

# Coastal Hypoxia Drives Microbial Diversity: Elucidation through 16S rRNA Amplicon Sequencing

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## Research Article

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# Abstract

The formation of oxygen-depleted zones in the bottom waters is one of the most widespread phenomena in coastal areas. Upwelling episodes occurring along the west coast of India due to the southwest monsoon lead to an increase in biological productivity which further lowers the dissolved oxygen in the upwelled waters, which intensifies annually between June and October. Here, we have determined the changes in the microbial community in response to the varying oxygen levels and other physicochemical parameters at the Candolim Time Series Station using high-throughput sequencing. Amplicon Sequence Variants across all the samples collected in different seasons were mostly affiliated to the phyla Proteobacteria, Actinobacteria, Bacteroidetes, Verrucomicrobia, Chloroflexi, Firmicutes and Planctomycetes, with the most dominant being Proteobacteria (21-41%). Statistical analysis revealed that microbial diversity differed significantly with changing DO, ammonia, nitrate and nitrite concentrations during different seasons. The microbial community shift due to seasonal hypoxia results in the differential biogeochemical cycling of essential nutrients with certain years seeing redox conditions up to sulphate reduction, while certain years seeing only nitrogen loss. Future scenario of global warming will serve as a big challenge for understanding the role of microbial diversity and its implications in the cycling of natural elements.

## Introduction

Coastal ecosystems play a pivotal role in regulating the global biogeochemical cycles. Some of the major processes in these ecosystems include the exchange of nutrients, carbon in the form of dissolved organic matter, reactive organic and inorganic trace gases, and various other physical and biogeochemical elements. Coastal ecosystems are significant as they are the areas with high productivity [Wiggert et al. 2005]. The increased biological productivity due to monsoonal upwelling leads to increased organic matter production, utilised by heterotrophic microorganisms. The intense decomposition of organic matter leads to rapid utilisation of *in situ* oxygen, leading to its depletion and forming hypoxic conditions in sub-surface waters [Gomes et al. 2019; Naqvi et al. 2000].

The west coast of India experiences coastal hypoxia due to upwelling, which is mainly influenced by the southward movement of the West Indian coastal current during the South West Monsoon (SWM) [Madhupratap et al. 1996]. The upwelled waters are already hypoxic ( $<2 \text{ mL L}^{-1}$ ) as these are from the thermocline region off the continental shelf of India [Naqvi et al. 2000]. During the SWM, the bottom waters having low oxygen levels profoundly impact the biogeochemistry and ecological functioning of the biome and the underlying sediments. Hypoxia can also be caused by the anthropogenic inputs of nutrients and organic matter, leading to a drastic decrease in DO due to eutrophication [Rabalias et al. 2002]. Such low oxygen levels threaten marine ecosystems and are also responsible for the loss of benthic microbial habitats. In addition, prolonged hypoxic conditions may change the microbial community [Crump et al. 2007] in response to oxygen depletion. In a case study carried out in the Black Sea, the presence or absence of oxygen in the water column has been suggested as the main reason for variations in microbial community structure and function [Thamdrup et al. 2000]. Beman and Carolan [2013] reported a non-linear relationship between the DO concentration and bacterial richness in the oxygen-deficient waters of the Eastern Tropical North Pacific Ocean.

Hypoxic events are often considered as one of the major global environmental problems [Diaz and Rosenberg 2008; Naqvi et al. 2010; Breitburg et al. 2018]. The seasonal coastal hypoxic zone of the Eastern Arabian Sea is by far the largest of all the known coastal hypoxic systems formed due to natural and anthropogenic activities [Naqvi et al. 2006; Sudheesh et al. 2020]. It is observed that if hypoxic conditions persist for a long time, then the organic matter and nutrients tend to accumulate in the sediments resulting in the expansion of the hypoxic zone. This leads to a further decrease in DO levels, thus establishing anoxic conditions, favouring the release of H<sub>2</sub>S by microorganisms [Diaz and Rosenberg 2008]. The progression of seasonal coastal hypoxia has been shown to follow a predictable pattern that starts with SWM's advent (June to September). Oxygen depletion at times might also begin in April-May and gradually intensify with time [Naqvi et al. 2006]. The CaTS-G5 station located on the West coast of India is being extensively monitored since 1997 to study the changes occurring due to coastal hypoxia. This could be considered a representative station to understand the oxygen deficiency in the coastal regions [Naqvi et al. 2006]. The oxygen-deficient conditions intensify towards the end of the southwest monsoon, whereas the water column remains well-oxygenated during other periods.

Prolonged oxygen-deficient episodes often change the bacterial community composition [Crump et al. 2007], affecting the ecosystem's functioning [Reed and Martiny 2013]. As oxygen is the most favourable electron acceptor in aerobic aquatic systems, its availability could influence gene expression of the bacterial community and affect respiratory metabolism. A shift in bacterial community composition during the development of hypoxia was reported by Beman and Carolan [2013] in the oxygen minimum zones of the Eastern North Pacific Ocean, where they recorded a non-linear relationship between bacterial richness and DO concentration. On the contrary, a negative correlation between bacterial richness and DO was reported by Spietz et al. [2015] in a hypoxic estuary. Various bacterial lineages belonging to the classes Alphaproteobacteria, Gammaproteobacteria and Deltaproteobacteria have been reported to be abundant in oxygen-deficient marine waters. Microbes reported from oxygen-depleted waters falling into these lineages are considered to be chemolithoautotrophic sulphur oxidisers that play a key role in sulfur and carbon cycling in the ocean ecosystem [Lipsewers et al. 2017]. Previous studies, carried out in the Northern Indian Ocean oxygen minimum zone (OMZ), based on metagenomic analysis, reported taxa belonging to Proteobacteria, Actinobacteria, Nitrospina, Planctomycetacia and SAR406 clade; however, the distribution varied along depths and sites sampled [Bandekar et al. 2018; Fernandes et al. 2019]. High throughput sequencing techniques and their applications have helped unravel the microbial diversity in environmental samples [Caporaso et al. 2012; Lynch and Neufeld 2015].

The present study was conducted to investigate the effect of hypoxia on the microbial diversity at the CaTS-G5 station in the Arabian Sea. The microbial communities were deduced by 16S rRNA amplicon sequencing from water samples collected during different seasons, i.e. pre-monsoon, monsoon and post-monsoon. The annual variation in the bacterial diversity was also studied, along with the distribution of bacterial communities in response to the physicochemical environmental variables and predictive functional analysis of the microbial communities.

## **Materials And Methods**

### **Sampling locations and sampling methodology**

Water samples were collected at the CaTS-G5 (15.50877° N, 73.6742°E) station along the CaTS transect in the coastal waters of the Eastern Arabian Sea during the following cruises and field trips: SSD052 ORV Sindhu Sadhana (05-07-2018), SSD057 ORV Sindhu Sadhana (20-09-2018), CaTS 157 field trip (04-10-2018) and CaTS 161 field trip (15-10-2019).

Samples were collected from 2 depths based on the oxygen profile, mid-depth and near bottom as 12 m (GV1) and 24 m (GV2) on SSD052, 13 m (GV3) and 25 m (GV4) on SSD057, 18 m (GV5) and 27 m (GV6) on CaTS 157, 18 m (GV7) and 27 m (GV8) on CaTS 161. Onboard the ship the temperature data of the seawater samples were obtained using a Conductivity-Temperature-Depth (Seabird Electronics SBE911) rosette system fitted Niskin bottles. During the field trips, it was obtained using a portable CTD (SeaBird Scientific SBE25 plus V2), and water samples were collected using 5 litre Niskin samplers. The DO samples were analysed following the Carpenter modification of the Winkler method [Carpenter 1965]. The titration was automated using ODF PC software compiled in LabView, in which the endpoint is UVC detected photometrically at 365 nm. For DNA extraction, ten litres of seawater was filtered through 0.22 µm Sterivex filters (Millipore, United States) using a peristaltic pump (Cole-Parmer India). The Sterivex filter cartridges were filled with DNA storage buffer (50 mM Tris pH 8.3, 40 mM EDTA, and 0.75 M sucrose) and stored at -80°C until further analysis.

## Dna Extraction And Sequencing

The Sterivex filter cartridge was cracked open aseptically using a clean plyer. The filter paper was cut with a sterile blade and added to a disruptor tube containing SLX Plus buffer, and DNA extraction was carried out as per the manufacturer's protocol (OMEGA BioTek, USA). The vacuum dried DNA samples were outsourced for sequencing to Eurofins Genomics (India). The QC passed samples were processed for amplicon generation followed by NGS library preparation using Nextera XT Index Kit (Illumina Inc.). The primers used to amplify the bacterial 16S V3-V4 region were 16S rRNA Forw GCCTACGGGNGGCWGCAG and 16S rRNA Rev ACTACHVGGGTATCTAATCC (Eurofins). The raw reads were used for further analysis.

## Sequence Analysis

High-quality clean reads obtained from Illumina were selected using Trimmomatic v 0.38 [Bolger et al. 2014], and the paired-end data was stitched into single-end reads using FLASH (v1.2.11) [Magoč and Salzberg 2011]. ASVs were generated using the DADA2 plugin in the QIIME2 pipeline [Caporaso et al. 2012], and the taxonomic identity was assigned using the Silva database (version 13\_8) [McDonald et al. 2012]. The resulting biome file was used for further analysis. Abundance estimation along with diversity analysis and rarefaction analysis was carried out using the QIIME program. The sequence data of the 16S rRNA gene was submitted to National Center for Biotechnology Information (PRJNA 706932; SUB9205387).

## Statistical Analyses

The variability in the bacterial classes was compared with 5 environmental variables, namely, temperature, DO, nitrate, nitrite and ammonia using Canonical Correspondence Analysis (CCA) using PAST-4 software V4.03

[Hammer et al. 2021]. QIIME2 pipeline was used for diversity analysis. Beta diversity at the phyla level between the depths and stations was calculated using multivariate scaling analysis (MDS) based on Bray-Curtis distance using Primer 6 software [Clarke et al. 2008]. The PCoA analysis was carried out and plots were obtained using Unweighted and weighted distance metrics in QIIME2.

## Predictive Functional Analysis

Predictive functional analysis of the microbial communities present in all eight samples was done using Tax4fun2 [Wemheuer et al. 2020]. The 16S rRNA sequences were compared with reference sequences in BLAST using the runRefBlast function. The predictive functions were calculated based on KEGG ortholog reference profiles using the Functional Prediction function. Heatmap and statistical analysis representing the functional predictions within the samples was done using the STAMP V2.1.3 software [Parks et al. 2014]. Tukey-Kramer test was carried out to check the differences in functional gene composition within the samples.

## Results

### Physico-chemical characteristics of the water samples

The temperature of the water samples during the study period varied between 21.8 and 32°C, whereas salinity varied between 33.83 and 35.78 PSU. The temperature at the bottom depths was lower than the upper depths during the months from July to Oct 2018, whereas no significant differences were observed in the salinity (Table 1). The DO levels showed a drastic change from July to October 2018, ranging from oxic conditions (2.3 - 4.4 mL L<sup>-1</sup>) in July 2018 followed by a hypoxic range of 0.1 - 0.5 mL L<sup>-1</sup> in September 2018, progressing towards suboxic conditions (<0.1 mL L<sup>-1</sup>) in upper depths and completely anoxic conditions in the near bottom during October 2018. The near-bottom depths experienced higher oxygen deficiency as compared to the mid-depths. In October 2018, the water samples collected from both mid and near-bottom depths exhibited anoxic conditions with DO levels in the range of 0 - 0.2 mL L<sup>-1</sup>. The nutrient concentrations also showed a seasonal variation like the DO values. In general, the nitrate concentrations varied between 0.1 and 8.3 µM, nitrite between undetectable levels and 1.6 µM and ammonia between 0.3 and 9.6 µM (Table 1). In 2018, higher nitrate concentrations (3.1 – 6.9 µM) were observed in September compared to other months. High nitrate was also observed in October 2019. In contrast to nitrate, higher ammonium concentrations (6.13 - 7.06 µM) were observed during October 2018, accompanied by low nitrate values (0.01 - 0.06 µM) and the absence of nitrite. High ammonium concentration (9.6 µM) was also observed in the near-bottom waters during July 2018. H<sub>2</sub>S (1.66 µM) was detected at the lower depth, indicating anoxic conditions in October 2018 (Table 1).

Table 1

Physico-chemical parameters at Candolim Time Series station (CaTS-G5) during the cruise and field trip

S.No.	Date	Cruise/FT	Sample ID	Depth (m)	DO (mL L <sup>-1</sup> )	Temperature (°C)	Salinity (PSU)	NO <sub>3</sub> (µM)	NO <sub>2</sub> (µM)	NH <sub>4</sub> (µM)
1	July 5, 2018	SSD-052	GV1	12	4.49	28.72	33.83	0.10	0.00	1.78
			GV2	24	2.34	28.02	34.76	2.81	0.76	9.60
2	Sep. 20, 2018	SSD-057	GV3	13	0.52	23.02	35.78	6.93	0.85	1.38
			GV4	25	0.16	21.82	35.59	3.05	0.40	1.75
3	Oct. 4, 2018	CaTS-157	GV5	18	0.02	23.27	35.49	0.01	0.00	7.06
			GV6	27	0.00	22.88	35.43	0.06	0.00	6.13
4	Oct. 15, 2019	CaTS-161	GV7	18	1.37	31.34	35.33	5.25	1.55	0.33
			GV8	27	0.52	31.96	34.97	8.28	1.36	0.35

An annual comparison of the physicochemical parameters between October 2018 and October 2019 sampling showed that the intensity of oxygen deficiency was more in October 2018, with oxygen concentration falling in the anoxic range compared to the conditions in October 2019. Although in 2019, the sampling site did not experience anoxia, the ammonia values were low (0.33 - 0.35 µM) compared to all the seasons in the previous year. The total organic carbon (TOC) was considerably high during October, with the highest value at near bottom depth, where the conditions were anoxic (Table 1).

## Prokaryotic Community Structure

A total of 28 bacterial phyla were identified across all the seasons sampled. The maximum number of ASVs were affiliated to 3 major bacterial phylogenetic groups, viz. Proteobacteria, Actinobacteria, and Bacteroidota (Fig. 1). Proteobacteria dominated across the mid-depth and near-bottom zones accounting for about 42.69 - 68.78% in almost all the seasons, except October 2018, where the mid-depths were dominated by the phylum Actinobacteria (33.2%). Bacteroidota showed a high abundance during September 2018 at both depths; however, it was less abundant during all other seasons. The phyla Planctomycetota and Verrucomicrobiota were evenly distributed across all the seasons at both mid and near-bottom depths. Two phyla, namely Spirochaetes and Nitrospirae, were abundant at lower depths in all the samples. The members of SAR406, Marinimicrobia phyla were present in all the samples.

Among the archaeal phyla, Thermoplasmatota showed dominance during July, October 2018 at both depths. However, during October 2019, Thermoplasmatota showed high abundance in the mid-depths. The two

euryarchaeal groups present in all the samples belonged to marine group II and marine group III, with the former being dominant in all the samples. In addition, archaeal phyla Crenarchaeota and Nanoarchaeota were present in most samples, albeit not so dominant.

A comparative analysis of relative abundance at the class level between mid and near-bottom depths across all the seasons highlighted that the class Acidobacteria belonging to phyla Actinobacteria showed high abundance in all samples. Among the Proteobacteria, the major classes were Alphaproteobacteria followed by Gammaproteobacteria (Fig. 2). It was noted that Alphaproteobacteria were abundant in mid-depth compared to the near-bottom depth in all the samples. Thermoplasmata belonging to the Phylum Thermoplasmata showed higher abundance in almost all samples except GV3, GV4 and GV8.

At the genus level, Candidatus Actinomarina, NS4 Marine group, NS5 Marine group, Marinimicrobia, clade 1a, AEGEAN-169 marine group, Marine group III, Sva0996 were ubiquitously present in all the samples. In addition, the SUP05 clade was also present in almost all the samples except GV2.

A comparison between the samples from mid-depths GV1, GV3, GV5 and GV7 showed a similar trend. Proteobacteria was the dominant phylum in almost all samples except for GV5, irrespective of sampling time. The Shannon alpha diversity analysis of surface depths, i.e. GV1, GV3, GV5 and GV7, indicated that GV3 exhibits higher alpha diversity followed by GV1, GV7 and GV5 (Table 2). Similarly, in the lower depths, Shannon alpha diversity analysis revealed that GV6 displayed higher diversity, followed by GV2, GV4 and GV8. MDS and PCoA analysis showed distinct grouping at phyla level at the mid-depths and near bottom depths.

Table 2  
Prokaryotic diversity estimates observed in mid and near bottom depths at Candolim Time Series station (CaTS-G5)

S. No.	Depth	Sample ID	Cruise no. and depth	Shannon (H)	Simpson (1-D)	Pielou's evenness (J')
1	Mid-depth	GV1	SSD052 (12 m)	6.6	0.878	0.907
2		GV3	SSD057 (13 m)	6.8	0.876	0.915
3		GV5	CaTS157 (18 m)	6.4	0.880	0.879
4		GV7	CaTS168 (18 m)	6.5	0.921	0.890
5	Near bottom	GV2	SSD052 (24 m)	6.6	0.905	0.898
6		GV4	SSD057 (25 m)	6.4	0.887	0.903
7		GV6	CaTS157 (27 m)	6.6	0.909	0.916
8		GV8	CaTS168 (27 m)	6.4	0.922	0.912

## Correlation Between Physicochemical Properties And The Microbial Community Structure

The effect of environmental variables on microbial community structure at the genus level was studied at the G5 station along both depths. Seasonal and annual variations in community structure with respect to 5 environmental variables were investigated using Canonical Correspondence Analysis (CCA). The triplot obtained reveals a strong positive correlation between dissolved oxygen, ammonia and the genus NS4\_marine group, Clade\_II and SAR 116 clade belonging to the class Alphaproteobacteria (Fig. 3). Archaeal marine group II also shows a slight positive correlation with these two parameters. This correlation was essentially observed in samples GV1 and GV2. NS5 marine group, Marinobacterium, clade 1a, PAUC34f and bacteroidetes showed a positive correlation with nitrate and negative correlation to temperature. Candidatus nitrosopelagicus, SAR202 clade, Marinimicrobia, SAR324, AEGEAN-169 marine group were all negatively correlated to nitrite concentration. Among all the environmental parameters analysed, DO, and ammonia significantly contributed to the variation in the community in the study area.

## Predictive Functional Analysis

The functions predicted by Tax4fun2 highlighted various diverse functions performed by the organisms that are essential for numerous ecological processes. The dominant pathways reported were involved in metabolism, such as carbohydrate, lipid, energy, nucleotide, amino acid metabolism, genetic information processing (Supplementary Fig. 3), cellular processes, and environmental information processing. Few of the abundant genes predicted in all the samples were sulfite reductase (K00392), thiosulfate sulfurtransferase (K01011), Nitrogen regulatory protein Pii1 (K02589), thioredoxin 1 (K03671), DNA repair and replication protein RecF (K03629) etc.(Supplementary Fig. 3).

## Discussion

The Western Shelf of India is considered one of the most productive areas of the Arabian Sea due to nutrient enrichment caused during the Southwest monsoonal upwelling [Naqvi et al. 2000, 2006; Shetye et al. 1990]. This area experiences considerable changes in the DO concentration throughout the year. It remains well-oxygenated from November - May, subsequently reaching hypoxic conditions by July and intensifying further during September-October. Hypoxia begins in June, followed by a decrease in the DO levels and an increase in denitrification during late August-early September, further progressing towards anoxia associated with sulphidic conditions during September-October [Naqvi et al. 2006, 2010].

The reduction of DO levels at the sampling site is a characteristic feature observed during the SWM [Naqvi et al. 2006] and is fairly evident in the study. Even in the present study, the conditions switched from hypoxic to suboxic to anoxic from July to October 2018. The reduction in the DO values may be attributed to the consumption of DO during remineralisation of the organic matter produced as a result of upwelling [Naqvi et al. 2000]. Concomitant with the reduction in the DO levels, nitrogen is also lost during September – October due to denitrification. This is evident from the loss of nitrate and nitrite during these months. These results correspond to the studies carried out by Naqvi et al. [Naqvi et al. 2006] at the same location. Unlike the open ocean Arabian Sea OMZ, which experiences only nitrogen loss due to denitrification and anammox [Ward et al. 2008, 2009; Lam et al. 2000], the Western Shelf of India also undergoes sulphate reduction (complete anoxia) as seen from the presence of H<sub>2</sub>S (1.66 µM) in the near-bottom depths during October 2018, which was also

reported previously [Naqvi et al. 2006; Shenoy et al. 2012, Pratihary et al. 2014]. One of the outcomes of such a condition is the accumulation of unprecedented amounts of nitrous oxide ( $\sim 1 \mu\text{M}$ , a greenhouse gas, [Naqvi et al. 2006]) and dimethylsulphide ( $\sim 0.5 \mu\text{M}$ , an anti-greenhouse gas) [Shenoy et al. 2007, 2012; Bepari et al. 2020]. However, the sulfidic conditions have been reported to occur during September-October, followed by the release of nitrogen from the decaying organic matter and its accumulation as ammonium [Naqvi et al. 2000, 2006].

Microbial communities being very complex in nature, their structure and functions tend to change in response to various physicochemical and environmental factors. The majority of the environmental factors affecting the microbial communities and their structural dynamics differ according to time and space [Fuhrman et al. 2015]. The effect of these variables on the diversity of prokaryotic communities is reported in the present study.

Prokaryotic communities in the study area have been known to exhibit a distinct seasonal pattern. Several environmental variables significantly influence the differences in abundance and distribution patterns. The bacterial communities observed in the study area were similar to those reported from coastal and open ocean oxygen-deficient regions [Wright et al. 2012; Fernandes et al. 2019, 2020]. Proteobacteria dominated the bacterial community structure at the phylum level in most samples, similar to the results obtained previously by Gomes et al. [2019]. The other phyla observed included Actinobacteria, Bacteroidetes, Chloroflexi, Planctomyces and were similar to those identified previously in oxygen minimum zones [Gomes et al. 2019; Fuhrman et al. 2015]. Bacteroidetes contribute significantly to the marine bacterial community and are dominant in nutrient-rich waters [Kirchman 2002]. Chloroflexi and Planctomycetes reported in the study area have been previously reported in the hypoxic regions and are known for their role in breaking down sulfated heteropolysaccharides, denitrification and carbon fixation, respectively [Ye et al. 2016; Yilmaz et al. 2016]. Verrucomicrobia, a minor group present in all the samples at both depths, is known to be ubiquitous in marine environments [Bergmann et al. 2011], and its higher relative abundance in July 2018 and October 2019 seems to indicate their affinity for oxic waters.

At the class level, Acidimicrobia belonging to phyla Actinobacteriota was dominant in both the depths in all the samples. The classes Alphaproteobacteria and Gammaproteobacteria were also ubiquitously present in all the samples. These Proteobacteria classes have been known to account for 30 - 50% of all bacteria in the surface waters in the Oceans [Gomes et al. 2019]. The presence of Firmicutes reported in the study coincides with the findings of Gomes et al. [2020], and these are known to encompass efficient denitrifiers. The predominant class, Acidobacteria, is known to be involved in nitrate reduction [Kielak et al. 2016].

The archaeal community was dominated by Euryarchaeal class Thermoplasmata, marine group II and marine group III in all the samples. A higher abundance of marine group II Euryarchaea observed in these hypoxic and anoxic waters were parallel to that reported in Arabian Sea OMZ, in the black sea, and anoxic Cariaco Basin wherein this phylum was present throughout the water column [Bandeekar et al. 2018; Madrid et al. 2001; Vetriani et al. 2003]. Marine group II archaea have been known to play an important role in carbon cycling [Lincoln et al. 2014; Zhang et al. 2015]. The higher relative abundance of Euryarchaea during October 2018 seems to indicate its affinity to thrive in anoxic waters, as reported previously by Belmar et al. [2011]. It has also been suggested that the concurrent aerobic and anaerobic processes in oxygen-deficient zones may lead

to higher archaeal diversity in those regions [Fussel 2013]. The presence of Nanoarchaeota was reported from all the water samples, though at very low abundance.

The various genera present in all the samples have been known to play a significant role in biogeochemical cycles. The OM60/ nor 5 clade belonging to the class Gammaproteobacteria are aerobic anoxygenic photoheterotrophs that play an important role in the carbon cycle [Yan 2009]. Similarly, the SAR324 clade of class Deltaproteobacteria are potential sulfur oxidisers and also participate in carbon fixation [Sheikh et al. 2014]. The presence of sulfur oxidising marine chemoautotrophs SUP05 clade in almost all are concurrent with the findings of Canfield et al. 2010 [Canfield et al. 2010]. This group of bacteria are known to dominate suboxic and anoxic waters and are assumed to take part in denitrification and annamox processes [Walsh et al. 2009; Hawley et al. 2014].

MDS and PCoA analyses based on Bray-Curtis distance at phyla level based on depth showed that the hierarchical clustering formed groups of mid and near-bottom depth samples (Fig. 4) and (Supplementary Fig. 1). In addition, the MDS analysis showed clustering of samples based on depths and DO levels. GV1, GV2, GV7 and GV8 are closer to each other and represent high oxygen content compared to GV3, GV4, GV5 and GV6. A similar partitioning pattern was observed between surface, oxygen minimum zone and near bottom depths in other known OMZs [Stevens and Ullao 2008; Podalska et al. 2012]. Among the five environmental variables used for correlation analysis, it was observed that DO, ammonia, nitrate and nitrite significantly affected the abundance of bacterial as well as the archaeal community. DO is considered crucial in determining changes in the microbial community composition in the marine environment [Stevens and Ullao 2008], which was also evident in this study with the community structure changing with the change in DO level. The functional analysis predicted using Tax4fun2 gave insights into the functional capabilities of microbial communities in the study area. This in turn highlighted the dominant pathways that are involved in various ecological processes, thus emphasizing on the significant role played by the bacterial communities in the coastal hypoxic conditions.

One of the largest naturally occurring oxygen-deficient zone develops during the late southwest monsoon, over the west coast of India, resulting from upwelling, which leads to depletion of DO in that area [Naqvi et al. 2000]. These low oxygen conditions are known to have severe effects on the coastal marine environment resulting in a loss of marine biodiversity. The prokaryotic communities are intensely affected by seasonal changes and respond by shifting community composition based on these environmental factors. Using high throughput sequencing methods, the abundance and distribution of various microbial lineages can be studied. These studies will serve as a useful tool to interpret the importance of various bacterial and archaeal groups in regulating biogeochemical cycling in hypoxic environments.

## Conclusion

Coastal ecosystems are greatly influenced by anthropogenic activities, which can be attributed to growing industrial development and pollution. These activities have the potential to influence the natural flow of events, such as the development of seasonal hypoxia along the west coast of India. This eventually adds to the declining oxygen concentration in the coastal environments and leads to changes in ecological functioning. High throughput sequencing techniques can be used to study the change in microbial diversity, which provides

insights into the microbial diversity as well as distribution of various microbial communities. Microorganisms contribute significantly to the biogeochemical cycling of several essential elements such as nitrogen, carbon, phosphate, and sulfur. Therefore, the influence of oxygen depletion on microbial community structure affects coastal environmental management and the global environment. Studying the implications of low oxygen concentration and other environmental variables on bacterial community structure provides insights into the processes that might be affected due to the development of hypoxia.

## Declarations

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### Competing interests

Vruti V. Naik, Samir R. Damare, Shruti S. Shah, Damodar M. Shenoy and Amara Begum Mulla declare that they have no conflict of interest.

### Author contributions

The study was conceptualized by the corresponding author (SD). The sampling and lab work was carried out by the first author (VN), third author (SS) and fourth author (ABM). SD, VN and SS carried out the amplicon sequence data analysis. The manuscript was written by SD, VN, and the last author (DMS). All authors have read and approved the submission of the manuscript.

### Ethics approval

Not applicable

### Consent to participate

Not applicable

### Consent to publish

Not applicable

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## Figures

Fig. 1.

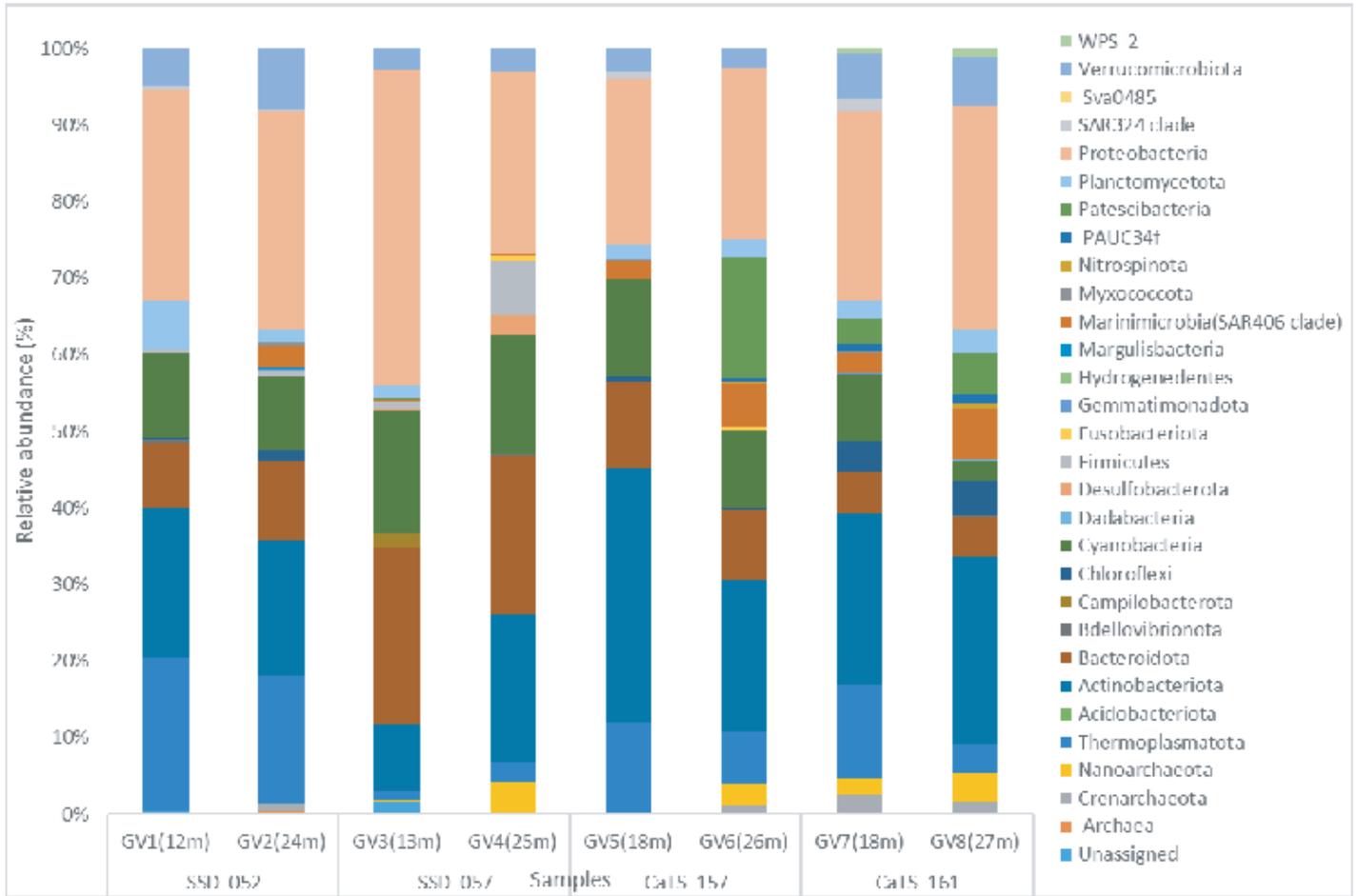


Figure 1

Percentages of relative abundance (Y-axis) and sampling cruise/field trip and depth-wise representation of phyla (X-axis) at the Candolim Time-series station (CaTS) G5.

Fig. 2.

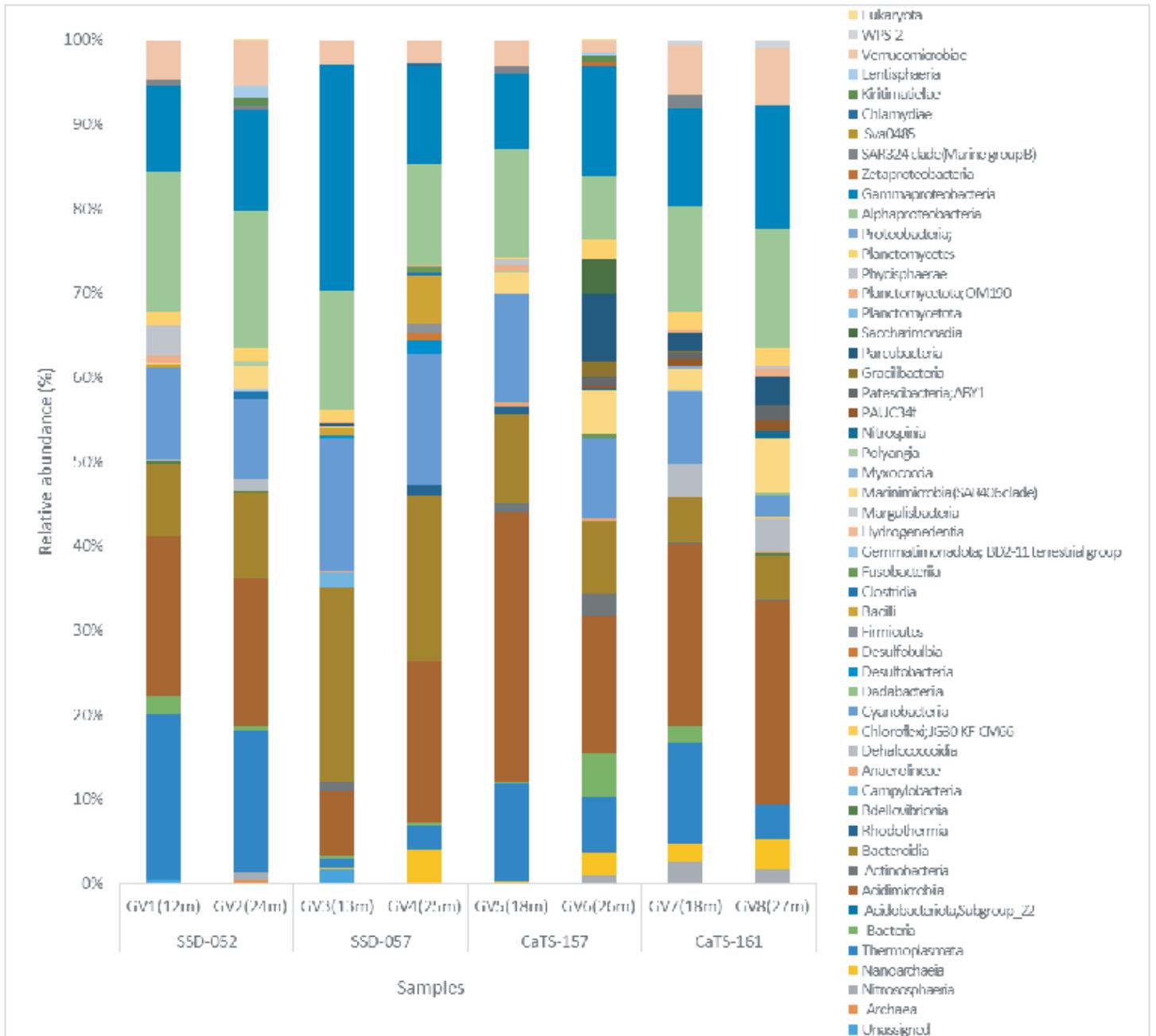
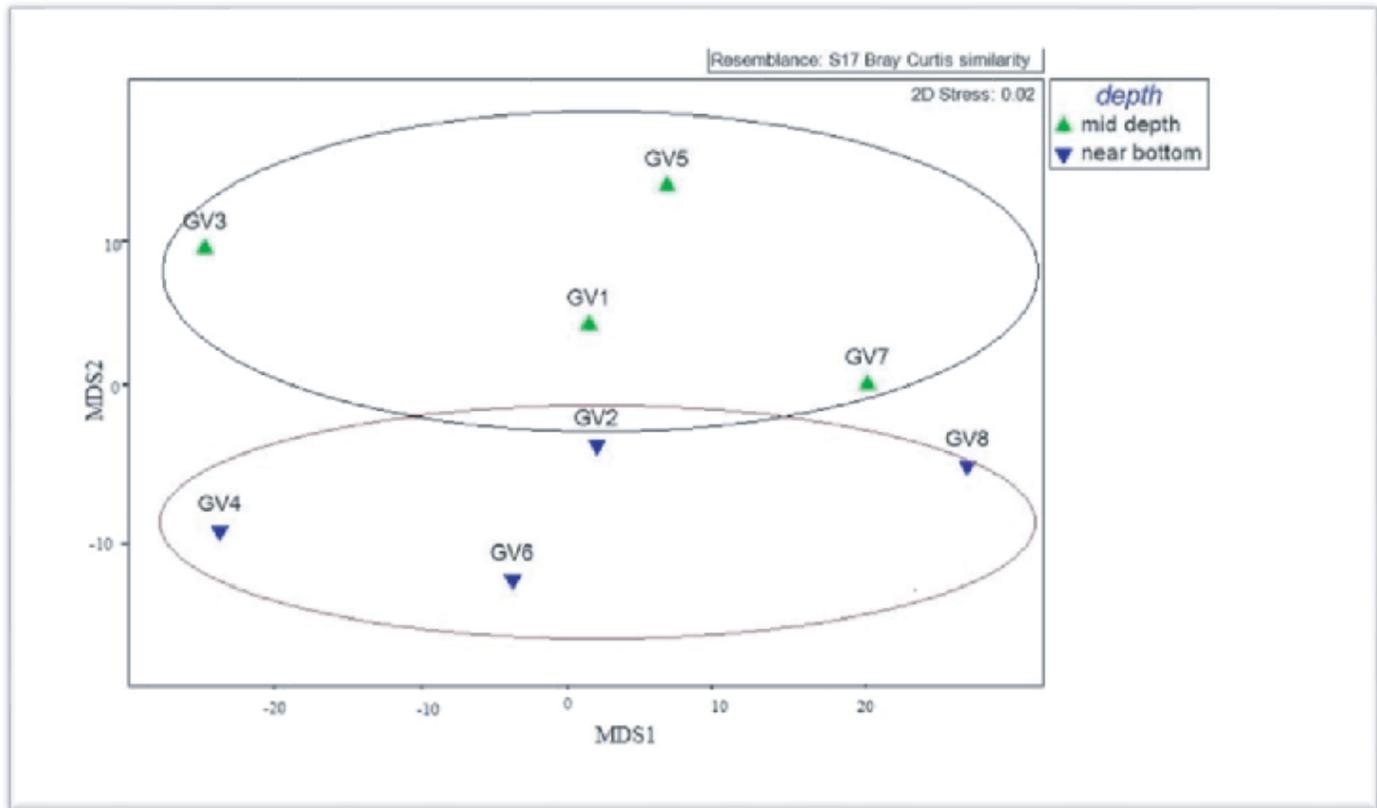


Figure 2

Percentages of relative abundance (Y-axis) and sampling cruise/field trip and depth-wise representation of class (X-axis) at the Candolim Time-series station (CaTS) G5.



*Fig. 4.*



**Figure 4**

MDS ordination plot based on Bray-Curtis distance.

## Supplementary Files

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- [Supplementarydata28112021.doc](#)