

Ribosomal Tag Pyrosequencing of DNA and RNA Reveals “Rare” Taxa with High Protein Synthesis Potential in the Sediment of a Hypersaline Lake in Western Australia

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ABSTRACT

Little is known about the potential activity of microbial communities in hypersaline sediment ecosystems. Ribosomal tag libraries of DNA and RNA extracted from the sediment of Lake Strawbridge (Western Australia) revealed bacterial and archaeal operational taxonomic units (OTUs) with high RNA/DNA ratios providing evidence for the presence of ‘rare’ but potentially “active” taxa. Among the ‘rare’ bacterial taxa *Halomonas*, *Salinivibrio* and *Idiomarina* showed the highest protein synthesis potential. Rare but ‘active’ archaeal OTUs were related to the KTK 4A cluster and the Marine-Benthic-Groups B and D. We present the first molecular analysis of the microbial diversity and protein synthesis potential of rare microbial taxa in a hypersaline sediment ecosystem.

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Introduction

Hypersaline environments, especially salt lakes, are widely distributed and occur in various climate zones on all continents (Williams 2002). Salt water contributes to a large fraction of the total inland water. The global volume of freshwater lakes is estimated to be $105 \times 10^3 \text{ km}^3$, while the total volume of inland saline water is with $85 \times 10^3 \text{ km}^3$ (Shiklomanov 1990) nearly as big. Since freshwater only contributes with 2.5% to the global water resources (Gleick 1993), salinisation of freshwater is a huge threat for water resources and ecosystems. Natural (primary) salinisation mainly occurs in closed endorheic drainage basins in semi-arid and arid regions (Williams 1999). These regions also suffer strongly from secondary salinisation which is induced anthropogenically. Raising water tables due to irrigation without proper drainage coupled with increasing soil salinity by evaporation, replacement of the natural vegetation with shallow rooted crops or introduction of wastewater by mining activities are the major reasons for secondary salinisation (Timms 2005; Williams 2002).

Several studies have reported on the vast diversity of microbial life in hypersaline waterbodies (Jiang et al. 2006; Makhdoumi-Kakhki et al. 2012; Mutlu et al. 2008; Parnell et al. 2010), sediments and soils (Jiang et al. 2006; Mesbah et al. 2007; Walsh et al. 2005; Youssef et al. 2012). Most of these studies are based on the comparative sequence analysis of phylogenetic marker genes, such as the 16S rRNA gene. They revealed that a large fraction of the identified microbial taxa have no close cultured representatives in public culture

collections and therefore not much is known about the physiology and actual *in situ* activity of these halophilic microorganisms (Oren et al. 2009). It is one of the major goals in microbial ecology to link the identification of microbial populations to information about their *in situ* metabolic state.

In recent years next generation sequencing technologies have expanded our knowledge on the diversity and composition of microbial communities in saline ecosystems (Bolhuis and Stal 2011; Bowen et al. 2012; Youssef et al. 2012). These massive parallel sequencing studies identified ‘rare’ taxa that have previously not been found in smaller clone libraries and many which are not closely related to cultivated strains. Thus, not much is known about the functional role of this rare microbial biosphere.

Nearly all sequencing studies of microbial communities are usually based on rRNA gene libraries constructed by amplifying DNA extracted from environmental samples. Environmental DNA extracts can contain DNA from active cells, inactive but viable cells, dormant, and dead cells. Moreover direct DNA extracts from environmental samples might also include extracellular DNA from degraded or lysed cells (Levy-Booth et al. 2007; Pietramellara et al. 2009) as well as structural exopolysaccharide DNA (Harmsen et al. 2010; Seper et al. 2011). Therefore analyses of microbial communities based on environmental DNA extracts convey information on active, inactive, and cell-free DNA and present a biased view of the actual community structure or metabolic state of microbial populations (Gentile et al. 2006).

In contrast environmental RNA extracts provide a more recent view on microbial activities because RNA has a rapid turnover rate within cells (Deutscher 2006) and a relatively short half-life compared to DNA once released from a cell (Novitsky 1986). Thus, pyrotag libraries from reverse transcripts (cDNA) of 16S rRNA are more representative for the ‘active’ fraction of the total microbial community (Blazewicz et al. 2013). The number of sequencing tags of a specific taxon in a RNA-based 16S rRNA library relative to that in a DNA-based 16S rRNA gene library can be used as a measure of potential activity. Therefore parallel sequencing and comparison of 16S rRNA tag libraries based on RNA and DNA can reveal if abundant taxa are also ‘active’ and help to identify “rare” but “active” taxa.

This approach has been used in previous studies of microbial communities in soils and lakes and provided the basis for generating and testing hypotheses on the complex interplay of microbial community structure and activity (Campbell et al. 2011; Gaidos et al. 2011; Hunt et al. 2013; Jones and Lennon 2010; Lanzén et al. 2011). While rRNA as an indicator of actual microbial activity in environmental communities has also limitations (Blazewicz et al. 2013), rRNA is a critical component of ribosomes and therefore required for protein synthesis. The ratio of 16S rRNA and 16S rRNA gene amplicons of a certain microbial taxa can therefore serve as an indicator of its protein synthesis potential.

Here we used parallel sequencing of 16S rRNA and 16S rRNA gene tag libraries of a sediment profile of the hypersaline Lake Strawbridge in Western Australia with the aim to (1) describe the archaeal and bacterial community composition, (2) compare the taxonomic richness and diversity of DNA- and RNA-derived 16S rRNA sequence tag libraries, (3) identify abundant and ‘rare’ taxa in the DNA-derived libraries that were also abundant in the RNA-derived libraries, and (4) evaluate if the RNA/DNA tag ratio as indicator of the protein synthesis potential provides new insights into the occurrence and distribution of ‘rare’ but ‘active’ taxa in a hypersaline sediment ecosystem.

Material and Methods

Lake Strawbridge (32.84455°S, 119.39780°E, WGS84) is a hypersaline lake located in south-west Australia in the Shire of Lake Grace (Figure 1). Sediment samples were taken from the northern shore line during a sampling campaign in March 2013. During sampling, the lake was nearly completely covered with water due to heavy rainfall. Three sediment layers could be distinguished: a top whitish layer (0–1 cm, dissolved salt crust), a thick blackish layer (1–10 cm) and a greyish layer (>10 cm). Samples for nucleic acid extraction of each layer were taken with a sterile spatula and immediately transferred to a 15-mL centrifuge tube containing 2 volumes of Life-Guard™ Soil Preservation Solution (MoBio Laboratories, Carlsbad, CA, USA) to stabilize nucleic acids. Samples were stored at 4°C until nucleic acid extraction. For geochemical analysis, sediment cores were taken by pushing a polypropylene tube (4.5-cm diameter) into the soft sediment. After removing the tube from the sediment both ends were sealed with rubber stoppers and cores were stored at 4°C until analysis.

Geochemical analysis

The water content was determined by weighing wet sediment samples from the three different horizons and subsequent drying at 105°C until weight stability. The samples were weighed at room temperature to determine the dry weight. For pH measurements, 10 g of air dried sediment were suspended in 25 mL of a 0.01 M CaCl₂ solution and pH was measured after 2 h. Total organic carbon (C_{org}) was quantified in samples that were dried at 60°C until weight stability with an Elementar Vario EL element analyzer (Elementar Analysensysteme GmbH, Hanau, Germany).

Due to the low water content and the consistency of the sediment, ion content and soluble organic carbon were quantified in reconstituted pore water (modified after (Emmerich et al. 2012)) as follows: 1 g of dried sediment was suspended in 20 mL deionized water and shaken for 24 h at 150 rpm. Samples were centrifuged for 5 minutes at 3750 g, and the

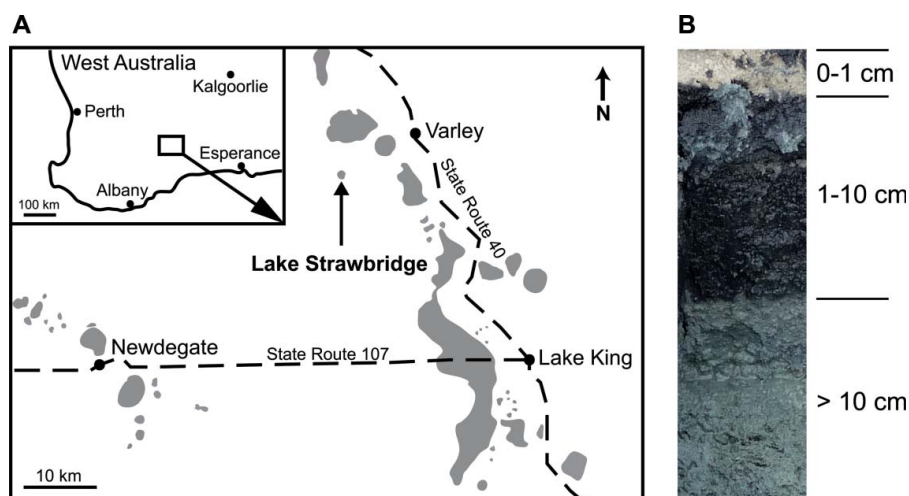


Figure 1. (A) Map of Western Australia and location of Lake Strawbridge. Grey shaded areas represent hypersaline lakes. (B) Sediment depth profile of the Lake Strawbridge sampling site.

supernatant was diluted 1:20 in deionized water before filtration through a 0.45 μm pore size cellulose ester filter (Millex HA filter, EMD Millipore Corporation, USA). Major ions were quantified by ion chromatography (Dionex DX 120, Thermo Scientific, Sunnyvale, CA, USA). Soluble organic carbon was measured using a High TOC Elementar system (Elementar Analysensysteme GmbH, Hanau, Germany).

Nucleic acid extraction and reverse transcription

For nucleic acids extraction sediment samples were treated as described previously (Emmerich et al. 2012). RNA and DNA were extracted in parallel using the RNA PowerSoil® Total RNA Isolation Kit (MoBio Laboratories, Carlsbad, CA, USA) in combination with the RNA PowerSoil® DNA Elution Accessory Kit (MoBio Laboratories, Carlsbad, CA, USA). For each sediment layer, three independent extractions were performed. Prior to reverse transcription, DNA was digested in the RNA extracts with the Ambion TURBO DNA-free™ Kit (Life Technologies, Carlsbad, CA, USA). Successful DNA removal was confirmed by PCR using the general 16S rRNA gene primers GM3 (5'-AGAGTTTGATCMTGGCTCAG-3') (Lane 1991) and 1392R (5'-ACGGGCGGTGTGTRC-3') (Lane 1991) with the following conditions: hot start at 70°C, 5 min at 95°C, 35 cycles with 1 min at 95°C, 1 min at 44°C and 3 min at 72°C followed by a final elongation step of 10 min at 72°C. If no PCR products were obtained, RNA extracts were used for reverse transcription.

The reverse transcription reaction mix (total volume 13 μL) contained 5 μL RNA (~0.5 to 1 μg), 8 μL DEPC-treated water, 2 mM dNTP mix (New England Biolabs, Ipswich, MA, USA) and 5 ng/ μL random primer (Invitrogen, Life Technologies, Carlsbad, CA, USA). The reaction mix was incubated for 5 min at 65°C before 1x First Strand buffer (Invitrogen, Life Technologies, Carlsbad, CA, USA), 5 mM DTT (Invitrogen, Life Technologies, Carlsbad, CA, USA), 2 U RNaseOUT™ Recombinant Ribonuclease Inhibitor (Invitrogen, Life Technologies, Carlsbad, CA, USA) and 10 U SuperScript® III Reverse Transcriptase (Invitrogen, Life Technologies, Carlsbad, CA, USA) were added. The reaction mix was then incubated for 5 min at 25°C, 60 min at 50°C and 15 min at 70°C in a S1000 thermal cycler (Bio-Rad Laboratories GmbH, Munich, Germany). cDNA was stored at -20°C until further use.

454 Pyrosequencing of Bacterial and Archaeal 16S rRNA Genes

For 454 pyrosequencing, three independent DNA extracts and cDNA samples per sediment layer were used. The bacterial 16S rRNA genes were amplified using primers 27F (5'-AGAGTTTGATCMTGGCTCAG-3') (Lane 1991) and 534R (5'-ATTACCGCGTCTGCTGGC-3') (Liu et al. 2007; Muyzer et al. 1993) targeting the V1-V3 region of the 16S rRNA gene. Archaeal 16S rRNA genes of the V6-V8 region were amplified using the primers 958F (5'-AATTGGANTCAACGCCGG-3') (DeLong 1992) and 1392R (5'-ACGGGCGGTGTGTRC-3') (Pace et al. 1986). The primers 27F and 958F contained Roche's 454 pyrosequencing barcodes and adaptor A, while primers 534R and 1392R contained adaptor B. PCR reactions for each

of the three DNA/cDNA samples were performed in duplicates with the FastStart High Fidelity PCR system (Roche, Mannheim, Germany).

The obtained PCR products were pooled in equimolar amounts. Quality of the amplified DNA was confirmed on an Experion® automated electrophoresis system (BioRad, Hercules, CA, USA). Prior to sequencing, the PCR products were quantified using the Quant-iT™ PicoGreen® dsDNA assay kit (Invitrogen, Eugen, OR, USA) and a QuantiFluor®-ST fluorometer (Promega, Madison, WI, USA). 454 pyrosequencing was performed on a Roche GS Junior Sequencer (454 Life Sciences, Branford, CT, USA) according to the manufacturer's instructions for amplicon sequencing. Pyrosequencing reads have been deposited in the ENA Sequence Read Archive (SRA) under accession number PRJEB7017.

Sequence analysis

Quality control, alignment and classification of the sequencing data were performed using the software package MOTHRU, version 1.33.3. (Schloss et al. 2009). Pyrosequencing noise and chimeras were removed with the in MOTHRU implemented algorithms PyroNoise (Quince et al. 2009) and UCHIME (Edgar et al. 2011) as described previously (Schloss et al. 2011). Sequences shorter than 200 bp and sequences with homopolymers longer than 8 bp were removed from the dataset. The remaining sequences were aligned against a seed alignment based on SILVA SSU Ref rRNA database (v.119) (Pruesse et al. 2007) and preclustered with the single-linkage algorithm applying a threshold of 2% (Huse et al. 2010).

A distance matrix was created and sequences were assigned to operational taxonomic units (OTUs) on the species level at 1.3% genetic distance (Yarza et al. 2014) using the average neighbor algorithm (Schloss and Westcott 2011). Sequences were classified using the Naïve Bayesian Classifier (Wang et al. 2007) and the SILVA reference taxonomy (v. 119). Because 16S gene copy numbers can vary in genomes of different organisms, relative abundances of DNA-derived OTUs were corrected based on the lineage specific gene copy number using the algorithm copyrighter (v. 0.46) (Angly et al. 2014).

Before alpha diversity analysis random subsampling was performed to normalize the dataset to the sample with the lowest number of reads. Rarefaction curves, Good's coverage estimator (GC), richness estimators (Chao1, ACE), and diversity indices (Shannon diversity, Simpson diversity) were calculated based on 1.3% genetic distance using MOTHRU's implementation of DOTUR (Schloss and Handelsman 2005). Cluster analysis on samples was performed using an Unweighted Pair Group Method with Arithmetic Mean (UPGMA) algorithm and was based on calculated Bray-Curtis dissimilarities between the samples (Bray and Curtis 1957).

Heatmaps were created with the ggplot2 plotting function in R (R Core Team 2013) based on the relative sequence abundances of the represented taxa. The relative sequence abundances of the relevant taxa in the different sediment layers were normalized by calculating Z-scores representing the number of standard deviations a certain value differs from the mean. Unfortunately, multivariate statistical analysis of the sequence data to infer correlations between taxa abundance and

geochemical parameters was hampered by the low number of samples ($n = 3$ sediment layers) and the reduced set of geochemical data. Furthermore, the focus of the study is on “rare” taxa with high protein synthesis. However, the temporal component of past, present, and potential future protein synthesis activity is not reflected in the geochemical data set. Therefore, even statistically significant correlations based on the available data could lead to false biological interpretations and speculations on interrelations that lack appropriate empirical support. We therefore did not include them in the discussion of our data.

Quantitative PCR of bacterial and archaeal 16S rRNA genes

Quantitative PCR (qPCR) of bacterial and archaeal 16S rRNA genes was performed using an iQ5 real-time PCR detection system (iQ5 optical system software, version 2.0, Bio-Rad). For bacterial 16S rRNA gene copy numbers general bacterial primers 341F (5'-CCTACGGGAGGCAGCAG-3') (Muyzer et al. 1995) and 797 R (5'-GGACTACCAGGTATCTAATCC-TGTT-3') (Nadkarni et al. 2002) were used with a 16S rRNA gene fragment of *Thiomonas* sp. cloned in a pCRTM 2.1* plasmid vector (Invitrogen, Darmstadt, Germany) as standard. Archaeal 16S rRNA gene copy numbers were obtained using the general archaeal primers 109F (5'-ACKGCTCAGTAA-CACGT-3') (Großkopf et al. 1998) and 915R (5' GTG-CTCCCCGCCAATTCCT-3') (Amann and Stahl 1991) with a 16S rRNA gene fragment of *Halobacterium salina* cloned in a pCRTM 4* plasmid vector (Invitrogen, Darmstadt, Germany) as standard. 20 μ L reaction volumes contained 1 \times SsoFastTM Eva Green[®] Supermix (Bio-Rad Laboratories GmbH, Munich, Germany), 2 μ L of hundredfold diluted DNA extract and 75 nM of primer 341F and 225 nM of primer 797R for bacterial 16S rRNA genes or 250 nM of archaeal 16S rRNA gene primers.

qPCR conditions for bacterial primers were 2 min at 98°C, 40 cycles of 5 s at 98°C and 12 s at 60°C. Conditions for archaeal primers were 3 min at 98°C, 40 cycles of 5 s at 98°C, 12 s at 52°C and 15 s at 72°C. Both protocols were followed by a melting curve analysis. For each qPCR three independent DNA extractions per sediment layer were measured in triplicates. The average cell numbers were calculated considering the average genomic 16S rRNA gene copy number retrieved from lineage specific copy number correction of the different microbial communities in each sediment layer by the algorithm copy-righter (Angly et al. 2014).

Results

Geochemical characteristics of sediment samples

Geochemical parameters quantified for the three sediment layers of Lake Strawbridge are summarized in Table 1. The sediments were slightly alkaline with pH 8.3 in the layer between 0–1 cm decreasing to pH 8.1 below 10 cm sediment depth. Sediment water content varied between 16.3% in the top layer and 23.8% in the layer from 1–10 cm depth. Usually, Lake Strawbridge sediments are permanently covered

by a centimeter thick salt crust. Due to severe rainfall prior to the sampling campaign in March 2013 the salt crust was mostly dissolved resulting in a pore water salinity of 185.6 g/L in the top layer between 0–1 cm. Following rainfall events evaporation quickly increases the salinity in this layer beyond saturation (Youssef et al. 2012). The salinity was 117.8 g/L NaCl in the sediment layer between 1–10 cm and 51.1 g/L NaCl below 10 cm depth. Comparable to the salinity, SO_4^{2-} concentrations decreased with sediment depth from 85.1 mM between 0–1 cm to 13.8 mM in the sediment sampled below 10 cm depth. Soluble and total organic carbon concentrations were highest in the layer between 1–10 cm depth with 5.9 ± 0.1 mg/L and $0.9 \pm 0.1\%$ (w/w), respectively.

General sequencing statistics

Archaeal and bacterial amplicons were sequenced in two separate GS junior sequencing runs yielding 173,976 archaeal and 190,034 bacterial raw sequence reads with an average read length of 372 ± 146 bp and 384 ± 186 bp, respectively. After quality processing with MOTHUR 71,186 archaeal and 79,772 bacterial high quality sequences remained. This resulted on average in $11,864 \pm 2,091$ archaeal and $13,295 \pm 2,042$ bacterial reads per RNA and DNA library for each sediment layer.

Alpha diversity of the microbial community

Rarefaction analysis of the archaeal DNA- and RNA-based OTU richness showed that the rarefactions curves did not reach an asymptote, and sampling did not recover the total estimated diversity in the libraries (Figure 2A). Richness estimators indicated that the observed richness covered on average 24 to 63% of the estimated total archaeal richness in the different sediment layers. Bacterial rarefaction analysis (Figure 2B) revealed a similar curve progression and the observed DNA- and RNA-based OTU richness covered on average 21 to 48% of the estimated total bacterial richness for all samples. In general, the observed and estimated richness (based on the Chao1 and ACE richness estimators) as well as the microbial diversity (based on the Shannon and Simpson indices) were higher in the bacterial than in the archaeal libraries for all sediment layers (Table 2). Good's coverage estimates ranged from 0.92 to 0.97 for the archaeal communities and from 0.83 to 0.96 for the bacterial communities.

In the RNA-based archaeal libraries, the sediment below 10 cm had the highest OTU diversity and richness while among

Table 1. Physical and chemical properties at different sediment depth of Lake Strawbridge.

Sediment depth(cm)	pH	Water content(%)	Salinity ^a (g/L)	SO_4^{2-} (mM)	Soluble organic carbon(mg/L)	C_{org} (%) ^b
0–1	8.3	16.3	185.6	85.1	2.2 ± 0.0	0.1 ± 0.0
1–10	8.2	23.8	117.8	21.1	5.9 ± 0.1	0.9 ± 0.1
>10	8.1	21.1	51.1	13.8	4.0 ± 0.0	0.1 ± 0.0

^a as g/L NaCl.

^b weight %.

Errors are given for duplicate measurements.

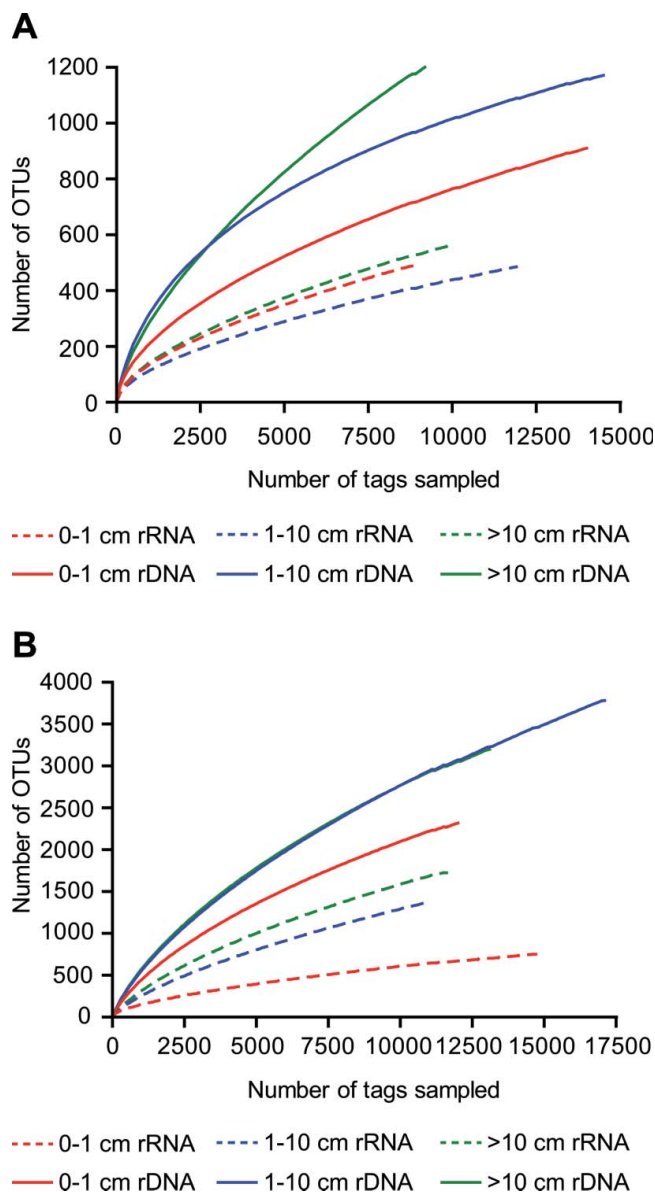


Figure 2. Rarefaction curves representing the number of observed OTUs in the archaeal (A) and bacterial (B) community based on OTU clustering at a genetic distance of 1.3%.

the DNA-based archaeal libraries, the layer between 1–10 cm had the highest OTU diversity and the sediment below 10 cm

the highest OTU richness. For both, the RNA- and DNA-based bacterial libraries the OTU richness and diversity were highest in the sediment below 10 cm. With the exception of the layer between 1–10 cm, in the RNA-based archaeal libraries the top layer had the lowest OTU richness and diversity in all archaeal and bacterial libraries independent of whether they have been derived from environmental RNA or DNA. The results of the alpha diversity calculations showed that the microbial diversity and richness increased with sediment depth. Rarefaction analysis as well as observed OTU numbers, estimated OTU richness, and calculated OTU diversity consistently revealed a lower microbial diversity in the RNA-based read libraries compared to the DNA-based libraries of archaeal and bacterial sequence tags.

Archaeal and bacterial cell numbers

Bacterial and archaeal cell numbers were derived from quantitative qPCR measurements. However, the application of quantitative PCR to estimate bacterial and archaeal cell numbers in environmental DNA extracts has limitations (to name a few: rRNA operon copy number variations, inhibitors, choice of primer binding sites and resulting amplicon length) (Smith and Osborn 2009), which is why results should be interpreted carefully. The amplification efficiencies of our Bacterial and Archaeal qPCR assays were 90.66% and 84.24%, respectively. The qPCR data is provided to ‘approximate’ total bacteria and archaea cell numbers per gram of sediment in order to place the sequencing data in relation to prokaryotic biomass in the analyzed sediments.

Bacterial cell numbers were higher in all sediment layers compared to archaeal cell numbers (Table 3). The highest cell numbers were found in the top layer for both, archaeal and bacterial cells with 3.87×10^8 and 7.56×10^8 cells per g dry sediment, respectively. Lowest cell numbers were observed in the layer between 1–10 cm for Archaea and Bacteria.

Archaeal taxonomic composition

All sediment layers of both, RNA- and DNA-based libraries were dominated by *Halobacteria* (average relative sequence abundance 78.4–99.8%) (Figure 3A). In the RNA-based libraries the *Thermoplasmata* accounted for 9.5 to 10.0% relative

Table 2. Richness estimators, diversity indices and observed OTU numbers of archaeal and bacterial communities based on OTU clustering at a genetic distance of 1.3%.

	Archaea						Bacteria					
	OTUs ^a	GC ^b	Richness		Diversity		OTUs ^a	GC ^b	Richness		Diversity	
			Chao1	ACE ^c	Shannon	Simpson			Chao1	ACE ^c	Shannon	Simpson
0–1 cm rRNA	488	0.97	1164	1884	3.38	0.103	657	0.96	1631	2671	3.73	0.088
1–10 cm rRNA	401	0.97	838	1524	3.20	0.116	1383	0.92	3593	6517	3.87	0.164
>10 cm rRNA	530	0.96	1164	1835	3.50	0.101	1690	0.90	3758	6334	4.13	0.184
0–1 cm rRNA gene	714	0.95	1537	2535	4.64	0.025	2249	0.88	5057	7712	5.88	0.019
1–10 cm rRNA gene	965	0.95	1522	1822	5.02	0.046	2931	0.83	6601	11120	6.57	0.010
>10 cm rRNA gene	1178	0.92	2794	4962	5.00	0.034	2944	0.84	6091	9631	6.67	0.007

^a Observed richness.

^b Good's coverage.

^c Abundance-based coverage estimator.

Prior to alpha diversity calculation random subsampling was performed to normalize the dataset to the sample with the lowest number of reads.

Table 3. Archaeal and Bacterial cell numbers based on 16S rRNA gene copy numbers with standard error at different sediment depths.

Cell numbers per g dry sediment		
Sediment depth(cm)	Archaea	Bacteria
0–1 cm	$3.87 \times 10^8 \pm 7.01 \times 10^7$	$7.56 \times 10^8 \pm 1.48 \times 10^8$
1–10 cm	$5.88 \times 10^7 \pm 4.73 \times 10^6$	$1.13 \times 10^8 \pm 2.88 \times 10^7$
> 10 cm	$9.91 \times 10^7 \pm 3.02 \times 10^7$	$4.73 \times 10^8 \pm 8.89 \times 10^7$

sequence abundance. The DNA-based community in the top sediment layer and the sediment below 10 cm mainly consisted of *Halobacteria* at relative sequence abundances of 99.8% and 98.4%, respectively, while the layer between 1–10 cm contained *Halobacteria* (78.4%) and *Thermoplasmata* (15.8%). The Bray-Curtis dissimilarities between the different sediment layers were higher among the DNA-based libraries, while the differences among the RNA-based communities with a maximum Bray-Curtis dissimilarity of 0.09 were quite small.

Bacterial taxonomic composition

The RNA-based libraries of all layers mainly consisted of *Proteobacteria* (50.0–66.2%), *Bacteroidetes* (14.1–28.3%), *Cyanobacteria* (2.1–7.9%) and *Firmicutes* (2.3–19.2%) (Figure 3B).

The DNA-based library of the top sediment layer was dominated by *Proteobacteria* (38.5%), *Bacteroidetes* (29.5%) and *Cyanobacteria* (11.5%). In the DNA-based library of the sediment layer between 1–10 cm *Proteobacteria* and *Firmicutes* were most abundant with 33.4% and 23.6%, respectively. The largest sequence fractions of the DNA-based library of the sediment below 10 cm depth were related to the *Proteobacteria* (33.1%), *Chloroflexi* (25.3%) and *Firmicutes* (11.6%). The similarity among the sediment layers was higher among the RNA-based bacterial libraries with Bray-Curtis dissimilarities ranging from 0.17 to 0.27, than the DNA-based libraries (dissimilarities 0.44–0.45).

Microbial community structure

Logarithmic rank abundance curves for archaeal RNA-based libraries (Figure 4A), archaeal DNA-based libraries (Figure 4B), bacterial RNA-based libraries (Figure 4C) and bacterial DNA-based libraries (Figure 4D) were plotted to visualize the distribution of abundant and rare OTUs among the different samples. An OTU was considered abundant when its relative sequence abundance was greater than 1%. Accordingly, “rare” OTUs had a relative sequence abundance of less than 1% (Campbell et al. 2011). All libraries shared a peak of highly abundant OTUs but the relative abundance of OTUs in the RNA-based libraries tailed off at lower rank numbers.

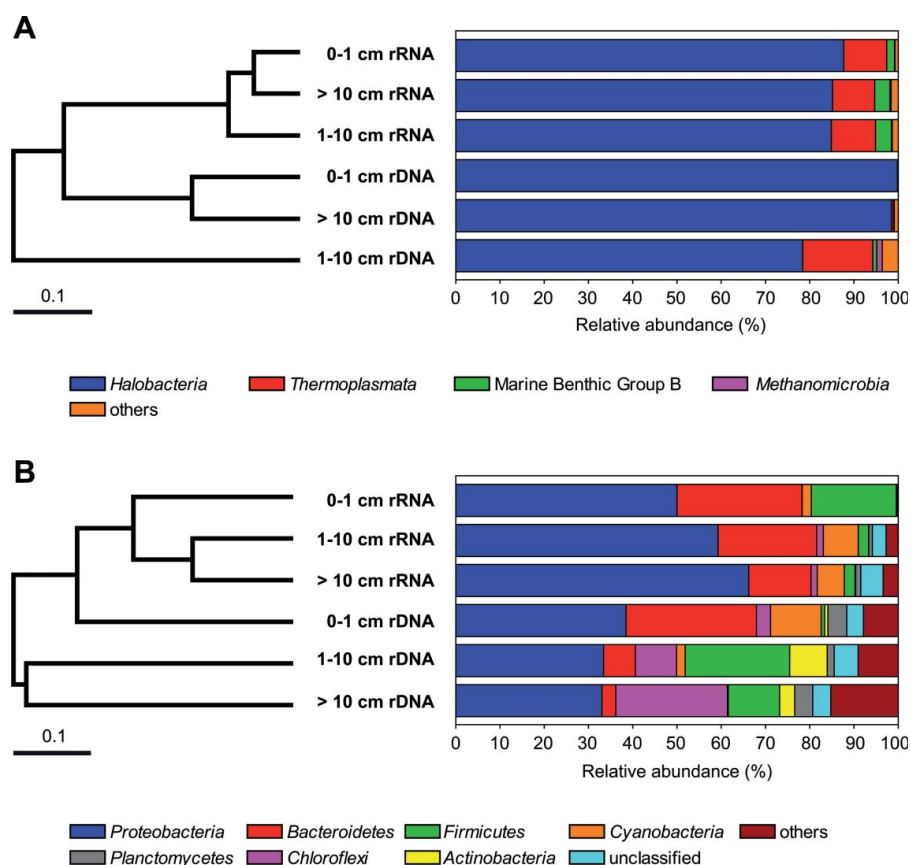


Figure 3. Relative abundances and hierarchical clustering of the archaeal (A) and bacterial (B) microbial communities in the different sediment layers of Lake Strawbridge. The cluster analysis is based on the Bray-Curtis dissimilarity at a OTU clustering reflecting 1.3% genetic distance. Scale bars indicate a dissimilarity of 0.1. Relative abundances of the dominant community members are shown on the phylum level for Bacteria and on the class level for Archaea. rRNA refers to the RNA-derived microbial community composition, whereas rDNA represents the DNA-based 16S rRNA gene microbial sequence diversity.

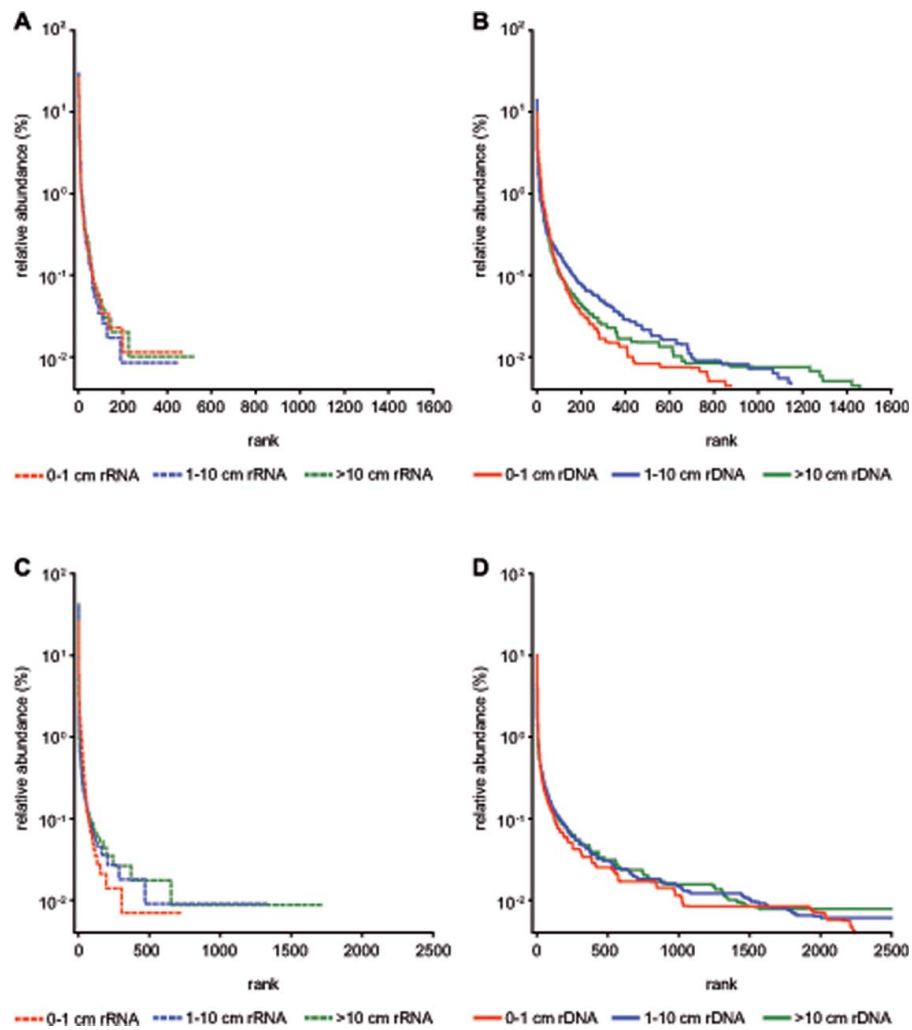


Figure 4. Rank abundance of archaeal RNA-based libraries (A), archaeal DNA-based libraries (B), bacterial RNA-based libraries (C) and bacterial DNA-based libraries (D) based on OTU clustering at a genetic distance of 1.3%. Continuous lines represent OTUs from DNA-based amplicon sequencing; dotted lines represent OTUs from RNA-based amplicon sequencing. For better comparability the rank number for the DNA-based bacterial library (D) was limited to 2500.

DNA-based libraries had considerable longer tails of low abundant OTUs compared to the RNA-based libraries.

To visualize differences in archaeal and bacterial community structure among the RNA and DNA-based sequence libraries from the different sediment depths, we generated heatmaps comprising all identified taxonomic groups with a relative sequence abundance of at least 1% in either of the libraries (Figures 5 and 6). The family *Halobacteriaceae* was the numerically dominant archaeal taxon in all sediment layers with relative abundances of 84.2–86.3% in the RNA-based and 78.3–99.8% in the DNA-based libraries. When we tried to increase the taxonomic resolution 11.5–40.0% of the *Halobacteriaceae* in the RNA-based libraries and 16.8–30.9% in the DNA-based libraries could not be classified on the genus level (Figure 5).

Among the *Halobacteriaceae* the genera *Halogranum* and *Haloarcula* were highly abundant in the RNA-based libraries with relative abundances of 30.2–62.7% and 5.1–7.1%, respectively. All other genera of the family *Halobacteriaceae* plotted in Figure 5 have mainly been found in the DNA-based libraries and were either completely absent or only present at low relative sequence abundances in the RNA-based libraries. The archaeal order *Thermoplasmatales*

including the Marine Benthic Group D and the family KTK 4A had a higher sequence abundance in the RNA-based libraries (9.5–10.0%) than in the DNA-based libraries with the exception of the layer between 1–10 cm (11.7%). Also the Marine Benthic Group B belonging to the *Thaumarchaeota* was more abundant in the RNA-based libraries compared to its abundance in the DNA-based libraries accounting for a relative sequence abundance between 1.8–3.7% across layers.

The dominant bacterial taxon in the RNA-based libraries was *Halomonas* with sequence abundances of 30.3–47.7% (Figure 6). Interestingly, in the DNA-based libraries *Halomonas* belonged to the rare taxa and was only detected at relative sequence abundances of 0.1% or lower. *Salinibacter* also showed high relative abundances in the RNA-based libraries (9.3–24.7%). Only in the top sediment layer did *Salinibacter* reach similarly high relative sequence abundance in the DNA-based library. The genera *Salinivibrio*, *Salisaeta*, *Idiomarina*, *Planococcus*, *Planomicrobium* and *Alkalibacterium* were mainly present in the RNA-based library of the top sediment layer, whereas in the DNA-based libraries they were either not present or occurred at sequence abundances below 0.1%. All other

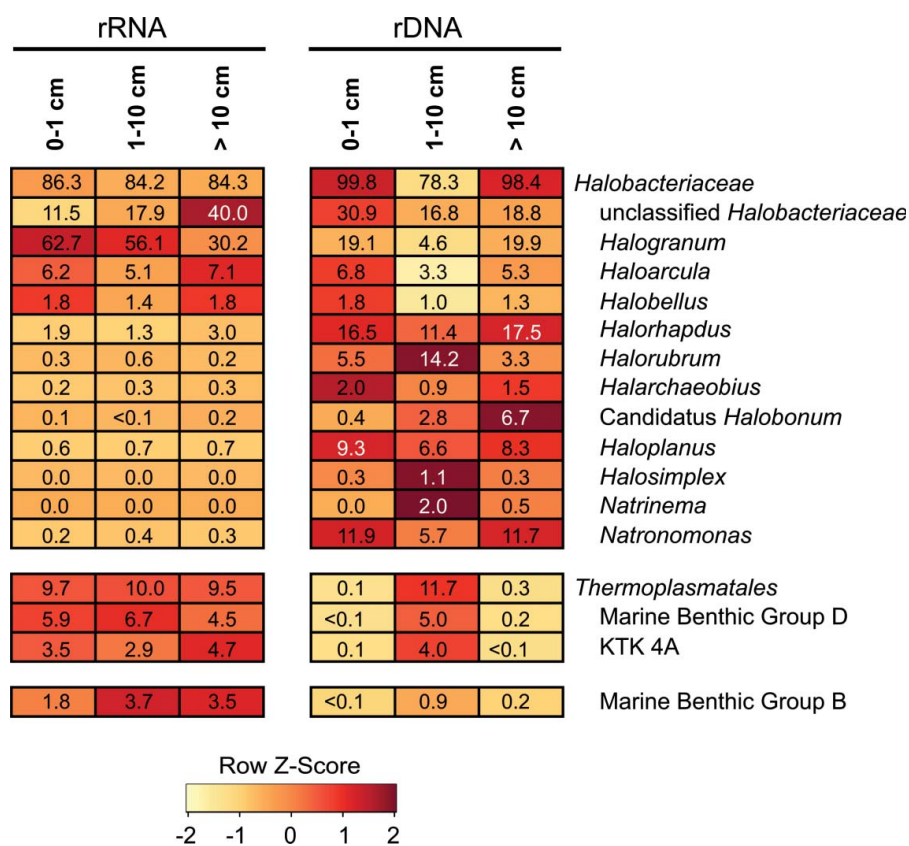


Figure 5. Heatmap showing changes in the relative abundance of dominant archaeal taxa (relative sequence abundance in at least one sample >1%) in the different sediment layers in the RNA- and DNA-based libraries. Z-scores represent the number of standard deviations a value differs from the mean and were used to normalize the relative taxa abundances in the different sediment layers. Positive Z-scores indicate values above the mean, negative Z-scores values below the mean. Numerical values in the heatmap represent the relative abundances of the respective taxa. A value <0.1 indicates that relative abundance was below 0.1%, whereas 0.0 indicates that no sequences for the respective taxonomic group were found. Taxonomic groups are given on the order, family or genus level.

bacterial groups listed in Figure 6 were present in higher relative abundances in the DNA-based libraries.

The rank abundance and rarefaction curves as well as the alpha diversity estimators revealed that the diversity and richness of the RNA-based libraries was consistently lower compared to the DNA-based libraries. This is also supported by direct comparison of the relative sequence abundances of selected taxa between the libraries across all sediment layers. The majority of taxonomic groups depicted in the heatmaps numerically dominated the DNA-based libraries while only a few individual taxa showed high relative sequence abundances in the RNA-based libraries.

To further illustrate the different abundance representation of individual taxa (>1%) in the RNA- and DNA-based tag libraries, we calculated RNA/DNA ratios of the identified archaeal and bacterial taxa and plotted their deviation from a 1:1 ratio in Figure 7. A 1:1 RNA/DNA ratio (solid line in Figure 7) means that a respective taxa is equally represented by its number of sequence tags in the RNA- and the DNA-derived libraries. A high RNA/DNA ratio will characterize low abundant but ‘active’ taxa (taxa with a high protein synthesis potential), while a low RNA/DNA ratio will describe taxa that were numerically prominent but ‘inactive’ (taxa with a low protein synthesis potential).

Overall the majority of the taxonomic groups were more abundant in the DNA-based libraries. The taxonomic groups that deviated most from a ratio of 1 have been highlighted in

Figure 7. The taxonomic groups *Orenia*, *Natronomonas*, *Haloanaerobacter*, *Candidatus Halobonum*, *Halorubrum*, *Caldithrix* and *Haloplanus* were characterized by a very low RNA/DNA ratio (0.008–0.09). In contrast the taxonomic groups *Halomonas*, *Halogramum*, *Idiomarina*, *Alkalibacterium*, *Salinivibrio*, Marine Benthic Group B, *Planococcus* and *Planomicrobium* were relatively ‘rare’ in the DNA-based libraries but showed a high sequence abundance in the RNA-based libraries with RNA/DNA ratios ranging from 6.15 to up to 1660.

Discussion

Geochemical characteristics

Lake Strawbridge sediments are permanently covered by a salt crust of several centimeters thickness. Due to severe rainfall prior to the sampling campaign in March 2013 the salt crust was mostly diluted. This resulted in lower salinity of the sediments as indicated by a porewater salinity of 185.6 g/L in the top layer, which is far below saturation. It is very likely that salinity is naturally restored by evaporation after rainfall events (Youssef et al. 2012)

Ribosomal RNA as an indicator for microbial activity

When using ribosomal tag sequencing of DNA and RNA in order to identify the ‘active’ fraction of a microbial

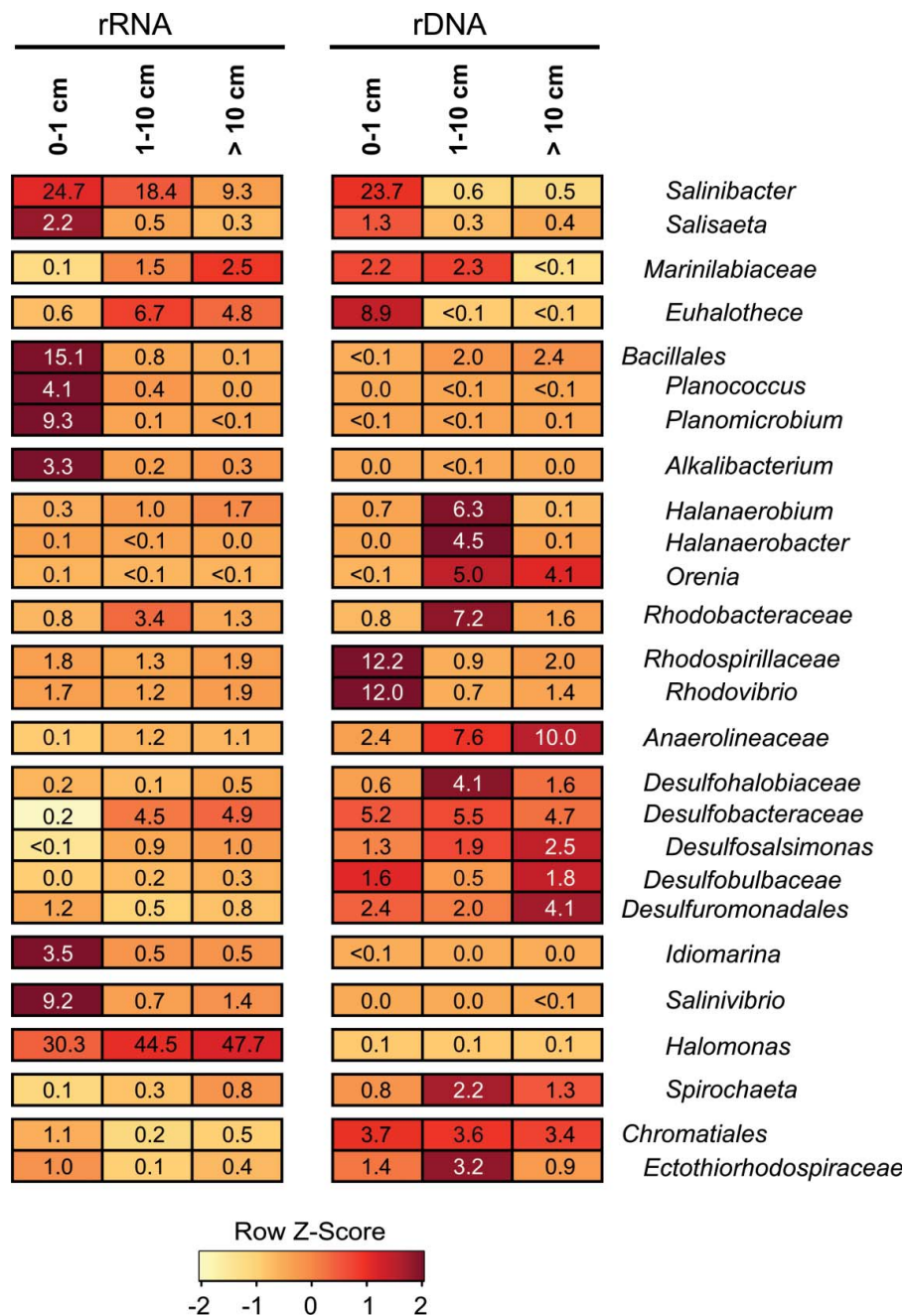


Figure 6. Heatmap showing changes in the relative abundance of dominant bacterial taxa (relative sequence abundance in at least one sample >1%) in the different sediment layers of the RNA- and DNA- based tag libraries. Z-scores represent the number of standard deviations a value differs from the mean and were used to normalize the relative taxa abundances in the different sediment layers. Positive Z-scores indicate values above the mean, negative Z-scores values below the mean. Numerical values in the heatmap represent the relative abundances of the respective taxa. A values <0.1 indicates that the relative sequence abundance was below 0.1% whereas 0.0 indicates that no sequences for the respective taxonomic group were found. Taxonomic groups are given on the order, family or genus level.

community it is important to consider the underlying assumptions and limitations of the approach in the interpretation of the obtained data. Currently there is not much known about the correlation of growth and nongrowth activities with cellular ribosomal RNA concentrations (Blazewicz et al. 2013). The correlation of ribosomal RNA content, growth rate and cellular activity is not directly proportional and can differ among taxa (Binnerup et al. 2001; Kemp et al. 1993; Rosset et al. 1966; Youssef et al. 2012). Recently, Blazewicz et al. (2013) evaluated rRNA as

an indicator of microbial activity in environmental communities.

The authors suggested to interpret cellular rRNA content as indicator of the protein synthesis potential of a cell with limited temporal resolution, meaning that a high rRNA content does not only indicate if cells are presently active but also if cells have recently been active or will become active in the future. Therefore, the relative abundance of ribosomes and thereby rRNA gives the maximum protein synthesis potential of a population at time of sampling but conveys no direct information

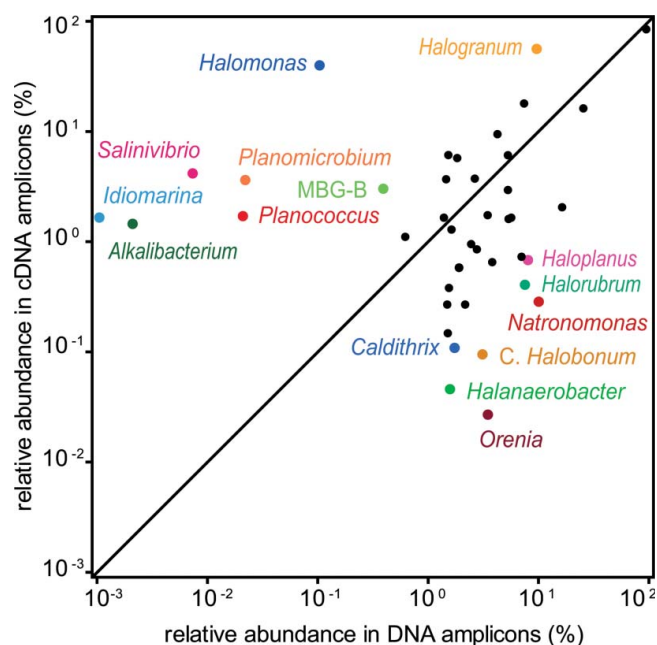


Figure 7. Relative abundance of taxa (>1%) in the microbial communities derived from DNA-based amplicon sequencing vs. relative abundance from RNA-based amplicon sequencing across all three sediment layers. Relative abundances are given on a logarithmic scale based on an OTU clustering at a genetic distance of 1.3%. The continuous line represents the 1:1 ratio, meaning that an OTU was equally abundant in the 16S rRNA gene tag libraries derived from both, DNA and RNA. MBG-B refers to Marine Benthic Group B.

on real-time protein synthesis. Nonetheless, parallel sequencing of ribosomal RNA sequence tags derived from environmental DNA and RNA pools reveal different microbial life strategies and can provide new insights into the complex interplay between cell abundance and activity in environmental microbial communities (Campbell et al. 2011; Gaidos et al. 2011; Hunt et al. 2013; Jones and Lennon 2010; Lanzén et al. 2011; Wilhelm et al. 2014).

Community composition and the 'rare' biosphere

OTU richness and diversity increased with sediment depth in both, the RNA- and DNA-based libraries of Bacteria and Archaea. One of the main factors limiting microbial diversity in hypersaline environments is salinity. Generally microbial diversity increases with decreasing salinity (Oren 2002) as also seen here for Lake Strawbridge sediment samples. Observed cell numbers in Lake Strawbridge sediment samples were in the same order of magnitude (10^7 – 10^8) as in other hypersaline lakes like Lake Quinghai (Dong et al. 2006) and Lake Chaka (Jiang et al. 2007). Based on cell numbers Bacteria were more abundant than Archaea in all sediment layers of Lake Strawbridge. This correlates with results for Lake Kasin (Emmerich et al. 2012) and La Salle del Rey sediments (Hollister et al. 2010). Other studies reveal that Archaea are the dominant organisms (Jiang et al. 2007; Swan et al. 2010). So far, the factors leading to the dominance of archaeal or bacterial cells remain unclear.

In the rank abundance analysis we observed an elongated tail of rare OTUs in the DNA-based libraries indicating the

existence of a 'rare' biosphere in the sediments of Lake Strawbridge which has also been reported for other environments previously (Elshahed et al. 2008; Galand et al. 2009; Pedrós-Alió 2011; Sogin et al. 2006). Because richness estimators and rarefaction analysis indicated that our sequencing approach covered 21–63% of the estimated total diversity, the true extent of Lake Strawbridge sediment's 'rare' biosphere remains to be explored. However, Good's coverage values, indicative of the percentage of estimated total phylotypes present in a sample, suggested that we covered the majority of archaeal and bacterial phylotypes in Lake Strawbridge. Alpha diversity measurements, rarefaction curves, and rank abundance curves revealed that OTU richness and diversity were lower in the RNA-based libraries compared to the DNA-based libraries indicative of the presence of taxa with low or no protein synthesis potential at the time of sampling. Such an 'inactive' community has also been described in other recent studies that compared RNA- and DNA-based sequence tag libraries (Gaidos et al. 2011; Lanzén et al. 2011).

Microbial life in saline environments faces thermodynamic limitations since it requires an adequate osmotic adaptation mechanism and a metabolism that generates enough energy for osmo-regulation and biomass synthesis (Oren 1999, 2011). On the other hand, microorganisms have the ability to endure unfavorable environmental conditions in a "nongrowth" state (dormancy, spore formation, inactive but viable cells) dividing again once environmental conditions become more favorable (Jones and Lennon 2010; Lennon and Jones 2011). Kulp et al. (2007) found that microorganisms that are adapted to low salinity conditions can remain viable at high salinities and rapidly repopulate sediments when water levels are rising and the salinity decreases, e.g. after rainfall.

The maximum dissimilarity between the sediment layers of the archaeal RNA-based libraries was very low (0.09). The decrease in salinity with increasing sediment depth seems to have no strong influence on the composition of the potentially 'active' archaeal community fraction. The majority of the sequences of the archaeal community were related to the *Halobacteriaceae*. These findings are consistent with several other studies on the archaeal community composition in hypersaline environments (Boutaiba et al. 2011; Mutlu et al. 2008; Ochsenreiter et al. 2002; Oren, Sørensen et al. 2009; Oueriaghli et al. 2013; Schneider et al. 2013; Youssef et al. 2012). Besides the *Halobacteriaceae*, the *Thermoplasmatales* presented a major fraction of the archaeal community in Lake Strawbridge. *Halobacteria* and *Thermoplasmata* have also recently been found to dominate the archaeal community fraction in the upper layers of a microbialite-forming microbial mat from a hypersaline lake of the Kiritimati Atoll in the Central Pacific (Schneider et al. 2013).

The dominant bacterial phyla observed in Lake Strawbridge were the *Proteobacteria*, *Bacteroidetes*, *Firmicutes*, *Cyanobacteria* and *Chloroflexi*. These phyla are often found in a diverse range of hypersaline environments such as salt lakes (Abed et al. 2012; Hollister et al. 2010; Mesbah et al. 2007), solar salt-erns (Sørensen et al. 2005), mangrove sediments (Andreote et al. 2012), salt marshes (Bowen et al. 2012) or hypersaline soils (Keshri et al. 2012). The bacterial community analysis also

revealed a lower dissimilarity among the RNA-based libraries compared to the DNA-based libraries suggesting that the protein synthesis potential of the bacterial community does not change much with sediment depth as also observed for the potentially 'active' archaeal community.

Identity and potential activity of abundant OTUs

In the RNA- and DNA-based libraries the majority of the archaeal OTUs were related to the *Halobacteriaceae*. We observed a fraction of *Halobacteriaceae* that could not be classified on the genus level in both the RNA-based (11.4–40.0% relative abundance) and the DNA-based (16.8–30.9% relative abundance) tag libraries. This is consistent with other 16S rRNA genes-based studies on the archaeal diversity in hypersaline environments that also described that a large fraction of the recovered archaeal sequences could not be classified (Caton et al. 2009; Mesbah et al. 2007; Oren, Sørensen et al. 2009; Youssef et al. 2012). Some halobacterial species, e.g. *Haloferax volcanii*, *Haloferax mediterranei* or *Halobacterium salinarium* are known to be polyploid carrying more than 10 copies of their chromosome (Lange et al. 2011; Zerulla and Soppa 2014). This could significantly increase the RNA/DNA ratio for individual genera and species belonging to the unclassified *Halobacteriaceae*. *Halobacteriaceae* are highly adapted to hypersaline environments and are the dominant heterotrophic microorganisms in hypersaline environments with a salinity of more than 250–300 g/L NaCl (Oren 1994).

They use the salt-in strategy to osmotically balance their cytoplasm with the surrounding medium (Christian and Waltho 1962). To do so, they possess a proteome enriched in acidic amino acids (Dennis and Shimmin 1997). They are mainly aerobic and heterotrophic organisms using a variety of different carbon sources (Oren 1994). Many *Halobacteriaceae* are pigmented causing a red coloring of many hypersaline waterbodies, also observed at Lake Strawbridge. The red cell pigmentation can be attributed to the presence of bacteriorhodopsins and halorhodopsins which enable the *Halobacteria* to use light energy for the generation of transmembrane proton and chloride gradients (Oren 1994; Oren and Rodríguez-Valera 2001). In both, the RNA- and DNA-based libraries *Halogranum* was the most abundant genus within the Archaea showing a high protein synthesis potential that decreases with sediment depth since *Halogranum* are aerobic and heterotrophic organisms preferring hypersaline conditions as present in Lake Strawbridge top sediment layer (Cui et al. 2011; Kim et al. 2011).

In contrast to the numerically dominant and potentially active *Halobacteriaceae* like *Halogranum* and *Haloarcula* we found that many other halobacterial taxa such as *Haloplanus*, *Halorubrum*, *Candidatus Halobonum* or *Natronomonas* only had a low RNA/DNA ratio in Lake Strawbridge attributing a low protein synthesis potential to these taxa at time of sampling.

In the bacterial community *Salinibacter* spp. were abundant in DNA-based libraries and simultaneously exhibited a high protein synthesis potential. *Salinibacter* inhabit hypersaline environments worldwide and share many properties with the archaeal family of the *Halobacteriaceae* (Oren 2013). They are

aerobic heterotrophs that form red colonies on agar plates due to their retinal pigments (Antón et al. 2002). Cell counts based on fluorescence *in situ* hybridization in solar salterns revealed that *Salinibacter* can account for up to 25% of the total bacterial community in hypersaline ecosystems (Antón et al. 2000).

Like the *Halobacteriaceae*, *Salinibacter* use the salt-in strategy for osmo-regulation that requires generally less metabolic energy compared to the synthesis of compatible solutes. *Salinibacter* have been reported to possess an acidic proteome in order to maintain their enzymatic activity under high salt conditions (Oren 2002; Oren and Mana 2002). In addition they possess xanthorhodopsin, a retinal pigment with a salinixanthin carotenoid antenna. The pigment enables *Salinibacter* to use light energy to generate a transmembrane proton gradient for energy conservation (Balashov and Lanyi 2007; Balashov et al. 2005; Lutnaes et al. 2002; Mongodin et al. 2005; Oren 2013).

In the bacterial community we found a variety of sulfate reducers belonging to the *Desulfhalobiaceae* (order *Desulfobrivales*), *Desulfobacteraceae* and *Desulfobulbaceae* (both order *Desulfobacterales*). In the RNA-based libraries of the layers 1–10 cm and below 10 cm the dominant sulfate reducing bacteria were *Desulfobacterales* with relative abundances of 4.5–4.9%, indicating that sulfate reduction occurs in these layers. Sulfate concentrations in Lake Strawbridge decreased with sediment depth (Table 1) supporting the theory of the activity of sulfate reducing bacteria.

Also the salinity decreases with sediment depth and it was shown previously that sulfate reduction rates were higher at lower salinity due to the higher cell numbers of sulfate reducing bacteria (Brandt et al. 2001). Nonetheless sulfate reduction was observed up to the highest salinities (Foti et al. 2007; Oren 2011; Porter et al. 2007) The order *Desulfobacterales* with the family *Desulfobacteraceae* were also observed to belong to the dominant sulfate reducers in hypersaline lakes (Brandt et al. 2001; Foti et al. 2007; Sorokin et al. 2010), hypersaline coastal pans (Porter et al. 2007) or deep sea basins (Borin et al. 2009).

Identity and potential activity of 'rare' OTUs

Archaeal OTUs of the Marine Benthic Group D and the KTK 4A cluster belonging to the *Thermoplasmatales* as well as the Marine Benthic Group B related to the *Thaumarchaeota* were characterized by a low abundance in the DNA-based libraries but a relatively high protein synthesis potential. From these archaeal taxa no pure cultures have so far been obtained. The KTK 4A cluster was first discovered by sequencing of 16S rRNA gene clones derived from saline brine sediments in the Red Sea (Eder et al. 1999). Here we provide the first evidence for the potential activity of KTK 4A-related archaea in the hypersaline sediments of Lake Strawbridge.

The Marine Benthic Groups B and D (MBG-B and -D) are among the most numerous archaea in saline ecosystems such as marine sub seafloor sediments (Lloyd et al. 2013; Sørensen and Teske 2006) or hypersaline microbial mats (Schneider et al. 2013). Jiang et al. also observed sequences related to the MBGs in sediments of hypersaline Qinghai Lake where they made up to 82% of the total archaeal community (Jiang et al.

2008). The archaeal MBGs B, C and D have also been described to inhabit the sediments of a meromictic lake located 1923 m above sea level in the southern Alps of Switzerland (Schubert et al. 2011).

Lake Strawbridge is, like most continental salt lakes in Western Australia, not of marine origin but emerged due to salinisation (Timms 2005). The occurrence of the archaeal MBGs B and D in Lake Strawbridge and other limnic ecosystems suggests that their environmental occurrence and distribution is not restricted to marine environments. The combined RNA and DNA tag sequencing approach used in this study further revealed that the identified archaeal MBGs were characterized by a high potential for protein synthesis. Previous studies relying on direct 16S rRNA analysis as indicator of potential activity have previously provided evidence for the potential activity of Marine Benthic Group B archaea in marine sedimentary subsurface ecosystems (Biddle et al. 2006; Sørensen and Teske 2006). Recently, the genomes of three isolated single cells of MBG-D related *Thermoplasmatales* have been sequenced (Lloyd et al. 2013). The single cell genomes encoded for extracellular peptidases suggesting that these uncultured archaea may have a previously undiscovered role in protein remineralization in anoxic sediments.

The bacterial taxon *Halomonas* showed the most prominent difference in relative sequence abundance between RNA- and DNA-based tag libraries. *Halomonas*-related sequences were 'rare' in the DNA-based libraries (0.1% abundance) but highly abundant in the RNA-based libraries (30.3–47.7% abundance) resulting in a RNA/DNA ratio of 403.8. This suggests that *Halomonas* exhibited a high protein synthesis potential in Lake Strawbridge sediments at the time of sampling. *Halomonas* are aerobic heterotrophs (García et al. 2004; Martínez-Cánovas et al. 2004b; Mata et al. 2002; Quesada et al. 2004; Xu et al. 2007). A possible explanation for their high activity could be their elaborate osmotic regulation mechanism. *Halomonas* synthesize the compatible solute ectoine.

Studies with *Halomonas elongata* revealed a low energy requirement for synthesis of ectoine from glucose with nearly 100% efficiency of carbon substrate conversion (Maskow and Babel 2001, 2002). However, constant osmo-regulation based on compatible solute production might require *Halomonas* to maintain a high ribosomal RNA content and protein synthesis potential. Although this interpretation requires further empirical evidence this example from our data nicely reinforces the value of simultaneous ribosomal tag sequencing of DNA and RNA to develop new testable hypothesis on the ecological role and functioning of rare taxa in complex microbial communities.

Other heterotrophic bacteria such as *Salinivibrio* (Amoozegar et al. 2008; Romano et al. 2011), *Alkalibacterium* (Ishikawa et al. 2009), *Idiomarina* (Brettar et al. 2003; Ivanova et al. 2000; Martínez-Cánovas et al. 2004a), *Salisaeta* (Vaisman and Oren 2009), *Planomicrobium* (Dai et al. 2005; Yoon et al. 2001) and *Planococcus* (Li et al. 2006; Miller and Leschine 1984) were also relatively "rare" in the DNA-based libraries, while possessing a high protein synthesis potential. These taxa were mainly present in the top layer suggesting that sufficient concentrations of organic carbon

and oxygen were present to sustain the activity of a diverse heterotrophic bacterial community.

Conclusions

Considering the limitations of using rRNA analysis as a general indicator of currently active microorganisms in environmental samples, the combined RNA- and DNA-based 16S rRNA gene tag sequencing approach presented in this study enabled new insights into the structure and protein synthesis potential of a microbial community in a hypersaline lake sediment.

Our study did identify the presence and composition of a rare biosphere in the DNA-based tag libraries of Archaea and Bacteria in Lake Strawbridge sediments. We also could show that several of the 'rare' bacterial and archaeal taxa such as *Halomonas*, *Salinivibrio* and the Marine benthic groups B and D exhibited a high protein synthesis potential (high relative sequence abundance in RNA-based libraries). This suggests that these 'rare' taxa are potentially active and contribute to biogeochemical cycling in Lake Strawbridge sediments what makes them interesting candidate taxa for further studies, e.g., their potential contribution to the formation of volatile halogenated hydrocarbons as recently investigated by Ruecker et al. (2014). The observation of 'rare' taxa that are potentially active is consistent to other studies on microbial communities in freshwater and marine ecosystems (Campbell et al. 2011; Jones and Lennon 2010; Wilhelm et al. 2014). The RNA/DNA ratio is a promising tool to estimate the protein synthesis potential of a microbial community and can help microbial ecologists to unravel the complex dynamics at play that shape the size and potential activity of microbial populations inhabiting hypersaline lake sediments.

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