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.

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 km³ and surface area of $3.8 \times 10^5$ km², 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
influential 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 (Lery 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 (Zorn 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 Haitanen 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 (Hassan-shahian et al., 2010, Hassanshahian, Emteizai 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 pre-combusted 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 × 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.

| Station   | Latitude (°N) | Longitude (°E) | Water depth (m) | Temp (°C) | Salinity (PSU) | pH | Oxygen concentration (mg L⁻¹) |
|-----------|---------------|----------------|-----------------|-----------|---------------|----|-------------------------------|
| Station 1 | 39.745        | 50.480         | 600             | 6.1       | 11.4          | 7.8 | 0.5                           |
| Station 2 | 39.990        | 51.500         | 205             | 6.6       | 11.3          | 8.0 | 3.3                           |
| Station 3 | 40.040        | 51.347         | 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 ‘135/138’ 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).

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 × 10⁶ cells pmol⁻¹ of PLFA (Mills et al., 2006), PLFA concentrations correspond to cell densities of 1.3 × 10⁶ to 7.6 × 10⁶ 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 u16:1 were found to be highest at Station 2 and lowest at Station 3 (Fig. S2, Supporting Information). 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).

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.
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, Desulfovibrionales (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 Oceanospirales (18%), Altermonadales (13%) and Xanthomonda (12%). At 4–8 cmbsf (at Station 3), Nitrospira (27%) was the most abundant phyla and a large proportion of sequences at this depth belonged to the order Thermodesulfovibrionales (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, Parvarchaeta (Fig. 3). Crenarchaeota (34%) was the most abundant phylum across all sediment samples, followed by Thaumarchaeota (15%), Euryarchaeota (12%) and Parvarchaeta (12%). Within Crenarchaeota, Marine Benthic Group B (MBGB) (26%) and Miscellaneous Crenarchaeotal Group (MCG) (11%) (also known as Batharchaeota, 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, however, it constituted 15% of assigned archaeal sequences at 0–4 cmbsf 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 Parvarchaeta were found in all sediment samples; the relative abundance of Parvarchaeta decreased with sediment depth at Station 1 and 2, while the opposite was true at Station 3.
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 Information)
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 and 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.
(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 subphyyla, Alteromonadales, Oceanospirillales and Thiotrichales were the most common orders detected. McCarren et al., (2010) found several phylogenetic groups within Alteromonadales 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 aero-
biologically degrade simple aliphatic hydrocarbons in marine envi-
ronments (Hazen et al., 2010). Single-cell sequencing and meta-
transcriptomic 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 de-
grading low- and high-MW organic matter in this system, par-
icularly in seafloor sediments where low levels of oxygen are
present in bottom waters.

Consistent with previous studies of marine sediments (Web-
ster et al., 2004; Fry et al., 2008; Blazekaj 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 propor-
tion (1–5%) of the microbial community. Even though Chloro-
flexi and JS1 often co-occur, it has been suggested that Chloro-
flexi dominate organic-rich, sandy seafloor sediments while
JS1 dominate strictly anoxic, organic-rich but poor quality re-
calcitrant carbon muddy sediments with low sulfate concentra-
tions (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 subsurface 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 fac-
ultative 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 im-
portant role in degrading organic matter at these deeper depths.

Sequences belonging to Thaumarchaeota accounted for a large
proportion of the archaeal sequences in surface sediments
across all stations. Thaumarchaeota are among the most abun-
dant 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 monoxygenase. 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 sedi-
ments 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 ob-
served 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 ma-
rine environments particularly in anoxic sediments (Inagaki
et al., 2003, 2006; Biddle et al., 2006; Fry et al., 2008). A higher rel-
ative 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 cmbsf 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 signifi-
cant role in degrading detrital proteins in anoxic marine sedi-
ments (Lloyd et al., 2013). Thus, the prevalence of MBGB and
MCG in these sediments indicates that they may also be con-
tributing 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, Parvar-
chaeota, 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 Par-
varchaeota in marine sediments and raises questions into their
metabolic function in marine environments.

Eutrophication and resulting deoxygenation of marine wa-
ters 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 sys-
tem are directly affected by depleting oxygen levels in over-
lying 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 com-
munities. Surface sediments collected from bottom waters that
had low levels of oxygen were dominated by Gammaproteobac-
tera, 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 wa-
ters become persistently hypoxic. Less evident taxonomic dif-
fferences 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. How-
ever, it is important to note that a large portion of archaeal se-
quences 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 micro-
bial 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 demon-
strated the dominance of a number of known heterotrophs sug-

gestinthatorganiccarbonisaprimar 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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