Composition of bacterial and archaeal communities in freshwater sediments with different contamination levels (Lake Geneva, Switzerland)

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ABSTRACT

The aim of this study was to compare the composition of bacterial and archaeal communities in contaminated sediments (Vidy Bay) with uncontaminated sediments (Ouchy area) of Lake Geneva using 16S rRNA clone libraries. Sediments of both sites were analysed for physicochemical characteristics including porewater composition, organic carbon, and heavy metals. Results show high concentrations of contaminants in sediments from Vidy. Particularly, high contents of fresh organic matter and nutrients led to intense mineralisation, which was dominated by sulphate-reduction and methanogenesis. The bacterial diversity in Vidy sediments was significantly different from the communities in the uncontaminated sediments. Phylogenetic analysis revealed a large proportion of Betaproteobacteria clones in Vidy sediments related to Dechloromonas sp., a group of dechlorinating and contaminant degrading bacteria. Deltaproteobacteria, including clones related to sulphate-reducing bacteria and Fe(III)-reducing bacteria (Geobacter sp.) were also more abundant in the contaminated sediments. The archaeal communities consisted essentially of methanogenic Euryarchaeota, mainly found in the contaminated sediments rich in organic matter. Multiple factor analysis revealed that the microbial community composition and the environmental variables were correlated at the two sites, which suggests that in addition to environmental parameters, pollution may be one of the factors affecting microbial community structure.

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1. Introduction

Untreated or only partly treated wastewaters including industrial, agricultural and domestic effluents constitute the main contamination sources in aquatic environments. Sediment contamination is usually due to inorganic and organic compounds including heavy metals (HMs) and hydrophobic organic compounds, such as polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs) (e.g. Koelmans et al., 2001; Eggleton and Thomas, 2004). The increasing contamination of sediments by inorganic and organic micro-pollutants is a big
concern in aquatic ecosystems (Förstner and Wittmann, 1979; Pardos et al., 2004; Schwarzenbach et al., 2006). Sediment contamination by HMs and other micro-pollutants might cause potential adverse effects to ecosystems and also pose human health risks (Salomons and Förstner, 1984; Verweij et al., 2004; Wang et al., 2004). The main environmental risk is remobilization of the contaminants and their return to the hydrosphere either by sediment re-suspension or by infiltration into the groundwater (Wildi et al., 2004). Therefore, the removal of HMs from wastewater or their accumulation in sediments should be examined extensively (e.g. Wang et al., 2004; Wildi et al., 2004). Microbial communities may be sensitive indicators for pollution in aquatic ecosystems and may be applied for biomonitoring and assessment purposes (Pronk et al., 2009). Heavy metal contamination, for example, may lead to a reduction of bacterial diversity (Sandaa et al., 1999). However, other studies observed either an increase in microbial diversity along with heavy metal contamination (Sorci et al., 1999) or no significant variation (Gillan et al., 2005). Other environmental factors as well as the time of exposure might explain these differences.

Lake Geneva is the largest freshwater reservoir of Western Europe with a volume of 89 km$^3$ and a maximum depth of 309 m. It is a monomictic temperate lake, with early spring overturn not occurring every year. The lake was considered eutrophic in 1970s and 1980s, but has become mesotrophic after drastic reduction of phosphorus inputs (Dorioz et al., 1998). Approximately 700,000 people are supplied with water from Lake Geneva. The city of Lausanne, located on the northern shore, discharges the largest volume of treated wastewater into the Bay of Vidy. The wastewater treatment plant (WWTP) of the city treats nowadays approximately 220,000 equivalent-inhabitants of wastewater. The WWTP effluent is released into the Bay of Vidy 700 m from the shore at 30 m depth. As a consequence, Vidy Bay is the most contaminated area of Lake Geneva. Published data document the accumulation of contaminants close to a recreational area and particularly around the WWTP discharge outlet.

Sediments are complex habitats densely colonised by diverse groups of microorganisms, which play key roles in biogeochemical cycling, aquatic food webs and the remobilization of heavy metals (Nealson, 1997; Lors et al., 2004; Ye et al., 2009). Many studies have been performed to examine the composition and variability of microbial communities in extreme or complex aquatic ecosystems. However, only a few studies compared microbial community structures in contaminated and uncontaminated sediments (Powell et al., 2003; Zhang et al., 2008). The aim of the present study was to compare the composition of the sediment-associated microbial communities in the contaminated Bay of Vidy with a closely less polluted site in the Ouchy area. The Bay of Vidy is currently used as a model system for several limnological, biogeochemical, and ecotoxicological studies. This research represents the first assessment of Bacteria and Archaea in contaminated and uncontaminated sediments of Lake Geneva and serves as important background information for these studies. For a better understanding of the microbial community structures, molecular analyses were complemented by a detailed physicochemical characterisation of the sediments.

2. Materials and methods

2.1. Study site description and sampling procedure

In August 2005, sediment was collected at two locations in Lake Geneva (Fig. 1): (i) within the Bay of Vidy near the outlet pipe of the WWTP of Lausanne (Swiss coordinates X: 534682, Y: 151410) and (ii) near the Ouchy area (Swiss coordinates X: 537985, Y: 150390). Sampling was done from R/V “La Licorne” using a core sampler (Benthos Inc, USA). Three cores (6.7 cm i.d., 1.5 m length) were retrieved from each site at a depth of 40 m. For microbiological analyses, the cores were opened longitudinally and sliced into 2 cm thick sections until 10 cm depth. The sediment samples were placed into sterile plastic containers, stored in an icebox and treated in the laboratory within 24 h. For chemical analysis, the intact sediment cores were transported to the laboratory and stored vertically in a cold-room at 4 °C until analysis.

2.2. Chemical analysis

Two cores per site were used for the chemical analyses: one was used to measure organic matter, nutrients and HMs contents and the other one was used to determine sulphur and iron concentrations and the porewater constituents.

Organic matter and nutrients: the cores were opened longitudinally and sliced every 2 cm down to a depth of 10 cm. Before analysis, sediment samples were air-dried at ambient room temperature. The particle grain size was measured with a laser Coulter® LS-100 diffractometer (Beckman Coulter, Fullerton, CA, USA), after a 5-min ultrasonic dispersal in deionised water according to the method described by Loizeau et al. (1994). The proportions of the three major size classes (clay < 2 µm; silt 2–63 µm; and sand > 63 µm) were determined from size distributions. Total organic matter content in sediments was estimated by loss on ignition (LOI) at 550 °C for 1 h in a Salvis oven (Salvis AG Emmenbrücke, Luzern, Switzerland) on 5 g of dried sediments. Total organic carbon (TOC) was determined by titrimetry following acid oxidation on 5 g of dried sediments. Total nitrogen (TN) was determined according to Kjeldahl (APHA, 1985) on 2 g of dried sediments. Total phosphorus (TP) and its different forms were measured on 150 mg of dried sediments with a spectrophotometer (Helios Gamma UV–Vis, Thermo Scientific, USA) at 850 nm, following the fractionation scheme of Williams et al. (1976) as modified by Burrell et al. (1990). The results are expressed in mg kg$^{-1}$ dry weight sediment (ppm).

Solid phases: sulphur and iron contents were determined every centimeter down to 10 cm depth. The sediment was sliced at room temperature in a N$_2$-filled glove bag and fixed in 50 mL tubes containing 10 mL zinc acetate solution (10%). Sulphide was extracted as acid volatile sulphur (AVS;
dissolved sulphide (H₂S) and iron sulphides (FeS₂) and chromium reducible sulphur [CRS; primarily pyrite (FeS₂), elemental sulphur (S₀), and some organic sulphur]. AVS and CRS were measured by a two step distillation process with cold 6 N HCl followed by boiling 1 M acidic CrCl₂ solution (Fossing and Jørgensen, 1989; Zopfi et al., 2008). Poorly crystalline Fe(III)oxides were extracted with 0.5M HCl according to Thamdrup et al. (1994).

Total contents of Cu, Cd, Cr, Zn, and Pb were determined by quadrupole-based Inductively Coupled Plasma—Mass Spectrometry (ICP—MS) (HP 4500, Agilent) following the digestion of 1 g of dried sediment in analytical grade 2 M HNO₃ (Pardos et al., 2004). Total Hg was quantified by atomic absorption spectrophotometry (Advanced Mercury Analyser; AMA 254, Altec, Czech Rep.) according to Hall and Pelchat (1997) and Ross-Barraclough et al. (2002). The method is based on sample combustion, gold amalgamation and atomic absorption spectrometry (AAS). Average values of triplicate measurements are expressed in mg kg⁻¹ dry weight sediment (ppm).

Porewater constituents: porewater was harvested by centrifugation (4000 rpm) under an N₂ atmosphere to avoid Fe²⁺ and H₂S oxidation. Samples for dissolved Fe²⁺ were acidified with 0.5 M HCl and analysed by the photometric Ferrozine method (Thamdrup et al., 1994). Dissolved sulphide was determined on Zn-acetate fixed samples using the colorimetric methylene-blue method (Cline, 1969; Zopfi et al., 2008). The major anions Cl⁻, SO₄²⁻, NO₃⁻, and PO₄³⁻ were determined by ion-chromatography on a DIONEX DX-120 system using an IonPac® AS14A anion exchange column, Na₂CO₃/NaHCO₃ (8 mM/1 mM) as eluent, an Anion Self-Regenerating Suppressor (ASRS® 300, 4 mm) module and a conductivity detector.

2.3. DNA extraction

The samples from 0 to 2 cm and 4—6 cm depth were used for microbiological analyses. Total DNA was extracted from 250 mg of sediment using the PowerSoil™ DNA Isolation Kit (Mo-Bio Laboratories, Carlsbad, CA, USA), according to the manufacturer’s instructions. The concentration of extracted DNA was measured spectrophotometrically (OD₆₀₀) and aliquots were used to estimate DNA quality by electrophoresis on 0.8% agarose gel stained with ethidium bromide (10 µg ml⁻¹, Sigma—Aldrich Chemie GmbH, Buchs, Switzerland). DNA extracts were stored at −20°C until used for PCR amplification.

2.4. PCR amplification

Nearly complete bacterial 16S rRNA genes were amplified using the primers 26f (5’-AGAGTTTGATCATGCTCA-3’) and 1392r (5’-GTGTGACGGGCGGTGTGTA-3’) (Brosius et al., 1981; Lane, 1991). Archaeal 16S rRNA genes were amplified using the primers 109f (5’-ACKGCTCAGTAACACGT-3’) and 915r (5’-GTGCTCCCCGGCACATTCTC-3’) (Görgkőpf et al., 1998; Stahl and Amann, 1991).

Both archaeal and bacterial PCR amplifications were performed separately using the Taq PCR Master Mix Kit (Qiagen,
Basel, Switzerland). Each PCR reaction was carried out in a volume of 50 μL, containing 1× PCR buffer, 1.5 mM of MgCl₂, 200 μM of each dNTP, 0.3 μM of each primer, 2.5 units of Taq DNA polymerase, 2.5 mg ml⁻¹ of bovine serum albumin (Invitrogen, Basel, Switzerland) and 2 μL of DNA (about 100 ng) of each sample. The following conditions were used for PCR amplification: initial denaturation at 94 °C for 5 min; 35 cycles of denaturation (94 °C for 30 s), annealing (52 °C for 30 s), extension (72 °C for 1 min) and a final extension step of 10 min at 72 °C. PCR products (10 μL) were separated by electrophoresis on 0.8% agarose gels and visualised by ethidium bromide staining and UV illumination.

2.5. Clone library construction and DNA sequencing

PCR products were purified using the NucleoSpin® Extract II Kit (Macherey–Nagel, Oensingen, Switzerland) according to the manufacturer’s instructions. The amplified DNA was then quantified using the PicoGreen® dsDNA Quantitation Reagent (Molecular Probes Inc., Eugene, OR, USA) and a TD-700 Fluorometer (Turner Designs Sunnyvale, CA, USA). Approximately 20–30 ng of amplified 16S rDNA were cloned into competent Escherichia coli cells using the TOPO TA cloning kit (Invitrogen, Basel, Switzerland) following the manufacturer’s recommendations. The transformed cells were plated on LB medium containing 50 mg L⁻¹ ampicillin, 60 mg L⁻¹ of IPTG (isopropyl-β-D-thiogalactoside), and 100 mg L⁻¹ of X-gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside), and incubated overnight at 37 °C. White recombinants were transferred to LB medium plates for 24 h. 80 and 40 recombinants were picked from samples taken at 0–2 cm and 4–6 cm, respectively, in each location, to constitute the Bacteria clone libraries. For the Archaea, only 2 recombinants (1 per site) were found at 0–2 cm and 34 colonies were picked from 4 to 6 cm. The insert size of all picked colonies, was determined by direct PCR using M13 forward and reverse primers included in the cloning kit. The products were subsequently purified with the NucleoSpin® Extract II Kit and sequenced using the BigDye® Terminator Cycle Sequencing Ready reaction kit (Applied Biosystems International, Inc., Rotkreuz, Switzerland). Sequences were obtained on an ABI PRISM® 310 Genetic Analyser, (Perkin Elmer, Schwerzenbach, Switzerland) using either the sequencing primer M13 for archaela clones or primer 27f for bacterial clones (Lane, 1991).

2.6. Phylogenetic analysis

The obtained sequences were checked and edited using the program EditSeq™ (DNAStar Inc., Madison, WI, USA). A chimera detection program was used to exclude chimeras (Maidak et al., 1999). NCBI BLAST (http://www.ncbi.nih.gov) was used to identify the most closely related 16S rRNA gene sequences. The partial 16S RNA gene sequences were then aligned in the Clustal W implementation of MEGA 3.0 (Kumar et al., 2004). The same program was used to produce neighbour-joining phylogenetic trees (Kimura-2 correction; bootstrap values for 500 replicates). The sequences were identified using the ribosomal database project classifier (Wang et al., 2007). Sequences from this study have been deposited with the EMBL database under accession numbers FN679050–FN679294.

The sequences were assigned to individual OTUs based on the 97% sequence similarity criterion. The number of OTUs and the rarefaction curves were estimated from the sequence data using Mothur v.1.12.3 (Schloss et al., 2009).

Comparison between the clone libraries from the two sites was done on the basis of genetic diversity by means of the parsimony test using Mothur v.1.12.3 (Schloss et al., 2009) and the the Fst-test using the program Arlequin, v.2.0 (Schneider et al., 2000).

A Mantel test using Spearman correlation (Manel, 1967) was applied to relate the microbial community compositions from the two sites with environmental variables. Multiple factor analysis (MFA) was done to obtain an integrative picture of the relationship between the bacterial community structures and the environmental factors across the 2 sites. The environmental parameters were separated into 2 matrices, one including organic matter contents and nutrient variables and one with heavy metal concentrations. The MFA allows the simultaneous ordination of a composite table obtained by the juxtaposition of the species and environmental datasets after weighting the different matrices (Escofier and Pages, 1994). The final ordination plot shows global points indicating the relative positions of the objects described by the combination of data-sets. Each global point is surrounded by partial points indicating the relative positions of the datasets taken separately (Escofier and Pages, 1994; Becue-Bertaut and Pages, 2008). These statistical analyses were carried out in “R”, a free software environment for statistical computing and graphics, using the Vegan library (R Development Core Team, 2005).

3. Results

3.1. Chemical analysis

Some general sediment characteristics including particle grain size, organic matter and nutrient contents are presented in Table 1. These sediments were mostly composed of silts,
approximately 65% and 80% for Vidy and Ouchy, respectively. Average organic matter contents in Vidy Bay sediments were much higher (21%) than in Ouchy sediments (4.5%). Average nutrient concentrations such as total nitrogen, ammonium and total phosphorus were also higher in Vidy sediments (12.6 ppm, 1.6 ppm and 8217 ppm, respectively) while they were considered low in the sediments from Ouchy (2 ppm, 0.5 ppm and 810 ppm, respectively). However, at both stations, no substantial differences in the nutrient contents between samples collected at 0–2 cm and 4–6 cm were observed.

Forewater concentrations of major ions, including Fe^{2+}, H$_2$S (=H$_2$S + HS$^-$ + S$^{2-}$) and SO$_4^{2-}$ are shown in Fig. 2. High concentrations of forewater PO$_4^{3-}$, reaching about 270 $\mu$M were only detected in the uppermost cm of Vidy Bay sediment samples (not shown). Nitrate was not detected in either one of the two cores. Forewater sulphate in the Ouchy sediment decreased from about 550 $\mu$M at the surface to 0 $\mu$M at 7 cm depth, whereas it was essentially depleted in the Vidy Bay sediment. Dissolved Fe$^{2+}$ concentrations were only high in Vidy sediment where they reached 260 $\mu$M. It is unlikely that under such Fe$^{2+}$-rich conditions free sulphide exists in forewater as it rapidly reacts with iron to form FeS. The measured forewater sulphide in the Vidy core represents thus mainly soluble FeS complexes or colloidal FeS phases. Minor amounts of poorly crystalline Fe(III)-oxides were only detected in the uppermost section of the Ouchy sediment core. Below 1 cm depth, and in the whole core from the Vidy Bay, HCl extractable Fe(III) was absent (Fig. 2). Differences in the iron-sulphur geochemistry exist between the two sites as indicated also by AVS and CRS data (Fig. 3). Unlike the Vidy sediments, solid phase sulphur species in the Ouchy sediments accumulated gradually with depth and reached a plateau at around 3 cm. Less reduced sulphur species such as S$^{2-}$, FeS$_2$, Fe$_3$S$_4$ may play a role as indicated by higher CRS contents. Iron contents in the Vidy sediments were about 50% higher than in the Ouchy site and a constant Fe/S ratio of 1 along the whole core suggests FeS as dominant phase.

The contents of heavy metals (in mg kg$^{-1}$) are reported in Table 2. All measured metal concentrations, except for Cr, were higher in the Vidy sediments, where peak concentrations reached 2.8 mg kg$^{-1}$ for Cd, 181.4 mg kg$^{-1}$ for Cu, 164.7 mg kg$^{-1}$ for Pb, and 2.3 mg kg$^{-1}$ for Hg.

### 3.2 Bacterial 16S rRNA gene clone libraries

At each site, sediment samples from 0 to 2 cm and 4–6 cm depth were used for clone library construction of bacterial 16S rRNA genes. Phylogenetic analysis showed that approximately 85% of the retrieved clones fell into known divisions with the rest remaining unclassified. Seven and twelve divisions were identified in the sediments from Vidy and Ouchy, respectively (Fig. 4). The dominant groups (Beta-, Gamma-, Delta-proteobacteria and Bacteroidetes) were found at both sites and all depths. Most sequences in the Bay of Vidy were affiliated with Betaproteobacteria (37%), Deltaproteobacteria (15%), Gammaproteobacteria (15%), and Bacteroidetes (23%). Also in the Ouchy sediments, most sequences were related to Betaproteobacteria (22%), Deltaproteobacteria (6%), Gammaproteobacteria (24%), and Bacteroidetes (12%).

The sequences were assigned to individual OTUs based on their phylogenetic positions and the 97% sequence similarity criterion. The sequences (n = 208) were grouped into 132 OTUs: 40, 26, 53, and 32 for Vidy 0–2 cm, Vidy 4–6 cm, Ouchy 0–2 cm, and Ouchy 4–6 cm, respectively (Table 3). The number of unique OTUs was higher in Ouchy than in Vidy sediments, 38% and 54% of the OTUs were exclusively found in Vidy and Ouchy, respectively. Only 8% of all OTUs were shared between the libraries from both sites. The OTU richness estimates based on rarefaction curves suggests a higher bacterial diversity for the sediments from Ouchy than from Vidy (Fig. 5).

By means of the parsimony test (P-Test) and the F$_{ST}$-test, a significant genetic differentiation was observed between the sediment bacterial communities of the two sites at all depths (0–2 and 4–6 cm), as well as between the two samples from Ouchy taken at different depths (Table 4). Significance for both tests signals less genetic diversity within each community than for two communities combined and that the different communities harbour distinct phylogenetic lineages (Martin, 2002).

### 3.3 Archaeal 16S rRNA gene clone libraries

All Archaea found in both sites fell into the Euryarchaeta division and most of them were from 4 to 6 cm of depth, except for 2 clones, which were retrieved from surface sediments: one from Vidy and one from Ouchy. The 36 obtained sequences were grouped into 18 OTUs: 1 OTU for Vidy 0–2 cm, 5 OTUs for Vidy 4–6 cm, 1 OTU for Ouchy 0–2 cm, and 12 for Ouchy 4–6 cm. A large proportion of these Euryarchaeta phylotypes, mostly originating from Vidy, were assigned to the methanogenic families Methanosetaeaceae and Methanomicrobiaceae (Fig. 9).

### 4 Discussion

Sediments were sampled in the area of Ouchy, a site close to the city of Lausanne but without known impact of contaminated water, and in the Bay of Vidy near the WWTP outlet pipe. The Bay of Vidy, contaminated with heavy metals, hydrophobic organic compounds and faecal bacteria, is currently the most polluted area of Lake Geneva (Pardos et al., 2004; Poté et al., 2008; Haller et al., 2009). Organic matter contents ranged from 18.7% (0–2 cm) to 23.7% (4–6 cm) and were much higher than in the Ouchy area (max. 5%) or...
Fig. 2 – Concentration profiles of porewater sulphate and sulphide in the sediments from Vidy and Ouchy (above). The concentrations of iron(II) in the porewater and contents of solid phase iron(III)oxides are shown below.
elsewhere in Lake Geneva (max. 5–8%) (Poté et al., 2008). The intense mineralisation of organic matter, indicated by high porewater phosphate, led to strongly reducing conditions in the Vidy sediments. Porewater sulphate was used up by sulphate-reducing bacteria and iron was essentially present as FeS. Due to the absence of any other significant oxidant, methanogenesis is suspected to be the major process for organic matter degradation at this site. The conditions were less reducing in the Ouchy sediments, where iron-reduction may have prevailed in the uppermost 1–2 cm of the sediment profile. Underneath, at 2.5 cm depth, the sediment was sulphidic due to the activity of sulphate-reducing bacteria. Below 7 cm depth, where sulphate was depleted, methanogenic conditions prevailed (Canfield and Thamdrup, 2009).

Heavy metal concentrations in the Bay of Vidy were up to six times higher than in Ouchy. According to the Canadian Sediment Quality Guidelines for the Protection of Aquatic Life (Conseil Canadien des Ministres de l’Environnement, 1999) the heavy metal concentrations in Vidy Bay were 2–8 times higher than reported PELs (probable effect levels). These results are in agreement with previous data from the same sampling site (Poté et al., 2008). In that survey, hydrophobic organic compounds, such as PAHs, PCBs and OCPs were investigated and concentrations of up to 2, 156 and 45 μg kg⁻¹ were determined, respectively. These values are considered high and above average levels for Lake Geneva (Corvi et al., 1986). It appears clearly that the sediments around the WWTP outlet pipe in the Bay of Vidy are heavily contaminated with many kinds of pollutants, possibly representing a significant source of toxicity for microbial communities and benthic organisms.

Results from this study indicate that the dominant phylogenetic groups at both sites were the Beta-, Gamma- and Delta-proteobacteria and Bacteroidetes, which is in agreement with 16S rRNA analyses of lake bacterioplankton (Zwart et al., 2002; Glöckner et al., 2000; Hiorns et al., 1997). Nevertheless, some differences between the two sites were observed in the relative proportion of the different Proteobacteria subdivisions. Proteobacteria accounted for 64% of the clones in Vidy and 55% of the clones in Ouchy. Proportions of Beta- and Deltaproteobacteria were much higher in Vidy Bay that in Ouchy sediments. In contrast, the Gammaproteobacteria were more abundant in Ouchy sediments (Fig. 4).

![Graph of depth profiles of acid volatile sulphur (AVS), chromium reducible sulphur (CRS), and total reducible sulphur (TRS) in the sediments from Vidy and Ouchy.](image_url)

**Fig. 3** – Depth profiles of acid volatile sulphur (AVS = H₂S + FeS), chromium reducible sulphur (CRS = S⁰ + FeS₂ + Fe₃S₄), and total reducible sulphur (TRS = AVS + CRS) in the sediments from Vidy and Ouchy.

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### Table 2 – Depth variation of heavy metal contents (mg kg⁻¹ dry weight sediment) in Vidy Bay and Ouchy sediments.

<table>
<thead>
<tr>
<th>Depth</th>
<th>Cu</th>
<th>Zn</th>
<th>Cd</th>
<th>Pb</th>
<th>Cr</th>
<th>Hg</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Ouchy</td>
<td>Vidy</td>
<td>Ouchy</td>
<td>Vidy</td>
<td>Ouchy</td>
<td>Vidy</td>
</tr>
<tr>
<td>0–2 cm</td>
<td>54.7</td>
<td>142.5</td>
<td>126.8</td>
<td>341.3</td>
<td>0.5</td>
<td>1.6</td>
</tr>
<tr>
<td>2–4 cm</td>
<td>68.3</td>
<td>135.9</td>
<td>155.1</td>
<td>327.8</td>
<td>0.7</td>
<td>1.5</td>
</tr>
<tr>
<td>4–6 cm</td>
<td>86.8</td>
<td>133.6</td>
<td>218.1</td>
<td>344.9</td>
<td>1.2</td>
<td>1.7</td>
</tr>
<tr>
<td>6–8 cm</td>
<td>135.5</td>
<td>181.4</td>
<td>305.5</td>
<td>446.8</td>
<td>1.7</td>
<td>2.3</td>
</tr>
<tr>
<td>8–10 cm</td>
<td>136.5</td>
<td>161.4</td>
<td>242.8</td>
<td>518.2</td>
<td>1.2</td>
<td>2.8</td>
</tr>
</tbody>
</table>

a Total variation coefficients for triplicate measurements were <15% for all elements.
Betaproteobacteria appear to be one of the major divisions present in freshwater systems (Zwart et al., 2002; Hahn, 2006). In this study they were also well represented in both locations (37% of clones in Vidy, 22% in Ouchy), particularly in surface sediments where they were dominant (Fig. 6). A prevalent group of bacteria in Vidy sediments, belonged to the Rhodocyclaceae, which are phenotypically and ecologically very diverse. Twenty clones of this family were affiliated to Dechloromonas, a genus containing many heterotrophic and facultative anaerobic chlorate-, perchlorate- or nitrate respiring bacteria (Wolterink et al., 2005). Members of this genus such as Dechloromonas aromatica are found in aquatic habitats and are capable of oxidising aromatic compounds such as toluene, benzene, and chlorobenzoate. D. aromatica is currently the only pure culture being able to degrade anaerobically benzene with nitrate as electron acceptor (Coates et al., 2001) A few clones, also found only in Vidy sediments, were related to Methylophilus (Methylophilaceae) a group of methylotrophic organisms. However, while methanol is oxidised as the sole carbon and energy source, some species may grow also on a limited range of other carbon compounds such as methylamines, formate, glucose, and fructose (Jenkins et al., 1987). Similar Methylophilaceae sequences were found in wastewater treatment pools in China (AY863077) and in freshwater calcareous mats (EF580978). The remaining Betaproteobacteria clones were found in both sites and included various genera. Several clones were affiliated to Propionivibrio (Rhodocyclaceae), which are aerotolerant or obligate anaerobic chemoorganotrophic bacteria. These bacteria are typical inhabitants of anaerobic, muddy freshwater sediments (Tanaka et al., 2003). Two further clones were related to Thiobacillus (Hydrogenophilaceae), which are ubiquitous in soils and sediments, and may grow on reduced sulphur compounds.

Compared to Betaproteobacteria, the Deltaproteobacteria subdivision (Fig. 7) was less abundant (15% and 6% of the clones in Vidy and Ouchy sediments, respectively). Most Deltaproteobacteria are sulphate-, iron- or proton-reducing (syntrophic) bacteria that play major roles in anoxic settings like meromictic lakes and sediments (Lehours et al., 2007; Karr et al., 2005). The large number of clones related to sulphate-reducing bacteria (Desulfobacteraceae) in Vidy Bay was not unexpected since sulphate consumption in the sediment was obvious from the porewater data. Moreover, the pool of total reduced sulphur (TRS) was quite high, especially in Vidy sediments. Several additional clones, only found in Vidy sediments, were related to Geobacter sp. (Geobacteraceae). These anaerobic bacteria have been isolated from freshwater sediments, soils and subsurface environments. They are traditionally considered chemooorganotrophic Fe(III)-reducing bacteria. However, most

<table>
<thead>
<tr>
<th>Table 3 – Distribution of Bacteria phylotypes in clone libraries from Vidy and Ouchy sediments (Lake Geneva). “A” stands for the 0–2 cm sediment section and “B” for the 4–6 cm sediment section.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vidy A</td>
</tr>
<tr>
<td>-------</td>
</tr>
<tr>
<td>Total no of sequences</td>
</tr>
<tr>
<td>Total no of OTUs</td>
</tr>
<tr>
<td>Percentage of unique OTUs</td>
</tr>
</tbody>
</table>

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<tr>
<th>Table 4 – Summary of F$_{ST}$ and P-test results for the comparison of microbial communities between Vidy and Ouchy sediments. “A” stands for the 0–2 cm sediment section and “B” for the 4–6 cm sediment section.</th>
</tr>
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<tbody>
<tr>
<td>Group</td>
</tr>
<tr>
<td>Vidy A vs Vidy B</td>
</tr>
<tr>
<td>Ouchy A vs Ouchy B</td>
</tr>
<tr>
<td>Vidy A vs Ouchy A</td>
</tr>
<tr>
<td>Vidy B vs Ouchy B</td>
</tr>
</tbody>
</table>

| a NS, not significant. |
Fig. 6 – Neighbour-joining phylogenetic tree of Betaproteobacteria 16S rRNA gene sequences retrieved from Vidy Bay and Ouchy sediments. “A” in clone names stands for the 0–2 cm sediment section and “B” for the 4–6 cm sediment section. Bootstrap values > 50% are shown (500 replicates). The scale bar represents 2% estimated sequence divergence.
species utilise a wide range of alternative electron acceptors, including NO$_3^-$, S$^0$, and other sulphur compounds (Coates et al., 1998). Similar sequences related to Geobacter sp. were retrieved from anthropogenically impacted urban creek sediment (EU284415) and from an aquifer where Fe(III) reduction was associated with aromatic hydrocarbon degradation (Nevin et al., 2005; AY653549). Another group of clones exclusively found in Vidy sediments belonged to the

Synthrophaceae, with one clone related to Smithella sp. Similar sequences were identified as core microorganisms involved in the anaerobic digestion of sludge (Riviere et al., 2009; CU922073).

The Gammaproteobacteria were more abundant in Ouchy sediments than in Vidy and were phylogenetically diverse (Fig. 8). Clones from both sites were affiliated to Methylobacter sp. (Methylcocccaceae), which are characterised by their

Fig. 7 – Neighbour-joining phylogenetic tree of Deltaproteobacteria 16S rRNA gene sequences from Vidy Bay and Ouchy sediments. “A” in clone names stands for the 0–2 cm sediment section and “B” for the 4–6 cm sediment section. Bootstrap values >50% are shown (500 replicates). The scale bar represents 2% estimated sequence divergence.
Fig. 8 — Neighbour-joining phylogenetic tree of Gammaproteobacteria 16S rRNA gene sequences from Vidy Bay and Ouchy sediments. "A" in clone names stands for the 0–2 cm sediment section and "B" for the 4–6 cm sediment section. Bootstrap values > 50% are shown (500 replicates). The scale bar represents 2% estimated sequence divergence.
Fig. 9 – Neighbour-joining phylogenetic tree showing 16S rRNA gene sequences of Archaea retrieved from Vidy Bay and Ouchy sediments. “A” in clone names stands for the 0–2 cm sediment section and “B” for the 4–6 cm sediment section. Bootstrap values >50% are shown (500 replicates). The scale bar represents 5% estimated sequence divergence.
specialised metabolism restricted to the oxidation of methane or methanol. Similar sequences were retrieved from a permafrost soil in Siberia (Liebner et al., 2009; EU124843) and an Arctic wetland soil (Wartiainen et al., 2006; AJ414655). Surprisingly, a few clones from Vidy sediments were related to phototrophic purple sulphur bacteria (Chromatiaceae) despite the fact that there is not much light to be expected at 30 m depth. However, some species can also grow under chemotrophic conditions in the dark, either autotrophically or heterotrophically using oxygen as terminal electron acceptor. Sequences similar to ours were retrieved from an anaerobic digestor (Riviere et al., 2009) and the sediment surface of Fayetteville Green Lake, USA (FJ437977). Several groups of Gammaproteobacteria clones remained unclassified but with similar sequences found in bacterioplankton communities of Lake Michigan (Mueller-Spitz et al., 2009. EU640647), river sediments (Li et al., 2008. EF590053), mangroves (EF125457) and agricultural soils (FJ444695).

A large number of sequences affiliated with the division Bacteroidetes (Cytophaga–Flexibacter–Bacteroidetes) were found in both sites, particularly in Vidy sediments. Bacteroidetes constitute the second largest group in Vidy sediments after their closest relatives were sequences found in freshwater lakes (Mueller-Spitz et al., 2009), anthropogenically impacted sediments, tundra soils (Liebner et al., 2008) and aquifers. Most of the sequences found in the 2 investigated sites remained unclassified. One clone found in Vidy sediments was affiliated to the genus Cytophaga sp. and one clone from Ouchy was related to the genus Flavobacterium sp.

A large proportion of the Euryarchaeota phylotypes, mostly retrieved from Vidy sediments, were related to methanogens like Methanoseta sp. (Methanosetaeae) and Methanomicrobiales (Fig. 9). A few species of Methanosaeta sp. have been isolated from anaerobic sewage digestors or sewage sludge (Zinder et al., 1984; Huser et al., 1982). Similar sequences to the clones found in this study, were retrieved from anaerobic sludge and a meromictic lake (Lehours et al., 2007). The rest of the archaeal sequences were only distantly related to any cultured species but similar to sequences retrieved from lake sediments (Pouliot et al., 2009; AY531743, EU782007), Arctic peat (AM712495) and the anoxic zone of a hydropower plant reservoir in the Brazilian Amazon (GU127420 and GU127500).

The two investigated sites differ clearly in terms of sediment chemical parameters and degree of pollution. It was therefore expected that the bacterial community composition would be different and reflect the differences in environmental conditions. Fig. 4 and Figs. 6–9 clearly show the diverse bacterial and archaeal lineages detected in the two sites. The results of the genetic diversity tests confirm the significant genetic differentiation of the sediment bacterial communities between the two sites at all depths. Seven and twelve divisions were identified in the sediments from Vidy and Ouchy, respectively. Among them Nitrospira, Planctomycetes, Verrucomicrobia and Gemmatimonadetes were only detected in Ouchy sediments. The apparent lower bacterial diversity in Vidy sediments may be explained by the high levels of a broad range of pollutants, which may induce adverse biological effects on microbial communities. The bacterial community composition changed with depth in the uncontaminated sediment. Conversely, no statistically significant variations were observed for the two sediment layers (0–2 and 4–6 cm) in the Bay of Vidy, which is explained by the high sedimentation rates and the non-consolidated nature of the sediment, permitting mobilisation and vertical mixing.

The microbial composition of both sites was correlated with the environmental variables, as shown by the Mantel correlation test (r = 0.9429, p = 0.044). This result suggests that the diversity of microbial communities may be affected by nutrients, organic matter as well as the degree of pollution. Many environmental variables are implicated, which is a situation inherent to all field studies. The Bay of Vidy is contaminated by all kinds of contaminants but also with high quantities of organic matter and nutrients. Under these conditions it is difficult to separate out the influence of the different environmental variables on microbial diversity and community composition. To learn more about the relative importance of individual environmental factors, microcosm studies will be required.

The integrative picture of the relationship between bacterial community structures and environmental factors at the two sites (Fig. 10) indicated that the sampling sites Ouchy and Vidy were clearly different with respect to both. Previous results already showed that the bacterial diversity in comparable contaminated and uncontaminated environments may differ significantly. The difference may be explained by the nature of pollution and a wide diversity of organic carbon

![Fig. 10 — The Multiple factor analysis (MFA) is a PCA-based technique allowing the simultaneous ordination of a composite table obtained by the juxtaposition of the species and the two environmental datasets, after weighting the different matrices. The superimposed representation shows one global point for each site, Vidy and Ouchy, at each depth (“A” stands for the 0–2 cm sediment section and “B” for the 4–6 cm sediment section). The three associated partial points correspond to the three datasets (microbial composition, organic matter and nutrients and heavy metals). The values on the axes indicate the percentage of total variation.](image-url)
sources (Sandaa et al., 1999; Sorci et al., 1999; Zhang et al., 2008). The polluted environment of Vidy Bay may have selected, among the dispersed microbes in sediments, certain functional bacterial communities which adapted to these conditions and became more dominant in that particular environment.

5. Conclusion

This is the first study reporting on the microbial community structures of Bacteria and Archaea in contaminated and uncontaminated sediments of Lake Geneva. Results show that the sediments of the two sites differed clearly in their organic matter and nutrient contents. Intense mineralisation of organic matter under sulphate-reducing and methanogenic conditions was indicated for the sediments from Vidy Bay. Furthermore, results confirm data of previous studies showing that the area around the WWTP outlet pipe in the Vidy Bay is heavily contaminated with various organic and inorganic pollutants. Phylogenetic analysis of sedimentary prokaryotes revealed that (i) archaeal and bacterial communities differed significantly between the contaminated and the non-contaminated sediments. (ii) For both sites, a correlation was observed between the microbial community structure and environmental variables suggesting that microbial diversity may be affected by nutrients, organic matter content and by the degree of pollution. (iii) Betaproteobacteria was the dominant bacterial group, representing more than 30% of the clones from surface sediments at both sites. (iv) A large proportion of Betaproteobacteria clones, mostly from Vidy sediments, were related to the reductively dechlorinating Dechloromonas sp. (iv) Consistent with geochemical data, Deltaproteobacteria including clones related to iron- (Geobacter sp.) and sulphate-reducing bacteria, were relatively more abundant in the contaminated sediments. (v) The archaeal communities were dominated by methanogenic Euryarchaeota, particularly in the organic matter-rich Vidy Bay sediments.

This study suggests that each site harbours a specific sediment microbial community. The apparent lower bacterial diversity in Vidy sediments may be explained by the significant concentrations of contaminants, which may induce adverse biological effects on benthic metazoans and microbes. However, given the long history of pollution in the bay, specific bacterial and archaeal communities may well have adapted to these particular conditions. Hence, more research on microbial community composition and specific activities of microorganisms inhabiting similar environments should be performed, in order to improve the understanding how pollution and eutrophication may affect microbial communities.

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