PRIMARY RESEARCH PAPER

Multi-scale phylogenetic heterogeneity of archaea, bacteria, methanogens and methanotrophs in lake sediments

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Abstract Understanding spatial microbial community variation is necessary to assess diversity patterns. In this study, we investigated spatial heterogeneity and variability of functional and total microbial benthic community structures in Lake Bourget, France. Community structure variability was determined vertically by comparing three sediment layers per core, and horizontally at the intra-site level (between up to three cores per sites) and between three sites. Bacterial, archaeal, methanotrophs and methanogens community structures were assessed by genotyping the 16S rRNA–23S rRNA intergenic spacer, 16S rRNA, *pmoA* and *mcrA* genes, respectively. The consequence of pooling DNA extracts before genotyping was tested. After pooling, the OTU number decreased for all communities, but it had no effect on structure. At the core scale, bacterial community structure significantly differed between the sediment layers, but archaeal, methanogens and methanotrophs community structures only differed significantly between the superficial and deeper layers. Changes in environmental conditions affected microbial community structures between sites (sediment carbonates, total carbon contents, median particle sizes, and water O_2 saturation), but not intra-site, as no significant changes were observed at the horizontal scale. These spatial scales should be considered to understand their importance for biogeochemical cycle when assessing benthic microbial community structure and diversity in lakes.

Keywords Spatial distribution · Microbial diversity · Freshwater sediment · Methanotrophy · Methanogenesis

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Introduction

Microorganisms are involved in the different biogeochemical cycles due to their metabolic activities (Fenchel et al., 1998; Madsen, 2011). In aquatic ecosystems, the benthic compartment represents a key area where microbes play crucial roles in the global

carbon cycle by releasing CO_2 and/or CH_4 into the atmosphere via decomposing organic material stored in sediments (Conrad, 1996; Paerl & Steppe, 2003; Schultz & Urban, 2008). Methanogenesis final product, methane, is an important greenhouse gas whose atmospheric concentration has doubled during the twentieth century (IPCC, 2007). Sediments are subjected to a strong redox gradient, and they become rapidly anoxic and favour methanogenesis as the final step of organic matter mineralization (Chaudhary et al., 2013). Methane produced in sediment is emitted to the water column and atmosphere by diffusion or bubbling, and it can be oxidized to carbon dioxide under favourable conditions (Krause et al., 2010). The net amount of methane released into the atmosphere is the result of the methanogenesis–methanotrophy activity equilibrium, and freshwater sediments contribute to up to 40–50% of the annual methane emission (Chaudhary et al., 2013). Archaea are responsible for methanogenesis under anoxic conditions (Garrity & Holt, 2001), whereas methanotrophy is generally carried out by bacteria under oxic conditions (Conrad, 2009), except for the anaerobic oxidation of methane (AOM), which is carried out by methanogenic archaea (Reeburgh, 2007; Knittel & Boetius, 2009; Crowe et al., 2011). Eukaryotes (amoebae, flagellates and ciliates) also play a key role in methane turnover and emission at the oxic/anoxic interface through the grazing of methanotrophic organisms (Murase & Frenzel, 2007).

Prior to the holistic study of an ecosystem, the scales of variability in the distribution of targeted communities must be determined to evaluate how common the diversity patterns are across space (Tšertova et al., 2013). Molecular methods targeting phylogenetic or functional gene markers allow for the efficient investigation of microorganism diversity. For instance, methanogens and methanotrophs diversity is relatively well known, but their distribution in freshwater sediment and their controlling environmental parameters are still under investigation (Deutzmann et al., 2011; Nolla-Ardèvol et al., 2012). The benthic compartment is very heterogeneous, resulting in variability and patchiness in microbial community composition at the horizontal, vertical and even temporal scales. Moreover, the dynamics of water column characteristics (e.g. nutrients and organic matter concentrations) are likely to generate spatial

and temporal heterogeneities for the sediments (Trolle et al., 2009).

Understanding the benthic microbial community structure and distribution, including specific functional communities, in lakes for which this knowledge is scarce, is an important challenge for sedimentary systems research. The present study aimed to gain insight into the spatial heterogeneity of microbial communities in the superficial sediment (0–20 cm) of Lake Bourget, considering total (bacteria and archaea) and functional (methanogens and methanotrophs) communities. In particular, we focused on potential horizontal variations in the microbial communities within sediments by considering 3 sites selected along a transect gradient from the main lake tributary to the deepest part of the lake. The specific objectives of this study were as follows: (i) analyse community structure heterogeneity at various horizontal scales: the intra-core scale, site scale and inter-site scale, considering the vertical distribution of microbial communities within sediment cores, (ii) investigate co-structuration between total and functional microbial communities.

Materials and methods

Study site and core collection

Lake Bourget is the largest natural lake in France (44.5 km^2, 18 km long and 3.4 km wide), with a maximum depth of 147 m and a watershed area of 560 km^2. Its major tributary is the river Leysse (Jacquet et al., 2012). Sample collection was carried out in December 2011 at three sampling sites: site 1 (E 5°52′6.77″–N 45°39′46.91″) is 65 m deep and located within the Leysse river plume, site 2 (E 5°52′11.55″–N 45°40′57.41″) is 110 m deep and located outside of the Leysse river plume and site 3 (E 5°51′37.10″–N 45°44′43.60″) is the deepest point of Lake Bourget (147 m) (Fig. 1). At sites 1 and 2, three replicate cores (C1, C2 and C3) were collected using a gravity multicorer (UWITEC, Mondsee, Austria). At site 3, due to windy conditions, only one core (C1) was sampled using a gravity corer (UWITEC, Mondsee, Austria). Water column temperature and dissolved oxygen profiles were carried out using a multiparameter probe (SBE 19+, Sea-Bird Electronics Inc., Washington, USA).
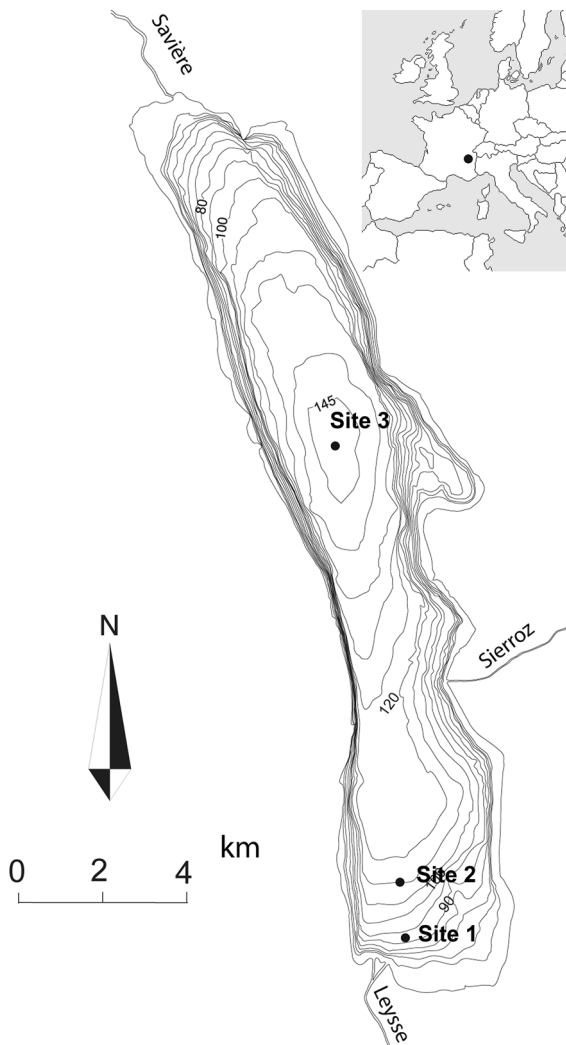
Fig. 1 Map showing the location of Lake Bourget and of sampling sites. Site 1 (E 5°52′6.77″–N 45°39′46.91″) is 65 m deep, site 2 (E 5°52′11.55″–N 45°40′57.41″) is 110 m deep, and site 3 (E 5°51′37.10″–N 45°44′43.60″) is the deepest point of Lake Bourget (147 m)

Sediment sample characterization

Sediment was sectioned into 1 cm slices at three depths (0–1 cm, 9–10 cm and 19–20 cm) to select contrasted redox conditions (Brandl et al., 1993). For each sediment layers, the centre of the section was sampled for DNA analysis and stored at −20°C. Sediment particle size distribution was determined by laser diffraction on dry sediments (Mastersizer S, Malvern, United Kingdom) and particles were grouped by the median particle grain size. The water

content of the sediment was estimated by drying at 60°C for 48 h. Organic matter and carbonate contents were measured by loss on ignition (Heiri et al., 2001). Total carbon and total nitrogen were determined using an elementary analyzer (Flash EA1112, ThermoFisher Scientific, Waltham, USA, Plateforme Analyses Environnementales, UMR 5553 LECA).

Total and functional microbial community structures from sedimentary DNA

DNA was extracted from 0.5 g of wet sediment using a NucleoSpin Soil kit (Macherey–Nagel EURL, Hoerdt, France), according to the manufacturer's protocol. For Lake Bourget sediment, DNA was extracted using SL1 lysis buffer and additive Enhancer SX buffer. The extracted DNA was quantified fluorometrically after staining with bisBenzimide (DNA Quantitation Kit, Fluorescence Assay, Sigma-Aldrich, St Louis, USA) using a Plate Chameleon fluorometer (Hidex, Turku, Finland; excitation: 340 nm, emission: 460 nm).

For core C1 from sites 1, 2 and 3, a total of ten replicates sub-samples were extracted for each of the three sediment layers, and the samples were genotyped to assess total and functional community structure variability at the inter-site scale (Fig. 2). The replicates from C1 cores were also used to study the consequence of pooling DNA extracts before amplification on total and functional community structures using the following procedure: DNA extracts of each sediment layer and site were pooled 2 by 2; a total of 36 pools were constituted (Fig. 2). For some samples ($n = 8$), there was not sufficient DNA to use for the pool analyses, preventing the realization of the four remaining pooled samples. More information on reproducibility of the genotyping techniques used is available in the supplementary information.

For cores C2 and C3 (sites 1 and 2 only), five replicate sub-samples per sediment layer were extracted and analysed. These samples, together with five replicates from the cores C1, were used to assess intra-site heterogeneity by comparing the results obtained for the 3 different cores originating for the same site (site 1 and site 2; Fig. 2).

Microbial community structures were assessed using two fingerprinting methods, providing peak profiles after capillary electrophoresis (CE) resolution of PCR-amplified phylogenetic or functional gene markers. Automated ribosomal intergenic spacer analysis
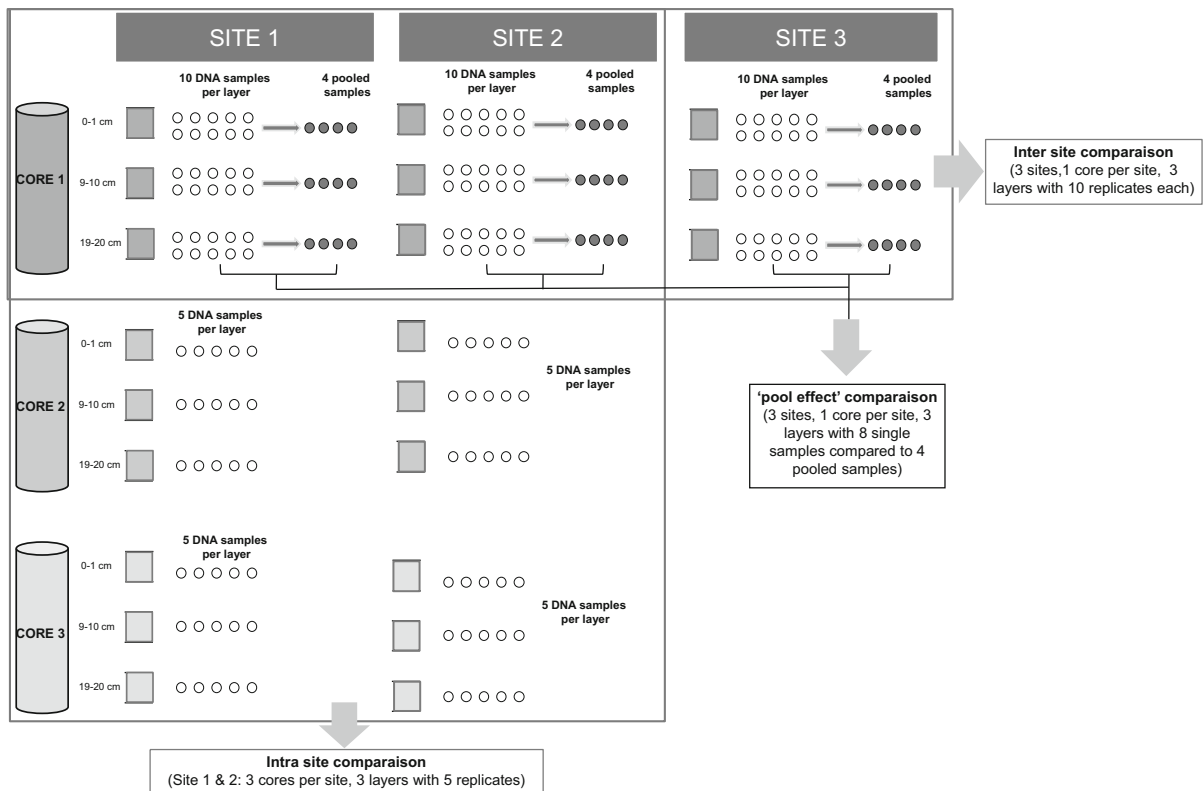
Fig. 2 Sampling design to compare the pooling effect (*black line*) benthic microbial community richness and structure, and their spatial variability of intra-sites (core 1, 2 and 3 for site 1 and 2, *grey dotted line*) and between sites (site 1, 2 and 3, *grey line*)

(ARISA) is based on the length polymorphism of intergenic spacer (ITS) sequences between 16S and 23S rRNA genes. Terminal-restriction fragment length polymorphism (T-RFLP) is based on the digestion of the total (16S rRNA gene) or functional (here *mcrA* and *pmoA* genes) gene marker following PCR amplification with a fluorescently labelled primer, so only the terminal-restriction fragment (T-RF) can be CE-resolved. ARISA was used for bacteria and T-RFLP was used for archaea (16S rRNA gene), because most archaea do not have intergenic spacers (Leuko et al., 2007). Functional microbial community structures were studied using T-RFLP analyses of two genes: *mcrA* (methyl coenzyme M reductase), which is involved in methanogenesis (Lueders & Friedrich, 2003) and *pmoA* (particulate methane monooxygenase), which is involved in methanotrophy (Costello & Lidstrom, 1999).

ITS were amplified using 5′-6-carboxyfluorescein (FAM)-labelled-S-D-Bact-1522-B–S-20 and L-D-Bact-132-a-A-18 (Normand et al., 1996) primers with 10 ng of template DNA and using a previously described

protocol (Lyautey et al., 2011). Archaeal 16S rRNA gene was amplified using 5′-6-FAM-labelled-340f and 1000r primers (Gantner et al., 2011) according to the authors' protocol, with slight modifications: the $MgCl_2$ concentration in the reaction mix was 1.5 mM and the hybridization temperature was increased to 60°C and 10 ng of extracted DNA was used as template. Functional genes were amplified using 10 ng of extracted DNA, using 5′-6-FAM-labelled-A189f and mb661r primers as described elsewhere (Bourne et al., 2001) for *pmoA* except that hybridization temperature was increased to 61°C, and using 5′-6-FAM-labelled-ME1 and ME2 primers (Hales et al., 1996) for *mcrA* as described previously (Roussel et al., 2009): however, the hybridization temperature was increased to 61°C. For each targeted gene, a positive control was used to verify amplification specificity (bacteria: DNA from an environmental isolate, archaea: *Halobacterium salinarum* (DSMZ 1616) and *Sulfolobus solfataricus* (DSMZ 3754) strains, methanotrophs: *Methylomonas methanica* (DSMZ 25384) strain and methanogens: DNA

from a methanization digester sludge). Amplification products were quantified on a 1.65% agarose gel using a MassRuler™ Express LR Forward DNA Ladder (Fermentas, Waltham, USA). For T-RFLP analyses, 50 ng of amplified DNA was digested with AluI (Promega Corporation, Madison, USA) for archaea 16S rRNA gene and MspI (Promega Corporation, Madison, USA) for *mcrA* and *pmoA* genes, for 4 h at 37°C. Restriction products and ARISA amplification products were purified using a AxyPrep PCR Clean-up Kit (Axygen, Tewksbury, USA) and separated on ABI 3730xl DNA Analyzer (BIOfidal DTAMB, IFR 41, Université Claude Bernard Lyon 1) using the internal size standard, GeneScan 1200 LIZ (Applied Biosystems, Waltham, USA) for bacteria intergenic spacer and *mcrA*, and Dye 5 ladder, 50–1000 bp (Gel company, San Francisco, USA), for archaea 16S rRNA gene and *pmoA*.

Data analysis

Physical and chemical parameters were compared (between sites and within sites) using Friedman test for all parameters, except for particle sizes, for which no data were available for site 3 samples. Site 1 and site 2 particle sizes data were compared using Wilcoxon test. All statistical tests were performed using Statistica software (Version 8.0, Statsoft, Inc., Tulsa, USA), and differences were considered significant at the 5% level.

T-RFLP and ARISA electropherograms were analysed using Applied Biosystems Peak Scanner software. Because two microplates per genes were needed to analyse the entire sample set, the threshold applied to each microplate was adjusted to minimize plate effects; internal standard peak heights were compared between plates, and a correction factor was calculated based on height differences. The correction factor was applied to samples that presented a low fluorescence signal, and the threshold from each well was divided by the correction factor. Samples with peak heights below the plate detection threshold were individually and manually adjusted. Fragments (T-RF or intergenic spacer) smaller than 160 bp for bacteria, 35 bp for archaea, 40 bp for *mcrA* and 50 bp for *pmoA* were considered as noise and were excluded from the analyses. Data were binned (i.e., electrophoretic profiles alignment) using the interactive binner script (Ramette, 2009) available online (http://www.ecology-research. com) and implemented using the R programming language (R Development Core Team, 2014; http:// cran.r-project.org/) with WS = 1 for bacteria and WS = 2 for others, Sh = 1 and an optimal divisor value of 1/1,000 (total communities) and 1/500 (functional communities). Peaks were scored as present or absent, and the data were exported as a presence/absence matrix. For pooled samples, a theoretical OTU number was computed from the profiles obtained from individual samples used in each pool, and then observed versus theoretic OTU number were compared using Wilcoxon test.

For all communities and pooled samples, profile similarity was computed using the Jaccard similarity index, and distances were represented by non-multidimensional scaling (nMDS, Clarke & Warwick, 2001) with R software (Vegan package, Oksanen et al., 2013). Significant differences between groups were determined from the nMDS plots, and ANalysis Of SIMilarity (ANOSIM, R software, Vegan package) was used to test significant differences between the following user-defined (a priori) groups: the intra-core scale between the three studied sediment layers, the intra-site scale between the three cores collected per site (site 1 and 2 only) and the inter-site scale between the three studied sites. ANOSIM was computed from the Jaccard similarity matrix, and a random permutation test (10,000 permutations) was applied. The test was considered significant at $P < 0.05$ after application of the Bonferroni correction (R software, stats package, p.adjust, http://cran.r-project.org/).

Redundancy analyses (RDA) were used to explore the relationships between environmental parameters and community structure variations. RDA was performed using R software (ade4 package, Dray & Dufour, 2007) with 1,000 Monte Carlo permutation tests.

Venn diagrams were used to enumerate shared or unique OTU between layers or sites. Diagrams were made using the online tool provided at the following web address: http://bioinfogp.cnb.csic.es/tools/venny/ index.html (Oliveros, 2007).

The relationships between total and functional community structures were evaluated using the Procrustean analysis (PROTEST software package—PROcrustean randomization test: http://labs.eeb.utoronto.ca/jackson/ pro1.html). Procrustean analyses superimpose, scale and rotate one data matrix upon the other until an optimal fit is found (Jackson & Harvey, 1993; Peres-Neto & Jackson, 2001; Heino et al., 2004). Estimated residuals

between the original values and the derived best-fit solution give the $m12$ statistic: a low $m12$ value indicates good correspondence between data matrices (Paavola et al., 2006). The PROTEST permutation procedure (10,000 permutations) was used to assess the statistical significance of the Procrustean fit between the two matrices (Peres-Neto & Jackson, 2001; Paavola et al., 2006).

Results

Study sites characterization

At the water–sediment interface, the temperatures were 5.8, 5.5 and 5.5°C, and the oxygen saturation rates were 63, 61 and 0%, at sites 1, 2 and 3, respectively. Based on the physical–chemical parameters measured in the sediments (Table 1), no intra-site difference was observed between cores C1, C2 and C3 collected at site 1 or 2 (Friedman test, $P > 0.05$ for all tested parameters). At the core scale, water content for site 1 ($P = 0.049$) and organic matter content for site 2 ($P = 0.049$) were significantly different between each layer. Between sites 1, 2 and 3 (as assessed based on comparing 1 core only per site), the water content significantly (Friedman test, $P = 0.049$) increased from $59 \pm 5\%$ (site 1) to $66 \pm 4\%$ (site 2) to $76 \pm 6\%$ (site 3). Particle sizes was significantly different between sites 1 and 2 (Wilcoxon test, $P = 0.021$), with a median grain size of 20 ± 3 μm at site 1 and 15 ± 3 μm at site 2. No significant differences were observed between the 3 sites for the other parameters (organic matter, carbonates, total nitrogen and total carbon contents, Friedman test, $P > 0.05$).

Effect of DNA extracts pooling on OTU number and structure

The bacterial, archaeal and methanogen OTU numbers between the pooled and single (non–pooled) samples were significantly different (Wilcoxon test, $P < 0.005$), with mean OTU number ($\pm$SD) of 45 ± 4, 10 ± 2 and 4 ± 1 OTUs per pools and of 50 ± 3, 12 ± 2 and 5 ± 1 OTUs per single sample, respectively. For methanotrophs, no significant difference was observed (Wilcoxon test, $P > 0.05$), with 6 ± 1 OTUs for both pooled and single samples. The OTUs lost by pooling exhibited a relative fluorescent intensity (RFI) $< 1\%$

(representing 91 and 62% of the "lost" OTUs for bacteria and archaea, respectively). No significant difference was observed between the community structures of pooled and single samples for total and functional communities within each site (see Fig. S1, ANOSIM, $P > 0.05$ for all cases).

Intra-site OTU number and structure heterogeneity

Structure and OTU number heterogeneity were assessed at the site scale within cores (five replicate samples from 1 cm sediment layers) and between cores (three cores for sites 1 and 2 only) (Fig. 2). Both for OTU number (Table S1) and for structure (Table S2) heterogeneity (as assessed by calculating $CV \pm SD$) was less important within cores compared to within sites (between cores of the same site) for the four studied communities (bacteria, archaea, methanotrophs and methanogens). No significant difference was observed within sites (site 1 or 2) for total (bacterial and archaeal) or functional (methanotrophs and methanogens) community structures between the three cores (ANOSIM, 10,000 permutations, $P > 0.05$ in all cases).

OTU number estimation and vertical and horizontal structure of communities

The total and functional community structure was assessed in the verticality of the sediment (across layers) and horizontally between sites. For bacteria, 185 distinct OTUs were detected (Table S3), $37 \pm 5\%$ were common between the three studied layers and $12 \pm 3\%$ were layer-specific. A total of 12% of the OTUs were common to the three sites, whereas 58% were site-specific (equally distributed in the 3 sites; Fig. 3A). For archaea, 63 distinct OTUs were detected (Table S4). On average, $37 \pm 11\%$ of the OTUs were shared between the three studied layers, and $15 \pm 7\%$ of the OTUs were layer-specific. Fourteen percent of the OTUs were common to the 3 sites, whereas 66% were site-specific (distributed as follows: 19% in site 1, 22% in site 3, and 25% in site 2; Fig. 3B). For methanotrophs, among the 15 distinct OTUs (Table S5), $53 \pm 12\%$ were shared between the three studied layers and $12 \pm 12\%$ were layer-specific. Forty percent of the OTU were common to the three sites, whereas a small number of OTUs were site-specific (27% Fig. 3C). For methanogen

Table 1 Physical and chemical description of Lake Bourget sediment for cores from sites 1, 2, and 3 (cores C1–C3 for sites 1 and 2, and core C1 for site 3)

Site	Layer (cm)	Core	Water content (%)	Organic matter (%)	Carbonates (%)	Median particle size (μm)	TN (%)	TC (%)
1	0–1	C1	60.73	8.24	24.71	17.62	0.24	9.92
		C2	62.58	8.51	24.33	18.18	0.25	9.71
		C3	62.80	5.45	16.03	16.93	0.25	9.77
		Mean ± SD	62.03 ± 1.14	7.40 ± 1.69	21.69 ± 4.91	17.58 ± 0.63	0.24 ± 0.01	9.80 ± 0.11
	9–10	C1	44.61	7.48	18.50	24.88	0.12	7.59
		C2	45.98	7.03	21.00	26.04	0.12	7.97
		C3	53.13	7.27	22.60	16.34	0.14	8.14
		Mean ± SD	47.09 ± 4.57	7.26 ± 0.23	20.70 ± 2.07	22.42 ± 5.30	0.13 ± 0.01	7.90 ± 0.28
	19–20	C1	55.93	7.79	22.66	22.61	0.19	9.48
		C2	57.53	8.84	24.75	21.97	0.20	9.50
		C3	59.56	7.84	24.55	18.89	0.21	9.22
		Mean ± SD	57.68 ± 1.82	8.15 ± 0.59	23.99 ± 1.15	21.16 ± 1.99	0.20 ± 0.01	9.40 ± 0.16
2	0–1	C1	70.67	8.56	27.83	18.51	0.33	11.45
		C2	67.57	10.24	20.23	13.45	0.24	8.54
		C3	70.08	9.11	26.99	17.18	0.35	11.32
		Mean ± SD	69.44 ± 1.65	9.30 ± 0.86	25.01 ± 4.17	16.38 ± 2.63	0.31 ± 0.06	10.44 ± 1.64
	9–10	C1	62.58	7.79	22.99	13.56	0.22	8.97
		C2	63.79	8.22	26.79	17.7	0.23	10.03
		C3	62.26	8.23	26.03	14.25	0.23	9.69
		Mean ± SD	62.88 ± 0.81	8.08 ± 0.25	25.27 ± 2.01	15.17 ± 2.22	0.23 ± 0.01	9.56 ± 0.54
	19–20	C1	64.02	7.09	27.43	14.47	0.24	10.00
		C2	59.80	7.98	26.23	11.37	0.16	9.34
		C3	65.00	7.52	29.43	17.49	0.20	10.40
		Mean ± SD	62.94 ± 2.76	7.53 ± 0.45	27.70 ± 1.62	14.44 ± 3.06	0.20 ± 0.04	9.92 ± 0.53
3	0–1	C1	82.83	9.31	27.49	NA	NA	NA
	9–10	C1	74.33	8.06	23.12	NA	0.25	9.40
	19–20	C1	71.50	8.50	28.39	17.03	0.38	10.97

Sediments from the 0–1 cm, 9–10 cm and 19–20 cm layers within cores were characterized for water content (%), organic matter content [% Dry Weigh (DW)], carbonates content (% DW), median particle size (μm), total nitrogen content (TN, % DW), and total carbon content (TC, % DW)

NA no data available

community, 11 distinct OTUs were detected (Table S6), $43 \pm 18\%$ were shared between the three studied layers and $10 \pm 10\%$ were layer-specific. Fifty-five percent of the OTUs were common to the three sites, and a small number were site-specific (27% in total; Fig. 3D).

Regarding the community structure, significant differences were observed between total and functional communities between the three sites (Fig. 4, ANOSIM, 10,000 permutations, $P < 0.001$, $R = 0.86$; $P < 0.001$, $R = 0.46$; $P < 0.005$, $R = 0.38$ and $P < 0.03$, $R = 0.29$ for bacteria, archaea, methanotrophs and

methanogens, respectively). For bacteria, the community structure was significantly different between the three layers (ANOSIM, 10,000 permutations, $P < 0.001$, $R = 0.82$). For archaea, methanotrophs and methanogens, the community structures within the cores were only significantly different in the surface layer versus deeper layers (9-10 and 19-20 cm) (ANOSIM, 10,000 permutations, $P < 0.001$, $R = 0.18$; $P < 0.005$, $R = 0.32$; and $P < 0.01$, $R = 0.27$, respectively). For all of the combinations tested (functional versus functional, total versus total or total versus functional), significant levels of concordance were

Fig. 3 Repartition of shared or unique OTU between sites (Venn diagram, Oliveros, 2007) for each microbial community: bacteria (**A**), archaea (**B**), methanotrophs (**C**) and methanogens (**D**)
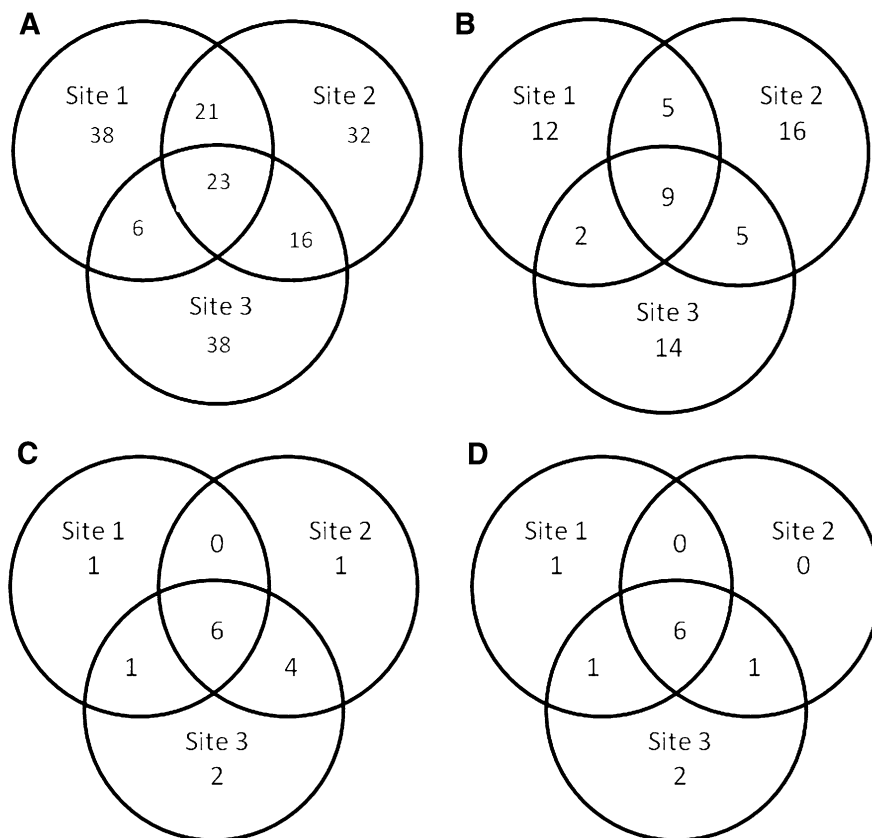
observed between microbial community structures (Procrustean analysis, two-dimensional representations not shown, $P < 0.001$ in all cases, Table 2).

Relationship between environmental parameters and community structures

The relationships between environmental parameters (from the water at the sediment interface and from sediment samples, as listed in Table 3) and microbial communities were explored by RDA. For the bacterial community structure, every parameter except the sediment organic matter content significantly explained the observed changes between sites ($P < 0.001$, Table 3). For archaea, only the median particle sizes and water O_2 saturation could explain changes in the community structure ($P < 0.001$, Table 3). For both methanogens and methanotrophs, the carbonate, total carbon contents, and median particle sizes, were significantly related with community structures (Table 3). The methanogens community structure was also significantly related to water O_2 saturation and sediment organic matter content.

Discussion

The main objective of this study was to investigate the spatial heterogeneity and variability of total and functional benthic microbial communities in a lacustrine environment using genotyping approaches (ARISA and T-RFLP). These approaches are widely used to characterize microbial community structure spatial and temporal changes (Schwarz et al., 2007; Deutzmann et al., 2011; Barbier et al., 2012), and T-RFLP can theoretically be used to affiliate T-RFs size and microbial taxa (TRiFLe software, Junier et al., 2008). Using this software, half of the detected T-RFs in the present work were related to the *Methanomicrobiales*, *Methanosarcinales*, *Methanococcales* and *Methanobacteriales* orders for methanogens, and the *Methylococcaceae*, *Methylocystaceae* and *Methylacidiphilaceae* families for
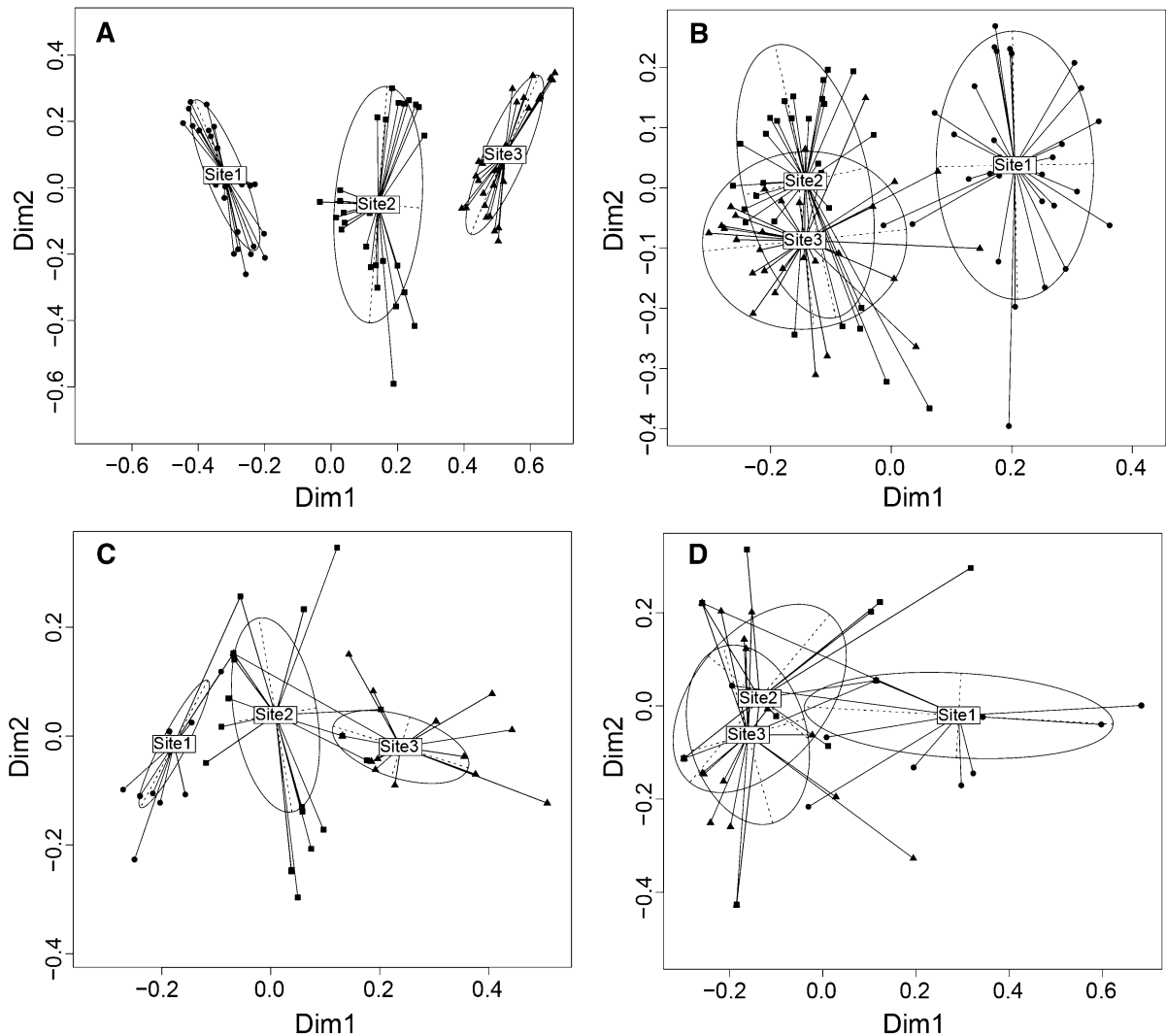
Fig. 4 nMDS representation of distances between bacterial (**A**, stress = 0.15), archaeal (**B**, stress = 0.36), methanotrophs (**C**, stress = 0.27) and methanogens (**D**, stress = 0.35) communities based on Jaccard similarity analysis (Site 1 *black circle*, Site 2 *black square*, Site 3 *black triangle*)

methanotrophs. These results are in accordance with other freshwater sediment-related literature, which recovered the same taxa (Borrel et al., 2011). However, further investigations (i.e., cloning and sequencing) are required here to identify the remaining T-RFs. Genotyping is a rapid and inexpensive tool for the analysis of a large number of samples. However, compared to next generation sequencing (NGS) techniques, the low resolution of genotyping prevents the detection of the rare biosphere (Gilbert et al., 2009; Bienhold et al., 2012; Jacob et al., 2013), but it still allows for the comparison of ecological trends (Gobet et al., 2014).

Consequently, this approach is perfectly suited to assess microbial community heterogeneity at different spatial scales.

At the site scale, the total and functional community structure heterogeneities increased from the intra- to the inter-core (i.e., intra-site) scale, confirming previous observations (Bowen et al., 2012; Jacob et al., 2013; Tšertova et al., 2013). At the micro-scale (sample replicates within 1-cm sediment layers), the occurrence of comparable physical and chemical conditions likely explains the observed similarity between community structures, especially because

Table 2 Procrustean analysis results between community 1 and 2 as indicated

Community 1	Community 2	m12	P
Bacteria	Archaea	0.596	<0.001
Bacteria	Methanotrophs	0.517	<0.001
Bacteria	Methanogens	0.843	<0.001
Archaea	Methanotrophs	0.693	<0.001
Archaea	Methanogens	0.876	<0.001
Methanotrophs	Methanogens	0.897	<0.001

The statistical significance of the test for 10,000 permutations is indicated by the P value, and m12 is a statistic indicator for the level of correspondence between the data matrixes (Jackson, 1995; Peres-Neto & Jackson, 2001)

genotyping approaches allowed for the detection of only intermediate to dominant populations. Pooling DNA extracts prior to genotyping total or functional microbial communities significantly decreased the observed OTU number, likely due to both method sensitivity and dilution effect, which both mask rare OTUs (Bowen et al., 2012; Gobet et al. 2014). The parallel analysis of several replicates was more powerful for analysing microbial community structure and OTU number, improving the richness estimation (Canning-Clode et al., 2008), and this approach will be considered for future works. For benthic microbial communities, ecological niche diversification occurs at the millimeter scale (Priscu et al., 1998; Nam et al., 2008), and it locally controls benthic prokaryotic communities (Bertics & Ziebis, 2009). As expected, niche differentiation was more important for microbial community structures vertically (in depth within cores) than horizontally (between the same layers of cores collected at a same site). Several authors have

reported the predominant role of sediment grain size on prokaryotic community structures (Hewson et al., 2007; Jackson & Weeks, 2008). Within cores, microbial community structures differed between layers according to their distance to the water column–sediment interface. This trait was common for bacteria and archaea (Edlund et al., 2008; Ye et al., 2009; Shivaji et al., 2011), as well as for methanogens and methanotrophs (Barbier et al., 2012; Borrel et al., 2012). Sediments are vertically stratified by the redox gradient (Fenchel & Finlay, 1995), controlling microbial metabolism and diversity. Here, we found that the community structure for bacteria was significantly different for each studied sediment layer. However, this was not the case for archaea, methanotrophs or methanogens. Swan et al. (2010) reported a similar trend hypothesizing prokaryotic communities that have different ecological requirements. They also highlighted that the T-RFs of many *Crenarchaeota* sequences are identical to those of the *Euryarchaeota*, potentially masking community structure changes. Different primers were used to amplify the archaeal 16S rRNA gene in the present study, and a resolution bias cannot be completely excluded from our approach.

For methane cycling micro-organisms, the community structure differences observed between the surface layer and the deeper layers are consistent with their ecological requirements. Methanotrophs are divided into 2 types depending on their morphological, physiological and ecological properties. Both types are frequently detected in freshwater sediments (Rahalkar et al., 2009; Biderre-Petit et al., 2011; Deutzmann et al., 2011). Several studies have suggested that type I methanotrophs (*Methylococcaceae*) may be more

Table 3 Redundancy analysis (RDA) performed to estimate environmental effects on spatial variability of community structures

Parameters	Bacteria	Archaea	Methanotrophs	Methanogens
Water content	**4.45*****	−1.68	1.65	1.40
Organic matter	−0.99	−0.66	−0.46	**−4.09*****
Carbonates	**4.18*****	−1.07	**2.06***	**2.42***
Median particles size	**8.93*****	**−3.335*****	**2.22***	**2.18***
Total nitrogen	**3.41*****	−1.29	1.43	1.50
Total carbon	**−4.39*****	1.93	**−2.15***	**−3.00****
O$_2$ saturation	**−6.51*****	**5.80*****	−1.04	**2.83***

For each community, t values were indicated, bold shown the significance

Significance levels are indicated as *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$

adaptable to changing environmental conditions such as methane and oxygen variations (Auman et al., 2000; Semrau et al., 2010; Ma et al., 2013). As previously suggested (Borrel et al., 2011), this might explain the dominance of type I methanotrophs in freshwater sediment which is a heterogeneous habitat. Methane and oxygen availability are important requirement for methanotrophy but methanotrophs can remain in a viable state under anaerobic conditions: they were still able to carry out methane oxidation after 1 month in anoxic conditions (Roslev & King, 1996). Thus, detecting methanotrophs when the water column is stratified and the epilimnion is anaerobic or anoxic at the water–sediment interface is consistent with their ecological tolerance toward O_2 limitation. However, detecting methanotrophs in the deeper sediment layers (9–10 and 19–20 cm), where anoxic conditions are permanent, seems surprising. Further work is needed to assess methanotrophic viability using RNA-based approaches (FISH or mRNA work). If they are not viable, it is possible that the detected DNA was a lacustrine record of past methanotroph communities, as previously demonstrated for Cyanobacteria in Lake Bourget, France (Domaizon et al., 2013, Savichtcheva et al., 2014), and methanogens in Lake Narlay (France, Belle et al., 2014). A general paradigm for methanogens is that they require anoxia, despite recent evidence that methanogenesis occurs in well-oxygenated lake water (Grossart et al., 2011, Tang et al., 2014). Two hypotheses can explain the detection of methanogens in the first cm of the Lake Bourget sediment column: (i) the sampling interval (1 cm) exceeded the oxygen diffusion depth (generally <5 mm, Viollier et al., pers. comm.), allowing for the presence of a methanogen community in anoxia, or (ii) the occurrence of methanogens, such as *Methanosarcina* or *Methanocella* allowed for the transcription of catalase genes to detoxify reactive oxygen species and produce methane, as previously demonstrated in soils (Angel et al. 2011).

Our sampling strategy was designed to ensure maximal reproducibility at the site scale (i.e, using a multicorer instead of carrying out three successive corings), whereas the three sampling sites were chosen due to their contrasting environmental characteristics. Site 1 is located within the plume of the main tributary of Lake Bourget. With a depth of 65 m, the deeper part of the water column is not subjected to seasonal hypoxia. Within this area, the sediment was gas-rich (Chapron et al., 2005), as determined using a seismic

reflection survey, suggesting the possible presence of dissolved methane in the interstitial water. Site 3 is located in the deepest part of the lake (depth of 147 m), and the deeper layers of the water column frequently undergo hypoxia (Jenny et al., 2013; INRA database Observatory on lakes—SOERE OLA http://www6.inra.fr/soere-ola). On the sampling date, the water–sediment interface of site 3 was located within the hypolimnion and was anoxic for almost 4 months (Jacquet et al. 2012). Located between sites 1 and 3, site 2 represents intermediate environmental conditions, as it is outside of the river plume and is characterized by intermediate depth. Despite these contrasting conditions, none of the physical and chemical descriptors, except sediment water content and oxygen concentration at the water–sediment interface, could be differentiated between the three sites. However, the four community structures patterns assessed in the present work differed significantly between sites. RDA analysis demonstrated that community structure variations could be explained by the following environmental conditions: median particle sizes for the four studied communities; carbonate and total carbon contents for all communities but archaea; water oxygen saturation for all communities but methanotrophs; total nitrogen and water contents for bacteria only, and organic matter content for methanogens only. Bacteria and methanotrophs community structures exhibited stronger site discrimination than archaea and methanogens, which is consistent with previous reports suggesting that archaeal and bacterial microorganisms respond differently to environmental conditions (Liu et al., 2009; Ye et al., 2009). In lake sediment, archaeal communities are frequently dominated by methanogens, as demonstrated on Lake Soyang (Korea, Go et al., 2000), on Lake Kinneret (Israel, Schwarz et al., 2007) or on Lake Pavin (France, Borrel et al., 2012). Other uncultured *Euryarchaeota* are also present in freshwater sediment such as Marine Benthic Group-D (MBG-D), Rice Cluster V (RC V) or the Lake Dagow Sediments (LDS) (Chan et al., 2005; Conrad et al., 2007; Ye et al., 2009; Conrad et al., 2010; Bhattarai et al., 2012; Borrel et al., 2012). *Creanarchaeota/Thaumarchaeota* lineages are also detected, such as Miscellaneous Crenarchaeotal Group (MCG), MBG-B (Conrad et al., 2010; Schubert et al., 2011; Borrel et al., 2012) for example. Bacterial community diversity and structure are correlated with pH, sediment organic matter,

carbon and nitrogen contents (Hollister et al., 2010), sediment pore water salinity (Swan et al., 2010), and water quality at the sediment water interface (Liu et al., 2009). Water chemistry (for pore water or at the sediment interface) was not assessed in the present work but it is likely that epi- and hypolimnetic waters were characterized by different concentrations of C and N forms. Both organic matter and carbon quantity and quality were previously related to changes in microbial community structure and function (Docherty et al., 2006). Here, the sampling site location (within/outside the river plume or in the deepest area) is probably responsible for the variability in the organic matter origin (allochthonous or autochthonous), explaining the community structure differences. Interestingly, the water content was significantly different between the three studied sites, and it was previously described as a controlling factor for benthic microbial communities (Wu et al., 2008; Röske et al., 2012). In sediment, the water content is correlated to porosity (Avnimelech et al., 2001) and it was suggested that its relationship with microbial community structure was a function of its influence on sediment oxygen concentrations, via diffusion mechanisms (Hollister et al. 2010), this would only be relevant for the few top millimeters of the sediment when the water column is oxygenated in this study.

Conclusion

The present work demonstrates that total and functional microbial community structures can be differentiated according to local environmental conditions (i.e., redox conditions), global environmental conditions (i.e., Lake Bourget stratification at sampling time), and geographical position within the lake relatively to major tributaries. Micro-scale heterogeneity did not affect the community structure within the same core, and it moderately affected heterogeneity between cores collected within a same sampling site. Pooling DNA extracts ahead of genotyping decreased the observed OTU number, suggesting that the independent characterization of 2–3 replicates would provide better insights into microbial communities. Total (bacteria and archaea) and functional (methanogens and methanotrophs) communities were controlled by environmental parameters, and bacteria seemed to be more prone to exhibit different

community structures in relation to depth within the sediment. In view of our results, analysing only one core per site seems sufficient to horizontally characterize microbial benthic community structures, but the vertical (depth-related) structure of functional communities requires detailed investigations within each core. The assessment of functional community structure and diversity in lakes should integrate all of these spatial scales and lake temporal dynamics to gain a better understanding of the microbial contribution to biogeochemical cycles.

References

Angel, R., D. Matthies & R. Conrad, 2011. Activation of methanogenesis in arid biological soil crusts despite the presence of oxygen. Plos One 6: e20453.

Auman, A. J., S. Stolyar, A. M. Costello & M. E. Lidstrom, 2000. Molecular characterization of methanotrophic isolates from freshwater lake sediment. Applied and Environmental Microbiology 66: 5259–5266.

Avnimelech, Y., G. Ritvo, L. E. Meijer & M. Kochba, 2001. Water content, organic carbon, and dry bulk density in flooded sediments. Aquacultural Engineering 25: 25–33.

Barbier, B. A., I. Dziduch, S. Liebner, L. Ganzert, H. Lantuit, W. Pollard & D. Wagner, 2012. Methane-cycling communities in a permafrost-affected soil on Herschel Island, Western Canadian Arctic: active layer profiling of *mcrA* and *pmoA* genes. FEMS Microbiology Ecology 82: 287–302.

Bhattarai, S., K. A. Ross, M. Schmid, F. S. Anselmetti & H. Bürgmann, 2012. Local conditions structure unique Archaeal communities in the anoxic sediments of meromictic Lake Kivu. Microbiology Ecology 64: 291–310.

Belle, S., C. Parent, V. Frossard, V. Verneaux, L. Millet, P.-M. Chronopoulou, P. Sabatier & M. Magny, 2014. Temporal changes in the contribution of methane-oxidizing bacteria to the biomass of chironomid larvae determined using stable carbon isotopes and ancient DNA. Journal of Paleolimnology 52: 215–228.

Bertics, V. J. & W. Ziebis, 2009. Biodiversity of benthic microbial communities in bioturbated coastal sediments is controlled by geochemical microniches. The ISME Journal 3: 1269–1285. doi:10.1038/ismej.2009.62.

Biderre-Petit, C., D. Jézéquel, E. Dugat-Bony, F. Lopes, J. Kuever, G. Borrel, E. Viollier, G. Fonty & P. Peyret, 2011.

Identification of microbial communities involved in the methane cycle of a freshwater meromictic lake. FEMS Microbiology Ecology 77: 533–545.

Bienhold, C., A. Boetius & A. Ramette, 2012. The energy-diversity relationship of complex bacterial communities in Arctic deep-sea sediments. The ISME Journal 6: 724–732.

Borrel, G., D. Jézéquel, C. Biderre-Petit, N. Morel-Desrosiers, J.-P. Morel, P. Peyret, G. Fonty & A.-C. Lehours, 2011. Production and consumption of methane in freshwater lake ecosystems. Research in Microbiology 162: 832–847.

Borrel, G., A.-C. Lehours, O. Crouzet, D. Jézéquel, K. Rockne, A. Kulczak, E. Duffaud, K. Joblin & G. Fonty, 2012. Stratification of Archaea in the deep sediments of a freshwater meromictic lake: vertical shift from methanogenic to uncultured Archaeal lineages. PLOS One 7: e43346.

Bourne, D. G., I. R. McDonald & J. C. Murrell, 2001. Comparison of *pmoA* PCR primer sets as tools for investigating methanotroph diversity in three danish soils. Applied and Environmental Microbiology 67: 3802–3809.

Bowen, J. L., H. G. Morrison, J. E. Hobbie & M. L. Sogin, 2012. Salt marsh sediment diversity: a test of the variability of the rare biosphere among environmental replicates. The ISME Journal 6: 2014–2023.

Brandl, H., K. W. Hanselmann, R. Bachoffen & J. Picard, 1993. Small-scale patchiness in the chemistry and microbiology of sediments in Lake Geneva, Switzerland. Journal of General Microbiology 139: 2271–2275.

Canning-Clode, J., N. Valdivia, M. Molis, J. C. Thomason & M. Wahl, 2008. Estimation of regional richness in marine benthic communities: quantifying the error. Limnology and Oceanography 6: 580–590.

Chan, O. C., P. Claus, P. Casper, A. Ulrich, T. Lueders & R. Conrad, 2005. Vertical distribution of structure and function of the methanogenic archaeal community in Lake Dagow sediment. Environmental Microbiology 7: 1139–1149.

Chapron, E., F. Arnaud, H. Noël, M. Revel, M. Desmet & L. Perdereau, 2005. Rhone River flood deposits in Lake Le Bourget: a proxy for Holocene environmental changes in the NW Alps, France. Boreas 34: 404–416.

Chaudhary, P. P., L. Brablcova, I. Buriankova & M. Rulik, 2013. Molecular diversity and tools for deciphering the methanogen community structure and diversity in freshwater sediments. Applied Microbiology and Biotechnology 97: 7553–7562.

Clarke, K. R. & R. M. Warwick, 2001. Change in Marine Communities: An Approach to Statistical Analysis and Interpretation, 2nd ed. PRIMER-E, Plymouth.

Conrad, R., 1996. Soil microorganisms as controllers of atmospheric trace gases (H_2, CO, CH_4, OCS, N_2O, and NO). Microbiology Reviews 60: 609–640.

Conrad, R., O. C. Chan, P. Claus & P. Casper, 2007. Characterization of methanogenic Archaea and stable isotope fractionation during methane production in the profundal sediment of an oligotrophic lake (Lake Stechlin, Germany). Limnology and Oceanography 52: 1393–1406.

Conrad, R., 2009. The global methane cycle: recent advances in understanding the microbial processes involved. Environmental Microbiology Reports 1: 285–292.

Conrad, R., P. Claus & P. Casper, 2010. Stable isotope fractionation during the methanogenic degradation of organic matter in the sediment of an acidic bog lake, Lake Grosse

Fuchskuhle. Limnology and Oceanography 55: 1932–1942.

Costello, A. M. & M. E. Lidstrom, 1999. Molecular characterization of functional and phylogenetic genes from natural populations of methanotrophs in lake sediments. Applied and Environmental Microbiology 65: 5066–5074.

Crowe, S. A., S. Katsev, K. Leslie, A. Sturm, C. Magen, S. Nomosatryo, M. A. Pack, J. D. Kessler, W. S. Reeburgh, J. A. Roberts, L. Gonzalez, G. Douglas Haffner, A. Mucci, B. Sundby & D. A. Fowle, 2011. The methane cycle in ferruginous Lake Matano. Geobiology 9: 61–78.

Deutzmann, J. S., S. Wörner & B. Schink, 2011. Activity and diversity of methanotrophic Bacteria at methane seeps in Eastern Lake Constance sediments. Applied and Environmental Microbiology 77: 2573–2581.

Docherty, K. M., K. C. Young, P. A. Maurice & S. D. Bridgham, 2006. Dissolved organic matter concentration and quality influences upon structure and function of freshwater microbial communities. Microbial Ecology 52: 378–388.

Domaizon, I., O. Savichtcheva, D. Debroas, F. Arnaud, C. Villar, C. Pignol, B. Alric & M. E. Perga, 2013. DNA from lake sediments reveals the long-term dynamics and diversity of *Synechococcus* assemblages. Biogeosciences 10: 3817–3838.

Dray, S. & A. B. Dufour, 2007. The ade4 package: implementing the duality diagram for ecologists. Journal of Statistical Software 22: 1–20.

Edlund, A., F. Hårdeman, J. K. Jansson & S. Sjöling, 2008. Active bacterial community structure along vertical redox gradients in Baltic Sea sediment. Environmental Microbiology 10: 2051–2063.

Fenchel, T. & B. J. Finlay, 1995. Ecology and Evolution in Anoxic Worlds. Oxford University Press, Oxford.

Fenchel, T., G. M. King & T. H. Blackburn, 1998. Bacterial BiochemistOry: The Ecophysiology of Mineral Cycling, 2nd ed. Academic Press, San Diego.

Gantner, S. A., A. F. Andersson, L. Alonso-Sáez & S. Bertilsson, 2011. Novel primers for 16S rRNA-based archaeal community analyses in environmental samples. Journal of Microbiological Methods 84: 12–18.

Garrity, G. M. & J. G. Holt, 2001. Phylum AII. Euryarchaeota phy. nov. In Boone, D. R., R. W. Castenholz & G. M. Garrity (eds), Bergey's Manual, 2nd ed. Springer, New York, NY: 211–355.

Gilbert, J. A., D. Field, P. Swift, L. Newbold, A. Oliver, T. Smyth, P. J. Somerfield, S. Huse & I. Joint, 2009. The seasonal structure of microbial communities in the Western English Channel. Environmental Microbiology 11: 3132–3139.

Go, Y. S., S. K. Han, I. G. Lee & T. I. Ahn, 2000. Diversity of the domain Archaea as determined by 16S rRNA gene analysis in the sediments of Lake Soyang. Archiv Fur Hydrobiologie 149: 459–466.

Gobet, A., A. Boetius & A. Ramette, 2014. Ecological coherence of diversity patterns derived from classical fingerprinting and next generation sequencing techniques. Environmental Microbiology 16: 2672–3681.

Grossart, H.-P., K. Frindte, C. Dziallas, W. Eckert & K. W. Tang, 2011. Microbial methane production in oxygenated water column of an oligotrophic lake. Proceedings of the National Academy of Sciences 108: 19657–19661.

Hales, B. A., C. Edwards, D. A. Ritchie, G. Hall, R. W. Pickup & J. R. Saunders, 1996. Isolation and identification of methanogen-specific DNA from blanket bog peat by PCR amplification and sequence analysis. Applied and Environmental Microbiology 62: 668–675.

Heino, J., P. Louhi & T. Muotka, 2004. Identifying the scales of variability in stream macroinvertebrate abundance, functional composition and assemblage structure. Freshwater Biology 49: 1230–1239.

Heiri, O., A. F. Lotter & G. Lemcke, 2001. Loss on ignition as a method for estimating organic and carbonate content in sediments: reproducibility and comparability of results. Journal of Paleolimnology 25: 101–110.

Hewson, I., M. E. Jacobson Meyers & J. A. Fuhrman, 2007. Diversity and biogeography of bacterial assemblages in surface sediments across the San Pedro Basin, Southern California Borderlands. Environmental Microbiology 9: 923–933.

Hollister, E. B., A. S. Engledow, A. J. M. Hammett, T. L. Provin, H. H. Wilkinson & T. J. Gentry, 2010. Shifts in microbial community structure along an ecological gradient of hypersaline soils and sediments. The ISME Journal 4: 829–838.

IPCC, 2007. Contribution of working group I to the Fourth Assessment Report of the IPCC. In Marquis, M., K. B. Averyt, M. Tignor & H. L. Miller (eds), Climate Change 2007: The Physical Science Basis. Cambridge University Press, Cambridge.

Jackson, C. R. & A. Q. Weeks, 2008. Influence of particle size on bacterial community structure in aquatic sediments as revealed by 16S rRNA gene sequence analysis. Applied and Environmental Microbiology 74: 5237–5240.

Jackson, D. & H. Harvey, 1993. Fish and benthic invertebrates: community concordance and community–environment relationships. Canadian Journal of Fisheries and Aquatic Sciences 50: 2641–2651.

Jackson, D. A., 1995. PROTEST: a Procrustean randomization test of community environment. Ecoscience 2: 297–303.

Jacob, M., T. Soltwedel, A. Boetius & A. Ramette, 2013. Biogeography of deep-sea benthic bacteria at regional scale (LTER HAUSGARTEN, Fram Strait, Arctic). PLOS One 8: e72779.

Jacquet, S., D. Barbet, S. Cachera, A. Caudron, M. Colon, C. Girel, J. Guillard, A. Hébert, F. Kerrien, L. Laine, J. Lazzarotto, J.-P. Moille, G. Paolini, M. E. Perga, P. Perney & F. Rimet, 2012. Suivi environnemental des eaux du lac du Bourget pour l'année 2011. Rapport INRA-CISALB-CALB: 220 pp.

Jenny, J. P., F. Arnaud, J. M. Dorioz, C. Giguet-Covex, V. Frossard, P. Sabatier, L. Millet, J. L. Reyss, K. Tachikawa, E. Bard, C. Pignol, F. Soufi & M. E. Perga, 2013. A spatiotemporal investigation of varved sediments highlights the dynamics of hypolimnetic hypoxia in a large hardwater lake over the last 150 years. Limnology and Oceanography 58: 1395–1408.

Junier, P., T. Junier & K.-P. Witzel, 2008. TRiFLe, a program for in silico terminal restriction fragment length polymorphism analysis with user-defined sequences sets. Applied and Environmental Microbiology 74: 6452–6456.

Knittel, K. & A. Boetius, 2009. Anaerobic oxidation of methane: progress with an unknown process. Annual Reviews of Microbiology 63: 311–334.

Krause, S., C. Lüke & P. Frenzel, 2010. Succession of methanotrophs in oxygen–methane counter-gradients of flooded rice paddies. The ISME Journal 4: 1603–1607.

Leuko, S., F. Goh, M. A. Allen, B. P. Burns, M. R. Walter & B. A. Neilan, 2007. Analysis of intergenic spacer region length polymorphisms to investigate the halophilic archaeal diversity of stromatolites and microbial mats. Extremophiles 11: 203–210.

Liu, F. H., G. H. Lin, G. Gao, B. Q. Qin, J. S. Zhang, G. P. Zhao, Z. H. Zhou & J. H. Shen, 2009. Bacterial and archaeal assemblages in sediments of a large shallow freshwater lake, Lake Taihu, as revealed by denaturing gradient gel electrophoresis. Journal of Applied Microbiology 106: 1022–1032.

Lueders, T. & M. W. Friedrich, 2003. Evaluation of PCR amplification bias by terminal restriction fragment length polymorphism analysis of small-subunit rRNA and *mcrA* genes by using defined template mixtures of methanogenic pure cultures and soil DNA extracts. Applied and Environmental Microbiology 69: 320–326.

Lyautey, E., A. Cournet, S. Morin, S. Boulêtreau, L. Etcheverry, J. Y. Charcosset, F. Delmas, A. Bergel & F. Garabetian, 2011. Electroactivity of phototrophic river biofilms and constitutive cultivable Bacteria. Applied and Environmental Microbiology 77: 5394–5401.

Ma, K., R. Conrad & Y. Lu, 2013. Dry/wet cycles change the activity and population dynamics of methanotrophs in rice field soil. Applied and Environmental Microbiology 79: 4932–4939.

Madsen, E. L., 2011. Microorganisms and their roles in fundamental biogeochemical cycles. Current Opinion in Biotechnology 22: 456–464.

Murase, J. & P. Frenzel, 2007. A methane-driven microbial food web in a wetland rice soil. Environmental Microbiology 9: 3025–3034.

Nam, Y. D., Y. Sung, H. W. Chang, S. W. Roh, K. H. Kim, S. K. Rhee, J. C. Kim, J. Y. Kim, J. H. Yoon & J. W. Bae, 2008. Characterization of the depth-related changes in the microbial communities in Lake Hovsgol sediment by 16S rRNA gene-based approaches. Journal of Microbiology 46: 125–136.

Nolla-Ardèvol, V., M. Strous, D. Y. Sorokin, A. Y. Merkel & H. E. Tegetmeyer, 2012. Activity and diversity of haloalkaliphilic methanogens in Central Asian soda lakes. Journal of Biotechnology 161: 167–173.

Normand, P., C. Ponsonnet, X. Nesme, M. Neyra & P. Simonet, 1996. ITS analysis of prokaryotes. In Akkermans, D. L., J. D. van Elsas & F. J. de Bruijn (eds), Molecular Microbial Ecology Manual. Kluwer Academic Publishers, Dordrecht: 1–12.

Oksanen, J., F. G. Blanchet, R. Kindt, P. Legendre, P. R. Minchin, R. B. O'Hara, G. L. Simpson, P. Solymos, M. H. H. Stevens & H. Wagner, 2013. vegan: Community Ecology Package. R package Version 2.0-6. http://CRAN.R-project.org/package=vegan.

Oliveros, J. C., 2007. VENNY. An interactive tool for comparing lists with Venn Diagrams. http://bioinfogp.cnb.csic.es/tools/venny/index.html.

Paavola, R., T. Muotka, R. Virtanen, J. Heino, D. Jackson & A. Maki-Petäys, 2006. Spatial scale affects community concordance among fishes, benthic macroinvertebrates, and

bryophytes in streams. Ecological Applications 16: 368–379.

Paerl, H. W. & T. F. Steppe, 2003. Scaling up: the next challenge in environmental microbiology. Environmental Microbiology 5: 1025–1038.

Peres-Neto, P. R. & D. A. Jackson, 2001. How well do multivariate data sets match? Evaluating the association of multivariate biological data sets: comparing the robustness of Mantel test and a Procrustean superimposition approach. Oecologia 129: 169–178.

Priscu, J. C., C. H. Fritsen, E. E. Adams, S. J. Giovannoni, H. W. Paerl, C. P. McKay, P. T. Doran, D. A. Gordon, B. D. Lanoil & J. L. Pinckney, 1998. Perennial antarctic lake ice: an oasis for life in a polar desert. Science 280: 2095–2098.

R Development Core Team, 2014. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna. http://www.r-project.org/.

Rahalkar, M., J. Deutzmann, B. Schink & I. Bussmann, 2009. Abundance and activity of methanotrophic bacteria in littoral and profundal sediments of Lake Constance (Germany). Applied and Environmental Microbiology 75: 119–126.

Ramette, A., 2009. Quantitative community fingerprinting methods for estimating the abundance of operational taxonomic units in natural microbial communities. Applied and Environmental Microbiology 75: 2495–2505.

Reeburgh, W. S., 2007. Oceanic methane biogeochemistry. Chemical Reviews 107: 486–513.

Röske, K., R. Sachse, C. Scheerer & I. Röske, 2012. Microbial diversity and composition of the sediment in the drinking water reservoir Saidenbach (Saxonia, Germany). Systematic and Applied Microbiology 35: 35–44.

Roslev, P. & G. M. King, 1996. Regulation of methane oxidation in a freshwater wetland by water table changes and anoxia. FEMS Microbiology Ecology 19: 105–115.

Roussel, E. G., A. L. Sauvadet, J. Allard, C. Chaduteau, P. Richard, M. A. Cambon Bonavita & E. Chaumillon, 2009. Archaeal methane cycling communities associated with gassy subsurface sediments of Marennes-Oléron Bay (France). Geomicrobiology Journal 26: 31–43.

Savichtcheva, O., D. Debroas, M. E. Perga, C. Arnaud, C. Villar, E. Lyautey, A. Kirkham, C. Chardon, B. Alric & I. Domaizon, 2014. Effects of nutrients and warming on *Planktothrix* dynamics and diversity: a paleolimnological view based on sedimentary DNA and RNA. Freshwater Biology. doi:10.1111/fwb.12465.

Schubert, C. J., F. Vazquez, T. Lösekann-Behrens, K. Knittel, M. Tonolla & A. Boetius, 2011. Evidence for anaerobic oxidation of methane in sediments of a freshwater system (Lago di Cadagno). FEMS Microbiology Ecology 76: 26–38.

Schultz, P. & N. R. Urban, 2008. Effects of bacterial dynamics on organic matter decomposition and nutrient release from sediments: a modeling study. Ecological Modelling 210: 1–14.

Schwarz, J. I. K., W. Eckert & R. Conrad, 2007. Community structure of Archaea and Bacteria in a profundal lake sediment Lake Kinneret (Israel). Systematic and Applied Microbiology 30: 239–254.

Semrau, J. D., A. A. DiSpirito & S. Yoon, 2010. Methanotrophs and copper. FEMS Microbiology Reviews 34: 496–531.

Shivaji, S., K. Kumari, K. H. Kishore, P. K. Pindi, P. S. Rao, T. N. R. Srinivas, R. Asthana & R. Ravindra, 2011. Vertical distribution of bacteria in a lake sediment from Antarctica by culture-independent and culture-dependent approaches. Research in Microbiology 162: 191–203.

Swan, B. K., C. J. Ehrhardt, K. M. Reifel, L. I. Moreno & D. L. Valentine, 2010. Archaeal and Bacterial communities respond differently to environmental gradients in anoxic sediments of a California hypersaline lake, the Salton Sea. Applied and Environmental Microbiology 76: 757–768.

Tang, K. W., D. F. McGinnis, K. Frindte, V. Brüchert & H.-P. Grossart, 2014. Paradox reconsidered: methane oversaturation in well-oxygenated lake waters. Limnology and Oceanography 59: 275–284.

Trolle, D., G. Zhu, H. Hamilton, L. Luo, C. McBride & L. Zhang, 2009. The influence of water quality and sediment geochemistry on the horizontal and vertical distribution of phosphorus and nitrogen in sediments of a large, shallow lake. Hydrobiologia 627: 31–44.

Tšertova, N., A. Kisand, F. Baty & V. Kisand, 2013. Homogeneous microbial diversity in the upper sediment layers of a shallow lake. Aquatic Microbial Ecology 70: 77–85.

Wu, L., L. Kellogg, A. H. Devol, J. M. Tiedje & J. Zhou, 2008. Microarray-based characterization of microbial community functional structure and heterogeneity in marine sediments from the Gulf of Mexico. Applied and Environmental Microbiology 74: 4516–4529.

Ye, W., X. Liu, S. Lin, J. Tan, J. Pan, D. Li & H. Yang, 2009. The vertical distribution of bacterial and archaeal communities in the water and sediment of Lake Taihu. FEMS Microbiology Ecology 70: 263–276.