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# Bacterial communities in sediments of Lake Baikal from areas with oil and gas discharge

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ABSTRACT: Diversity and abundance of bacterial communities were investigated in methane hydrate- and oil-bearing sediments with different composition of pore waters at 6 sites in Lake Baikal, Siberia, using massively parallel sequencing (the Roche 454 platform). Sequences of *Proteobacteria* (17–48%), *Actinobacteria* (19–44%) and *Cyanobacteria* (17–48%) dominated the communities at all sites. Phylogenetic analysis of cyanobacterial sequences showed a large contribution of planktonic species and presence of uncultured lineages, whose representatives were detected in sediment communities of freshwater lakes. The ratio of different classes of *Proteobacteria* varied considerably: the *Epsilon-* and *Delta-* classes were the least represented, while the *Alpha-, Beta-* and *Gamma-* classes were the most represented. Some samples also showed a significant contribution of *Bacteroidetes* (7–13%), *Chloroflexi* (13%), candidate division OP10 and unclassified sequences (up to 16%). Other taxa made up less than 1%. Bacterial communities included a large number of unique phylotypes, whereas only few conventional phylotypes were detected. The phylotypes dominating the communities belonged to several taxa with cosmopolitan distributions. Their closest homologues involved liquid and gaseous hydrocarbons from deep sediments in biological cycles. Bacterial communities in Lake Baikal possessed moderate bacterial richness compared with other lake ecosystems.

KEY WORDS: Lake Baikal · Sediments · Methane hydrates · Oil and methane seeps · Mud volcano · Bacterial communities · Pyrosequencing



## INTRODUCTION

Bacterial communities play an important role in sediments of freshwater lakes by contributing to the transformation of organic and inorganic matters (Nealson 1997, Newton et al. 2011, Zhang et al. 2015). The composition of bacterial communities in lake sediments can be determined by a variety of environmental factors, such as sediment depth (Nam et al. 2008, Lim et al. 2011, Shivaji et al. 2011), salinity (Edlund et al. 2006, Dai et al. 2013), nutrients (Nelson et al. 2007), phosphorus (Zeng et al. 2009, Song et al. 2012), organic matter (Zeng et al. 2009), pH (Zeng et al. 2009, Xiong et al. 2012) and pollution (Haller et al. 2011). According to Zhang et al. (2015), total organic carbon (TOC), total nitrogen and water depth control the abundance of bacteria, while nitrate nitrogen is an important determinant of bacterial diversity. Recent findings suggest that fluctuations of key microbial taxa reflect the dynamics of important biogeochemical processes (McCalley et al. 2014, Ruff et al. 2015). Insights into environmental microbiomes have tremendously improved next-generation sequencing methods and global databases, which have advanced microbial ecology from the identification of rare members of microbial communities (Hugoni et al. 2013) to global microbial distribution patterns (Zinger et al. 2011, Sul et al. 2013).

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Molecular methods have been applied in recent studies of bacterial communities in bottom sediments of many lakes, including the North American Great Lakes (Winters et al. 2014), 13 freshwater lakes on the Yunnan Plateau, China (Zhang et al. 2015), Lake Geneva, Switzerland (Haller et al. 2011), and Lake Bourget, France (Billard et al. 2015). Unfortunately, little is known about the structure of bacterial communities in deep freshwater sediments.

Lake Baikal, Siberia, is the oldest and deepest lake in the world, with an estimated age of 30–40 million years and sedimentary deposits of over 7 km thick (Golmshtok et al. 2000). It is a rift lake with geological faults through which fluids (Golubev 1993, Pogodaeva et al. 2007, Granina 2008, Zemskaya et al. 2010) and hydrocarbon gases and oil (Kalmychkov et al. 2006, Khlystov et al. 2007, 2013) discharge to the surface from the deep zone of sediments. Moreover, the near-surface sediment layers in Lake Baikal contain gas hydrates (GHs) (Kuzmin et al. 1998, Klerkx et al. 2003). Fluids, oil, methane and other hydrocarbon gases in Lake Baikal can be a virtually unlimited source of carbon and energy for microorganisms providing transformation of hydrocarbons and their involvement in food webs. A wide range of geochemical parameters can ensure the existence of different microbial taxa, including unique ones detected by massively parallel sequencing in the archaeal and bacterial communities associated with hydrate-containing sediments near the St. Petersburg methane seep (Central Baikal) (Kadnikov et al. 2012). Ruff et al. (2015) reported that in marine environments, the key functional taxa varied in relative sequence abundance between different seeps due to environmental factors, sediment depth and seafloor temperature. In the present study, we deal with taxonomic diversity and structure of bacterial communities in the sediments of Lake Baikal, where oil- and gas-bearing fluids come from the deep zone of sedimentary strata. We also discuss the key bacterial taxa and their implied role in the degradation of hydrocarbons depending on the specific geochemical factors of the environment.

## MATERIALS AND METHODS

### Study areas

In total, 18 sediment samples were collected during 2006–2010 at 6 geomorphologically distinct sites: oil seeps at Gorevoy Utes (820–872 m water depth) and Tolsty (250 m) in Central Baikal, a low-temperature vent in Frolikha Bay in Northern Baikal (420 m), a methane seep Posolsk Bank (400 m), a mud volcano Malenky (~1 371 m) in Southern Baikal and a reference site near Barguzin Bay (Central Baikal, ~1061 m) (Fig. 1, Table 1). Direct observations from the deep-sea manned submersible 'MIR' revealed the discharge of methane and other gases at 5 sites, as well as liquid oil at Gorevoy Utes (Site 1) and Tolsty (Site 2). Layers of

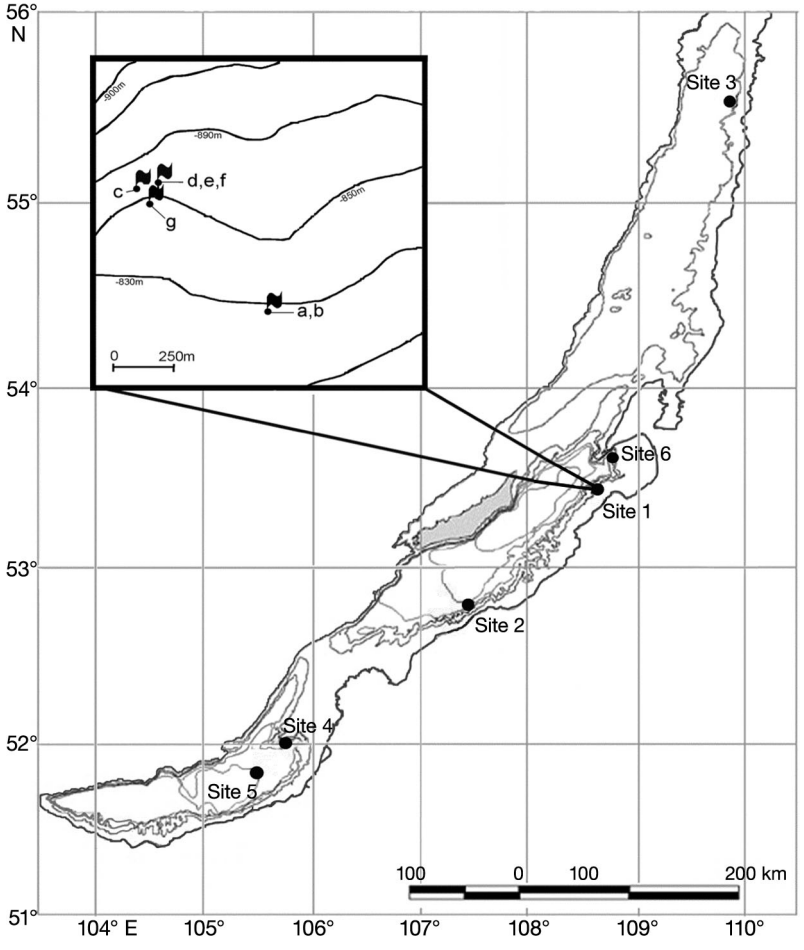


Fig. 1. Sampling sites in Lake Baikal. Based on the map presented De Batist et al. (2002). Oil seeps, Site 1: Gorevoy Utes (53.30° N, 108.39° E), Site 2: Tolsty (52.38° N, 107.21° E); a low-temperature vent, Site 3: Frolikha Vent (55.30° N, 109.45° E); methane seep, Site 4: Posolsk Bank (52.03° N, 105.84° E); mud volcano, Site 5: the Malenky (51.92° N, 105.63° E); a reference site, Site 6: Barguzin Bay (53.26° N, 108.35° E). Sampling sites (Samples 1a to g) studied in different years (Table 1) are indicated by flags in the oil seep Gorevoy Utes

Table 1. Characterisation of the studied sites and the chemical composition of pore water in the sediments. TOC: total organic carbon, $Fe_{tot\ diss}$: total dissolved Fe, $\Sigma_i$: total ions, nd: not detected, –: no data, GC: gravity cores, BC: benthic cores, Gr: grab sampler, GH: gas hydrate

| Date of sampling (mo-yr), sediments, sampler | Water depth (m) | Sample ID | Concentration (mg $l^{-1}$) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | TOC (%) | $HCO_3^-$ | $CH_3COO^-$ | $Cl^-$ | $NO_3^-$ | $SO_4^{2-}$ | $Fe_{tot\ diss}$ | $Na^+$ | $K^+$ | $Ca^{2+}$ | $Mg^{2+}$ | $\Sigma_i$ |
| **Site 1: Gorevoy Utes** | | | | | | | | | | | | | | |
| 07-2006 (0–1 cm) thin oxic silt, GC | 820 | 1a | 2.71 | 183 | 0.8 | 0.6 | nd | nd | 4.7 | 15 | 3 | 39 | 4 | 250 |
| 07-2006 (180–190 cm) anoxic diatomic clay, GC | 820 | 1b | 0.97 | 108 | 0.5 | 0.2 | nd | nd | 3.8 | 7 | 2 | 25 | 3 | 150 |
| 08-2007 (0–1 cm) thin oxic silt, oil, Gr | 872 | 1c | 3.95 | 76 | 1.2 | 2.2 | 0.7 | 1.3 | 5.9 | 4.8 | 2.1 | 18 | 2.1 | 114 |
| 08-2007 (0–1 cm) thin oxic silt, GC | 872 | 1d | 2.73 | 52 | 4.4 | 3.4 | 0.5 | 5.6 | 5.3 | 5.4 | 2.9 | 12 | 2,2 | 94 |
| 08-2007 (70–75 cm) anoxic clay, GH monolith with oil inside, GC | 872 | 1e | 98.4 | 68 | 1.1 | 0.7 | nd | 1.1 | 3.6 | 5.7 | 1.6 | 17 | 2.9 | 102 |
| 08-2007 (115–116 cm) anoxic diatomic clay, oil, GC | 872 | 1f | 1.24 | 126 | 5.2 | 0.44 | 0 | 0.9 | 2.3 | 7.2 | 3.3 | 26 | 4.1 | 175 |
| 08-2008 (0–1 cm) thin oxic silt, 'MIR' (submersible) | 841 | 1g | 2.12 | 97 | 17 | 1,5 | nd | 3.3 | 1.8 | 4.6 | 2.4 | 22 | 0.83 | 150 |
| **Site 2: Tolsty** | | | | | | | | | | | | | | |
| 08-2007 (0–1 cm) thin oxic silt, detritus, thin oil films, BC | 250 | 2a | 9.24 | 64 | 1.3 | 4.1 | 0.5 | 1.9 | 6.6 | 4.7 | 2.5 | 12 | 0.62 | 98 |
| 08-2007 (10–15 cm) thin oxic silt, detritus, thin oil films, BC | 250 | 2b | 8.10 | 79 | 0.0 | 3.8 | 0.6 | 0.2 | 8.1 | 5.4 | 3.8 | 13 | 0.52 | 114 |
| **Site 3: Frolikha Vent** | | | | | | | | | | | | | | |
| 07-2010 (0–1 cm) bacterial mats, light-brown silty oxic sand, 'MIR' | 420 | 3 | 1.00 | 167 | 54 | 12 | 0.1 | 16.8 | 2.5 | 29 | 18 | 46 | 10 | 355 |
| **Site 4: Posolsk Bank** | | | | | | | | | | | | | | |
| 08-2010, (0–1 cm), bacterial mats, anoxic diatomic clay, GC | 400 | 4 | 2.68 | 49 | 3.1 | 1.1 | 0.3 | 62 | 0.3 | 10 | 6.1 | 25 | 5.0 | 162 |
| **Site 5: Malenky** | | | | | | | | | | | | | | |
| 09-2010 (0–1 cm) anoxic black coarse sand, GC | 1371 | 5a | 0.29 | 131 | 0 | 0.7 | 0 | 90 | 0.1 | 7.2 | 4.7 | 51 | 5.6 | 290 |
| 09-2010 (60–65 cm) anoxic diatomic clay, hydrotroilite, GC | 1371 | 5b | 1.32 | 227 | 0 | 0.5 | 0 | 46 | 0.5 | 5.54 | 3.2 | 52 | 13 | 348 |
| 09-2010 (130–131cm) anoxic diatomic clay, hydrotroilite, above GH layer, GG | 1371 | 5c | 1.18 | 247 | 0 | 0.5 | 0 | 33 | 1.1 | 5.8 | 3.6 | 57 | 13 | 361 |
| 09-2010 (132–139 cm) GH layer with anoxic clay interlayers inside, GC | 1371 | 5d | 0.97 | 85 | 0 | 0.5 | 0 | 122 | 0.2 | 6 | 3.9 | 51 | 9.6 | 278 |
| 09-2010 (140–146 cm) anoxic clay with ologonite inside, GC | 1371 | 5e | 1.24 | 163 | 0 | 0.6 | 0 | 363 | 0.2 | 7.8 | 7.0 | 155 | 19 | 716 |
| 09-2010 (145 cm) oligonite–Fe(Mn,Zn) $(CO_3)_2$[a], GC | 1371 | 5f | 1.12 | 526 | 0 | 0.6 | 0 | 114 | 2.2 | 11,5 | 9.2 | 165 | 16 | 845 |
| **Site 6: Barguzin Bay** | | | | | | | | | | | | | | |
| 08-2008 (0–1 cm) oxic silt, GC | 1061 | 6 | 0.73 | 49 | nd | 1.6 | 0.6 | 5.2 | 0.5 | 4.4 | 2.6 | 12 | 0.97 | 77 |
| Baikal water (Grachev et al. 2004) | | | | 67 | – | 0.4 | 0.1 | 5.3 | – | 3.5 | 0.9 | 16 | 3 | 96 |

[a]Oligonite – Fe(Mn, Zn)$(CO_3)_2$. A.I. Sapozhnikov (A.P. Vinogradov Institute of Geochemistry SB RAS) detected the structure of the carbonate using the JCXA733 microprobe

methane hydrates occurred in the sediments of the mud volcano Malenky (Sample 5d, See Table 1) and the oil seep Gorevoy Utes (Sample 1e). A reference site was taken in an area with normal sedimentation (Site 6. Fig. 1). Chemical composition of pore waters in sediments at the reference site corresponded to Baikal water (Grachev et al. 2004). Bottom sediments were collected using benthic (BC) and gravity corers (GC), and an ocean grab (Gr) sampler from board the RV 'Vereshchagin', as well as with handlers of the 'MIR' submersibles (MIR-1 and MIR-2) during the 2008–2010 dives.

Sediment samples lifted on board were immediately packaged in sterile foil and put into liquid nitrogen until the laboratory analysis. DNA was isolated in the laboratory immediately after the fieldwork; it was then stored at −70°C. Pore waters were centrifuged on board directly after sampling. Chemical analysis of pore waters was carried out in the laboratory according to previously reported methods (Zemskaya et al. 2010). TOC was measured with a VARIO TOC/TNB cube (ELEMENTAR Analysensysteme).

### DNA extraction and metagenomic sequencing

Total DNA was isolated from the bottom sediments by phenol–chloroform extraction (Shubenkova et al. 2005). The V3 region of the 16S rRNA gene was amplified using the universal primers U341f (CCT ACG GGR SGC AGC AG) and U515r (TTA CCG CGG CKG CTG VCA C) (Kadnikov et al. 2012) in an automated thermocycler BIS-M 111 (BIS-N). Amplicons were made in 4 technical replicates of 20 µl and pooled in 1 sample to justify the potential PCR-related stochastic bias. Pyrosequencing was performed on a GS FLX 454 genome sequencer (Roche) using titanium chemistry according to the manufacturer's recommendation.

### Pyrosequencing data analysis

Metagenomic analysis of taxonomic composition and diversity of microbial communities from bottom sediments was carried out using MOTHUR v.1.31.2 (Schloss et al. 2009). The sequences of the 16S rRNA gene fragments underwent conventional filtration selecting the reads which did not contain ambiguous nucleotides. The obtained sequences were processed by the PyroNoise algorithm (Quince et al. 2011) to remove sequencing errors; sequences longer than 100 bp with homopolymer tracts less than 6 bp were then selected and aligned with the bacterial 16S rRNA gene sequences from the SILVA database (www.mothur.org/wiki/Silva_reference_files). The NAST algorithm with a k-mer length of 8 bp was employed for sequence alignment. Sequences shorter than 130 bp that did not map to the V3 region of the 16S rRNA gene (positions 6428–11 892 relative to the initial SILVA alignment) were excluded from further analysis. Chimeric sequences were detected using the UCHIME algorithm (Edgar et al. 2011) with standard parameters. Nucleotide sequences of the 16S rRNA were deposited in the NCBI Short Read Archive (SRA id: SRP045338).

When calculating the genetic distance matrix, multiple insertions or deletions represented by consecutive gaps were assumed to be the result of a single mutation event. Sequence clustering was based on UPGMA analysis of genetic distances. After clustering, the operational taxonomic units (OTUs) containing only 1 sequence upon clustering at a genetic distance level of 0.03 (singleton OTU 0.01) were discarded.

Taxonomic complexity of the community was assessed using rarefaction implemented in MOTHUR by the analysis of curves depicting dependence of the number of detected phylotypes (i.e. clusters) on the number of analysed sequences for the 1, 3 and 5% genetic distances (see Fig. S1 in the Supplement, available at www.int-res.com/articles/suppl/a76p095_supp.pdf). To characterise the molecular-genetic diversity, we calculated the observed OTUs: species richness (*S*), Good's coverage, Chao1 and ACE indices, and Simpson's inverse index at a genetic distance level of 0.03. Comparison with the known 16S rRNA sequences was performed using BLASTN (Altschul et al. 1990). The composition of bacterial communities in relation to specific chemical parameters was analysed by non-metric multidimensional scaling and plotted with Grapher 9. Venn diagrams were generated using MOTHUR to show both unique and shared OTUs in each sample.

Taxonomic identification of the $OTU_{0.03}$ representative sequences was performed by comparison of these 16S rRNA sequences in several databases: SILVA (http://arb-silva.de), Greengenes, RDP, and NCBI, using BLASTN. When the detected 16S rRNA sequence of a validated microorganism had more than 97% homology, the cluster was assigned to the corresponding genus. Nucleotide sequences for molecular phylogenetic analysis were aligned using CLUSTALW. Phylogenetic trees were constructed by the neighbour-joining (NJ) and Kimura 2-parameter methods implemented in MEGA version 4.0 (Tamura et al. 2007).

## RESULTS

### Sediment pore water chemistry

Table 1 shows the chemical composition of pore waters in the sediments. At the reference site (Site 6), the chemical composition and total ions ($\Sigma_i$) of pore waters were consistent with the values identified in lake sediments with a normal sediment rate, and their chemical composition was similar to Baikal water (Grachev et al. 2004). TOC content was 0.73%.

The samples from the oil seep (Site 1) showed the most significant variations of TOC, $\Sigma_i$ and composition of the major ions. Pore waters of Samples 1a and 1b were rich in bicarbonate, calcium, sodium and iron ions, with $\Sigma_i$ reaching 150 to 250 mg $l^{-1}$, which is higher than values at the reference site. Analysis of the samples collected at the same coordinates in 2007 (Samples 1c–f), indicated that $\Sigma_i$ of pore waters varied from 94 to 175 mg $l^{-1}$ and was generally comparable to or slightly higher than the values obtained for the reference site. Notably, bicarbonate and acetate ions in Sample 1f, and chlorine and total dissolved Fe ($Fe_{tot\ diss}$) ions in Sample 1c were high. In the latter sample, oil covered the surface and was observed in the sediments to a depth of 50 cm. In most samples, TOC content ranged from 1.24 to 3.95%, reaching 98.4% in the oil-saturated Sample 1e (Table 1).

In Sample 1g from the area with bitumen structures (Khlystov et al. 2007), TOC was 2.12%; $\Sigma_i$ of pore waters was 150 mg $l^{-1}$ due to the increased concentrations of bicarbonate, calcium, sodium and potassium ions, as well as acetate and $Fe_{tot\ diss}$ (Table 1). The chemical composition of pore waters from another oil seep (Samples 2a and 2b) was not substantially different from that of the reference site, except for the slightly increased content of bicarbonate, chlorine and $Fe_{tot\ diss}$ ions, as well as the estimated TOC of 9.24% in Sample 2a and 8.10% in Sample 2b.

Pore waters of Site 3 had high $\Sigma_i$ values (355 mg $l^{-1}$), which is typical of the sediments in this area (Granina 2008, Zemskaya et al. 2012). Concentrations of practically all ions (i.e. bicarbonate, chloride, acetate, sodium, calcium and magnesium ions) were high when compared to the reference site, while TOC content was 1%.

The sample from Site 4, collected in the zone of microbial mats, mainly consisted of filamentous bacteria which are morphologically similar to representatives of the genus *Thioploca* from the bacterial mats of the Frolikha Vent (Namsaraev et al. 1994). The chemical composition of pore waters of this sample in the first 10 cm of sediments showed increased values of sulphate (up to 62 mg $l^{-1}$) and acetate ions (3.1 mg $l^{-1}$), and TOC content was 2.68%.

All samples from Site 5 also had high $\Sigma_i$ (Table 1) and almost the same TOC content (0.97–1.32%), except for the surface layer, which had a TOC of 0.29%. Sulphate concentrations in pore waters ranged from 33 mg $l^{-1}$ (Sample 5c) to 363 mg $l^{-1}$ (Sample 5f). The $\Sigma_i$ of the latter sample reached 843 mg $l^{-1}$. The major cations of pore waters in this core were calcium ions (up to 165 mg $l^{-1}$ vs. 12 mg $l^{-1}$ at the reference site) and magnesium ions (up to 19 mg $l^{-1}$ vs. 0.97 mg $l^{-1}$ at the reference site). Such concentrations of some ions are typical for zones with the most intense fluid discharge in this area (Zemskaya et al. 2010).

### Diversity of 16S rRNA gene sequences

Pyrosequencing of the V3 region of the 16S rRNA gene yielded 135 148 sequences. After initial filtering, alignment of reads and removal of chimeric sequences and contaminants, we analysed 99 801 reads with an average length of 150–155 bp. The number of sequences varied from 2310 to 10 895 per sample (Table 2).

Analysis of taxonomic complexity (Chao1 index), including the rarefaction curves constructed for genetic distances at 1, 3 and 5% (Fig. S1), showed that all rarefaction curves in the observed data sets did not reach the plateau, likely due to insufficient sequencing, which impairs the completeness of diversity characteristics of communities, and sequencing errors that were not removed during the filtration. Based on the results of the rarefaction analysis, we conclude that the sampling conditions for Samples 5d and 1b differed from those for other communities. In other samples, the rarefaction curves were close together, indicating similar sampling and sequencing conditions, and a possible similarity between the ratios of reads from different phylotypes in the examined communities. Notably, the majority of the sequences in the analysed libraries were highly similar (99 and 100%) to the 16S rRNA of uncultured bacteria, which complicated their identification even at the genus level. Good's coverage values varied from 67.9% (Sample 1d) to 97.7% (Sample 5d, Table 2), confirming the incomplete assessment of taxonomic diversity.

The number of $OTUs_{0.03}$ in the samples ranged from 116 to 1681 (Table 2). The highest values of Chao, ACE (richness) and Simpson's inverse index (diversity) were recorded in Samples 1d and 2b from the oil seeps (Table 2). Rarefaction curves constructed from $OTU_{0.03}$ (Fig. S1) showed species variability and rich-

Table 2. Sample coverage, species richness and species diversity indices. OTU: operational taxonomic unit. Parentheses: lower and upper confidence limits, respectively

| Sample ID | Number of sequences | Genetic distance for OTU clustering: 0.03 | | | | | |
|---|---|---|---|---|---|---|---|
| | | Good's coverage | Number of OTUs | Shannon | ACE | Chao1 | Simpson's inverse index |
| 1a | 9846 | 93.4 | 1047 | 4.7 (4.6, 4.8) | 4949 (4611, 5319) | 2707 (2358, 3149) | 36.0 (34.6, 37.4) |
| 1b | 10205 | 94.1 | 1024 | 4.9 (4.8, 4.9) | 4083 (3801, 4393) | 3163 (2663, 3813) | 38.9 (37.1, 41.0) |
| 1c | 2310 | 79.7 | 691 | 5.1 (5.1, 5.3) | 3316 (3028, 3641) | 1806 (1536, 2155) | 42.3 (38.0, 47.7) |
| 1d | 3642 | 67.9 | 1681 | 6.8 (6.8, 6.9) | 7985 (7504, 8505) | 4497 (4047, 5031) | 379.7 (338.4, 432.5) |
| 1e | 7344 | 96.6 | 407 | 2.7 (2.6, 2.7) | 1745 (1554, 1967) | 1142 (905, 1491) | 4.2 (4.0, 4.3) |
| 1f | 10895 | 96.3 | 637 | 3.3 (3.3, 3.4) | 3422 (3127, 3752) | 1793 (1486, 2212) | 5.9 (5.7, 6.2) |
| 1g | 5262 | 93.0 | 610 | 3.7 (3.6, 3.8) | 2329 (2122, 2564) | 1360 (1167, 1619) | 7.9 (7.4, 8.4) |
| 2a | 3397 | 86.3 | 652 | 4.3 (4.2, 4.4) | 3771 (3412, 4176) | 2035 (1687, 2499) | 17.2 (16.0, 18.7) |
| 2b | 7236 | 90.3 | 1069 | 5.3 (5.2, 5.3) | 7232 (6758, 7746) | 3381 (2884, 4015) | 62.2 (58.9, 65.9) |
| 3 | 5338 | 93.6 | 551 | 4.0 (3.9, 4.0) | 2370 (2145, 2629) | 1281 (1085, 1547) | 15.7 (14.9, 16.6) |
| 4 | 4197 | 93.9 | 395 | 3.4 (3.3, 3.4) | 1795 (1593, 2031) | 995 (811, 1260) | 10.1 (9.6, 10.7) |
| 5a | 4253 | 92.8 | 481 | 3.6 (3.5, 3.6) | 2226 (2001, 2485) | 1305 (1064, 1645) | 7.9 (7.4, 8.4) |
| 5b | 3867 | 94.4 | 386 | 4.0 (3.9, 4.0) | 1385 (1237, 1558) | 874 (716, 1109) | 16.0 (15.0, 17.1) |
| 5c | 6310 | 91.8 | 776 | 3.8 (3.7, 3.9) | 4078 (3755, 4437) | 2089 (1785, 2484) | 10.2 (9.7, 10.7) |
| 5d | 2780 | 97.7 | 116 | 2.2 (2.2, 2.3) | 379 (305, 483) | 250 (183, 385) | 5.0 (4.8, 5.3) |
| 5e | 2566 | 94.0 | 274 | 3.7 (3.6, 3.7) | 957 (835, 1104) | 647 (507, 870) | 12.0 (11.1, 13.1) |
| 5f | 3966 | 88.7 | 804 | 5.4 (5.4, 5.5) | 2294 (2114, 2497) | 1635 (1442, 1887) | 72.2 (64.4, 79.1) |
| 6 | 6387 | 92.6 | 800 | 4.9 (4.8, 4.9) | 3281 (3033, 3557) | 2102 (1852, 2665) | 38.7 (36.5, 41.2) |

ness in these samples. The communities in the sediments containing GHs (Sample 5d) showed a minimal number of phylotypes (OTU: 116 and Chao1: 250) and low diversity indices, while the communities from carbonate-bearing sediments (Sample 5f) had the maximum values (OTU: 804 and Chao1: 1635). Bacterial communities in some samples from oil seeps had the same taxonomic complexity as from the reference site (OTU: 800 and Chao1: 2102), whereas the samples from bacterial mats (Sites 3 and 4) had lower taxonomic complexity indices (OTU: 551 and 395; Chao1: 1281 and 995).

Venn diagrams were constructed for bacterial communities in the surface (Fig. 2a,c) and subsurface (Fig. 2b,d,e) sediments, as well as in the sediment layers from different depths (Fig. 2b,d,e). At all sites, we recorded many unique sequences (Fig. 2). Analysis of the libraries of the 16S rRNA gene sequences from the different layers of sediments from oil seeps in different years (Fig. 2a,b) showed that 46 to 80% of the communities consisted of unique OTUs and only 1 to 2% were shared. The analysis of communities from the reference site (Site 6), oil seep site (Sample 1g) and bacterial mats (Sites 3 and 4) also indicated a majority of unique OTUs (60 to 70%), and only 2% of shared OTUs (Fig. 2c). Communities at Samples 5a, b, d and f (Fig. 2d) contained 4% of shared OTUs, while unique OTUs ranged from 44 to 71%. The comparison of 16S rRNA gene libraries of Samples 5c and 5e to the communities from above and below these samples (Fig. 2e) indicated that 0.5% were shared OTUs and from 51 to 85% were unique. Composition analysis of the unique OTUs revealed that they are normally represented in all libraries by a small number of sequences, whereas the sequences comprising shared OTUs were numerous in the majority of the reads. These are members of *Cyanobacteria* (subsection I), *Actinobacteria*, marine group (family *Acidimicrobiaceae*), hgcI clade (family *Sporichthyaceae*), *Bacteroidetes* (genus *Flavobacterium*), *Alphaproteobacteria* (clade SAR11, family *Acetobacteraceae*, family *Sphingomonadaceae*), *Betaproteobacteria* (genus *Rhodoferax,* family *Methylophilaceae*), *Gammaproteobacteria* (family *Methylococcaceae*), *Verrucomicrobia* (genus *Opitutus*) and unclassified sequences, which were found in all analysed communities.

## Community analysis

Fig. 3a,b shows different taxa in bacterial communities of the samples. Representatives of 3 phyla, *Proteobacteria* (36%), *Actinobacteria* (21%), *Chloroflexi* (13%) and unclassified sequences (9%) dominated the community of surface sediments from the reference site. *Acidobacteria*, *Firmicutes, Chlorobi*, candidate division, *Gemmatimonadetes*, BHI80-139, *Caldiserica, Chlamydiae, Deferribacteres, Deinococcus-Thermus, Fibrobacteres, Lentisphaerae, Fusobacteria, Spirochaetes*, TA06 and *Planctomycetes* were minor.

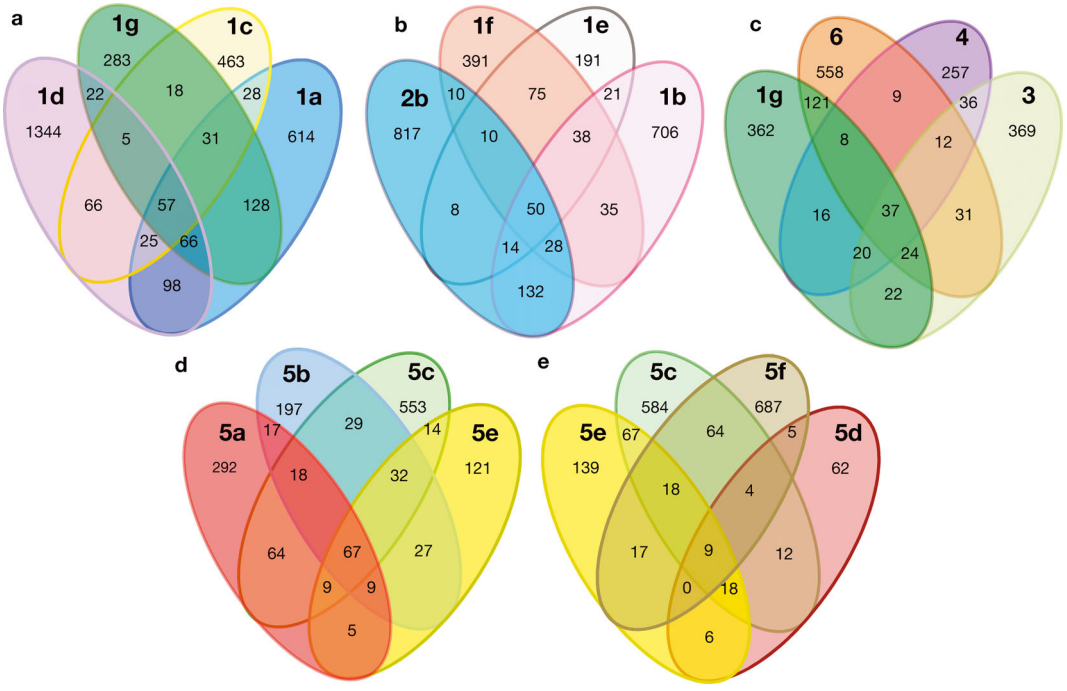


Fig. 2. Unique and shared bacterial operational taxonomic units (OTUs, at a distance of 0.03) in the bacterial communities: (a) surface sediments from Site 1, (b) subsurface sediments from Sites 1 and 2, (c) surface samples from Sites 1, 3, 4 and 6, and (d,e) samples in different layers of Site 5. See Fig. 1 for site locations

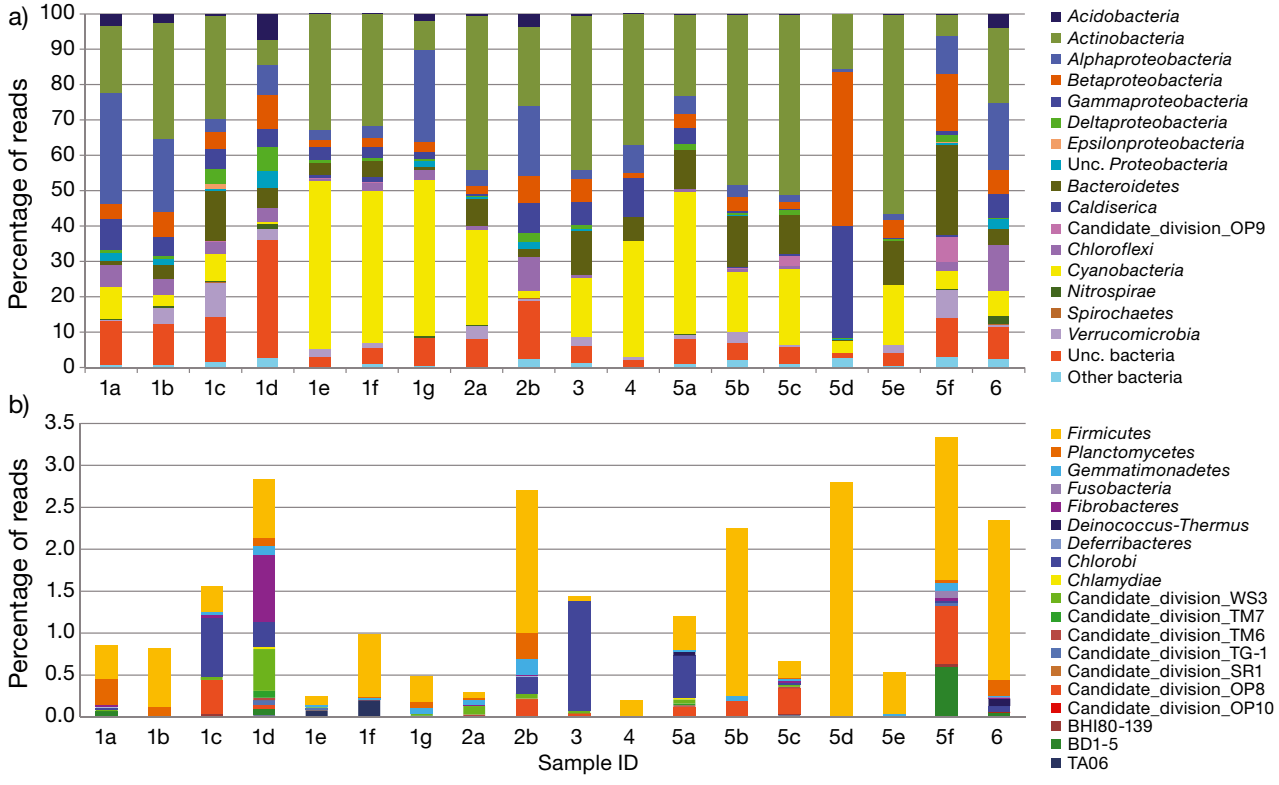


Fig. 3. Taxonomic composition of bacterial communities from bottom sediments: (a) >96.5% reads; (b) <3.5% reads. Based on data from a metagenomic analysis of the V3 loop of the 16S rRNA gene and bacterial classification from the SILVA database with a confidence threshold of 80%. Unc.: unclassified. See Table 1 for definitions of Sample IDs

*Acidimicrobiaceae* (marine group) and *Sporichthyaceae* (hgcl clade) sequences represented *Actinobacteria*; sequences of the family *Propionibacteriaceae* and the order *Rubrobacteridae* were less represented. Their closest relatives have been registered in different rivers, lakes, snow, sediments and soils worldwide (Table S1 in the Supplement). *Cyanobacteria* dominating among other communities were observed in 7%, representing a small range of taxa. Their closest homologues were mainly assigned to uncultured bacteria, and they were found in communities of Baikal sponges and biofilms of Lake Taihu (China) and Lake Michigan (USA) (Table S2). Only this sample (Site 6) indicated a small number of sequences of *Cyanobium* sp., which are planktonic species (Belykh et al. 2011). Notably, the bulk of the *Cyanobacteria* sequences in all of the examined 16S rRNA gene libraries were closest to the planktonic members of the genus *Synechococcus* (Fig. S2).

In the community of the sample from the reference site, *Alphaproteobacteria* dominated (19%) and were mainly (5%) related to the uncultured *Xanthobacteraceae*. The number of *Deltaproteobacteria* detected was minor, with a predominance of unclassified sequences of the order *Syntrophobacterales.* The families *Comamonadaceae* and *Methylophylaceae* (4%), as well as unclassified sequences (2%) mainly represented *Betaproteobacteria* (7%). *Gammaproteobacteria* in the community comprised 7%. Of these, 3% were assigned to unclassified representatives of the order *Methylococcales.* Interestingly, the representatives of *Anaerolineaceae* identified in the community of the reference site had homologues in the community of the cold seep of the mud volcano (Maignien et al. 2013).

Similar to those from other types of geological structures, the communities from oil seeps (Sites 1 and 2) were dominated by representatives of 3 phyla: *Actinobacteria*, *Cyanobacteria* and *Proteobacteria.* In the surface sediments, they comprised 19–44, 44 and 33–48%, and in the reduced sediments they represented 22–33, 43–48 and 36–41%, respectively. In addition, the community of Sample 2b contained many unclassified sequences (16%, Fig. 3a). The closest homologues of *Actinobacteria* in bacterial communities of the oil seeps included the sequences from bottom sediments and the water column of marine and freshwater ecosystems, permafrost soils, communities of Baikal sponges, and other environments (Table S1). Phylogenetic analysis of *Actinobacteria* sequences from the sediments of different geological structures demonstrated their maximum diversity in the sediments of the oil seep Gorevoy Utes (Site 1).

Ratios and composition of different *Proteobacteria* classes in sediments at oil seeps varied significantly. In Sample 1a, *Alphaproteobacteria* constituted 32%, while in Sample 1c collected at the same coordinates, they were only 4%. All samples had various proportions of the *Xanthobacteraceae, Acetobacteraceae, Methylocystaceae*, SAR11 and unclassified sequences. Some sequences of *Alphaproteobacteria* were highly similar to representatives of the genus *Pelagibacter*, which are widespread in fresh and salt waters. They are likely to dominate in ecosystems that are poor in nutrients (Giovannoni et al. 1990, Morris et al. 2002, Tripp 2013).

*Betaproteobacteria* and *Gammaproteobacteria* ranged from 1 to 10 and 0.3 to 9%, respectively, in the surface sediments of the oil seeps. For *Betaproteobacteria*, the most representative were the sequences of the following taxa: *Variovorax, Rhodoferax*, *Methylophilaceae* (1%), sequences TRA3-2 0 (2%), *Nitrosomonodaceae* family (0.5%), and representatives of unclassified lineages. Among the latter, *Methylococcales* (2–8%) dominated, while in some samples, unclassified sequences prevailed (2%).

*Deltaproteobacteria* in communities from the surface sediment layers varied from 0.3 to 7%, and in the lower layers from 0.7 to 2%. There were mainly sequences of the orders *Syntrophobacterales, Myxococcales* and *Desulfobacterales*, and unclassified sequences. *Syntrophobacterales*, specifically *Syntrophus* and *Smithella* spp., are common in methanogenic hydrocarbon-degrading communities, including methanogenic oil-sand tailings, oil-sand tailings enrichment cultures, hydrocarbon-contaminated sediments and aquifers, methanogenic hexadecane-degrading consortia, oil field production water, methanogenic coal seam groundwater and coal-impacted wetlands (Johnson et al. 2015). Minor sequences of the class *Epsilonproteobacteria* the genera *Sulfurimonas* and *Sulfuricurvum* were detected only in several communities (Samples 1c, 3 and 5c and 5f). Members of this class are chemolithoautotrophs and are able to use molecular sulphur or its compounds as electron donors, nitrates and nitrites as acceptors, and $CO_2$ as the sole carbon source (Inagaki et al. 2003, Kodama & Watanabe 2004).

In the sediments of Frolikha Vent (Site 3), *Actinobacteria* comprised nearly half of the community (44%). The proportion of *Proteobacteria* and *Cyanobacteria* was smaller (17 and 16%, respectively); the community also contained *Bacteroidetes* (13%) and *Verrucomicrobi*a (2%) sequences. Approximately 5% of the sequences were not related to known phyla and were assigned as unclassified (Fig. 3). The

composition of *Actinobacteria* was close to that of the community from the reference site dominated by representatives of the 2 taxa hgcI_clade and marine group. *Cyanobacteria* included the sequences related to the Baikal plankton species and those found among communities from soils and lakes. The sequences of the *Beta-* and *Gammaproteobacteria* classes dominated; *Alpha-* and *Deltaproteobacteria* were less represented (7, 6, 3 and 1%, respectively). The composition of *Gammaproteobacteria* was more homogenous: 4% of the sequences belonged to the family *Methylococcaceae*, while 2% did not have homologues and were considered unclassified. Among *Betaproteobacteria*, *Rhodocyclaceae* and *Methylophilaceae* members comprised 1.5 and 0.5%, respectively. Among *Deltaproteobacteria*, 0.5% belonged to the order *Syntrophobacterales*, including 0.2% of the sequences assigned to *Geobacteracea*, 0.2% to *Desulfobacteraceae*, and 0.2% were unclassified. The phylum *Bacteroidetes* contained representatives of the orders *Sphingobacteriales* (4%) and *Flavobacteriales* (7%), with a significant number of sequences belonging to the genus *Flavobacterium* (4%).

*Actinobacteria* (37%), *Cyanobacteria* (33%), *Proteobacteria* (20%), and *Bacteroidetes* (7%, Fig. 3) dominated the bacterial community in Sample 4 from the zone with bacterial mats, where the concentrations of sulphate and acetate ions were high. The taxonomic composition of *Actinobacteria* did not significantly differ from the composition of the reference site. We can point out a small number of the sequences of the order *Rubrobacteridae*, the genus *Propionibacterium*, and the presence of sequences of the genus *Microbacterium*. The Gram-positive and catalase-positive strains of the genus *Microbacterium* participate in the nitrogen cycle (Kageyama et al. 2007). The closest homologues of these *Actinobacteria* were isolated from river sediment and an unidentified hydroid.

*Cyanobacteria* were rather diverse in the sample. We registered sequences that were closely related to the planktonic species and typical of the areas with gas-bearing fluid seepages. Their closest homologues were identified in different lakes and soils (Table S2).

In this sample, *Proteobacteria* were dominated by *Gammaproteobacteria* (11%) and *Alphaproteobacteria* (8%). The former class was mainly represented by sequences of the genus *Caulobacter* (6%), and the latter by sequences of the family *Pseudomonadaceae*. We did not detect any sequences of the genus *Thioploca* that formed bacterial mats at the sampling site. As mentioned above, Good's coverage coefficients and rarefaction curves confirmed the incomplete characteristics of the bacterial diversity in the analysed sample. *Betaproteobacteria* contributed only 1% to the community (Fig. 3), whereas their diversity was rather high. In the community of this area, we did not detect *Deltaproteobacteria*, although studies of sediments at this site revealed sulphate-reducing bacteria of the genera *Desulfobulbus* and *Desulfosporosinus* (Pimenov et al. 2014).

In different samples from Site 5, the structure of the bacterial communities was fairly similar, except for communities from Samples 5d and 5f. *Actinobacteria* increased (23–56%) with sediment depth, whereas *Cyanobacteria* (40–17%) and *Proteobacteria* (15–8%) decreased (Fig. 3). In the surface layer of sediments (Sample 5a), *Actinobacteria* were dominated by the families *Acidimicrobiaceae* and *Sporichthyaceae*. None of the detected sequences had cultured homologues; their closest relatives were found in Yellowstone Lake (USA), the community of Baikal sponges, underground mine waters in Canada and in Lakes Kanagawa (Japan) and Damariscotta (USA) (Table S1). Many sequences of the family *Sporichthyaceae* were close to those in the deep sediment layers of stratified lakes and communities from Arctic lakes with elevated methane concentration, as well as to bacteria involved in the degradation of oil hydrocarbons in the Baltic Sea. In the deeper sediment layers of Samples 5b and 5d, we detected sequences of the genus *Propionibacterium* en masse. Their cultured representatives isolated from different environments were capable of producing lactic acid, propionic acid and acetic acid from glucose (Cumminis & Johnson 1986). Uncultured sequences of the group OPB41 represented over 70% of the *Actinobacteria* sequences in the bacterial community of Sample 5f, whereas few representatives of the order *Acidimicrobidae* were identified in other layers of the core. The community of Sample 5a differed in composition from the phylum *Verrucomicrobia*, making up approximately 70% of the genus *Luteolibacter*. Cultured representatives of this genus are obligate aerobes (Jiang et al. 2012); therefore, their massive presence and functional role in the community of Sample 5f with a carbonate layer remains unclear. *Proteobacteria* (77%) dominated the community of Sample 5d.

The community of Sample 5f with the carbonate layer had a different composition of taxa, including *Proteobacteria* (31%), *Bacteroidetes* (26%, genera *Paludibacter, Flavobacterium*, order *Sphingobacteriales*), *Verrucomicrobia* (8%) and candidate division OP10 (or JS1) (7%) (Fig. 3). Representatives of the latter taxon were previously registered in the gas-

hydrate sediment layer of the St. Petersburg methane seep (Kadnikov et al. 2012) and in various freshwater sediments. Members of the OP10/JS1 group were key representatives in areas with marine methane hydrates, making up 50% of the clone libraries (Inagaki et al. 2006). Moreover, they were detected both in deep and subsurface marine sediments (Teske 2006), and in some other oxygen-free environments, offshore and onshore mud volcanoes (Reed et al. 2002, Alain et al. 2006). Virtually all sequences of the genus *Sediminibacterium* (*Bacteroidetes*) were similar to those from cold-water ecosystems. Bacteria of this phylum can degrade complex organic compounds (Mobberley et al. 2012). In addition to the above taxa, 11% of the sequences from the community of Sample 5f were only distantly related to the cultured organisms and could not be classified, even at the phylum level.

*Proteobacteria* in different sediment layers of Site 5 were distributed as follows: the percentage of *Betaproteobacteria* increased with depth from 4% in the surface layer to 44% in the gas-hydrate layer, while *Alphaproteobacteria* varied from 1 to 10%. In the community of sediments containing GHs (Sample 5d), *Betaproteobacteria* (44%) and *Gammaproteobacteria* (32%) prevailed, and *Alpha-* (11%) and *Betaproteobacteria* (16%) dominated the community of Sample 5f. In the latter community, *Betaproteobacteria* were mainly represented by the family *Oxalobacteraceae*, unlike in other communities where the family *Comamonadaceae* dominated, which developed massively in the near-bottom area of the St. Petersburg methane seep (Kadnikov et al. 2012). In Samples 5d and 5f, almost all or half of the *Gammaproteobacteria* sequences belonged to the order *Pseudomonadales*, whereas in Samples 5c and 5e, the majority of sequences belonged to the order *Methylococcales.* Their closest homologues isolated from aerobic and microaerophilic biocoenoses (Bowman 2006) can use methane and $C_1$ compounds. Despite the increased sulphate ions in all sediment layers from Site 5, the number of *Deltaproteobacteria* sequences in Sample 5d was small (0.3%), while in Samples 5c and 5e it was 1.2 to 2%. Among the dominating taxa of this class were representatives of *Myxococcales* and unclassified *Syntrophobacterales*.

Using principal component analysis, we studied the effect of TOC concentration, $\Sigma_i$, and ions such as bicarbonate, iron and sulphate on the distribution of individual phylotypes ($OTU_{0.03}$) from all studied libraries (Fig. 4). We analysed only $OTUs_{0.03}$ which included 50 or more sequences and determined their taxonomic affiliation. Hence, the values reported have $p < 0.05$. In the surface layers of sediments of all of the examined structures (Fig. 4c), the component composition of pore waters did not affect the distribution of the majority of OTUs. TOC content, $\Sigma_i$ and members of the phyla *Cyanobacteria* and *Actinobacteria* (hgcI_clade), as well as the class *Deltaproteobacteria* showed a reliable correlation. Moreover, ion concentrations of chlorine, sulphate and iron in the surface sediment layers were reliably associated with *Cyanobacteria* forming a cluster with unclassified sequences. Ion concentrations of $Fe_{tot\ diss}$ and bicarbonate reliably correlated with the distribution of scanty 43F-1404R (*Deltaproteobacteria*) and SAR11 cluster, which were not always dominant among *Alphaproteobacteria*.

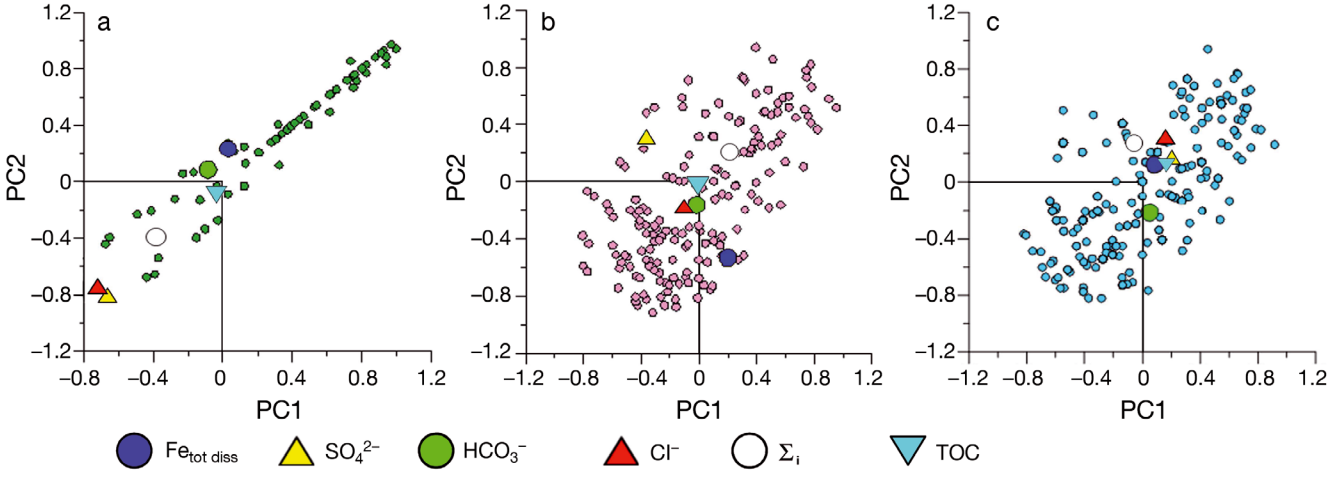


Fig. 4. Principal Components Analysis (PCA) plot showing the relationship between the bacterial operational taxonomic unit (OTU) composition and the environmental factors in sediments from different sites of Lake Baikal: (a) Site 5, (b) Sites 1 and 2, (c) upper layers of all sites. TOC: total organic carbon. See Fig. 1 for site locations

In sediments of oil seeps, we observed the most variable content of some ions and $\Sigma_i$; the dispersion of phylotypes was similar to that from the surface sediment layers (Fig. 4b). In these areas, $\Sigma_i$ and TOC contents were reliably associated with the distribution of the sequences of the taxa *Acidobacteria* (*Acidobacteriaceae*), *Betaproteobacteria* (*Comamonadaceae, Methylophilaceae*), and *Alphaproteobacteria* (*Acetobacteraceae*, wr0007). Sulphate ions, the concentrations of which in pore waters from the sediments of oil seeps were close to or lower than the reference site, had no effect on the taxonomic composition, with the exception of *Betaproteobacteria* (*Comamonadaceae*) and *Actinobacteria* (hgcI_clade). The content of bicarbonate ions was associated with the distribution of taxa such as *Alphaproteobacteria* (*Rhodospirillalis*), *Actinobacteria* (hgcI_clade) and *Betaproteobacteria* (*Alcaligenaceae, Methylophilaceae*).

The dispersion of individual OTUs in the samples from Site 5, characterised by high $\Sigma_i$ and sulphate ion concentration, differed from that mentioned above (Table 1, Fig. 4a). TOC content, concentration of specific ions and $\Sigma_i$ in pore waters did not have a reliable effect on the distribution of individual OTUs in different samples from this station; in all cases, the p-value was $>0.05$.

## DISCUSSION

The data obtained in this study from sediments of oil seep, mud volcano, methane seep, vent and reference sites show in the detected Baikal bacterial communities a wide spectrum of taxa with different types of metabolism. In each sample, at the phylotype level, communities were diverse and unique. In all examined communities, taxa of the phyla *Actinobacteria* and *Proteobacteria* typical of freshwater lakes (Newton et al. 2011) dominated, with their ratio varying in different samples. A wide representation of *Actinobacteria* in the examined communities was expected, since the bacteria belonging to this phylum are known degraders of hydrocarbons of different origins, including natural and anthropogenic (Kampfer 2010); they are also capable of utilising oil and other hydrocarbons coming from deep layers of sediments. Previously, we have shown the ability of natural microbial communities (Pavlova et al. 2008) and the *Rhodococcus* sp. strain (Likhoshvay et al. 2013) isolated from the oil seep area to degrade different hydrocarbons. Additionally, we have detected the presence of *alk*-genes (Likhoshvay et al. 2014, Lomakina et al. 2014).

*Proteobacteria*, which are the dominant bacterial phylum in the marine and freshwater metagenome (Haller et al. 2011, Song et al. 2012, Dai et al. 2013, Eiler et al. 2014, Zhang et al. 2015), constituted a considerable part of the communities in samples from the surface layers of all study areas, but their maximum was observed in the communities of reduced sediments from the mud volcano Malenky adjacent to GHs (Sample 5d). Among *Proteobacteria*, we identified representatives of various classes involved in different stages of degradation of organic matter entering surface layers of the sediments after spring and autumn growth peaks of diatoms (Votintsev et al. 1975), as well as those that are capable of the aerobic degradation of aromatic hydrocarbons and alkanes (Kadnikov et al. 2013). Similarly to the area of the St. Petersburg methane seep (Kadnikov et al. 2012), *Proteobacteria* included methano- and methylotrophic bacteria of the genus *Methylobacter* and families *Comamonadaceae*, *Methylophilaceae* and *Methylocystacea* (Table S3). Sequences of these taxa were most representative in the surface sediment layers, where methane was actively oxidised under aerobic conditions followed by formation of microbial mats whose activity is based on methyl-methanotrophy (Zemskaya et al. 2015). The sequences that are most similar to the methanotrophs from Lake Baikal have a wide distribution; they are found in the sediments of Lakes Washington, Biwa, Michigan and Geneva, an Arctic lake, Damariscotta Lake, Yellowstone Lake, Lake Taihu, freshwater iron-rich microbial mats and hydrothermal vents of Yellowstone Lake (Table S3).

Unlike the areas with deep-sea hydrothermal fields of the Suiyo Seamount dominated by *Gammaproteobacteria* (Kato et al. 2013), the discharge zones of gas-bearing fluids in Lake Baikal were mostly dominated by *Alphaproteobacteria*. In the sediment communities from oil seeps, representatives of the SAR11 clade prevailed. These are widespread in seawater, where they oxidise sulphur and carbon (Tripp 2013). Aerobic heterotrophs (*Sphingomonas* and *Caulobacter*) capable of degrading aromatic hydrocarbons were detected among the *Alphaproteobacteria*. In some communities, we found a high percentage of *Betaproteobacteria*. Unclassified sequences with unidentified phylogenetic affiliations comprised up to 2–5% of bacteria in the communities from oil seeps. Some *Betaproteobacteria* are known to execute the anaerobic degradation of aromatic compounds and alkanes (Zedelius et al. 2011), using nitrate as an electron acceptor. Population analysis has shown that the distribution of individual taxa of *Alpha-* and *Betaproteobacteria* was reliably associated with the TOC

content, the concentrations of which were rather high in areas with oil seeps. Our results correlated with those from studies on the communities found in deep-sea sediments of the Gulf of Mexico and the organic-rich Qiongdongnan Basin in the South China Sea (Hu et al. 2010, Kimes et al. 2013), where organic carbon was shown to be the most unimportant parameter. Phylotypes related to other uncultured environmental clones in *Alphaproteobacteria* and *Gammaproteobacteria* were relatively abundant in the sand, rock, sulphide mound and chimney samples (Kato et al. 2013).

In a variety of deep-subsurface environments, *Deltaproteobacteria* participate in the anaerobic biodegradation of hydrocarbons (Lloyd et al. 2010, Orcutt et al. 2010). At our studied sites, representatives of this class contributed significantly to the bacterial communities only in a few samples of sediments from oil seeps and the mud volcano Malenky. Members of the genus *Geobacter* capable of degrading aromatic hydrocarbons (Lovley & Phillips 1989) were associated with a reduction of iron oxide Fe (III). Formation of hydrogen sulphide is insufficient in the sediments of Lake Baikal due to low intensity of sulphate reduction (Pimenov et al. 2014). Therefore, in the lake sediments, oxygenated compounds of Fe and Mn may persist long enough in the oxidised form and be involved in microbial metabolism.

Members of the phylum *Bacteroidetes* were found in the communities of almost all samples with a maximum contribution in Sample 5f. Representatives of this phylum can degrade and grow on a variety of complex substrates including cellulose, chitin and agar (Kharade & McBride 2014). Therefore, they were numerous in bacterial communities from various biocoenoses of Lake Baikal (Kadnikov et al. 2013, Zakharova et al. 2013), where diatoms and other organic substrates accumulate. Almost all of them were microorganisms of several uncultured lineages of 16S rRNA sequences close to those detected in studies of microbial communities of soils and lacustrine sediments.

Additionally, some communities showed a high percentage of *Cyanobacteria*, which prevailed in the communities of surface and subsurface sediments at different sites. Previously, we also observed a high percentage of *Cyanobacteria* in surface sediments of the St. Petersburg methane seep. Although microscopic examinations detected their physiologically active state (Kadnikov et al. 2012), phylogenetic analysis (Fig. S2) indicated that the bulk of *Cyanobacteria* sequences had the highest similarity with planktonic species of *Cyanobacteria* from Lake Baikal and other lakes and hence, they cannot be considered active members of benthic microbial communities. *Cyanobacteria* in the examined communities, which are closely related to the uncultured sequences from bottom sediments and soils, were less numerous (Table S2). They are likely additionally involved in the fixation of molecular nitrogen using carbon dioxide produced during the destruction of methane by methanotrophic bacteria.

The structure of the communities in the gas hydrate-bearing layers of sediments differed from that in the sediments above and below these layers, which was previously shown in the study of methane hydrate-bearing sediments in the Ulleung Basin, in the East Sea of Korea (Lee et al. 2013). The dominant taxa in Lake Baikal included sequences of the OP10/JS1 group and *Chloroflexi* typical of communities from marine sediments containing methane hydrates and communities from the sediments adjacent to GHs near the site of the St. Petersburg methane seep (Kadnikov et al. 2012). These groups were normally associated with organic matter fermentation and methanogenesis. *Chloroflexi* were mainly obtained from anoxic and organic-rich environments, such as sediments that are rich in organic matter but lack hydrates, hot springs and anaerobic wastewater sludge (Sekiguchi et al. 2003, Inagaki et al. 2006). *Chloroflexi* dominated the communities in sediments from a few lakes (Song et al. 2012, Zhang et al. 2015).

Bacterial communities in the study areas of Lake Baikal demonstrate moderate bacterial richness compared with other lake ecosystems (Glöckner et al. 2000, Newton et al. 2011, Song et al. 2012, Zhang et al. 2015). Similarly to the microbial communities from sites with methane seeps in marine environments (Ruff et al. 2015), bacterial communities in Lake Baikal overlap at the phylum level, but show increasing dissimilarity at increasing phylogenetic resolution below the class level, with clear distinctions between oil and methane seeps, surface and subsurface, and carbonate and GH communities. Our results indicate that different geochemical parameters along vertical and horizontal gradients affect the specific bacterial taxa. Bacterial communities from oil seeps have higher species richness and more unclassified sequences compared to the communities from other samples. A few cosmopolitan taxa were also the most sequence-abundant organisms and seem to involve gaseous and liquid hydrocarbons from deep sediments in biological cycles. The constant supply of various hydrocarbons from deep Lake Baikal sediments and their involvement in food webs could ensure survival of various benthic organisms in periods of unfavourable climates in this ancient ecosystem (Grachev et al. 1995).

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