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Microbial diversity and long-term geochemical trends in the euxinic zone of a marine, meromictic lake☆

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ABSTRACT

Hypoxic and anoxic niches of meromictic lakes are important sites for studying the microbial ecology of conditions resembling ancient Earth. The expansion and increasing global distribution of such environments also means that information about them serves to understand future phenomena. In this study, a long-term chemical dataset (1996–2015) was explored together with seasonal (in 2015) information on the diversity and abundance of bacterial and archaeal communities residing in the chemocline, monimolimnion and surface sediment of the marine meromictic Rogoznica Lake. The results of quantitative PCR assays, and high-throughput sequencing, targeting 16S rRNA genes and transcripts, revealed a clear vertical structure of the microbial community with *Gammaproteobacteria* (*Halochromatium*) and cyanobacteria (*Synechococcus* spp.) dominating the chemocline, *Deltaproteobacteria* and *Bacteroidetes* dominating the monimolimnion, and significantly more abundant archaeal populations in the surface sediment, most of which affiliated to *Nanoarchaeota*. Seasonal changes in the community structure and abundance were not pronounced. Diversity in Rogoznica Lake was found to be high, presumably as a consequence of stable environmental conditions accompanied by high dissolved carbon and nutrient concentrations. Long-term data indicated that Rogoznica Lake exhibited climate changes that could alter its physico-chemical features and, consequently, induce structural and physiological changes within its microbial community.

Introduction

Meromictic lakes account for <1% of the world's lakes [4,38], providing unusual, and rare, ecosystems suitable for studying microbial ecology in redox transition zones. The expansion and increasing global distribution of hypoxic and anoxic environments relates to anthropogenic environmental impact and climate change phenomena, and warrants study. Lakes, in general, are quite susceptible to climate change as their volume and salinity gradient depend on hydrological conditions [95].

Water profiles in meromictic lakes are strongly stratified chemically due to incomplete mixing over multi-year intervals [5,23,38,58]. Reduced vertical mixing typically results in anoxia in deeper water layers, and in the accumulation of sulfide, nutrients and organic matter in the lower, anoxic layer (monimolimnion) [38,74]. In Rogoznica Lake, which was used as a study site in this work, an oxic surface water layer (mixolimnion) and a deeper, euxinic (anoxic and sulfidic) monimolimnion layer prevail throughout the year. At the mixolimnion–monimolimnion interface, a steep chemical gradient, the chemocline, is formed [55]. Such redox transition zones in marine environments are typically characterized by diverse, abundant, and highly active prokaryotic communities [27,32], and they have unique microbial communities at distinct layers consistent with geochemical and physical partitioning [23,36,44,73]. Cyanobacteria usually dominate the oxic surface [56,68]; anaerobic, phototrophic bacteria occupy the chemocline; and sulfate-reducers and -oxidizers dominate deep, anoxic lay-

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☆ Raw sequence files supporting the results of this article are available in the European Nucleotide Archive under the project accession number PRJEB29356.

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ers [23,54,92]. Meromictic lakes with their shallow phototrophic chemocline communities, extreme geochemical gradients, and laminated sedimentary records under conditions of rapid sedimentation that tend to preserve biosignatures [65] are of particular interest for geochemical and microbial research.

Development of high-throughput sequencing (HTS) has considerably improved the knowledge of the diversity and functions of complex microbial communities [78]. However, an important challenge in microbial ecology is to link the diverse, uncultivated majority with their ecological role and ecosystem function. This is best achieved by studying both total community potential (DNA-based studies) as well as its active fraction (RNA-based studies) [46]. Indeed, compared to other well-explored freshwater and marine environments, relatively little is known about the microbial ecology of meromictic lakes [4,28,44].

The aims of this study, therefore, were to: (1) characterize the multi-year, depth-resolved geochemistry; (2) identify dominant members of the active prokaryotic communities of the hypoxic, anoxic and euxinic niches during the investigated seasons; and (3) describe the ecophysiological interactions underpinning the biogeochemistry in marine, meromictic Rogoznica Lake. A long-term (1996–2015) sampling campaign was undertaken to comprehensively characterize and monitor the biogeochemistry of the lake. Studies on the microbial ecology of the lake are meagre [12,69,70,71], especially for the anoxic monimolimnion and sediment layers. HTS and quantitative PCR assays were applied, targeting 16S rRNA (DNA and RNA) phylogenetic markers, in order to evaluate seasonal differences in the microbial ecology of the chemocline, monimolimnion and surface sediment of Rogoznica Lake.

Materials and methods

Study site

Rogoznica Lake (known as “Dragon Eye”) is situated on the central part of the eastern Adriatic coast (Croatia) on the Gradina peninsula (43°32′N 15°58′E). The lake is relatively small (10.276 m^2) and shallow (maximum depth, 15 m). It is surrounded by karstic cliffs (4–23 m) that prevent wind-shear mixing. During the year, the water column is chemically and physically stratified into an oxic mixolimnion, and a euxinic chemocline and monimolimnion. The depth of the chemocline is influenced by the prevailing meteorological conditions (i.e. temperature and rainfall), and it is characterized by a sharply increasing concentration gradient of nutrients, organic matter, and sulfide [18]. Accumulation and mineralization of allochthonous organic matter is characteristic of the monimolimnion, as indicated by high concentrations of dissolved organic carbon (DOC up to 6 $mg\,L^{-1}$), iodine species (up to 1 μM) and nutrients (NH_4^+ up to 150 μM; PO_4^{3-} up to 22 μM; SiO_4^{4-} up to 400 μM). As a result of biological sulfate reduction, the monimolimnion is rich in reduced sulfur species (RSS), mainly in the form of sulfide that can reach 5 mM [12,20,24,96]. Sediment in Rogoznica Lake is laminated, and composed of poorly sorted silt and clay silt, such as biogenic carbonates. The most abundant mineral is calcite followed by aragonite, quartz, dolomite and pyrite [12,19].

Sampling, and measurements of physico-chemical parameters

Long-term data were collected from the middle of the lake at the deepest point, during regular seasonal sampling between 1996 and 2015. Temperature (T), sulfide (H_2S) and ammonium (NH_4^+) concentrations were measured as described below [20]. To determine any possible long-term trends of these concentrations in the monimolimnion (12 m depth), a linear regression method fit was used comprising a linear trend in time together with seasonal cycles (only for temperature). Chemical parameters did not follow a seasonal cycle, and so an additional term representing yearly variability was not used. Grouping data by seasons significantly reduced the number of measurements, thus compromising statistical confidence. Therefore, whole time series were used, which was necessary in order to avoid uneven sample datasets (there were more data during the summer season).

Samples for microbiological analysis were taken seasonally – in January, April, July and September – in 2015. Water samples were taken from the chemocline (8.5 m depth) and monimolimnion (12 m depth), using 5-L Niskin bottles, which were immediately placed on ice until they were filtered (0.22 μm MCE) within 2–3 h of sampling. Filters were immediately flash-frozen in liquid nitrogen. Surface sediment samples were obtained using a gravity corer (to 10 cm) and frozen (−80 °C) until further analysis. Reduced sulfur species (RSS) were measured immediately after sampling using an electrochemical method [9,16]. Dissolved and particulate organic carbon (DOC and POC) were measured in filtered samples using standard high-temperature catalytic oxidation (TOC-VCPH-5000 solid sample TOC analyzer, Shimadzu, Japan). Temperature (T), salinity (S), dissolved oxygen (O_2), oxidation-reduction potential (ORP) and pH were measured in situ by a HQ40D multimeter probe (Hach Lange, Germany).

Samples (50 mL) for ammonium (NH_4^+) measurements were stabilized by adding 2 mL phenol (1 M dissolved in 95% v/v ethanol) [47] before being stored in the dark at 4 °C. Samples (500 mL) for measurements of other nutrients were directly stored at −20 °C. The concentrations of nitrate (NO_3^-), nitrite (NO_2^-), ammonium (NH_4^+) and orthophosphate (PO_4^{3-}) were determined according to the approach of Strickland and Parsons [84], but without filtration.

Nucleic acid co-extraction, and cDNA synthesis

All solutions for use in nucleic acid purifications were prepared with diethylene pyrocarbonate-treated (DEPC) water to inactivate RNase. Nucleic acids were co-extracted according to a modified version of the method described by Griffiths et al. [37]. Filters – in case of water samples – or 0.2 g sediment were mixed with 0.5 mL 2% (w/v) cetyl trimethylammonium bromide (CTAB) buffer and 0.5 mL phenol:chloroform:isoamyl alcohol (25:24:1). Samples were lysed by bead beating for up to 4 min. After centrifugation (13,300 rpm, 10 min), the supernatant was transferred to a new tube together with an equal volume of chloroform:isoamyl alcohol (24:1), before being mixed gently and then centrifuged (13,300 rpm, 10 min). Total nucleic acids were precipitated from the extracted aqueous layer with 0.1 volume 3 M sodium acetate and 1 volume of ice-cold isopropanol, followed by centrifugation (12,500 rpm, 30 min). Pelleted nucleic acids were then washed in ice-cold 70% (v/v) ethanol and air-dried prior to resuspension in 50 μL DEPC-treated water. The quality of DNA and RNA was determined using a NanoDrop spectrophotometer (NanoDrop Technologies, Wilmington, USA). The concentrations were determined using a Qubit fluorometer (Invitrogen, USA), as well as by electrophoretic separation on 1% (w/v) agarose gels.

Total RNA was treated with Turbo DNase (2 U μL^{-1}, Ambion) at 37 °C for 30 min. The DNase was inactivated at room temperature for 5 min. Possible DNA contamination of the RNA templates was routinely monitored using PCR assays targeting 16S rRNA genes and using RNA aliquots as template. No contaminating DNA was detected in any of these reactions. To construct complementary DNA (cDNA), aliquots of 1.5 μL 10 mM deoxyribonucleoside triphosphate (dNTP) mix (New England Biolabs), 0.75 μL 50 mM $MgCl_2$ (Sigma Aldrich, USA) and 2.25 μL random primers (60 μM) (New England Biolabs) were added to 16 μL RNA samples, before incubation at 75 °C for 4 min followed by chilling on ice. Aliquots of 3 μL 10× first-strand buffer [500 mM Tris–HCl (pH 8.3), 750 mM KCl, 30 mM $MgCl_2$, 50 mM DTT], 1 μL SUPERase-In RNase inhibitor (10 U μL^{-1}, New England Biolabs), and 1.5 μL Moloney murine leukemia virus–reverse transcriptase (M-MuLV) (200 U μL^{-1}, New England Biolabs) were added to provide a final reaction volume of 25 μL. The mixture was incubated at 25 °C for 10 min and 42 °C for 50 min, and then at 90 °C for 10 min to inactivate the reverse transcriptase (RT) [50]. The concentration and quality of cDNA were determined using NanoDrop and Qubit spectrophotometers, as before.

Quantitative PCR

Reaction mixtures (20 μL) for quantitative PCR (qPCR) assays targeting 16S rRNA genes contained 10 μL TaqMan Mastermix (Roche Applied Science, Switzerland), 0.2 μM of each primer with the corresponding hydrolysis (TaqMan®) probe, and 5 μL template DNA or cDNA. Primers targeting bacterial 16S rRNA genes were BAC338F (5′-ACTCCTACGGGAGGCAG-3′) and BAC805R (5′-GACTACCAGGGTATCTAATCC-3′), with the hydrolysis probe BAC516F (5′-TGCCAGCAGCCGCGGTAATAC-3′). Archaeal 16S rRNA genes were targeted using ARC787F (5′-ATTAGATACCCSBGTAGTCC-3′) and ARC1059R (5′-GCCATGCACCWCCTCT-3′) with hydrolysis probe ARC915F (5′-AGGAATTGGCGGGGGAGCAC-3′), as previously described [94]. Reactions were performed in a LightCycler® 480 (Roche Applied Science, Switzerland) under the following conditions: 94 °C for 10 min; 45 cycles of 94 °C for 10 s and 60 °C for 30 s [64]. A control without DNA template was included in each qPCR assay, and for each primer and probe set, and all experiments were undertaken in triplicate (n = 36).

To obtain standards, 16S rRNA genes were amplified and ligated into the pCR 4-TOPO Vector (Invitrogen, USA), which was used to transform One-Shot OmniMAX 2 T1R competent *E. coli* cells (Invitrogen, USA), according to the manufacturer's instructions. Plasmid DNA was extracted from cultures of isolated transformants using the Isolate II Plasmid Mini Kit (Bioline, UK), and then sequenced. BLASTn was used to identify bacterial and archaeal 16S rRNA gene sequences against the NCBI database according to a minimum identity of 97%. The concentration of plasmid DNA was determined with a Qubit fluorimeter (Invitrogen, USA) and 16S rRNA gene concentrations in plasmid DNA were extrapolated. A series of standard samples was produced by ten-fold serial dilution of bacterial (4.14×10^{10} to 4.14×10^{1}) and archaeal (3.78×10^{10} to 3.78×10^{1}) plasmid DNA preps. Standard curves, derived from qPCR assays using the standard series, were observed to have $R^2 > 0.99$. The qPCR efficiencies (E), calculated using the formula $E = 10^{(-1/\text{slope})} - 1$ (where slope is the slope of the standard curve), were 90% for bacterial and 91.6% for archaeal assays.

Amplicon sequencing and bioinformatics

Samples of DNA and cDNA were used for high-throughput sequencing of partial 16S rRNA genes on an Illumina MiSeq platform by FISABIO (Valencia, Spain). Forward and reverse primers for PCR were 515f and 806r, respectively [13]. A total of 1,684,033 raw sequences were obtained from 24 samples. Sequence screening, alignment to the Silva (v123) database, clustering, chimera-removal, and taxonomic classification were performed using Mothur v1.39.3 [76]. Sequences were clustered into operational taxonomic units (OTUs) at a 97% similarity level based on the distance matrix and Shannon-Wiener diversity. Chao1 (bias-corrected species richness estimator) and ACE (non-parametric abundance-based coverage estimator) coverage measurements were calculated after normalization due to the unequal numbers of sequences in the samples.

Data are presented as the mean ± standard deviation (SD) and were performed on 24 samples (n). Normality was tested using Statistica 7 software, and differences in the abundance of bacterial and archaeal 16S rRNA genes were evaluated using a Kruska–Wallis test. For statistical non-metric multidimensional scaling (nMDS), the PRIMER 6 v.6.1.11. and PERMANOVA + v.1.0.1. statistical software packages were used [21]. Similarities were calculated for each abundance resemblance matrix by means of the Bray-Curtis similarity index [22]. Canonical correspondence analysis (CCA) and Monte Carlo permutation tests were used to analyze the relationships between the lake's community structure and environmental factors using Canoco version 5 software [60]. Spearman's rank correlation test was used to define the degree of dependence between the variables and was performed with Statistica 7 software. Analyses were undertaken using only classes assigned as dominant (frequency of appearance >1% and at least in one sample), and both relative abundance and environmental data from individual sampling depths were used. Rare species (frequency of appearance <1% in all samples) were not included so as to minimize the number of regressions and avoid the influence of false positives that may occur due to automatic annotations.

Results

Environmental parameters

Environmental conditions in the small, marine meromictic Rogoznica Lake (eastern Adriatic Coast, Croatia) were investigated seasonally in 2015. Temperature, dissolved oxygen, pH, oxidation-reduction potential (ORP), salinity, reduced sulfur species (RSS), nutrients, and the organic matter profiles of the water column indicated thermohaline stratification tightly coupled to chemical stratification with the chemocline spanning from 8–9 m during all sampled seasons (Fig. 1). Temperature in the mixolimnion ranged from 7.3 °C in the winter to 28.9 °C in the summer season. Salinity fluctuations were also observed in the mixolimnion (14.5 to 32.1 psu in the winter and summer seasons, respectively). Seasonal differences were less pronounced in the chemocline and monimolimnion. Temperature ranged from 21.7 to 29.5 °C in the chemocline, and from 18.8 to 20.9 °C in the monimolimnion, during the winter and summer seasons, respectively. Salinity was 34.2 psu in the chemocline during spring and 40.3 psu during winter sampling, whereas in the monimolimnion it ranged from 33 psu during autumn to 40.4 psu in the winter. pH was constant throughout the year (7.5–8.0 in the mixolimnion), with the lowest pH occurring in the monimolimnion (6.82–7.0 in the summer and spring seasons, respectively) (Fig. 1). The mixolimnion was well oxygenated (6.6 and 12.3 mg L^{-1} in the autumn and winter seasons, respectively), with rapid drawdowns through the chemocline to the monimolimnion, resulting in decreasing redox potential. The concentration of reduced sulfur and nitrogen species followed these gradients. Highest sulfide (maximum 2.97 mM in the autumn) and ammonium concentrations (up to 329 μM in the winter) were observed in the monimolimnion. NO_2^- and NO_3^- concentrations were quite low, decreasing along the redox gradient with lowest NO_2- in the spring sampling of the monimolimnion (0.03 μM) whilst NO_3^- was below detection limits beneath the mixolimnion. Vertical profiles of particulate (POC) and dissolved organic carbon (DOC) concentrations sharply increased in the chemocline layer, suggesting pronounced bacterial production and accumulation. POC (3.6 mg L^{-1}) and DOC (4.8 mg L^{-1}) concentrations reached respective maxima in the summer monimolimnion (Fig. 1).

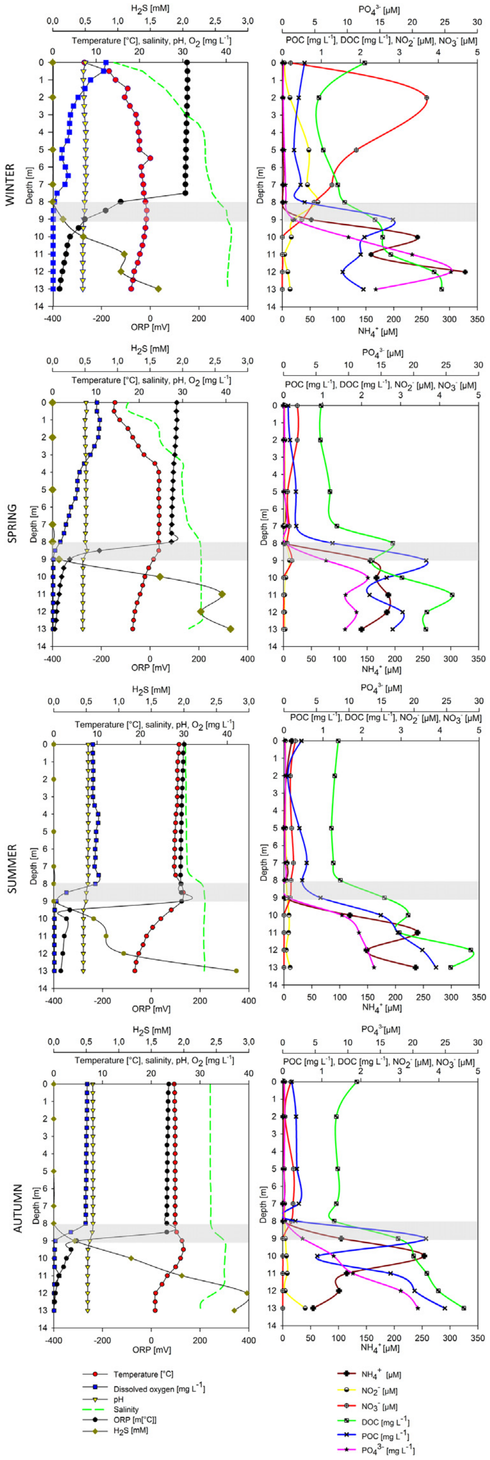

Fig. 1. Vertical profiles of physico-chemical parameters in Rogoznica Lake during the investigated seasons. Samples for high-throughput 16S rRNA gene sequencing were taken from the chemocline (shadowed areas) and monimolimnion (12 m).

Long-term trends of temperature, sulfide and ammonium profiles

Correlation and statistical justification of the method for long-term, monimolimnion temperature data (1996–2015) analysis were high ($r^2 = 0.5$ at 95% significance; *p* value, 3.0×10^{-18}) and indicated a clear trend of increasing temperature by 0.70 °C ± 0.73 in 10 years (Fig. 2a).

For sulfide at 12 m, $r^2 = 0.3$ (at 95% significance; *p* value, 1.0×10^{-8}) with the linear trend of increased values being $8.0 \times 10^{-4} \pm 3.0 \times 10^{-4}$ mM/10 y (Fig. 2b). The analyses were focused on the first 10 years, when there was less variability and the trend was still evident (7.9×10^{-4} mM/10 y). In the second half (decade) of the measurements (2006–2015), the variability was larger and the linear trend of 1.2×10^{-3} mM/10 y was more pronounced (marginal *p* value similar to the confidence interval value of 0.05; Fig. 2b).

In the case of ammonium at 12 m, the linear trend of increasing values across the entire period of 20 years was 60 ± 34 µM/10 y (Fig. 2c), which was more variable (correlation coefficient, $r^2 = 0.18$) than for sulfide but was statistically relevant (*p* value, 8.2×10^{-4}).

Taxonomic structure of the prokaryotic community

The taxonomic structure of the microbial communities in the euxinic zones of Rogoznica Lake was assessed using phylogenetic annotations of the 16S rRNA gene (DNA) sequence reads generated from HTS (Fig. 3). *Bacteria* – as distinct from *Archaea* – comprised 93.2–99.7% of the reads from the water column and >80% from the sediment. Among bacterial sequences, *Cyanobacteria* (most of which were related to the *Synechococcus* sp. CC9902 strain) represented a plurality of designated sequences from the chemocline layer. They dominated the chemocline during winter (40.8%) and spring (20.2%), comprising on average 23.6 ± 11.7% of the total chemocline community. *Synechococcus*-related sequences were also frequently retrieved from the monimolimnion samples (5.5–7.6% in the spring and autumn, respectively; average, 6.5 ± 0.9%), while in the surface sediment they comprised <0.4% of DNA sequences. The second most abundant class in the chemocline was *Gammaproteobacteria* (represented exclusively by *Chromatiaceae*), which accounted for 7% in the winter to 16.2% in the summer season (average, 14 ± 5%), whereas in the monimolimnion they comprised on average 11.2 ± 5.42%. *Chromatiaceae* were lowly represented in the surface sediment (average, 1.3 ± 1.2%). The next abundant class throughout the investigated zones was *Deltaproteobacteria*, accounting on average for 9.4 ± 3.1% of the total community in the chemocline, 12.4 ± 2.4% in the monimolimnion and 14.5 ± 2.9% in the surface sediment. Within *Deltaproteobacteria*, the dominant genus was *Desulfatiglans* followed by unclassified *Desulfobacteraceae*. *Bacteroidetes* (represented by *Sphingobacteriales* and *Bacteroidetes* VC2.1 Bac22) was the most abundant phylum in the monimolimnion (average, 19.7 ± 2%), whereas they were less abundant in the chemocline (average, 12.1 ± 3.3%) and sediment (average, 2.5 ± 0.9%). The proportion of the community represented by *Anaerolineaceae* was highest in the monimolimnion (3.9% in the spring and 8.4% in the autumn; average, 6.1 ± 1.8%). In other layers they accounted on average for 2.8 ± 2.1%, except in the winter sediment sample, when they comprised 16.6% of DNA sequences. Often retrieved sequences were also affiliated to *Alphaproteobacteria* (*Rhodobacterales*, *Rhodospirillales* and *Rhizobiales* orders), which enriched the chemocline community in the winter (13.7%), and *Planctomycetes* (average, 5.6 ± 3.9%; maximum in winter sediment 13.4%). Other taxa comprising >1% of sequences at least during one season included *Chlorobia* (average, 0.5 ± 0.4%), *Cloacimonetes* (more abundant in water layers; average, 5.2 ± 2.3%), *Dehalococcoidia* (prevalent in the surface sediment; average, 14.7 ± 11.9%), *Kiritimatiellae* (prevalent in the monimolimnion; average, 3.9 ± 1.4%), *Latescibacteria* (found only in autumn and summer sediment samples), *Omnitrophicaeota* (the highest abundance in the surface sediment; average, 2.7 ± 1.3%), *Patescibacteria* (prevalent in both water layers; average, 2.7 ± 1.3%) and *Spirochaetia* (detected in all samples; average, 1.4 ± 0.6%). Among bacterial DNA, 3.3 ± 0.4% of chemocline reads, 5.6 ± 0.8% of monimolimnion reads, and 8.8 ± 1.2% of sediment reads were designated as unclassified sequences.

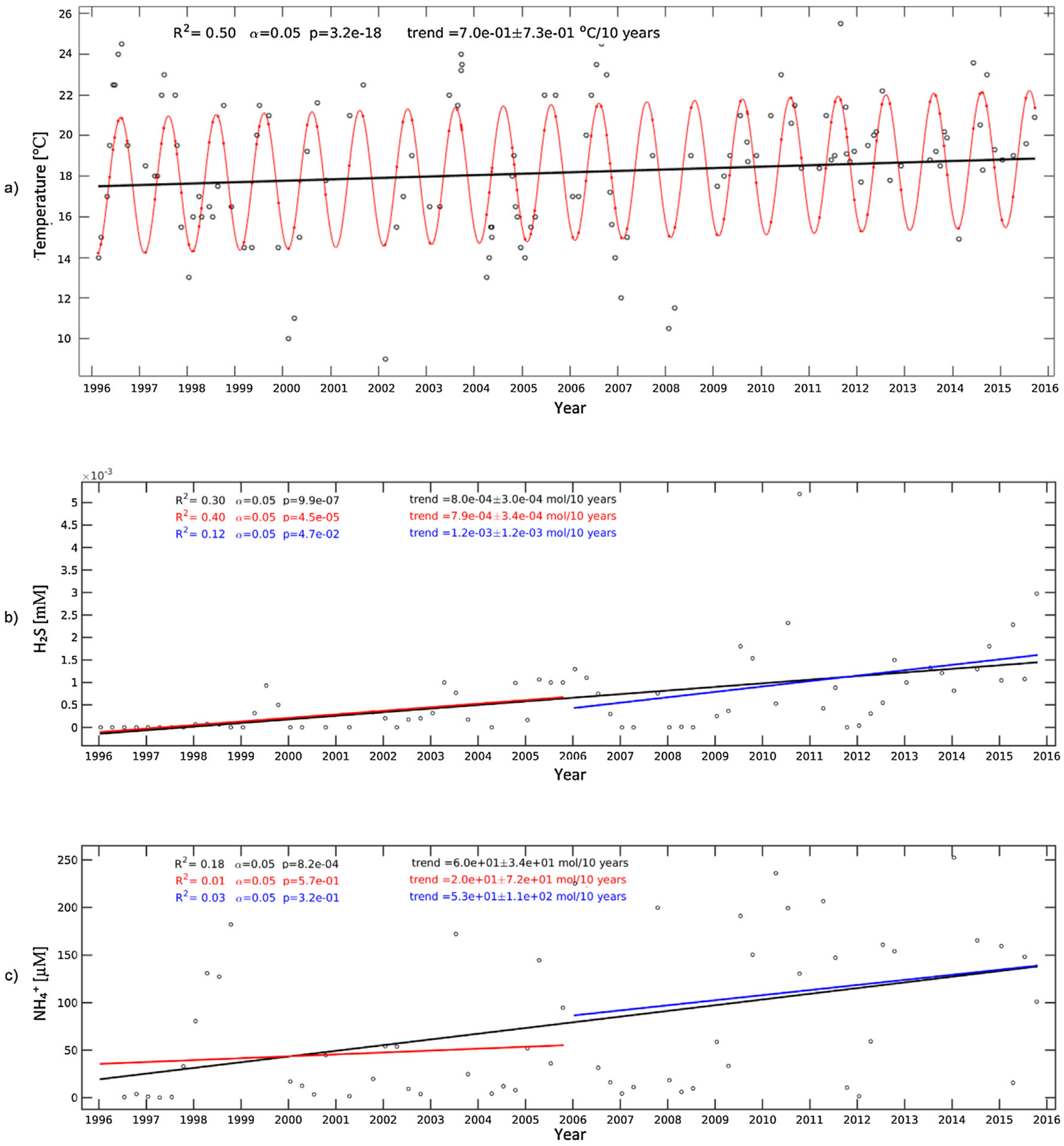

Fig. 2. Long-term (1996–2015) trends of temperature (a), sulfide (b) and ammonium (c) in the monimolimnion layer of Rogoznica Lake at a depth of 12 m.

Archaeal sequences from the chemocline and monimolimnion DNA were affiliated to *Euryarchaeota* (average, 1.1 ± 0.83%) and *Nanoarchaeota*, comprising on average 6.5 ± 5.3% of the prokaryotic community from the water column. In addition to *Euryarchaeota* (average, 4.9 ± 2%) and *Nanoarchaeota* (average, 8.5 ± 5.2%) in the surface sediment, *Asgardaeota* was also detected but accounted for only 0.6 ± 0.4% of DNA sequences. Taxonomically unclassified *Archaea* comprised on average 1.8 ± 1.6% of reads in the DNA pool.

Structure of the active fraction of the prokaryotic community

Bacterial sequences accounted for >96% of the active microbial community (based on analysis of cDNA sequencing) in the water column and >77% in the sediment samples. *Archaea* comprised <1% of the active fraction of the microbial community in the chemocline and, on average, 0.6 ± 0.5% in the monimolimnion. *Archaea* appeared more relatively abundant in the active fraction of surface sediment samples, ranging from 11% in the winter to 23% in the summer. Unclassified *Bacteria* comprised average proportions of 0.7 ± 0.2% of the active chemocline community, 2.3 ± 0.3% of the active monimolimnion community, and 8.2 ± 1.7% of the active surface sediment community. Meanwhile, unclassified *Archaea* comprised an average proportion of 1.4 ± 0.3% of the active sediment community.

In the surface sediment, the most frequently retrieved sequences affiliated to *Nanoarchaeota* (from 6.1% in the winter to 11.6% in the summer; average, 7.95 ± 2.5%). *Euryarchaeota* comprised an average 4.8 ± 2.1% of total reads. Within the active community, *Asgardaeota* comprised up to 5% of the surface sediment community, while it was absent from the water layers. The most relatively abundant recovered bacterial 16S rRNA sequences (average, 54.4% ± 19%) indicated the presence of *Gammaproteobacteria* in the chemocline layer. *Gammaproteobacteria* accounted for 6.1% (winter) to 50.1% (summer) of the active monimolimnion community (average, 23 ± 19%), but only 0.7 ± 0.4% in the surface sediment samples. Interestingly, *Gammaproteobacteria* were represented exclusively by the family *Chromatiaceae* (i.e. *Halochromatium*). A considerable fraction (average, 30 ± 22%) of the cDNA

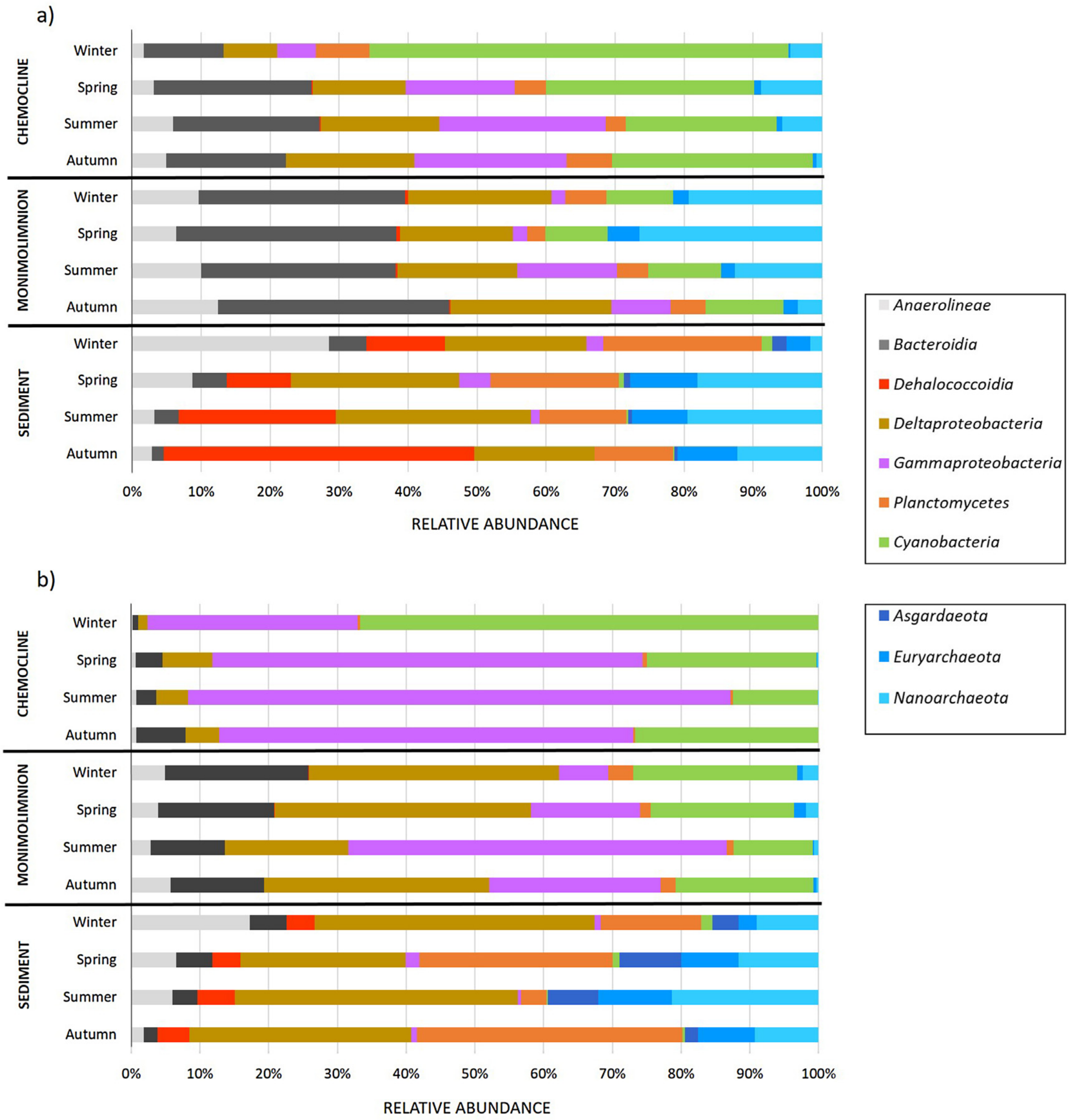

Fig. 3. Relative abundance of bacterial and archaeal community composition in the chemocline, monimolimnion and surface sediment samples over the sampling seasons based on DNA (a) and cDNA (b) sequences of the 16S rRNA gene. Represented taxa occurred on average at >3% abundance in at least one sample. Relative abundances of rare and unclassified taxa are given in Supplementary Table S1.

sequences in the chemocline belonged to *Cyanobacteria* (i.e. *Synechococcus* sp. CC9902-related cyanobacteria). All other bacterial taxa in the chemocline accounted for 12% of the community. *Synechococcus*-related sequences were also prominent in the monimolimnion and comprised from 10% in the summer to 20.4% in the winter (average, 16.7 ± 4.3%). *Deltaproteobacteria* (a majority of which belonged to the *Desulfatiglans* family, with a minor proportion of unclassified *Desulfobacteraceae*) was the most abundant class in the monimolimnion, comprising on average 27.2 ± 7.3% of active bacteria. Meanwhile, they accounted for 14% (spring) to 27.6% (winter) in the surface sediment (average, 22.3 ± 5.9%). *Bacteroidia* was enriched in the monimolimnion and ranged from 9.8% in the summer to 17.8% in the winter (average, 13.5 ± 3.4). The surface sediment samples were abundant in *Planctomycetacia* sequences that comprised from 2% in the summer to 30% in the autumn season (average, 14.6 ± 11.9%), whereas *Anaerolineaceae* were present in the monimolimnion (average, 3.8 ± 1%) and in sediment samples (average, 5 ± 4.5%). Other taxa that comprised >1% of sequences at least in one season were *Chlorobia* (up to 1.1% in the chemocline), and *Alphaproteobacteria* (*Rhodobacterales, Rhodospirillales* and *Rhizobiales*) that comprised ~1% of the active community in the chemocline and monimolimnion. *Cloacimonetes* and *Kiritimatiellae* comprised up to 2.9% and 1.3% of the community in the monimolimnion, respectively, while *Dehalococcoidia* (up to 3.6%), *Latescibacteria* (up to 1.4%), *Omnitrophicaeota* (up to 2.2%) and *Spirochaetia* (up to 1.7%) were found only in the surface sediment. Overall, the most relatively abundant recovered rRNA sequences were related to *Gammaproteobacteria* (*Chromatiaceae*, average, 54.4 ± 26%), followed by *Deltaproteobacteria* (average, 17.9 ± 11.5%) and *Cyanobacteria* (average, 15.9 ± 17.6%). These populations appeared to be spatially, rather than temporally, distributed, with clear structural differences between the investigated layers.

Prokaryotic diversity in Rogoznica Lake

After the quality check, 1,290,344 sequences (average length, 254 ± 11 bp) were used to generate OTUs (3% distance cut-off level). Rarefaction curves derived from the samples did not reach an asymptote, since the number of OTUs increased as more sequences

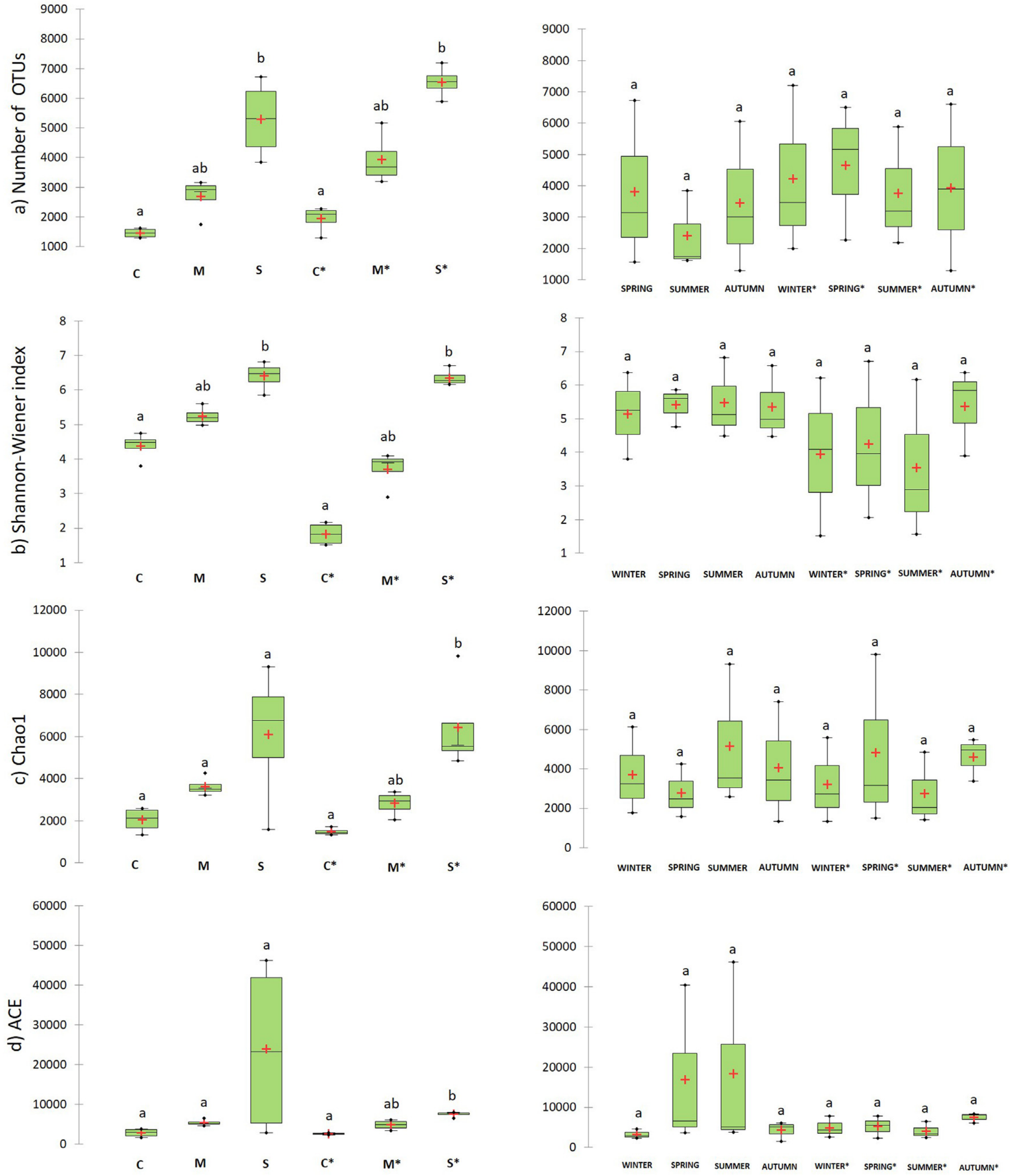

Fig. 4. Spatial (left) and temporal (right) differences in OTU number, Shannon-Weaver index, Chao1 and ACE estimators in the chemocline (C), monimolimnion (M) and surface sediment (S) of Rogoznica Lake. cDNA samples are marked with an asterisk (*). Identical letters above the bars indicate that differences were not significant according to the Kruskal–Wallis test ($p < 0.05$).

were added (Supplementary Fig. S1) even in the samples with most sequences. Although rarefaction curves did not reach an asymptote, coverage was >90% in all seasonal samples. The fewest OTUs were in DNA and cDNA samples from the chemocline (average, 1,456 ± 158 in DNA and 1,939 ± 442 in cDNA), followed by the monimolimnion samples (average, 2,688 ± 644 in DNA and 3,934 ± 873 in cDNA), while significantly more (Kruskal–Wallis; $p < 0.05$) OTUs were found in surface sediment in both DNA (average, 5,297 ± 1,330) and cDNA (average, 6,553 ± 534) pools (Fig. 4). Accordingly, Shannon-Wiener indices (Fig. 4) increased with depth and were significantly (Kruskal–Wallis; $p < 0{,}05$) higher in the surface sediment. In DNA samples, the diversity index ranged from 4.4 ± 0.4 in the chemocline, 5.2 ± 0.3 in the monimolimnion, to 6.4 ± 0.4 in the sediment. A similar pattern was observed from cDNA samples. In the chemocline, the Shannon-Wiener index was 2.9 ± 0.3, in the monimolimnion 3.7 ± 0.6, and in the sediment 6.2 ± 0.4. A similarly increasing gradient with depth was found for Chao1 and ACE (Fig. 4), although none of these diversity descriptors showed significant ($p < 0.05$) seasonal differences.

Abundance of bacterial and archaeal 16S rRNA genes

The abundance of bacterial 16S rRNA genes in the chemocline followed an increasing trend from winter to summer when it reached a maximum of $4.23 \pm 3.7 \times 10^8$ genes mL^{-1}, although it was slightly lower in autumn (Fig. 5). Generally, fewer bacteria were apparent in the monimolimnion, and $1.46 \times 10^7 \pm 2.88 \times 10^6$ genes mL^{-1} were detected in the winter and $1.01 \times 10^8 \pm 4.42 \times 10^6$

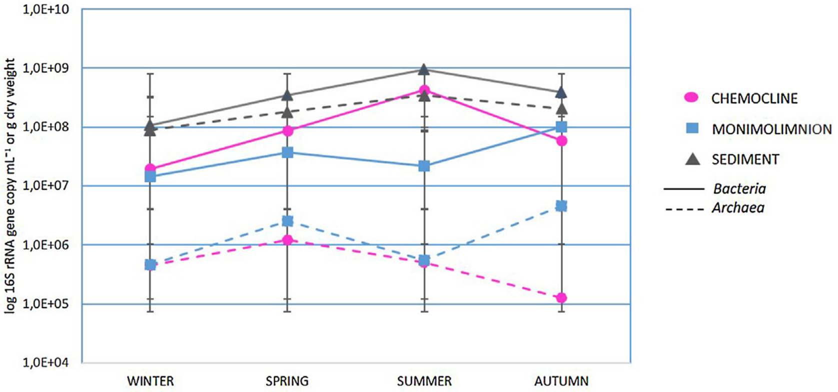

Fig. 5. Abundance of bacterial (solid line) and archaeal (dashed line) 16S rRNA genes in the chemocline, monimolimnion and surface sediment of Rogoznica Lake determined by qPCR. Vertical error bars represent the standard deviation.

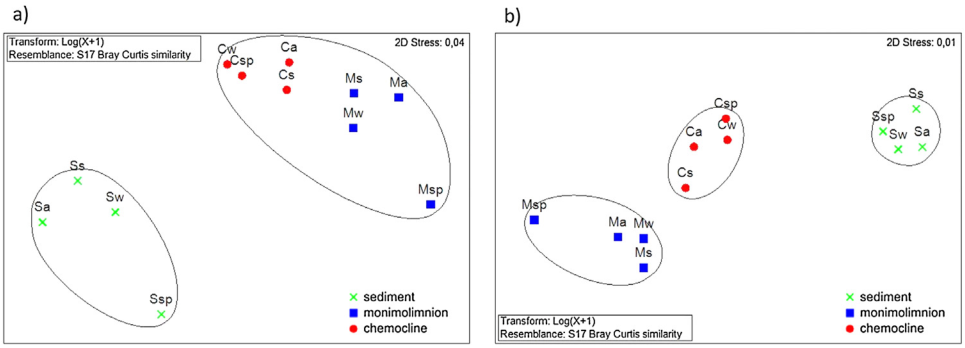

Fig. 6. nMDS plot for relative bacterial abundances (class level) from different sampling seasons and depths. Two distinct groups of samples are separated based on the DNA sequences (a) and three groups based on cDNA sequences (b). Results of the cluster analysis are based on a similarity level of 70%, and are superimposed on the MDS ordination. The capital letters C, M and S represent the chemocline, monimolimnion and sediment, respectively. The lower case letters stand for: w-winter; sp-spring; s-summer; a-autumn.

genes mL^{-1} in the autumn. Bacteria were significantly ($p < 0.05$) more abundant in the surface sediment samples, where a similar seasonal trend to the chemocline layer was seen, with maximum abundance in the summer ($9.48 \pm 4.3 \times 10^8$ genes g^{-1} dry weight).

The lowest abundance of archaeal 16S rRNA genes was observed in the chemocline that, in contrast to bacterial abundance, reached the maximum in spring ($1.21 \times 10^6 \pm 1.1 \times 10^5$ genes mL^{-1}). In the monimolimnion, a maximum of archaeal 16S rRNA genes was observed in the autumn ($4.64 \times 10^6 \pm 1.59 \times 10^5$ genes mL^{-1}). Sediment harbored significantly higher archaeal abundance ($p < 0.05$) that followed the same trend as for bacteria, increasing from winter to summer ($8.89 \times 10^7 \pm 2.51 \times 10^6$ genes g^{-1} dry weight) and decreasing in the autumn.

According to Kruskal–Wallis tests, there were no significant ($p > 0.05$) seasonal differences in the abundance of bacteria or archaea. However, on average, significant differences in archaeal abundance ($p < 0.05$) were observed between the chemocline, monimolimnion and surface sediment, whilst there were no significant ($p > 0.05$) seasonal differences between average bacterial and archaeal abundances.

Community structure in relation to the environmental variables

Non-metric multidimensional scaling (nMDS) was performed in order to compare the differences in bacterial community composition between the three regions of the lake. In nMDS and cluster analysis, relative abundance data relating to all bacterial classes from samples collected at two different water depths, and from sediment, were integrated for each sampling season. Analysis of spatio-temporal succession of the total (DNA) bacterial and archaeal community using nMDS and cluster analysis (similarity level 70%) clustered data into two groups and showed clearly separated water (chemocline and monimolimnion) and sediment samples (Fig. 6). nMDS analyses of the active (cDNA) community fraction, however, produced higher resolution and clustered data into three groups at each of the sampling depths, indicating a vertical change in the composition of the active community (Fig. 6).

Collinearity of environmental parameters was checked using Spearman's rank correlation, and selected for canonical correspondence analysis (CCA), in order to establish the relationships between the environmental factors and the main bacterial taxa based on DNA and cDNA sequences (Fig. 7). CCA analyses were plotted to show relative abundance data together with environmental data (including temperature, sulfide, particulate and dissolved organic carbon, phosphate, and ammonium). Using Monte Carlo permutation tests (499 permutations), significant relationships between environmental variables and canonical axes were analyzed using a Canoco program. Based on the 5% level in a partial Monte Carlo permutation test, the community structure was significantly linked ($p < 0.05$) to the physico-chemical characteristics of the water. Results of the CCA analysis of the total (DNA) community explained 65.8% variation in the first axis and 16.8% in the second axis. The most important factors influencing the community were H_2S ($F = 6.6$; $p = 0.004$), DOC ($F = 4.4$; $p = 0.026$), and PO_4^{3-} ($F = 3.2$; $p = 0.049$), while T ($F = 3.1$; $p = 0.062$), NH_4^+ ($F = 0.4$; $p = 0.756$) and POC ($F = 0.3$; $p = 0.794$) were not significant. CCA results of the active community explained 28.4% and 47.9% of the variation in the first and second axis, respectively. T ($F = 5.3$; $p = 0.044$) was the most important factor on the first

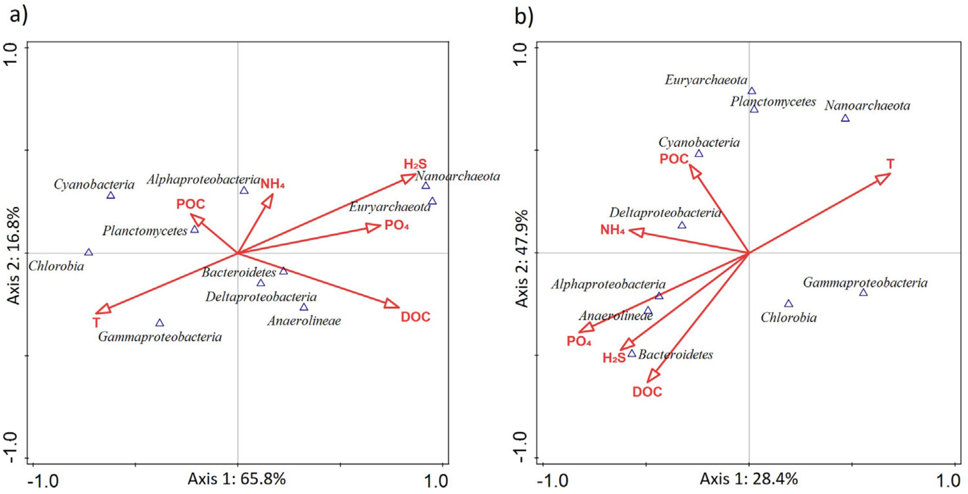

Fig. 7. Canonical correspondence analysis (CCA) ordination diagram of bacterial taxa related to the environmental variables of temperature (T), particulate (POC) and dissolved organic carbon (DOC), sulfide (H_2S), phosphate (PO_4^{3-}) and ammonium (NH_4^+) based on DNA (a) and cDNA (b) sequences. Eigenvalues (a): Axis 1: 0.2256, Axis 2: 0.0577, Axis 3: 0.0233, Axis 4: 0.0028. Eigenvalues (b): Axis 1: 0.2104, Axis 2: 0.1436, Axis 3: 0.0606, Axis 4: 0.0033. Environmental variables are presented as arrows.

axis, whereas positive loadings on the second axis were observed for DOC (F = 4.2.6; $p = 0.037$) and H_2S (F = 3.7; $p = 0.049$). NH_4^+ (F = 1.3; $p = 0.342$) and POC (F = 0.4; $p = 0.754$) were not found to be significant. Furthermore, some specific bacterial groups correlated closely with particular environmental variables in the CCA plot. *Gammaproteobacteria* were associated with temperature, whereas *Anaerolineae*, *Alphaproteobacteria* and *Bacteroidetes* were associated with dissolved organic carbon, sulfide and phosphates, indicating their role in organic matter degradation.

Discussion

In this study, the spatio-temporal distribution of prokaryotic communities occupying the hypoxic/anoxic (i.e. euxinic) niche of Rogoznica Lake was investigated. High-throughput 16S rRNA gene sequencing of DNA and cDNA, together with qPCR data and physico-chemical parameters, were used to describe the dynamics of the microbial community in the lake. Physico-chemical measurements revealed the water column was vertically stable throughout the year as a result of the thermohaline and chemical stratification, which is typical for meromictic lakes. Seasonal changes were less apparent in the chemocline (which was established at a depth of 8–9 m) and in the monimolimnion, which represented relatively stable and conserved regions. In accordance with the stability of the water column, long-term data gathered from the monimolimnion over the past two decades clearly showed increasing temperature, sulfide and ammonium concentrations, indicating that the temperature had increased throughout the entire water column of the lake. However, the warming rate of the anoxic layer was lower compared to the surface layer, although it was greater than in the monimolimnion of stratified Lake Kivu [53]. It has been shown that even such a small increase in temperature can greatly influence the microbial food web in the adjacent Adriatic Sea [79]. Two periods could be characterized by sulfide and ammonium concentrations, which were less variable during the first decade and more variable in the second decade (i.e. more extreme values were observed), as the main indicators of climate change.

In 2015, the highest POC concentrations were observed in the chemocline, indicating pronounced accumulation and production of organic carbon in this layer. On the other hand, phosphates, ammonium and DOC concentrations reached maximum values in the monimolimnion, suggesting mineralization was an important process under anoxic conditions. This was further confirmed by CCA analysis that identified sulfide, together with DOC, phosphates and temperature, as the most significant factors influencing the abundance of dominant taxa, which was similarly observed in the anoxic zones of other meromictic lakes [4]. Interestingly, although seasonal fluctuations are typically recorded in bacterioplankton communities of eutrophic lakes and coastal waters [25,29,77], the prokaryotic community structure and abundance of Rogoznica Lake in this current study were found to be relatively stable year-round within the layers studied, based on both DNA and RNA gene pools. Neither diversity nor the abundance of these communities varied significantly ($p > 0.05$) over the sampling seasons. As demonstrated by nMDS clustering, a clear vertical zonation was observed in the community composition, which was consistent with physico-chemical gradients. In the investigated period, and based on primers used for HTS, phototrophic *Gammaproteobacteria* and *Cyanobacteria* were predominant in the chemocline, and sulfate-reducing *Deltaproteobacteria* in the monimolimnion, whereas a significantly higher proportion of *Archaea* was found in the surface sediment.

qPCR analyses confirmed that prokaryotic abundance did not vary significantly ($p > 0.05$) over the sampling seasons, neither in the water layers nor the surface sediment, reflecting the prevailing stable environmental conditions. Furthermore, qPCR revealed that *Bacteria* were numerically more important than *Archaea* in all the samples. Archaeal abundance increased with depth, and was significantly higher in the sediment than in the water layers. Enrichment of *Archaea* in deeper layers confirmed earlier findings from marine systems and other meromictic lakes, supporting the idea that such sediments are low-energy environments [12,55,87,90].

DNA and cDNA sequencing similarly revealed the dominance of *Bacteria* over *Archaea*. Based on OTUs, Shannon-Wiener indices, and all other diversity descriptors, diversity increased with depth but was seasonally stable. This trend was consistent with findings from similar environments, although the diversity in Rogoznica Lake was higher, especially in the surface sediment [3,4,14,31,54,59]. To the best of our knowledge, this is one of the highest levels of microbial diversity recorded in the literature. High prokaryotic diversity in Rogoznica Lake could be supported by its small volume and by stable environmental conditions (i.e. stable stratification), as well as high nutrients and DOC concentrations, thus promoting a variety of mineralization processes requiring complex community interactions [4,56].

In addition to the increase of diversity, dominance of the individual communities was depth-specific. Based on the results in this study, it is proposed that carbon fixation was conducted by phototrophic *Gammaproteobacteria* (i.e. *Chromatiaceae* and *Synechococcus*-related cyanobacteria) in the chemocline, which corresponded to the highest POC concentrations observed in this layer. Phototrophs comprised >80% of the active chemocline community. *Chromatiaceae* dominated the community during most of the sampling periods, except during the winter season, when *Synechococcus* became prevalent even in the monimolimnion. Although *Synechococcus* strains are ubiquitous in marine environments, including microbial mats [75] and anoxic estuarine sediments [26], their presence below the oxycline in meromictic lakes is not usual [11]. Two *Synechococcus* strains have been isolated recently from a dark euxinic water layer of the Black Sea. A metagenomic study has shown that under these extreme conditions isolated strains possessed heterotrophic ability and were potentially capable of fermenting polyglucose to lactate and use various nitrogen source, all of them converging to ammonia [10]. This might also be one of the reasons for the high ammonium concentrations recorded in the monimolimnion of Rogoznica Lake. The large supply of H_2S fuels *Gammaproteobacteria* (*Chromatiaceae*), typical anoxygenic, phototrophic sulfide-oxidizers that have been observed near the oxic–anoxic interfaces of other stratified systems [8,30,39,57,62]. They were also found to be active in the monimolimnion, especially during the summer season, where they accounted for 54% of retrieved sequences. This may be due to increased insolation supporting their growth in deeper layers, and, indeed, also confirms their obligatory photosynthetic nature. Capacity for oxidation of reduced sulfur compounds has also been linked to *Alphaproteobacteria* [92], which were represented mainly by *Rhodobacterales* and *Rhodospirillales* (purple non-sulfur bacteria), but comprised a minor fraction (generally around 1%) of the active community within the studied water layers. Although green sulfur bacteria (GSB, i.e. *Chlorobia*) were previously shown to be the most abundant anoxygenic phototrophs in the lake during the stratified periods of 2011 and 2012 [69], in the present study, *Chlorobia* represented only a minor fraction (up to 2.6% in the chemocline during the autumn season) from both DNA and RNA pools. One of the reasons for such a discrepancy could be the primer set used in this study that can overestimate *Gammaproteobacteria* [67]. Furthermore, in the current study, the upper hypoxic part of the chemocline was sampled, whereas the other study focused on the lower, anoxic part. Taking into account the changes of many environmental conditions over a short vertical distance in this layer, changes in the microbial community structure can also be expected. Despite the different sampling and methodological approaches, we believe that anoxygenic phototrophs in Rogoznica Lake exhibit temporal shifts, as recorded similarly in permanently stratified Lake Cadagno [88]. Observed shifts in Rogoznica Lake are likely to be related to the POC concentration, which was consistently higher (up to six times) in the mixolimnion in 2011/2012 than in 2015. This must have increased light scattering and attenuation in this layer and reduced light intensity in the chemocline and monimolimnion. Subsequently, low light availability in 2011/2012 enabled development of GSB that can grow at lower light intensity than PSB [7]. On the other hand, PSB (*Chromatiaceae*) were also found dominant in the chemocline of the lake in 1997 [17] when the POC concentrations were quite similar to the ones recorded in this study. Thus, it seems that the productivity of the oxygenated mixolimnion plays an important role in shaping the anoxygenic phototrophic populations in the chemocline and monimolimnion of Rogoznica Lake [12].

Most of the lake's sulfide production occurred in the monimolimnion and surface sediment where the highest H_2S concentrations were recorded. Although high, these concentrations did not exceed the threshold concentration thought to inhibit sulfate reduction (~16 mM) [72]. The potential for dissimilatory sulfate reduction is presumed to originate from *Deltaproteobacteria*, the majority of which belonged to the *Desulfatiglans* lineage and a few unclassified *Desulfobacteraceae*, which comprise all of the known SRB that oxidize acetate completely during sulfate reduction (although members of this group may carry out both reductive and oxidative parts of the sulfur cycle) [62,80]. Active *Deltaproteobacteria* members were detected in the chemocline layer, but were more prominent in the monimolimnion and the surface sediment, where they are known to account for >50% of organic carbon mineralization [49]. *Desulfatiglans*-related populations are typical and abundant in marine sediments [1,85] where they occur with increasing sulfate and organic matter concentration. Their physiology is poorly understood, however, isolated strains are sulfate-reducers that can grow on aromatic hydrocarbons, which can explain their high abundance in sediments [48]. Taking into account high H_2S concentrations and their sharp gradient between the monimolimnion and chemocline recorded in this study and previously [16], sulfur disproportionation would be energetically unfavorable compared to sulfate reduction [35,86]. Therefore, and as already confirmed by multiple sulfur isotope fractionations, disproportionation does not appear to be a dominant process within the sulfur cycle in Rogoznica Lake [51]. High concentrations of organic matter can also be degraded by a dense population of *Bacteroidetes*, which were most abundant in the monimolimnion of the lake in both DNA and RNA gene pools over the sampling seasons. Members of this phylum are commonly found in the anoxic zones of meromictic lakes [4,39], and also in marine environments where they can comprise up to 30% of total bacterial counts [2]. It has been shown that marine *Bacteroidetes* possess abundant glycosyl transferases and a large number of polymer degrading enzymes, confirming their role in the degradation of particulate organic matter, especially proteins [34]. Members of *Anaerolineaceae* were identified as potential fermentative bacteria in the monimolimnion of the lake, and have been found to decompose carbohydrates by fermentation [93]. Although they were lowly represented in all DNA samples, RNA analysis suggested that they comprised a considerable fraction of the active community in the monimolimnion and surface sediment, especially in the winter.

In contrast to the investigated water layers, surface sediment harbored a considerable fraction of *Planctomycetes* (mostly assigned as the AKAU3564 sediment group) and *Chloroflexi* (*Dehalococcoidia*-related sequences). This heterotrophic *Planctomycetes* group was first described in methane hydrate-bearing deep marine sediments [45] and a recent study showed their strong association to *Deltaproteobacteria* as part of a methane-oxidizing consortia [89]. *Dehalococcoidia* species are widespread in marine sediments [52,91] and anoxic zones of meromictic lakes [6] where they are usually linked to reductive dehalogenation, but their metabolic versatility also indicates an important role in the chlorine and carbon cycles within uncontaminated environments [91]. In contrast to *Planctomycetes* that were less represented in the DNA gene pool, *Dehalococcoidia*-related sequences were frequently recovered from DNA samples (up to 30%), but they comprised only a minor (up to 4%) fraction of the active community. However, the exact role of these communities in Rogoznica Lake should be investigated further.

Archaeal abundance was significantly higher in the sediment and the majority of archaeal sequences were affiliated to *Nanoarchaeota*. Members of this phylum have been found in various extreme environments [15,40,42,61], but to the best of our knowledge this is its first record in a meromictic lake. These organisms are obligate symbionts/parasites found in association with chemolithotrophic crenarchaeon *Ignicoccus hospitalis*, which reduces sulfur [43,66]. Since neither *I. hospitalis* nor other *Crenarchaeota* have been detected in this study, it is possible that the *Nanoarchaeota* in Rogoznica Lake are associated with some other sulfur-respiring *Euryarchaea* or unclassified *Archaea*, as noticed similarly in Yellowstone National Park hot springs [10], however, its ecology in the lake should be elucidated in detail. Archaeal sequences that were often retrieved, but only in the sediment samples, affiliated with *Asgardaeota* (i.e. recently described phylum *Lokiarchaeota*) found in hydrothermal vents [82]. Its genome harbors a complete tetrahydromethanopterin (H_4MPT)-dependent Wood–Ljungdahl pathway and enzymes, revealing it to be a hydrogen-dependent, strictly anaerobic and very probably autotrophic archaeon [81].

Interestingly, few very specific bacterial populations, comprising only a minor fraction within the active bacterial community, were characteristic for the specific investigated layer of the Lake. These bacteria included *Latescibacteria* (WS3), *Omnitrophicaeota* and *Spirochaetia*, which were found to be enriched in the surface sediment, as well as *Cloacimonetes* and *Kiritimatiellae* found more abundant in the monimolimnion. These bacteria were detected in different anoxic environments including meromictic systems. However, their ecological roles within these environments are not entirely clear since many species lack cultured representatives [33,39,63,83]. Although lowly represented, their ecological contribution to the functioning of the investigated zones in Rogoznica Lake cannot be neglected since rare taxa are often highly active [41].

Overall, high-throughput 16S rRNA gene sequencing revealed a clear differentiation of complex microbial communities between the chemocline, monimolimnion and surface sediment of Rogoznica Lake. The extreme euxinic chemistry highly influenced their distribution. This was characterized by photosynthetic bacteria in the chemocline, anaerobic *Deltaproteobacteria* and *Bacteroidetes* in the monimolimnion, and archaeal populations characteristic for the sediment. Nevertheless, in order to make quantitative estimates of the exact microbial role in a specific metabolic pathway and to learn more about their interdependencies, metagenomic analyses, accompanied by incubation experiments, are needed and planned for future study. This would be especially important for abundant and active *Synechococcus*-related species below the chemocline, as well as for *Nanoarchaeota*, in order to predict their exact niches and physiological features under these extreme conditions.

Most importantly, the results of this study clearly emphasized the need for further, comprehensive and long-term investigations on the interaction between different physico-chemical and biological components of the Rogoznica Lake ecosystem in order to fully understand its functioning, behavior and population dynamics. Rogoznica Lake can serve as a good model for both hypoxia-anoxia appearance and an environment threatened by climate changes. Observed long-term changes (warming, sulfide and ammonium accumulations) are typical indicators of global change that may adversely affect the physical stability of the water column and, subsequently, nutrient cycling and ecosystem productivity.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.syapm.2019.126016.

References

[1] Ahn, Y.B., Chae, J.C., Zylstra, G.J., Haggblom, M.M. (2009) Degradation of phenol via phenylphosphate and carboxylation to 4-hydroxybenzoate by a newly isolated strain of the sulfate-reducing bacterium *Desulfobacterium anilini*. Appl. Environ. Microbiol. 75, 4248–4253.

[2] Alonso-Saez, L., Gasol, J.M. (2007) Seasonal variations in the contributions of different bacterial groups to the uptake of low-molecular-weight compounds in northwestern mediterranean coastal waters. Appl. Environ. Microbiol. 73, 3528–3535.

[3] Andrei, A.S., Robeson, M.S., Baricz, A., Coman, C., Muntean, V., Ionescu, A., Etiope, G., Alexe, M., Sicora, C.I., Podar, M., Banciu, H.L. (2015) Contrasting taxonomic stratification of microbial communities in two hypersaline meromictic lakes. ISME J. 9, 2642–2656.

[4] Baatar, B., Chiang, P.W., Rogozin, D.Y., Wu, Y.T., Tseng, C.H., Yang, C.Y., Chiu, H.H., Oyuntsetseg, B., Degermendzhy, A.G., Tang, S.L. (2016) Bacterial communities of three saline meromictic lakes in Central Asia. PLoS One, 11.

[5] Biderre-Petit, C., Jezequel, D., Dugat-Bony, E., Lopes, F., Kuever, J., Borrel, G., Viollier, E., Fonty, G., Peyret, P. (2011) Identification of microbial communities involved in the methane cycle of a freshwater meromictic lake. FEMS Microbiol. Ecol. 77, 533–545.

[6] Biderre-Petit, C., Dugat-Bony, E., Mege, M., Parisot, N., Adrian, L., Mone, A., Denonfoux, J., Peyretaillade, E., Debroas, D., Boucher, D., Peyret, P. (2016) Distribution of *Dehalococcoidia* in the anaerobic deep water of a remote Meromictic Crater Lake and detection of *Dehalococcoidia*-derived reductive dehalogenase homologous genes. PLoS One, 11.

[7] Brocks, J.J., Love, G.D., Summons, R.E., Knoll, A.H., Logan, G.A., Bowden, S.A. (2005) Biomarker evidence for green and purple sulphur bacteria in a stratified Palaeoproterozoic sea. Nature 437, 866–870.

[8] Buhring, S.I., Sievert, S.M., Jonkers, H.M., Ertefai, T., Elshahed, M.S., Krumholz, L.R., Hinrichs, K.U. (2011) Insights into chemotaxonomic composition and carbon cycling of phototrophic communities in an artesian sulfur-rich spring (Zodletone, Oklahoma, USA), a possible analog for ancient microbial mat systems. Geobiology 9, 166–179.

[9] Bura-Nakić, E., Helz, G.R., Ciglenečki, I., Ćosović, B. (2009) Reduced sulfur species in a stratified seawater lake (Rogoznica Lake, Croatia); seasonal variations and argument for organic carriers of reactive sulfur. Geochim. Cosmochim. Acta 73, 3738–3751.

[10] Callieri, C., Slabakova, V., Dzhembekova, N., Slabakova, N., Peneva, E., Cabello-Yeves, P.J., Di Cesare, A., Eckert, E.M., Bertoni, R., Corno, G., Salcher, M.M., Kamburska, L., Bertoni, F., Moncheva, S. (2019) The mesopelagic anoxic Black Sea as an unexpected habitat for *Synechococcus* challenges our understanding of global “deep red fluorescence”. ISME J. 13, 1676–1687.

[11] Camacho, A., Picazo, A., Miracle, M.R., Vicente, E. (2003) Spatial distribution and temporal dynamics of picocyanobacteria in a meromictic karstic lake. Algol. Stud. 109, 171–184.

[12] Čanković, M., Petrić, I., Marguš, M., Ciglenečki, I. (2017) Spatio-temporal dynamics of sulfate-reducing bacteria in extreme environment of Rogoznica Lake revealed by 16 S rRNA analysis. J. Mar. Syst. 172, 14–23.

[13] Caporaso, J.G., Lauber, C.L., Walters, W.A., Berg-Lyons, D., Lozupone, C.A., Turnbaugh, P.J., Fierer, N., Knight, R. (2011) Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. Proc. Natl. Acad. Sci. U. S. A. 108, 4516–4522.

[14] Casamayor, E.O., Triado-Margarit, X., Castaneda, C. (2013) Microbial biodiversity in saline shallow lakes of the Monegros Desert, Spain. FEMS Microbiol. Ecol. 85, 503–518.

[15] Casanueva, A., Galada, N., Baker, G.C., Grant, W.D., Heaphy, S., Jones, B., Ma, Y.H., Ventosa, A., Blamey, J., Cowan, D.A. (2008) Nanoarchaeal 16S rRNA gene sequences are widely dispersed in hyperthermophilic and mesophilic halophilic environments. Extremophiles 12, 651–656.

[16] Ciglenečki, I., Ćosović, B. (1997) Electrochemical determination of thiosulfate in seawater in the presence of elemental sulfur and sulfide. Electroanalysis 9, 775–780.

[17] Ciglenečki, I., Kodba, Z., Viličić, D., Ćosović, B. (1998) Seasonal variation of anoxic conditions in the Rogoznica Lake. Croat. Chem. Acta 71, 217–232.

[18] Ciglenečki, I., Carić, M., Kršinić, F., Viličić, D., Ćosović, B. (2005) The extinction by sulfide-turnover and recovery of a naturally eutrophic, meromictic seawater lake. J. Mar. Syst. 56, 29–44.

[19] Ciglenečki, I., Pichler, S., Prohić, E., Ćosović, B. (2006) Distribution of redox-sensitive elements in bottom waters, porewaters and sediments of Rogoznica Lake (Croatia) in both oxic and anoxic conditions. Water Air Soil Pollut. 6, 534–545.

[20] Ciglenečki, I., Janeković, I., Marguš, M., Bura-Nakić, E., Carić, M., Ljubešić, Z., Batistić, M., Hrustić, E., Dupčić, I., Garić, R. (2015) Impacts of extreme weather events on highly eutrophic marine ecosystem (Rogoznica. Lake, Adriatic coast). Cont. Shelf Res. 108, 144–155.

[21] Clarke, K.R., Gorley, R.N. 2006 PRIMER V6: User Manual-Tutorial, Plymouth Marine Laboratory.

[22] Clarke, K.R., Warwick, R.M. 1994 An approach to statistical analysis and interpretation. In: Change in Marine Communities, 2.

[23] Comeau, A.M., Harding, T., Galand, P.E., Vincent, W.F., Lovejoy, C. (2012) Vertical distribution of microbial communities in a perennially stratified Arctic lake with saline, anoxic bottom waters. Sci. Rep., 2.

[24] Ćosović, B., Ciglenečki, I., Viličić, D., Ahel, M. (2000) Distribution and seasonal variability of organic matter in a small eutrophicated salt lake. Estuar. Coast. Shelf Sci. 51, 705–715.

[25] Crump, B.C., Hobbie, J.E. (2005) Synchrony and seasonality in bacterioplankton communities of two temperate rivers. Limnol. Oceanogr. 50, 1718–1729.

[26] Crump, B.C., Peranteau, C., Beckingham, B., Cornwell, J.C. (2007) Respiratory succession and community succession of bacterioplankton in seasonally anoxic estuarine waters. Appl. Environ. Microbiol. 73, 6802–6810.

[27] Daffonchio, D., Borin, S., Brusa, T., Brusetti, L., van der Wielen, P.W.J.J., Bolhuis, H., Yakimov, M.M., D'Auria, G., Giuliano, L., Marty, D., Tamburini, C., McGenity, T.J., Hallsworth, J.E., Sass, A.M., Timmis, K.N., Tselepides, A., de Lange, G.J., Hubner, A., Thomson, J., Varnavas, S.P., Gasparoni, F., Gerber, H.W., Malinverno, E., Corselli, C., Garcin, J., McKew, B., Golyshin, P.N., Lampadariou, N., Polymenakou, P., Calore, D., Cenedese, S., Zanon, F., Hoog, S., Party, Biodeep Sci, (2006) Stratified prokaryote network in the oxic-anoxic transition of a deep-sea halocline. Nature 440, 203–207.

[28] Degermendzhy, A.G., Zadereev, E.S., Rogozin, D.Y., Prokopkin, I.G., Barkhatov, Y.V., Tolomeev, A.P., Khromechek, E.B., Janse, J.H., Mooij, W.M., Gulati, R.D. (2010) Vertical stratification of physical, chemical and biological components in two saline lakes Shira and Shunet (South Siberia, Russia). Aquat. Ecol. 44, 619–632.

[29] Diao, M., Sinnige, R., Kalbitz, K., Huisman, J., Muyzer, G. (2017) Succession of bacterial communities in a seasonally stratified lake with an anoxic and sulfidic hypolimnion. Front. Microbiol., 8.

[30] Diao, M.H., Huisman, J., Muyzer, G. (2018) Spatio-temporal dynamics of sulfur bacteria during oxic-anoxic regime shifts in a seasonally stratified lake. FEMS Microbiol. Ecol., 94.

[31] Dirnitriu, P.A., Pinkart, H.C., Peyton, B.M., Mormile, M.R. (2008) Spatial and temporal patterns in the microbial diversity of a meromictic soda lake in Washington State. Appl. Environ. Microbiol. 74, 4877–4888.

[32] Edlund, A., Hardeman, F., Jansson, J.K., Sjoling, S. (2008) Active bacterial community structure along vertical redox gradients in Baltic Sea sediment. Environ. Microbiol. 10, 2051–2063.

[33] Farag, I.F., Youssef, N.H., Elshahed, M.S. (2017) Global distribution patterns and pangenomic diversity of the candidate phylum "*Latescibacteria*" (WS3). Appl. Environ. Microbiol., 83.

[34] Fernandez-Gomez, B., Richter, M., Schuler, M., Pinhassi, J., Acinas, S.G., Gonzalez, J.M., Pedros-Alio, C. (2013) Ecology of marine *Bacteroidetes*: a comparative genomics approach. ISME J. 7, 1026–1037.

[35] Finster, K., Liesack, W., Thamdrup, B. (1998) Elemental sulfur and thiosulfate disproportionation by *Desulfocapsa sulfoexigens* sp. nov., a new anaerobic bacterium isolated from marine surface sediment. Appl. Environ. Microbiol. 64, 119–125.

[36] Garcia, S.L., Salka, I., Grossart, H.P., Warnecke, F. (2013) Depth-discrete profiles of bacterial communities reveal pronounced spatio-temporal dynamics related to lake stratification. Environ. Microbiol. Rep. 5, 549–555.

[37] Griffiths, R.I., Whiteley, A.S., O'Donnell, A.G., Bailey, M.J. (2000) Rapid method for coextraction of DNA and RNA from natural environments for analysis of ribosomal DNA- and rRNA-based microbial community composition. Appl. Environ. Microbiol. 66, 5488–5491.

[38] Gugliandolo, C., Lentini, V., Maugeri, T.L. (2011) Distribution and diversity of bacteria in a saline meromictic lake as determined by PCR-DGGE of 16S rRNA gene fragments. Curr. Microbiol. 62, 159–166.

[39] Hamilton, T.L., Bovee, R.J., Sattin, S.R., Mohr, W., Gilhooly, W.P., Lyons, T.W., Pearson, A., Macalady, J.L. (2016) Carbon and sulfur cycling below the chemocline in a meromictic lake and the identification of a novel taxonomic lineage in the FCB superphylum, Candidatus *Aegiribacteria*. Front. Microbiol., 7.

[40] Han, R., Zhang, X., Liu, J., Long, Q.F., Chen, L.S., Liu, D.L., Zhu, D.R. (2017) Microbial community structure and diversity within hypersaline Keke Salt Lake environments. Can. J. Microbiol. 63, 895–908.

[41] Hausmann, B., Knorr, K.H., Schreck, K., Tringe, S.G., del Rio, T.G., Loy, A., Pester, M. (2016) Consortia of low-abundance bacteria drive sulfate reduction-dependent degradation of fermentation products in peat soil microcosms. ISME J. 10, 2365–2375.

[42] Hohn, M.J., Hedlund, B.P., Huber, H. (2002) Detection of 16S rDNA sequences representing the novel phylum "Nanoarchaeota": indication for a wide distribution in high temperature biotopes. Syst. Appl. Microbiol. 25, 551–554.

[43] Huber, H., Hohn, M.J., Rachel, R., Fuchs, T., Wimmer, V.C., Stetter, K.O. (2002) A new phylum of *Archaea* represented by a nanosized hyperthermophilic symbiont. Nature 417, 63–67.

[44] Humayoun, S.B., Bano, N., Hollibaugh, J.T. (2003) Depth distribution of microbial diversity in Mono Lake, a meromictic soda lake in California. Appl. Environ. Microbiol. 69, 1030–1042.

[45] Inagaki, F., Nunoura, T., Nakagawa, S., Teske, A., Lever, M., Lauer, A., Suzuki, M., Takai, K., Delwiche, M., Colwell, F.S., Nealson, K.H., Horikoshi, K., D'Hondt, S., Jorgensen, B.B. (2006) Biogeographical distribution and diversity of microbes in methane hydrate-bearing deep marine sediments, on the Pacific Ocean Margin. Proc. Natl. Acad. Sci. U. S. A. 103, 2815–2820.

[46] Inceoglu, O., Lliros, M., Crowe, S.A., Garcia-Armisen, T., Morana, C., Darchambeau, F., Borges, A.V., Descy, J.P., Servais, P. (2015) Vertical distribution of functional potential and active microbial communities in meromictic Lake Kivu. Microb. Ecol. 70, 596–611.

[47] Ivančić, I., Degobbis, D. (1984) An optimal manual procedure for ammonia analysis in natural-waters by the Indophenol Blue Method. Water Res. 18, 1143–1147.

[48] Jochum, L.M., Schreiber, L., Marshall, I.P.G., Jorgensen, B.B., Schramm, A., Kjeldsen, K.U. (2018) Single-cell genomics reveals a diverse metabolic potential of uncultivated desulfatiglans-related deltaproteobacteria widely distributed in marine sediment. Front. Microbiol., 9.

[49] Jorgensen, B.B. (1990) The sulfur cycle of fresh-water sediments - role of thiosulfate. Limnol. Oceanogr. 35, 1329–1342.

[50] Juusola, J., Ballantyne, J. (2003) Messenger RNA profiling: a prototype method to supplant conventional methods for body fluid identification. Forensic Sci. Int. 135, 85–96.

[51] Kamyshny, A., Zerkle, A.L., Mansaray, Z.F., Ciglenečki, I., Bura-Nakić, E., Farquhar, J., Ferdelman, T.G. (2011) Biogeochemical sulfur cycling in the water column of a shallow stratified sea-water lake: speciation and quadruple sulfur isotope composition. Mar. Chem. 127, 144–154.

[52] Kaster, A.K., Mayer-Blackwell, K., Pasarelli, B., Spormann, A.M. (2014) Single cell genomic study of *Dehalococcoidetes* species from deep-sea sediments of the Peruvian Margin. ISME J. 8, 1831–1842.

[53] Katsev, S., Aaberg, A.A., Crowe, S.A., Hecky, R.E. (2014) Recent warming of Lake Kivu. PLoS One, 9.

[54] Klepac-Ceraj, V., Hayes, C.A., Gilhooly, W.P., Lyons, T.W., Kolter, R., Pearson, A. (2012) Microbial diversity under extreme euxinia: Mahoney Lake, Canada. Geobiology 10, 223–235.

[55] Kubo, K., Kojima, H., Fukui, M. (2014) Vertical distribution of major sulfate-reducing bacteria in a shallow eutrophic meromictic lake. Syst. Appl. Microbiol. 37, 510–519.

[56] Lauro, F.M., DeMaere, M.Z., Yau, S., Brown, M.V., Ng, C., Wilkins, D., Raftery, M.J., Gibson, J.A.E., Andrews-Pfannkoch, C., Lewis, M., Hoffman, J.M., Thomas, T., Cavicchioli, R. (2011) An integrative study of a meromictic lake ecosystem in Antarctica. ISME J. 5, 879–895.

[57] Lay, C.Y., Mykytczuk, N.C.S., Yergeau, E., Lamarche-Gagnon, G., Greer, C.W., Whyte, L.G. (2013) Defining the functional potential and active community members of a sediment microbial community in a high-arctic hypersaline subzero spring. Appl. Environ. Microbiol. 79, 3637–3648.

[58] Lehours, A.C., Bardot, C., Thenot, A., Debroas, D., Fonty, G. (2005) Anaerobic microbial communities in Lake Pavin, a unique meromictic lake in France. Appl. Environ. Microbiol. 71, 7389–7400.

[59] Lehours, A.C., Evans, P., Bardot, C., Joblin, K., Gerard, F. (2007) Phylogenetic diversity of *Archaea* and bacteria in the anoxic zone of a meromictic lake (Lake Pavin, France). Appl. Environ. Microbiol. 73, 2016–2019.

[60] Lepš, J., Šmilauer, P. 2003 Multivariate Analysis of Ecological Data Using CANOCO, Cambridge University Press.

[61] Lipsewers, Y.A., Hopmans, E.C., Damste, J.S.S., Villanueva, L. (2018) Potential recycling of thaumarchaeotal lipids by DPANN *Archaea* in seasonally hypoxic surface marine sediments. Org. Geochem. 119, 101–109.

[62] Llorens-Mares, T., Yooseph, S., Goll, J., Hoffman, J., Vila-Costa, M., Borrego, C.M., Dupont, C.L., Casamayor, E.O. (2015) Connecting biodiversity and potential functional role in modern euxinic environments by microbial metagenomics. ISME J. 9, 1648–1661.

[63] Lloyd, K.G., Steen, A.D., Ladau, J., Yin, J.Q., Crosby, L. (2018) Phylogenetically novel uncultured microbial cells dominate earth microbiomes. Msystems, 3.

[64] McKeown, R.M., Scully, C., Enright, A.M., Chinalia, F.A., Lee, C., Mahony, T., Collins, G., O'Flaherty, V. (2009) Psychrophilic methanogenic community development during long-term cultivation of anaerobic granular biofilms. ISME J. 3, 1231–1242.

[65] Meyer, K.M., Kump, L.R. (2008) Oceanic euxinia in Earth history: causes and consequences. Annu. Rev. Earth Planet. Sci. 36, 251–288.

[66] Paper, W., Jahn, U., Hohn, M.J., Kronner, M., Nather, D.J., Burghardt, T., Rachel, R., Stetter, K.O., Huber, H. (2007) *Ignicoccus hospitalis* sp. nov., the host of '*Nanoarchaeum equitans*'. Int. J. Syst. Evol. Microbiol. 57, 803–808.

[67] Parada, A.E., Needham, D.M., Fuhrman, J.A. (2016) Every base matters: assessing small subunit rRNA primers for marine microbiomes with mock communities, time series and global field samples. Environ. Microbiol. 18, 1403–1414.

[68] Peura, S., Eiler, A., Bertilsson, S., Nykanen, H., Tiirola, M., Jones, R.I. (2012) Distinct and diverse anaerobic bacterial communities in boreal lakes dominated by candidate division OD1. ISME J. 6, 1640–1652.

[69] Pjevac, P., Korlević, M., Berg, J.S., Bura-Nakić, E., Ciglenečki, I., Amann, R., Orlić, S. (2015) Community shift from phototrophic to chemotrophic sulfide oxidation following anoxic holomixis in a stratified seawater lake. Appl. Environ. Microbiol. 81, 298–308.

[70] Pjevac, P., Dyksma, S., Goldhammer, T., Mujakić, I., Koblizek, M., Mussmann, M., Amann, R., Orlić, S. (2019) In situ abundance and carbon fixation activity of distinct anoxygenic phototrophs in the stratified seawater lake Rogoznica. Environ. Microbiol., http://dx.doi.org/10.1111/1462-2920.14739.

[71] Rastelli, E., Corinaldesi, C., Petani, B., Dell'Anno, A., Ciglenečki, I., Danovaro, R. (2016) Enhanced viral activity and dark CO2 fixation rates under oxygen depletion: the case study of the marine Lake Rogoznica. Environ. Microbiol. 18, 4511–4522.

[72] Reis, M.A.M., Almeida, J.S., Lemos, P.C., Carrondo, M.J.T. (1992) Effect of hydrogen-sulfide on growth of sulfate reducing bacteria. Biotechnol. Bioeng. 40, 593–600.

[73] Rogozin, D.Y., Trusova, M.Y., Khromechek, E.B., Degermendzhy, A.G. (2010) Microbial community of the chemocline of the meromictic Lake Shunet (Khakassia, Russia) during summer stratification. Microbiology 79, 253–261.

[74] Romero, J.R., Melack, J.M. (1996) Sensitivity of vertical mixing in a large saline lake to variations in runoff. Limnol. Oceanogr. 41, 955–965.

[75] Ruffroberts, A.L., Kuenen, J.G., Ward, D.M. (1994) Distribution of cultivated and uncultivated *Cyanobacteria* and *Chloroflexus*-like bacteria in hot-spring microbial mats. Appl. Environ. Microbiol. 60, 697–704.

[76] Schloss, P.D., Westcott, S.L., Ryabin, T., Hall, J.R., Hartmann, M., Hollister, E.B., Lesniewski, R.A., Oakley, B.B., Parks, D.H., Robinson, C.J., Sahl, J.W., Stres, B., Thallinger, G.G., Van Horn, D.J., Weber, C.F. (2009) Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl. Environ. Microbiol. 75, 7537–7541.

[77] Shade, A., Kent, A.D., Jones, S.E., Newton, R.J., Triplett, E.W., McMahon, K.D. (2007) Interannual dynamics and phenology of bacterial communities in a Eutrophic lake. Limnol. Oceanogr. 52, 487–494.

[78] Simon, C., Daniel, R. (2011) Metagenomic analyses: past and future trends. Appl. Environ. Microbiol. 77, 1153–1161.

[79] Šolić, M., Grbec, B., Matić, F., Šantić, D., Šestanović, S., Gladan, Z.N., Bojanić, N., Ordulj, M., Jozić, S., Vrdoljak, A. (2018) Spatio-temporal reproducibility of the microbial food web structure associated with the change in temperature: long-term observations in the Adriatic Sea. Prog. Oceanogr. 161, 87–101.

[80] Sorokin, D.Y., Berben, T., Melton, E.D., Overmars, L., Vavourakis, C.D., Muyzer, G. (2014) Microbial diversity and biogeochemical cycling in soda lakes. Extremophiles 18, 791–809.

[81] Sousa, F.L., Neukirchen, S., Allen, J.F., Lane, N., Martin, W.F. (2016) *Lokiarchaeon* is hydrogen dependent. Nat. Microbiol., 1.

[82] Spang, A., Saw, J.H., Jorgensen, S.L., Zaremba-Niedzwiedzka, K., Martijn, J., Lind, A.E., van Eijk, R., Schleper, C., Guy, L., Ettema, T.J.G. (2015) Complex *Archaea* that bridge the gap between prokaryotes and eukaryotes. Nature 521, 173.

[83] Spring, S., Bunk, B., Sproer, C., Schumann, P., Rohde, M., Tindall, B.J., Klenk, H.P. (2016) Characterization of the first cultured representative of *Verrucomicrobia* subdivision 5 indicates the proposal of a novel phylum. ISME J. 10, 2801–2816.

[84] Strickland, J.D.H., Parsons, T.R. 1972 A Practical Handbook of Seawater Analysis.

[85] Suzuki, D., Li, Z.L., Cui, X.X., Zhang, C.F., Katayama, A. (2014) Reclassification of *Desulfobacterium anilini* as *Desulfatiglans anilini* comb. nov. within *Desulfatiglans* gen. nov., and description of a 4-chlorophenol-degrading sulfate-reducing bacterium, *Desulfatiglans parachlorophenolica* sp. nov. Int. J. Syst. Evol. Microbiol. 64, 3081–3086.

[86] Thamdrup, B., Finster, K., Hansen, J.W., Bak, F. (1993) Bacterial disproportionation of elemental sulfur coupled to chemical-reduction of iron or manganese. Appl. Environ. Microbiol. 59, 101–108.

[87] Tiodjio, R.E., Sakatoku, A., Issa, Fantong, W.Y., Tchakam, K.B., Tanyileke, G., Hell, V.J., Ohba, T., Kusakabe, M., Tanaka, D., Nakamura, S., Ueda, A. (2016) Vertical distribution of bacteria and *Archaea* in a CO2-rich meromictic lake: a case study of Lake Monoun. Limnologica 60, 6–19.

[88] Tonolla, M., Peduzzi, R., Hahn, D. (2005) Long-term population dynamics of phototrophic sulfur bacteria in the chemocline of Lake Cadagno, Switzerland. Appl. Environ. Microbiol. 71, 3544–3550.

[89] Trembath-Reichert, E., Case, D.H., Orphan, V.J. (2016) Characterization of microbial associations with methanotrophic *Archaea* and sulfate-reducing bacteria through statistical comparison of nested Magneto-FISH enrichments. PeerJ, 4.

[90] Tuomi, P., Torsvik, T., Heldal, M., Bratbak, G. (1997) Bacterial population dynamics in a meromictic lake. Appl. Environ. Microbiol. 63, 2181–2188.

[91] Wasmund, K., Schreiber, L., Lloyd, K.G., Petersen, D.G., Schramm, A., Stepanauskas, R., Jorgensen, B.B., Adrian, L. (2014) Genome sequencing of a single cell of the widely distributed marine subsurface *Dehalococcoidia*, phylum *Chloroflexi*. ISME J. 8, 383–397.

[92] Yau, S., Lauro, F.M., Williams, T.J., DeMaere, M.Z., Brown, M.V., Rich, J., Gibson, J.A.E., Cavicchioli, R. (2013) Metagenomic insights into strategies of carbon conservation and unusual sulfur biogeochemistry in a hypersaline Antarctic lake. ISME J. 7, 1944–1961.

[93] Yi, J., Dong, B., Jin, J.W., Dai, X.H. (2014) Effect of increasing total solids contents on anaerobic digestion of food waste under mesophilic conditions: performance and microbial characteristics analysis. PLoS One, 9.

[94] Yu, Y., Lee, C., Kim, J., Hwang, S. (2005) Group-specific primer and probe sets to detect methanogenic communities using quantitative real-time polymerase chain reaction. Biotechnol. Bioeng. 89, 670–679.

[95] Zadereev, E.S., Boehrer, B., Gulati, R.D. (2017) Introduction: meromictic lakes, their terminology and geographic distribution, in: Gulati, R.D., Zadereev, E.S., Degermendzhi, A.G. (Eds.), Ecology of Meromictic Lakes, vol. 1, Springer International Publishing, Switzerland, pp. 1–11.

[96] Žic, V., Carić, M., Ciglenečki, I. (2013) The impact of natural water column mixing on iodine and nutrient speciation in a eutrophic anchialine pond (Rogoznica Lake, Croatia). Estuar. Coast. Shelf Sci. 133, 260–272.