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Distinctly different bacterial communities in surface and oxygen minimum layers in the Arabian Sea

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Abstract

Contributions of microbial communities to biogeochemical processes in oxygen minimum oceanic zones are being realized through the applications of molecular techniques. To understand seasonal and depth-wise variations in bacterial community structure (BCS) in the Arabian Sea oxygen minimum region, extensive sampling and molecular analyses were carried out. 16S rRNA gene sequencing was done to profile the BCS from five depths, surface (5m), deep chorophyll maximum (43-50m, DCM), 250m, 500m and 1000m during Spring intermonsoon (SIM), Fall intermonsoon (FIM), and Northeast monsoon (NEM) seasons. Sequencing of 743 chimera-free clones revealed a clear vertical partitioning of BCS between the surface (surface + DCM) and OMZ (250 + 500 + 1000m) layers. There was no distinct seasonal difference in the BCS. Most 16S rRNA gene sequences were affiliated to *Gammaproteobacteria* (39.31%), *Alphaproteobacteria* (23.56%) and *Cyanobacteria* (20.2%). Higher diversity and OTUs in OMZ predominantly consisting of *Alteromonodales*, *Sphinogomonadales*, *Rhodobacterales*, *Burkholderales*, and *Acidimicrobiales* we observed might be due to their microaerophilic metabolism, ability to degrade recalcitrant substrates and assimilate sinking particulate matter. Further hitherto undescribed diversity both in surface and OMZ layers was evidenced. Implicit role of extant bacterial community in denitrification and anammox and in sulphur oxidation is highlighted.

Keywords: Oxygen Minimum Zone, Bacterial diversity, 16S rRNA gene, Gammaproteobacteria, Cyanobacteria, Arabian Sea

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1 Introduction

Poor ventilation of intermediate layers, higher microbial respiration and organic matter oxidation within the water column (Wyrtki 1962) lead to oxygen minimum zones (OMZ) with dissolved oxygen (DO) concentrations often below < 20µM L^{-1} (Lisa 2003). Major OMZs are found in the intermediate depths of eastern tropical North Pacific (ETNP, Wyrtki 1966), eastern tropical South Pacific (ETSP, Wyrtki 1966), eastern Arabian Sea (Wyrtki 1973; Madhupartap et al. 1996; Naqvi and Jayakumar 2000) and eastern South Atlantic (Karstensen et al. 2008). Within the OMZ, intense anaerobic and related processing of nitrogenous compounds (Stramma 2008) lead to loss of fixed nitrogen to the atmosphere. Denitrification (Naqvi 1994) and anaerobic oxidation of ammonia (anammox, Dalsgaard et al. 2012) are the major pathways for this nitrogen loss. Hypoxic conditions often select resilient microbes and restrict their vertical distribution (Wishner et al. 1995).

The OMZs, earlier believed to occur mostly in nutrient rich upwelling regions, are currently expanding and/or intensifying due to anthropogenic impacts (Diaz and Rosenberg 2008). The expansion of OMZs is affecting benthic ecosystems and marine fisheries due to habitat alterations and/or changes in nutrient cycling (Stramma et al. 2008). Further, the OMZ expansion is ascribed to increased production of climate active trace gases including carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O). Microbes are involved in all such processes but little is known about their community structure and metabolism within OMZs, in particular from the Arabian Sea-OMZ (AS-OMZ).

Coinciding with an active denitrification zone (Naqvi 1994; Naqvi et al. 1998) the AS-OMZ is among the largest anoxic regions in the oceans with DO levels < 20µM, sometimes dipping as low as 0.1 µM (Naqvi 2006; Paulmier and Ruiz-Pino 2009). This alone accounts for 20% of the oceanic denitrification (Codispoti et al. 2001) and contributes to 40% of the global pelagic dinitrogen (N_2) production (Naqvi et al. 2008). Previous studies mostly focused on delineating the role of denitrifying and anammox bacteria in the loss of fixed nitrogen in the AS-OMZ (Jayakumar et al. 2009; Bulow et al. 2010; Pitcher et al. 2011; Newell et al. 2011; Bouskill et al. 2012). However, detailed attempts to understand bacterial community structure and/or bacterial diversity of the AS-OMZ and its overlying surface waters are a few and far in between

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(Fuchs et al. 2005; Jain et al. 2014). The present study thus aimed to delineate phylogenetic diversity of the overall bacterial communities in the AS-OMZ, as well as the diversity of bacterial phylotypes contributing to temporally stable bacterial community structure (BCS) in the OMZ (vide Jain et al. 2014). For this, we analyzed small subunit ribosomal RNA gene (16S rRNA or SSU rRNA) clone libraries prepared from water samples collected from five depths from the Arabian Sea Time Series station (ASTS; 17°0.126'N, 67°59.772'E), during three different seasons. ASTS is akin to well known HOTS (Hawaii Ocean Time-Series) in the Pacific Ocean and BATS (Bermuda Atlantic Time-Series) in the Altantic Ocean.

2 Materials and methods

2.1 Sampling

Water samples were collected under the SIBER program in May 2012 (Spring intermonsoon [SIM]), September 2012 (Fall intermonosson [FIM]), and February 2013 (Northeast monsoon [NEM]), as described previously by Jain et al (2014). In brief, samples for DNA extractions were collected using pre-cleaned Niskin bottles on a CTD rosette. Samples from surface (0.5m), deep chlorophyll maxima (DCM) (~35-50m), intense denitrification zone (250m, 500m) and deep denitrification zone (1000m) at the ASTS station (17°0.126' N, 67°59.772'E) were strained through 200µm pore sized bolting silk, immediately after collection, 2.5 L of seawater sample from each depth was filtered peristatically through a Sterivex cartridge fitted with 0.22 µm pore size membrane filter (Millipore, USA). The Sterivex cartridge was then filled with 1.8 ml of lysis buffer (50 mM 131 Tris pH 8.3, 40 mM EDTA and 0.75 M sucrose), sealed, and stored frozen at -80°C until nucleic acid extraction was performed in the laboratory.

2.2 DNA extraction and PCR amplification of 16s rRNA genes

DNA was extracted from Sterivex filters using using the modified method of Ferrari & Hollibaugh (1999). The precipitated DNA was hydrated in 50 µl sterile deionised water. All DNA samples were subjected to PCR amplification using universal 16S rRNA primers 27F and 1492R (to confirm that the DNA was of PCR quality. The 16S rRNA gene was amplified following conditions mentioned by Sambrook (1989). PCR amplification was performed in a final volume of 50 µl in a thermocycler (Applied Biosystems, USA) and correct amplification was ensured by checking for the amplicons electrophoretically.

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95 **2.3 Clone library construction and DNA sequencing**

96 PCR amplified 16S rRNA gene products were purified using Axyprep-96 PCR Clean up
97 kit (Axygen, Biosciences), cloned into pCR4-TOPO vector using a TOPO-TA cloning kit for
98 sequencing (Invitrogen, USA) and transformed by chemical transformation into TOP-10 cells as
99 per manufacturer's instructions. As the transformation efficiency was low to moderate, at least
100 thrice cloning trials were repeated to collect a minimum of 65 clones for further analyses. All
101 positive clones/transformants from each sample were picked out, grown overnight at 37°C on LB
102 plates and subjected to the colony-PCR with primers sets pucM13F/pucM13R using temperature
103 conditions as per manufacturer's instructions. PCR products were purified with the Axyprep-96
104 PCR Clean up kit (Axygen, Biosciences) and then sequenced using an ABI 3130XL genetic
105 analyzer (Applied Biosystems, USA). Clone libraries were constructed from particular depths
106 keeping DO profile in mind. However, for the purpose of comparison, clone libraries from
107 surface and DCM depth were considered as surface group (SIM, FIM and NEM). Similarly,
108 clone libraries from 250, 500 and 1000m were considered as OMZ group for each season
109
110 **2.4 Phylogenetic analysis of clone sequences**

111 The sequences were assembled into contigs using DNA Baser sequence assembly
112 software version 2 (DNA baser, USA). Vector contamination was removed from the sequences
113 using the VecScreen tool (http:// www. ncbi. nlm. nih. gov /tools/vecscreen/). Only consensus
114 sequences without vector and primer residues and with a quality score of 20 (which translates
115 into more than 99.5% correct bases, Allex 1999), were used for further analyses. Chimeric
116 sequences were excluded using a chimera detection program
117 (http://decipher.cee.wisc.edu/FindChimeras.html). It is to be noted that close to 15% of the total
118 sequences were chimeric. Phylogenetic affiliation of proper sequences was determined using the
119 naïve Bayesian classifier of the RDP sequence classifier tool (Wang et al. 2007) using the 16S
120 rRNA Training Set 14 (updated released on 26 May 2015;
121 https://rdp.cme.msu.edu/classifier/classifier.jsp). The classification of sequences was done using
122 1,000 pseudo-bootstrap replications at a bootstrap value of 80%, which results in a standard error
123 of only 1.3%. The sequences were also compared with other databases including SILVA, NCBI
124 and Greengenes, using MOTHUR. The sequences were assigned to a phylum if their identity was
125 > 90% in the different databases searched. The sequences obtained and described in this study

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were submitted to the NCBI GenBank database and are available under accession numbers KJ589647 to KJ590044, KR269603 to KR269693, KR919859 to KR920002, KR919859 to KR920002 and KR673365 to KR819266.

2.5 Determination of OTUs

16S rRNA gene sequences were aligned using ClustalX and the alignment was curated using Gblocks (Castresana 2000) to remove poorly aligned positions and divergent regions. Distance matrices were created from the curated alignments with Phylip 3.66. The sequences were then assigned into phylotypes (operational taxonomic units, OTUs) using MOTHUR by applying the average neighbor rule (Schloss and Westcott 2011). 97% cut-off for sequence similarity was used to delimit an OTU.

2.6 Estimation of shared and unique OTUs

In order to elucidate seasonal and depth wise differences in bacterial phylotypes, the fraction of shared and unique OTUs was estimated using MOTHUR. Further, the numbers of shared and unique OTUs between all three seasons surface samples (SIM-surface, FIM-surface, NEM-surface) and all three seasons OMZ samples (SIM-OMZ, FIM-OMZ, NEM-OMZ) were also estimated.

2.7 Construction of Phylogenetic tree

A phylogenetic tree of representative shared OTUs from the clone libraries was constructed to visualize their relationship and affiliations with the closest relative sequences from the database (Kemble et al. 2011). The tree was constructed as per MEGA-6 using maximum composite likelihood as substitution model and bootstrap values were calculated using neighbor-joining method with a resampling size of n = 500.

2.8 Statistical analysis of clone libraries

The clone libraries from the ASTS were analysed statistically using RDP Library Compare (http://rdp.cme.msu.edu/wiki/index.php/Lib_Compare) and ∫ LIBSHUFF analyses. The Ribosomal Database Project (RDP) II library compare tool uses the naive Bayesian rRNA Classifier Version 1.0 at an 80% confidence threshold (Wang et al. 2007; Cole et al. 2007) to

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157 calculate the difference between two libraries. In brief, the RDP LibCompare provides P values
158 for determining statistical significance of abundance differences for individual taxa instead of
159 estimating overall difference between samples. This tool uses the RDP Classifier to assign
160 sequences to taxa. Depending on the abundance of sequences assigned to each taxon, one of two
161 statistical tests is used to compute a P value to determine if a taxon is differentially represented
162 in the libraries. The ∫ LIBSHUFF program was applied to compare bacterial community structure
163 between libraries in a phylogenetic context. It measures differences between communities based
164 on differences between sequences using Monte Carlo test (Schloss et al. 2004; Schloss 2008). ∫
165 LIBSHUFF implements the Cramer-von Mises statistic to test the generic hypothesis that two
166 communities are the same (Singleton et al. 2001; Schloss et al. 2004). All statistical analyses
167 were performed using MOTHUR software, except RDP.
168
169 **2.9 Diversity and richness estimation**
170 The indices for diversity (Simpson's and Shannon's indices), richness estimates
171 (Jackknife, Chao 1 and ACE), rarefaction and collectors curve were performed using MOTHUR
172 (Schloss et al. 2009). Total richness of the clone libraries was extrapolated from the observed
173 number of OTUs using the three nonparametric richness estimators.
174
175 # 3 Results
176 **3.1 Physico-chemical characteristics of the sampling site**
177 Physico-chemical characteristics (i.e. temperature, salinity, dissolved oxygen, pH, nitrite,
178 nitrate, ammonium, silicate, phosphate and total organic carbon) at the ASTS are described
179 earlier in Jain et al. (2014) and vertical distribution of dissolved oxygen (DO), nitrite (NO_2) and
180 nitrate (NO_3) during three seasons is shown in **Figure 1**. In general, surface and DCM depths
181 (43-50 m) are well oxygenated followed by a steep oxycline between DCM and 250 m. The
182 average DO concentration ranged from 185.24 ± 31.1 μM L^{-1}at DCM to 5.56 ± 5.5 μM L^{-1} at
183 250m. The DO concentrations were slightly more at 1000 m. The nitrite concentration was 0.29
184 in the upper thermocline region (50m) with higher oxygen concentrations (during FIM) and 2.5
185 μM L^{-1} in the intermediate depths (250m) with low oxygen (during NEM). Surface waters
186 during SIM and FIM were devoid of nitrate and it was quite low during the NEM.
187

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188

189 **3.2 Bacterial Community Structure**

190 Seasonal distribution pattern of clone sequences from surface and OMZ (**Figure 2**)
191 indicate that maximum numbers of clone sequences were affiliated to three major phylogenetic
192 groups. These are *Gammaproteobacteria* (39.31%), *Alphaproteobacteria* (23.56%) and
193 *Cyanobacteria* (20.2%). These groups represented up to 82% of the usable sequences. The
194 relative proportions of sequences in these groups varied temporally, as well as spatially, with
195 depth. The percentages of *Gamma-* and *Alphaproteobacteria* were much higher in the OMZ than
196 in the surface layers. During NEM the percentages of *Cyanobacteria* and *Gammaproteobacteria*
197 were the highest at the surface and in the DCM. Notably, the percentage of
198 *Gammaproteobacteria* within OMZ (250, 500 and 1000m) did not vary much between seasons.
199 The distribution of *Alphaproteobacteria* in OMZ was quite similar during FIM and NEM. The
200 highest proportion of unclassifiable bacterial sequences in the surface layers, as well as OMZ
201 depths, was observed during SIM, followed by FIM and NEM.

202

203 **3.3 Shared and Unique OTUs**

204 The shared and unique OTUs between all three seasons in the surface and OMZ samples
205 were divided into four categories: (1) OTUs common to all seasons, (2) OTUs common to two
206 seasons, (3) season-specific OTUs having >1 sequence, and (4) season-specific OTUs having
207 only one sequence (singleton). Three OTUs common to all season's within surface (Common to
208 all season's surface-OTUs, CTASS-OTU) was represented by a minor fraction (11-17 %) of the
209 sequences in individual seasons (**Figure 3**). Further, 20 OTUs (Common to 2 seasons surface,
210 CT2SS-OTU) were shared within surface during two of the three seasons and represented by 19
211 to 38 % of the sequences in individual seasons. The proportion of season-specific OTUs
212 (containing only one sequence) in the surface was the largest (43-73%) and represented by 19-
213 57% of sequences in individual season.

214

215 A total of eight OTUs were common within OMZ (Common to all seasons OMZ,
216 CTASO-OTU) during three seasons and represented by 14-37% of individual season sequences.
217 Further, 20 OTUs (Common to 2 seasons OMZ, CT2SO-OTU) shared within OMZ during two
218 of the three seasons represented 15-20 % of the individual season sequences. The proportion of

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season-specific OTUs in the OMZ was the largest (50-63%) and represented by 28-46% of the individual sequences from any of the seasons. More than half (63%) of season-specific OTUs in the OMZ are represented by single sequences.

3.4 Phylogenetic affiliation and phylogenetic tree of shared OTUs

The relative abundance and phylogenetic affiliation of the OTUs shared by all seasons and any of the two seasons in surface and OMZ are listed in **Table 1(a)** and **Table 1(b)**. To study the evolutionary differences between shared OTUs, representative sequences were subjected to phylogenetic analysis and the relationship with the database sequences was depicted using a phylogenetic tree for surface (**Figure 4**) and OMZ (**Figure 5**). All CTASS-OTUs (CTASS-OTU-1, 2 and 3) represented 13% of all surface sequences and were related to the *Cyanobacteria*, more precisely to the order *Synechococcales.* On the phylogenetic tree CTASS-OTU-1 and CTASS-OTU-3 appears on the same branch while CTASS-OTU-2 branches independently from them and their closest relatives are sequences from Red Sea. The OTUs shared between two of the three seasons (CT2SS-OTUs) were mainly affiliated with the *Cyanobacteria*, *Proteobacteria* and *Acidobacteria*. SIM-Surface and FIM-Surface shared OTUs were related to *Gammaproteobacteria* (CT2SS-OTU-4, 9, 11 and 13), *Betaproteobacteria* (CT2SS-OTU-10), *Alphaproteobacteria* (CT2SS -OTU-14 and 15), *Cyanobacteria* (CT2SS-OTU-8 and 16) and *Acidobacteria* (CT2SS-OTU-20). FIM-Surface and NEM-Surface shared ten OTUs, of which six were affiliated to *Cyanobacteria* (CT2SS-OTU-3, 5, 12, 17 and 18), two to *Gammaproteobacteria* (CT2SS-OTU-2 and 21) and one each to *Alphaproteobacteria* (SS-OTU-1) and *Betaproteobacteria* (CT2SS-OTU-19). SIM-Surface and NEM-Surface shared only one OTU affiliated to *Gammaproteobacteria* (CT2SS-OTU-6).

Of the eight CTASO-OTUs, the two most abundant ones, namely CTASO-OTU-1 and CTASO-OTU-2, represented 16% of all OMZ sequences and were related to *Gammaproteobacteria*, in the Order *Alteromonadales*. CTASO-OTU-1 and -2 branched independently from each other and their closest relatives are sequences from Arctic Ocean, Ishigaki Jima Island (Japan), South China Sea and Red Sea. The next most abundant CTASO-OTU-3 and CTASO-OTU-4 represented 5% of all OMZ sequences and belong to the Order *Sphingomonadales* of Class *Alphaproteobacteria.* Both these aligned on the same branch in the

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tree and are related to sequences from South Atlantic Ocean and South Pacific gyre. The CTASO-OTU-5, CTASO-OTU-6, CTASO-OTU-7 and CTASO-OTU-8, represented 1% of the total OMZ sequences. They are affiliated with *Burkholderilaes*, *Acidimicrobiales*, uncultured *Gammaproteobacteria*, and *Rhodobacterales*, respectively. The closest relatives of CTASO-OTU-5, 6, 7, and 8 are reported from Sri Lanka thermal spring, Indian Ocean, Red Sea, and Submarine basalt loihi Seamount, respectively.

The OTUs shared in the OMZ between two seasons were mainly from three different Phyla, including *Proteobacteria*, *Actinobacteria* and marine group A (MGA). SIM-OMZ and FIM-OMZ were found to share OTUs related to *Gammaproteobacteria* (CT2SO-OTU-2, 3, 10, 11, 12, and 15), MGA (CT2SO-OTU-12 and 15) and *Alphaproteobacteria* (SO-OTU-11). The FIM-OMZ and NEM-OMZ shared four OTUs. Of those, three were affiliated to *Alphaproteobacteria* (CT2SO-OTU-1, 14, and 16) and one to *Actinobacteria* (CT2SO-OTU-20). The SIM-OMZ and NEM-OMZ shared 10 OTUs. Four of them were affiliated with to *Alphaproteobacteria* (CT2SO-OTU-6, 7, 8, and 18), another four to *Gammaproteobacteria* (CT2SO-OTU-9, 13, 17, and 20), and one each to MGA (CT2SO-OTU-4) and *Actinobacteria* (CT2SO-OTU-5).

3.5 Comparison of clone libraries

Using the RDP classifier, it was seen that at Class level, the surface samples during all three seasons had more representatives of *Cyanobacteria* than in the OMZ samples. This was entirely different in the OMZ where *Alphaproteobacteria, Gammaproteobacteria* and *Marinimicrobia* were the dominant groups (**Table 2**). Further, from the RDP libcompare it was clear that the abundance of *Gammaproteobacteria,* (*Altermonodales*), *Alphaproteobacteria* (*Spingomondales*), and SAR11 differed significantly between seasons in the surface clone libraries. In contrast, the abundance of *Cyanobacteria* and *Alphaproteobacteria* was significantly lower and different in the OMZ (**Table 3**). The LIBSHUFF ($p < 0.0016$) analysis, used to note that bacterial community, was significantly different between surface and OMZ during all three seasons. The bacterial community in surface layers differed significantly between seasons (LIBSHUFF, $p < 0.0001$), whereas no such significant seasonal difference was found in the bacterial community extant in the OMZ (**Table 4**).

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3.6 Analysis of clone diversity and richness

Shannon and Simpson indices, as well as rarefaction curves, clearly indicate that at an evolutionary distance of 3% the bacterial diversity in both surface and OMZ was the highest during SIM followed by NEM and FIM (**Table 5**). However, during each season, higher diversity indices were evident in the OMZ than in the surface layers. The nonparametric Jackknife, Chao 1 and ACE estimators also revealed that the estimated OTUs are much higher in the OMZ than the observed number of OTUs during all the seasons. Rarefaction (**Figure 6a**) and collector curves (**Figure 6b**) implied that no saturation was reached either at sequence or OTU level at an evolutionary distance of 1% and 3%, respectively. However, at Class or Family level, at distances of 10%, the rarefaction implied some saturation.

4 Discussion:

Traditionally the OMZs were seen as regions dominated by heterotrophic denitrification fueled by sinking of organic matter produced via photosynthesis in the sunlit surface ocean. They were also considered to process a fundamentally different microbial community and operate on a different biogeochemistry. The discovery Anammox, and active but cryptic sulfur cycle in anoxic OMZs have significantly shifted the old paradigms. For almost a decade now the OMZ gene surveys have focused extensively on microbes performing denitrification and anaerobic ammonia oxidation (anammox) with less emphasis on the overall microbial community. Microbial communities within OMZs play central roles in ocean, yet we still lack a fundamental understanding of how microbial biodiversity is distributed across the OMZs. In this regard, our efforts are useful in providing some novel insights. Arabian Sea is modulated seasonally by upwelling, winter cooling (Prasanna Kumar et al. 2001) and semi-annual reversal of monsoonal winds (Madhupratap et al. 1996). This greatly influences primary production, organic carbon concentration and flux (Hansell and Peltzer 1998) and bacterial abundance (Ramaiah et al. 1996, 2000; Jain et al. 2014). In spite of the global biogeochemical and climatic importance of AS-OMZ, spatio-temporal variation (Riemann et al. 1999; Fuchs et al. 2005; Jain et al. 2014) and phylogenetic diversity of the microorganisms inhabiting therein are only sparsely addressed (Riemann et al. 1999; Jayakumar et al. 2009; Divya et al. 2010, 2011). Oxygen deficient waters in the intermediate depths is a perennial feature of the Northeastern Arabian Sea which gets intensified during winter or NEM season due to poor water circulation and high surface

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312 productivity (Naqvi et al. 1990; Prasanna Kumar and Prasad 1996). Lower DO level and higher
313 nitrite concentration at the intermediate depth during NEM season signifies intense
314 denitrification processes and the presence of nitrite in the thermocline region signifies the
315 nitrification process (Sen Gupta et al. 1976). During intermonsoon (SIM and FIM) periods, the
316 intense solar heating and weak winds stratify the Northeastern Arabian Sea surface layer, leading
317 to depletion of nitrate in the upper euphotic zone (Muraleedharan and Prasanna Kumar 1996).
318
319 The SIM is a transition period from winter to summer and is generally known to be a
320 period of low primary productivity caused by water column stratification and oligotrophic
321 conditions (Madhupratap et al. 1996). During this period, the bacterial community is mainly
322 sustained by the slow-to-degrade dissolved organic carbon (DOC) from earlier phytoplankton
323 blooms of the NEM (Ramaiah et al. 2000). Despite these environmental conditions we observed
324 higher bacterial diversity during SIM (both in surface as well as OMZ).Most microbial habitats
325 are spatially heterogeneous (Kassen and Rainey 2004) and can contain a large number of
326 potential niches. Previous studies reported a positive correlation between habitat heterogeneity
327 ("patchiness") and the phylogenetic diversity of bacteria (Korona et al. 1994; Rainey et al. 2000;
328 Kassen et al. 2000; Zhou et al. 2002). We find that the bacterial diversity at the ASTS varies
329 seasonally in the surface layers and in OMZ and is at its highest during SIM. High rates of
330 denitrification might be among the many possible causes for this higher diversity. Further, the
331 high proportion of unclassified bacteria may also contribute to the increased diversity to some
332 extent as observed during SIM.
333
334 Though over 60 clones were finally available from each depth for sequencing, they do
335 not capture the full bacterial diversity in the AS-OMZ. This notwithstanding, our efforts are
336 useful to suggest that overall bacterial diversity at Phylum and OTU level in the ASTS is higher
337 in OMZ than in the surface layers. Interestingly, the depth-dependent variation of OMZ bacterial
338 diversity is not consistent among different studies (Ganesh et al. 2014). Further, Bryant et al
339 (2012) reported a consistent decline in the bacterial diversity within the ETSP-OMZ. Similarly,
340 Zaikova et al (2010) observed low diversity associated with the seasonal OMZ off British
341 Columbia. In contrast, Stevens and Ulloa (2008), based on 16S rRNA clone libraries identified
342 higher OTU diversity at the ETSP-OMZ, a pattern consistent with the observation in our study.

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Similarly, Brown et al (2009) and Kemble et al (2011) showed elevated OTU richness to coincide with the zone of minimum oxygen at the HOTS. Bryant et al (2012) and Jain et al (2014) attributed lower diversity in the OMZ to competition for limited resources, environmental filtering, and lower redox potential and less readily available organic matter. On the other hand, higher diversity in the OMZ has been linked to the use of a wider range of terminal oxidants compared to the oxic depths where oxygen is the dominant electron acceptor (Stevens and Ulloa 2008). Most clades of *Gammaproteobacteria* are known to denitrify using nitrate as electron acceptor (Miller et al. 2010). Among them, the predominant *Alteromonodales* in the AS-OMZ appears to be a proficient denitrifier group.

Bacterial community structure at the ASTS is dominated by phylotypes affiliated with three major phylogenetic groups viz., *Gammaproteobacteria*, *Alphaproteobacteria* and *Cyanobacteria*. The dominance of *Alphaproteobacteria* and *Gammaproteobacteria*, has been reported earlier from a variety of pelagic marine environments (Giovannoni and Rappé 2000), including the OMZ of the ETSP (Stevens and Ulloa 2008; Ganesh et al. 2014) and the Southern Arabian Sea (Fuchs et al. 2005). Our results suggest seasonal variation in the abundance of certain major bacterial groups mainly in the surface layers than in the OMZ depths. As Hansell & Peltzer (1998) suggested, the organic carbon concentration and changes in primary productivity patterns in the surface layers may be responsible for variation in the abundance of *Gammaproteobacteria* (*Altermonodales*) and *Alphaproteobacteria* (*Spingomondales*). Higher abundance of *Gammaproteobacteria* (*Altermonodales*) and *Alphaproteobacteria* (*Spingomondales*) and SAR11 in the OMZ libraries might be supported by microaerophilic metabolism, particle associated lifestyle and/or their ability to use abundantly available nitrate as their terminal electron acceptor for energy generation.

The total number of common OTUs shared within surface clone libraries was significantly lower than that in the OMZ. All common surface OTUs constitute only a minor fraction (13 %) of the total surface sequences and were mostly affiliated with *Cyanobacteria*, more precisely to the genus *Synechococcus* and *Prochlorococcus.* Interestingly, majority of surface OTUs (shared between two of the three clone libraries) were also affiliated with *Cyanobacteria*, specifically to *Prochlorococcus. Cyanobacteria* of the genera *Prochlorococcus*

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and *Synechococcus* have been found inhabiting the upper lit part of OMZs (Johnson et al. 1999; Goericke et al. 2000; Ulloa et al. 2006; Galán et al. 2009). However, phylotypes *of Synechococcus* are often restricted to the upper part of the euphotic zone and do not show a clear vertical partitioning with depth as *Prochlorococcus* does. Moreover *Prochlorococcus* is dominant in the oligotrophic waters (Olson et al. 1990; Li 1995; Liu et al. 1997a; Grob et al. 2007). The dominance of *Prochlorococcus* over *Synechococcus* in the OMZ is presumably a result of specific physiological adaptation to the prevailing light and nutrient conditions within the region. Even though few *Prochlorococcus* ecotypes are able to utilize oxidized forms of nitrogen, particularly nitrite (Moore et al. 2002), the biological processes of nitrate and nitrite reduction carried out by the local OMZ microbial community (Farías et al. 2007) could support their dominant presence in the AS-OMZ upper stratum.

It is apparent that the higher number of common OTUs affiliated with *Alteromonodales* (16% of the total OMZ sequences) and *Sphinogomonadales* (5% of the total OMZ sequences) might contribute to the temporally stable BCS in the OMZ. However, it should be noted that proportions of phylotypes affiliated with *Alteromonodales* and *Sphinogomonadales* also varied seasonally in the surface layers. *Alteromonas macleodii* clustered into two major genotypic groups or ecotypes, one found in the upper water column and another in the deep water column. Martinez et al (2008) reported that the deep ecotype is better suited to microaerophilic conditions and for the degradation of recalcitrant compounds and colonizes relatively large particles that sink rapidly to meso and bathypelagic depths. On the other hand, the surface ecotype, a typical r-strategist (investing most energy in multiplying fast) has more potential for regulation and degradation of sugars and amino acids, and specializes in colonizing smaller particulate organic matter with much slower sinking rates (Perez et al. 2012). Therefore, it is possible that the persistence of *Alteromonodales* throughout the year in the AS-OMZ could be due to its microaerophilic metabolism and due to its ability to degrade recalcitrant compounds as well as to be able to degrade large sinking particulate matter, for eg. transparent exopolymeric particles (Dileep kumar et al. 1998 and Ramaiah et al. 2005). *Alteromonadales*, particularly in the genera *Idiomarina* and *Alteromonas* are known to metabolize both labile and semi-labile high molecular weight dissolved organic matter (McCarren et al. 2010). Presence of *Sphingomonadales* in the upper OMZ has been reported earlier by Riemann et al (1999), and although their ecological

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significance in OMZ is not clear, *Sphingomonadales* has been documented to have wide metabolic capabilities (Miller et al. 2010) and can degrade aromatic compounds (Fredrickson et al. 1995). Thus, their abundance, especially of *Novosphingobium* and *Sphingobium*, in the AS-OMZ may be driven by high concentrations of phosphate and ammonia as postulated by Liu et al (2015).

The SAR11 group is known to limit the flux of sulfur from the ocean by demethylating dimethylsulfoniopropionate (DMSP), which is usually degraded to volatile dimethyl sulfide (Moran et al. 2003; Howard et al. 2006). Demethylated DMSP can serve as a substrate in sulfur oxidation in the deeper layer. This group is also known to assimilate labile amino acids, thereby playing a significant role in C, N, and S cycling (Malmstrom et al. 2004). The other common OMZ-OTUs were those *Burkholderilaes*, *Acidimicrobiales*, uncultured *Gammaproteobacteria*, and *Rhodobacterales* seems to contribute to the stable BCS in the AS-OMZ. It is interesting to note that only a few of the common OMZ OTUs affiliated to known denitrifiers, suggesting that denitrification is spatially heterogenous (niche segregation). Many of the other shared OMZ OTUs (between any two seasons) and season specific OMZ OTUs were affiliated with *Nitratireducter*.sp, a known denitrifying bacterium.

One of the most significant findings in OMZ biogeochemistry and microbiology has been the recognition of a cryptic pelagic sulphur cycle. Traditionally, it was believed that sulphur reduction would not initiate until the oxygen and nitrate/nitrite is fully consumed (Froelich et al. 1979). Surprisingly, recent metagenomic studies have discovered an abundant and diverse sulfur-oxidizing microbial community in the OMZ layers. This community is particularly enriched in *Deltaproteobacteria* related to sulfur-oxidizing symbionts of deep sea bivalves (Canfield et al. 2010). Radiolabeled sulfate tracer experiments from the OMZ off the Chilean coast revealed significant sulfate reduction in the upper reaches of the OMZ water (Canfield et al. 2010). Sulphur oxidising bacterial clades, such as *Thiotrichales* and *Desulfobacteriales*, were present in the libraries made from 250m and 500m, although represented by only a few sequences. These are the very first indications of the existence of sulphur metabolizing bacterial community in the AS-OMZ.

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Based on diversity and richness indicators, the estimated total numbers of OTUs for all samples from the AS-OMZ were much higher than the observed number of OTUs, indicating that additional sampling would have revealed greater diversity. The same trend was observed when rarefaction and collector curves were calculated as no stationarity was reached at levels below Phylum approximated by an evolutionary distance of 10%. Together, the richness estimators ACE and Chao 1, and the three different diversity indices indicate that there is a high degree of undescribed diversity in both surface and OMZ, a large fraction of which likely belong to clusters with sequences whose taxonomic resolution is finer than that defined for species.

Results of this study demonstrate that the OMZ bacterial community is diverse yet distinctively different communities predominate in the surface and OMZ layers. Many of the groups that have adapted to low oxygen are likely to have important roles in C, N and S cycling within the OMZs. Organisms contributing to high species-richness may act as regulators of fluxes of C, N and S into and out of the OMZ. Their rates of cycling and metabolism in the OMZ seem to be stable. While seasonal variation in the OMZ bacterial community is minimal, the diversity in the OMZ depths is greater than that in the surface layers, with deep-branching groups and many novel and as yet uncultivated clades. Also, the sulphur oxidation appears to be operated by certain microbes in OMZs exemplied by sparse yet obvious presence of *Thiotrichales* and *Desulfobacteriales* in the AS-OMZ. Prevalence of denitrification, anammox and sulphur oxidation brings forth the role of microbes in the AS-OMZ ecosystem functioning.

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812 **Table 1 (a)**. Percentage of OTUs from Arabian Sea Time Series (ASTS) location being common to all three seasons and in two
813 seasons in surface during different season and the phylogenetic affiliation of the representative sequence from each OTU.

Surface								
Sampling season	**SIM**	**FIM**	**NEM**	**Total seqs**	**Phylogenetic affiliation**	**Accession number**	**nt**	**% similarity**
OTUs from surface common to all seasons								
CTASS-OTU-01 (KJ589701)	**4.8**	**101**	**1.1**	21	*Uncultured Cyanobacterium*	EF123580.1	Caribbean sea	97%
CTASS -OTU-02 (KJ589666)	**5.9**	**5.7**	**5.3**	19	*Uncultured Prochlorococcus sp.*	JN547420.1	Red sea	99%
CTASS -OTU-03 (KJ590008)	2.4	0.2	4.3	8	*Uncultured Prochlorococcus sp.*	KF001530.1	South tyrrhenian sea	98%
OTUs from surface found in any two seasons								
CT2SS-OTU-01 (KJ590006)	0.0	0.7	2.2	3	*Uncultured alphaproteobacterium*	JF824769.1	northern gulf of Mexico	100%
CT2SS-OTU-02 (KJ589966)	0.0	8.2	2.2	15	*Uncultured Alteromonas sp.*	HQ161387.1	Eastern Mediterranean Sea	99%
CT2SS-OTU-03 (KJ589895)	0.0	6.7	3.3	14	*Uncultured Synechococcus sp.*	AY125366.1	Oligotrophic oceanic waters	99%
CT2SS-OTU-04 (KJ589963)	4.8	3.1	0.0	9	*Alteromonas sp.*	FJ170025.1	South China Sea	97%
CT2SS-OTU-05 (KJ589897)	0.0	2.5	4.3	8	*Uncultured Prochlorococcus sp.*	JN547417.1	Red Sea	99%
CT2SS-OTU-06 (KR919951)	1.2	0.0	7.7	8	*Uncultured Alteromonas sp.*	KF545037.1	Deep sea sediment	98%
CT2SS-OTU-07 (KJ589680)	1.2	2.5	0.0	5	*Uncultured bacterium*	EF574727.1	Coco's Island	99%
CT2SS-OTU-08 (KJ589670)	1.2	2.5	0.0	5	*Uncultured gammaproteobacterium*	GQ250617.1	Gulf of Mexico	97%
CT2SS-OTU-09 (KJ589891)	2.4	1.3	0.0	4	*Burkholderia sp.*	KT159931.1	Swamp forest	99%
CT2SS-OTU-10 (KJ589935)	2.4	1.3	0.0	4	*Halophilic bacterium*	AB042502.2	Antarctic habitats	99%
CT2SS-OTU-11 (KJ589940)	0.0	0.7	2.2	3	*Uncultured cyanobacterium*	HQ242063.1	North East Pacific	99%
CT2SS-OTU-12 (KJ589978)	1.2	1.3	0.0	3	*Idiomarina baltica*	AJ440215.1	Central Baltic Sea	98%
CT2SS-OTU-13 (KJ589695)	1.2	1.3	0.0	3	*Uncultured alpha proteobacterium*	HQ242175.1	North East Pacific	91%
CT2SS-OTU-14 (KJ589652)	1.2	0.7	0.0	2	*Alpha proteobacteria*	JQ515459.1	Caribbean reef	96%
CT2SS-OTU-15 (KJ589945)	1.2	0.7	0.0	2	*Uncultured cyanobacterium*	EU980186.1	Lake Taihu China	97%
CT2SS-OTU-16 (KJ590032)	0.0	0.7	1.1	2	*Uncultured bacterium*	JX668703.1	Polar marine water	97%
CT2SS-OTU-17 (KJ589948)	0.0	0.7	1.1	2	*Uncultured cyanobacterium*	GQ348575.1	Oceanic dead zones	93%
CT2SS-OTU-18 (KJ589900)	0.0	0.7	1.1	2	*Beta proteobacterium*	HM163254.1	Sediment sample	99%
CT2SS-OTU-19 (KJ589725)	1.2	0.7	0.0	2	Uncultured Acidobacterium	KF183225.1	Soil Bacterial Community	94%
CT2SS-OTU-20 (KR919954)	0.0	0.7	1.1	2	*Alteromonas macleodii*	KC871610.1	Atlantic Ocean	98%

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814 **Table 1 (b)**. Percentage of OTUs from Arabian Sea Time Series (ASTS) location being common to all three seasons and in two
815 seasons in OMZ during different season and the phylogenetic affiliation of the representative sequence from each OTU.

OMZ								
Sampling season	**SIM**	**FIM**	**NEM**	**Total seqs**	**Phylogenetic affiliation**	**Accession number**	**Environment**	**% similarity**
OTUs from OMZ common to all seasons								
CTASO-OTU-01 (KJ589883)	**7.8**	**15.4**	**12.2**	46	Uncultured *Alteromonas* sp.	AB262378.1	Ishigaki jima Island, Japan	99%
CTASO-OTU-02 (KR673368)	**0.7**	**7.7**	**5.3**	17	*Alteromonas* sp.	JX533666.1	South china sea	100%
CTASO-OTU-03 (KJ589777)	0.7	2.6	3.8	9	*Erythrobacter* sp.	AM990645.1	South Pacific Gyre	96%
CTASO-OTU-04 (KR269635)	1.3	3.4	2.3	9	Uncultured bacterium clone	KM110216.1	South Atlantic Ocean	99%
CTASO-OTU-05 (KR919998)	0.7	2.6	0.8	5	Uncultured *Burkholderia* sp.	KF305507.1	Sri Lanka thermal spring	100%
CTASO-OTU-06 (KJ589826)	0.7	0.9	1.5	4	Uncultured bacterium clone	JX441387.1	Indian Ocean	100%
CTASO-OTU-07 (KJ589739)	1.3	0.9	0.8	4	Uncultured *Proteobacterium* clone	KM018890.1	Red Sea	99%
CTASO-OTU-08 (KR269655)	0.7	0.9	0.8	3	*Methylarcula* sp.	DQ412076.1	Submarine basalt loihi Seamount	99%
OTUs from OMZ found in any two seasons								
CT2SO-OTU-01 (KR919865)	0.0	0.9	3.1	5	*Sphingobium* sp.	KF672731.1	Haihe estuary	100%
CT2SO-OTU-02 (KJ589884)	1.3	2.6	0.0	5	Uncultured *gamma proteobacterium* clone	JN232986.1	Continental slope Cape Lookout	98%
CT2SO-OTU-03 (KJ589779)	2.6	0.9	0.0	5	Uncultured marine microorganism clone	JN166297.1	Subtropical North Pacific	96%
CT2SO-OTU-04 (KJ589746)	2.0	0.0	0.8	4	Uncultured bacterium clone, Marine Group A,	HQ674579.1	Northeast subarctic pacific Ocean	98%
CT2SO-OTU-05 (KJ589789)	1.3	0.0	0.8	3	*Euzebya* sp.	KP735966.1	Pacific Ocean	96%
CT2SO-OTU-06 (KR819220)	1.3	0.0	0.8	3	*Nitratireductor* sp.	EU440986.1	Southwest Indian Ridge	97%
CT2SO-OTU-07 (KJ589735)	1.3	0.0	0.8	3	Uncultured marine microorganism clone	JN166353.1	Subtropical North Pacific	98%
CT2SO-OTU-08 (KJ589873)	1.3	0.0	0.8	3	Uncultured bacterium clone	JN165748.1	Subtropical North Pacific	
CT2SO-OTU-09 (KR673384)	0.7	0.0	0.8	2	Uncultured bacterium clone	JX391520.1	Marine sediments	98%
CT2SO-OTU-10 (KR919866)	0.7	0.9	0.0	2	*Oleibacter marinus* strain 2O1,	NR_112787.1	Tropical ocean	99%
CT2SO-OTU-11 (KR269632)	0.7	0.9	0.0	2	Uncultured *alpha proteobacterium* clone	GQ337156.1	Deep Arctic Ocean	99%
CT2SO-OTU-12 (KR269629)	0.7	0.9	0.0	2	Uncultured bacterium clone	HQ674365.1	Pacific Ocean	99%
CT2SO-OTU-13 (KJ589749)	0.7	0.0	0.8	2	Uncultured bacterium clone	JX441447.1	Indian Ocean	95%
CT2SO-OTU-14 (KR919982)	0.0	0.9	0.8	2	*Alterierythrobacter* sp.	EU440971.1	Southwest Indian Ridge	98%

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CT2SO-OTU-15 (KJ589784)	0.7	0.9	0.0	2	Uncultured bacterium clone, Marine Group A	HQ673196.1	Northeast subarctic pacific Ocean	99%
CT2SO-OTU-16 (KR819230)	0.0	0.9	0.8	2	Uncultured bacterium clone	KF146540.1	Yellow Sea	98%
CT2SO-OTU-17 (KR673387)	0.7	0.0	0.8	2	Uncultured *gamma proteobacterium* clone	KP076569.1	Coastal Arabian Sea	97%
CT2SO-OTU-18 (KJ589880)	0.7	0.0	0.8	2	Uncultured bacterium clone	M110216.1	South Altantic Ocean	98%
CT2SO-OTU-19 (KJ589799)	0.7	0.0	0.8	2	Uncultured bacterium clone	AY375071.1	Deep sea sediments pacific ocean	97%
CT2SO-OTU-20 (KR919989)	0.0	0.9	0.8	2	Uncultured *actinobacterium*	FJ615153.1	Saanich Inlet OMZ	99%

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842 **Table 2**. RDP library comparison results for the groups of significantly differing ($p < 0.01$) sequences between surface and OMZ clone
843 libraries and their phylogenetic affiliations according to RDP library compare (Cole et al 2007). The more abundant group is shown in
844 bold.

RDP classifier taxonomic rank for sequences			
Spring Inter-monsoon (SIM)	**Surface**	**OMZ**	**p-value**
Phylum *Cyanobacteria*	**41**	9	6.00E-14
class *Cyanobacteria*	**34**	8	1.05E-11
Phylum *Proteobacteria*	39	**120**	4.23E-06
class *Gammaproteobacteria*	16	**72**	4.13E-05
Fall Inter-monsoon (FIM)			
Phylum *Cyanobacteria*	**66**	0	1.50E-16
class *Cyanobacteria*	**56**	0	3.82E-14
Phylum *Proteobacteria*	87	**105**	1.06E-09
class *Alphaproteobacteria*	28	**33**	3.85E-02
order *Sphingomonadales*	0	**9**	3.89E-04
class *Gammaproteobacteria*	40	**62**	2.60E-06
order *Alteromonadales*	32	**40**	9.32E-03
Northeast Monsoon (NEM)			
Phylum *Cyanobacteria*	**31**	2	1.10E-10
class *Cyanobacteria*	**29**	1	6.10E-11
Phylum *Proteobacteria*	55	**109**	3.40E-04
class *Alphaproteobacteria*	15	**46**	2.98E-03

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855 **Table 3.** RDP library comparison results for the groups of significantly differing ($p < 0.01$)
856 sequences within surface and OMZ clone libraries obtained during different seasons and their
857 phylogenetic affiliation according to RDP library compare (Cole et al 2007). The more abundant
858 group is shown in bold.

RDP classifier taxonomic rank for sequences			p-value
	SIM-surface	**FIM-surface**	
Phylum *Proteobacteria*	39	**87**	1.71E-1
class *Gammaproteobacteria*	16	**40**	2.50E-1
order *Alteromonadales*	5	**32**	4.77E-3
	FIM-surface	**NEM-surface**	
Phylum *Proteobacteria*	**87**	55	3.03E-1
class *Alphaproteobacteria*	**28**	15	8.65E-3
order *Sphingomonadales*	0	**5**	4.38E-3
family *SAR11*	**16**	0	1.01E-3
class *Gammaproteobacteria*	**40**	39	2.78E-3
	NEM-surface	**SIM-surface**	
Phylum *Proteobacteria*	**55**	39	3.49E-2
class *Gammaproteobacteria*	**39**	16	4.00E-4
order *Alteromonadales*	**29**	5	3.97E-5
	SIM-OMZ	**FIM-OMZ**	
Phylum *Cyanobacteria*	**9**	0	8.05E-3
	FIM-OMZ	**NEM-OMZ**	
NA	NA	NA	NA
	NEM-OMZ	**SIM-OMZ**	
Class *Alphaproteobacteria*	**46**	33	6.34E-3

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870 **Table 4**. ∫-LIBSHUFF comparisons of clone libraries constructed from the surface and OMZ
871 depths during spring intermonsoon (SIM), fall intermonsoon (FIM), and northeast monsoon
872 (NEM).

		Surface			OMZ		
		SIM-S	**FIM-S**	**NEM-S**	**SIM-O**	**FIM-O**	**NEM-O**
	SIM-s	0	0.2783	0.0525	0.0902	**<0.0001**	0.0001
Surface	**FIM-s**	**<0.0001**	0	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
	NEM-s	**<0.0001**	**<0.0001**	0	0.0006	**<0.0001**	**<0.0001**
	SIM-o	**<0.0001**	**<0.0001**	**<0.0001**	0	0.0036	0.0022
OMZ	**FIM-o**	**<0.0001**	**<0.0001**	**<0.0001**	0.0262	0	0.0352
	NEM-o	**<0.0001**	**<0.0001**	**<0.0001**	0.0615	0.0071	0

873 With an experiment wise error rate of 0.05 and taking into account a Bonferroni's correction for
874 multiple comparisons, the libraries were considered significantly different when either of the two
875 *P* values generated for an individual pairwise comparison was lower than 0.0016 (significant
876 values are marked in bold).

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888 **Table 5**. Comparison of bacterial diversity in clone libraries constructed from the surface layers
889 and OMZ during spring intermonsoon (SIM), fall intermonsoon (FIM), and northeast monsoon
890 (NEM) at the Arabian Sea Time Series (ASTS) location.

	No. of sequences	No. of OTUs	Richness estimation				
			Shannon	Simpson	Chao	ACE	Jackknife
Surface							
SIM	84	60	3.86	0.02	405.50	1350.94	322.88
FIM	158	59	3.57	0.04	106.75	150.83	114.32
NEM	93	58	3.82	0.02	158.33	310.95	152.12
OMZ							
SIM	160	119	4.63	0.01	378.67	1052.11	374.61
FIM	117	68	3.79	0.04	162.23	171.30	169.81
NEM	131	92	4.21	0.02	425.67	1004.50	958.52

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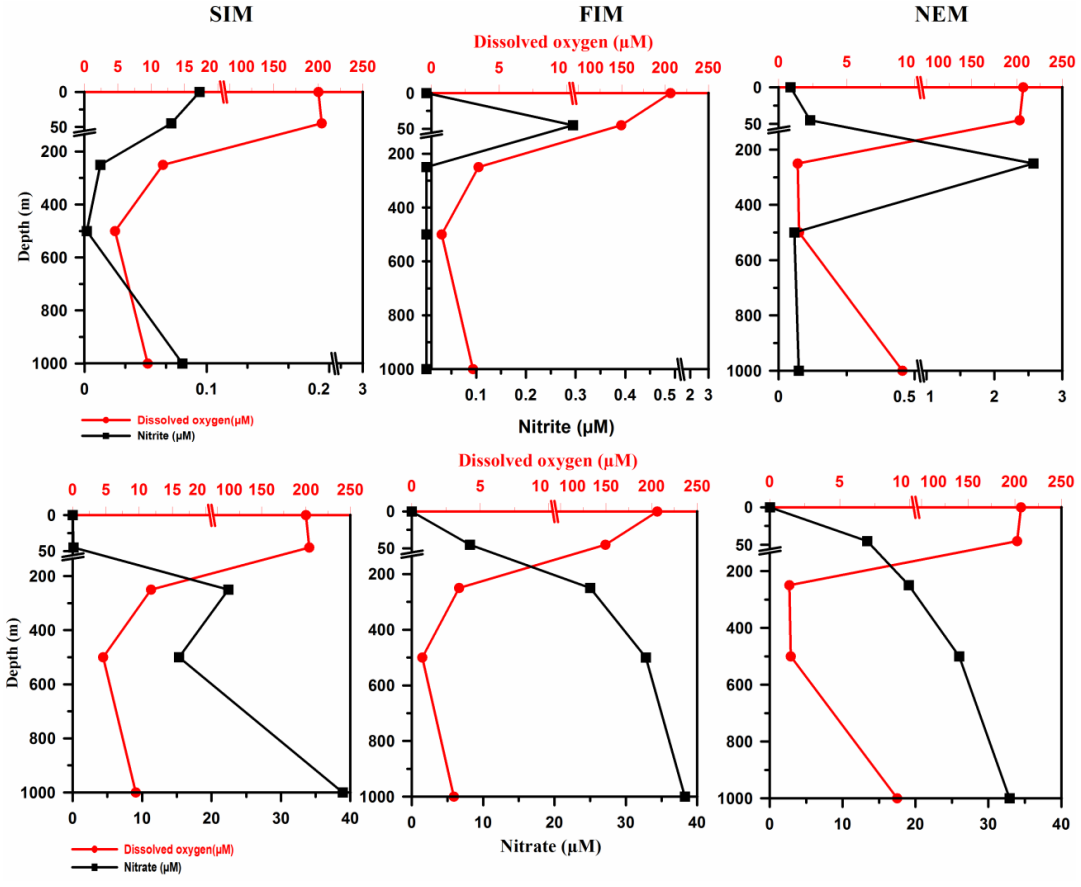

Figure 1 Vertical distribution of dissolved oxygen (DO), nitrate ($NO_{3)}$ and nitrite (NO_2) concentrations during spring intermonsoon (SIM), fall intermonsoon (FIM), and northeast monsoon (NEM) at the Arabian Sea Time Series (ASTS) location.

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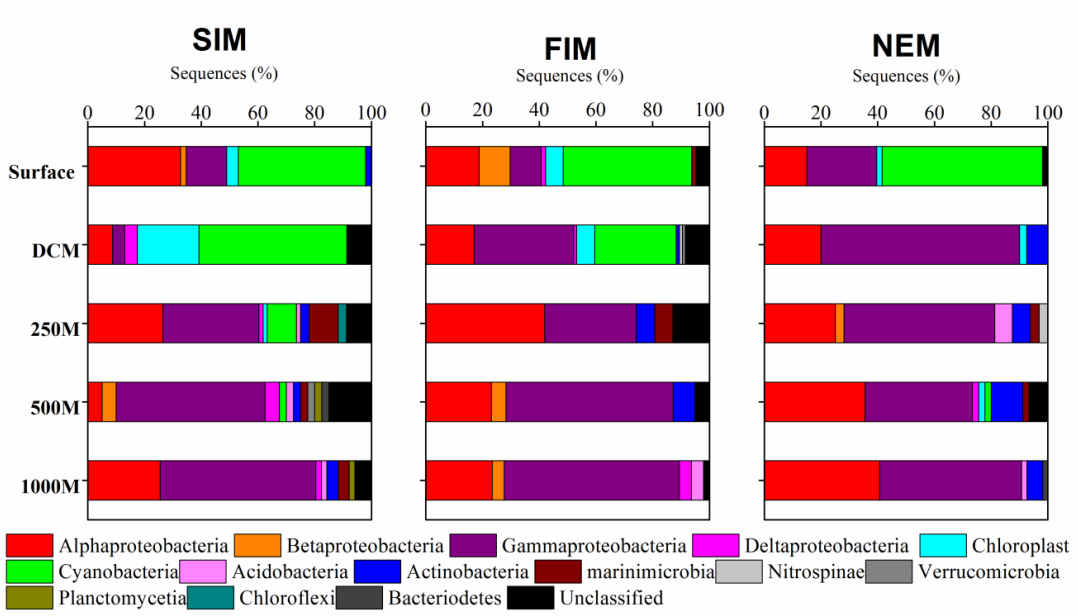

Figure 2 Vertical distribution of major bacterial groups at the Arabian Sea time series (ASTS) location during spring intermonsoon (SIM), fall intermonsoon (FIM), and northeast monsoon (NEM).

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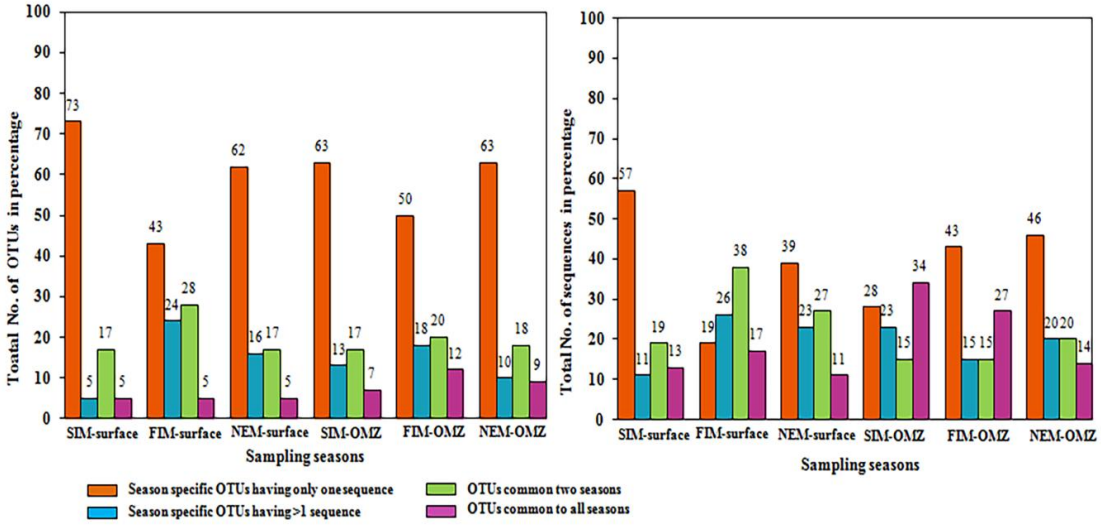

Figure 3 Percentage of shared and unique OTUs in the surface and OMZ clone libraries obtained during spring intermonsoon (SIM), fall intermonsoon (FIM), and northeast monsoon (NEM) at the Arabian Sea Time Series (ASTS) location.

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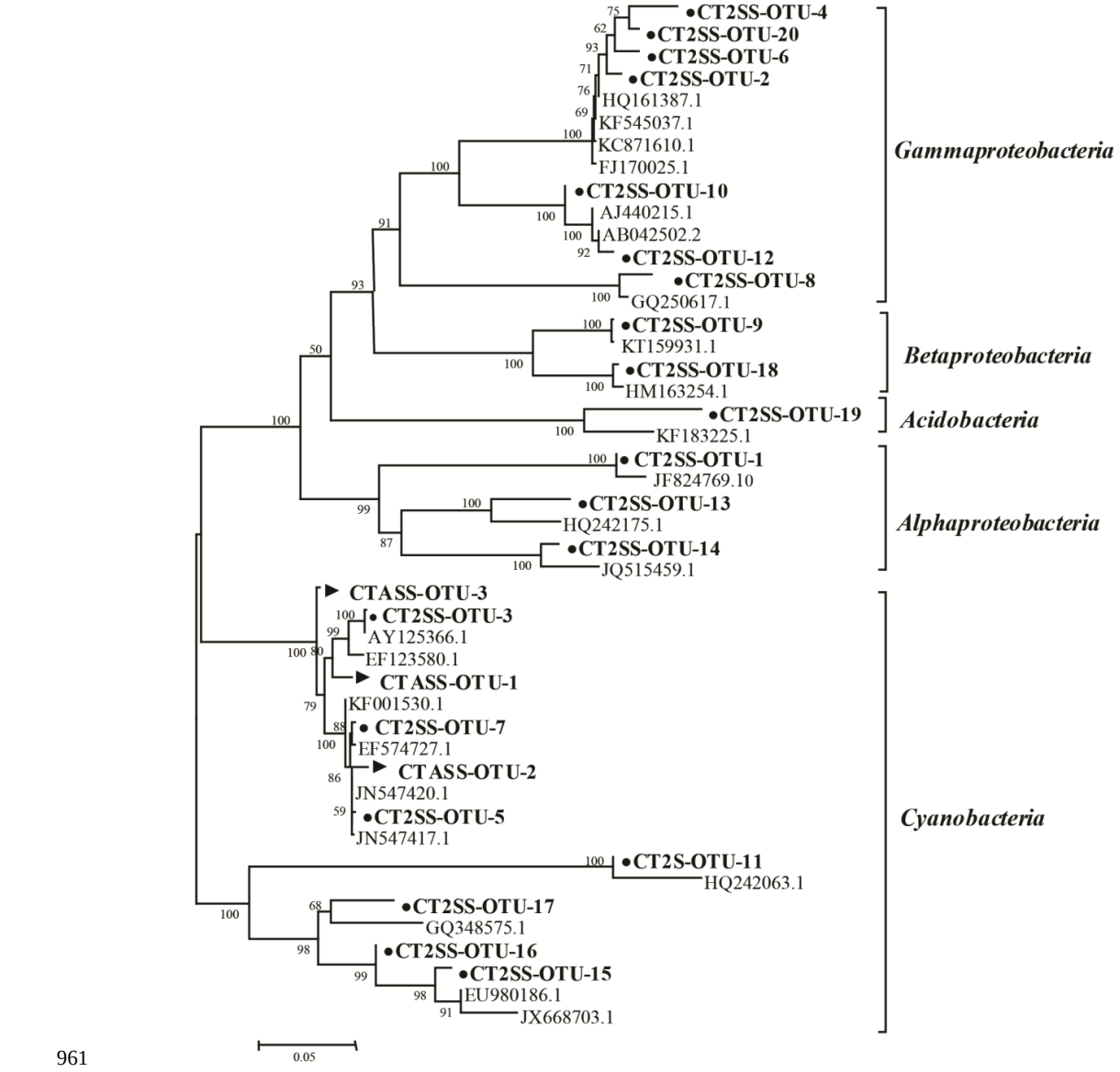

Figure 4 Phylogenetic tree of representative sequences of the OTUs common between surface clone libraries obtained during spring intermonsoon (SIM), fall intermonsoon (FIM), and northeast monsoon (NEM).

CTASS- Common to all season surfaces, **CT2SS**- Common to two season surface

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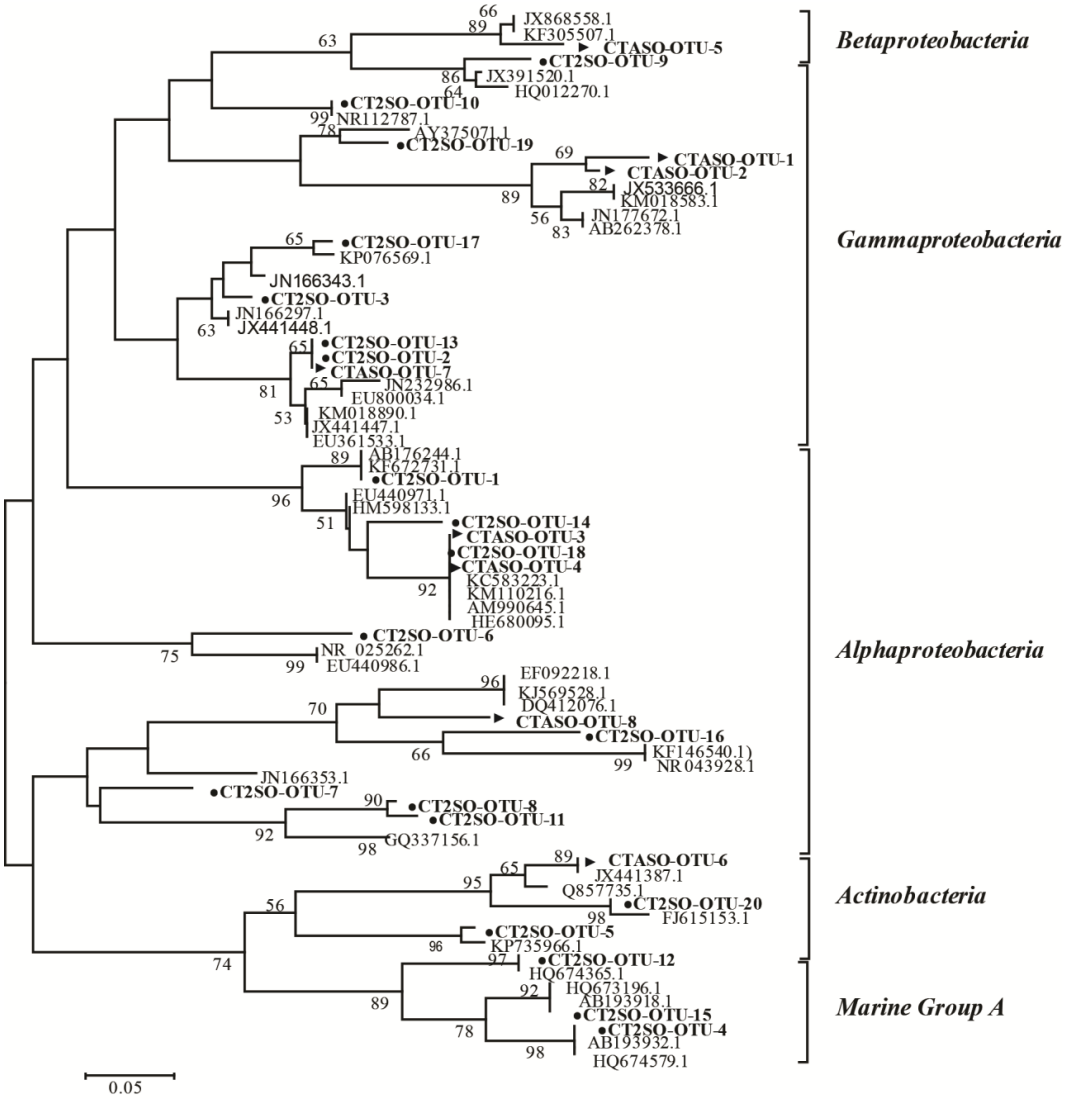

Figure 5 Phylogenetic tree of representative sequences of the OTUs common between OMZ clone libraries obtained during spring intermonsoon (SIM), fall intermonsoon (FIM), and northeast monsoon (NEM).

CTASO- Common to all season OMZ, **CT2SO**- Common to two season OMZ

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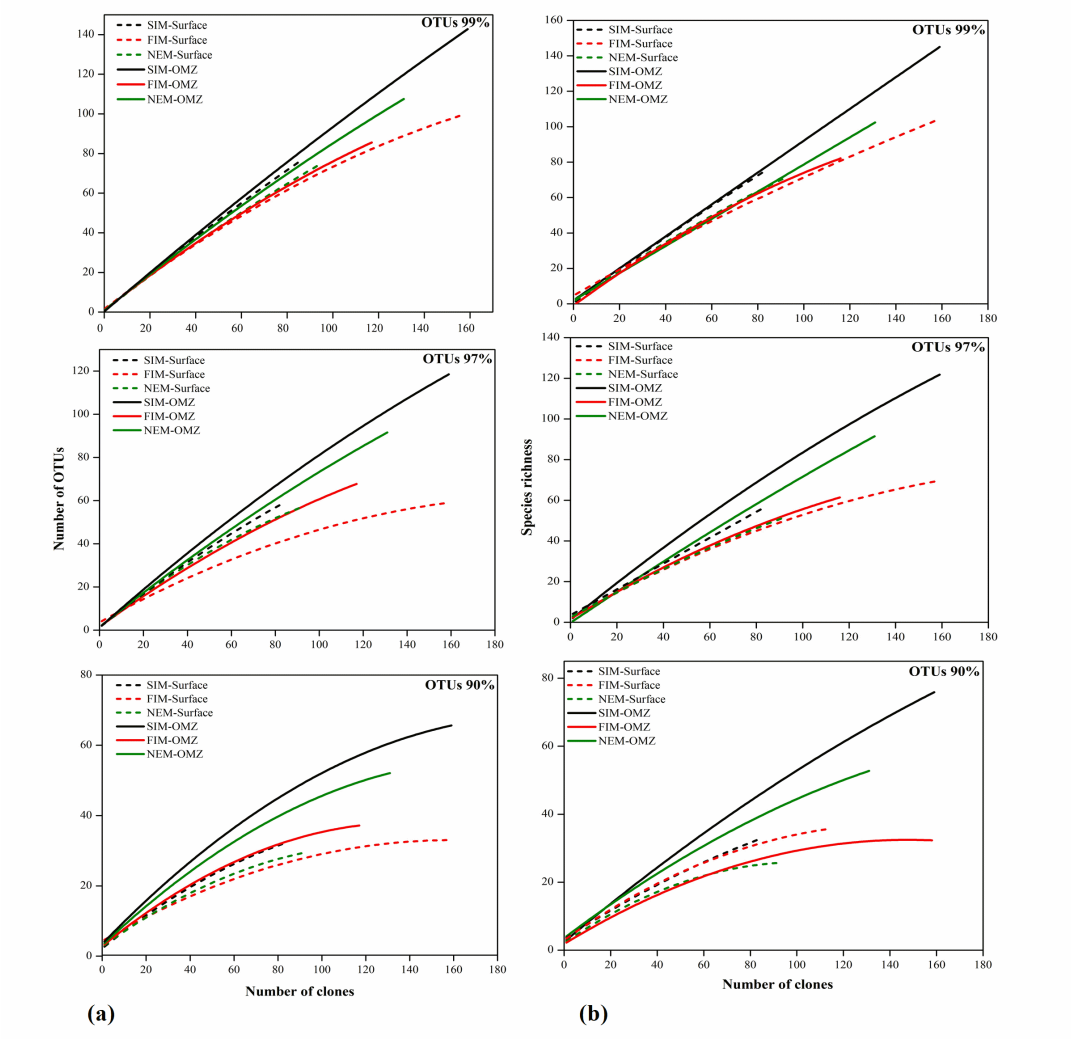

Figure 6 Rarefaction (a) and Collectors (b) curves for the surface and OMZ clone libraries at 90%, 97%, and 99% similarity level obtained during spring intermonsoon (SIM), fall intermonsoon (FIM), and northeast monsoon (NEM) at the Arabian Sea Time Series (ASTS) location