatial and temporal scales.

### Nature and quantity of organic matter are significant drivers shaping sediment microbial communities in Lake Remoray

A variance partitioning analysis was applied to identify and rank the contribution of organic matter inputs in the distribution of sediment microbial communities. In our analysis, we also took into account water depth since it allows integrating numerous abiotic parameters not measured in this study such as dissolved oxygen, light intensity, temperature, nutrient availability (Böer et al. 2009). Our approach allowed us to explain 12–53% of microbial parameter variation (diversity index (Fig. 3), genetic structure (Fig. 5) and relative abundance of bacterial and archaeal taxa (Fig. 7)).

Among the parameters selected in our model, water depth of Lake Remoray was the best driver explaining the variation of sediment microbial communities. These results are consistent with several studies characterizing distribution of sediment microbial community of freshwater and marine ecosystems (Edlund et al. 2006; Hewson, Jacobson-Meyers and Fuhrman 2007; Zhang et al. 2014; Ding et al. 2015; Ruuskanen et al. 2018; Wu et al. 2019). Although the abiotic or biotic factors associated with water depth are unknown, this result shows that it is a relevant proxy parameter to explain sediment microbial communities' variation. In a recent study, Wu et al. (2019) notably used water depth gradient to explain the variation of dominant bacterial phyla abundance. Their results showed different abundance patterns depending on water depth, highlighting the existence of different microbial niches along the lake water depth gradient.

The quality of the sediment organic matter, represented by C/N ratio, was the second best driver of microbial community and contributed significantly to the variation of microbial genetic structure and the relative abundance of nine bacterial and archaeal taxa. Previous works have already reported significant correlation between C/N ratio and bacterial composition change (Zhang et al. 2014; Xiong et al. 2015; Dai et al. 2016; Wu et al. 2017), confirming the importance of this factor in the distribution of sediment microbial community within freshwater ecosystems. Interestingly, the increase of C/N ratio in sediment positively influenced the relative abundance of Bacteroidetes and Gammaproteobacteria, two bacterial groups commonly found in organic-rich sediment (Newton et al. 2011). The high abundance of these taxa in C/N-rich sediments could be explained by their high abilities to degrade complex molecules into simple compounds (Mühlenbruch et al. 2018). In

contrast, high C/N ratio negatively influenced the relative abundance of cyanobacteria species, as observed in the two freshwater lakes from the Yunnan Plateau (southwestern China) by Dai and colleagues (2016).

As observed by others studies in marine sediments (Polymenakou et al. 2005; Bienhold, Boetius and Ramette 2012; Fagervold et al. 2014), the origin of organic matter also largely accounted for the distribution of sediment microbial taxa in lake Remoray, as much as C/N ratio. Here, the sediment carotenoids concentration representing an input of organic matter of phytoplanktonic origin explained the spatial variation of 5 microbial taxa. Similar results were observed by Xiao et al. (2017) in a work evaluating the spatial changes of sediment bacterial communities in a eutrophic urban river (Taihu Basin, China). In their study, sediment chlorophyll-a concentration was positively correlated to the abundance of 16 microbial genera, including genera affiliated to Chloroflexi phylum. In agreement with our third hypothesis, our results therefore show that the sources and quality of organic matter inputs from Lake Remoray are structuring drivers of benthic microbial communities.

### CONCLUSION

Geographical approach combined with microbial and organic matter sediment characterization used in this study provided us substantial information about microbial communities inhabiting sediment. In Lake Remoray, organic matter mainly comes from algal overproduction during summer period which could be related to an excess of nutrients due to the close lake-watershed connectivity. But, in some areas of the lake, organic matter is mainly originating from the watershed.

In this study, hypoxia consequences were indirectly assessed on the diversity of sediment microbial communities after the hypoxia event that occurred in 2016. To our knowledge, it was the first study deciphering accurately the horizontal variation of sediment microbial communities following such an event. The resulting extensive picture of microbial taxa provided us interesting information on the lake areas where hypoxia could occur. The presence and abundance variation of some anaerobic sediment microbial groups across Lake Remoray such as Dehalococcoidia, Thermotogae and Methanomicrobia could be directly related to the hypoxia prevailing in the deepest layers of the water column. We also confirmed that quantity, origin and quality of organic matter are important drivers of sediment microbial communities' geographical distribution. Further investigations need to be addressed to characterize more accurately the sediment microbial response induced by the hypoxia period and the resulting consequences on the global functioning of Lake Remoray.

### SUPPLEMENTARY DATA

Supplementary data are available at FEMSEC online.

### ACKNOWLEDGEMENTS

This work was supported by the Pole Research & Development on Lacustrine Ecosystems (ECLA) of the French Biodiversity Agency (OFB) and the site Zone Atelier Arc Jurassien <https://zaaj.univ-fcomte.fr>. We thank the Lake Remoray nature reserve centre (RNN46) for giving us authorization to perform sediment sampling in the lake. The authors are grateful to the INRAE MIGALE bioinformatics platform (<http://migale.jouy.inra.fr>) for providing



computing and storage resources. We thank Sandrine Vix for technical support and Victor Frossard for his help in implementing the statistical approach.

**Conflicts of interest.** None declare.

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