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RESEARCH ARTICLE

Microbial community composition and diversity in Caspian Sea sediments

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One sentence summary: This study describes microbial biomass, community composition and diversity in Caspian Sea sediments using lipid and genomic techniques.

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ABSTRACT

The Caspian Sea is heavily polluted due to industrial and agricultural effluents as well as extraction of oil and gas reserves. Microbial communities can influence the fate of contaminants and nutrients. However, insight into the microbial ecology of the Caspian Sea significantly lags behind other marine systems. Here we describe microbial biomass, diversity and composition in sediments collected from three sampling stations in the Caspian Sea. Illumina sequencing of 16S rRNA genes revealed the presence of a number of known bacterial and archaeal heterotrophs suggesting that organic carbon is a primary factor shaping microbial communities. Surface sediments collected from bottom waters with low oxygen levels were dominated by *Gammaproteobacteria* while surface sediments collected from bottom waters under hypoxic conditions were dominated by *Deltaproteobacteria*, specifically sulfate-reducing bacteria. *Thaumarchaeota* was dominant across all surface sediments indicating that nitrogen cycling in this system is strongly influenced by ammonia-oxidizing archaea. This study provides a baseline assessment that may serve as a point of reference as this system changes or as the efficacy of new remediation efforts are implemented.

Keywords: Caspian Sea; marine sediments; bacteria; archaea; Illumina; PLFA

INTRODUCTION

With a volume of 78 000 km^3 and surface area of 3.8×10^5 km^2, the Caspian Sea is one of the largest inland bodies of water on earth (Dumont 1998). Like the Aral and Black Seas, it was once connected to oceans but has been landlocked for the past five million years. Water depths vary across the Caspian Sea with the northern portion exhibiting a maximum depth of 20 m while the southern portion has a maximum depth of 1025 m (Kosarev and Yablonskaya 1994). Approximately, 130 rivers drain into the Caspian Sea with the Volga River accounting for the majority of

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influent waters (Kosarev 2005). Due to the influx of freshwater, the salinity of the Caspian Sea is approximately one-third of the salinity of seawater making it a lacustrine brackish body of water (Leroy *et al.*, 2007).

Environmental pollution has become a growing problem in this landlocked basin in which contaminants can accumulate and persist. Nutrient-rich industrial and agricultural effluents from rivers have steadily increased over recent years leading to eutrophication (Zonn 2005). Consequently, bottom waters are oxygen deficient and hypoxic zones exist, particularly in the summer months (Diaz 2001). Hypoxic zones can have detrimental effects including mass mortality of benthic organisms, losses in biodiversity and changes in biogeochemical cycles (Diaz and Rosenberg 1995; Wu 2002). The Caspian Sea has also seen substantial expansion of oil and gas exploration and production with the finding of significant reserves, comparable to that of the North Sea (Effimoff 2000). Hydrocarbon concentrations in sediments exceed the background for other fishery water bodies by almost 2-fold (Tolosa *et al.*, 2004). Similarly, concentrations of organochlorinated compounds and heavy metals have been found to exceed sediment quality guidelines (de Mora *et al.*, 2004a, b).

Microbial communities play a pivotal role in the fate of contaminants and nutrients. However, sufficient baseline data is required to establish a foundation to gauge community shifts in response to environmental perturbation or anthropogenic pollution. Sediment microbial communities are particularly important since they have greater cell density and taxonomic diversity than planktonic communities and can respond rapidly to their surrounding environmental conditions (Jørgensen and Boetius 2007; Lozupone and Knight 2007; Gibbons *et al.*, 2014). The growth and distribution of sediment microbial communities is strongly affected by the availability of carbon sources and electron acceptors. Persistent hypoxia can lead to changes in the structure and function of microbial communities within sediments (Meyer-Reil and Köster 2000; Kristiansen, Kristensen and Jensen 2002; Jäntti and Hietanen 2012; Hou *et al.*, 2014).

While there have been studies into the geophysical and hydrological aspects of the Caspian Sea (Peeters *et al.*, 2000; Diaconescu, Kieckhefer and Knapp 2001), the microbial ecology of the Caspian Sea is less well characterized than other marine systems. Previous microbial studies have focused solely on isolating and characterizing oil-degrading microorganisms (Hassanshahian *et al.*, 2010, Hassanshahian, Emtiazi and Cappello 2012). The primary aim of this study was to describe the biomass, diversity and composition of sediment microbial communities in the Caspian Sea using a combination of lipid and genomic techniques. The secondary aim was to characterize seafloor microbial communities across varying hypoxic conditions to determine how oxygen levels of bottom waters affect microbial community structure. This study is one of the first to survey microbial communities in the Caspian Sea and provides a baseline description that may serve as a point of reference as this system changes. This is particularly important if efforts are undertaken to reduce hypoxia and pollution since shifts in microbial populations are rapid and sensitive indicators of change.

MATERIALS AND METHODS

Site characterization and sample collection

Three sediment cores were collected at three different sampling stations during a research cruise in July 2013 using a Multicorer (Fig. S1, Supporting Information). All sampling stations were located in the southern basin of the Caspian Sea, which has been categorized as persistently hypoxic (<2 mg L^{-1} of dissolved oxygen) (Diaz and Rosenberg 1995). Temperature, salinity, pH and oxygen concentrations of bottom waters were measured approximately 15 m from the seafloor with a MIDAS CTD + sensor array (Valeport Ltd, St. Peter's Quay, UK). Stations 1, 2 and 3 had water depths of 600, 205 and 141 m, respectively. Sediment cores ranged in length from 8 to 36 centimeters below seafloor (cmbsf). Following collection, intact cores were sectioned at 4 cm depth intervals and homogenized. Sediment samples were stored at -20°C on ship and transported to the University of Tennessee for further analysis.

Sediment analysis

Sediment samples were sent to SOEST Laboratory for Analytical Biogeochemistry (University of Hawaii) for total organic carbon (TOC) analyses. Sediment samples were decarbonated with HCl and percentages of total organic carbon (%TOC) were determined using the Shimadzu TOC-L combustion analyzer (Kyoto, Japan). Sediment grain size distributions for each section were determined using a Beckman Coulter LS 230 laser particular size analyzer (Brea, CA, USA) and textures were assessed using the standard textural triangle.

PLFA extraction and analysis

For each sediment sample, 30 to 50 g was extracted using a modified Bligh and Dyer method as per Mahmoudi *et al.*, (2013a). Sediments were extracted using 2:1 methanol/dichloromethane (DCM) and filtered into separatory funnels using 0.45 μm precombusted glass fiber filters (GF/G, Whatman). Following phase separation using nanopure water, the organic phase was collected and separated into three fractions by gravity column chromatography using fully activated silica (precombusted at 450°C for 8 h) and DCM, acetone and methanol to elute non-polar, neutral and polar fractions, respectively. The polar fraction, which contained phospholipids, was evaporated to dryness under a stream of nitrogen gas and reacted to fatty acid methyl esters (FAMEs) via a mild alkaline methanolysis reaction. Identification and quantification of FAMEs utilized an Agilent 6890 gas chromatograph coupled to an Agilent 5973 mass spectrometer (equipped with a 60 m $\times$ 0.25 mm Rtx®-1 column). The temperature program for the GC oven was 60°C for 2 min, ramp to 150°C at 10°C min^{-1} and then ramp to 312°C at 4°C min^{-1}. FAMEs were identified using a bacterial reference standard (Bacterial Acid Methyl Esters CP, Mix, Matreya Inc.), mass-fragmentation patterns and retention times and quantified using external calibration standards (which contained FAMEs of various chain length).

Genomic DNA extraction and PCR amplification

Genomic DNA was extracted in triplicate from each sediment sample using the PowerSoil DNA Isolation kit (MoBio Laboratories Inc., Carlsbad, CA, USA) according to manufacturer's protocol. Triplicate DNA extracts were subsequently further purified using the Genomic DNA Clean and Concentrator kit (Zymo Research, Irvine, CA, USA). 16S rRNA genes were amplified in triplicate using primer pair 515F and 806R (Caporaso *et al.*, 2012). The reverse primers included a 12-bp barcode for multiplexing of samples during sequencing analysis.

Table 1. Coordinates of sampling stations and environmental parameters of bottom waters.

	Latitude	Longitude	Water depth (m)	Temp (°C)	Salinity (PSU)	pH	Oxygen concentration (mg L^{-1})
Station 1	39.745 59	50.480 600	600	6.1	11.4	7.8	0.5
Station 2	39.990 26	51.500 808	205	6.6	11.3	8.0	3.3
Station 3	40.040 59	51.347 301	141	6.9	11.3	8.1	4.8

Barcoded amplicon sequencing of bacterial and archaeal SSU genes and sequence analysis

16S libraries were prepared according to Caporaso *et al.*, (2012). Briefly, 16S amplicons were pooled together and analyzed by Bioanalyzer (Agilent Technologies) to assess quality and size of amplicons. Following dilution, libraries were subjected to quantitative-PCR to ensure accurate quantification of purified amplicons. 16S libraries were sequenced using an Illumina MiSeq (San Diego, CA, USA) platform at the University of Tennessee. The forward read data (trimmed to 250 bp) was used for analysis due to poor quality and short read length of the reverse reads. Sequences were processed and quality controlled through a combination of the UPARSE and QIIME pipelines (Caporaso *et al.*, 2010a; Edgar 2013). The QIIME (v1.7; Caporaso *et al.*, 2010a) script, split_libraries_fastq.py, was used to demultiplex the sequence data with the quality filter set to zero. The generation of OTUs (97% sequence similarity) and quality control processing was carried out via the UPARSE pipeline (Edger 2013), including *de novo* and reference-based chimera detection. The resulting OTU table was converted to BIOM format (McDonald *et al.*, 2012). Taxonomy was assigned using the RDP classifier (Wang *et al.*, 2007) against the updated May 2013 '13_5/13_8' Greengenes database (DeSantis *et al.*, 2006; McDonald *et al.*, 2012; Werner *et al.*, 2012) via QIIME (Caporaso *et al.*, 2010a). A phylogeny was constructed using FastTree (Price, Dehal and Arkin 2010) from a masked PyNAST (Caporaso *et al.*, 2010b) alignment. The resulting phylogeny was manually rooted to Archaea via Dendroscope (v3; Huson and Scornavacca 2012). Any OTU that comprised less than 0.005% of the total data set was removed to limit the effect of spurious OTUs on analysis (Bokulich *et al.*, 2013; Navas-Molina *et al.*, 2013). Finally, various analyses, evenness and alpha-diversity metrics were calculated after pooling the technical replicates and rarefying the samples to the same sequencing depth using QIIME (Caporaso *et al.*, 2010a), R v3.1 (R Core Team 2013) and Phyloseq (McMurdie and Holmes 2013). After merging replicates, bacterial data were rarefied to ~22 900 reads and archaeal data were rarefied to ~230 reads for non-metric multidimensional scaling (NMDS), principal coordinates analysis and alpha-diversity analysis. For ADONIS analysis individual samples (i.e. all replicates) were rarefied to ~2775 and ~1000 reads for bacteria and archaea, respectively. The sequence data generated in this study were deposited in GenBank under BioProject PRJNA261725 (BioSample Accessions: SAMN03074722-SAMN03074762).

Statistical analyses

Microbial communities across sediment samples were compared using weighted UniFrac (Lozupone and Knight 2005) based on the phylogenetic relationship of representative reads from different sediment samples. Variation in microbial communities over sampling locations and sediment depth was assessed using both a UPGMA tree and ADONIS analysis of all triplicates. Patterns in microbial community structure in relationship to environmental variables were visualized using NMDS plot based on a weighted UniFrac distance matrix. A stress function was used to assess the goodness-of-fit of the ordination. Stress values range from 0 to 1; values below 0.2 suggest that the ordination accurately represents the observed dissimilarity between samples (Clarke 1993). Environmental variables were fitted to the NMDS ordinations as vectors with the 'envfit' function of the 'vegan' package in R v3.1 (Oksanen *et al.*, 2013).

RESULTS

Environmental parameters of sampling stations and sediment properties

Temperature, pH and salinity of bottom waters (as defined by measurements taken at the bottom meter of a CTD cast) at the three sampling stations were similar; however, considerable variations in oxygen concentrations between stations were observed (Table 1; Fig. S2, Supporting Information). Bottom waters at Station 1 had the lowest concentrations of dissolved oxygen (0.5 mg L^{-1}), indicative of severe hypoxia. The upper limit for hypoxia in marine environments is approximately 3 mg L^{-1} of dissolved oxygen (Hofmann *et al.*, 2011). Therefore, bottom waters at Station 2 were mildly hypoxic (3.3 mg L^{-1}), while bottom waters at Station 3 were under low oxygen saturation (4.8 mg L^{-1}). Salinity at all three stations was approximately one-third of the mean ocean salinity value of 35 practical salinity units (PSU) (Wilson 1975). TOC varied between sediment cores and was found to be lowest at Station 3 and highest at Station 2 (Table S1, Supporting Information). The TOC values observed at Station 3 were similar to those previously measured in the Caspian Sea (Parr *et al.*, 2007). In contrast, TOC values measured at Station 1 and 2 were higher and consistent with those found in organic-rich, anoxic marine sediments such as those from the Black Sea (Xu *et al.*, 2001). Particle size analysis revealed that the majority of sediment samples were primarily composed of silt and clay particles such that they are classified as having a texture consistent with a silt loam (Table S1, Supporting Information).

Microbial PLFA concentrations and distributions

Microbial biomass, estimated from PLFA concentrations, was found to be highest at Station 2 and lowest at Station 3 (Fig. 1). Using a conversion factor of 5.9×10^4 cells $pmol^{-1}$ of PLFA (Mills *et al.*, 2006), PLFA concentrations correspond to cell densities of 1.3×10^6 to 7.6×10^8 cells g^{-1} (Table S2, Supporting Information). Microbial biomass was correlated with TOC; however, some fluctuations were observed at certain depths (Fig. 1). The distribution of PLFAs for all sediments samples was dominated by monounsaturated and n-saturated PLFAs, as expected for sediments (Table S3, Supporting Information) (Zelles 1999). Overall, 16:0 was the most dominant PLFA while 18:0, i15:0 and

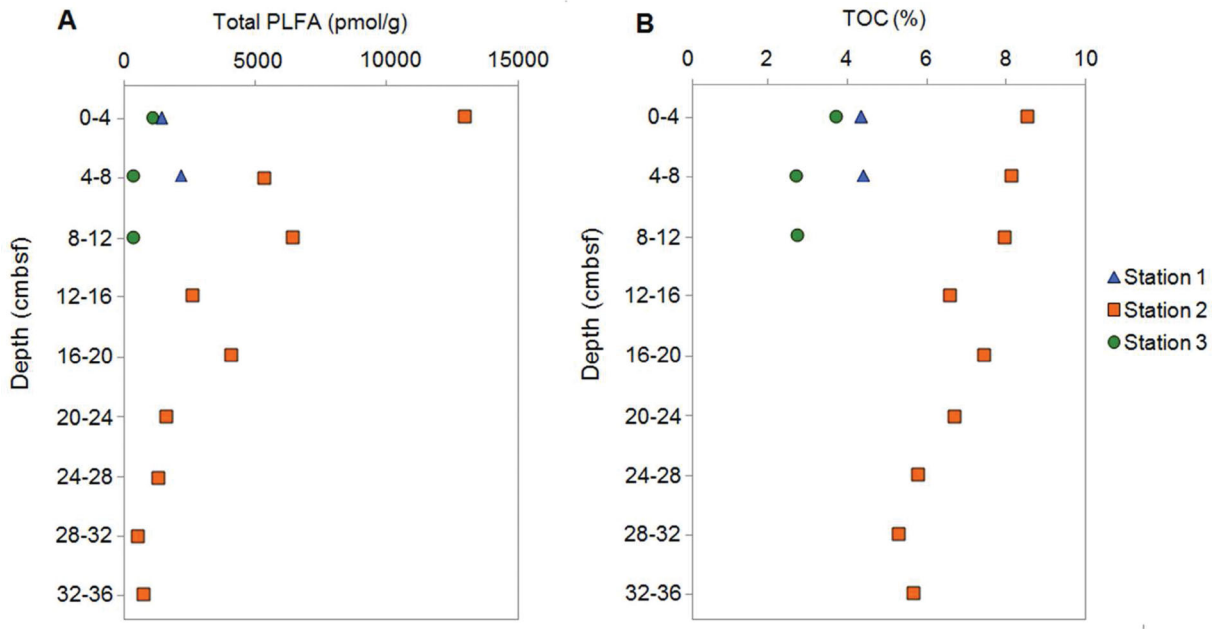

Figure 1. Depth distribution of total PLFA concentrations (**A**) and percent TOC (**B**) in sediment cores collected from the Caspian Sea.

a15:0 were also present. Monounsaturated PLFAs are associated with Gram-negative bacteria (Zelles 1999) such as *Proteobacteria*, *Chloroflexi* and *Planctomycetes*, which were abundant in the sequencing results. Terminally branched PLFA are indicative of Gram-positive bacteria and were present at low levels in all sediment samples (<25%). Mid-branched saturated PLFA were also found at low levels; these are typical of sulfate-reducing bacteria (SRB) and *Actinomycetes*, which were present at all stations based on the sequencing results. Interestingly, a microeukaryotic biomarker PLFA, 18:2, was observed in surface sediments at Station 2.

Bacterial 16S rRNA analysis

Illumina-based analysis of 16S rRNA amplicons recovered a total of 3 377 889 (2278 OTUs) of bacterial 16S and 343 848 (178 OTUs) of archaeal 16S sequences with an average length of 250 bp. Fifty-five different bacterial phyla were detected across all sediment samples (Fig. 2). The most abundant bacteria were *Proteobacteria* (33% of bacterial reads on average), followed by *Planctomycetes* (14%) and *Chloroflexi* (12%). Within *Proteobacteria*, the majority of sequences were assigned to the classes, *Deltaproteobacteria* (16%) and *Gammaproteobacteria* (12%). *Deltaproteobacteria* dominated the upper sediment layers (0–16 cmbsf) Station 1 and 2; the predominant order at these depths was the sulfate-reducing family, *Desulfobacterales* (12%). Further, the relative abundance of sequences belonging to *Planctomycetes* and *Chloroflexi* increased with sediment depth at Station 1 and 2. The most dominant orders within *Planctomycetes* and *Chloroflexi* were *Phycisphaerae* (11%) and *Anaerolineae* (9%), respectively. The majority of sequences in surface sediments (0–4 cmbsf) at Station 3 belonged to *Gammaproteobacteria* (60%). Within *Gammaproteobacteria*, the dominant orders at this depth were *Oceanospiralles* (18%), *Altermonadales* (13%) and *Xandomondales* (12%). At 4–8 cmbsf (at Station 3), *Nitrospirae* (27%) was the most abundant phyla and a large proportion of sequences at this depth belonged to the order *Thermodesulfovibrionaceae* (27%), which was present in relatively low abundances at Station 1 and 2. Finally, *Planctomycetes* (42%) was the most abundant phyla at 8–12 cmbsf at Station 3 with *Phycisphaerae* (39%) comprising the majority of assigned sequences at this depth.

Archaeal 16S rRNA analysis

Four different archaea phyla were detected across sediment samples including the newly designated phylum, *Parvarchaeota* (Fig. 3). *Crenarchaeota* (34%) was the most abundant phylum across all sediment samples, followed by *Thaumarchaeota (15%)*, *Euryarchaeota* (12%) and *Parvarchaeota* (12%). Within *Crenarchaeota*, *Marine Benthic Group B* (MBGB) (26%) and *Miscellaneous Crenarchaeotal Group* (MCG) (11%) (also known as *Bathyarchaeota*, Meng *et al.*, 2013) had the highest relative abundance. At Stations 1 and 2, the relative abundance of sequences corresponding to MBGB increased with sediment depth such that it was the most dominant order from 12 to 36 cmbsf. Sequences belonging to MCG did not account for a large portion of the archaeal sequences at Station 1 and 2 (4%); however, it constituted 15% of assigned archaeal sequences at 0–4 cmsbf and 47% at 4–8 cmbsf in Station 3. Cenarchaeales was the most abundant class within *Thaumarchaeota*; this class was found to be predominant in surface and upper layers of sediments at all three sampling stations and decreased with sediment depth. Among *Euryarchaeota*, the majority of sequences were assigned to *Thermoplasmata*, particularly in deeper sediments layers (24–32 cmbsf). *Euryarchaeota* sequences corresponding to anaerobic methanotrophic archaea were absent at all sampling stations. Further, sequences belonging to methanogenic groups such as Methanobacteria were present in fairly low abundances (1% on average). Sequences corresponding to *Parvarchaeaota* were found in all sediment samples; the relative abundance of *Parvarchaeota* decreased with sediment depth at Station 1 and 2, while the opposite was true at Station 3.

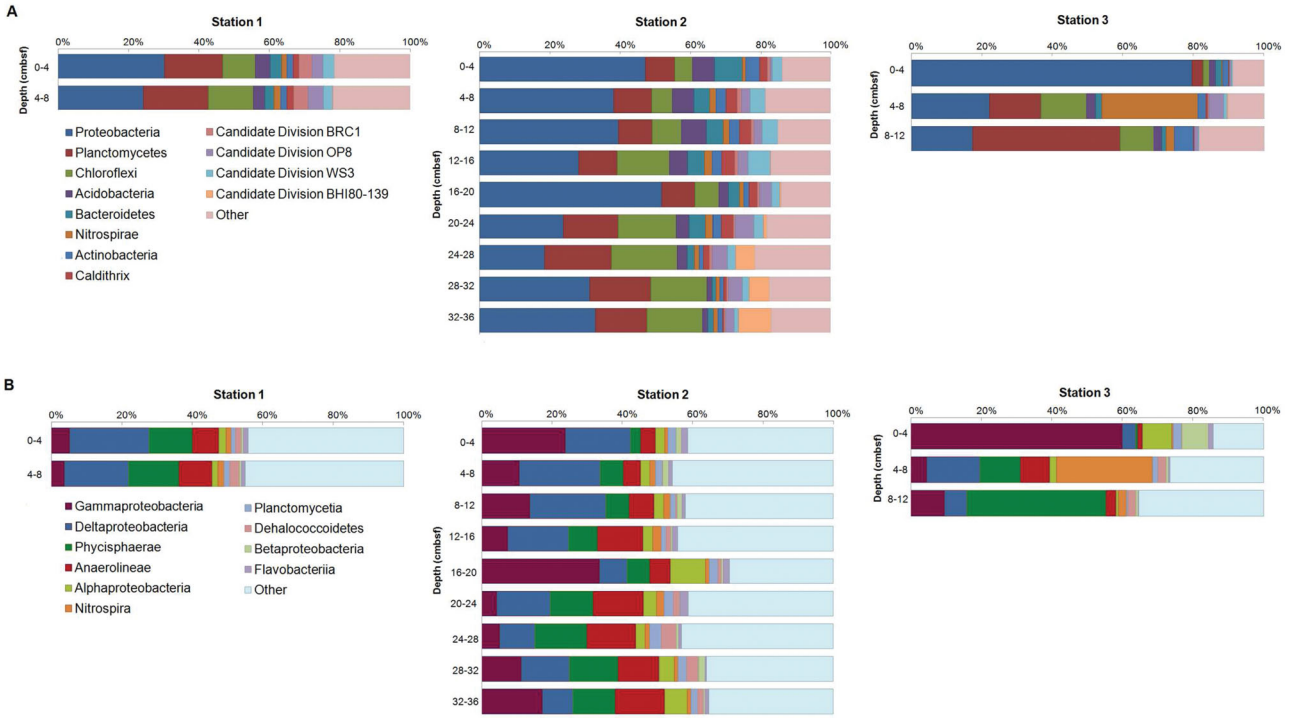

Figure 2. Relative abundance of dominant bacterial groups observed in sediment cores collected from the Caspian Sea. Taxonomic distributions are depicted for the ranks of Phylum (**A**) and Class (**B**).

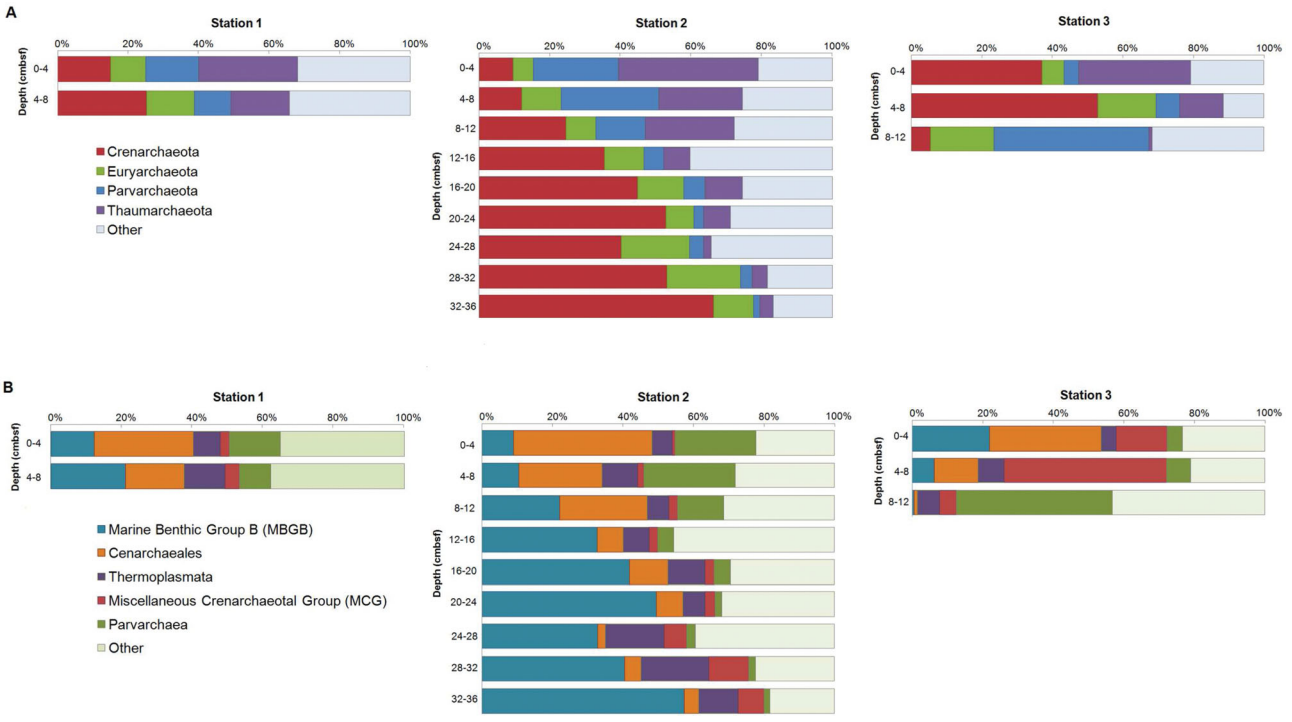

Figure 3. Relative abundance of dominant archaeal groups observed in sediment cores collected from the Caspian Sea. Taxonomic distributions are depicted for the ranks of Phylum (**A**) and Class (**B**).

Statistical comparison of 16S amplicons among sediment samples

The similarity and dissimilarity of 16S sequences across sediment samples were measured using weighted UniFrac distance metric (Fig. S3, Supporting Information). These analyses showed similar trends for bacterial and archaeal communities such that sediment samples from Station 1 and 2 showed strong clustering, while sediment samples from Station 3 had a dispersed distribution. Hierarchical cluster analysis (Fig. S4, Supporting

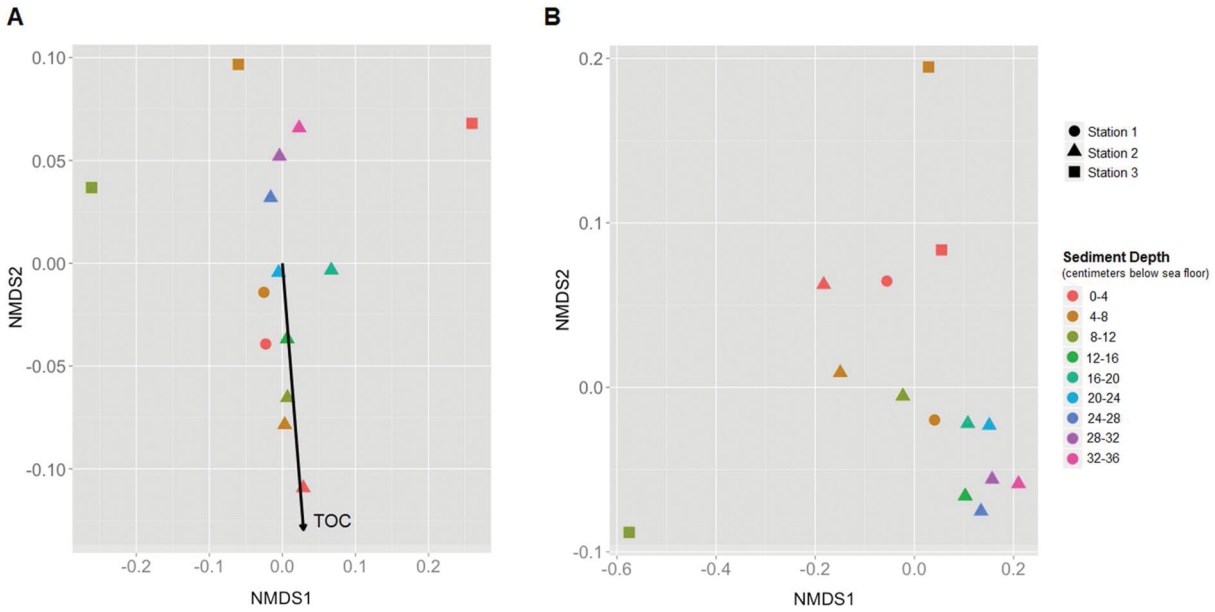

Figure 4. Community analysis using NMDS of weighted UniFrac distance matrix for bacteria (**A**) and archaea (**B**). Only statistically significant fitted ($P < 0.05$) environmental variables (TOC) are shown as vectors (arrows).

Information) of bacterial and archaeal communities indicated that sediment depth and sampling location may be factors contributing to variability in community structure. Accordingly, ADONIS analysis confirmed that sediment depth ($R^2 = 0.39$, $P = 0.0001$, strata = location) and sampling location ($R^2 = 0.27$, $P = 0.0001$, strata = depth) affected the observed variation among bacterial communities. Further, ADONIS analysis indicated that sediment depth ($R^2 = 0.61$, $P = 0.0001$, strata = location) but not sample location ($R^2 = 0.29$, $P = 0.07$, strata = depth) affected the observed taxonomic distribution of archaeal communities among samples.

NMDS analysis also showed that bacterial communities at Station 1 and 2 were more taxonomically similar compared to Station 3 (Fig. 4a, 2D stress: 0.065). Sediment samples within Station 3 were much more taxonomically different from one another compared to Station 1 and 2, highlighting the heterogeneity of this sediment core. Fitting of environmental variables to the bacterial NMDS ordination revealed a significant relationship ($P < 0.05$) between the observed pattern of taxonomic clustering with TOC ($R^2 = 0.64$, $P < 0.01$). This is consistent with the notion that organic carbon is one of the most fundamental factors shaping microbial communities in marine sediments (Jørgensen *et al.*, 2012). NMDS of archaeal communities showed some clustering of sediment samples from Station 1 and 2 (Fig. 4b; 2D stress: 0.035), although more dispersion was observed. Fitting of environmental variables to the archaeal NMDS ordination found no significant correlations ($P > 0.05$), suggesting that the observed pattern of taxonomic clustering for archaea was likely influenced by environmental factors not accounted for in this study.

Microbial diversity

Species richness, coverage and diversity indices were calculated for each sediment sample (Table S4a and b, Supporting Information; Fig. 5). Good's coverage ranged from 98 to 99% for bacteria and 88 to 97% for archaea, indicating that the rarified sequencing depth represented the majority of 16S rRNA sequences in each sample. Chao1 values revealed that bacterial richness was significantly higher than archaeal richness across all sediment samples (T-Test, P values < 0.05). Correspondingly, each sediment sample on average contained 1630 and 120 bacterial and archaeal OTUs, respectively. Both bacterial and archaeal diversity (based on Shannon Index and Faith's PD) was greater at Station 1 and 2 relative to Station 3 and peaked in surface sediments. In contrast, bacterial and archaeal diversity was significantly lower at Station 3 and peaked at 4–8 cmbsf rather than in surface sediments as observed at Station 1 and 2.

DISCUSSION

Sediment microbial communities are vital components of marine environments and play important roles in nutrient cycling, organic matter remineralization and degradation of contaminants. Here, we combined lipid and DNA-based approaches to characterize the biomass, composition and distribution of sediment microbial communities in the Caspian Sea.

Prokaryotic cell density in marine sediments is typically 10^8 to 10^9 cells g^{-1} at the surface and decreases with depth (Parkes, Cragg and Wellsbury 2000). The cell densities observed here for Caspian Sea sediments are consistent with those reported for marine sediments around the world (Parkes *et al.*, 2000; Reed *et al.*, 2002; Carr *et al.*, 2013). A strong correlation between PLFA concentrations and TOC content in our study supports the notion that heterotrophy is dominant in marine sediments (D'Hondt *et al.*, 2004; Biddle *et al.*, 2006). PLFA concentrations were found to decrease with sediment depth; however, some increases were observed relative to the previous depths. These increases in cell density may be attributed to the increased TOC also observed at these depths. Increases in cell densities in different sediment layers have previously been observed for other marine sediments (Inagaki *et al.*, 2003; Schippers *et al.*, 2012; Giovannelli *et al.*, 2013).

Microbial sulfate reduction is the major pathway for anaerobic degradation of organic matter in marine sediments

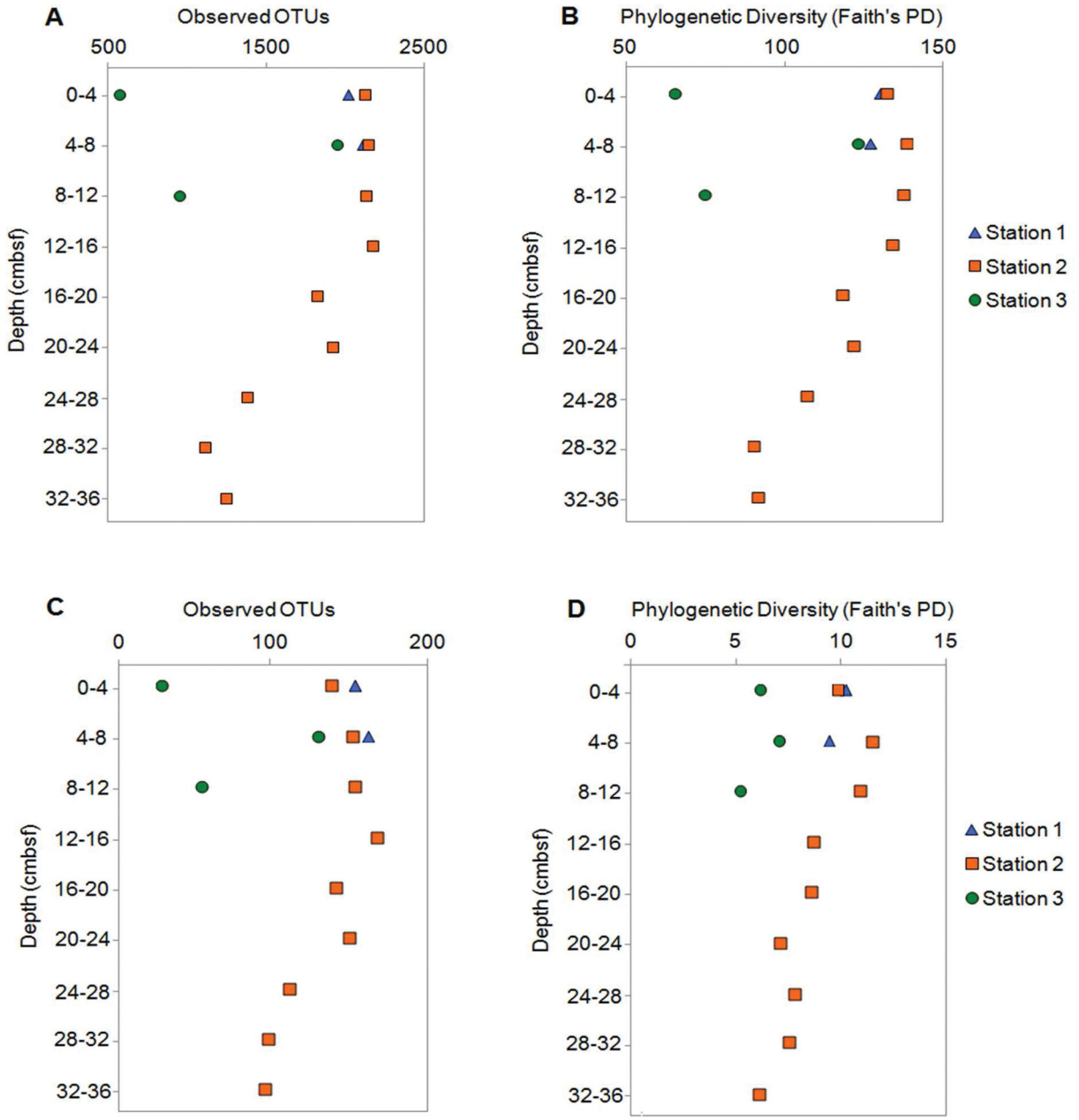

Figure 5. Changes in observed OTU richness and Faith's phylogenetic diversity (PD) with sediment depth for bacteria (**A**; **B**) and archaea (**C**; **D**).

(Jørgensen 1982; Canfield *et al.*, 1993) and is often mediated by groups belonging to *Deltaproteobacteria*, specifically *Desulfobacterales* (Orphan *et al.*, 2001; Leloup *et al.*, 2009). In this study, *Desulfobacterales* and *Desulfarculales* were the dominant groups in surface and upper sediments at sampling stations with hypoxic bottom waters, Station 1 and 2 (Table S5, Supporting Information). SRB belonging to *Deltaproteobacteria* were present in lower abundances in surface sediments (0–4 cmbsf) at Station 3. However, the anaerobic, sulfate-reducing genus *Thermodesulfovibrio* (belonging to the phylum *Nitrospirae*) dominated sediments at Station 3 at 4–8 cmbsf, consistent with probable oxygen depletion within a few centimeters of the sediment-water interface (Jørgensen 1983; Revsbech, Madsen and Jørgensen 1986). The relative abundance of known SRB sequences across all sampling stations suggests that dissimilatory sulfate reduction may be a primary pathway for anaerobic carbon degradation in Caspian Sea sediments.

Gammaproteobacteria have been found to be one of the most abundant bacterial groups in marine sediments (Inagaki *et al.*, 2003; Polymenakou *et al.*, 2005; Feng *et al.*, 2009; Mahmoudi *et al.*, 2013b; Ruff *et al.*, 2013). *Gammaproteobacteria* were present in all sediment cores in this study and accounted for a particularly large proportion of bacterial sequences in surface sediments at Station 3. Within this subphyla, *Altermonadales*, *Oceanospirillales* and *Thiotrichales* were the most common orders detected. McCarren *et al.*, (2010) found several phylogenetic groups within *Alteromondales* and *Thiotrichales* to be stimulated with the addition high-molecular-weight (HMW) dissolved organic matter in seawater incubations indicating that these groups may play a role in the degradation of HMW-organic

matter. In contrast, *Oceanospirillales* have been shown to aerobically degrade simple aliphatic hydrocarbons in marine environments (Hazen *et al.*, 2010). Single-cell sequencing and metatranscriptomic investigations of *Oceanospirillales* demonstrated that members of this order have genes coding for n-alkane and cycloalkane degradation (Mason *et al.*, 2012). Groups within *Gammaproteobacteria* may be playing an important role in degrading low- and high-MW organic matter in this system, particularly in seafloor sediments where low levels of oxygen are present in bottom waters.

Consistent with previous studies of marine sediments (Webster *et al.*, 2004; Fry *et al.*, 2008; Blazejak and Schippers 2010), the relative abundance of *Chloroflexi* and *Planctomycetes* increased with sediment depth such that these groups were dominant in deeper sediments layers (20–36 cmbsf). Little is known about the physiology of *Chloroflexi*, although they are presumed to be heterotrophic (Webster *et al.*, 2011). Members of the candidate division JS1 are key bacterial representatives associated with methane hydrates and commonly co-occur with *Chloroflexi* in anoxic sediment zones (Fry *et al.*, 2008; Jørgensen *et al.*, 2012). In this study, sequences belonging to JS1 comprised a small proportion (1–5%) of the microbial community. Even though *Chloroflexi* and JS1 often co-occur, it has been suggested that *Chloroflexi* dominate organic-rich, sandy seafloor sediments while JS1 dominant strictly anoxic, organic-rich but poor quality recalcitrant carbon muddy sediments with low sulfate concentrations (Inagaki *et al.*, 2006; Webster *et al.*, 2007).

Planctomycetes have been found in high numbers (10^8 cells mL^{-1}) in intertidal marine sediments (Musat *et al.*, 2006) and are often detected in deep-sea and subseafloor sediments (Reed *et al.*, 2002; Inagaki *et al.*, 2006; Harrison *et al.*, 2009). Similar to *Chloroflexi*, their metabolic function in the environment is largely unexplored (Fuerst and Sagulenko 2011). The majority of *Planctomycetes* sequences detected in this study were assigned to the newly designated class, *Phycisphaerae*, which contain facultative fermentative heterotrophs (Fukunaga *et al.*, 2009). The higher relative abundance of both *Planctomycetes* and *Chloroflexi* in deeper sediment layers indicates that they may play an important role in degrading organic matter at these deeper depths.

Sequences belonging to *Thaumarcheaota* accounted for a large proportion of the archaeal sequences in surface sediments across all stations. *Thaumarchaeota* are among the most abundant archaea on earth and have been detected in soils, marine waters and sediments (Francis *et al.*, 2005; Leininger *et al.*, 2006; Wuchter *et al.*, 2006). Thus far, all organisms of this group have been identified as aerobic ammonia-oxidizers and possess the key enzyme, ammonia monooxygenase. Due to their ubiquity in marine environments, it is thought that *Thaumarchaeota* play a major role in global nitrification (Stahl and De la Torre 2012). To date, all known *Thaumarchaeota* have been found to be obligate aerobes; however, we detected *Thaumarchaeota* in sediments collected from sampling stations with hypoxic bottom waters. Similarly, *Thaumarchaeota* have been observed in other anoxic environments including oxygen-deficient waters (Peng, Jayakumar and Ward 2013; Parsons *et al.*, 2014) suggesting that this group may possess alternative physiologies. Regardless, the higher prevalence of *Thaumarchaeota* in surface sediments observed here suggests that this group may play a significant role in nitrogen cycling in the Caspian Sea.

MBGB and MCG have been shown to be ubiquitous in marine environments particularly in anoxic sediments (Inagaki *et al.*, 2003, 2006; Biddle *et al.*, 2006; Fry *et al.*, 2008). A higher relative abundance of MCG to total microbes has been observed in marine sediments where sulfate penetrates at least 10 cm (Kubo *et al.*, 2012). MCG accounted for the majority of assigned archaeal sequences at 4–8 cmsbf at Station 3; correspondingly, SRB were the most abundant bacterial group at this depth as well. In contrast, MBGB were present in fairly high abundances in all sediment samples and their relative abundance increased with sediment depth. Biddle *et al.*, (2006) hypothesized that MBGB and MCG are anaerobic heterotrophs that consume buried carbon. More recently, it was shown that MCG play a significant role in degrading detrital proteins in anoxic marine sediments (Lloyd *et al.*, 2013). Thus, the prevalence of MBGB and MCG in these sediments indicates that they may also be contributing to the anaerobic degradation of organic matter in the Caspian Sea.

We also detected a large number of sequences belonging to the newly assigned phyla, *Parvarchaeota*. Recently, phylogenetic analysis of 201 single-cell genomes enabled characterization of a new super phylum, DPANN, containing *Diapherotrites*, *Parvarchaeota*, *Aenigmarchaeota*, *Nanohaloarchaeota* and *Nanoarchaeota* (Rinke *et al.*, 2013). Members of this super phylum have small cell and genome sizes; little is known about their physiology or metabolism. To our knowledge, this is the first report of *Parvarchaeota* in marine sediments and raises questions into their metabolic function in marine environments.

Eutrophication and resulting deoxygenation of marine waters can alter the oxidation–reduction balance in sediments as well as associated biogeochemical processes. It is difficult to discern whether sediment microbial communities in this system are directly affected by depleting oxygen levels in overlying waters or by associated geochemical changes resulting from this depletion. Deoxygenation of overlying waters may lead to greater preservation and thus, input of organic carbon to seafloor sediments. In this study, microbial biomass in surface sediments ranged from 10^7 to 10^8 cells g^{-1} across sampling stations, which suggests that hypoxic bottom waters may not lead to substantial decreases in biomass of seafloor microbial communities. Surface sediments collected from bottom waters that had low levels of oxygen were dominated by *Gammaproteobacteria*, while surface sediments collected from hypoxic bottom waters were dominated by *Deltaproteobacteria*, specifically SRBs. Thus, microbial sulfate reduction may become an increasingly important metabolism in seafloor sediments where bottom waters become persistently hypoxic. Less evident taxonomic differences were observed for archaeal groups in surface sediments across sampling stations, which suggests that these groups may be less sensitive to levels of oxygen in overlying water. However, it is important to note that a large portion of archaeal sequences were not assigned to any phyla emphasizing how little we know about archaea in marine sediments. Nevertheless, our study provides the first baseline assessment of sediment microbial communities in the Caspian Sea, which could serve as the foundation for future investigations into key microbial groups and their biogeochemical role in this system.

CONCLUSION

Environmental pollution has become a significant problem in the Caspian Sea due to increased oil and gas exploration and discharge of agricultural and industrial wastewaters. Little is known about the function of even the most dominant microbial groups in the Caspian Sea, thereby limiting our understanding of the microbial metabolic potential. In this study, we demonstrated the dominance of a number of known heterotrophs suggesting that organic carbon is a primary factor shaping microbial

communities. Further, nitrogen cycling in seafloor sediments may be influenced by *Thaumarchaeota*, based on their relative abundance in surface sediments. Future studies using RNA or isotopic approaches may reveal the extent to which different groups identified in this study may be driving nutrient cycling as well as influencing the fate of contaminants.

SUPPLEMENTARY DATA

Supplementary data is available at FEMSEC online.

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