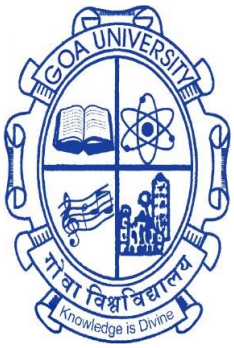
Assessment of the heterotrophic bacterial community structure and its activity along the West coast of India.

A THESIS SUBMITTED IN PARTIAL FULFILLMENT FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

IN THE SCHOOL OF EARTH, OCEAN, AND ATMOSPHERIC SCIENCES

GOA UNIVERSITY



By

Ashutosh Shankar Parab

CSIR-National Institute of Oceanography,

Dona Paula, Goa, India.

August *2024*

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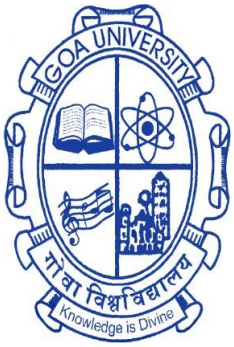

By

Ashutosh Shankar Parab

CSIR-National Institute of Oceanography,

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Under the Guidance of

Dr. Cathrine Sumathi Manohar

CSIR-National Institute of Oceanography, Dona Paula, Goa, India.

August *2024*

Declaration

As required under the University Ordinance OA—19A-1, I Ashutosh Shankar Parab, here by declare that the thesis entitled **"Assessment of the heterotrophic bacterial community structure and its activity along the West coast of India."** submitted to the Goa University in partial fulfillment of the requirement for the award of the degree of Doctor of Philosophy in Marine Sciences, is a record of original and independent research work done by me under the supervision and guidance of Dr. Cathrine Sumathi Manohar, Senior Principal Scientist, Biological Oceanography Division, CSIR-National Institute of Oceanography, Dona Paula, Goa and it has not submitted for the award of any Degree/Diploma/Associateship/Fellowship or other similar title of any candidate of any University. The literature related to the problem analysed and investigated has been appropriately cited. Due acknowledgements have been made wherever required.

Place: Dona Paula

Date: 02 August 2024 Ashutosh Shankar Parab

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(Council of Scientific & Industrial Research)
Dona Paula, Goa - 403 004, India

Certificate

This is to certify that, the thesis entitled **"Assessment of the heterotrophic bacterial community structure and its activity along the West coast of India."**, submitted to the Goa University, in partial fulfillment of the requirement for the award of the degree of Doctor of Philosophy in **Marine Sciences**, is a record of original research work done by **Mr. Ashutosh Shankar Parab**, under my supervision and guidance, and the thesis or any part has not been previously submitted for the award of any Degree/Diploma/Associateship/Fellowship or other similar title of any candidate of any University.

Place: Dona Paula
Date: 02 August 2024

Dr. Cathrine Sumathi Manohar,
Senior Principal Scientist,
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Dona Paula, Goa – 403004.

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Dedicated...

To my **parents**, for their endless love and support; to my **mentor**, for wisdom and guidance; to my **sister**, for her constant encouragement; to my **wife**, for her patience and understanding; and to my **friends**, for their unwavering companionship. Each of you has been a vital part of this journey, and to you, I dedicate this work with heartfelt gratitude.

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Chapter 1.

Introduction: Significance of the marine microbial loop along the west coast of India.

1.1. Introduction

NIO extends from 30ºN to 10ºS and 50ºE to 100ºE and comprises three distinct oceanographic regions which include the Arabian Sea (AS) and Bay of Bengal (BoB) to the west and east of the Indian sub-continent and the Equatorial Indian Ocean (EIO) to the south (Figure 1.1). All these three regions are unique and have distinct biogeochemical characteristics owing to the contrasting hydrography of these regions (Prasanna Kumar et al. 2009). Over the past few decades, our understanding of the biogeochemical processes and microbiological diversity in the NIO has significantly deepened since the initiation of the International Indian Ocean Expedition (IIOE) between 1962 and 1965. This was largely driven by the establishment of the oceanographic research institutes in India, within the Council of Scientific and Industrial Research-National Institute of Oceanography (CSIR-NIO) in 1966 and the Ministry of Earth Science in 1981. Numerous significant research initiatives have been undertaken to further our understanding of the biogeochemical processes and microbiological diversity in the NIO. These projects include the Global Ocean Ecosystem Dynamics (GLOBEC: 1992 - 2009), Joint Global Ocean Flux Study (JGOFS(India): 1992 - 1997), Bay of Bengal Process Studies (BOBPS: 2001 - 2006), Poly-metallic nodules–Environmental Impact Assessment (PMN-EIA: 1982 onwards), Indian Ocean Global Ocean Observing System (IOGOOS: 2001 onwards), Sustained Indian Ocean Biochemical Biogeochemical and Ecological Research (SIBER: 2006 - onwards) and various others (Hood et al., 2009; Prasanna Kumar et al., 2009). The oceanographic process in the NIO has been significantly advanced through the generous funding provided by international organisations such as the Scientific Committee on Oceanic Research (SCOR) and the Intergovernmental Oceanographic Commission (IOC) of UNESCO and national scientific bodies (https://incois.gov.in/). Research activities at universities and industry-sponsored studies have been crucial in advancing our understanding of marine ecosystems by pioneering innovative methodologies and exploring understudied aspects of marine biology (Hood et al., 2009).

Unlike the Atlantic or the Pacific Ocean, the Indian Ocean is landlocked in the north. It is shown to experience seasonally changing monsoonal circulation which is characteristic of this region and causes the coastal and open ocean upwelling in the central AS (Banse, 1959; Wyrtki, 1973; Shetye et al. 1991; Smitha et al. 1998; Madhupratap et al.1996). In addition, winter cooling during the northeast monsoon (December-February)

also adds up to the variability of physical features (Prasanna Kumar et al. 2001). This leads to seasonal differences in primary production, with very high productivity in the AS, compared to the BoB or EIO regions (Banse 1968; Sarma et al. 2003). Therefore, seasonal differences in biological production greatly influence the biogeochemistry and sedimentation rates (Bourgoin and Tremblay, 2010). Another characteristic feature in the NIO is the seasonal coastal denitrification along the WCI, an extremely well-developed, perennial, open ocean, oxygen minimum zone (OMZ) (Naqvi et al. 2000) in mid-depths of the AS and a shallow oxygen-depleted environment in the BoB (Azhar et al. 2017).

The eastern region of the Indian Ocean, the BoB, is a unique embayment with many large rivers such as Irrawaddy, Brahmaputra, Ganges, Mahanadi, Godavari, Krishna and Cauvery which discharges its riverine inputs. The upper 30 - 40 m column in most of the region north of the BoB remains almost perennially stratified (Prasanna Kumar et al. 2002) due to excessive precipitation and lower evaporation, accompanied by low-saline surface waters and warmer sea-surface temperatures (Han and Webster, 2002). Because of heavy loads of suspended terrestrial sediment, the surface waters are rather perennially turbid shallowing the euphotic layer. This along with the existence of low subsurface chlorophyll maxima leads to low photosynthetic production in this region (Prasanna Kumar et al. 2002; Madhupratap et al. 2003). The EIO, which is south of the Indian sub-continent, is primarily oligotrophic in the absence of equatorial upwelling. These open regions experience expectedly low primary productivity due to the combination of very low nutrient concentrations and minimal coastal influence (Schott et al. 2002). However, the relatively higher ratio between the bacterial and primary productivity signifies the importance of heterotrophic bacteria in the food web dynamics in these regions of low autotrophic production (Ramaiah et al. 2009).

1.2. Ecological significance of microbes in the marine biogeochemical process

The ocean serves as a major repository for anthropogenic CO_2 and the current global awareness of climate change has prompted intense interest in studying carbon biogeochemistry in marine habitats. Nearly one-half of global photosynthesis occurs in the ocean and this results in the production of huge amounts of organic matter. Various biological, chemical and physical forces in the oceanic realm modify, decompose and

redistribute it within diverse time frames ranging from a few hours to long periods extending up to hundreds of years (Hansell and Carlson, 2013). Heterotrophic bacteria, the largest component of the microbial community, account for the substantial transformation of particulate organic matter in aquatic ecosystems into dissolved organic matter and run the microbial loop. Viral breakdown and heterotrophic activity by fungi, yeasts and other protists also play an important role in the marine biogeochemical cycle through the microbial loop (Buchan et al. 2014). Studies have shown that the microbial diversity and community of the heterotrophic microbes change with the availability of dissolved organic matter (Giovannoni et al. 2005; Jiao et al. 2010). The microbial ecology investigations in the latter part of the twentieth century have proved beyond doubt that microbial communities play a central role in organic matter recycling in marine ecosystems (Castillo et al. 2022). Gaining a thorough understanding of the bacterial communities and their activities in the NIO, which is one of the productive marine regions is essential for comprehending their significance in the present-day context which is witnessing a major shift due to climate change (Dalpadado et al. 2023; Sridevi et al. 2023).

Microbial communities are of prime ecological importance as the transformation of organic matter from labile to recalcitrant states is known to occur through the microbial carbon pump. A proportion of this transformed organic matter is not mineralized but is instead stored for hundreds of years in the form of refractory organic matter in the ocean. The modest fluctuations in the ocean's carbon reservoir can wield a substantial influence on the atmospheric carbon dioxide reservoir. As a result, research efforts have been sparked to investigate the contribution of microbial communities in the biotransformation of organic matter (Carlson et al. 2011; Castillo et al. 2022). Understanding these processes is crucial for comprehending the mechanisms by which carbon is transformed in the ocean as there are numerous unanswered questions from the microbial carbon pump perspective, especially from the NIO. This basin is unique as it is one of the highly productive regions due to the semi-annual, monsoonal influence and harbours one of the major pelagic oxygen-depleted environments. Hence it is imperative to get a complete understanding of the scientific information available on the bacterial abundance, composition and distribution of the different phylogenetic taxa and their functional importance from this region.

1.3. Understanding of bacterial ecology in the NIO during the pre-genomics era using conventional tools

The pioneering works on marine microbial ecology were carried out in the NIO regions during the IIOE and from coastal regions along the Indian coastline were reviewed by Iyer and Pillai (1976). The paper provides an extensive analysis of the work carried out from 1950 to 1970, utilizing the available conventional tools of that time, offering valuable insights into the research conducted during that era. It includes the first studies which showed the presence of autochthonous bacteria from these regions and the diversity of bacterial and fungal flora associated with many marine organisms is also summarised (Iyer and Pillai, 1976). Studies carried out to understand the bacterial community from the latter part of 1970 up to the 1990s, were largely based on culture-based techniques and plate counts to estimate the bacterial CFUs (Figure 1.2). In these studies, the microbial groups present in the marine habitats were determined based on the biochemical assays to identify the bacteria and determine their activity (Appendix 1.1).

The studies from this period largely were from nearshore and coastal regions (Nair et al. 1978; Nair and Loka Bharathi 1982; Loka Bharathi and Chandramohan 1990; Ramaiah and Chandramohan 1992a). Ship-based observations were also carried out to determine the vertical distribution of heterotrophic bacteria (Nair et al. 1979; Chandramohan et al. 1987; Loka Bharati et al. 1992; Duklow, 1993). The physiological activities of the bacterial isolates from the southwest coast of India and Lakshadweep regions in the Arabian Sea region were carried out based on biochemical assays (Appendix 1.1). The authors reported considerable monthly changes in the bacterial populations, indicating that terrestrial runoff influences bacterial dispersion. Proteobacteria, Firmicutes, Bacteroidetes and Actinobacteria were the only bacterial phyla identified during this time frame, based on biochemical assays (Figure 1.3). The carbohydrase, lipolytic and proteolytic activities and the role of bacteria in the marine, nitrogen and sulfur cycle were also characterized based on biochemical tests (Figure 1.4 and Appendix 1.1).

Studies on the novel and unique groups such as the photosynthetic bacteria were characterized from the sediments of the Lakshadweep lagoons of Agatti atoll by Loka Bharathi and Chandramohan (1986), who identified them as *Prosthecochloris* spp. and *Chromatium* spp. using electron microscopy.

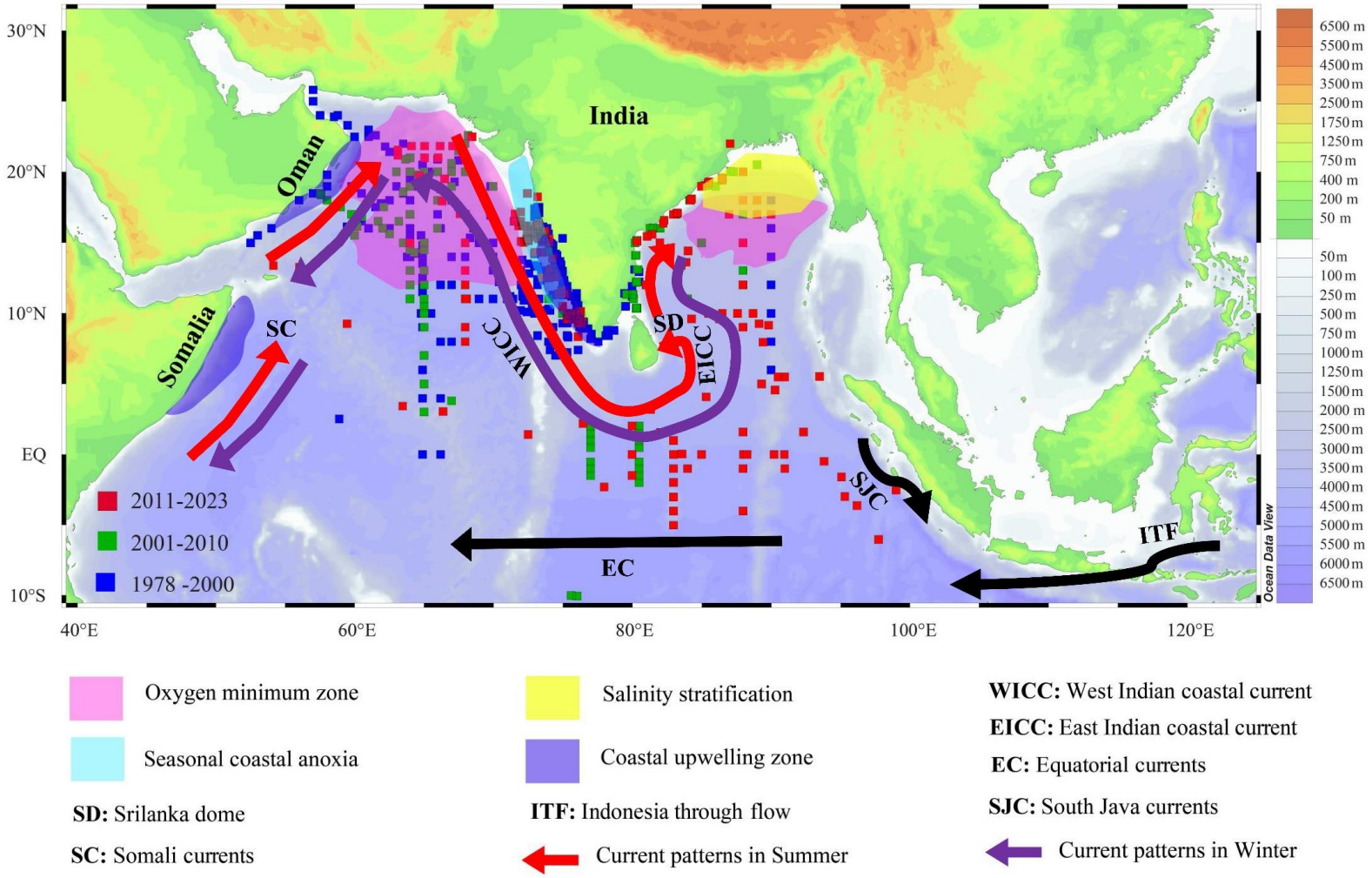


Figure 1.1: Map showing sampling locations of bacterial studies in different periods in the Northern Indian Ocean.

Limited research has been conducted on the photosynthetic non-sulfur bacteria belonging to the Rhodospirillaceae family in this region. Similarly, studies on luminous bacteria, which employ various enzymes to produce light energy are distinctive and widespread in marine environments, including the NIO region. These luminous bacteria occupy diverse ecological niches within the marine environment. Despite their significance, comprehensive investigations on these bacteria in tropical seas are lacking, leaving a gap in our understanding. To date, there have been no reported studies specifically focused on the production of enzymes by luminous bacteria. Ramaiah and Chandramohan (1992b) investigated the activity of L-asparaginase in marine photosynthetic bacteria collected from different marine ecosystems. While reports on these bacterial groups have emerged from the Andaman Sea, shedding light on their mechanisms of bioluminescence in marine habitats (Zari et al. 2020), there is still much to explore regarding many of these novel marine bacterial groups and their distribution patterns. The current understanding remains incomplete, and further research is needed to delve into the exploration of these bacteria and gain a more comprehensive understanding of their diversity and ecological distribution. In the pre-genomic era, studies to understand the microbial process relied on quantifying colony-forming units (CFUs) which were supported with microscopic observations to determine bacterial abundance and estimation of productivity using radiolabelled compounds (Figure 1.2). Studies to determine the bacterial abundance based on microscopic analysis using chromogenic dyes such as Acridine orange are reported from the early 1990s in the Indian Ocean region (Appendix 1.1). Since then, there have been significant advancements in the approach to determining microbial abundance (Ramaiah et al. 1996b; Hoppe and Ullrich 1999; Pomroy and Joint 1999; Koppelmamn et al. 2005). Estimating bacterial abundance remains a critical parameter that continues to be studied extensively even till the present day using molecular probes (Figure 1.2). Starting from the early 1990s, studies on bacterial productivity in the open ocean regions of the Arabian Sea and BoB, including the oligotrophic waters of the EIO, were conducted using labelled thymidine or leucine (Figure 1.2). These methods were employed to assess and quantify bacterial productivity in these marine environments. The focus of these studies was to gain insights into the metabolic activity and growth rates of bacteria in the oligotrophic waters of the EIO, as well as in the AS and BoB basins (Ducklow 1993; Ramaiah et al. 1996b; Hope and Ullrich; Pomroy and Joint 1999; Prasanna Kumar et al. 2000; Gauns et al. 2005; Gonsalves et al. 2009; Basu et al. 2013).

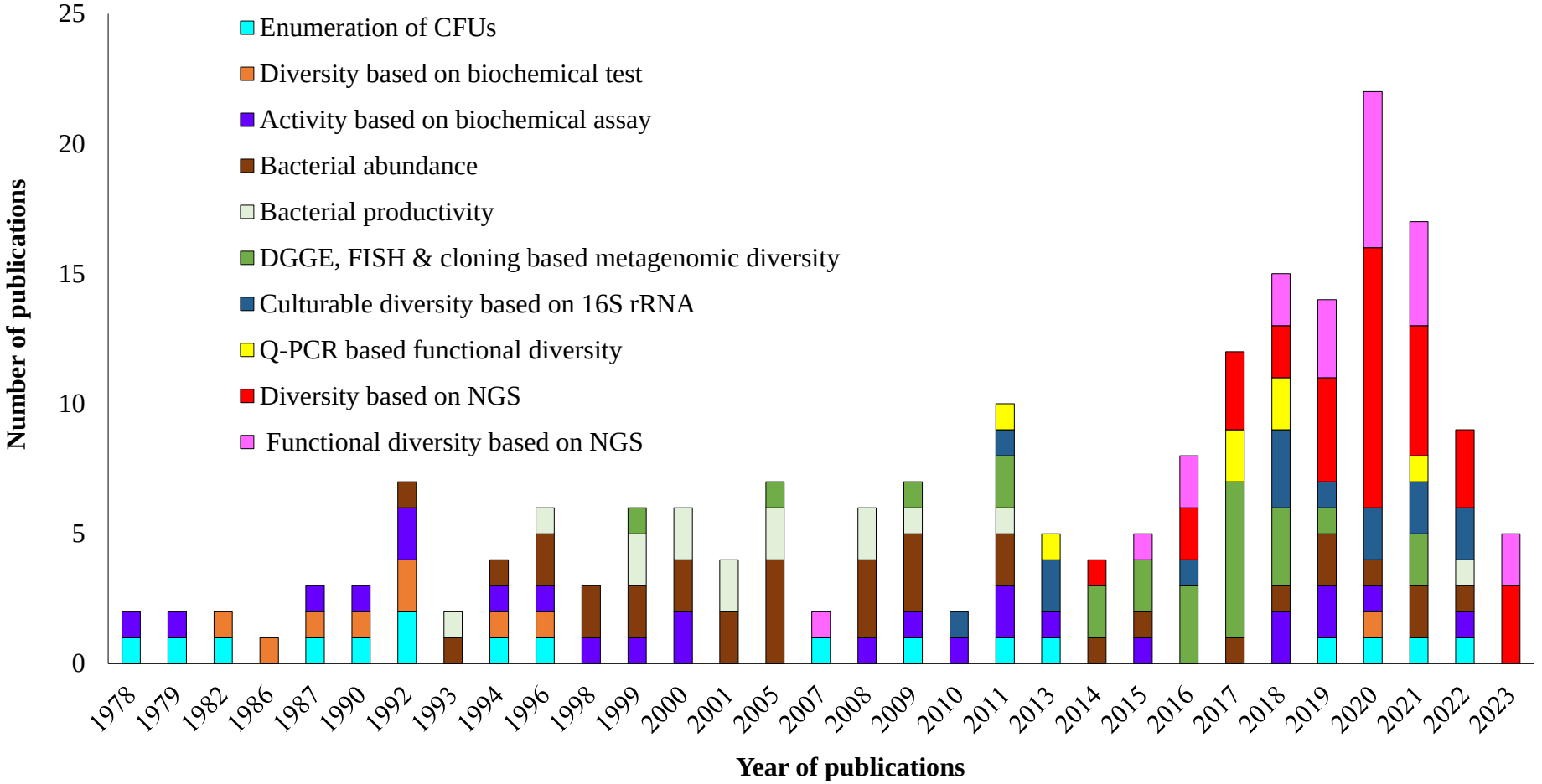


Figure 1.2: Year-wise studies on the bacterial abundance, diversity and activity from pre-genomics to OMICs era from the Northern Indian Ocean.

In a study conducted by Ramaiah et al. (1996a), the researchers examined the variations in bacterial abundance and productivity across different seasons and locations in the central and eastern regions of the Arabian Sea. The findings highlighted a significant turnover of bacteria during the inter-monsoon period in April-May. This turnover was suggested to play a crucial role in supporting the dominance of the 'microbial loop' within the food web and as a source of dissolved organic carbon. These results shed light on the seasonal and spatial dynamics of bacterial communities, emphasizing their importance in nutrient cycling and carbon fluxes in the Arabian Sea ecosystem. Furthermore, this study established the significant role of microbes in biogeochemical processes by elucidating the relationship between transparent exopolymer particle concentrations, labile particulate organic carbon, and total organic carbon. The findings highlighted the importance of microbial activity in the production and degradation of organic matter, which are known to influence the cycling of carbon in marine ecosystems. This relationship provides valuable insights into the intricate interactions between microbes and organic carbon pools, further emphasizing the essential role of microbes in biogeochemical processes.

Monsoon season and increased primary productivity are characteristic features of the NIO, hence many studies tried to establish the correlation between primary and bacterial productivity. The researchers evaluated the biological response to physical forcing and its impact on the primary productivity and the biological pump of the northern Arabian Sea during the northeast monsoon of 1997 (Dileep Kumar et al. 1998; Prasanna Kumar et al. 2001). Studies suggest that the average bacterial production in the global oceans is approximately 30 % of the primary production (Cole et al. 1988). However, bacterial productivity is reported to be up to 40 % of primary production during the summer monsoon in the eastern half of the AS, along the west coast of India (Ramaiah et al. 1996a). The average ratios of bacterial to primary production accounted for about 29 % in the central BoB and 31 % in the western BoB, along the east coast of India. Additionally, the study demonstrated that the relationship between phytoplankton and bacterial production differs in open ocean and coastal waters. The heterotrophic bacteria are also shown to utilize *in situ* dissolved organic carbon, rather rapidly and it is also apparent that they use allochthonous, organic matter to meet their carbon demands (Fernandes et al. 2008). The autotrophic production in the ultra-oligotrophic EIO is poor, however, the higher bacterial productivity reported in these regions is a critical link in the trophodynamics, as the bacterial biomass production generates food for phagotrophic heterotrophs through the microbial loop and mineralization provides nutrients for autotrophs (Damare and Raghukumar 2008; Fernandes et al. 2008). The classical 'Arabian Sea

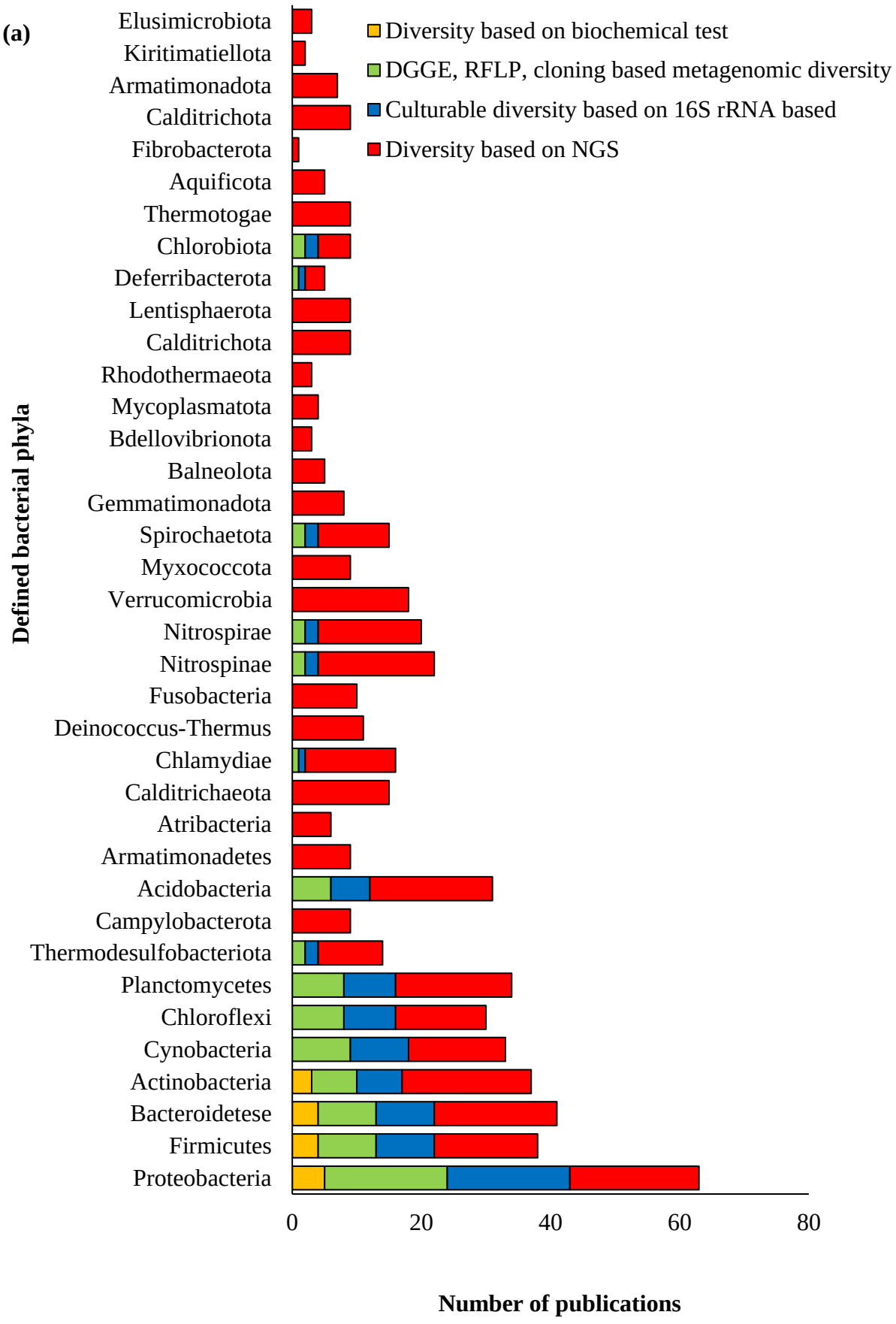
mesozooplankton stable-biomass paradox' through the microbial loop was established based on the studies by quantifying the abundance of heterotrophic bacteria, protists, microzooplankton and bacterial growth rates in the northern AS. This showed that heterotrophic bacteria play a key role in supporting microzooplankton growth, particularly in the mesopelagic zone (Ramaiah et al. 2005). Such important microbial processes were established based on the abundance, biochemical tests and productivity measurements (Figure 1.2 and Appendix 1.1) which have shown the role played by the bacterial community in the NIO. These methods, although limited by their reliance on cultivable organisms, were instrumental in studying microbial processes before the emergence of genomic techniques.

1.4. Improved understanding based on genomic and metagenomic studies

Molecular tools to study the bacterial diversity from various niches including marine habitats were established in the late 1990s (DeLong, 1997; Hugenholtz et al. 1998). The first reports of bacterial diversity, based on Denaturing Gradient Gel Electrophoresis (DGGE) analysis from the NIO region were reported in the same year by the international community (Riemann et al. 1999) from the central AS and along the Oman coast (Figure 1.1 and Appendix 1.2). However, detailed studies using molecular tools were carried out from 2009 onwards which targeted the microbial community from the AS oxygen minimum zone (OMZ) (Jayakumar et al. 2009). They investigated the bacterial diversity and community structure in these regions by analysing the 16S rRNA gene based on Restriction Fragment Length Polymorphism (RFLP). Along with it, they also characterized the qPCR-based functional markers to understand the denitrifying and anammox bacterial community (Pitcher et al. 2011; Jay et al. 2013). Following this, the use of 16S rRNA gene sequence for the identification of cultivable bacterial groups was reported (Figure 1.2). Studies have also described the taxonomic and functional diversity of culturable bacteria in the sediments underlying the OMZ in the AS based on the amplified rDNA restriction analysis (ARDRA) along with 16SrRNA sequencing for genotypic characterization (Divya et al. 2010).

The cultivable bacterial diversity was studied for analysis of the phylogenetic diversity from the OMZ (Figures 1.2 and 1.3). Most of the studies also characterized their ecological importance based on the analysis of their functional characteristics to study their role in the nitrogen and sulfur cycle (Amberkar et al. 2018; Mulla et al. 2018; Fernandes et al. 2019;

Menezes et al. 2020; Bhattacharya et al. 2020). Studies were also carried out in the open ocean region to study the cultivable diversity and bacterial markers such as fatty acid methyl esters (FAME) were also used for taxonomic characterization and comparative analysis (Isaccs et al. 2021). Khandeparker et al. (2011) investigated the abundance and phylogenetic diversity of culturable bacteria in coastal, estuarine habitats and their ability to produce carbohydrate-degrading enzymes was reported (Figure 1.4). The study showed that 65 % of isolated bacteria were able to hydrolyse complex marine carbohydrates. Many coastal studies were carried out using these molecular tools and they also investigated the secondary metabolite production of the cultivable bacteria along with their taxonomic characterisation (Rajyasabhapathi et al. 2013; Anas et al. 2016; Pawar et al. 2018). These studies on the cultivable diversity of bacteria using 16S rRNA sequence analysis grouped the isolates into the major five bacterial phyla Proteobacteria, Firmicutes, Bacteroidetes, Actinobacteria and Chloroflexi which increased to about 15 recognised and 7 candidate bacterial phyla based on metagenomic analysis using different molecular tools like DGGE, RFLP, cloning and sequencing technique (Appendix 1.2 and Figure 1.3). Metagenomic, 16S rRNA clone library analysis, characterized the bacterial community structure and diversity in the OMZ sediments of the eastern AS which showed the presence of unidentified candidate bacteria phyla, Microgenomates, Omnitrophica and Aminicenantes for the first time from this region (Figure 1.3). Proteobacteria were shown to be the most prevalent taxa, followed by Planctomycetes and Chloroflexi; other bacterial phyla such as Spirochetes, Firmicutes, Acidobacteria, Verrucomicrobia and Thermodesulfobacteriota were also reported. The bacterial dynamics determined based on the variations in the OMZ intensity between the slope and offshore stations showed that anaerobic bacteria are the dominant community (Gonsalves et al. 2011). The presence of the fermenters, nitrate-reducing, ammonia-oxidizing, nitrite-oxidizing and sulfate-reducing bacterial populations in the OMZ in these studies suggests that these bacterial groups have an impact on the OMZ and lead to the formation of a more intense and thicker OMZ in the eastern AS than in the western AS based on biochemical assay (Appendix 1.2). DGGE-based investigations to study the influence of seasonal variations in physicochemical features and phylogenetic makeup of bacterial communities from the OMZ region of AS and BoB have reported, seasonal fluctuations in the bacterial groups belonging to Alphaproteobacteria, Gammaproteobacteria, Cyanobacteria, Bacteroidetes, Chlamydiae, Chloroflexi, Planctomycetes and candidate phyla such as Latescibacteria (Jain et al. 2014; Divya et al. 2017).


(a)
Diversity based on biochemical test
DGGE, RFLP, cloning based metagenomic diversity
Culturable diversity based on 16S rRNA based
Diversity based on NGS
Defined bacterial phyla
Elusimicrobiota
Kiritimatiellota
Armatimonadota
Calditrichota
Fibrobacterota
Aquificota
Thermotogae
Chlorobiota
Deferribacterota
Lentisphaerota
Calditrichota
Rhodothermaeota
Mycoplasmatota
Bdellovibrionota
Balneolota
Gemmatimonadota
Spirochaetota
Myxococcota
Verrucomicrobia
Nitrospirae
Nitrospinae
Fusobacteria
Deinococcus-Thermus
Chlamydiae
Calditrichaeota
Atribacteria
Armatimonadetes
Acidobacteria
Campylobacterota
Thermodesulfobacteriota
Planctomycetes
Chloroflexi
Cynobacteria
Actinobacteria
Bacteroidetese
Firmicutes
Proteobacteria
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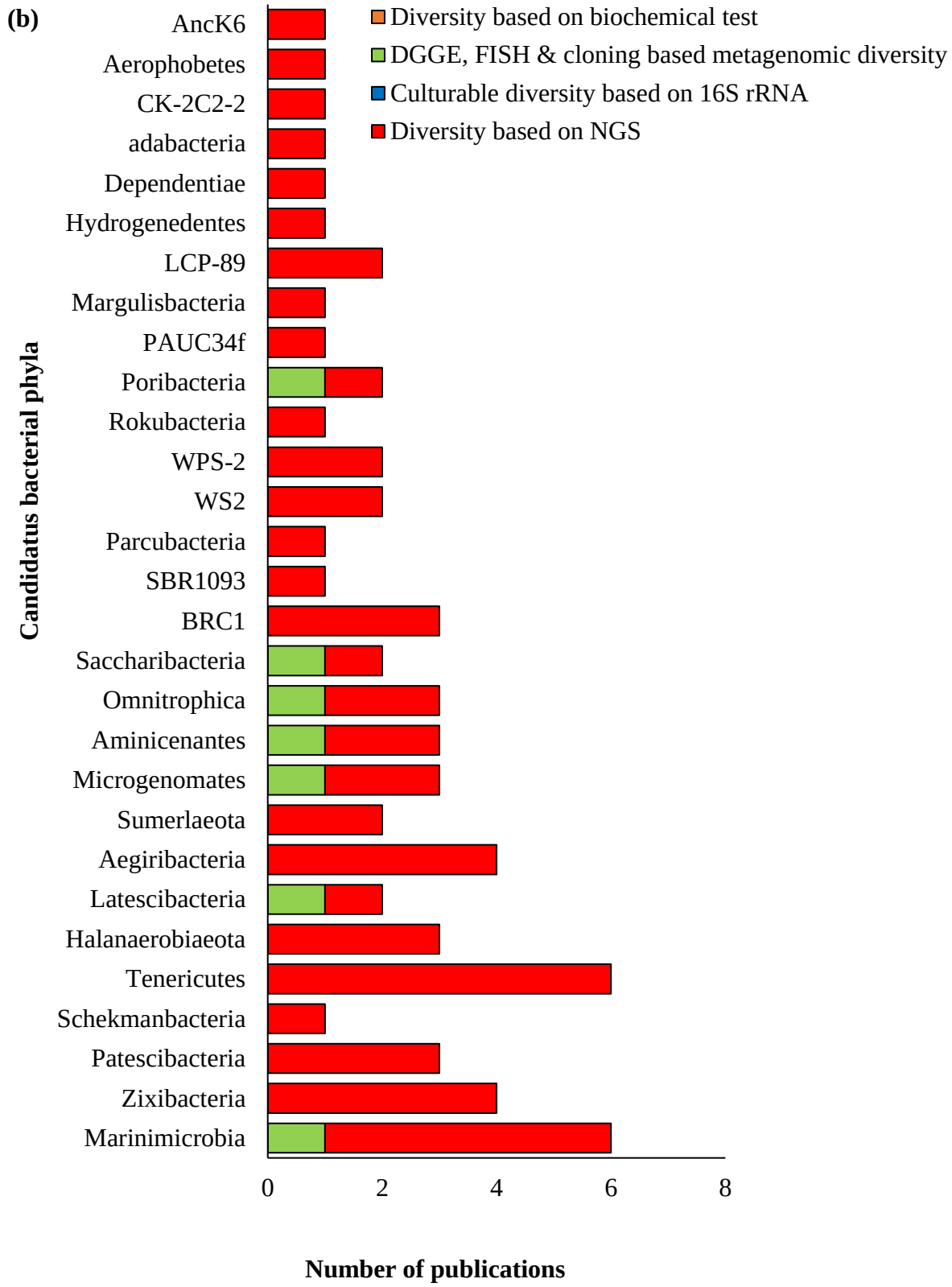


Figure 1.3: The number of publications reporting bacterial diversity as (a) defined phyla and (b) candidatus phyla using different techniques in the Northern Indian Ocean.

The AS-OMZ characterized based on 16S rRNA cloning and sequencing studies on the depth-wise profiles of the bacterial community, showed that Proteobacteria and Cyanobacteria contributed to the maximum bacterial diversity and taxa such as Alteromonodales, Sphinogomonadales, Rhodobacterales, Burkholderales and Acidimicrobiales. They were shown to contribute to the microaerophilic metabolism and ability to degrade and assimilate particulate organic matter (Bandekar et al. 2016; 2017). Further previously undescribed diversity both in surface and OMZ layers was also reported with bacterial communities that were shown to be involved in major biogeochemical processes such as denitrification, anammox and sulfur oxidation within the OMZ (Gomes et al. 2015; Gomes et al. 2017). Studies on the functional diversity from this region were also carried out to quantify the denitrification and anammox activity using markers genes such as 16Sr RNA, nitrate (*narG*), nitrite (*nirS*), nitrous oxide (*nosZ*) reductase and anammox-specific *hzs* and *hzo* genes (Wang et al. 2017; Qian et al. 2018; Bandekar et al. 2018; Lincy & Manohar 2020; Lincy & Manohar 2021). These studies have elucidated the diversity and abundance of both denitrifying and anammox bacteria which indicates that both the denitrification and anammox processes are occurring simultaneously. Functional profiles of bacterial communities showed that these reductases were present in a diverse range of taxonomic groups, mostly well-known denitrifiers and many novel assemblages of candidate bacterial groups (Figure 1.3). The findings show that the bacterial populations are varied, with various communities predominating at different depths in the OMZ with greater diversity in the surface layers from the AS and BoB regions (Appendix 1.2).

Bacterial diversity from the coastal regions along the WCI from Maharashtra, Karnataka, Goa and Kerala including the mud bank areas off Cochin were also studied using DGGE analysis. These studies carried out along the southwest coast of India have shown that upwelling influences the bacterial community structure (Singh and Ramaiah 2011; Veettil et al. 2015; Anas et al. 2018; Anas et al. 2021). Some studies used these techniques to investigate the microbial dynamics in the open ocean regions of the NIO to understand the seasonal changes (Riemann et al. 1999; Jing et al. 2016). These studies based on DGGE largely help in the identification of the dominant bacterial groups which was a major drawback. Hence 16S rRNA clone library and sequencing-based studies were much preferred, which brought to light the phylogenetic diversity of the total bacterial communities at a higher taxa level (Khandeparkar et al. 2014; Nair et al. 2016). Studies in the upwelling regions along the WCI have reported a significant seasonal variation and a positive correlation between primary and bacterial productivity. Studies with the advent of genomic and metagenomic tools paved the

way to understand the bacterial dynamics in the NIO region in unparalleled detail. Before the genomic era, the understanding of bacterial communities in this region was largely based on culture-dependent biochemical methods, which could only account for a fraction of the actual bacterial diversity (Figure 1.3). With the introduction of genomic techniques, researchers were able to identify individual bacterial strains, leading to a better understanding of their physiology and ecological roles.

1.5. Present understanding based on metagenomic studies using Next Generation Sequencing (NGS) tools

In recent years, the NGS approaches have exponentially increased the understanding of the bacterial community and introduced an increased number of Candidatus taxa that are still known only based on their genomic makeup. Since then, there has been an exponential increase in reports on the identification of bacterial phyla (Figure 1.3). The application of these tools has brought to light a greater understanding of the microbial community and their contribution to the marine biogeochemical process. These NGS-based studies were carried out from the coastal habitats such as the tropical estuaries of Mandovi and Zuari situated in Goa, port areas, coastal regions along the WCI from Gujarat to Kerala and from the east coast of India (Gomes et al. 2017; Parvati et al. 2019; Nathan et al. 2020; Parvati et al. 2020; Mote et al. 2021). The impact of the estuarine and coastal activities on the bacterial community structure was also studied using these latest high throughput sequencing techniques (Khandeparker et al. 2017; Sachithanandam et al. 2020; Jasna et al. 2020; Kuchi et al. 2021). The association of the microbial communities investigated using the NGS method showed the presence of archaeal and bacterial groups. The distinct microbial communities identified, suggest differing microbial activity contributes to the sediment nutrient cycling. Studies from the mangrove habitats and characterizing the anthropogenic factors show that they have a significant role in community composition and ecosystem functioning. The bacterial diversity studied from coastal habitats and associated with pollutants using metagenomics tools have also identified the bacterial communities that can be used for the bioremediation of harmful pollutants such as hydrocarbons (Fernandes et al. 2019).

The NGS-based studies on 16S rRNA sequence are used to study only the bacterial diversity and some studies have used 16S rRNA primers to study the specific functional groups like the annamox community (Villanueva et al. 2014; Lincy & Manohar 2021). These NGS-

based studies were also carried out in the open ocean region, however as most of these studies provide information only on bacterial diversity (Sujith et al. 2017; Gao et al. 2021), studies are often supplemented with functional diversity analysis using shotgun metagenomic sequencing analysis (Appendix 1.1 and Figure 1.2). In 2017, Verma et al. explored the bacterial communities in the deep-sea sediments of the Bay of Bengal and the volcanic Barren Island in the Andaman Sea. The research revealed that Actinobacteria emerged as the dominant phylum across all stations. An intriguing finding was the influence of sediment texture on the differences observed in bacterial composition between the two locations. The study also delved into the genetic variations within the sediment bacteria, which might play a crucial role in enabling their adaptation to the extreme and extremophilic conditions of the deep-sea environments. Recent studies for understanding bacterial communities and metabolic potentials from the open ocean regions contribute significantly to our understanding of bioprospecting efforts and the intricate geochemical cycling processes occurring in the deep sea (Wu et al. 2019; Ding et al. 2021). Another study by Zhu et al. (2022) studied the EIO, with a particular focus on microbial communities in deep-sea sediments. The findings reported the dominance of bacterial phyla such as Proteobacteria, Firmicutes and Chloroflexi, alongside abundant archaeal (Thaumarchaeota and Euryarchaeota) and fungal (Ascomycota and Basidiomycota) taxa. Interestingly, a significant portion of the archaeal and fungal communities remained unclassified in existing databases, shedding light on the vast unexplored territory of microbial life in this inaccessible marine ecosystem. Moreover, the study revealed that bacterial communities exhibited higher dynamics compared to archaeal and fungal communities, suggesting unique ecological roles for each group in this less-explored marine ecosystem. In the same year, Marimuthu et al. (2022) studied a deep-sea region of the BoB, employing the Nanopore sequencing technique. They investigated the microbial diversity and metabolic potentials of deep-sea sediment samples. Their findings showcased the dominance of Proteobacteria in sediment samples, with depth playing a crucial role in determining taxonomic diversity. Notably, these bacterial communities demonstrated potential for carbon dioxide fixation, nitrogen, and sulfur metabolism, as well as bioremediation capabilities for various pollutants (Figure 1.4). The genomic tools are used not only to understand the diversity but also to elucidate their functional characteristics based on transcriptomic and proteomic analysis (Bhattacharya et al. 2020; Bhattacharya et al. 2021; Xing et al. 2021). However, reports of these latest approaches are very few from the NIO region.

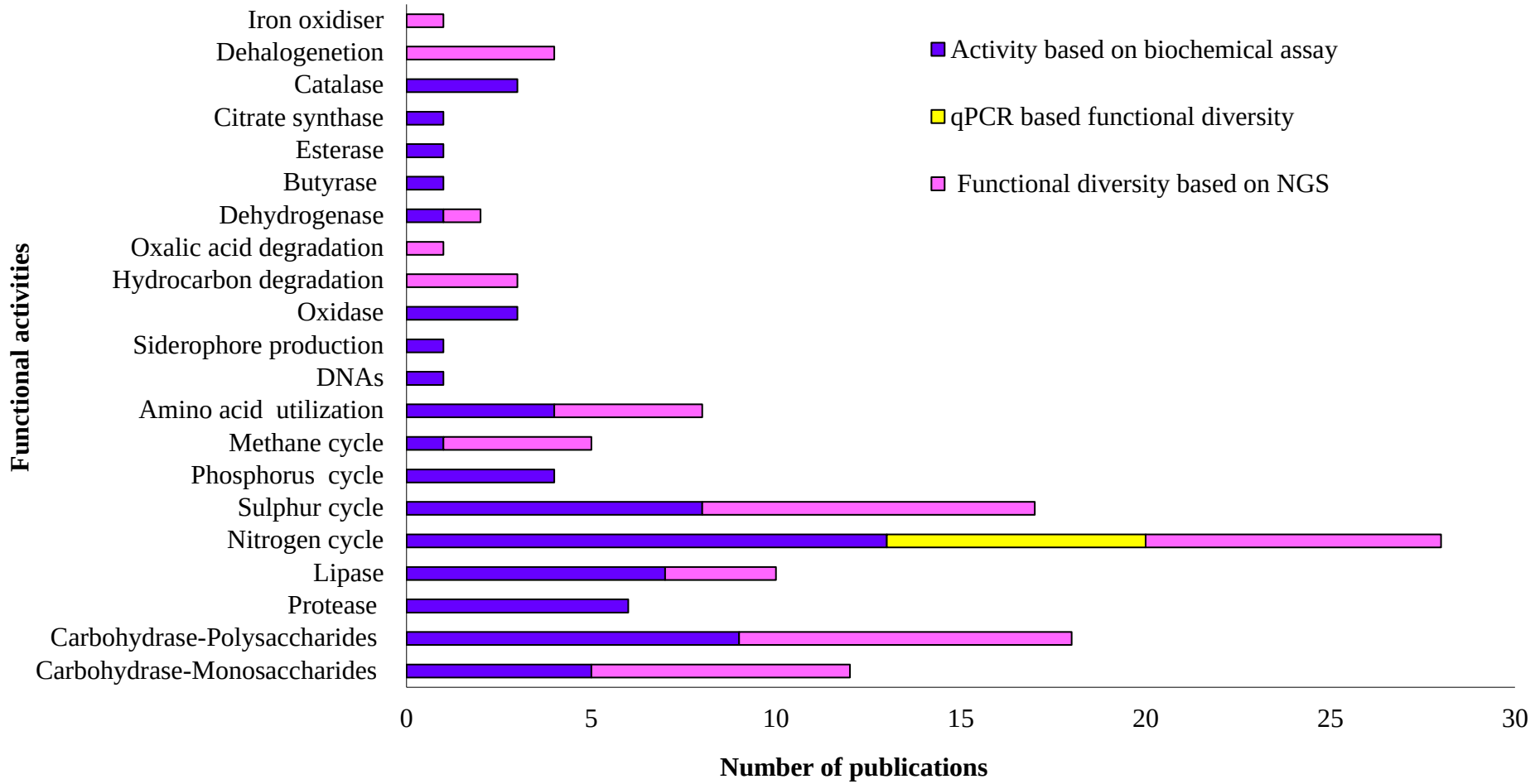


Figure 1.4: Number of publications reporting functional activities of bacterial communities using different techniques in the Northern Indian Ocean.

In conclusion, NIO is one of the most characteristic regions with diverse oceanographic features. Understanding of the microbial community structure in these unique habitats has rapidly increased in the last decade, especially with the advent of molecular tools and the application of NGS technologies. Early studies on marine microbial ecology in the NIO regions date back to the 1950s-1990s. These pioneering studies utilized culture techniques, qualitative plate assays and biochemical tests to identify and understand bacterial activity. Autochthonous bacteria were discovered in these marine regions, with specific studies focusing on their physiological activities and involvement in marine nitrogen and sulphur cycles. Techniques before the genomic era relied on quantifying colony-forming units (CFUs) and microscopic observations. These earlier methods, although limited by their reliance on cultivable organisms, were essential in studying microbial processes. Molecular tools, introduced in the late 1990s, significantly enhanced the understanding of bacterial diversity. Techniques such as DGGE, RFLP, ARDRA and cloning-dependent 16S rRNA gene sequencing were employed. These molecular tools revealed the presence of many more bacterial phyla and provided insights into their functional characteristics, especially concerning nitrogen and sulphur cycling (Figure 1.4). Metagenomic studies further expanded the understanding of bacterial community structures, especially in the oxygen minimum zones (OMZ) and deep-sea sediments. These tools have identified bacterial taxa in-depth, revealing the existence of numerous Candidatus taxa known only by their genomic makeup. Also, studies to characterize the functional diversity of bacterial communities have been consistently used to understand the metabolic processes and functional characteristics in these habitats. Despite advancements, a large part of the bacterial diversity from the NIO remains unexplored, with many unanswered questions related to microbial carbon pumps, especially from the biogeochemically active upwelling regions. This can be addressed by mapping the abundance, composition and distribution of the microbial parameters along with the transcriptome studies. Studies based on their metabolomics need to be carried out to improve our understanding of the microbial processes in the NIO, which is a dynamic, oceanographic habitat. Hence in this study, the relationship between the physicochemical characteristics and primary productivity on the bacterial abundance, cultivable diversity, its activity and metagenomics-based phylogenetic and functional profiles along the WCI during the non-monsoon and monsoon seasons were studied based on the following objectives:

1. To investigate the spatial variations in bacterial abundance and activity along the west coast of India.

2. To map the bacterial community structure along the west coast of India.
3. To determine the functional diversity of the bacterial groups along the west coast of India.

1.6. Study area and sampling strategy

For this research work, sampling was carried out on oceanographic cruises during the non-monsoon season (Cruise SSK113-January 2018 and SSK125-February 2019) and monsoon seasons (Cruise SSD053-July 2018 and SSK131-September 2019). Samples were collected from both these seasons along the WCI at three stations, off Goa, Mangalore and Trivandrum, which lie between 8 and 16˚N and 72 to 76˚E (Figure 1.5). The three locations at each station are (i) in the coastal, continental shelf region at ~ 30 m water depth, (ii) in the slope along the ~300 m contour and (iii) in the off-shore location at ~ 600 m water depth. The sampling stations off Goa from the continental shelf, slope and off-shore region are designated as G30, G300 and G600 respectively, similarly, sampling stations off Mangalore are designated as M30, M300 and M600 and off Trivandrum station as T30, T300 and T600 (Figure 1.5).

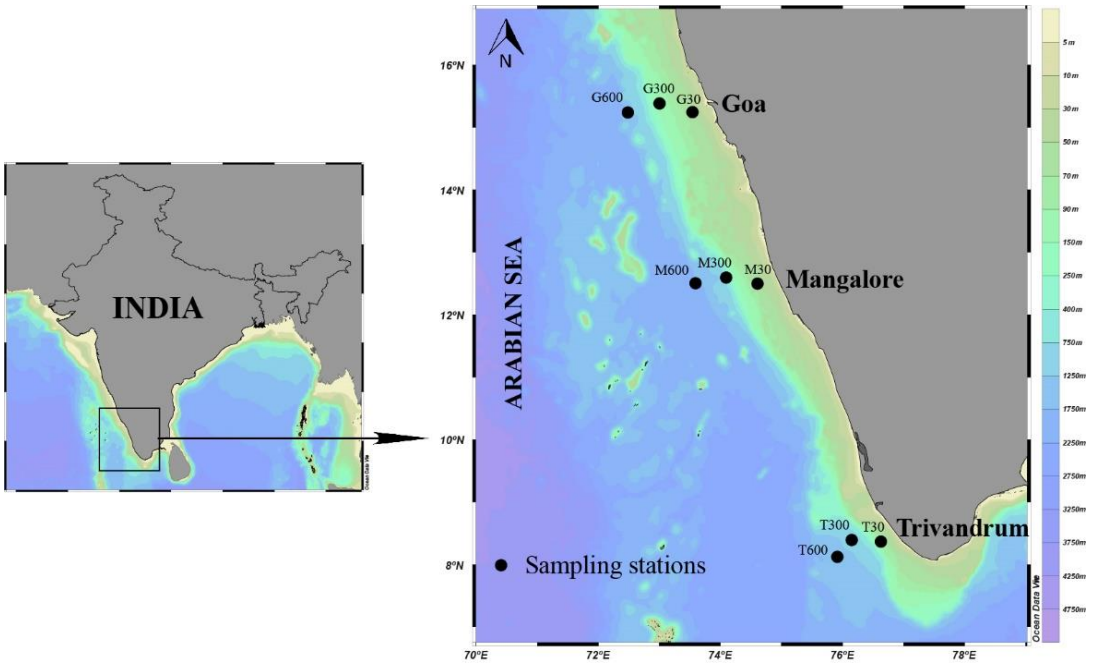


Figure 1.5: Study area and sampling stations along the west coast of India at Goa, Mangalore and Trivandrum.

Chapter 2.

Bacterial dynamics and community structure based on culturable diversity along the west coast of India.

Abstract

The West Coast of India (WCI) is influenced largely by semi-annual changes in the wind pattern and monsoonal currents. This is reported to cause upwelling which increases the phytoplankton growth in this region during the summer monsoon and it is recorded to be one of the world's highest primary productivity. Increase in the phytoplankton productivity is known to influence the microbial activity which is one of the key biogeochemical processes of the marine ecosystem as it plays an important role in the biotransformation of organic matter. However, the changes in the bacterial community structure and their heterotrophic enzyme activity with increasing primary production are not clearly understood from this region. Hence in this study, the bacterial dynamics along the WCI at Goa, Mangalore and Trivandrum, were investigated from the continental shelf, slope and off-shore region during the non-monsoon and monsoon season. Seasonal changes in the bacterial parameters such as abundance, viable counts and radioactivity based in-situ primary and bacterial productivity were estimated, along with the physicochemical characteristics. Statistical analysis shows a significant ($p < 0.05$) correlation between primary and bacterial productivity. Significant seasonal variations could also be established for the physicochemical parameters such as dissolved oxygen, nitrate, nitrite and phosphate, fluorescence values representing the chlorophyll concentration, bacterial abundance, viable counts and productivity. Taxonomic diversity studies on the cultivable bacterial morphotypes have shown that Proteobacteria, Firmicutes and Actinobacteria were the major phyla found during both the non-monsoon and monsoon season and Bacteroidetes, was represented only in the non-monsoon season. The bacterial community structure at phylum, genus and operational taxonomic unit levels also changed with higher diversity in the monsoon season. However, the hydrolytic enzyme activity of the bacterial morphotypes was comparatively lower during the monsoon season probably due to the active primary production. Though this study has improved our understanding of the monsoonal influence on the bacterial dynamics along the WCI, further studies based on metagenomic and transcriptome analysis are required to get a better understanding of the role played by the bacterial community from this region.

2.1. Introduction

Nearly one-half of the global photosynthesis occurs in the ocean due to the carbon fixation by the phytoplankton community and this leads to the production of huge amounts of organic matter (OM) (Karl et al. 1998; Mühlenbruch et al. 2018). Various physical, chemical and biological processes such as viral lysis, microbial activities and protistan grazing of phytoplankton cells; modify, decompose and redistribute the OM within diverse time frames ranging from a few hours to millennia (Robinson and Ramaiah, 2011; Stoecker et al. 2017). The carbon fixed by the phytoplankton can be categorized into two distinct oceanic carbon pools: particulate organic matter and dissolved organic matter (Jiao et al. 2014). The phytoplankton biomass, zooplankton, nektons and their degraded cells, and the microbial communities constitute the particulate organic matter, estimated to be approximately 30×10^{15} g C (Hansell and Carlson, 2001). Most of it is immediately respired back as CO_2 by diverse biota and a small fraction is actively transported to the seafloor by higher organisms through the 'biological pump' and the 'microbial carbon pump' by the heterotrophic microbial communities (Ducklow et al. 2001; Jiao et al. 2011; Enke et al. 2018). The heterotrophic activity of the microbes, especially the enzymatic breakdown of OM, is the major pathway that produces dissolved OM and drives the oceanic carbon cycle (Fang et al. 2015; Moran et al. 2022).

Understanding the importance of microbial activity has increased significantly after the role of microbial transformation of particulate OM to labile and refractory forms in the oceanic habitats was characterized (Jiao et al. 2014; Lian et al. 2021). Though most of the OM would be readily respired, a small fraction is known to be converted into refractory organic matter which has a turnover time of millennia (Hansell and Carlson, 2013; Siegel et al. 2021). Recent studies indicate that specific bacterioplankton lineages are responsible for remineralizing the OM (Landa et al. 2016). It is also evident that many of the bacterial communities are structured with unique metabolic capabilities that are important in determining the fate of the exported OM and its lability (Robinson and Ramaiah, 2011). Heterotrophic bacteria utilize the OM through the hydrolytic activity of the extracellular enzymes and the ability of the bacterial groups to utilize the OM varies considerably. Reports have shown that Alpha, Beta and Gamma Proteobacteria and Bacteroidetes have the metabolic genes necessary to produce and secrete a variety of extracellular hydrolytic enzymes (Zimmerman et al. 2013; Teeling et al. 2016; Mahmoudi

et al. 2019). This implies that the ability to produce these enzymes is a functionally distinct rather than a functionally redundant feature and that changes in the taxonomic composition will affect the hydrolytic activity (Mahmoudi et al. 2020). The ability of microbial extracellular hydrolytic enzymes to access and break down complex organic substances as a source of carbon and energy is the central metabolic process of the marine habitat as it can, in turn, control the production of the primary producers (Arnosti, 2011; Amin et al. 2015; Bolch et al. 2017).

The West Coast of India (WCI) extends from 7°N to 25°N bordering the eastern Arabian Sea covering a total length of approximately 3010 km coastline of the Indian sub-continent. This region is influenced largely by semi-annual changes in the climate, wind pattern and monsoonal currents. The Arabian Sea and its coastal regions are known to be one of the highly productive regions of the World Ocean (Sarma et al. 2003; Dalabehara and Sarma, 2021). Studies have shown that the average primary productivity (PP) along the WCI is calculated to range from about 210 mg C m^{-2} d^{-1} and it increases close to 950 mg C m^{-2} d^{-1} during the summer or southwest monsoon season (Gauns et al. 2005). During this season, the upwelling of nutrient-rich subsurface waters leads to an increase in the phytoplankton biomass and this is known to control the production of OM in these regions (Ram et al. 2003; Jacob et al. 2009; Shanthikrishnan et al. 2021). Bacterial abundance and activities are also shown to have a similar changing pattern with low abundance and reduced bacterial productivity, during the winter (December-February) which increases during the summer monsoon (June-September) (Ramaiah et al. 2009). However, the bacterial activity of the communities that also proportionately increases during the monsoon season is not elucidated thoroughly from this region. Hence this study aimed to understand the relationship between the physicochemical characteristics and PP on the bacterial abundance, cultivable diversity and its activity during the non-monsoon and monsoon season along the WCI representing the continental shelf, slope and off-shore regions.

2.2. Methodology

2.2.1. Study area, sample collection and measurement of physicochemical parameters

Sampling was carried out during the non-monsoon season onboard RV Sindhu Sankalp (Cruise # SSK-113) in January 2018 and the productive, summer monsoon season onboard RV Sindhu Sadhana (Cruise # SSD-053) in July 2018. In total 42 samples were collected from each seasons along the WCI at three stations, off Goa, Mangalore and Trivandrum, which lie between 8°N to 16°N and 72°E to 76°E (Figure 1.5). The three locations at each station were (i) in the coastal, continental shelf region at ~ 30 m water depth, (ii) in the slope along the ~ 300 m contour and (iii) in the off-shore location at ~ 600 m water depth. The sampling stations off Goa from the continental shelf, slope and off-shore region are designated as G30, G300 and G600 respectively, similarly, sampling stations off Mangalore are designated as M30, M300 and M600 and off Trivandrum station as T30, T300 and T600 (Figure 1.5).

The conductivity-temperature-depth (CTD) underwater profiler (Sea-Bird, USA, model: SBE-911 plus, sensor accuracy of ± 0.0003 S/m, ± 0.001°C and ± 0.015 % for conductivity, temperature and depth respectively) was used to measure the water column physicochemical properties temperature, salinity, dissolved oxygen (DO) and fluorescence representing the chlorophyll concentration using the respective electronic probes at all the nine stations in the January and July 2018 sampling. Water samples from the shelf stations G30, M30 and T30 were collected from the surface, chlorophyll maxima depth (C-Max), based on the CTD profile; and bottom waters, at about 3 m above the seafloor. Similarly, at the slope stations, G300, M300 and T300 samples were collected at the surface, C-Max, 100 m, 200 m and bottom water depths (~300 m). In the off-shore stations, along the 600 m contour, samples were collected from the surface, C-Max, 100, 200, 300, 400 m and bottom depth (~ 600 m). The C-Max depth varied for each station and it was determined based on the values obtained from the fluorescence sensor representing the chlorophyll concentration during the downward haul of the CTD profiler and the water samples were collected at discrete depths during the upward haul, using pre-cleaned Niskin samplers connected to the underwater CTD unit (Sea-Bird, USA). The water samples collected were immediately sub-sampled and stored appropriately for further analysis. Nutrients such as nitrate, nitrite, phosphate and silicate levels in the water samples were analyzed using an automated continuous flow analyzer (SKALAR 5000, Netherlands).

2.2.2. Primary productivity measurements

The PP measurements in the water samples from the coastal, shelf and off-shore stations in both seasons were measured using the ^{14}C-labelled inorganic C, $NaH^{14}CO_3$ as per JGOFS protocols (UNESCO 1994). For this water samples were collected from the surface, C-Max, and 30 m depth at the shelf stations and from the surface, C-Max, 100 m and 200 m of the off-shore stations in three light and one dark, 300 ml polycarbonate bottles (Nalgene, Germany). One ampoule of $NaH^{14}CO_3$ (Board of Radiation and Isotope Technology, Mumbai; Specific activity 185 kBq) was added to each bottle and incubated *in-situ* using a mooring system for 12 hours from sunrise to sunset. At the end of the incubation period, water samples were filtered through GF/F 47 mm diameter, 0.7 µm pore size filters (Whatman, USA) and stored in the dark in scintillation vials. For analysis, filters were dissolved overnight in concentrated HCl, followed by incubation in a liquid scintillation cocktail (Sisco Research Laboratory, Mumbai). The radioactivity was measured in the scintillation counter (PerkinElmer Wallac 1409 DSA, USA) and expressed as mg C m^{-3} d^{-1}.

2.2.3. Estimation of bacterial abundance, viable counts, productivity, respiration and growth efficiency

Bacterial abundance (BA) was determined following the modified method of Porter and Feig (1980). In brief, subsamples of ~20 ml water, from each depth were preserved with buffered formalin (2 % final concentration) immediately after sampling and stored at room temperature in the dark for further analysis. One to five ml aliquots of the preserved samples in triplicates were incubated with 4'6'-diamidino-2-phenylindole (DAPI) at the final concertation of 1 µg ml^{-1} and filtered onto black, 0.22 µm pore size polycarbonate membrane filters (Millipore, U.S.A). Counts were made from 10 - 20 microscopic fields with an epifluorescence microscope (Olympus BX-51, Japan). The final bacterial counts were expressed as numbers L^{-1} of the water sample.

Total viable counts (TVC) of bacteria were enumerated from water samples following the spread plate technique on Zobell Marine Agar (ZMA) microbiological plates. For this, seawater samples of 100 µL aliquots were plated aseptically on ZMA plates in triplicates and incubated aseptically for 24 - 48 hours at 28°C along with appropriate controls to minimize contamination. Bacterial colony counts were determined

at the end of the incubation and expressed as colony forming units (CFUs). Bacterial colonies grown were characterized based on their colony morphology and grouped into morphotypes. One representative colony for each morphotype was streak-purified on ZMA plates for further analysis.

The bacterial production (BP) rate was determined based on the ^{3}H-thymidine incorporation rates in the water samples following the method described in JGOFS Protocols (UNESCO, 1994). BP was estimated at shelf, slope and off-shore stations from all the depths during both seasons. For this, 20 ml of water samples in triplicates were incubated in the dark with ^{3}H-thymidine (specific activity 18 Ci mmole^{-1}, Bhabha Atomic Research Centre, Mumbai) for 2 hours at ambient temperature. The incubation was terminated by the addition of filtered formalin and the samples were filtered through 0.22 µm cellulose acetate, 25 mm diameter filters (Millipore India, Bangalore). The filters were rinsed with cold trichloroacetic acid (10 % w/v) followed by ethanol (70 % v/v) and stored in scintillation vials for further analysis. To measure the radioactivity, the filter papers were incubated overnight in ethyl acetate, followed by the addition of a liquid scintillation cocktail (Sisco Research Laboratory, Mumbai) in the recommended volume; and measured in a scintillation counter (PerkinElmer Wallac 1409 DSA, USA) and expressed as mg C m^{-3} d^{-1}.

Community respiration (TR) rates were estimated from the decrease in DO concentration in water samples incubated in pre-cleaned 125 ml of BOD bottles for 24 hours. For this water samples were collected in BOD bottles in two sets as triplicates, the DO in the first set was immediately fixed with Winkler reagents to estimate the initial concentration. The DO in the second set was quantified at the end of the incubation period in the dark for 24 hours and the decrease in DO concentration was estimated. DO was determined using the Dosimat-865 automated titrator following the Winkler titration method modified by Carpenter (1965). Bacterial respiration (BR) was calculated from the ratio of BR to TR of 0.45. Bacterial growth efficiency (BGE) was estimated as the ratio of net bacterial production (BP) to gross bacterial production (BP + BR) (Kim et al. 2017).

2.2.4. Molecular identification of bacterial morphotypes and phylogenetic analysis

Bacterial genomic DNA was extracted from pure cultures of all the bacterial morphotypes grown for 24 hours in Zobell Marine Broth (ZMB) at 28℃ using a high salt concentration buffer followed by chloroform-phenol extraction and precipitation with isopropanol. The DNA extracted was subjected to PCR amplification of the 16S rRNA gene using the universal primers 27F (5'AGAGTTTGATCMTGGCTCAG3') and 1492R (5'TACGGYTACCTTGTTACGACTT-3'). The 25 µL PCR reaction mixture included genomic DNA, 1X PCR buffer, 1.5 mM $MgCl_2$, 200 µM concentration of each deoxy nucleotide triphosphate, 0.5 µM concentration of each oligonucleotide primer and the final volume was adjusted to 25 µL with PCR-grade water. The amplification was performed using initial denaturation at 94°C for 5 min, followed by 30 cycles of 94°C for 50 sec, 54°C for 45 sec and 72°C for 1 min, with a final extension at 72°C for 7 min with 1U Taq DNA polymerase (Sigma Aldrich) in an Eppendorf vapour protect master cycler. The confirmation of 16S rRNA amplification was done by agarose gel electrophoresis in 1 % agarose gel prepared using 1X TAE buffer and results were visualized in the SYGENE Gel documentation system. The PCR product was purified using the HiPurA™ PCR purification kit (HiMedia, India). The PCR products were sequenced in Applied Biosystems (ABI) 3730 DNA Stretch Sequencer, with the XL Upgrade and the ABI Prism BigDye Terminator version 3.1 Cycle Sequencing Ready Reaction Kit. They were processed to remove primers and were also screened for quality using Chromas version 2.6.6. These sequences were analyzed in the NCBI database using the bioinformatics tool BLAST (Basic local alignment search tool) to confirm their taxonomic and phylogenetic similarity. GenBank accession numbers of sequences deposited of the bacterial morphotypes are MW785066-MW785148 and MW795735-MW795873.

The 16S rRNA sequences of the bacterial morphotypes obtained from January and July 2018 sampling were aligned against the SILVA reference alignment in MOTHUR version 1.46.1 to obtain pairwise alignment. Based on the distance matrix the sequences were assigned into operational taxonomic units (OTUs) in MOTHUR using the cluster command and the average neighbour rule at a cut-off of 0.1 sequence similarity. A total of 222 sequences obtained from both the samplings were assigned to 111 OTUs. A representative sequence from each OTU was identified using the get.oturep command (Schloss et al. 2009) and a phylogenetic tree of representative OTUs was built in MEGA-X using the maximum likelihood method and Kimura 2-parameter substitution model, with bootstrap values calculated using the neighbour-joining method with a resampling size of n = 1000.

2.2.5. Estimation of hydrolytic enzyme activity

The different morphotypes isolated were analyzed for their carbohydrase, protease and lipase activity based on qualitative substrate utilization assay following standard protocols. The utilization of monosaccharides by bacterial morphotypes was studied by inoculating each bacterial morphotype in media containing pH indicator (Bromocresol purple) and supplemented with different monosaccharides such as dextrose, arabinose, rhamnose and galactose individually (Reiner, 2016). The monosaccharide utilization ability was estimated based on the colour change from purple to yellow due to a change in pH due to substrate utilization after 48 hours of incubation at 28°C. Extracellular enzyme activity for hydrolysis of polysaccharides such as starch (1 %), carboxyl methylcellulose (CMC 1 %), agar (2 %), alginate (0.5 %) and xylan (0.5 %) was also carried out by using each of the substrates as a carbon source in basal salt agar plates for estimating the carbohydrase activity (Meddeb-Mouelhi et al. 2014; Sawant et al. 2015; Khalifa and Kldayel, 2019). Protease and lipase activity were tested on basal salt agar plates supplemented with Skim Milk (SM) and Tween 80, respectively (Fenice et al. 2007). Each of the bacterial morphotypes was grown on the substrate plates and incubated at 28°C for 48 hours and observed for their zones of hydrolysis around bacterial colonies. The hydrolytic activity of amylase, agarose and alginate lyase was determined by staining with Gram's iodine for 10 min at the end of the incubation period. Cellulase and xylanase activity was determined by staining with 0.5 % Congo red for 15 min followed by destaining with 1N NaCl for 10 min. The protease and lipase activity were estimated by measuring the zone of hydrolysis around bacterial colonies. The bacterial morphotypes showing hydrolytic enzyme activity (%) were categorized into no activity or minimum activity for monosaccharide substrates. For the polysaccharide, protease and lipase activity (%) the morphotypes could be grouped based on the zone of hydrolysis exhibited into isolates with very good activity (21-30 mm), good activity (11-20 mm), minimum activity (1-10 mm) and no activity group. Hydrolytic enzyme activity (%) on different substrates by the bacterial morphotypes within each phylum was also estimated.

2.2.6. Statistical analysis

The significance of distribution between physicochemical and biological characteristics in the two seasons was determined using a single factor ANOVA. Pearson's correlation analysis was performed to interpret relationships within physicochemical and biological parameters between two seasons at the 5% ($p < 0.05$) level of significance. Statistical analyses were performed using SPSS 21.

2.3. Results

2.3.1. Physicochemical characteristics, primary productivity and bacterial dynamics

Seasonal changes were observed in the physicochemical parameters determined from the sampling locations along the WCI. The average sea surface temperature (SST) was 28 ± 1°C in all the stations during the non-monsoon season in January 2018 sampling; and during the July 2018 monsoon season, the SST reduced to 27 ± 1°C in almost all the stations, except at Trivandrum shelf station, where the lowest temperature of 22°C was recorded. In all the shelf (30 m) stations the temperature was almost uniform throughout the water column in non-monsoon season. However, during the monsoon season, there was a reduction in temperature by 2 to 4°C from the surface to the bottom waters in the shelf stations. In the 300 m and 600 m stations, the thermocline was up to ~ 100 m during the non-monsoon season and in the monsoon season the thermocline depth was reduced to ~ 50 m at Goa and Mangalore and in the southern station at Trivandrum, it was shallow at ~ 25 m depth (Figure 2.1). The sea surface salinity during the non-monsoon season was 34 ± 2 PSU and the values were uniform throughout the water column in the shelf stations. During the monsoon season, in the shelf stations, the salinity varies from the surface to the bottom from ~30 to 34 PSU at Goa and ~33 to 35 PSU at Mangalore. However, there was no change at Trivandrum (30 m) station. The surface salinity at the 300 m (slope station) and 600 m station (off-shore station) were 34 ± 2 PSU in both seasons and did not show any seasonal changes. The halocline depth, in the slope and off-shore station, was similar to the thermocline depth at 100 m during the non-monsoon season and is reduced to 25 - 50 m depth in the monsoon season (Figure 2.1).

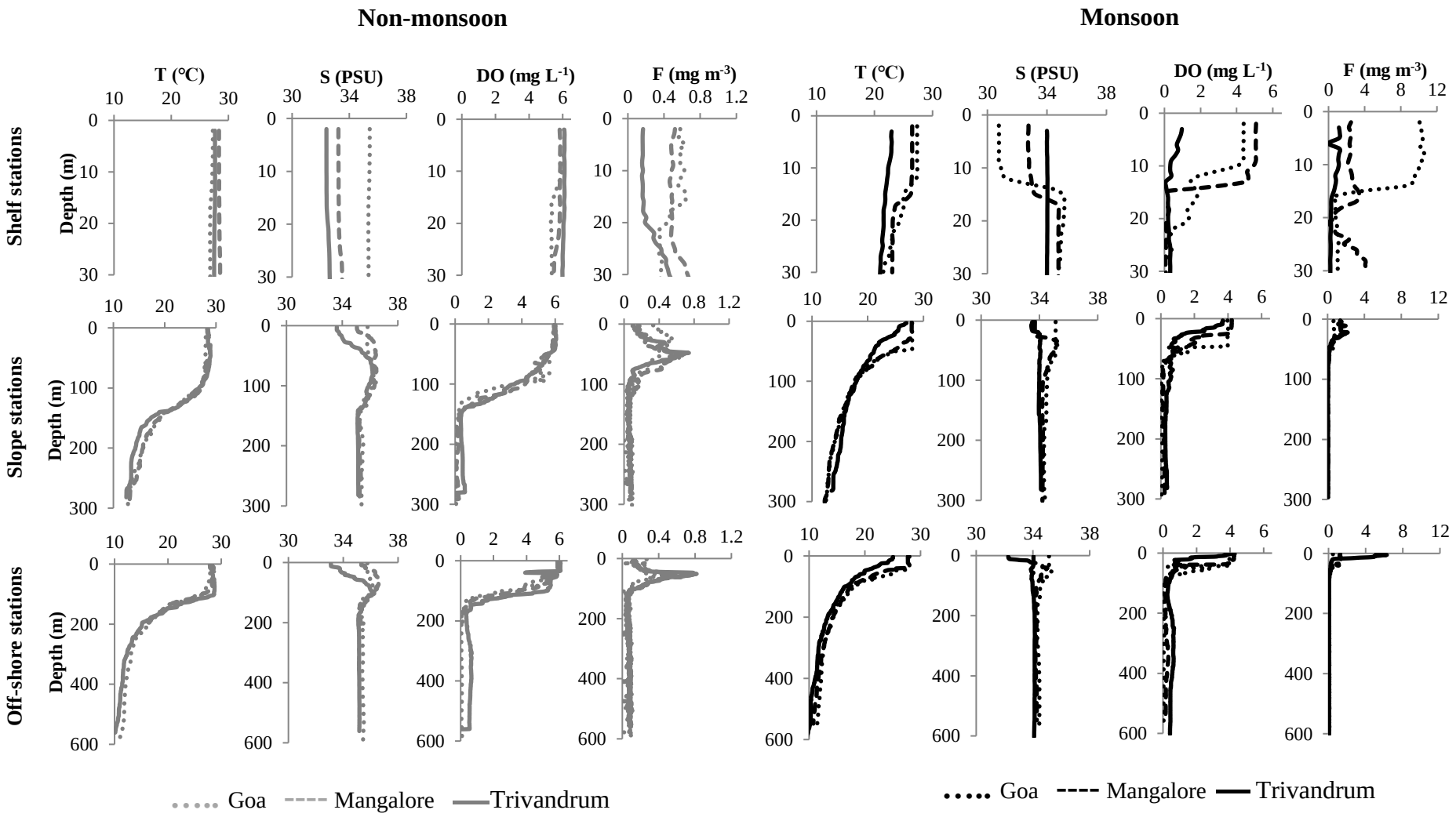


Figure 2.1: Profiles of physiochemical variables temperature (T, °C), salinity (S, PSU), dissolved oxygen (DO, mg L^{-1}) and fluorescence (F, mg m^{-3}) in the water column of the shelf, slope and off-shore stations during non-monsoon and monsoon season.

The surface DO values during the non-monsoon season were ~ 6 mg L^{-1} at the shelf, slope and off-shore station. In the productive monsoon season, the surface DO values dropped down to ~ 4 mg L^{-1} in all the stations and at the shelf region, in Trivandrum station T30, the value was < 1 mg L^{-1}. The DO values in the 30 m stations were uniform throughout the water column during the non-monsoon season, but the values at Goa and Mangalore dropped to < 1 mg L^{-1} at mid-depth during the monsoon season. At the 300 m and 600 m stations, the mixed layer depth (MLD) based on the DO concentration was at 100 m, where the levels reached close to anoxic conditions. During the monsoon season, the MLD was reduced to a shallower depth (25-50 m) like the other hydrographic features observed based on the temperature and salinity profiles (Figure 2.1). The chlorophyll concentration was determined using the fluorescence sensor mounted on the underwater CTD unit in all the locations sampled along the WCI. High surface fluorescence values of 10 mg m^{-3} were observed at the G30 station off Goa and 6 mg m^{-3} at the off-shore station, T600 during the southwest monsoon season. Average Chlorophyll maxima observed during the monsoon season was at 15 m depth and in the non-monsoon season, it was at 25 m on the shelf and 50 m on the slope and off-shore stations. The chlorophyll maxima were observed to be shallower during the monsoon season than during the non-monsoon season. Average fluorescence values recorded during the productive monsoon season in the water column were ~ 0.92 mg m^{-3} which was comparatively higher than the non-monsoon season where the average water column value was ~ 0.24 mg m^{-3} (Figure 2.1). Nitrate concentration, in all the stations in the WCI during the non-monsoon season was an average of ~ 13 µM and it reduced to ~ 0.2 µM in the monsoon season (Figure 2.2). The average nitrite concentration along all the stations was only ~ 0.1 µM in the non-monsoon season and it increased to ~ 6.6 µM in the monsoon season. The average phosphate and silicate concentration in the water column during the non-monsoon season and monsoon season was ~ 2 and ~ 10 µM respectively. The concentration of phosphate and silicate did not vary much between both seasons (Figure 2.2).

The average PP in the water column during the non-monsoon season was ~ 48 mg C m^{-3} d^{-1} and the values increased during the monsoon to ~ 63 mg C m^{-3} d^{-1} (Table 2.1). A similar observation was recorded in the BP measured during both seasons.

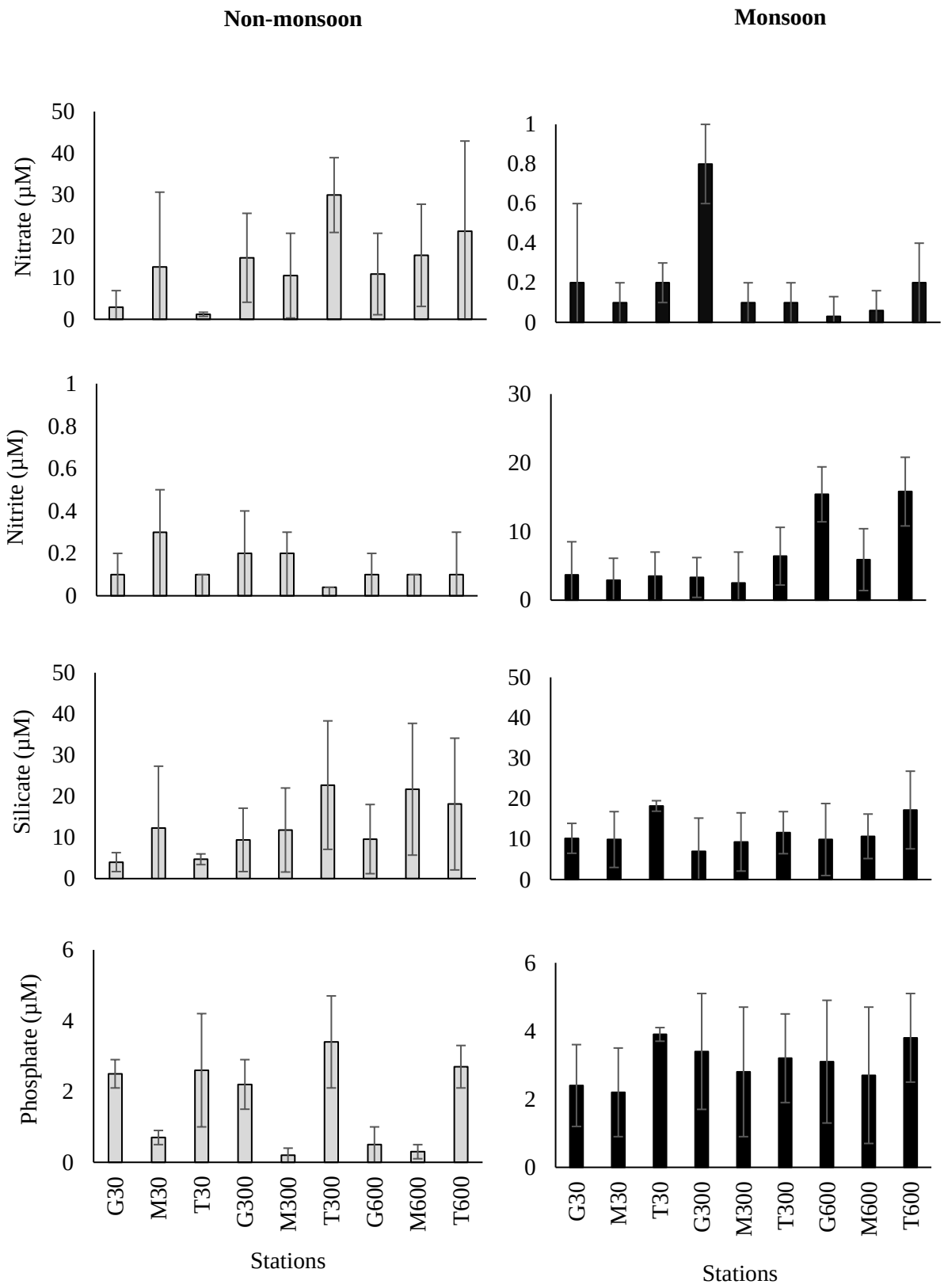


Figure 2.2: Station-wise variables of the chemical parameters nitrate (NO_3^-), nitrite (NO_2), silicate (SiO_3^{2-}) and phosphate (PO_4^{3-}) measured during non-monsoon and monsoon season in the shelf, slope and off-shore station.

Table 2.1: Station-wise profiles of primary productivity (PP), bacterial productivity (BP), total respiration (TR), bacterial respiration (BR) and bacterial growth efficiency (BGE) during the non-monsoon and monsoon season.

Station	PP*	BP*	TR*	BR*	BGE (%)
Non-monsoon					
G30	36.8 ± 61	5.1 ± 1.4	60.5 ± 88	27.2 ± 40	46 ± 48
G300	ND	1.2 ± 1.3	9.4 ± 15	4.2 ± 7	68 ± 45
G600	20.7 ± 36	1.8 ± 2.0	298.2 ± 421	134.2 ± 190	41 ± 51
M30	111.1 ± 165	2.5 ± 2.1	2.6 ± 4	1.2 ± 2	78 ± 39
M300	ND	3.1 ± 4.9	29.5 ± 23	13.3 ± 10	13 ± 13
M600	37.0 ± 48	6.4 ± 5.2	18.2 ± 26	8.2 ± 12	60 ± 44
T30	60.3 ± 41	1.2 ± 0.9	153.5 ± 263	69.1 ± 118	52 ± 50
T300	ND	4 ± 3.5	57.8 ± 76	26 ± 34	35 ± 24
T600	24.0 ± 27	1.2 ± 1.8	27.1 ± 25	12.2 ± 11	25 ± 38
Monsoon					
G30	76.6 ± 95	10.9 ± 0.8	55.4 ± 80	24.9 ± 36	56 ± 41
G300	ND	7.9 ± 1.6	241.7 ± 333	108.8 ± 150	29 ± 25
G600	22.9 ± 35	8.5 ± 1.1	166.7 ± 243	75 ± 109	308 ± 662
M30	93.2 ± 91	9.1 ± 0.4	1.5 ± 3	0.7 ± 1	28 ± 48
M300	ND	8 ± 0.6	168.2 ± 133	75.7 ± 60	13 ± 7
M600	58.2 ± 65	8.9 ± 1.8	91.9 ± 124	44.4 ± 57	23 ± 22
T30	89.1 ± 76	10.5 ± 2.3	130.8 ± 6	58.9 ± 3	15 ± 2
T300	ND	7.2 ± 2.1	75.3 ± 55	33.9 ± 25	26 ± 18
T600	38.3 ± 40	8.4 ± 2.3	193.6 ± 240	90.7 ± 107	14 ± 8

ND- No data; * (mg C m^{-3} d^{-1})

BP was lower during the non-monsoon season in the range of 1.2 to 6.4 mg C m^{-3} d^{-1}, as compared to the monsoon season which was in the range of 7.2 to 10.9 mg C m^{-3} d^{-1}. The average TR during the non-monsoon season was ~ 73 mg C m^{-3} d^{-1} across the sampling stations and in the monsoon season, it was ~ 125 mg C m^{-3} d^{-1}. A similar trend was observed in the BR and BGE calculated. During the non-monsoon season and monsoon season, the BR was 33 and 57 mg C m^{-3} d^{-1} respectively.

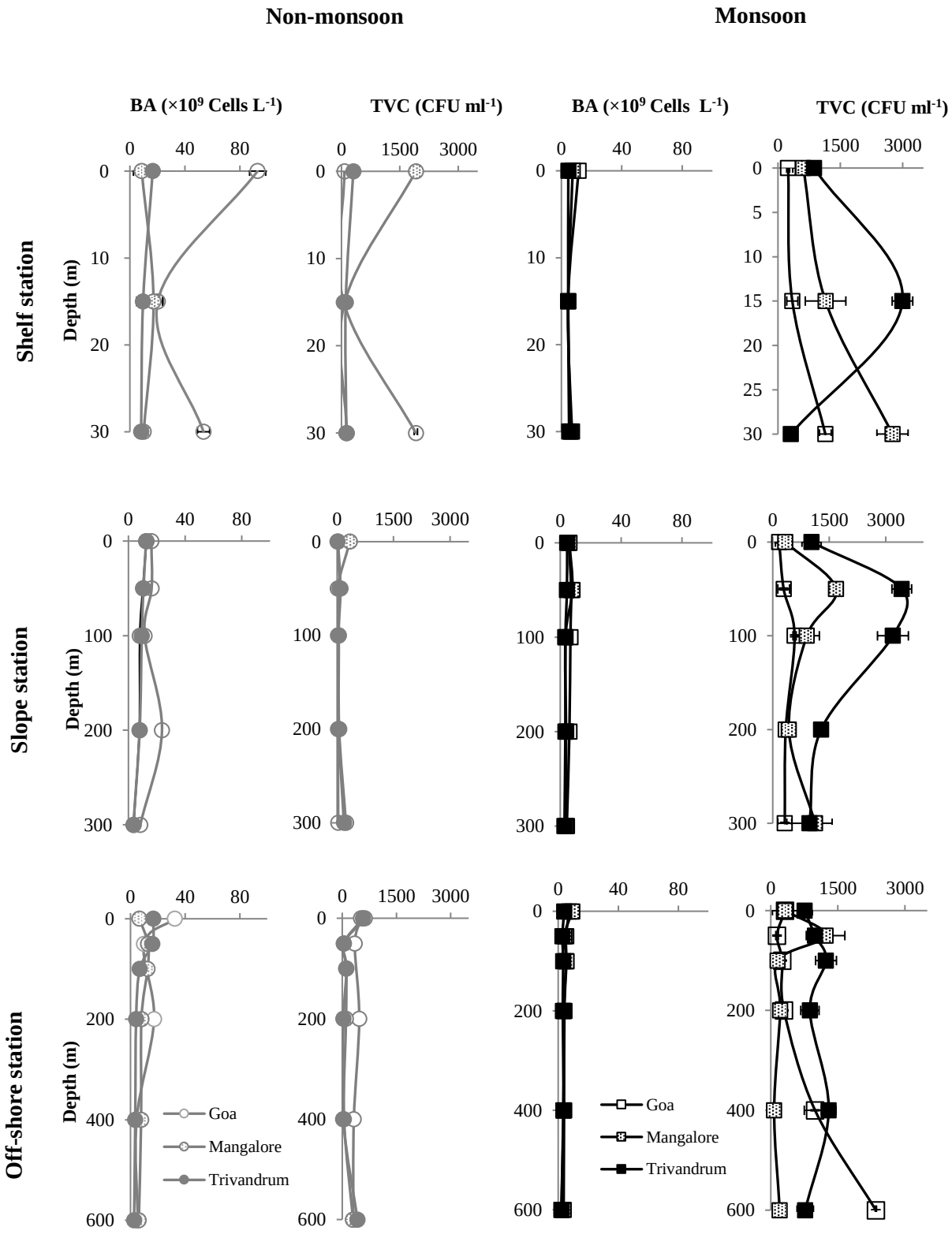


Figure 2.3: Profiles of bacterial abundance (BA) and total viable counts (TVC) in the shelf, slope and off-shore stations during non-monsoon and monsoon season.

The average BGE was 46 % during the sampling in the non-monsoon season and increased to 57 % during the monsoon season (Table 2.1). The average BA, which is the count of free-living bacteria in the water samples during the non-monsoon season was 13.6 $\times 10^9$ counts L^{-1} and in the monsoon season, the average water column bacterial cell counts were 4.9 $\times 10^9$ cells L^{-1}. BA in the water column was observed to be uniform in the order of 10^9 counts L^{-1} in all the stations during both seasons (Figure 2.3). Total viable counts of cultivable bacteria determined based on the CFU, estimated during the non-monsoon season were in the range of 10 to 1910 CFU ml^{-1} and during monsoon sampling, it was in the range of 75 to 3425 CFU ml^{-1}. Average viable counts during the non-monsoon season were 224 CFU ml^{-1} and 928 CFU ml^{-1} in the monsoon season (Figure 2.3).

Statistical correlation of the physicochemical, autotrophic and heterotrophic, bacterial parameters in both seasons was studied. DO and Temperature show a very good correlation with a significance level of 0.01 (Table 2.2). Temperature influences Fluorescence, PP, TR and BR significant correlation at 0.05 level. DO also showed a significant correlation with the PP, TR and BR. PP parameters, fluorescence and PP showed a correlation with very good significance (Table 2.2). A negative correlation with good significance between DO and bacterial parameters, BP and TVC and between nitrate concentration with BP, BR and TVC observed, could be due to the impact of the OMZ (Gonsalves et al. 2011). Denitrifying conditions are reported from these stations and the positive correlation between nitrite and BP could be because of the secondary nitrite maxima reported from these regions due to bacterial activity (Lincy and Manohar, 2021). Correlation between PP and BP with good significance shows that the bacterial parameters are influenced by the changes in the PP (Table 2.2). ANOVA analysis of different parameters between both seasons shows variations with good significance for DO and nutrients, such as nitrate, nitrite and phosphate which proves the influence of upwelling on the autotrophic activity (Fluorescence). Significant seasonal variations were also observed for bacterial parameters BA, TVC and BP (Table 2.3). These results suggest that the bacteria utilize labile OM which is available in abundance during the productive season (Ramaiah et al. 2005; Ramaiah et al. 2009).

Table 2.2: Pearson's correlation matrix of physicochemical and biological variables, Temperature (T), dissolved oxygen (DO), nitrate (NO_3^-), nitrite (NO_2^-), Fluorescence (F), primary production (PP), bacterial production (BP), Total respiration (TR), bacterial respiration (BR), Bacterial growth efficiency (BGE) and total viable counts (TVC).

	T	**DO**	**NO_3^-**	**NO_2^-**	**F**	**PP**	**BP**	**TR**	**BR**	**BGE**	**TVC**
T	1										
DO	0.87**	1									
NO_3^-	-0.20*	-0.09	1								
NO_2^-	-0.12	-0.11	-0.20*	1							
F	0.29*	0.15	-0.17	0.04	1						
PP	0.39*	0.24*	-0.07	0.09	0.46**	1					
BP	-0.15	-0.32*	-0.37*	0.29*	0.24*	0.23*	1				
TR	0.31*	0.27*	-0.02*	0.14	0.18	0.12	0.01	1			
BR	0.30*	0.27*	-0.22*	0.14	0.18	0.12	0.02	0.99**	1		
BGE	-0.18	-0.11	-0.02	0.00	-0.07	-0.16	0.11	-0.15	-0.15	1	
TVC	-0.12	-0.31*	-.32*	0.14	0.17	0.22*	0.42**	0.05	0.05	0.21*	1

**Correlation is significant at the 0.01 level (2-tailed), * correlation is significant at the 0.05 level (2-tailed).

Table 2.3: ANOVA: single factor to show the significance of distribution between various parameters, Temperature (T), dissolved oxygen (DO), nitrate (NO_3^-), nitrite (NO_2^-), Fluorescence (F), primary production (PP), bacterial production (BP), Total respiration (TR), bacterial respiration (BR), Bacterial growth efficiency (BGE) and total viable counts (TVC) during two seasons.

Variables	Df	F value	F critical	p-value
Temperature	1	2.22	3.96	0.14
DO	1	19.08	3.96	< 0.001***
Salinity	1	0.36	3.96	0.55
Fluorescence	1	6.19	3.96	0.01**
NO_3-	1	34.74	3.96	< 0.001***
NO_2-	1	12.27	3.96	< 0.001***
PO_4^{3-}	1	22.47	3.96	< 0.001***
SiO_3^{2-}	1	0.84	3.96	0.36
BA	1	12.88	3.96	< 0.001***
TVC	1	34.92	3.96	< 0.001***
PP	1	0.49	4.08	0.49
BP	1	91.24	3.96	< 0.001***
TR	1	2.14	3.96	0.15
BR	1	2.30	3.96	0.13
BGE	1	0.28	3.96	0.60

Df: degree of freedom, F value greater than F critical value indicates statistical significance, ***significant at 0.1%, **significant at 1%.

2.3.2. Culturable bacterial diversity and community structure

Unique morphotypes were characterized based on the 16S rRNA sequence for diversity analysis from non-monsoon and monsoon season sampling. A total number of 83 and 139 unique morphotypes were picked from the non-monsoon and monsoon sampling, respectively. Proteobacteria, Firmicutes and Actinobacteria were the major phyla found during both the non-monsoon and monsoon seasons and Bacteroidetes were

represented only in the non-monsoon season (Figure 2.4). The relative abundance of Firmicutes phylum was highest (55 %) in January 2018, during the non-monsoon season, followed by Proteobacteria (42 %), Actinobacteria and Bacteroidetes phyla contributed to 1 % each in the non-monsoon season (Figure 2.4a). During the monsoon season, the bacterial communities included Firmicutes, which was represented with the highest relative abundance of 53 % followed by Proteobacteria with 27 %. The contribution of Proteobacteria was relatively lower in monsoon compared to non-monsoon sampling (Figure 2.4). However, Actinobacterial diversity increased to 20 % in the monsoon sampling and Bacteroidetes were completely absent. Phylum-wise distribution of the bacterial morphotypes in the shelf, slope and off-shore locations at each sampling station was also determined. The results show that in the non-monsoon sampling, the distribution of bacterial morphotypes from the major phyla Proteobacteria and Firmicutes were uniform in all the stations. Actinobacteria and Bacteroidetes which were represented by 1 % each were identified from the Trivandrum shelf (T300) and Goa shelf location (G30) respectively (Figure 2.4a). The major phyla, during the monsoon season, included morphotypes from Firmicutes, Proteobacteria and Actinobacteria. The distribution of these morphotypes was almost uniform across the shelf, slope and off-shore stations at all the locations (Figure 2.4b).

The 16S rRNA sequences of 83 and 139 morphotypes from non-monsoon and monsoon seasons were analyzed together using MOTHUR and clustered into 111 OTUs which are sequentially represented from BWCI1 to BWCI111. Phylogenetic analysis of the OTUs shows that 49 OTUs belonged to Proteobacteria which was clustered within Alpha, Beta and Gamma Proteobacteria class. Firmicutes were represented by 41 OTUs, Actinobacteria by 20 OTUs and Bacteroidetes by only one OTU (Figure 2.5a-d). Bacteroidetes were represented by one OTU (BWCI91) only from the non-monsoon season and clustered with strong bootstrap support with *Flavobacterium* sp. (Figure 2.5a). Actinobacteria was represented by 1 % only during the non-monsoon season and it increased (20 %) during the monsoon season. Within, Actinobacteria, OTU, BWCI81 *Micrococcus luteus* was the only representative in the non-monsoon season and during the monsoon season, OTUs BWCI83 and BWCI22 clustered with *Micrococcus* sp. sequences. Other Actinobacterial OTUs belonged to major genera such as *Aeromicrobium* sp., *Corynebacterium* sp., *Brachybacterium* sp., *Pseudoclavibacter* sp. and *Actinobacteria bacterium*.

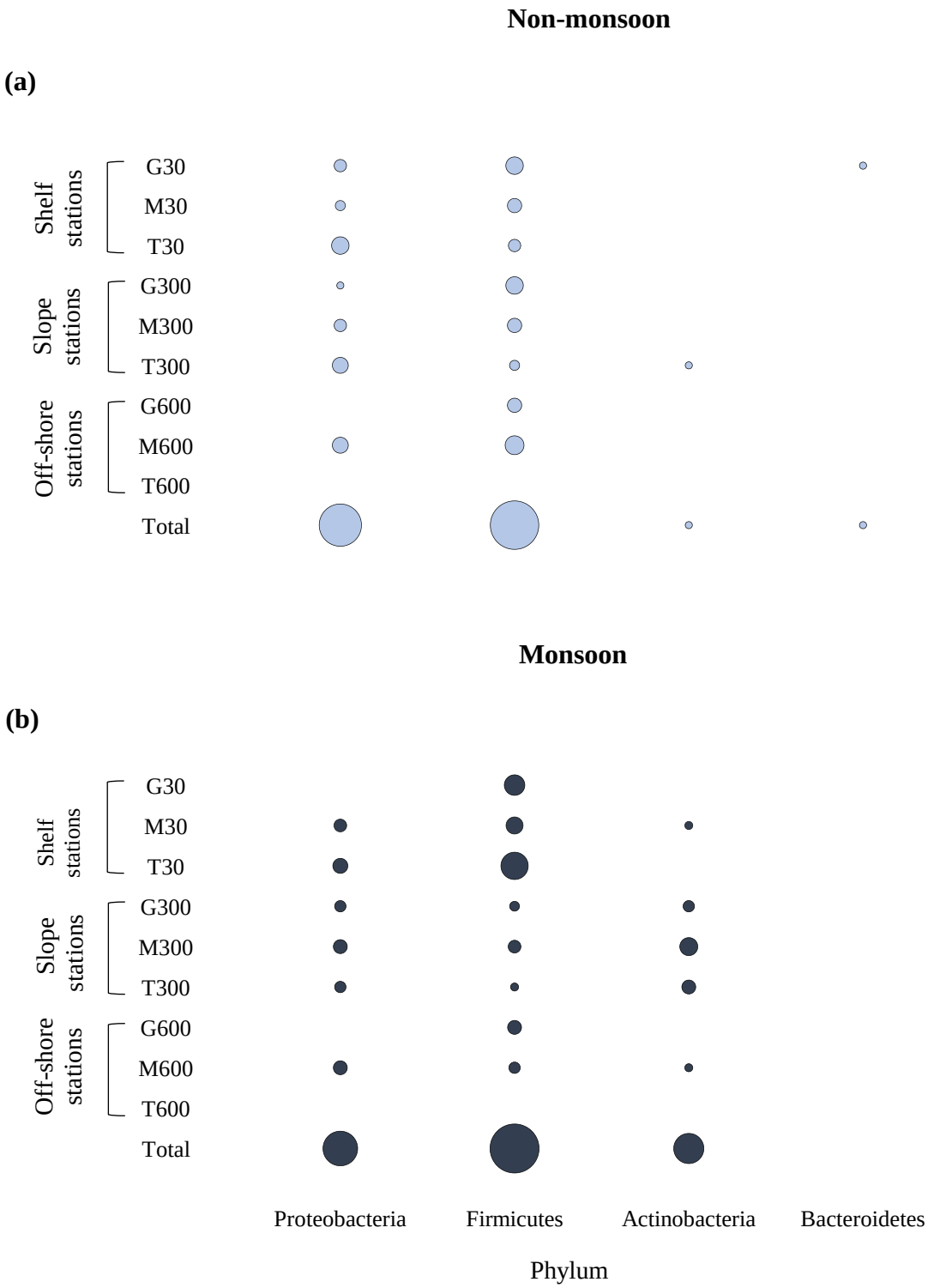


Figure 2.4: Phylum-wise distribution of the bacterial morphotypes (%) along the shelf, slope and off-shore stations during (a) non-monsoon and (b) monsoon season.

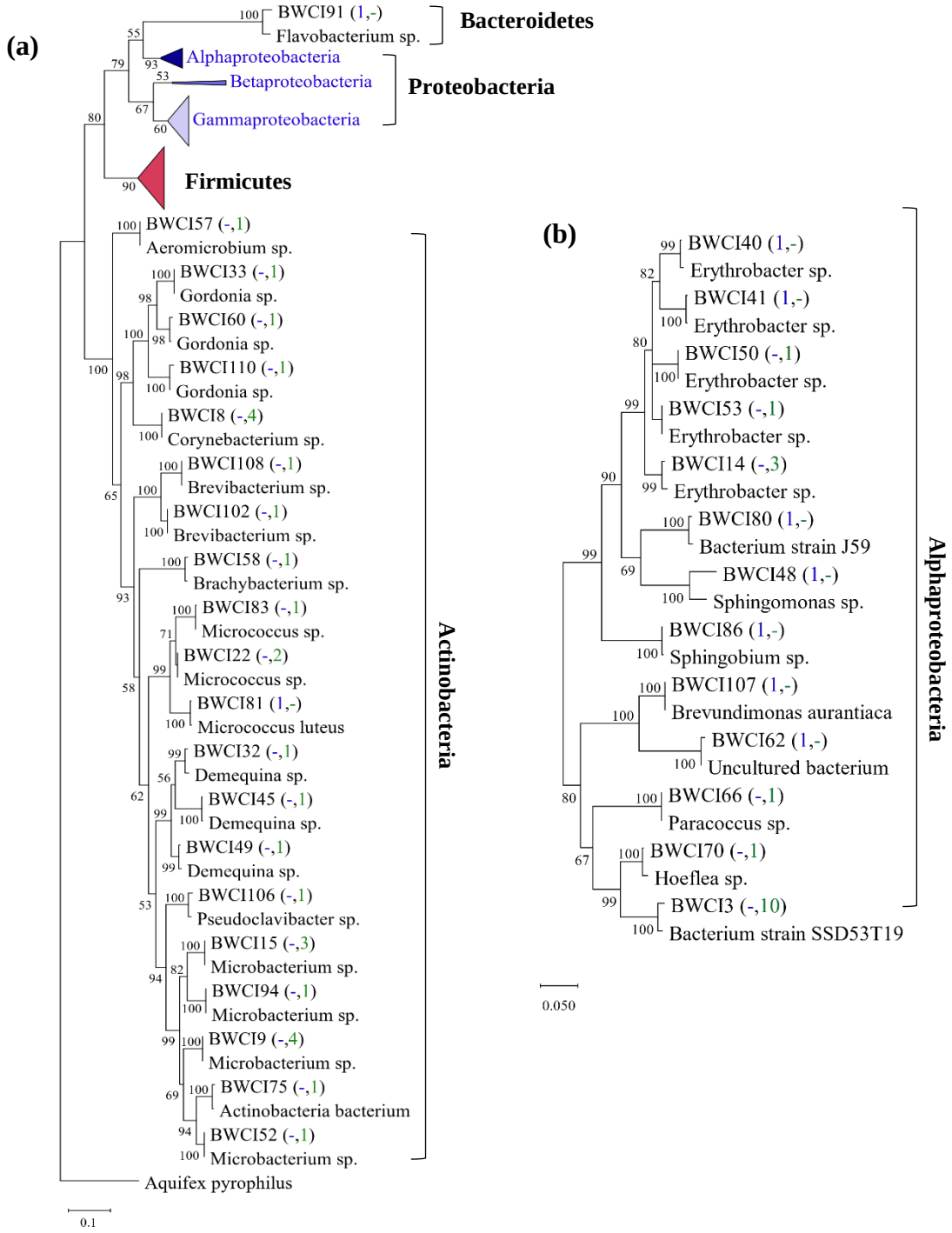
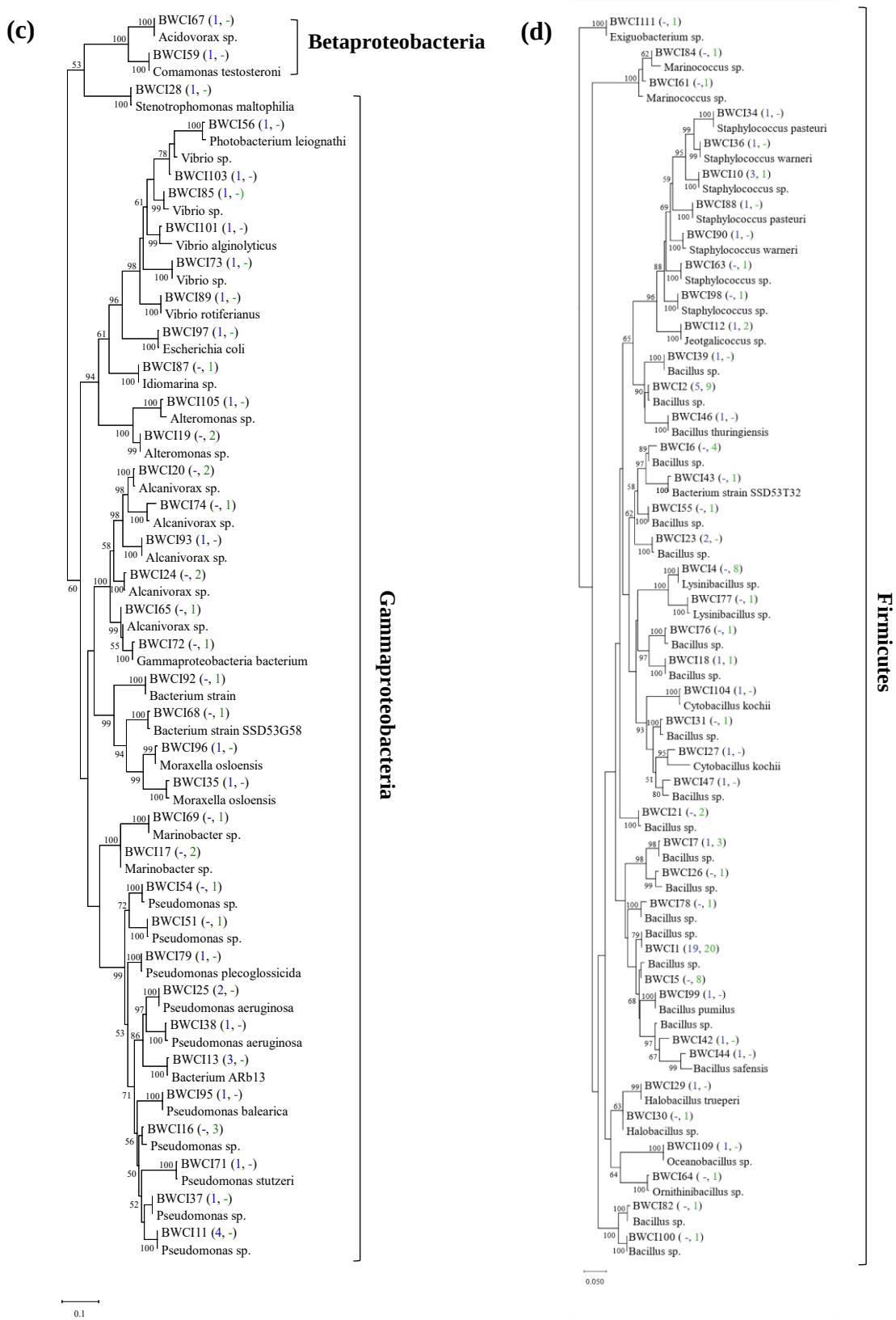


Figure 2.5: Maximum-likelihood-based phylogenetic tree of 16 rRNA gene sequences of representative OTUs belonging to (a) Actinobacteria and Bacteroidetes, (b) Alphaproteobacteria, (c) Gammaproteobacteria and Betaproteobacteria and (d) Firmicutes. The number in parentheses following OTU names indicates the number of sequences obtained from the non-monsoon and monsoon season.

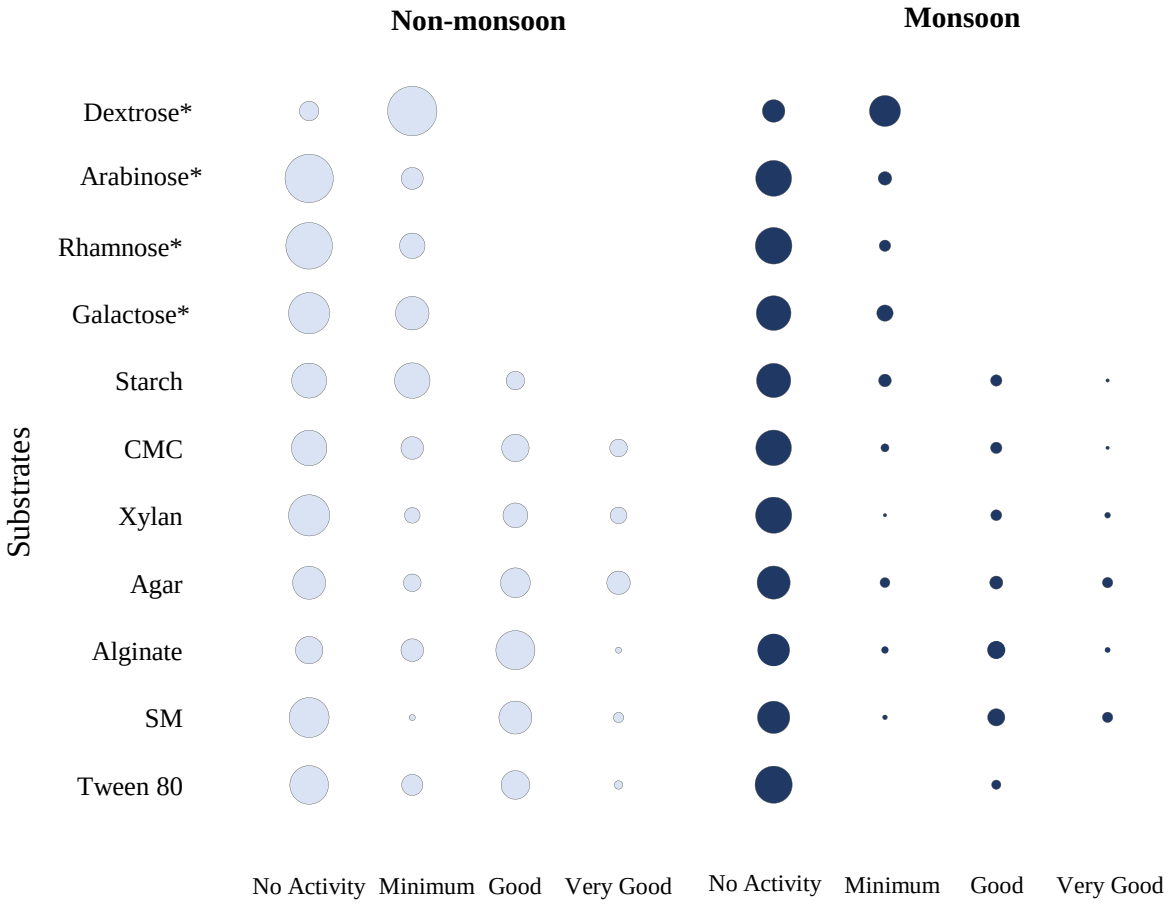

(c)
BWCI67 (1, -)
Acidovorax sp.
BWCI59 (1, -)
Comamonas testosteroni
Betaproteobacteria
BWCI28 (1, -)
Stenotrophomonas maltophilia
BWCI56 (1, -)
Photobacterium leiognathi
Vibrio sp.
BWCI103 (1, -)
BWCI85 (1, -)
Vibrio sp.
BWCI101 (1, -)
Vibrio alginolyticus
BWCI73 (1, -)
Vibrio sp.
BWCI89 (1, -)
Vibrio rotiferianus
BWCI97 (1, -)
Escherichia coli
BWCI87 (-, 1)
Idiomarina sp.
BWCI105 (1, -)
Alteromonas sp.
BWCI19 (-, 2)
Alteromonas sp.
BWCI20 (-, 2)
Alcanivorax sp.
BWCI74 (-, 1)
Alcanivorax sp.
BWCI93 (1, -)
Alcanivorax sp.
BWCI24 (-, 2)
Alcanivorax sp.
BWCI65 (-, 1)
Alcanivorax sp.
BWCI72 (-, 1)
Gammaproteobacteria bacterium
BWCI92 (-, 1)
Bacterium strain
BWCI68 (-, 1)
Bacterium strain SSD53G58
BWCI96 (1, -)
Moraxella osloensis
BWCI35 (1, -)
Moraxella osloensis
BWCI69 (-, 1)
Marinobacter sp.
BWCI17 (-, 2)
Marinobacter sp.
BWCI54 (-, 1)
Pseudomonas sp.
BWCI51 (-, 1)
Pseudomonas sp.
BWCI79 (1, -)
Pseudomonas plecoglossicida
BWCI25 (2, -)
Pseudomonas aeruginosa
BWCI38 (1, -)
Pseudomonas aeruginosa
BWCI13 (3, -)
Bacterium ARb13
BWCI95 (1, -)
Pseudomonas balearica
BWCI16 (-, 3)
Pseudomonas sp.
BWCI71 (1, -)
Pseudomonas stutzeri
BWCI37 (1, -)
Pseudomonas sp.
BWCI11 (4, -)
Pseudomonas sp.
Gammaproteobacteria
0.1
(d)
BWCI111 (-, 1)
Exiguobacterium sp.
BWCI84 (-, 1)
Marinococcus sp.
BWCI61 (-,1)
Marinococcus sp.
BWCI34 (1, -)
Staphylococcus pasteuri
BWCI36 (1, -)
Staphylococcus warneri
BWCI10 (3, 1)
Staphylococcus sp.
BWCI88 (1, -)
Staphylococcus pasteuri
BWCI90 (1, -)
Staphylococcus warneri
BWCI63 (-, 1)
Staphylococcus sp.
BWCI98 (-, 1)
Staphylococcus sp.
BWCI12 (1, 2)
Jeotgalicoccus sp.
BWCI39 (1, -)
Bacillus sp.
BWCI2 (5, 9)
Bacillus sp.
BWCI46 (1, -)
Bacillus thuringiensis
BWCI6 (-, 4)
Bacillus sp.
BWCI43 (-, 1)
Bacterium strain SSD53T32
BWCI55 (-, 1)
Bacillus sp.
BWCI23 (2, -)
Bacillus sp.
BWCI4 (-, 8)
Lysinibacillus sp.
BWCI77 (-, 1)
Lysinibacillus sp.
BWCI76 (-, 1)
Bacillus sp.
BWCI18 (1, 1)
Bacillus sp.
BWCI104 (1, -)
Cytobacillus kochii
BWCI31 (-, 1)
Bacillus sp.
BWCI27 (1, -)
Cytobacillus kochii
BWCI47 (1, -)
Bacillus sp.
BWCI21 (-, 2)
Bacillus sp.
BWCI7 (1, 3)
Bacillus sp.
BWCI26 (-, 1)
Bacillus sp.
BWCI78 (-, 1)
Bacillus sp.
Bacillus sp.
BWCI1 (19, 20)
Bacillus sp.
BWCI5 (-, 8)
BWCI99 (1, -)
Bacillus pumilus
Bacillus sp.
BWCI42 (1, -)
BWCI44 (1, -)
Bacillus safensis
BWCI29 (1, -)
Halobacillus trueperi
BWCI30 (-, 1)
Halobacillus sp.
BWCI109 (1, -)
Oceanobacillus sp.
BWCI64 (-, 1)
Ornithinibacillus sp.
BWCI82 (-, 1)
Bacillus sp.
BWCI100 (-, 1)
Bacillus sp.
Firmicutes
0.050

Brevibacterium spp. was represented by 2 OTUs, *Gordonia* spp. and *Demequina* spp., with 3 OTUs and *Microbacterium* spp. with 4 OTUs with a total representation of 19 OTUs belonging to the monsoon season and there was no shared OTUs within this cluster (Figure 2.5a). The 49 OTUs of phyla Proteobacteria grouped within the Alpha, Beta and Gamma clusters (Figure 2.5b, c). No representatives were observed belonging to the Delta and Epsilon Proteobacteria class from both the samplings. The OTUs belonging to Proteobacteria clustered within Alphaproteobacteria with 13 OTUs (Figure 2.5b), the Betaproteobacteria cluster had only 2 OTUs and the Gamma cluster had the maximum representation with 34 OTUs belonging to well-classified bacterial genera (Figure 2.5c). Alphaproteobacteria and Gammaproteobacteria had representative OTUs from both seasons which included major representatives grouping with *Erythrobacter* spp., from the former and *Alteromonas* spp., *Alcanivorax* spp. and *Pseudomonas* spp. from the latter. Betaproteobacteria OTUs clustering with *Acidovorax* sp. and *Comamonas* sp. were represented only from the non-monsoon season and none of the OTUs were common to both the sampling seasons (Figure 2.5b, c). OTUs belonging to Firmicutes were represented uniformly in both seasons and of the 41 OTUs clustered within this phylum, 6 OTUs were shared between both seasons. The majority of the OTUs clustered with the *Bacillus* spp. followed by *Staphylococcus* spp. within the Firmicutes cluster (Figure 2.5d).

2.3.3. Hydrolytic enzyme activity of culturable bacterial morphotypes

Characterization of the hydrolytic enzyme activity was determined in all the representative 83 and 139 morphotypes. The results showed that the bacterial morphotypes were able to utilize all three major groups of substrates as they exhibited carbohydrase, protease and lipase enzyme activity. It was observed that monosaccharide glucose was the most utilized substrate in both seasons (Figure 2.6). The bacterial morphotypes from the non-monsoon sampling showed higher hydrolytic enzyme activity with only an average of 50 % which showed no activity and during the monsoon season sampling, an average of 78 % of the bacterial morphotypes did not show activity in any of the substrates tested (Figure 2.6). Based on the zone of hydrolysis, the percentage of morphotypes from the non-monsoon season showed activity in alginate >agar > starch > CMC >Tween 80 > SM > xylan (Figure 2.6a). The activity of the morphotypes was relatively lower in the morphotypes from the monsoon season and the hydrolytic enzyme

activity was characteristically distinct, especially the protease activity which was relatively the highest. The morphotypes showed activity in SM >alginate >agar > starch > CMC > xylan> Tween 80 (Figure 2.6b). A small percentage of morphotypes were largely *Bacillus* spp. or *Pseudomonas* spp. exhibited very good activity in the non-monsoon and monsoon seasons (Figure 2.6). This shows that the isolates from the non-monsoon season exhibited better hydrolytic enzyme activity.



*Monosaccharide activity is categorized only into no activity or minimum activity.

Figure 2.6: Hydrolytic enzyme activity of bacterial morphotypes (%) isolated during the non-monsoon and monsoon season.

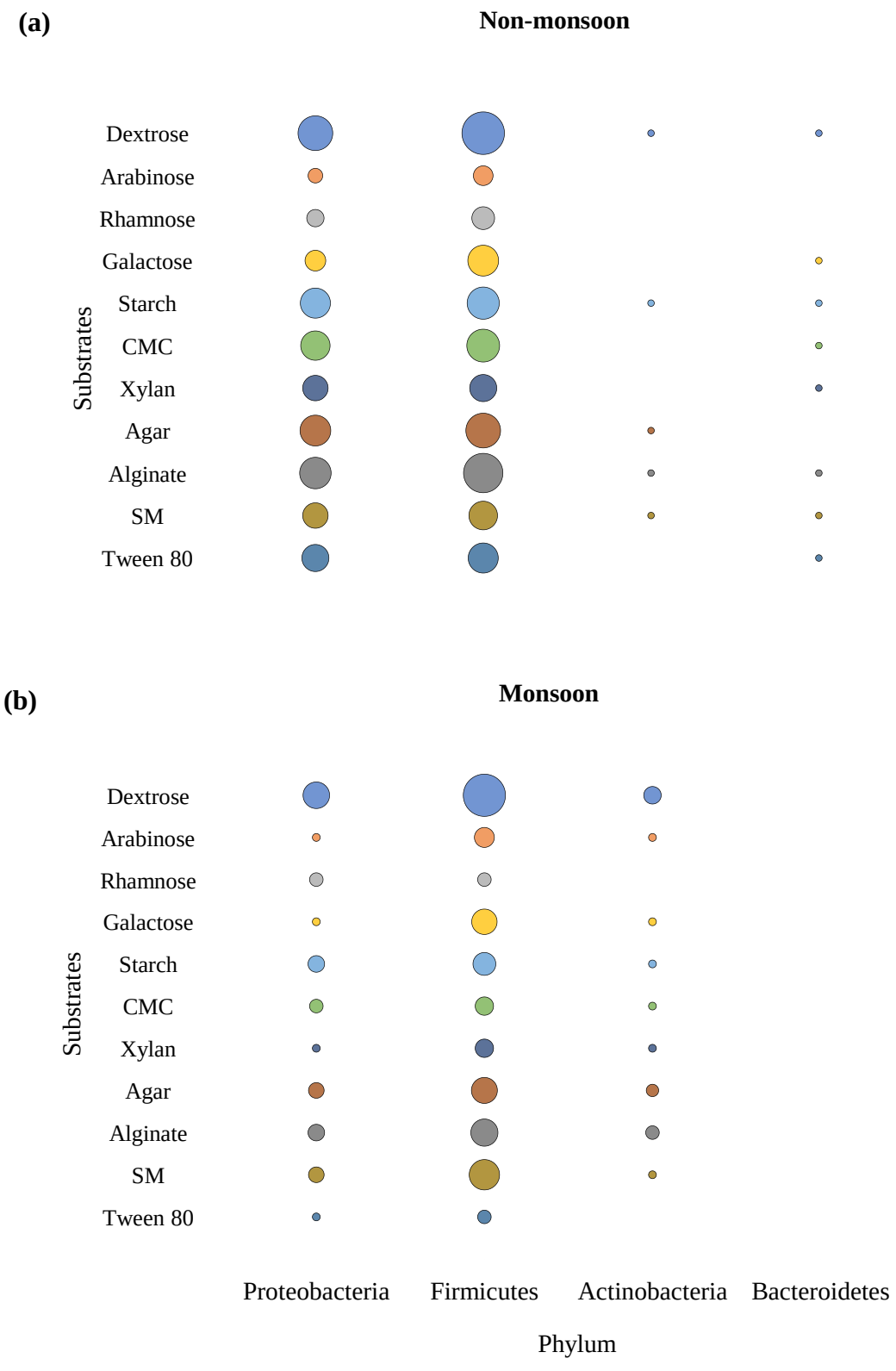


Figure 2.7: Hydrolytic enzyme activity of bacterial morphotypes (%) of different phyla isolated during (a) non-monsoon and, (b) monsoon season.

The hydrolytic enzyme activity of the morphotypes belonging to each phylum was studied and the results show that bacterial isolates from phylum Firmicutes followed by Proteobacteria from both the sampling season exhibited good hydrolytic activity and were able to utilize all the 11 substrates tested. Actinobacteria was represented by 1 % only at phylum and genus level during the non-monsoon season, which increased up to 20 % during the monsoon season. However, its activity increased marginally from 1 to 8 % during the monsoon season (Figure 2.7). Bacteroidetes which were represented only by 1% in the non-monsoon season showed minimum activity in major carbohydrate substrates along with protease and lipase activity. Results show that Firmicutes exhibited good hydrolytic enzyme activity in both seasons (Figures 2.6 and 2.7).

2.4. Discussion

2.4.1. Relationship between physicochemical characteristics, primary productivity and bacterial dynamics

The northwestern region of the Indian Ocean, the Arabian Sea, especially the WCI has higher biological productivity than the eastern, Bay of Bengal or the central, Equatorial Indian Ocean regions. Studies have shown that the semi-annual reversal of monsoonal winds annually from June to September induces open ocean upwelling and divergence in the Arabian Sea. These seasonal features regulate near-surface currents, influence mixed layer development and affect the nutrient supply to this region (Shetye et al. 1991; Sarma et al. 2003; Kumar et al. 2007). In addition, winter cooling during the northeast monsoon (December-February) also adds up to the variability of physical features (Kumar et al. 2001). The predominant characteristics which include strong winds, cooler waters, heavy rainfall and a significant increase in phytoplankton production during the monsoon and warm waters, weak winds, low rainfall and low phytoplankton production from the non-monsoon season have been reported from the coastal, southwest region (Santhikrishnan et al. 2021; Kottayil et al. 2022). In this study, the consequence of the seasonal variations that influence primary productivity and their impact on bacterial dynamics was investigated.

The water column characteristics assessed during the monsoon and non-monsoon seasons revealed distinct variations during both seasons. The temperature and

salinity values characteristically influenced the MLD in the continental slope and off-shore station, which was observed to be at ~ 100 m depth in the non-monsoon season and it was shallower at 25 to 50 m depth in the monsoon season. A similar trend was seen in the oxycline depths in both seasons (Figure 2.1), shallow MLD which is an upwelling phenomenon was observed during the monsoon season as previously described (Smitha et al. 2008). A significant increase in fluorescence value was recorded during the monsoon season (Figure 2.1). Earlier studies from the WCI have shown that the average PP increases during the monsoon season (June-September) due to upwelling (Ramaiah et al. 2005). The concentration of the essential nutrient, nitrate reduced significantly during the monsoon season (Figure 2.2), which could be due to the active PP which could have depleted the nitrates. An increase in nitrite concentration in the monsoon season (Figure 2.2) is possibly due to the nitrite build-up from denitrification activity in the coastal stations reported occurring from seasonal hypoxia along the WCI (Naqvi et al. 2010; Gonsalves et al. 2011; Lincy and Manohar, 2021). TR representing the community respiration, PP measuring the autotrophic activity and heterotrophic activity based on BP, BR and BGE all show a significant increase in the monsoon in comparison with non-monsoon season (Table 2.1). BA estimated during this study was in the order of 10^9 cells ml^{-1} during both seasons. However, the bacterial CFUs during the monsoon increased in comparison to the non-monsoon season. Increased bacterial abundance during the monsoon season highlights the bacterial dependence on phytoplankton-derived organic matter (Molina et al. 2020). These results are consistent with those of the previous studies from the Arabian Sea suggesting that increases in primary productivity during the monsoon influence bacterial numbers (Ramaiah et al. 2005; Fernandes and Bogati, 2022). However, there are no reports on the seasonal variations of the bacterial community and its activity which is characterised in this study to understand their ecological role during the monsoon season with increasing productivity.

2.4.2. Seasonal variations in the bacterial community structure

Bacterial diversity from water samples assessed by 16S rRNA sequencing during this study revealed that the morphotypes belonged to four major phyla, Proteobacteria, Firmicutes, Actinobacteria and Bacteroidetes which are identified as the predominant culturable bacterial groups from marine habitats (Amberkar et al. 2021). Proteobacteria

contributed about 42 % of the bacterial diversity during the non-monsoon season and was reduced to 27 % during the monsoon season. Gammaproteobacteria was the dominant class found within the Proteobacteria phylum during both seasons (Figure 2.4). Proteobacteria is the major phylum represented in many marine habitats such as the coastal regions (Khandeparker et al. 2017), upwelling zones (Li et al. 2014; Vijayan et al. 2021) and OMZ (Bandekar et al. 2018; Mulla et al. 2018). Bacterial diversity at the OTU level showed that Proteobacteria had maximum representation in this study with 49 OTUs from Alpha, Beta and Gamma classes. There were no shared OTUs within the Proteobacteria cluster and their numbers reduced from 29 to 20 OTUs in the monsoon season. OTUs clustering with *Pseudomonas* spp., *Vibrio* spp., *Alteromonas* spp. and *Alcanivorax* spp. were the major Gammaproteobacteria identified. Recent studies have shown that Gammaproteobacteria of the phylum Proteobacteria are the dominant groups during algal blooms. Within Gammaproteobacteria *Vibrio* spp. and *Psudoalteromonas* spp. were the major genera contributing to the bacterial communities associated with algal bloom (Chen et al. 2021).

Firmicutes contributed almost equally during both seasons, with a representation of four genera *Staphylococcus* spp., *Bacillus* spp., *Halobacillus* spp. and *Jeotgalicoccus* sp. from both seasons and a few genera which was exclusively isolated from the non-monsoon and monsoon season (Figure 2.5). Major bacterial genera such as *Staphylococcus* which had a major contribution during the non-monsoon season reduced considerably during the monsoon season, which is one of the major pathogenic groups of bacteria (Isaacs et al. 2021). They could not proliferate during the monsoon season due to the presence of active phytoplankton communities which are known to produce anti-bacterial compounds to prevent infection (Shannon and Abu-Ghannam, 2016; Mühlenbruch et al. 2018). This prevents the growth of pathogenic bacteria and in turn, paves the way for the proliferation of native bacterial communities such as *Marinococcus* spp. which are represented in higher numbers during the productive monsoon season (Figure 2.5). Studies have reported that *Bacillus* spp. and *Staphylococcus* spp. are the major culturable bacteria associated with the phytoplankton bloom (Basu et al. 2013). Their dominance was also reported during the phytoplankton bloom decay period (Zhao et al. 2017; Gómez-Consarnau et al. 2019). Many of the Firmicutes bacteria are also known to be euryhaline, which can degrade OM efficiently in marine habitats (Margesin and Schinner, 2001).

Genus *Micrococcus* was the only representative of phylum Actinobacteria in the non-monsoon season. It increased considerably in the monsoon season and was represented by genera such as *Microbacterium*, *Corynebacterium*, *Aeromicrobium* and *Gordonia* (Figure 2.5). Actinobacteria are also reported in the OMZ, mesopelagic and deep-sea regions (Wright et al. 2012; Rodriguez-Mora et al. 2013; Kamjam et al. 2017) from culturable and metagenomics studies (Ghai et al. 2013). The seasonal appearance of Actinobacteria in many marine habitats is often attributed to the presence of phytoplankton blooms (Basu et al. 2013; Parulekar et al. 2017; Zhou et al. 2018). Recent research also suggests that changes in the phytoplankton communities have a significant impact on the dynamics of heterotrophic bacterial diversity in coastal and oceanic waters. Which is reported to be influenced by the autochthonous organic matter generated by the changing phytoplankton composition (Landa et al. 2016; Aytan et al. 2018; Arandia-Gorostidi et al. 2022). Marine Bacteroidetes are known to express the polysaccharide utilization loci which facilitate the degradation of algal polysaccharides (Krüger et al. 2019; McKee et al. 2021). Genus *Flavobacterium* sp. was the only Bacteroidetes isolated from the non-monsoon season and this group was not represented in the monsoon season (Figure 2.5). They are reported to be one of the major contributors to the remineralization of carbohydrates leading to refractory carbon storage in the ocean (Zhang et al. 2015). Many marine bacterial genera such as *Idiomarina*, *Marinococcus* and *Marinobacter* were exclusively obtained only during the monsoon season which is majorly known for the degradation of OM (Lucas et al. 2016; Gomes et al. 2019; Mukherjee et al. 2020). Studies have suggested that the bacterial metabolic processes and products of remineralization can also influence the phytoplankton community structure (Bolch et al. 2017). More detailed studies on this could help us to understand the complex bacteria phytoplankton interactions.

2.4.3. Seasonal variations in the bacterial hydrolytic enzyme activity

Hydrolytic enzyme activities of bacteria and their enzymatic potential are studied in various coastal regions (Khandeparker et al. 2011; Singh and Ramaiah 2011). However, comparative studies on the hydrolytic enzyme activities of bacteria from the coastal/shelf, slope and open ocean water column are not addressed to understand the seasonal difference in the BA, community structure and hydrolytic enzyme activities.

Different types of carbohydrates, lipids and protein substrates were tested in this study to characterize the hydrolytic enzyme activity of bacteria during the non-monsoon season and productive monsoon season (Figure 2.7). Bacterial communities play an important role by synthesizing extracellular enzymes for the remineralization of oceanic OM which is composed of carbohydrate polysaccharides, monosaccharides, lipids and protein polymers (Traving et al. 2015; Bolch et al. 2017). Carbohydrates make up a 90 % share of phytoplankton-derived OM including various polysaccharides and monosaccharides (Underwood et al. 2010), neutral monosaccharides like glucose, arabinose, rhamnose and galactose are the major contributors to the phytoplankton derived OM (Passow et al. 2007; Engel and Händel, 2011). Results from this study show that 50 % of bacterial morphotypes exhibit enzyme activity during the non-monsoon season and from monsoon season an average of only 20 % of morphotypes showed activity. This result suggests the metabolic potential of microbes was higher during the non-monsoon season, which could be due to the availability of DOM which is sustained for an extended period in the water column and could be available for bacterial utilization (Smriga et al. 2016). The low hydrolytic activity during the monsoon season suggests that culturable bacteria isolated during the monsoon season did not express enzymes to utilize fresh and complex OM generated from phytoplankton blooms. Studies have also found that the enzyme activity of bacteria exhibited variation based on the availability and nature of OM, implying an unequal distribution of enzyme activity in different seasons and regions (Basu et al. 2013; Khodse and Bhosle, 2013, Mahmoudi et al. 2020).

Proteobacteria, such as *Stenotrophomonas* sp. and *Alcanivorax* spp., that degrade organic substances are shown to have made a significant contribution to bacterial communities; a similar finding was obtained in recent research in the Southern East China Sea, where the existence of these groups was recognized (Yang et al. 2020). Recent research also shows that the production of extracellular enzymes in bacteria increases in harsh environments to recycle available organic materials (Bolch et al. 2017; Showalter and Deming, 2021). Bacterial numbers and extracellular enzyme activity were found to be sustained even at low oxygen concentrations in recent investigations in the eastern tropical South Pacific OMZ, implying that bacteria can efficiently recycle OM in the low oxygen environment (Maßmig et al. 2020). Our results accordance with previous studies which have also found that the dominant class Gammaproteobacteria synthesizes enzymes that hydrolyse diverse types of polysaccharides enzymes (Alderkamp et al. 2007; Teeling et al. 2016). The shift in the bacterial community suggests that the

availability of OM is the major factor that controls the expression of extracellular enzymes in bacterial groups. It is also reported that among Firmicutes, *Bacillus* spp. which was highest during both seasons; can play an important role in OM degradation in marine environments (Divya et al. 2010). Actinobacteria can produce extracellular enzymes like cellulase, and chitinase, as well as Actinobacteria, which are also known for degrading recalcitrant OM (Manucharova, 2009). Recent studies have documented that Actinobacteria from marine sediment possess enzymes to degrade refractory OM as well as different polysaccharide compounds (Chen et al. 2016). Significant seasonal fluctuations in bacterial communities reported in the southern Benguela upwelling region imply that bacterial communities alter depending on the nature of the OM present in the water column (Rocke et al. 2020). Recent metagenomic studies have revealed that microbial communities and metabolism potentials differ between the depth zones of the water column, with microbial communities in the mesopelagic zone having a higher metabolic potential to degrade amino acids, lipids, nucleotides and xenobiotics (Sripan et al. 2021). Though metagenomics approaches have greatly enhanced our understanding of bacterial diversity, there is still a large diversity that is still unexplored. It is also very vital to cultivate native, bacteria from marine habitats using media to mimic the native habitat and to understand the metabolic processes (Giovannoni and Stingl, 2007). Such studies can help in establishing the importance of phylogenetic diversity to its ecological functioning.

2.5. Conclusion

This study investigated the bacterial dynamics along the WCI at Goa, Mangalore and Trivandrum, from the continental shelf, slope and off-shore region during the non-monsoon and monsoon seasons. Seasonal variations in the bacterial parameters such as abundance, viable counts and radioactivity-based *in-situ* primary and bacterial productivity were estimated, along with the physicochemical characteristics. Statistical analysis shows a significant correlation between primary and bacterial productivity. Significant seasonal variations could also be established for the physicochemical parameters such as dissolved oxygen, nitrate, nitrite and phosphate, fluorescence values representing the chlorophyll concentration, bacterial abundance, viable counts and productivity. Taxonomic diversity studies on the cultivable bacterial morphotypes have shown that Proteobacteria, Firmicutes and Actinobacteria were the major phyla found

during both the non-monsoon and monsoon seasons and Bacteroidetes, was represented only in the non-monsoon season. The bacterial community structure at phylum, genus and operational taxonomic unit levels also changed with higher diversity in the monsoon season. However, the hydrolytic enzyme activity of the bacterial morphotypes was comparatively lower during the monsoon season probably due to the active primary production. However, we still lack an understanding of the factors that influence extracellular enzyme activity in marine habitats. It is interesting to note that monsoon influences the abundance and culturable diversity of heterotrophic bacteria positively and decreases bacterial activity along the WCI. Though this study has improved the understanding of the monsoonal influence on the bacterial dynamics along the WCI, further studies based on metagenomic and transcriptome analysis are required to get a better understanding of the role played by the bacterial community from this region.

Chapter 3.

Bacterial dynamics and community structure based on unculturable diversity along the west coast of India.

Abstract

An increase in primary productivity is recorded annually during the southwest monsoon season in the West coast of India (WCI). The influence of seasonal variations in primary productivity on bacterial dynamics in coastal and offshore regions off Goa, Mangalore and Trivandrum at chlorophyll maxima (C-Max) depths was explored during the non-monsoon and productive monsoon season. The physicochemical characteristics exhibited significant seasonal differences, shallow mixed layer depth and higher primary productivity was recorded during the monsoon season. A significant positive correlation between the in situ primary productivity, estimated using the radiotracer method and bacterial parameters shows the influence of the former on the latter in the study region. Based on metagenomic analysis, bacterial diversity was assessed in the C-Max depths, which recorded the highest viable counts during both seasons. Taxonomic profiles showed that the most dominant bacterial communities identified from the study region include the Proteobacteria and Firmicutes during the non-monsoon season and Cyanobacteria followed Proteobacteria, Bacteroidetes, Actinobacteria and candidatus phyla during the monsoon season. Canonical correspondence analysis (CCA) showed the influence of primary productivity parameters on bacterial communities with distinct seasonal variations. Seasonal variations in the primary productivity also significantly influenced the functional profiles of the bacterial communities. It was evident that pathways related to heterotrophic metabolism were represented in the non-monsoon and monsoon season, however, during the monsoon season prokaryotic autotrophic activities were predominantly higher. These observations underscore the significant influence of primary productivity variations on bacterial abundance and community composition in the WCI.

3.1. Introduction

Heterotrophic bacteria are known to contribute significantly to the marine food webs and biogeochemical process as they play a significant role in recycling the organic matter, which is fixed by the primary producers (Lønborg et al. 2019; Heinrichs et al. 2020). Through the 'microbial loop,' they break down the particulate organic matter into the dissolved form, thus providing necessary nutrients for the growth and proliferation of phytoplankton, which forms the basis of the marine food web (Wiggert et al. 2005; Azam and Malfatti, 2007). Bacterial extracellular enzymes initiate microbial remineralization throughout the water column and sediment. They catalyze the breakdown of high molecular weight organic matter into smaller compounds that can be effectively re-utilized (Hach et al. 2020; Liu & Liu, 2020). A portion of the re-mineralized organic matter is integrated into the biomass of various marine biological entities, another portion is converted to CO_2 and a smaller fraction is converted into refractory organic matter (Jiao et al. 2011). Evaluating the processes that influence the structure of bacterial communities on spatial and temporal scales is essential for understanding the recycling of organic matter in marine ecosystems (Danovaro et al. 2017).

Studies on the bacterial dynamics associated with the organic matter generated due to varying primary productivity from upwelling regions such as the Benguela upwelling system, Northern South China Sea and Ría de Vigo, Spain, using genomics tools have shown the association of dominant bacterial communities, including Alphaproteobacteria, Gammaproteobacteria, Bacteroidetes and Planctomycetes (Kuypers et al. 2005; Cai et al. 2007; Alonso-Gutiérrez et al. 2009). Recent Next-generation sequencing (NGS) technologies have provided valuable advancements in understanding bacterial communities and allowing an in-depth examination of the functional profiles and their dynamics. These studies have found bacterial communities including Proteobacteria, Cyanobacteria, Bacteroidota, Firmicutes, Actinobacteria and certain previously unidentified Candidatus phyla prevalent in diverse regions such as the South China Sea (Jing et al. 2013; Liu et al. 2022), northern Benguela upwelling (Bergen et al. 2015) and Canary current system (Thiele et al. 2019). These studies highlight the participation of specific bacterial communities in the breakdown of phytoplankton biomass. NGS technologies have enhanced the understanding of the active groups and have revealed the ecological significance of rare phylotypes such as SAR11,

Alteromonadales and Vibrionales in their response to organic matter availability (Gutiérrez-Barral et al. 2021; Mena et al. 2022; Gong et al. 2022).

The West coast of India (WCI), is influenced by semi-annual changes in the wind pattern which leads to an increased primary productivity, annually from June to September, during the southwest monsoon season (Smitha et al. 2008; Gupta et al. 2016). Despite technological advancements, a large part of the bacterial diversity from the WCI remains unexplored, with many unanswered questions related to the microbial carbon pump. Studies on the bacterial communities from this region have primarily focused on specific regions, such as the oxygen minimum zones (Bandekar et al. 2018; Fernandes et al. 2020; Lincy and Manohar, 2021), the mud bank regions (Anas et al. 2021; Vijayan et al. 2022) and estuarine habitats (Khandeparker et al. 2017; Kuchi et al. 2021). Studies have shown the influence of monsoon and seasonal variations of primary productivity on the bacterial community structure based on culturable approaches in the estuarine, coastal and open ocean waters along the WCI (Anas et al. 2021; Vijayan et al. 2022; Parab et al. 2022). Prior investigation, on the culturable bacterial diversity along the WCI, showed higher bacterial abundance during the monsoon season and with the highest colony-forming units (CFUs) from the C-Max depths (Parab et al. 2022). The C-Max is a prominent feature of tropical marine environments occurring between 20-100 m water depth with maximum primary productivity, increased organic load and higher bacterial activity (Cullen, 2015). However, there is limited research from the WCI, on the impact of seasonal variations of primary productivity on the bacterial communities using high-throughput sequencing techniques. To address this knowledge gap, this study aimed to comprehensively analyze the impact of increased productivity on the bacterial dynamics and functional profiles along one of the most productive zones, the WCI, during non-monsoon and monsoon seasons.

3.2. Methodology

3.2.1. Study location, sample collection and assessment of physicochemical parameters

Sampling was conducted during oceanographic cruises onboard RV Sindhu Sankalp during the non-monsoon season in February 2019, Cruise # SSK-125 and in the

southwest monsoon season in September 2019, Cruise # SSK-131 along the WCI. Samples were collected between 8°N and 16°N and 72°N to 76°E, at three stations: off Goa, Mangalore and Trivandrum (Figure 1.5). At each station, samples were collected from a coastal location at 30 m water depth and an off-shore location at 600 m depth. The sampling locations in the coastal and off-shore regions of Goa are designated as G30 and G600 respectively. Similar to this, sampling locations off Mangalore and Trivandrum stations are designated as M30, M600 and T30, T600 respectively (Figure 1.5). At each of the six sampling locations, the physicochemical characteristics, including water column temperature, salinity, dissolved oxygen (DO) and fluorescence, were measured using the conductivity-temperature-depth (CTD) underwater profiler (Sea-Bird, SBE-911 plus, USA) in both the seasons following standard methodologies as described in Chapter 2, section 2.2.

3.2.2. Estimation of chlorophyll and primary productivity

Chlorophyll-a (Chl-a) concentrations in the water column at discreet depths were determined fluorometrically following a standard protocol of the Joint Global Ocean Flux Study (JGOFS, UNESCO 1994). For this, 1 L of seawater sample from each depth was filtered using a 0.4 µm, 47 mm GF/F filter (Whatman, USA) in triplicates. Chl-a was extracted from the filters using 10 mL of 90 % acetone through overnight incubation at 20°C in the dark. After the incubation, the samples were vortexed and centrifuged to remove cellular debris. The clear supernatant was collected and used for the estimation of chl-a concentration on a Trilogy Laboratory Fluorometer (Turner Designs, USA). The water column chl-a concentration from the euphotic zone was determined through the integration of chl-a values from the surface to 100 m depth and was expressed as mg m^{-2}. Primary productivity (PP) was assessed in water samples from coastal and off-shore locations in both seasons based on the *in-situ* radiotracer method using labelled inorganic C, $NaH^{14}CO_3$ following the standard protocol of the JGOFS (UNESCO 1994) as described in Chapter 2, section 2.2.

3.2.3. Estimation of bacterial productivity and dynamics

The rate of bacterial productivity (BP) at coastal and off-shore stations, was determined from all the sampling depths during both seasons based on the radiotracer method as outlined in the JGOFS protocols (UNESCO 1994). Bacterial abundance was estimated using epifluorescence microscopy. The bacterial counts were also converted to bacterial carbon (BC) using the conversion factor of 20 fg cell $^{-1}$ (Ducklow et al. 1995) and reported as mg C m^{-3}. The total viable counts (TVC) of heterotrophic bacteria present in the water samples were also determined based on the spread plate technique on Zobell Marine Agar (ZMA) expressed as colony-forming units (CFUs mL^{-1}) as described in Chapter 2, section 2.2.

3.2.4. Bacterial community and functional diversity analysis

Extraction of metagenomic DNA, library preparation and Illumina sequencing

For extraction of metagenomic DNA, 20 L of water samples from C-Max depths at each location were filtered through 0.22 mm Durapore filters (Millipore, USA) in duplicates using a Peristaltic pump (Millipore, USA) on board the research vessel. The filters were immediately stored in cryovials at -80°C in a DNA storage buffer containing 0.75M sucrose, 40 mM EDTA and 50 mM Tris (pH 8.3). DNA extraction was carried out using a FastDNA^{TM} SPIN kit (MP Biomedical, USA) according to the manufacturer's protocol. The quality of the metagenomics DNA extracted from each filter paper was tested, pooled together and a sufficient quantity of DNA was utilized for library preparation, which targeted the 16S rRNA gene V3-V4 region using specific proprietary primers (16S amplicon PCR forward primer: 5' TCGTCGGCAGCGTCAGATGTGTAT AAGAGACAGCCTACGGGNGGCWGCAG 3' and 16S amplicon PCR reverse primer: 5' GTCTCGTGGGCTC GGAGATGTGTATAAG AGACAGGACTACHVGGGTATC TAATCC 3'). Illumina PCR sequencing adapter sequences used were, Index 1 Read 5′ CAAGCAGAAGACGGCATA CGAGAT[i7]GTCTCGTGGGC TCGG and Index 2 Read 5′ AATGATACGGCGACC ACCGAGATCTACAC [i5] TCGTCGGCAGCGTC (Qu et al. 2018; Bora et al. 2022). The 16S rRNA gene libraries generated were sequenced on the NovaSeq 6000 v1.5 Illumina sequencing platform (Illumina Inc., USA) at the National Institute of Biomedical Genomics, West Bengal, India. The sequences of the amplicons of about 250 bp obtained from the C-Max depths from all the locations during both seasons were processed with a standard pipeline using the QIIME2 tool (Bolyen et

al. 2019). The raw reads were initially quality filtered and primers were removed using Cutadapt v4.2. After quality filtration, the ASV file was generated using the DADA2 tool v3.16 and taxonomic profiling of the operational taxonomic units (OTU) was carried out against the V3-V4 classifier.

Functional diversity analysis

Functional diversity of bacterial communities was derived from marker gene sequences using the PICRUSt2 software, version 2.4.1. These acquired gene profiles were subsequently categorized into KEGG pathways (Douglas et al. 2020). The PICRUSt tool facilitated the prediction of potential functionalities from 16S rRNA sequence data. Employing the KEGG database, closed reference OTU picking was performed against the Greengenes taxonomy. The predicted functional profiles with gene profiles were further grouped into KEGG pathways, following PICRUSt protocols. Additionally, to measure the variance between reference genomes and the inferred metagenome, NSTI (Nearest Sequenced Taxon Index) scores were determined.

3.2.5. Data analysis

Statistical analyses were performed using SPSS v26, to investigate the significance of the distribution between physicochemical and biological characteristics in the two seasons using a single-factor analysis of variance (ANOVA). Pearson's correlation analysis was employed to interpret relationships within the physicochemical and biological parameters between the two seasons, at a significance level of 5 % ($P < 0.05$). Alpha diversity indices and Beta diversity between two seasons at the OTU level were calculated using Non-metric Multidimensional Scaling Analysis (NMDS) based on Bray–Curtis distance using the R package Phyloseq v1.22.3 (McMurdie and Holmes, 2013). The significance of the distribution of bacterial community between non-monsoon and monsoon season were estimated using STAMP bioinformatics software v2.1.3 (Parks et al. 2014). Heatmap of bacterial communities at a class level was plotted using hierarchical clustering analysis based on the Euclidean similarity index in R package Pheatmap v1.0.12. The R packages: ggplots2 and were used for the visualization of the data. All these analyses were performed in R studio v3.5.1 (R Core Team, 2015). Bacterial community structure at the genera level, along with environmental variables of the non-

monsoon and monsoon season, were analyzed based on canonical correspondence analysis (CCA) using PAST software v3.23 (Hammer et al. 2006).

3.3. Results

3.3.1. Physicochemical parameters

The physicochemical parameters varied significantly between the non-monsoon and monsoon seasons. During the non-monsoon season, the temperature in the water column remained constant at coastal stations. In the monsoon season, the temperature decreased by 2 to 4 °C from the surface to the bottom of the water column at coastal stations. In the off-shore stations, the thermocline depth was observed between 80 and 100 m during the non-monsoon season and it was shallower at 25 to 50 m, during the monsoon season (Figure 3.1). During the non-monsoon season, halocline depth was observed at 80 ± 20 m in the off-shore stations, which decreased to 25 to 50 m during the monsoon season similar to the thermocline depth (Figure 3.1). The influence of salinity on the water column density was also studied based on Brunt-Vaisala frequency from both seasons (Appendix 3.1). Analysis of these physiochemical parameters clearly shows that the monsoon season has a shallower mixed layer depth (30 ± 20 m) than the non-monsoon season (80 ± 20 m). The average sea surface DO concentration at the coastal and off-shore locations was 4 ± 2 mg L^{-1} during the non-monsoon and monsoon seasons. In the coastal stations at 30 m, DO values were consistent throughout the water column during the non-monsoon season and in the monsoon season the DO values dropped to < 1 mg L^{-1} at mid-depth and nearly reached anoxic conditions at the bottom depths. In the off-shore stations (600 m depth) DO concentration reached close to anoxic conditions at the mixed layer depths (MLD) in both the the non-monsoon and monsoon seasons (Figure 3.1).

Fluorescence values recorded during the non-monsoon season throughout the water column was an average ~ 0.4 ± 0.3 mg m^{-3} and it reached a maximum of around 1 mg m^{-3} at the C-Max depth. In the monsoon season, the average value of fluorescence in the water column was ~ 0.9 ± 1 mg m^{-3} and it reached a maximum of ~ 6 mg m^{-3} at the C-Max depth (Figure 3.1). In the monsoon season, C-Max in coastal and off-shore stations was observed at approximately 10 m and 30 m respectively.

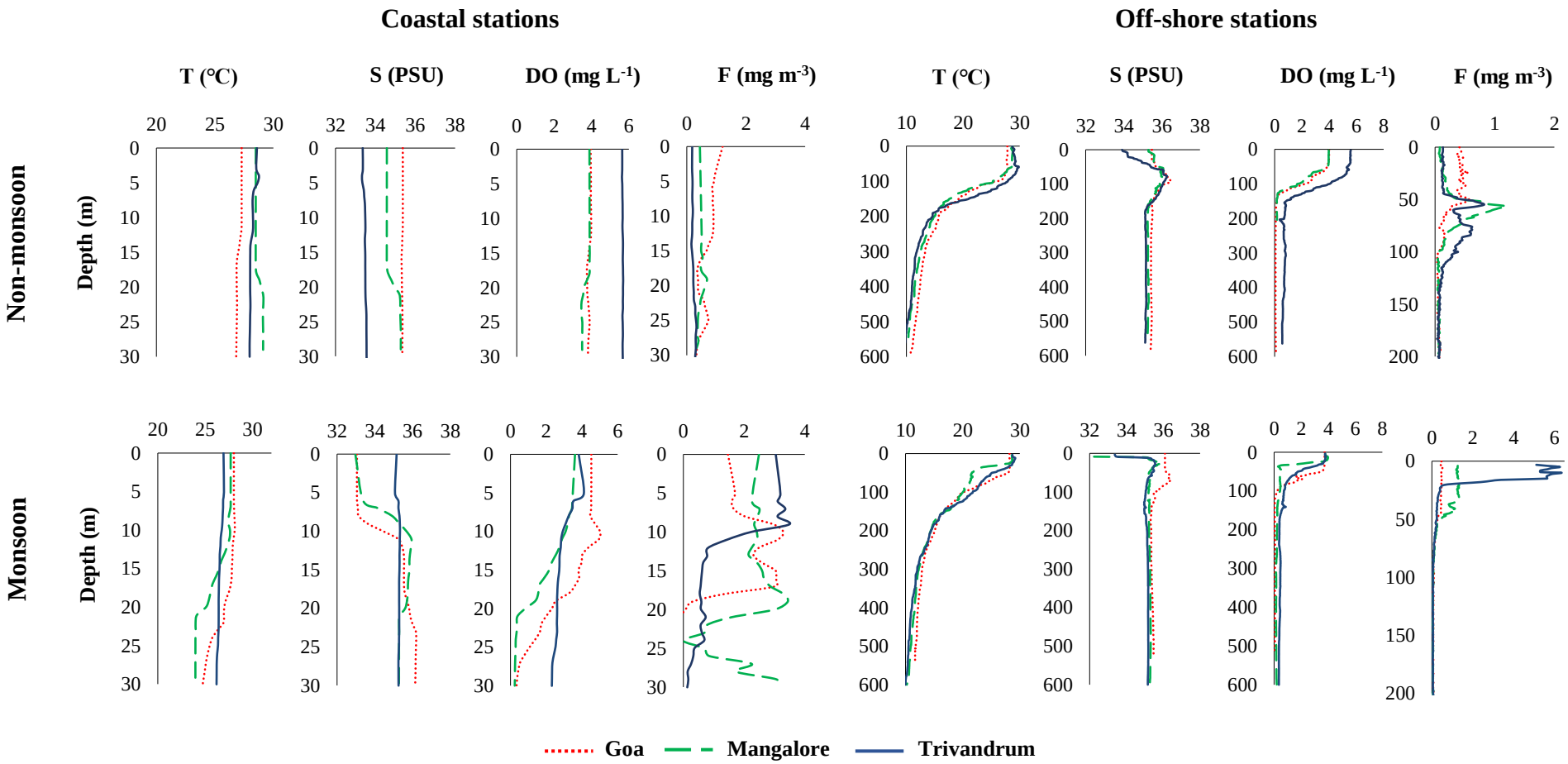


Figure 3.1: Profiles of physiochemical variables temperature (T), salinity (S), dissolved oxygen (DO) and fluorescence (F) in the water column of the coastal and off-shore stations during non-monsoon and monsoon season along the west coast of India.

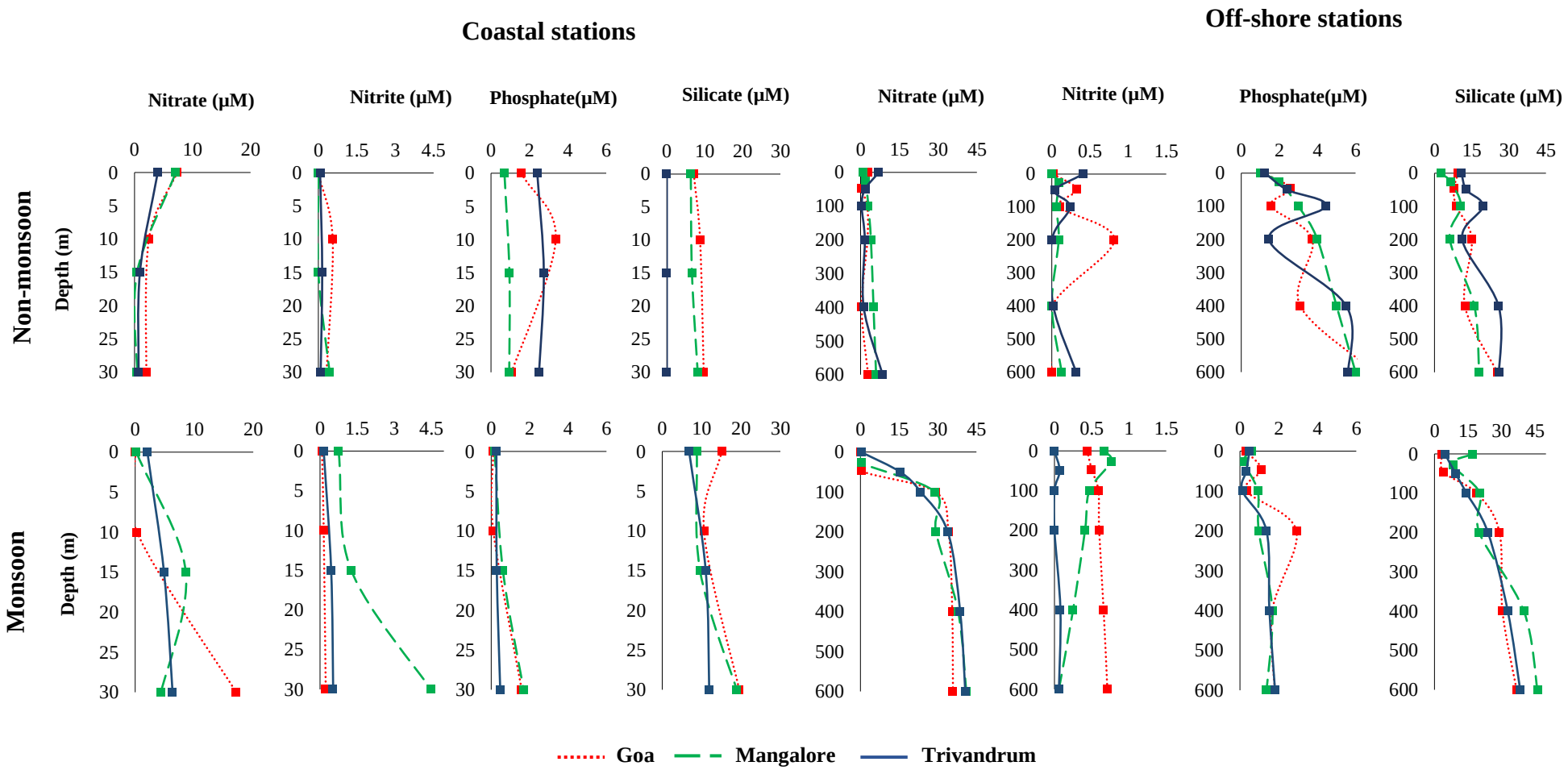


Figure 3.2. Profiles of nitrate, nitrite, phosphate and silicate in the water column of the coastal and off-shore stations during non-monsoon and monsoon season along the west coast of India.

C-Max depths in the water column varied during both seasons, which were about 15 m and 55 m during the non-monsoon season in the coastal and off-shore stations respectively. Higher nutrient concentration was also observed in the monsoon season in both the coastal and off-shore stations. The nitrate concentration, measured at all stations during the non-monsoon season, had an average of around 2.55 ± 2.47 µM, which increased to ~ 17.37 ± 16.14 µM in the monsoon season (Figure 3.2). The average nitrite concentration along all stations was ~ 0.16 ± 0.2 µM during the non-monsoon season and increased in the monsoon season to ~ 0.53 ± 0.85 µM. The average phosphate concentration in the water column during the non-monsoon and monsoon seasons was ~ 3 ± 2.8 µM and 0.92 ± 0.73 µM respectively (Figure 3.2). The average silicate values detected during the non-monsoon and monsoon seasons were ~10.83 ± 7.30 µM and ~18.93 ± 12 µM, respectively.

3.3.2. Primary productivity and bacterial dynamics

Chl-a concentration estimated fluorometrically from discreet water depths ranged from 0.002 to 0.16 mg m^{-3} in the non-monsoon and monsoon season it was estimated to be 0.003 to 1.05 mg m^{-3} (Appendix 3.4) Column integrated chl-a in the euphotic zone calculated for coastal and off-shore stations was observed to be 5.64 ± 2 mg m^{-2} in the non-monsoon and it increased to 11.42 ± 6 mg m^{-2} during the monsoon seasons (Figure 3.3a). The PP in the water column during the non-monsoon season varied between 1.20 to 145.38 mg C m^{-3} d^{-1}, while during the monsoon season, it ranged from 29.62 to 266.24 mg C m^{-3} d^{-1} (Appendix 3.4). The column-integrated PP in the euphotic zone was 800.31 ± 538 mg C m^{-2} during the non-monsoon season and the PP estimated during the monsoon season was higher and estimated to be 3355.60 ± 1608 mg C m^{-2} (Figure 3.3b). The bacterial activity in both seasons was estimated by quantifying the bacterial abundance and productivity. The estimated BP did not vary much between the seasons which was about 10 ± 2 mg C m^{-3} d^{-1} in the coastal and off-shore stations (Table 3.1). Bacterial counts were determined microscopically and were expressed as bacterial carbon, which was estimated to be 209.88 ± 47 mg C m^{-3} during the non-monsoon season and it increased to an average of 1017.62 ± 132 mg C m^{-3} in monsoon season (Table 3.1).

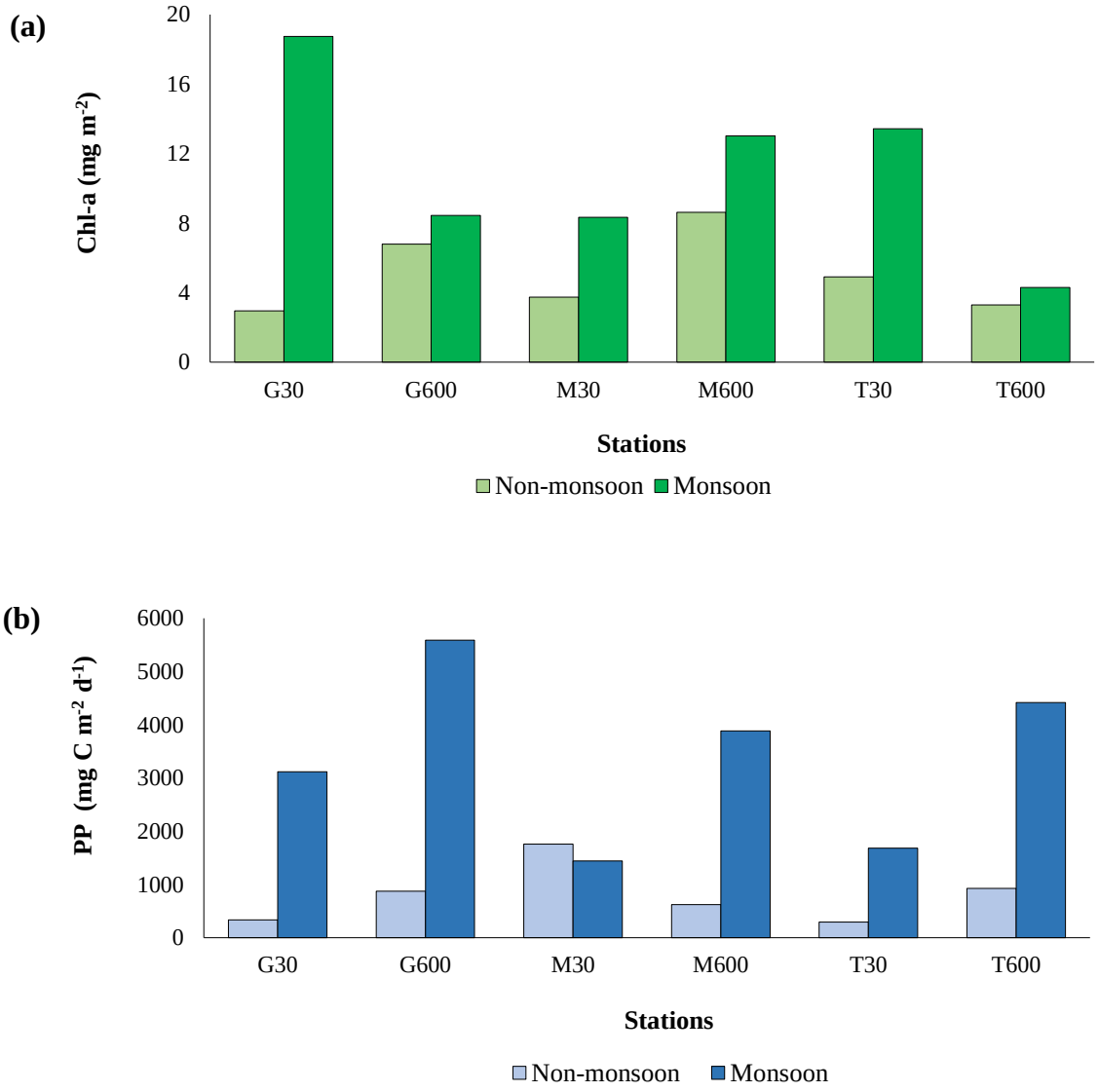


Figure 3.3: Column integrated (a) chlorophyll-a (Chl-a) concentration and (b) primary productivity (PP) in the water column of the coastal and off-shore stations during non-monsoon and monsoon season along the west coast of India.

TVC was enumerated to estimate the viable bacterial counts in the water column from all the stations. An average of 4.16 $\times 10^3$ CFU mL^{-1} in the non-monsoon season was recorded and it increased to 11.39 $\times$ 10^3 CFU mL^{-1} during the monsoon season (Figure 3.4). The highest bacterial CFUs were obtained from the C-Max depths from almost all the locations sampled along the WCI (Figure 3.4). An increasing trend of the bacterial carbon biomass and CFUs could be due to the higher primary productivity observed during the monsoon season. Statistical analysis based on the ANOVA test was carried out to evaluate the significant changes in different parameters during both seasons (Table 3.2).

Table 3.1: Average bacterial productivity (BP), bacterial abundance (BA) and bacterial carbon biomass (BC) in the water column of the coastal and off-shore stations during non-monsoon and monsoon season.

Stations	BP (mg C m^{-3} d^{-1})	BA (Cells × 10^9 L^{-1})	BC (mg C m^{-3})
Non-monsoon season			
G30	4.30 ± 5.19	10.64 ± 2.68	212.87 ± 53.52
G600	2.95 ± 1.33	11.31 ± 4.37	226.11 ± 87.48
M30	3.79 ± 2.72	13.65 ± 0.78	272.99 ± 15.65
M600	25.92 ± 10.99	6.36 ± 2.06	127.23 ± 41.14
T30	6.75 ± 0.93	10.97 ± 0.09	219.30 ± 1.73
T600	17.80 ± 9.53	10.04 ± 4.10	200.78 ± 82
Monsoon season			
G30	13.89 ± 0.79	52.94 ± 14.27	2657.00 ± 1436.22
G600	7.34 ± 2.26	57.09 ± 40.05	1682.00 ± 293.67
M30	7.13 ± 0.44	55.51 ± 10.33	1923.33 ± 158.31
M600	7.91 ± 2.04	41.25 ± 11.19	1580.00 ± 394.08
T30	14.51 ± 2.35	54.45 ± 26.72	2226.67 ± 156.86
T600	10.93 ± 2.27	44.05 ± 26.75	1756.50 ± 399.50

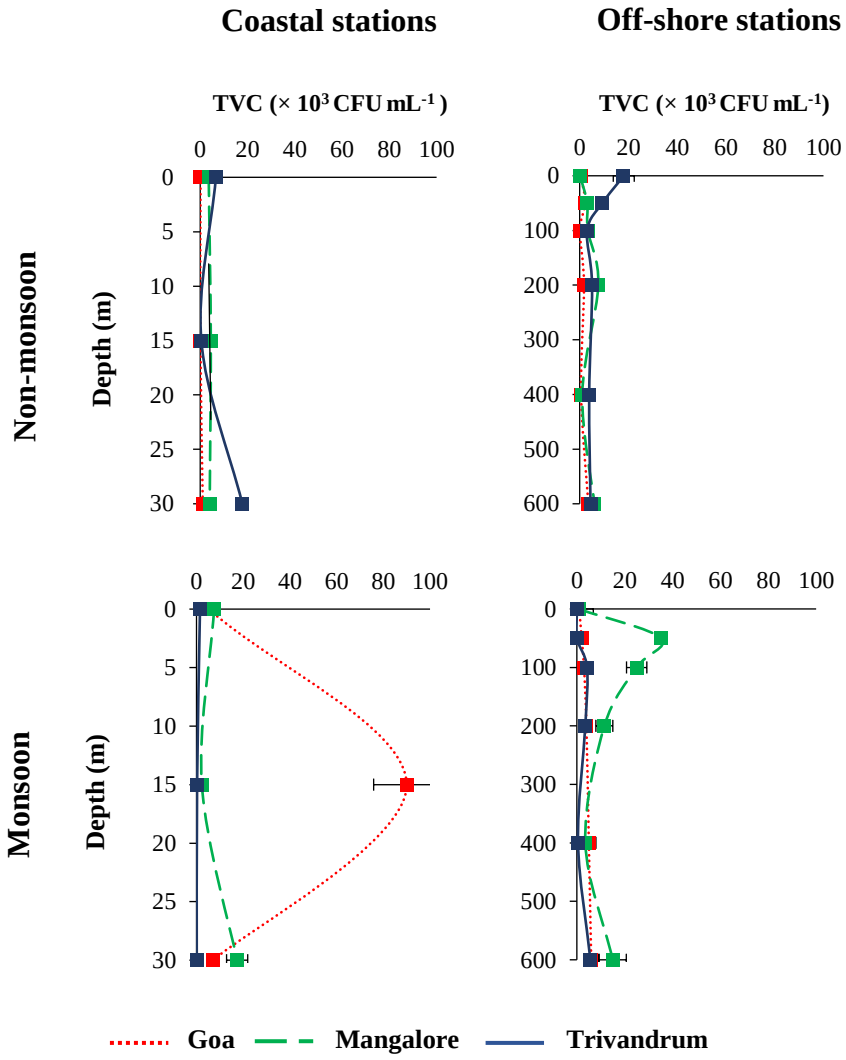


Figure 3.4: Total viable counts (TVC) of culturable bacteria in the water column of the coastal and off-shore stations during non-monsoon and monsoon season along the west coast of India.

Estimated environmental parameters including DO, fluorescence, nitrate, nitrite, phosphate, silicate, chl-a, PP, BC and TVC showed significant variations between seasons ($p < 0.05$). Correlation between environmental variables was also determined using Pearson's correlation matrix for the physicochemical and biological variables measured from water samples during non-monsoon and monsoon seasons (Table 3.3). A significant correlation was observed between the different environmental variables estimated during both seasons. Temperature and DO had a significant positive correlation with fluorescence. *In situ,* fluorescence concentration determined showed a significant positive correlation with PP and BC. Among the nutrients, nitrate and silicate were

positively correlated with Chl-a and PP showed a positive correlation with TVC and BC respectively (Table 3.3). These results indicate temperature has a significant impact on the fluorescence values which is a measure of the phytoplankton abundance. A significant positive correlation between the primary productivity variables and bacterial abundance and counts shows the influence of the former on the latter in the study area.

Table 3.2: Analysis of variance (ANOVA: single factor) assessing the seasonal difference in the physicochemical and biological parameters: temperature, salinity, dissolved oxygen (DO), fluorescence, nutrients, chlorophyll-a (chl-a), primary productivity (PP), bacterial productivity (BP), bacterial abundance (BA), bacterial carbon (BC) and total viable counts (TVC) between non-monsoon and monsoon season.

	df	**F**	**F crit**	**P-value**
Temperature	1.00	0.57	4.03	0.45
Salinity	1.00	0.02	4.03	0.88
DO	1.00	8.68	4.03	0.001**
Fluorescence	1.00	4.58	4.03	0.04*
Nitrate	1.00	22.34	4.03	< 0.001**
Nitrite	1.00	4.86	4.03	0.03*
Phosphate	1.00	14.39	4.03	< 0.001**
Silicate	1.00	9.14	4.03	< 0.001**
Chl-a	1.00	5.41	4.04	0.02*
PP	1.00	11.45	4.08	< 0.001**
BP	1.00	0.96	4.03	0.33
BA	1.00	70.05	4.03	< 0.001**
BC	1.00	70.05	4.03	< 0.001**
TVC	1.00	3.78	4.03	0.06*

Df: degree of freedom, F value greater than F critical value indicates statistical significance.

*: Significant at 0.1%., **: Significant at 1%.

Table 3.3: Pearson's correlation matrix of physicochemical and biological parameters, temperature (T), salinity (S), dissolved oxygen (DO), fluorescence (F), nutrients, Chlorophyll-a (Chl-a), primary production (PP), bacterial production (BP), bacterial carbon biomass (BC) and total plates counts (TVC) during non-monsoon and monsoon season.

	T	S	DO	F	Nitrate	Nitrite	Phosphate	Silicate	Chl-a	PP	BP
T	1										
S	-0.06	1									
DO	**.80****	-0.16	1								
F	**.43***	**-.40***	**.39***	1							
Nitrate	**-.72****	-0.01	**-.58****	-0.30	1						
Nitrite	-0.03	-0.01	-0.26	-0.05	-0.05	1					
Phosphate	0.01	0.31	0.16	-0.25	-0.14	-0.05	1				
Silicate	**-.59****	0.08	**-.45***	-0.35	**.76****	0.22	0.24	1			
Chl-a	-0.06	0.14	0.04	-0.02	0.15	-0.12	-0.10	0.11	1		
PP	0.13	**-.37***	0.003	**.72****	0.04	0.05	**-.44***	-0.03	0.24	1	
BP	-0.013	0.23	-0.03	-0.03	-0.18	-0.26	0.25	-0.16	0.04	-0.12	1
BC	-0.01	-0.30	-0.17	**.58****	0.24	0.17	**-.42***	0.13	0.14	**.90****	-0.23
TVC	0.00	-0.01	0.04	-0.11	-0.02	0.07	-0.17	0.03	**.55****	0.26	-0.04

**Correlation is significant at the 0.01 level (2-tailed). *Correlation is significant at the 0.05 level (2-tailed).

3.3.4. Alpha and Beta diversity of bacteria from the C-Max along the WCI

The bacterial diversity during the non-monsoon and monsoon seasons was assessed from the C-Max depths, which had the highest bacterial CFUs from almost all the locations sampled along the WCI (Figure 3.4) in this study. The bacterial sequences generated using next-generation Illumina sequencing techniques show that the number of reads was nearly similar during both seasons. However, OTUs generated after denoising the sequence reads were higher in the monsoon season (Appendix 3.5). The rarefaction curves, based on observable features, were curved toward the saturation plateau at the 97 % cut-off value, indicating that the sampling sizes were enough for the community analysis (Appendix 3.2). The average OTU abundance in the non-monsoon season was 646 OTUs and it increased in the monsoon season, to an average of 2286 OTUs (Appendix 3.5). For both non-monsoon and monsoon seasons, various alpha diversity indices for diversity and richness were calculated. Shannon, Chao1 and ACE indices were significantly higher during the monsoon season. The coastal stations showed considerably higher Shannon, Chao-1 and ACE diversity indices than the off-shore stations (Figure 3.5a). Statistical analysis of the diversity indices calculated also shows significant seasonal differences (Appendix 3.6).

Beta diversity analysis of bacterial communities during non-monsoon and monsoon seasons was performed based on the NMDS method. NMDS ordination differentiates the samples into abundance-weighted community compositions based on the Bray-Curtis distance. The bacterial communities were grouped based on the seasons and locations and NMDS analysis showed that sampling locations during the non-monsoon season were divided into two apparent groups (Figure 3.5b). The bacterial communities from the non-monsoon season of the Goa and Mangalore locations clustered together and a similar grouping was evident in the Trivandrum coastal and off-shore communities.

3.3.5. Bacterial community structure at C-Max along the WCI

Bacterial OTUs generated in Illumina sequencing were classified based on the Silva V3-V4 classifier for taxonomic profiling.

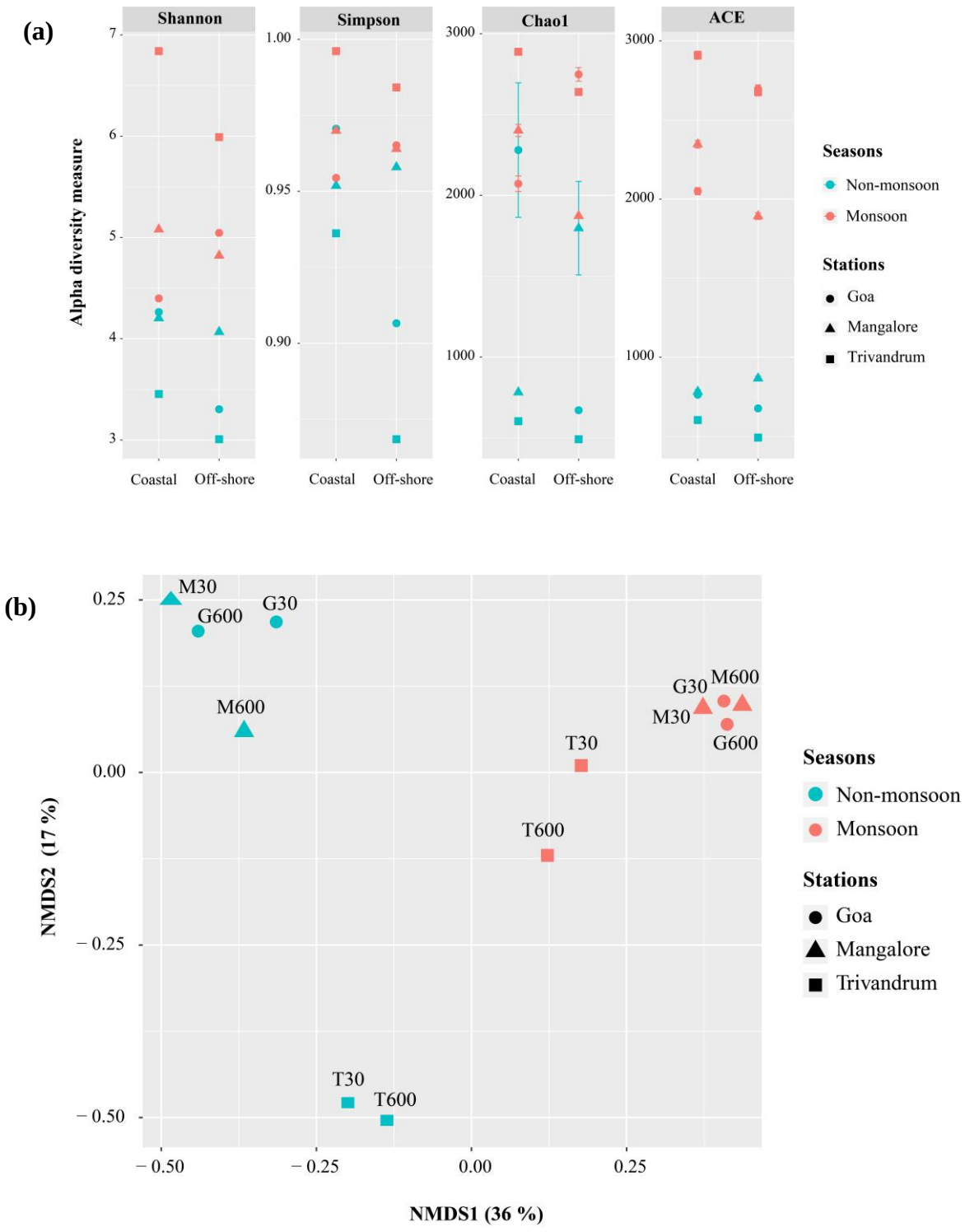
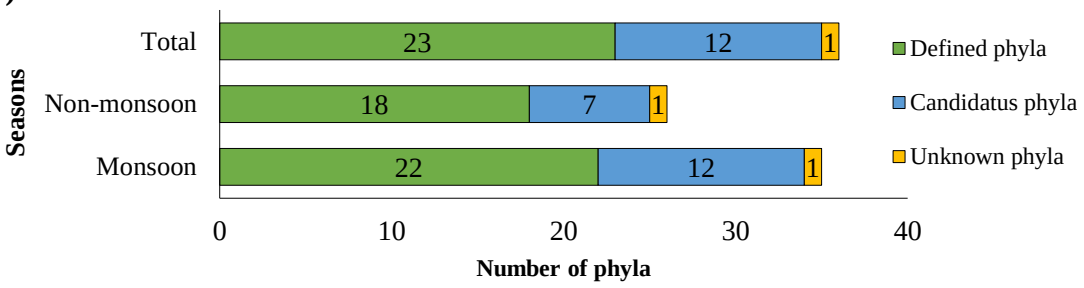


Figure 3.5: (a) Alpha diversity measure and (b) Non-metric multidimensional scaling (NMDS) plot (axis defines 2D space that allows the best spatial representation of sample distance, based on Bray–Curtis distance with stress = 0.08) depicting variations in the bacterial operational taxonomic units (OTUs) in the coastal and off-shore stations during monsoon and non-monsoon season along the west coast of India.

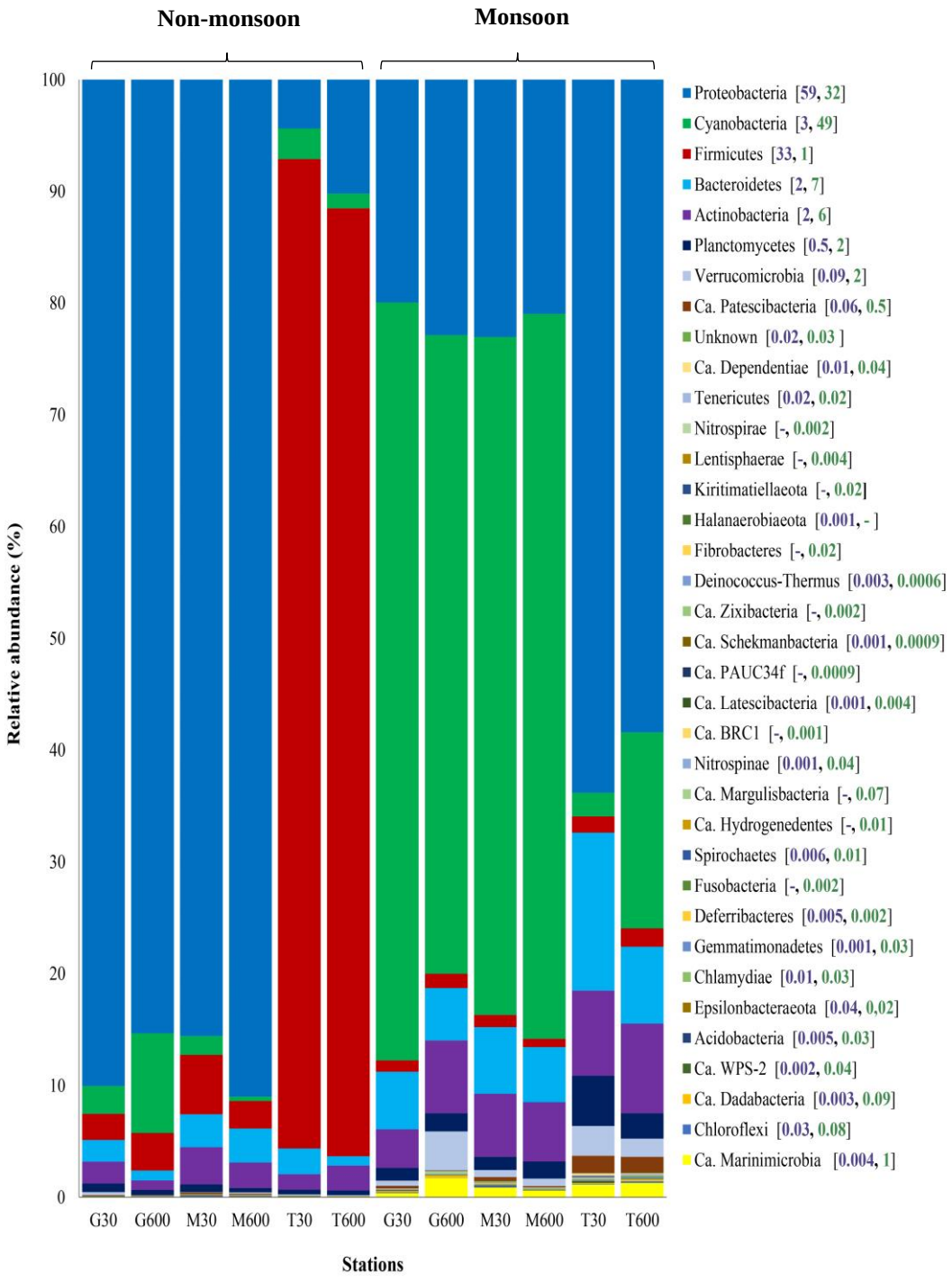
For bacterial classification, the sklearn classifier within QIIME was utilized, which operated on a confidence score range of 0.7 to 1. Based on taxonomic annotation, OTUs generated during both seasons belonged to 36 bacterial phyla in total, of which 26 phyla were present during the non-monsoon season and the number of phyla increased during the monsoon season to 35 (Figure 3.6a). In the non-monsoon season, 18 of the 26 phyla belonged to well-defined (Based on List of Prokaryotic names with Standing in Nomenclature (LPSN), 7 of them were of candidatus status and 1 uncharacterized phylum was identified. During the monsoon season, the number of well-defined phyla increased to 22, candidatus to 12 and one uncharacterized phyla was reported (Figure 3.6a). The dominant phyla observed in the C-Max depth in the non-monsoon season were Proteobacteria (59 %) and Firmicutes (33 %), while in the monsoon season, it was Cyanobacteria (49 %) followed by Proteobacteria (32 %) (Figure 6b). The proportion of Cyanobacteria communities increased significantly from 3 % in the non-monsoon season to 49 % during the monsoon season. Though the relative abundance of phylum Proteobacteria decreased during the monsoon season, it remained the second most dominant phyla. The relative abundance of phylum Firmicutes decreased considerably from 33 to 1 % during monsoon season. Bacteroidetes, Actinobacteria, Planctomycetes, Verrucomicrobia and candidatus phylum Marinimicrobia were also among the top 10 phyla observed during both seasons and their abundance increased by two to three folds during the monsoon season.

Among the well-defined phyla, about 5 bacterial phyla including Nitrospirae, Lentisphaerae, Kiritimatiellaeota, Fibrobacteres and Fusobacteria were specific to the monsoon season and only one phylum, Halanaerobiaeota was specific to the non-monsoon season. Higher bacterial diversity in the monsoon season was evident with an increase in the relative abundance of bacterial phyla, namely Nitrospinae, Spirochaetes, Gemmatimonadetes, Chlamydiae, Acidobacteria and Chloroflexi. A higher diversity of candidatus phyla was also recorded in the monsoon, which accounted for about 7 and 12 phyla from the non-monsoon and monsoon seasons respectively. The candidatus phyla including Ca. Patescibacteria, Ca. Dependencies, Ca. Schekmanbacteria, Ca. Latescibacteria, Ca. WPS-2, Ca. Dadabacteria, Ca. Marinimicrobia was found during both seasons. Most of the candidatus phyla that were commonly identified in both seasons also showed an increase in their relative abundance during the monsoon season.

(a)


Total
Non-monsoon
Monsoon
Seasons
23
12
1
18
7
1
22
12
1
Defined phyla
Candidatus phyla
Unknown phyla
0
10
20
30
40
Number of phyla

(b)


Non-monsoon
Monsoon
Relative abundance (%)
100
90
80
70
60
50
40
30
20
10
0
G30
G600
M30
M600
T30
T600
G30
G600
M30
M600
T30
T600
Stations
Proteobacteria [59, 32]
Cyanobacteria [3, 49]
Firmicutes [33, 1]
Bacteroidetes [2, 7]
Actinobacteria [2, 6]
Planctomycetes [0.5, 2]
Verrucomicrobia [0.09, 2]
Ca. Patescibacteria [0.06, 0.5]
Unknown [0.02, 0.03]
Ca. Dependentiae [0.01, 0.04]
Tenericutes [0.02, 0.02]
Nitrospirae [-, 0.002]
Lentisphaerae [-, 0.004]
Kiritimatiellaeota [-, 0.02]
Halanaerobiaeota [0.001, -]
Fibrobacteres [-, 0.02]
Deinococcus-Thermus [0.003, 0.0006]
Ca. Zixibacteria [-, 0.002]
Ca. Schekmanbacteria [0.001, 0.0009]
Ca. PAUC34f [-, 0.0009]
Ca. Latescibacteria [0.001, 0.004]
Ca. BRC1 [-, 0.001]
Nitrospinae [0.001, 0.04]
Ca. Margulisbacteria [-, 0.07]
Ca. Hydrogenedentes [-, 0.01]
Spirochaetes [0.006, 0.01]
Fusobacteria [-, 0.002]
Deferribacteres [0.005, 0.002]
Gemmatimonadetes [0.001, 0.03]
Chlamydiae [0.01, 0.03]
Epsilonbacteraeota [0.04, 0.02]
Acidobacteria [0.005, 0.03]
Ca. WPS-2 [0.002, 0.04]
Ca. Dadabacteria [0.003, 0.09]
Chloroflexi [0.03, 0.08]
Ca. Marinimicrobia [0.004, 1]

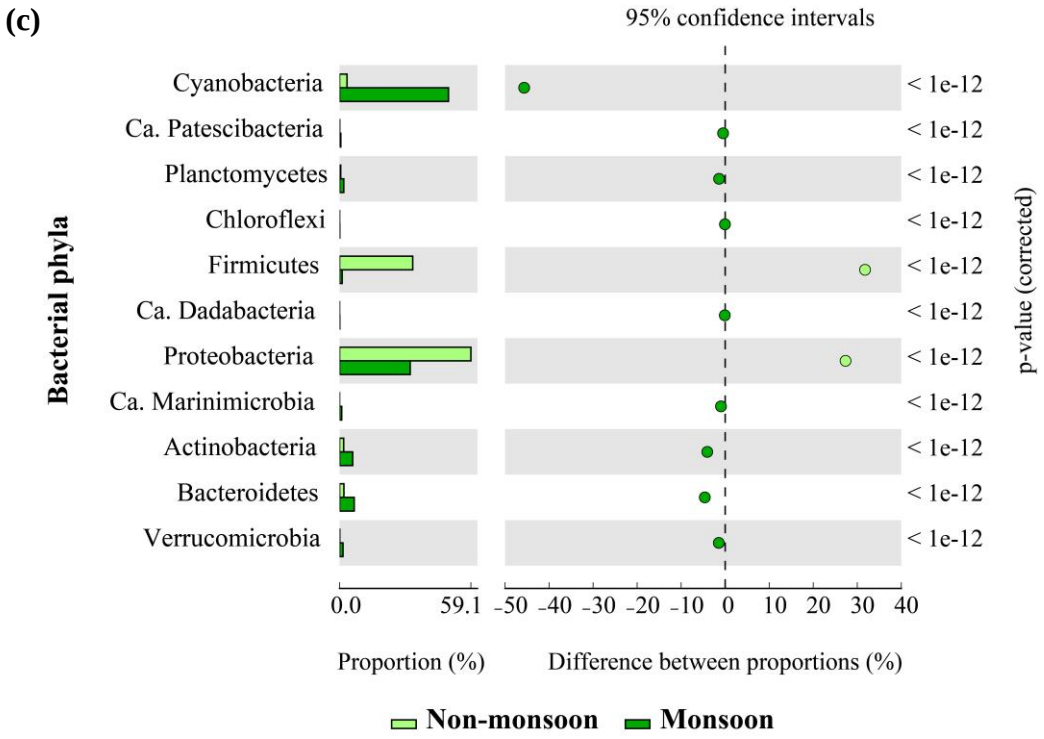


Figure 3.6: (a) Distribution of total bacterial phyla as Defined, Candidatus and Unknown taxa, (b) Relative abundance of bacterial phyla (number in parentheses indicates the relative abundance in the non-monsoon and monsoon season) and (c) Distribution of bacterial phyla showing significant statistical difference during the non-monsoon and monsoon season.

Candidatus phyla including Ca. Zixibacteria, Ca. PAUC34f, Ca. BRC1, Ca. Margulisbacteria and Ca. Hydrogenedentes were exclusively present during the monsoon season (Figure 3.6b). These results suggest that the monsoon season had a higher diversity of both defined and Candidatus phyla (Figure 3.6). The temporal differences in bacterial communities were observed in 8 well-defined and 3 candidtus phyla. Proteobacteria and Firmicutes were identified as the dominant phyla during the non-monsoon season with statistical significance ($p < 0.001$). Cyanobacteria, Actinobacteria, Bacteroides, Verrucomicrobia, Planktomycates, Cloroflexi and candidatus phyla Patescibacteria, Dadabacteria and Marinimicrobia are shown to have a major contribution during the monsoon season with a statistical significance of $p < 0.001$ (Figure 3.6c).

Distribution of the bacterial OTUs at the class level was also investigated using hierarchical clustering of the heatmap, based on the Euclidian similarity index for the top 5 phyla (Appendix 3.3:). Within the Proteobacteria phylum, the bacterial class

Gammaproteobacteria was dominant during both seasons, followed by Alphaproteobacteria and Deltaproteobacteria. Although Gammaproteobacteria was dominant during both seasons, their relative abundance reduced from 48 to 17 % during the monsoon season and there was a marginal increase in the abundance of Alphaproteobacteria and Deltaproteobacteria. The diversity of phylum Firmicutes decreased during the monsoon season and distinct differences in the class level were also observed with increased representation of class Bacilli and Clostridia during the non-monsoon which decreased during the monsoon season.

The monsoon season was marked by a higher abundance of phylum Cyanobacteria, which was represented by class Melainabacteria, Oxyphotobacteria and Sericytochromatia (Appendix 3.3). Representation of classes of phyla Actinobacteria (classes Acidimicrobiia, Actinobacteria, Coriobacteriia, Nitriliruptoria, Rubrobacteria, Thermoleophilia and an unknown class) and phylum Bacteroidetes (class Bacteroidia, Ignavibacteria and Rhodothermia) also increased during the monsoon season (Appendix 3.3). The other predominant representatives were classes Planctomycetacia and Verrucomicrobiae of phylum Planctomycetes and Verrucomicrobia respectively in both seasons. A few classes belonging to phylum Planctomycetes and Candidatus phylum Marinimicrobia (SAR406 clade), namely Ca. Patescibacteria and Ca. Margulisbacteria were exclusively present during the monsoon season.

A significant difference in the distribution of the bacterial classes was observed between both seasons, with higher diversity in the monsoon season (Appendix 3.3). To further investigate the community structure of bacterial populations, bacterial communities were categorized at order and family levels. During the non-monsoon season, the most abundant bacterial family was Moraxellaceae, belonging to the order Pseudomonadales of Gammaproteobacteria. During the monsoon season, order Synechococcales of the family Cyanobiaceae belonging to phylum Cyanobacteria was the predominant autotrophic community. The most abundant heterotrophic bacterial community at the order level in this season was the SAR86 clade from Gammaproteobacteria and Flavobacteriales from phylum Bacteroidetes.

3.3.6. Correlation between environmental variables and bacterial communities

The influence of environmental and primary productivity parameters on bacterial community structure at the genera level was evaluated for both the non-monsoon and monsoon seasons using CCA (Figure 3.7).

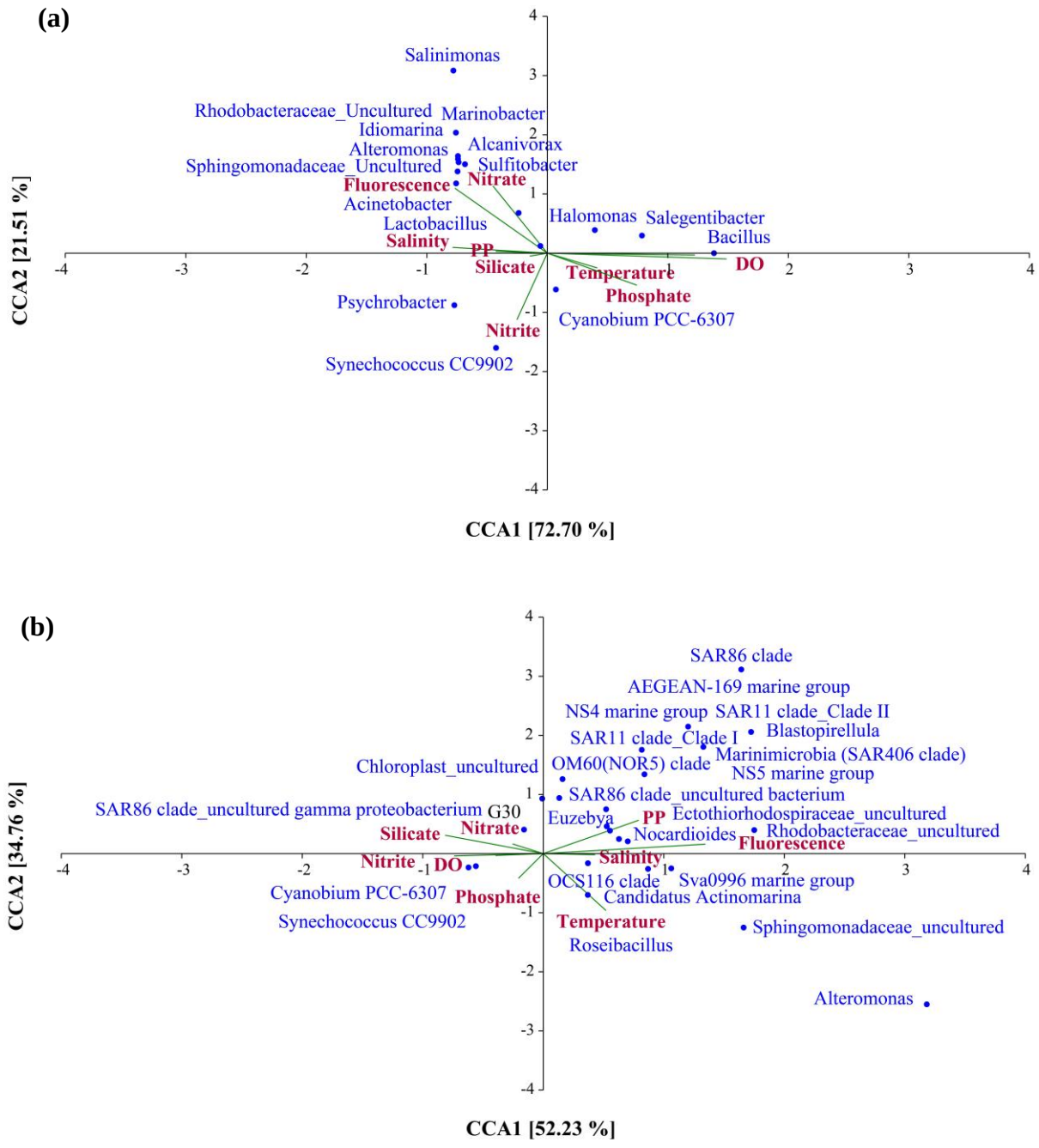


Figure 3.7. Canonical correspondence analysis (CCA) ordination plot of dominant bacterial genera and environmental variables temperature, salinity, dissolved oxygen (DO), fluorescence, chlorophyll-a (chl-a), primary productivity (PP) and nutrients during (a) non-monsoon and (b) monsoon season.

For the analysis, bacterial genera with a relative abundance of more than 0.5 % were plotted along with temperature, salinity, DO, fluorescence and nutrient concentrations measured during both seasons. Based on the CCA ordination results, the environmental factors considered in this study accounted for 94.21 % and 88.99 % of the variation in the community during the non-monsoon and monsoon seasons respectively. In both seasons, nutrients correlated with the autotrophic bacterial genera such as *Cyanobium* PCC-6307, *Synechococcus* CC9902 and Chloroplast_unclassified belonging to Cyanobacteria phylum. The heterotrophic community was influenced by the primary productivity in both seasons, but there were significant seasonal differences in the bacterial groups. During the non-monsoon season, the dominant bacterial genera included *Salinimonas, Marinobacter*, *Idiomarina*, *Alcanivorax*, *Alteromonas*, *Acinetobacter* from Gammaproteobacteria; *Sulfitobacter, Rhodobacteraceae_uncultured* and *Sphingomonadaceae_uncultured* of Alphaproteobacteria *Bacillus* and Lactobacillus of phylum Firmicutes and S*alengentibacter* of Bacteroidetes were also among the major contributing genera during the non-monsoon season (Figure 3.7a). During the monsoon season, the majority of the bacterial genera belonged to candidatus or unclassified novel marine groups. This included including SAR86 clade, OM60 (NOR5), Ectothiorhodospiraceae_uncultured, from class Gammaproteobacteria; AEGEAN-169 marine group, SAR11 clade_Clade I, Rhodobacteraceae_uncultured, from class Alphaproteobacteria of Proteobacteria. Alteromonas, a Gammaproteobacteria was the only taxa that was listed as the major contributor from both seasons. Representatives of phylum Bacteroidetes including NS4 and NS5 marine groups; bacterial genera Sva0996 marine group and CA. *Actinomarina*, from phylum Actinobacteria; Roseibacillus of phylum Verrumicrobia and Ca. Marinimicrobia (SAR406 clade) were the dominant contributing taxa during the monsoon season (Figure 3.7b).

3.3.7. Functional diversity at C-Max along the WCI

The functional profiles of the bacterial communities showed that the predicted pathways belonged to a variety of vital classes of functions like metabolism, processing of genetic and environmental information and pathways for cellular processes during both seasons (Figure 3.8a). Significant variations in the abundance of functional genes were also evident between the non-monsoon and monsoon seasons. Further investigations into

seasonal changes in the gene profiles show that, during the non-monsoon season, there was a significant increase in the abundance of metabolic pathways related to carbohydrate, lipid and amino acid metabolism, glycan biosynthesis and xenobiotic degradation. Pathways related to energy metabolism including, carbon fixation by prokaryotes, photosynthesis and membrane transport and biosynthesis of secondary metabolites such as antibiotics were abundant in the monsoon season (Figure 3.8b and c). Functional profiling of the pathways related to the cellular processes revealed that signalling pathways, biofilm formation and pathways related to cellular prokaryotic communities were abundant during the non-monsoon season. Pathways related to cell growth and death, cell motility and prokaryotic quorum sensing were uniformly distributed in both seasons (Figure 3.8c). Based on the functional gene profiles, it is evident that pathways related to heterotrophic metabolism were represented in the non-monsoon and monsoon season, however during monsoon season prokaryotic autotrophic activities were predominantly higher than in the non-monsoon season.

3.4. Discussion

Physicochemical characteristics determined in the study region along the WCI during the non-monsoon and monsoon seasons varied significantly. The water column temperature and salinity variations depicted that the mixed layer depth was much shallower in the monsoon season than in the non-monsoon season. This is a typical characteristic of the Arabian Sea region due to the seasonal monsoonal winds, which leads to increased primary productivity and accumulation of organic matter in this region (Smitha et al. 2008; Gupta et al. 2021). An increase in primary productivity parameters followed by high microbial abundance was observed in the monsoon samples from the study region. Studies have suggested that increased microbial activity was evident in the regions of high primary productivity for sustaining the food web and ecosystem functioning (Gauns et al. 2005; Lamont et al. 2014; Reji et al. 2020). The increase in viable bacterial counts during the monsoon season in the C-Max depths from the study suggests a strong dependence of the bacterial community on organic matter derived from phytoplankton.

(a)

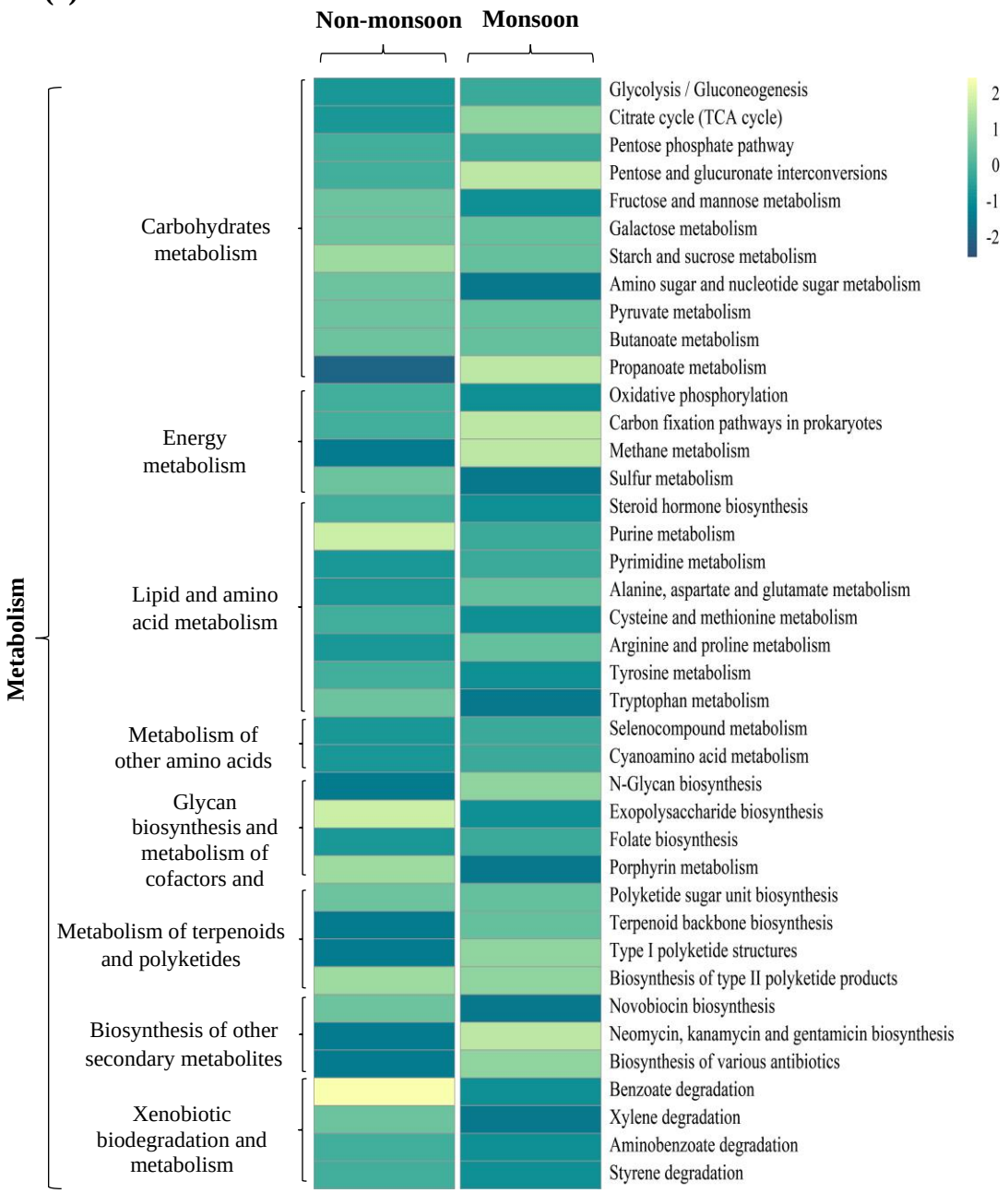

Non-monsoon
Monsoon
Metabolism
Carbohydrates metabolism
Energy metabolism
Lipid and amino acid metabolism
Metabolism of other amino acids
Glycan biosynthesis and metabolism of cofactors and
Metabolism of terpenoids and polyketides
Biosynthesis of other secondary metabolites
Xenobiotic biodegradation and metabolism
Glycolysis / Gluconeogenesis
Citrate cycle (TCA cycle)
Pentose phosphate pathway
Pentose and glucuronate interconversions
Fructose and mannose metabolism
Galactose metabolism
Starch and sucrose metabolism
Amino sugar and nucleotide sugar metabolism
Pyruvate metabolism
Butanoate metabolism
Propanoate metabolism
Oxidative phosphorylation
Carbon fixation pathways in prokaryotes
Methane metabolism
Sulfur metabolism
Steroid hormone biosynthesis
Purine metabolism
Pyrimidine metabolism
Alanine, aspartate and glutamate metabolism
Cysteine and methionine metabolism
Arginine and proline metabolism
Tyrosine metabolism
Tryptophan metabolism
Selenocompound metabolism
Cyanoamino acid metabolism
N-Glycan biosynthesis
Exopolysaccharide biosynthesis
Folate biosynthesis
Porphyrin metabolism
Polyketide sugar unit biosynthesis
Terpenoid backbone biosynthesis
Type I polyketide structures
Biosynthesis of type II polyketide products
Novobiocin biosynthesis
Neomycin, kanamycin and gentamicin biosynthesis
Biosynthesis of various antibiotics
Benzoate degradation
Xylene degradation
Aminobenzoate degradation
Styrene degradation
2
1
0
-1
-2

Figure 3.8: Heatmap showing functional pathways during the non-monsoon and monsoon season, (a) related to metabolism and (c) pathways related to genetic, environmental information processes and cellular processing.

3.4.1. Seasonal variation in the bacterial diversity and community structure

Metagenomic analysis of the bacterial diversity showed that, during the non-monsoon season, only 26 of the total 36 phyla identified in this study were represented and about 35 phyla were represented in the monsoon season. The diversity indices also showed that the monsoon season had a higher diversity in the coastal and offshore stations (Fig. 4). The analysis of the bacterial community showed significant seasonal variations at the Phylum, class and genus level determined in this study. The most dominant bacterial communities identified from the study region include the Proteobacteria and Firmicutes during the non-monsoon season and Cyanobacteria, Proteobacteria followed by Bacteroidetes, Actinobacteria and candidatus phyla during the monsoon season (Fig. 5). Proteobacteria, the most prevalent heterotrophic bacterial group exhibited community shift and significant seasonal variations with Gammaproteobacteria being the dominant class during the non-monsoon season. Reports from various marine ecosystems have observed an increase in the relative abundance of Gammaproteobacteria which can be attributed to their remarkable ability to adapt and thrive under conditions of limited organic matter availability through the secretion of extracellular hydrolysis enzymes, enabling them to access and metabolise complex substrates present in the marine environment (Spring et al., 2015; Liu and Liu, 2020, (Cho and Giovannoni, 2004). Additionally, these bacteria possess the capacity to utilize alternative sources of energy for growth (Cho et al., 2007; Chen et al., 2021). Proteobacterial class, Alphaproteobacteria and Deltaproteobacteria abundance increased significantly during the monsoon season (Fig. S5). These groups have been identified as particle-degrading microorganisms, which suggests their potential significance as key contributors to the biogeochemical cycling of carbon fixed by primary producers during upwelling. Their mutualistic interactions show that they play an important role during the monsoon season in the breakdown of organic matter and enriching the nutrient cycles (Sheik et al., 2013; Aldunate et al., 2018; Bachmann et al., 2018; Sun et al., 2022). Firmicutes were the second dominant phyla in the non-monsoon season represented by major genera *Bacillus* and *Lactobacillus* and their total abundance decreased significantly in the monsoon (Table S4). The dominance of this group during the non-monsoon season could be due to their ability to efficiently utilize and grow in the presence of limited organic matter. They are also known to be metabolically versatile and can adapt to different environmental

conditions (Siezen and van Hylckama Vlieg, 2011; Kadnikov et al., 2020). These bacteria have evolved strategies to thrive well in these conditions by employing various mechanisms such as sporulation and biofilm formation (Kadnikov et al., 2020).

The dominant bacterial groups during the monsoon season were from the autotrophic bacterial phyla, the Cyanobacteria which increased by three folds during the monsoon season. The significant increase in the abundance of Cyanobacteria from 3 % during the non-monsoon season to 49 % during the monsoon season implies that this group of bacteria may have a competitive advantage over other bacterial groups during the upwelling season (Wasmund et al., 2012; Liu et al., 2022). Cyanobacteria are known to form blooms in nutrient-rich waters and thus increase in their abundance as compared to non-monsoon season (James et al., 2022). During both seasons, *Synechococcus* and *Cyanobium* were the dominant genera of Cyanobacteria observed. These dominant forms are considered significant Cyanobacteria due to their important ecological role in oceanic upwelling regions, specifically in carbon fixation and trophic networks (Dore et al., 2022). During the monsoon season, the presence of bacterial phyla such as Actinobacteria, Bacteroidetes, Planctomycetes, Verrucomicrobia and various candidatus phyla significantly increased as compared to non-monsoon (Fig. 4b). Actinobacteria and Bacteroidetes are known to encompass diverse microbial taxa with versatile metabolic capabilities (Bunse et al., 2016). Their reduced abundance during the non-monsoon season suggests a potential limitation in their ability to thrive under nutrient-limited conditions (Ho et al., 2017). These bacterial groups may rely on higher organic matter availability, which is typically associated with the monsoon period due to increased primary production (Alonso et al., 2006; Schattenhofer et al., 2009). They are also reported to be important players in the breakdown of particulate organic materials in the water column (Kirchman, 2002; Pinhassi et al., 2004; Bergen et al., 2015 (Morris et al., 2012; Montes et al., 2020). In addition to the well-defined phyla observed in the study region, the presence of various candidatus phyla was also detected. During the monsoon season, candidatus phyla including Ca. Marinimicrobia, Ca. Patescibacteria and Ca. Dadabacteria exhibited a significant increase in their relative abundance (Fig. 4b and Fig. S4). Their potential roles in the decomposition and utilization of the abundant organic matter generated during this period highlight their ecological significance in nutrient cycling and carbon metabolism within the study region (Mestre et al., 2018; Frank et al., 2016; Yilmaz et al., 2016). Further investigations into the functional potential and ecological contributions of these candidatus phyla are warranted to elucidate their specific

roles in microbial community dynamics and ecosystem functioning during the monsoon season.

3.4.2. Influence of environmental variables on the bacterial community composition

CCA aided in understanding the influence of environmental variables, especially DO, nutrients and primary productivity parameters on the bacterial communities at the genera level. The analysis showed, that DO and nutrient levels exhibited a significant correlation with the Cyanobacterial community which included the *Synecoccocus*, *Cynanobium PCC-6307* and Cloroplast_*unclassified* in both seasons. Physicochemical factors such as temperature and DO availability play a crucial role in shaping the diversity, abundance and activity of bacterial communities in the marine ecosystem, particularly in upwelling regions (Sun et al., 2022). In the non-monsoon season, the majority of the dominant taxa exhibited a significant correlation with primary productivity and fluorescence (Fig. 6a). However, during the monsoon season there was a significant shift in the bacterial heterotrophic groups, which included a majority of candidatus bacterial communities. The bacterial communities in the monsoon season included SAR86, OM60 (NOR5) of Gammaproteobacteria; NS4 and NS5 marine groups of Bacteroidetes, Actinobacterial genera Sva0996 marine groups, Ca. *Actinomarina* and Ca. *Marinimicrobia* was observed. The enhanced availability of nutrients and labile carbon sources during the productive monsoon season likely stimulates the growth and metabolic activities of these bacterial phyla (Hug et al., 2015; Reji et al., 2020; Graham and Tully, 2021). These bacterial groups have previously been shown to be associated with high-productivity environments and their presence may reflect an increase in nutrient availability due to upwelling (Bachmann et al., 2018; Reji et al., 2020; James et al., 2022). This provides a vivid understanding that the seasonal variations in the primary production in the study region influenced the autotrophic and heterotrophic bacterial communities.

3.4.3. Seasonal variation in the bacterial functional profiles

Seasonal variations in the primary productivity also exerted a significant influence on the predicted functional profiles of the bacterial communities. Predictive functional profiling showed autotrophic activities including, prokaryotic carbon fixation activities and cellular catabolism increased during the monsoon season. This could be due to the significant increase in Cyanobacterial populations during the upwelling season. Studies suggest that the temporal variations in functional profiles observed using metagenomics approaches may be influenced by the high abundance of genes associated with photosynthesis from Cyanobacteria (Ward et al., 2017; Matsumoto et al., 2021). Understanding the metabolic interactions between primary producers, such as photoautotrophic species like phytoplanktons and Cyanobacteria and heterotrophic organisms, such as bacteria and archaea, is essential for understanding how ecosystems work (Caroppo et al., 2022). In this study, observation-based functional profiles underscore the significant impact of primary productivity variations, particularly those associated with upwelling events, on the composition of bacterial communities within the WCI region. Studies indicate that during the monsoon season, the metabolism of microbial communities is particularly active in breaking down complex organic compounds such as carbohydrates, proteins and polysaccharides (Caroppo et al., 2022). Bacteria are also shown to exhibit increased enzymatic activity and cellular activity such as quorum sensing and biofilm formation allowing them to efficiently break down available organic matter and obtain essential nutrients for growth (Lever et al., 2015; Prüß, 2017), which was also evident from our study (Fig. 7). Further investigations into the functional potential and ecological contributions of these bacterial communities are essential to understanding their ecological significance.

3.5. Conclusion

This study presents a detailed account of the bacterial community structure in C-Max depths during the non-monsoon and monsoon seasons along the WCI. An increase in primary productivity is recorded annually during the southwest monsoon season along the west coast of India. The influence of seasonal variations in primary productivity on bacterial dynamics was explored along with the physio-chemical parameters during the non-monsoon and productive monsoon seasons. The physicochemical characteristics exhibited significant seasonal differences and the water column temperature and salinity variations depicted that the mixed layer depth was much shallower with higher primary

productivity in the monsoon season. A significant positive correlation between the *in situ* primary productivity, estimated using the radiotracer method and bacterial parameters shows the influence of the former on the latter in the study area. Bacterial diversity based on next-generation, metagenomic analysis was assessed in the chlorophyll maxima depths, which recorded the highest viable counts during both seasons. Taxonomic profiles showed that the most dominant bacterial communities identified from the study region include the Proteobacteria and Firmicutes during the non-monsoon season and Cyanobacteria, Proteobacteria followed by Bacteroidetes, Actinobacteria and candidatus phyla during the monsoon season. The abundance and composition of these phyla significantly varied between both seasons, which was evident through Canonical correspondence analysis. It showed the influence of primary productivity parameters on the bacterial communities at the genera level with distinct seasonal variations. In the non-monsoon season, the dominant taxa were *Idiomarina, Alteromonas, Marinobacter, Alcanivorax* belonging to Gammaproteobacteria; *Sulfitobacteria,* Sphingomonadaceae_ *uncultured,* Rhodobacteraceae_*uncultured* from Alphaproteobacteria and *Lactobacillus* of Firmicutes which exhibited a significant correlation with primary productivity. However, during the monsoon season, there was an increase in the autotrophic communities with *Cyanobium* and *Synechococcus* genera of Cyanobacteria and the heterotrophic groups, included a majority of candidatus bacterial taxa. This included SAR86, OM60 (NOR5) of Gammaproteobacteria; NS4 and NS5 marine groups of Bacteroidetes, Actinobacterial genera Sva0996 marine groups, Ca. *Actinomarina* and Ca. *Marinimicrobia*. Further investigations into the functional potential and ecological contributions of these candidatus phyla are required to elucidate their importance in the study region. Seasonal variations in the primary productivity also exerted a significant influence on the predicted functional profiles of the bacterial communities. These observations underscore the significant impact of primary productivity variations on bacterial abundance and community composition along the west coast of India.

Chapter 4.

Insights into the functional profiles of bacteria along the west coast of India.

Abstract

The west coast of India (WCI) is known for its unique oceanographic features such as the seasonal monsoonal winds, upwelling of nutrient-rich waters and a significant increase in primary productivity during the monsoon season. In this study, we utilised the shotgun metagenomics approach to determine the seasonal variations in bacterial taxonomic and functional profiles during the non-monsoon and monsoon seasons in the WCI. Significant seasonal variations in the bacterial community structure were observed at the phylum and genera levels. These finding also correspond with seasonal shifts in the functional profiles of the bacterial communities based on the variations of genes encoding enzymes associated with different metabolic pathways. Pronounced seasonal variation of bacterial taxa was evident with an increased abundance of *Idiomarina*, *Marinobacter*, *Psychrobacter* and *Alteromonas* of Proteobacteria, Bacillus and Staphylococcus of Firmicutes during the non-monsoon season. These taxa were linked to elevated nucleotide and amino acid biosynthesis, amino acid and lipid degradation. Conversely, during the monsoon, the taxa composition changed with *Alteromonas*, Candidatus *Pelagibacter* of Proteobacteria and Cyanobacteria *Synechococcus*; contributing largely to the amino acid and lipid biosynthesis, fermentation and inorganic nutrient metabolism which was evident from functional analysis. Regression analysis confirmed that increased seasonal primary productivity significantly influenced the abundance of genes associated with carbohydrate, protein and lipid metabolism. These highlight the pivotal role of seasonal changes in primary productivity in shaping the bacterial communities, their functional profiles and driving the biogeochemical cycling in the WCI.

4.1. Introduction

Marine bacteria are fundamental players in the marine biogeochemical cycles, decomposing organic matter and recycling the nutrients essential for sustaining the ecosystem. This breakdown is facilitated by the bacterial extracellular enzymes, such as carbohydrases, proteases and lipases, which break down the organic matter (Mühlenbruch et al. 2018; Dittmar et al. 2021; Dithugoe et al. 2023). In addition to supporting the marine food web, the recycling of organic matter by bacterial communities also plays a key role in regulating the global carbon cycle by facilitating the long-term storage of carbon in recalcitrant forms (Jiao & Zheng, 2011; Moran et al. 2016; Heinrichs et al. 2020; DeVries, 2022). In marine ecosystems, the taxonomic composition and functional abilities of bacterial communities are influenced by various environmental factors such as water temperature, nutrients and organic matter availability (Zhou et al. 2021; Eigemann et al. 2023). Bacterial activity in marine environments demonstrates a marked variability, which appears to be influenced by the availability and characteristics of organic matter, suggesting a distinct distribution of enzymatic activities across seasons and regions (Basu et al. 2013; Arnosti et al. 2021). The diversity and composition of these bacterial communities are crucial in determining the rate and efficiency of organic matter recycling (Zhou et al. 2021). Hence, understanding the bacterial community structure and their heterotrophic functional activities is of global importance in the present climate change scenario.

The study region, WCI is characterized by semi-annual changes in the climate, wind pattern, monsoonal currents and upwelling, which plays a significant role in shaping the phytoplankton productivity dynamics along this region (Vinayachandran et al. 2021; Gupta et al. 2022; Albin et al. 2022). Increased productivity during southwest monsoon leads to the generation of substantial amounts of organic matter in subsequent concentration along the WCI (Shetye et al. 2022; Nandakumar et al. 2023). Seasonal variations strongly influence the distribution of nutrients and organic matter in this region, with the southwest monsoon playing a crucial role in driving enhanced productivity. During the monsoon season, intense rainfall and terrestrial inputs further contribute to the organic matter dynamics, fuelling the growth of marine microorganisms (Sarma et al. 2014; Nageswar Rao et al. 2019; Silori et al. 2022). However, during the non-monsoon season, the WCI experiences a relatively low-productive environment (Santhikrishnan et

al. 2021; Albin et al. 2022). Understanding the intricate dynamics of productivity, organic matter generation and its relation to bacterial functional activities is crucial to comprehending the complex ecological processes along WCI. Despite the importance of bacterial communities in biogeochemical cycling in marine ecosystems, little is known about their composition and diversity along the WCI. Previous studies have primarily focused on the physio-chemical factors influencing bacterial diversity and community structure in specific regions such as oxygen minimum zones, mud banks regions and sediment cores (Vijayan et al. 2023 and reference therein), however, the specific bacterial communities and their functional diversity involved in the biogeochemical processes have not been studied thoroughly from this region. Furthermore, there is limited knowledge about how bacterial communities vary during different seasons, which is important for understanding the temporal dynamics of bacterial functional activities recycling organic matter. Based on the culturable bacterial diversity along the WCI, it was observed that there was a pronounced increase in bacterial abundance during the monsoon season. The chlorophyll maxima depths (C-Max) consistently reported the most significant colony-forming units (CFUs) (Parab et al. 2022). The C-Max, located between 20-100 m in marine water columns, is known for high productivity due to conditions favourable for phytoplankton growth (Cullen, 2015). This depth offers abundant organic matter, supporting bacterial heterotrophic activities. Despite its importance, there is limited research in WCI's about how changes in primary productivity affect bacterial functions in C-Max zones, especially using advanced metagenomics sequencing methods.

To address this, the present study used high-throughput shotgun sequencing to investigate the bacterial taxonomy and functional diversity at C-Max depths along the WCI during non-monsoon and monsoon seasons. Shotgun metagenomics represents a robust and superior approach for functional analysis in microbial ecology and environmental studies (Quince et al. 2017), unlike targeted methods like 16S rRNA sequencing that focus on specific conserved regions. By sequencing all genetic material present in environmental samples, shotgun metagenomics enables the comprehensive exploration of microbial diversity and the identification of functional genes and pathways (Galloway-Peña & Hanson, 2020). Thus, this study will provide an in-depth report of bacterial functional diversity based on shotgun metagenomics, offering a comprehensive view of the genetic potential within diverse microbial communities during non-monsoon and monsoon seasons. This research contributes to a better understanding of the

ecological significance of bacterial communities in this region and will provide valuable insights into seasonal variations in bacterial heterotrophic activities.

4.2. Methodology

4.2.1. Sample collection and DNA extraction

Sampling was conducted on board RV Sindhu Sankalp in the study region along the WCI, during the non-monsoon season in February 2019 (Cruise # SSK-125) and southwest monsoon season in September 2019 (Cruise # SSK-131). Samples were collected from coastal (30 m) and off-shore (600 m) locations off Goa, Mangalore and Trivandrum along the WCI (Figure 1.5). Water samples were collected at discrete depths in all the locations including the C-Max depth, immediately sub-sampled and stored for subsequent analysis. Water samples collected at C-Max depths from all 6 stations during both cruises were filtered using a peristaltic pump on board the research vessel for metagenomic DNA extraction. Approximately 20 L of water sample in triplicates was filtered through 0.22 mm Durapore filters (Millipore, USA) and stored in cryovials containing DNA storage buffer, consisting of 0.75M sucrose, 40 mM EDTA and 50 mM Tris (pH 8.3) and was maintained at -80°C. Subsequently, DNA extraction was performed using the FastDNA^{TM} SPIN kit (MP Biomedical, USA), from the triplicate samples following the manufacturer's instructions and DNA was pooled together for further analysis. The extracted metagenomic DNA was assessed for quality and an adequate amount of DNA was obtained for subsequent sequencing.

4.2.2. Shotgun sequencing and assembly

DNA samples were sequenced using the shotgun metagenomics technique at the National Institute of Biomedical Genomics (NIBG, West Bengal, India). For this, library preparation was carried out using TruSeq DNA PCR-Free Library Prep Kit (Illumina, USA) and the library was sequenced on the NovaSeq 6000 system (v1.5, Illumina, USA) according to Illumina's recommended protocols (Illumina, USA). Quality control and assembly of the shotgun sequencing reads were performed using a series of bioinformatics tools. Raw sequencing reads were initially assessed for quality using

FastQC v0.12.0. Low-quality bases and adapter sequences were removed from the reads using Trimmomatic v0.39, (Bolger et al. 2014). To eliminate potential contamination from untargeted DNA, Kneaddata v0.11.0 was used. The resulting high-quality reads were then subjected to de novo assembly using MEGAHIT v1.2.8 (Li et al. 2016), resulting in the generation of contigs. Assembly quality was evaluated using QUAST v5.1.0 (Gurevich et al. 2013), while BUSCO v5.4.7 (Manni et al. 2021) was used to assess the completeness of the assembly. Post-assembly processing involved read mapping using Bowtie2 v2.5.1 (Langmead and Salzberg, 2012).

4.2.3. Taxonomic and functional annotation

For taxonomic assessment, MetaPhlAn2 was utilized to profile the metagenomic content of the assembly, enabling the identification and quantification of microbial species within the dataset (Truong et al. 2015). For functional diversity assessment, an open reading frame (ORF) was predicted using Prokka v1.14.6 for comprehensive genome annotation (Seemann, 2014). For each of the samples, gene clustering based on sequence identity, CD-HIT v 4.8.1 was employed (Li et al. 2012). For evaluation of the overall functional diversity of bacterial metabolic, cellular and carbohydrate utilization processes; predicted ORFs annotated against reference databases like KEGG (Kyoto Encyclopedia of Genes and Genomes), EggNOG (Evolutionary Genealogy of Genes: Non-supervised Orthologous Groups) and CAZy (carbohydrate-active enzymes). KEGG annotation was conducted utilizing kofamscan v1.3.0 to assign KEGG orthologs terms to the predicted ORFs. Functional classification based on orthologous groups from the EggNOG database was performed using eggnog-mapper v2.1.9 (Cantalapiedra et al. 2021). Identification of CAZymes within the ORFs was achieved by mapping the data set against the CAZy database using run_dbcan v2.0.0 (Zhang et al. 2018).

To further investigate the organic matter degradation potential, information on genes encoding enzymes involved in carbohydrate, protein and lipid degradation was extracted from annotation data. Carbohydrate-degrading enzymes were assessed by estimating the abundance of various Glycoside Hydrolase (GH) families from the CAZy annotated data. Protein degradation was studied by manually searching for peptidase families, with a particular emphasis on bacterial extracellular peptidases. The InterPro database (Paysan-Lafosse et al. 2023), which provides a classification of protein families

and the MEROPS database, dedicated to peptidase classification, were utilized for this purpose (Rawlings et al. 2018). The abundance of genes encoding peptidases of interest was extracted from the EggNOG annotated data. For lipid degradation analysis, ORFs were annotated against the KEGG database to extract the abundance of pathways responsible for fatty acid degradation processes. In the analysis, functional assignment profiles from the KEGG, EggNOG and CAZy annotation output files were imported into R Studio v3.5.1. These files were manually sorted and searched to identify specific genes and pathways associated with carbohydrate, protein and lipid degradation processes. Custom searches, filtering and sorting operations were performed on the functional annotation data to extract the relevant information (Tseng et al. 2015).

4.2.4. Statistical analysis

The data was analysed statistically to evaluate the significance level of the differences in the bacterial community and functional distribution between non-monsoon and monsoon seasons. The assessment of bacterial alpha diversity indices was estimated using PAST v4.03 (Paleontological statistics software for analysis and education) software (Hammer and Harper, 2006). The t-test analysis was performed using Microsoft Excel (2016) to evaluate significant differences in bacterial alpha diversity indices between both seasons. For beta diversity assessment, non-metric multidimensional scaling (NMDS) analysis, was conducted utilizing the R package BiodiversityR (Kindt and Coe, 2005). Additionally, the permutational multivariate analysis of variance (PERMANOVA) was performed using PAST v4.03, to evaluate the statistical significance of diversity distribution across different sampling stations and seasons. Hierarchical clustering analysis based on the Euclidean similarity index was performed using the R package Pheatmap v1.0.12 to generate a heatmap of bacterial communities. Statistical significance between taxonomic and functional gene abundance was assessed using STAMP (statistical analysis of taxonomic and functional profiles) bioinformatics software v2.1.3 (Parks et al. 2014). Data visualization for various statistical tests was performed using the R package ggplot2 (R Core Team, 2015).

4.3. Results

4.3.1. Bacterial distribution and taxonomic composition

Shotgun sequences obtained from the six, water samples collected from C-Max depth from each season, were in the range of about 10 to 18 × 10^6 raw reads. These sequences were denoised and filtered, and an average of about 5 to 9 × 10^5 filtered reads was used for the analysis, which grouped into about 750 unique features (Appendix 4.3). A rarefaction curve was plotted to assess the sequencing depth and its efficacy in capturing the microbial diversity. The plateauing of the rarefaction curve suggested that the sequencing depth was optimal, with further sequencing likely to add limited new taxa (Appendix 4.1). Based on the diversity indices calculated, the mean Simpson's index was 0.36 and 0.42 during the non-monsoon and monsoon seasons respectively and the mean Shannon diversity index was about 0.80 in the samples analysed (Table 4.1). During the non-monsoon, the evenness values ranged 0.12 to 0.24 and in the monsoon, a wider range of values from 0.22 to 1.00 was estimated, which showed that there was significant temporal difference in this season. The Chao-1 index exhibited significant seasonal variations, with higher values (12.50) in the non-monsoon; than in the monsoon season (6.33). The t-test analysis performed to evaluate the differences in diversity indices showed significant seasonal variations in the Evenness and Chao-1 index ($p < 0.05$) (Table 4.1).

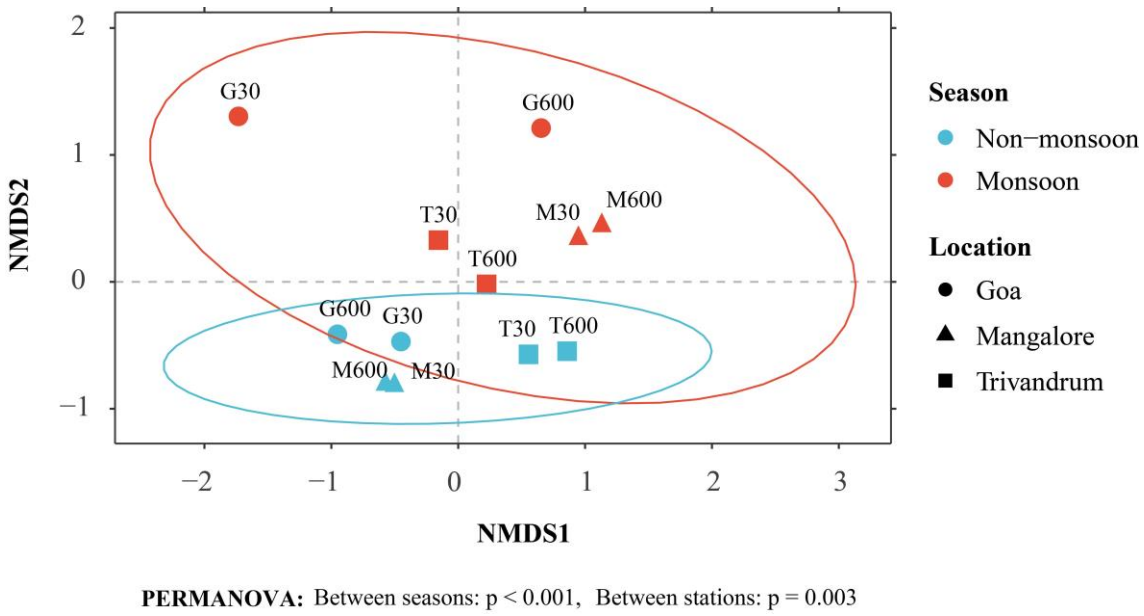
Based on the NMDS analysis, a distinct seasonal variation in the bacterial communities from the sampling locations, off Goa, Mangalore and Trivandrum along the WCI was observed. The non-monsoon samples from coastal (30 m) and off-shore (600 m) stations of Goa and Mangalore, displayed a closer clustering pattern, suggesting a higher degree of similarity in comparison to the Trivandrum samples (Figure 4.1). During the monsoon season, there were wider variations in the diversity patterns between the bacterial communities from the sampling stations. This pattern suggests that environmental factors associated with the monsoon season, such as increased productivity and nutrient influx, may significantly influence and diversify the bacterial diversity and community structure along WCI.

Table 4.1: Diversity indices estimated during the non-monsoon and monsoon seasons along the west coast of India.

Stations	**Diversity indices**			
	Simpson	**Shannon**	**Evenness***	**Chao-1***
Non-monsoon season				
G30	0.58	1.26	0.24	15.00
G600	0.27	0.67	0.12	16.00
M30	0.53	1.22	0.24	14.00
M600	0.40	0.94	0.16	16.00
T30	0.17	0.36	0.18	8.00
T600	0.19	0.38	0.24	6.00
Mean	0.36	0.81	0.20	12.50
Monsoon season				
G30	0.00	0.00	1.00	1.00
G600	0.79	1.60	0.99	5.00
M30	0.48	0.90	0.31	8.00
M600	0.58	1.01	0.55	5.00
T30	0.31	0.71	0.25	8.00
T600	0.37	0.88	0.22	11.00
Mean	0.42	0.85	0.55	6.33

*Significant at 0.05 level (t-test)

The two-way PERMANOVA analysis validated the NMDS results, showing significant seasonal variation ($p < 0.001$) and temporal variation ($p = 0.003$) in the bacterial community structure. The bacterial taxonomic composition in the water column at C-Max depths revealed the presence of bacterial phyla including Proteobacteria, Bacteroidetes, Firmicutes and Actinobacteria during the non-monsoon season and in the monsoon season Cyanobacteria was also reported (Figure 4.2). The relative abundance of bacterial genera within these bacterial phyla exhibited distinct patterns between the non-monsoon and monsoon seasons.



PERMANOVA: Between seasons: $p < 0.001$, Between stations: $p = 0.003$

Figure 4.1: NMDS analysis illustrating seasonal differences in bacterial community composition between non-monsoon and monsoon seasons.

In the Proteobacteria phylum, within the Gammaproteobacteria class, pronounced seasonal variation was observed which includes genera *Idiomarina*, whose relative abundance significantly ($p < 0.001$) decreased from 53.06 % in the non-monsoon season to 2.96 % in the monsoon season (Figure 4.2). In contrast, *Alteromonas* significantly ($p < 0.001$) increased from 28.17 % in the non-monsoon season to 58.49 % in the monsoon season (Figure 4.2). Proteobacterial genera *Marinobacter, Nitratireductor* and *Pelagibaca* also displayed an increase in their relative abundance during the monsoon season. Significant differences were also observed in the Actinobacteria phylum within the Actinomycetes class, with *Nocardioides* genera increasing from a relative abundance of 0.48 % in the non-monsoon season to 5.76 % in the monsoon season ($p < 0.001$) (Figure 4.2). Within the Bacteroidetes and Firmicutes phylum, several genera present during the non-monsoon season were not detected in the monsoon season (Figure 4.2). Within the Cyanobacteria phylum, significant variation was seen in the genus *Synechococcus*, which was not detected during the non-monsoon season but contributed to a relative abundance of 21.65 % during the monsoon season (Figure 4.2).

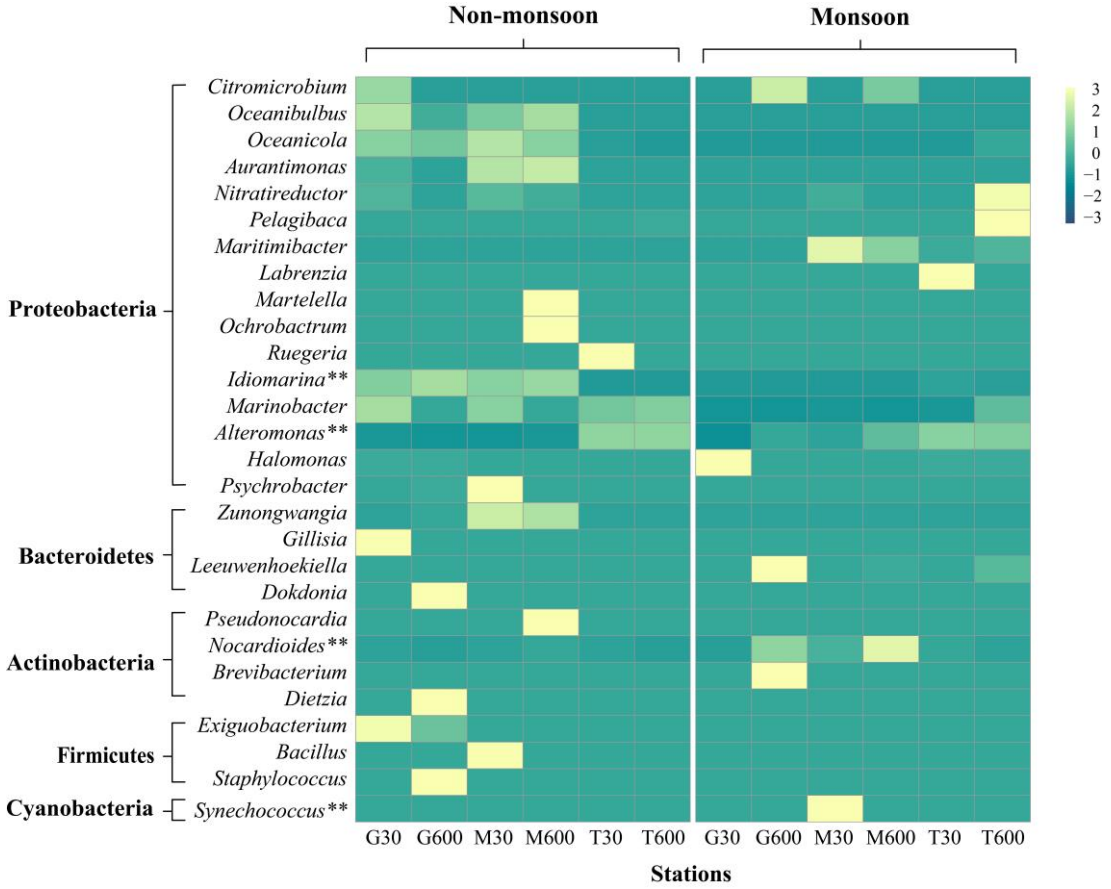


Figure 4.2: Heatmap displaying the relative abundance of bacterial taxa during non-monsoon and monsoon seasons along the west coast of India, the significance of seasonal variations at $p < 0.001$** is also represented for specific bacterial genera.

4.3.2. Functional insight into metabolism and cellular processes

The results of the KEGG and EggNOG pathway analysis provided valuable insights into the functional profiles of bacterial communities along the WCI. Among the six major KEGG pathways, metabolism emerged as the predominant functional category, constituting ~ 55 % during the non-monsoon and monsoon seasons (Figure 4.3). A relative abundance of genetic information processing pathways and cellular processes was represented around 8.5 % during both seasons. Environmental information processing pathways contributed about 11 % during the non-monsoon season and marginally reduced in the monsoon season (9 %). Pathways associated with organismal

systems and human diseases exhibited a proportion of ~ 4 % and 12.5 % during both the non-monsoon season and monsoon season respectively (Figure 4.3). Within the metabolic pathways, the relative abundance of energy metabolism increased significantly ($p < 0.001$) during the monsoon season. A similar significant increase in relative abundance was also observed in pathways for amino acid metabolism and metabolism of cofactors and vitamins during monsoon season (Figure 4.4 and Appendix 4.4). There was a notable increase in the pathways for folding, sorting and degradation under the genetic information processing pathways, such as viral information processing ($p = 0.004$) and translation process ($p < 0.001$) in the monsoon season (Figure 4.4 and Appendix 4.5).

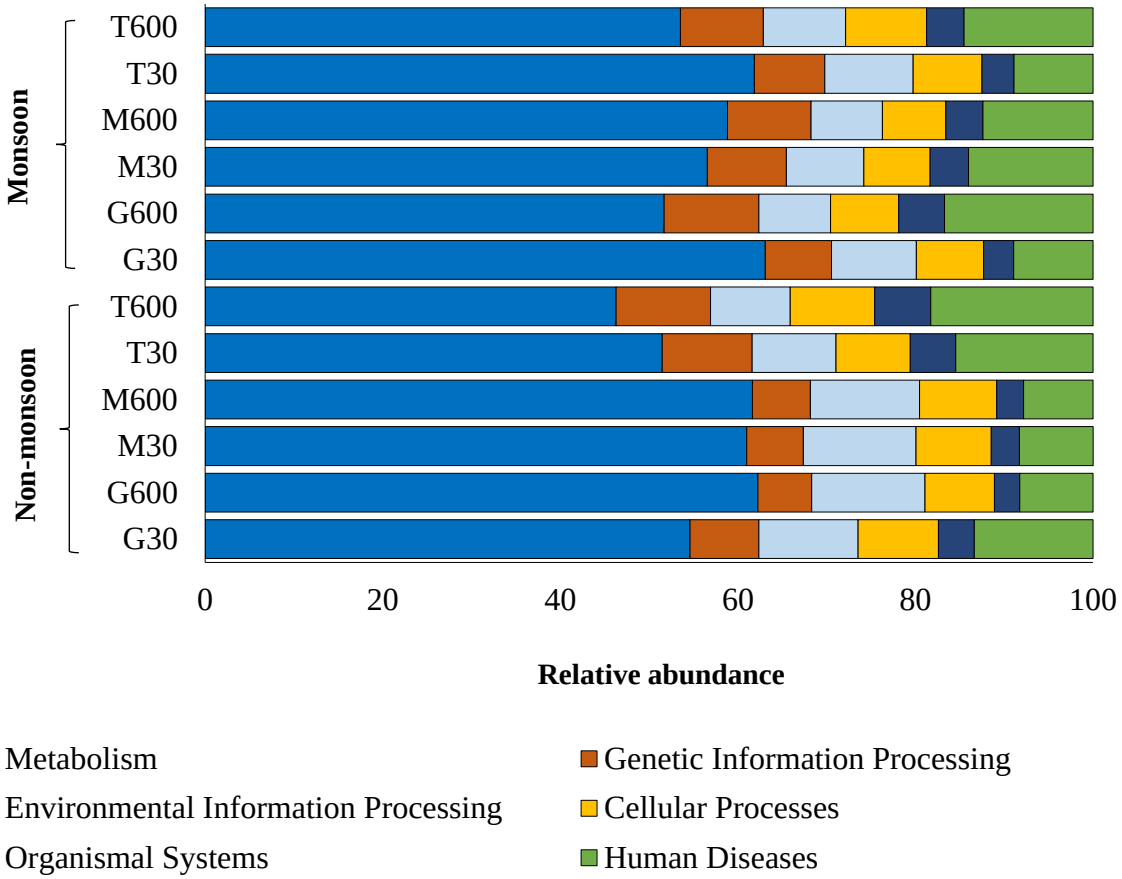


Figure 4.3: Relative abundance of major KEGG pathways across all stations during the non-monsoon and monsoon seasons.

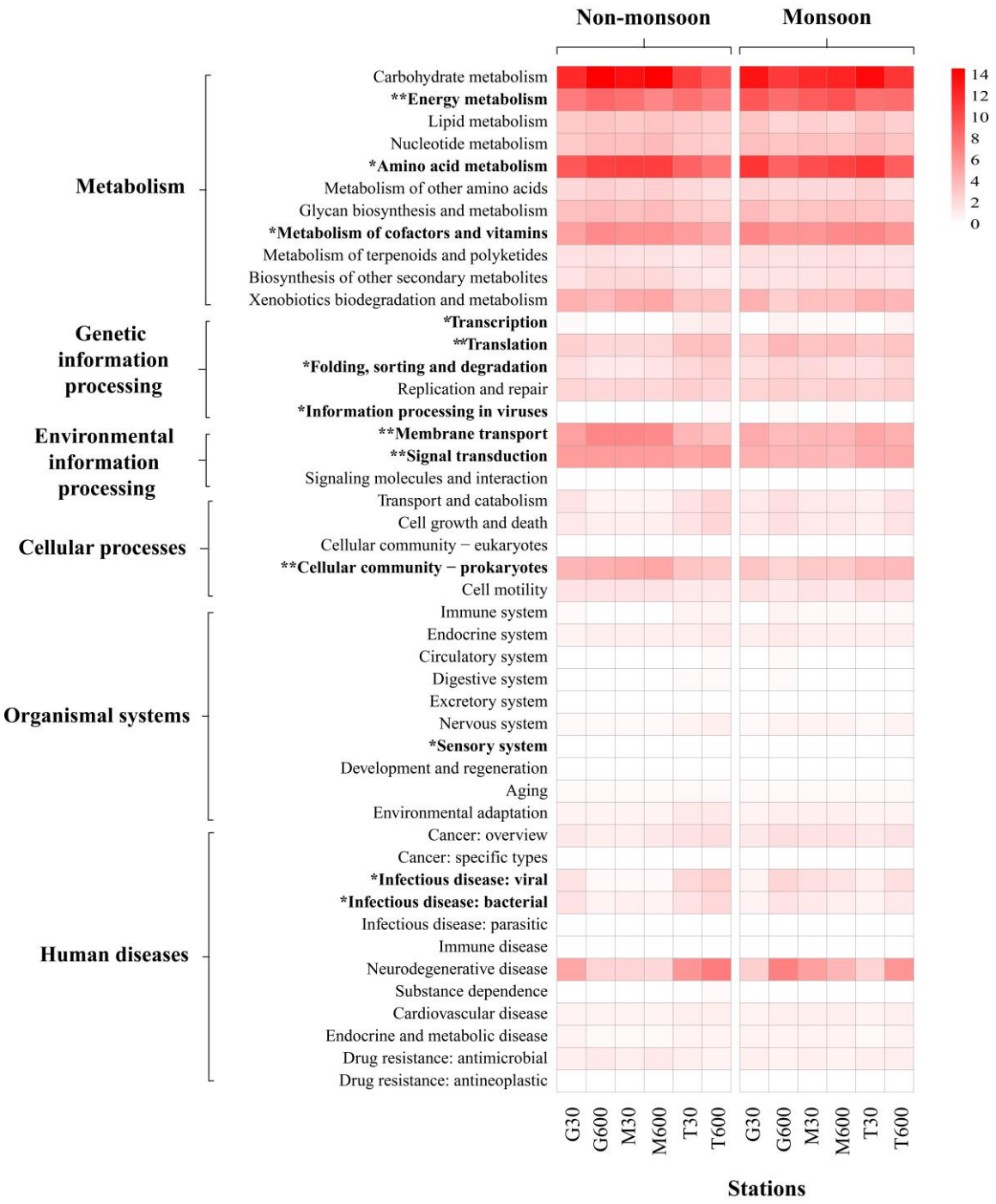


Figure 4.4: Comparative heatmap of all major pathways across all stations during the non-monsoon and monsoon seasons, the significance of seasonal variations at $p < 0.05$* and $p < 0.001$** is also represented for specific pathways.

Seasonal variations were also observed in the pathways for the environmental information processing category, with a significant increase in both membrane transport and signal transduction processes ($p < 0.001$) during non-monsoon season. Within the cellular processes pathways, the non-monsoon season exhibited an increase in relative abundance in the cellular community of prokaryotes ($p < 0.001$) and sensory system process ($p < 0.05$), within organismal systems pathways (Figure 4.4 and Appendix 4.4).

The relative abundance of various metabolism and cellular process pathways annotated against the EggNOG database was also analysed. Results obtained were similar to the analysis based on KEGG annotations with a higher relative abundance of several pathways including those involving energy production and amino acid metabolism in the monsoon season. However, pathways related to RNA processing and modification decreased significantly during the monsoon season. A significant increase in pathways with unknown functions and those related to cell motility was also observed during the monsoon and non-monsoon seasons respectively (Appendix 4.2 and Appendix 4.4).

4.3.3. Functional insight into carbohydrate, protein and lipid utilization

Carbohydrate utilization

The comparative analysis of the ORFs belonging to the CAZymes families exhibited distinct variations in the non-monsoon and monsoon seasons. Within the CAZymes, Glycosyltransferases (GTs), responsible for synthesizing the glycosidic bond in carbohydrates, emerged as the predominant group during both seasons with a relative abundance of ~ 48 %. Following it, the Glycoside Hydrolases (GHs), key enzymes in hydrolysing the glycosidic bond between two or more carbohydrates, showed a marginal increase in relative abundance during the monsoon season (Figure 4.5a). Similarly, Carbohydrate Esterases (CEs), which cleave linkages of carbohydrate esters, observed a significant increase in their relative abundance during the monsoon ($p < 0.001$). In contrast, the abundance of Auxiliary Activities (AAs) enzymes, enzymes pivotal in lignin degradation and modification and Carbohydrate-Binding Modules (CBMs), crucial for targeting enzymes to their complex carbohydrate substrates evidenced a marginal decline in the monsoon (Figure 4.5a). The relative abundance of polysaccharide lyases (PLs), involved in the non-hydrolytic cleavage of polysaccharides, remained steady at about 1 % throughout both seasons.

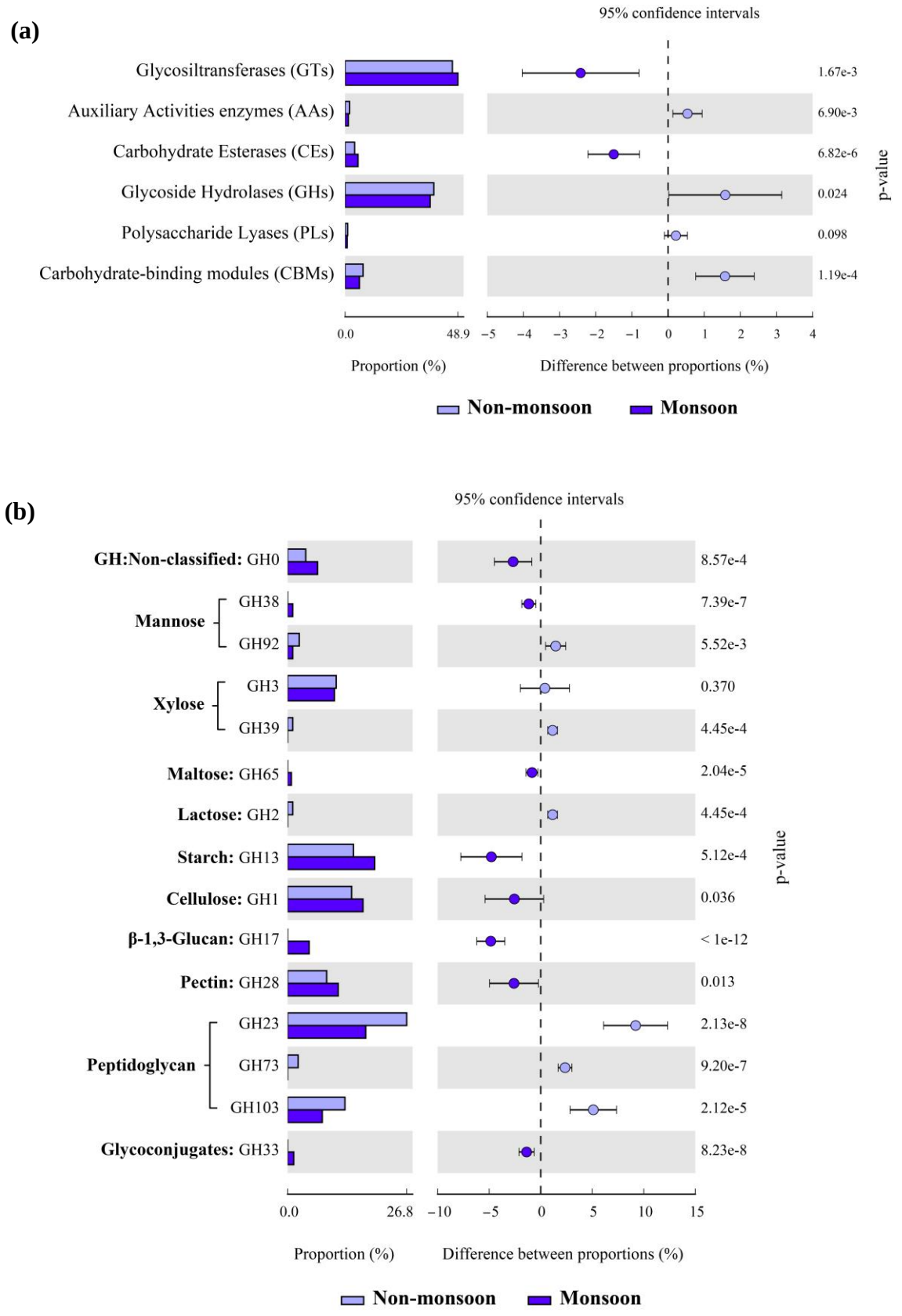


Figure 4.5: Extended error bar plot of (a) major CAZy enzyme families and (b) glycoside hydrolases (GHs) during non-monsoon and monsoon season.

To further understand carbohydrate degradation, the glycoside hydrolase subfamily was examined, revealing distinct distribution patterns across sampling seasons (Figure 4.5b). During the non-monsoon season, GH0, a non-classified glycoside hydrolase, exhibited a relative abundance of 4.07 %, which significantly increased to 6.74 % in the monsoon season ($p < 0.001$). Enzyme subfamilies, GH92 and GH38, both associated with mannose hydrolysis, presented contrasting dynamics. Relative abundance of GH92 was higher in the non-monsoon season, however, GH38 was increased during the monsoon season. Enzymes catalysing xylose degradation, such as GH3 and GH39 varied in their seasonal trends with higher relative abundance during non-monsoon. Similarly, GH2 enzymes, which are associated with lactose hydrolysis were only detectable during the non-monsoon season (Figure 4.5b). Seasonal variations were also observed in peptidoglycan hydrolases such as GH23, GH73 and GH103. Among these, GH23 significantly decreased from 26.81 % during the non-monsoon to 17.60 % in the monsoon ($p < 0.001$), while GH103 exhibited a decline from 12.90 % to 7.80 % ($p < 0.001$). However, GH73 was only detectable in the non-monsoon season. During monsoon season, the relative abundance of maltose hydrolysing enzymes such as GH65 and starch-degrading enzyme GH13 also increased significantly ($p < 0.001$). Similarly, GH1 associated with cellulolytic activities and GH28 enzyme, associated with pectin hydrolysis increased significantly during the monsoon season (Figure 4.5b). Furthermore, the relative abundance of β-1, 3-Glucan targeting enzymes GH17 and GH33, associated with glycoprotein and glycolipid degradation was only detected during monsoon season.

Protein utilization

The analysis of peptidase enzymes revealed the occurrence of various peptidases across sampling seasons. Aminopeptidases, which are associated with the cleaving of amino acids from the N-terminal end of proteins showed significant seasonal variation. Aminopeptidase N (Peptidase M1), aminopeptidase Y (Peptidase M3) and glycyl aminopeptidase (Peptidase M61) were significantly higher during the non-monsoon season ($p < 0.001$) (Figure 4.6). Carboxypeptidases, essential for cleaving amino acids from the C-terminal end, such as carboxypeptidase A (Peptidase M14) and LD-carboxypeptidase (Peptidase S66) detected during the non-monsoon season, but their relative abundance significantly decreased in the monsoon season ($p < 0.001$). The relative abundance of endopeptidase pitrilysin (Peptidase M16) was also significantly higher during non-monsoon season. Several enzymes with specialized functions such as

imelysin (Peptidase M75) and prepilin peptidase (Peptidase A24), were only detected during the non-monsoon season (Figure 4.6). During monsoon season, aminopeptidases such as methionine aminopeptidase 1 (Peptidase M24) marked a significant increase in the relative abundance of 22.73 % ($p < 0.001$). Along with it, the relative abundance of glutamate carboxypeptidase (Peptidase M20) also increased significantly during the monsoon season ($p = 0.003$). Within the endopeptidase category, which acts on internal peptide bonds, the relative abundance of beta-lytic metallopeptidase (Peptidase M23) and prolyl oligopeptides (Peptidase S9) significantly ($p < 0.001$) increased during the monsoon season.

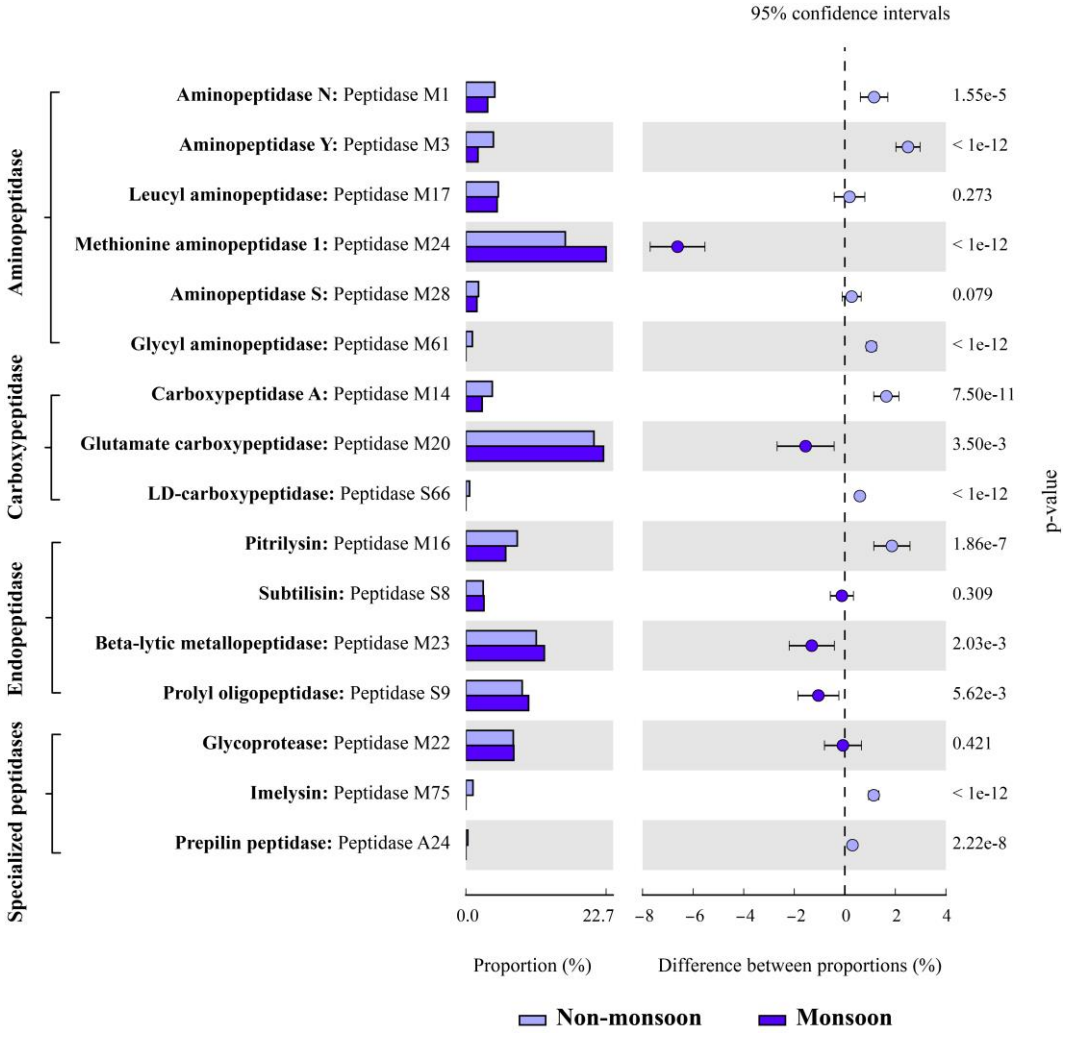


Figure 4.6: Extended error bar plot of peptidase enzymes, along with significance levels during non-monsoon and monsoon season.

Lipid utilization and fermentation

The analysis of pathways responsible for lipid degradation revealed significant differences in the abundance of specific enzymes during the non-monsoon and monsoon seasons (Figure 4.7). During the non-monsoon season, enzymes responsible for the initial activation pathways of fatty acid degradation such as long-chain fatty acid ligase had a significantly higher relative abundance of 9.59 % ($P < 0.001$). Similarly, the β-Oxidation cycle enzymes presented significant activity with a marginally higher abundance of 3-hydroxybutyryl-CoA epimerase enzyme ($p = 0.01$).

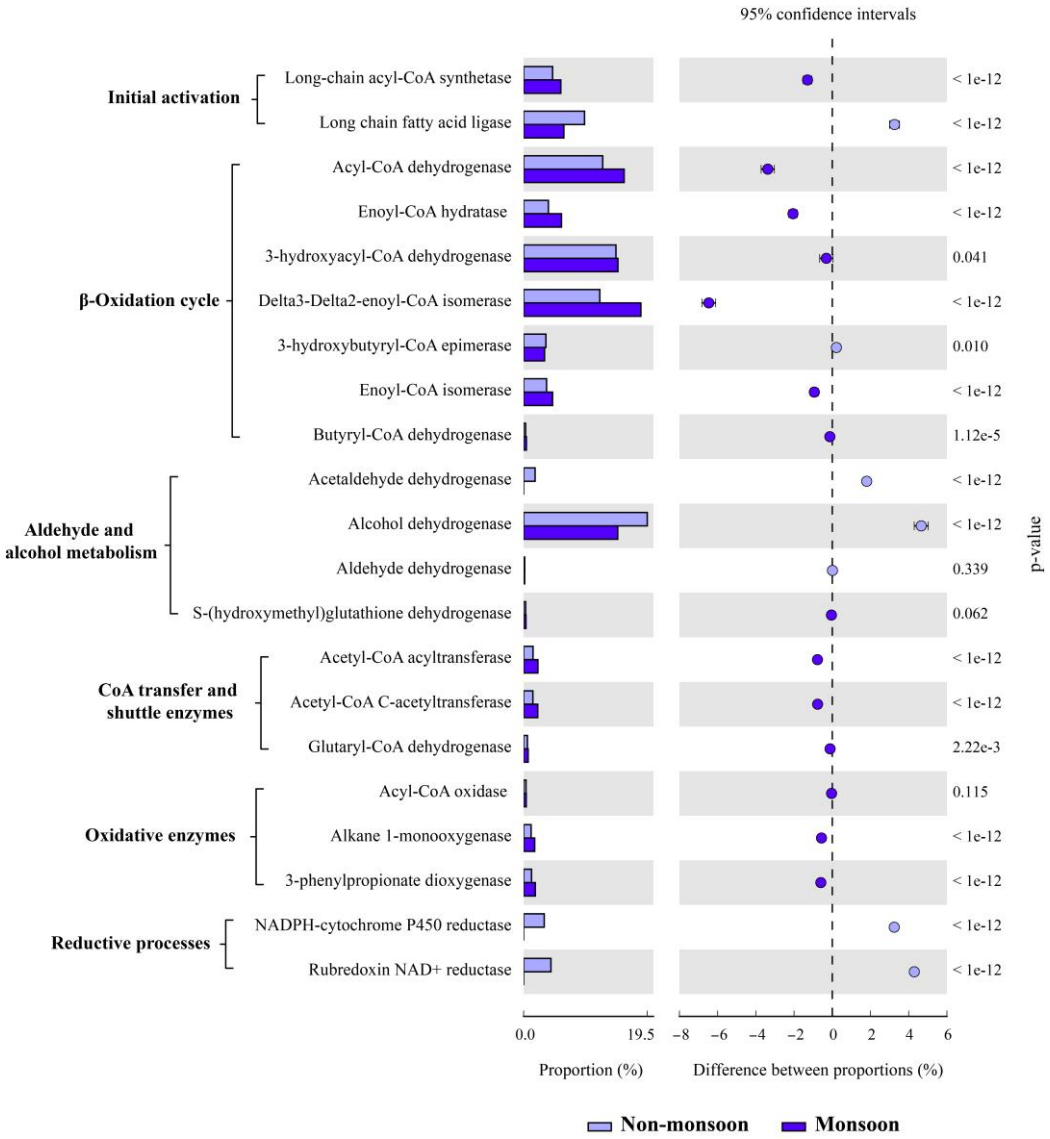


Figure 4.7: Extended error bar plot of enzymes involved in lipid metabolism along with significance levels during non-monsoon and monsoon season.

Enzymes associated with the aldehyde and alcohol metabolism, such as acetaldehyde dehydrogenase and alcohol dehydrogenase exhibited a higher relative abundance during the non-monsoon season ($p < 0.001$). The monsoon season witnessed a shift in enzymatic profiles, with an evident surge in the β-Oxidation cycle enzymes during the monsoon season (Figure 4.7). Notably, acyl-CoA dehydrogenase, enoyl-CoA hydratase and Delta3-Delta2-enoyl-CoA isomerase exhibited a significantly higher relative abundance of 15.82 %, 5.95 % and 18.45 %, respectively ($P < 0.001$). Other β-Oxidation enzymes such as Enoyl-CoA isomerase and butyryl-CoA dehydrogenase also showed a significant increase in their relative abundance during monsoon season (Figure 4.7). Relative abundance of Long-chain acyl-CoA synthetase was also detected to be higher during monsoon season. Similarly, the enzymes involved in CoA transfer and shuttle such as Glutaryl-CoA dehydrogenase ($p = 0.002$), acetyl-CoA acyltransferase, acetyl-CoA C-acetyltransferase and 3-phenylpropionate dioxygenase exhibited a significant increase in relative abundance ($P < 0.001$) during monsoon season.

4.4. Discussion

4.4.1. Seasonal variation in bacterial diversity and composition

Bacterial composition and taxonomic diversity assessed based on shotgun metagenomics at C-Max depths along the WCI during the non-monsoon and monsoon seasons revealed a significant seasonal variation. Bacterial diversity studies along the WCI, based on cultivable bacterial assessments (Gomes et al. 2019; Anas et al. 2021; Isaacs et al. 2021; Vijayan et al. 2022; Parab et al. 2022) as well as through 16S amplicon sequencing-based metagenomics studies (Vijayan et al. 2022), have revealed that the primary productivity influences the bacterial diversity in this region. However, shotgun-based, whole bacterial genome metagenomic analysis in these regions is almost negligible. This is the first study to elucidate the seasonal variations of primary productivity on both the taxonomic and functional diversity of the bacterial communities from the WCI. During monsoon season, nutrient availability in the euphotic zone fosters increased phytoplankton activity, leading to the generation of a higher amount of organic matter along WCI, compared to non-monsoon season (Shetye et al. 2022; Nandakumar et al. 2023). Recent research along the WCI also indicates the prominent presence of

phytodetritus in the particulate organic matter influenced by physical forces such as upwelling and monsoonal terrestrial runoff (Pradhan et al. 2014; Silori et al. 2021; 2023). During the non-monsoon season, bacterial diversity in the coastal locations displayed notable similarities with the off-shore locations. As the monsoon season progressed, a distinct separation between coastal and off-shore bacterial populations was observed. This was confirmed through statistical tests and two-way PERMANOVA analysis, emphasizing the influence of both seasonality and sampling locations (Figure 4.1). The influence of the monsoon is reported to be more pronounced near the coast compared to deeper waters from across the globe (Parvathi et al. 2019; Anas et al. 2021; James et al. 2022; Castillo et al. 2022).

During both the non-monsoon and monsoon seasons, four dominant bacterial phyla were observed including Proteobacteria, Bacteroidetes, Firmicutes and Actinobacteria. These are largely reported from various marine regions and are known to play pivotal roles in various biogeochemical recycling processes (Fuhrman et al. 2015; Sun et al. 2020; Castillo et al. 2022; Arandia-Gorostidi et al. 2022). The Proteobacteria phylum, specifically the Gammaproteobacteria class, displayed the most substantial seasonal variation. Genera like *Idiomarina* and *Marinobacter* saw significant reductions in their relative abundance from the non-monsoon to the monsoon season (Figure 4.2). Such declines in specific taxa could be attributed to various factors, including competition for resources, predation, or changes in water parameters brought about by the monsoon. Conversely, certain genera within the Alphaproteobacteria class, like *Nitratireductor* and *Pelagibaca*, exhibited an increased abundance during the monsoon season, suggesting that these taxa might have ecological or metabolic advantages under increased organic load during monsoonal conditions (Labbé et al. 2004; Tseng et al. 2015; Thiele et al. 2018). Notably, seasonal shifts were also observed in Bacteroidetes genera *Zunongwangia*, whose abundance increased during the non-monsoon season, highlighting its ability to break down complex organic matter and providing new insights into the adaptations and functionalities during low nutrient conditions (Qin et al. 2010; Williams et al. 2013). On the other hand, Bacteroidetes genera *Leeuwenhoekiella* increased during the monsoon season, which shows its resilience or adaptability to changing conditions (Gifford et al. 2020). Within the Actinobacteria phylum, the increase in *Nocardioides*' relative abundance from the non-monsoon to the monsoon season could imply a role for this genus in decomposing complex organic matter, a function often associated with Actinobacteria in marine environments (Yaradoddi et al. 2021). Bacterial

genera from phylum Firmicutes were only detected during the non-monsoon season. In contrast, the absence of genera like *Exiguobacterium* and *Staphylococcus* from Firmicutes phylum during the monsoon season could be due to environmental stresses or competitive interactions (Hede & Khandeparker, 2020). Interestingly, the Cyanobacteria phylum, represented by the genus *Synechococcus*, increases during the monsoon season (Figure 4.2). This observed increase in Cyanobacterial genera was also evident in previous studies based on 16S rRNA amplicon sequencing (Anas et al. 2021). As primary producers, Cyanobacteria play a crucial role in marine carbon cycling (Huisman et al. 2018; Anas et al. 2021; Liu et al. 2022). Their absence during the non-monsoon season and subsequent surge in the monsoon season suggests that these organisms might be responding to increased nutrient availability or light conditions favourable for photosynthesis during the monsoon (Huisman et al. 2018; Bachmann et al. 2018; Liu et al. 2022). The observed seasonal variations in bacterial diversity and distribution underscore the dynamic nature of microbial communities in marine ecosystems (Broman et al. 2021; Arandia-Gorostidi et al. 2022; Saunders et al. 2022). These shifts can offer valuable insights into the ecological and biogeochemical processes that govern these environments.

4.4.2. Seasonal variation in metabolism and cellular processes

The functional metabolic diversity of marine bacterial communities is influenced by various environmental forces they encounter, especially in regions subject to pronounced seasonal variations in primary productivity dynamics along the WCI. Shotgun metagenomics based on KEGG and EggNOG pathway analyses has shown that metabolism was among the highest functional categories in both seasons, emphasizing the central role bacteria play in marine biogeochemical cycles. The surge in energy metabolism pathways (Figure 4.4 and S2) during the monsoon season suggests an intensified energy demand, possibly due to the increased autotrophic and heterotrophic activity. A significant increase in energy production and conversion could reflect the increased availability of organic substrates (Traving et al. 2017), possibly due to increased productivity as well as organic matter deposition from terrestrial runoff along WCI during monsoon season (Maya et al. 2011; Pradhan et al. 2014). This surge in metabolic activity might facilitate the microbial communities' ability to rapidly exploit available resources

during the monsoon. A significant increase in amino acid transport and metabolism during the monsoon season could indicate a heightened demand for protein synthesis, aligning with the observed increase in translation processes (Figures 4.3 and 4.4). This reiterates the notion that the monsoon season, with its characteristic environmental conditions, drives microbial communities towards an intensified metabolic state, optimizing growth and proliferation (Dang & Lovell, 2015; Smriga et al. 2016; Traving et al. 2017; Enke et al. 2018).

The heightened representation of genetic information processing during the monsoon season may suggest increased genomic activity, possibly due to higher bacterial replication and repair processes during the dynamic monsoonal conditions (Smriga et al. 2016; Jurgensen et al. 2022). The increase in translation activity (Figure 4.4) emphasizes an upscaling of protein synthesis, key to meeting the monsoonal metabolic demands (Zhang et al. 2022). The functional category of the EggNOG database, 'function unknown' displayed a higher percentage during the monsoon season (Appendix 4.1). This category's prominence underscores the vast uncharacterised microbial functional genomics (Galperin et al. 2015; Thomas & Segata, 2019). The variations across seasons in this category suggest that many microbial processes integral to monsoonal adaptations remain poorly understood, necessitating further exploratory research.

4.4.3. Seasonal variation in organic matter utilization potential

Marine phytoplankton are a significant source of abundant organic carbon in the ocean. Nevertheless, the increased dissolved organic matter and its implications for the biological pump and microbial loop largely rest on the degradation processes carried out by heterotrophic bacteria and the specific nature of the organic matter (Mühlenbruch et al. 2018; Kieft et al. 2021; Izabel-Shen et al. 2021; Moran et al. 2022). This relationship and impact have been explored and discussed in multiple studies over the years along WCI (Bhaskar & Bhosle, 2008; Khandeparker et al. 2011; Khodse & Bhosle, 2011, 2013; Basu et al. 2013). Assessments of heterotrophic enzyme activities have been utilized as tools to establish links between organic matter degradation and bacterial interactions along the WCI (Divya et al. 2010; Kamaleson et al. 2019; Parab et al. 2022). Building upon this, shotgun metagenomics analyses have facilitated in-depth functional

assessments, elucidating a variety of enzymes and pathways involved in organic matter recycling, thereby enhancing understanding of microbial functionalities in these regions.

Carbohydrate-active enzymes (CAZymes) serve as the primary biochemical tool harnessed by marine microbial communities to decompose and break the complex polysaccharides (Sosa et al. 2017; Drula et al. 2022; Eigemann et al. 2023). The seasonal variations in CAZyme profiles within the sampling regions along the WCI offered valuable insights into the carbohydrate metabolism of the bacterial communities, which have not been previously investigated through metagenomics approaches. The consistent presence of Glycosyltransferases, which increase during the monsoon season, underscores their pivotal role in marine carbohydrate dynamics, possibly facilitating the synthesis of complex polysaccharides as a carbon storage strategy (Thrash et al. 2017; Wei et al. 2021). This surge might be linked to a higher production of extracellular polymeric substances (EPS) observed in late phytoplankton blooms, a phenomenon prevalent in the southwest monsoon season in WCI (Ramaiah et al. 2002; Bhaskar & Bhosle, 2006) aiding in carbon sequestration through the creation of both easily degradable and more resistant EPS (Decho & Gutierrez, 2017; Mari et al. 2017).

Glycoside hydrolases, critical in breaking down glycosidic bonds, showed considerable fluctuation between non-monsoon and monsoon seasons, highlighting a likely increase in phytoplankton-derived organic matter degradation during the latter (Figure 4.5b). This can be attributed to the enhanced activities of enzymes GH13 and GH1, which are responsible for targeting starch and cellulose, respectively. This phenomenon supports the notion that the monsoon season fosters a richer carbohydrate environment through primary productivity and terrestrial influences (Sarma et al. 2014; Nageswar Rao et al. 2019), demanding more robust enzymatic breakdown processes (Arnosti et al. 2021). The abundance of enzymes like GH38 and GH65, which target mannose and maltose respectively, could be indicative of the degradation of specific phytoplankton-derived monosaccharides in the water column during monsoon season (Mühlenbruch et al. 2018; James et al. 2019). Furthermore, terrestrial run-offs, heightened during monsoon due to increased rainfall, introduce a range of organic compounds into the marine system along WCI (Khodse & Bhosle, 2013; Sarma et al. 2014; Nageswar Rao et al. 2019). The presence of enzymes like GH17, targeting β-1, 3-Glucan and GH28, acting on pectin, can be linked to the decomposition of terrestrial organic matter introduced to the marine system (Figure 4.5). The steady occurrence of Polysaccharide Lyases in both seasons highlights their ongoing role in breaking down polysaccharides

non-hydrolytically, including those in algal cell walls such as pectin and alginate (Thrash et al. 2017).

The analysis of peptidase enzymes has shown significant variations among peptidase types across monsoon and non-monsoon seasons. The surge in the enzymatic activities of methionine aminopeptidase 1 in monsoon season reveals that microbes are involved in the targeted cleavage of prevalent amino acids (Li et al. 2016; Cabello-Yeves et al. 2021; Kilgour et al. 2022). Levels of glutamate carboxypeptidase increased during the monsoon season, potentially signalling an adaptation to changes in the composition of organic matter. Carboxypeptidases, known to associate with sinking marine particles, play a substantial role in the degradation of labile organic matter and facilitate carbon and nitrogen cycling in the ocean (Liu & Liu, 2018; Dithugoe et al. 2023). The significant presence of lipid metabolism enzymes, such as acetaldehyde dehydrogenase and alcohol dehydrogenase during the non-monsoon season (Figure 4.7); underscores their role in the conversion of recalcitrant organic compounds to more labile forms, such as carboxylic acid (Landry et al. 2017; Thrash et al. 2017; Saw et al. 2020). These could play a pivotal role in energy production during the non-monsoon season when primary productivity might be lower, making readily available carbon sources scarcer. However, the pronounced increase in the activity of β-oxidation cycle enzymes during monsoon season highlights the microbial community's transition towards more extensive fatty acid degradation (Liu & Liu, 2018; Usman et al. 2022; Loza et al. 2022). Overall, these findings emphasize the complex interaction between seasonal shifts and enzyme activity, revealing a heightened role in the biogeochemical cycling of organic matter (James et al. 2019; Inomura et al. 2022; Jenkinson, 2023). The observed functional shifts with increased carbohydrase, peptidase and lipase activity in the monsoon season, highlight the increased microbial activity, ensuring ecological stability in marine biogeochemical processes.

4.5. Conclusion

In this study, shotgun metagenomics was employed to uncover the bacterial diversity and their functional dynamics along WCI. Seasonal shifts, especially from non-monsoon to monsoon season were observed in bacterial taxonomic composition and their subsequent metabolic pathways. The increased degradation activities during the monsoon

season can be attributed to the increased primary productivity. Bacterial community analyses show a significant increase in the abundance of bacterial genera *Idiomarine* in non-monsoon and *Alteromonas, Nocardioides* and *Synechococcus* during monsoon season. The increased microbial role in the monsoon season was evident from various metabolic pathways, CAZymes, peptidases and lipid utilization processes. This study provides an in-depth assessment of bacterial functional diversity contributing to the understanding of the microbial processes associated with increased primary productivity along the WCI. While shotgun metagenomics offers an in-depth view of microbial functional diversity, it is not without limitations. The inherent biases, potential loss of low-abundance taxa and challenges in precise taxonomic assignment warrant complementary methodologies for a complete understanding. In this regard, RNA-based transcriptomic studies would be essential, which can provide insights into the actively transcribed genes, transcriptomics can provide real-time snapshots of microbial activity, revealing the immediate metabolic responses to environmental fluctuations. Further studies based on metabolomics and transcriptomics would enhance our understanding of these pathways' broader implications on organic matter recycling, sequestration and associated microbial ecology along WCI during these contrasting seasons.

Chapter 5.

Summary and conclusion

5.1. Summary

The West Coast of India (WCI) is influenced largely by semi-annual changes in the wind pattern and monsoonal currents. This is reported to cause upwelling which increases the phytoplankton growth in this region during the summer monsoon and it is recorded to be one of the world's highest primary productivity regions. Studies have shown that the average primary productivity (PP) along the WCI is calculated to be about 210 mg C m^{-2} d^{-1} and it increases close to 950 mg C m^{-2} d^{-1} during the summer or southwest monsoon season as compared to less productive non-monsoon season. During the southwest monsoon season, the upwelling of nutrient-rich subsurface waters leads to an increase in the phytoplankton biomass and this is known to control the production of OM in these regions. These seasonal changes in primary productivity have been shown to influence bacterial abundance and productivity, with increased bacterial activity during the summer monsoon. An increase in phytoplankton productivity is known to influence microbial activity which is one of the key biogeochemical processes of the marine ecosystem as it plays an important role in the biotransformation of organic matter. However, the seasonal influence of varying primary productivity on the bacterial communities, their activity and functional profiles are not elucidated thoroughly from these regions. Hence, during this study, the relationship between the physicochemical characteristics and PP on the bacterial abundance, cultivable and metagenomic diversity and functional profiles based on Illumina NGS and shotgun sequencing approach during the non-monsoon and monsoon season along the WCI representing the continental shelf, slope and off-shore regions was studied.

The influence of varying primary productivity during the non-monsoon and monsoon seasons on the culturable bacterial diversity was studied based on the bacterial abundance, viable counts and radioactivity-based *in-situ* productivity measurements, along with the physicochemical characteristics. Statistical analysis showed a significant ($p < 0.05$) correlation between primary and bacterial productivity. Significant seasonal variations could also be established for the physicochemical parameters such as dissolved oxygen, nitrate, nitrite and phosphate, fluorescence values representing the chlorophyll concentration, bacterial abundance, viable counts and productivity. Taxonomic diversity studies on the cultivable bacterial morphotypes had shown that Proteobacteria, Firmicutes and Actinobacteria were the major phyla found during both the non-monsoon and

monsoon seasons and Bacteroidetes, was represented only in the non-monsoon season. The bacterial community structure at phylum, genus and operational taxonomic unit levels also changed with higher diversity in the monsoon season. However, the hydrolytic enzyme activity of the bacterial morphotypes was comparatively lower during the monsoon season probably due to the active primary production (Figure 5.1). Though this study has improved our understanding of the monsoonal influence on the bacterial dynamics along the WCI, further studies based on metagenomic analysis were required to better understand the role played by the bacterial community from this region.

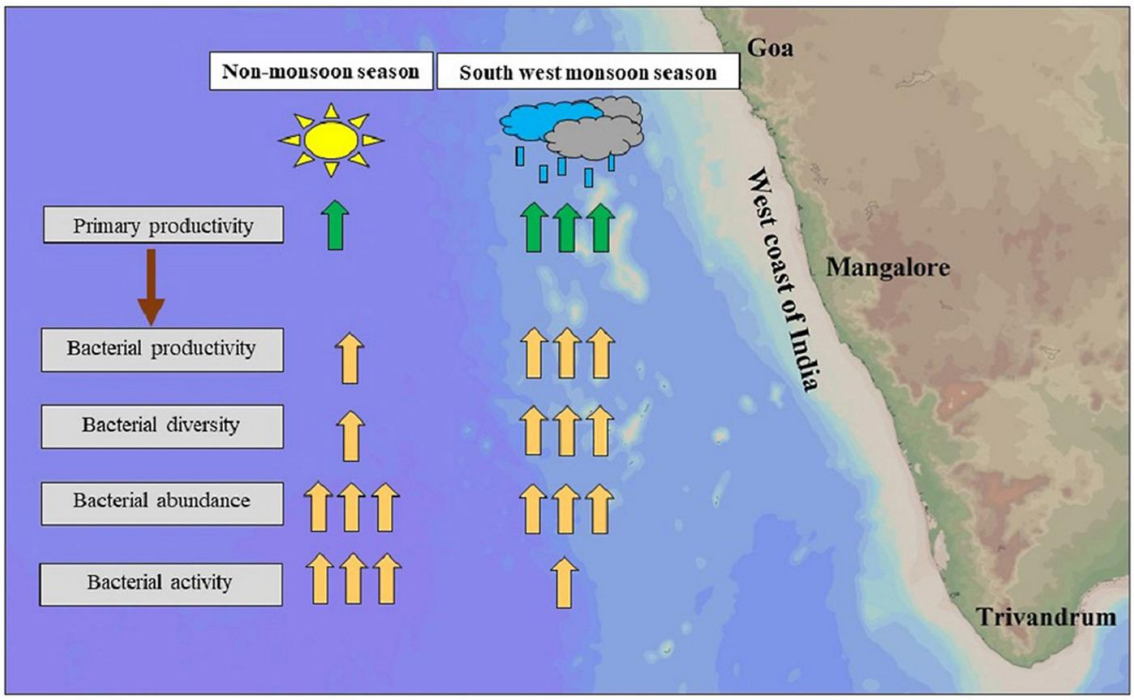


Figure 5.1: Summary of results from culturable diversity study during non-monsoon and monsoon season along the west coast of India.

Results from the culturable bacterial diversity studied along the WCI showed higher bacterial abundance during the monsoon season and with the highest colony forming units (CFUs) from the C-Max depths. The C-Max is a prominent feature of tropical marine environments occurring between 20-100 m water depths with maximum primary productivity, due to the optimal physicochemical conditions for the growth of phytoplankton, leading to a very high availability of organic substrate for bacteria. High-throughput sequencing of 16S rRNA amplicons was carried out to study the impact of increased productivity on the bacterial dynamics along the WCI, during non-monsoon and monsoon seasons.

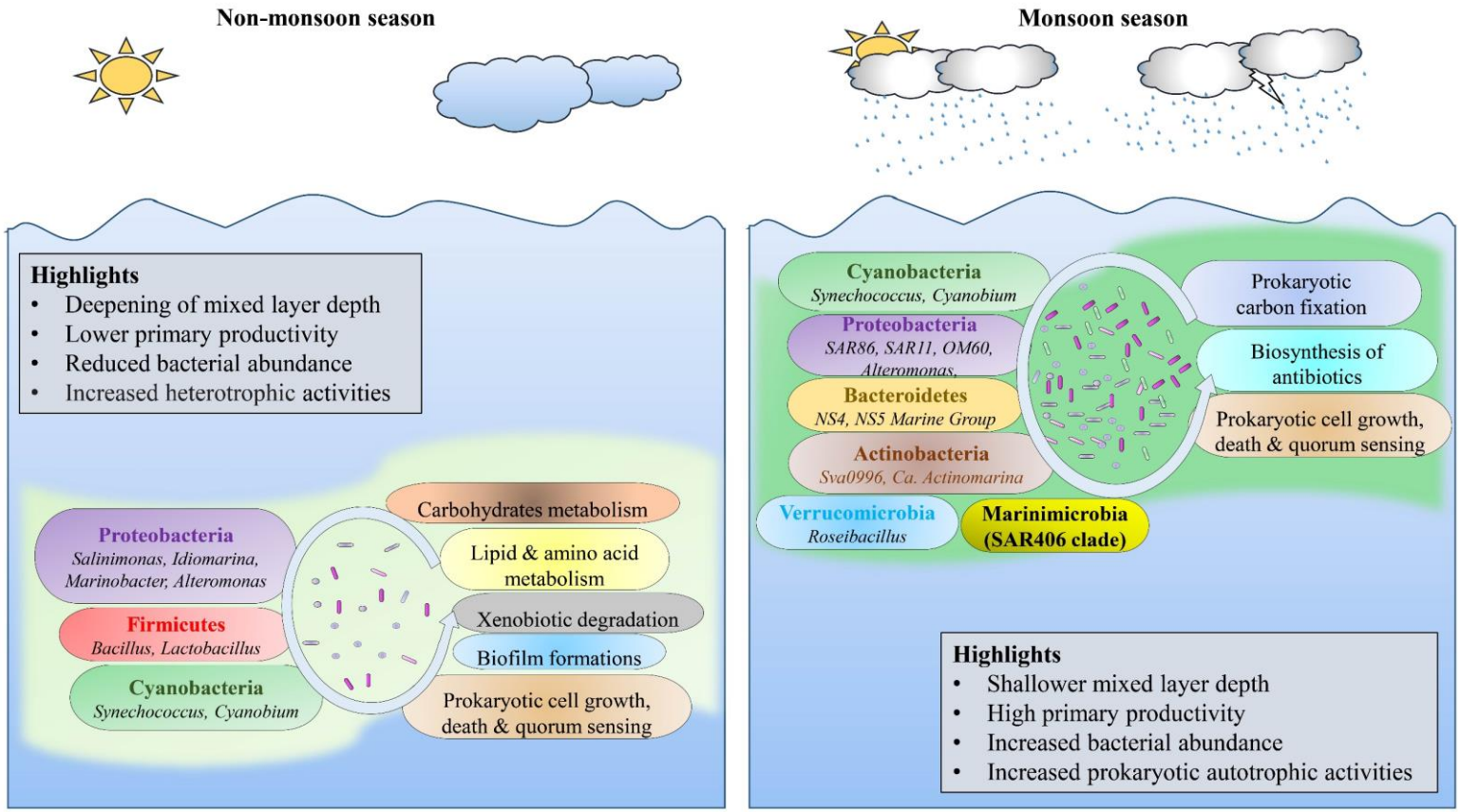


Figure 5.2: Summary of results from metagenomic-based 16S diversity studies during non-monsoon and monsoon seasons along the west coast of India.

The taxonomic profiling revealed distinct dominant bacterial groups in both seasons. During the non-monsoon season, Proteobacteria and Firmicutes were prevalent, whereas in the monsoon season, Cyanobacteria and Proteobacteria were more prominent, followed by Bacteroidetes, Actinobacteria, and various candidatus phyla. Canonical correspondence analysis highlighted the impact of primary productivity on bacterial genera, revealing clear seasonal variations. In the non-monsoon season, key taxa included *Idiomarina*, *Alteromonas*, *Marinobacter*, and *Alcanivorax* from Gammaproteobacteria; *Sulfitobacteria* and various uncultured members of Sphingomonadaceae and Rhodobacteraceae from Alphaproteobacteria; and *Lactobacillus* from Firmicutes. Contrastingly, the monsoon season exhibited an increase in autotrophic communities, including the *Cyanobium* and *Synechococcus* genera of Cyanobacteria, and an increase in heterotrophic groups, predominantly candidatus bacterial taxa. This included SAR86, OM60 (NOR5) from Gammaproteobacteria, NS4 and NS5 marine groups from Bacteroidetes, and Actinobacterial genera like Sva0996 marine groups, Ca. *Actinomarina*, and Ca. *Marinimicrobia*. The study suggests that further research into the functional roles and ecological significance of these candidatus phyla is needed to fully understand their role in the marine environment. The analysis also showed that seasonal shifts in primary productivity significantly affect the functional profiles of these bacterial communities (Figure 5.2).

The influence of seasonal changes in primary productivity on bacterial functions along the WCI was also studied using the deep sequencing technique, based on shotgun metagenomics. This approach generated extensive metagenomics data, which were then thoroughly analysed for both taxonomic and functional diversity, utilizing databases like KEGG, EggNOG and CAZy. The functional analysis of the metagenomic datasets revealed a broad spectrum of functional capabilities within the bacterial communities in the WCI. This included genes related to vital processes such as energy metabolism, amino acid metabolism, and cellular processes, showing significant variation between seasons. Furthermore, notable differences were observed in the abundance of CAZy enzymes, particularly Glycoside hydrolase. The diverse array of CAZymes, peptidases, and lipid utilization pathways highlighted in the study underscores the integral roles these bacterial communities play in marine biogeochemical cycles along the WCI. These findings demonstrate that bacterial communities not only exhibit significant taxonomic diversity but also possess a wide range of functional capabilities that vary seasonally (Figure 5.3).

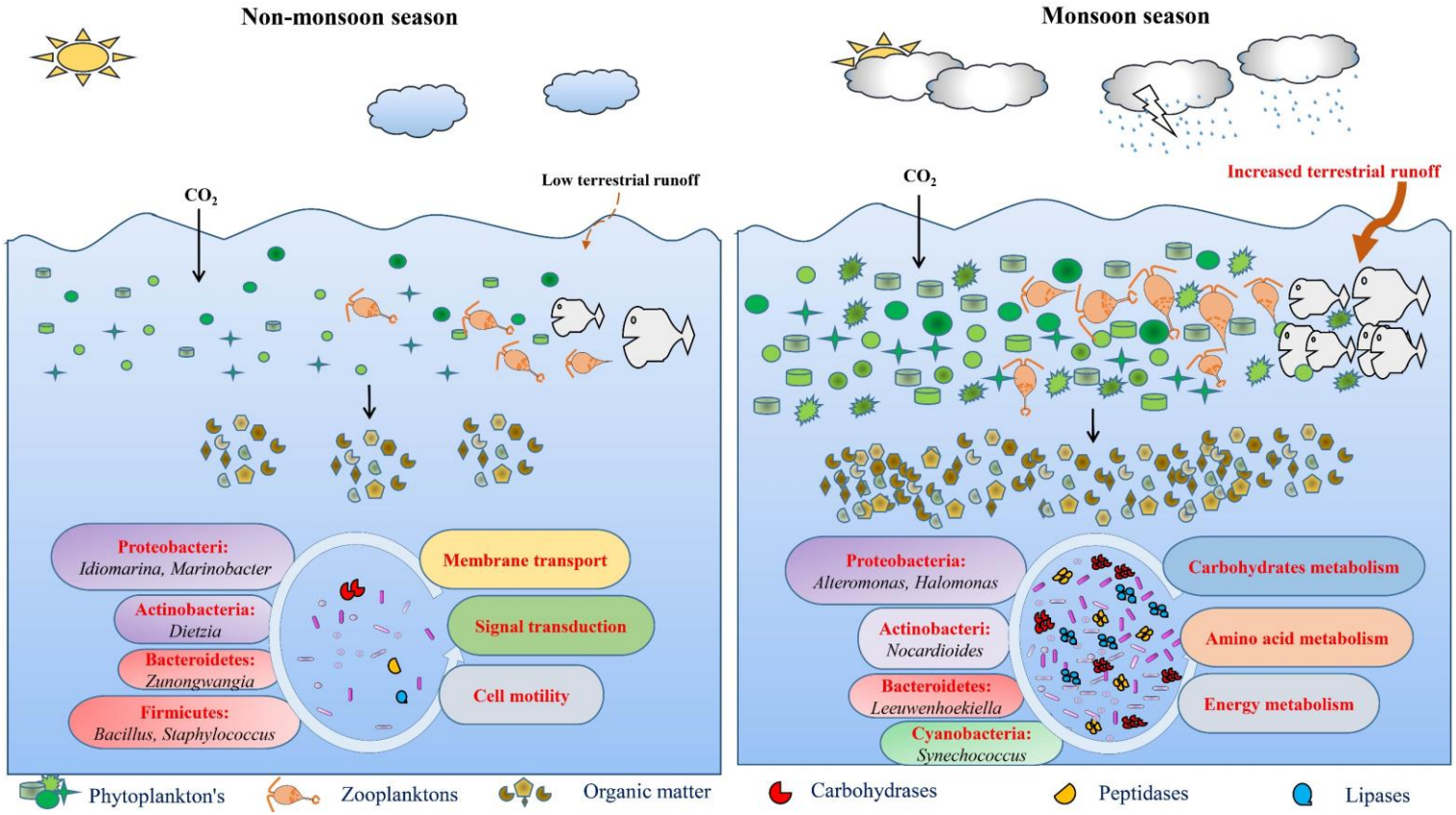


Figure 5.3: Summary of results from Shotgun metagenomic-based functional diversity studies during non-monsoon and monsoon season along the west coast of India.

5.2. Conclusion

This study has shown that primary productivity significantly influences bacterial productivity, viable counts and diversity in the monsoon. The difference in the bacterial diversity characterized shows distinct phyla, genera and OTUs during the non-monsoon and monsoon seasons with a very low percentage of overlap. Taxonomic diversity studies based on cultivable methods showed that Proteobacteria, Firmicutes, Actinobacteria and Bacteroidetes were the major phyla found during both seasons. Increased diversity was retrieved based on metagenomic studies from both seasons, however, the predominant heterotrophic phyla were Proteobacteria, Firmicutes, Actinobacteria and Bacteroidetes. Along with this, the autotrophic bacterial community, Cyanobacteria increased significantly during the monsoon season. An increased abundance of candidatus phyla was also reported in the monsoon season and a large majority of the core microbiome genera belonged to the candidatus taxa in the monsoon season. Studies on the substrate utilization capacity from the bacterial morphotypes obtained from both seasons showed that the morphotypes from the non-monsoon season showed higher hydrolytic enzyme activity. The decrease in bacterial activity in the monsoon could be due to the active phytoplankton community in this season. However, we still lack an understanding of the factors that influence extracellular enzyme activity in marine habitats. Major implications from this study are based on the observed taxonomic and functional diversity patterns, combined with physicochemical factors, due to primary productivity variations and relationships among bacterial communities. Study using Next-generation sequencing methods has provided a deeper understanding of the community structure and diversity associated with seasonal changes in primary productivity, along the WCI. The level of taxonomic resolution provided by Illumina sequencing allowed us to build upon previous research and uncover, seasonal gradients of the in-depth bacterial taxonomic assessment, revealing spatiotemporal variations at the genera level, from this region. Additionally, Illumina sequencing has enabled us to understand the presence of candidatus phylotypes that are part of the dominant genera which could not be characterized by conventional culture-based methods. The seasonal transitions along the WCI and the dynamics of the bacterial community composition are most likely the outcome of increased primary productivity and associated seasonal environmental factors.

The functional profiles of the cultivable bacterial community were higher in the non-monsoon season compared to the monsoon season. The NGS-based predictive functional profiles of the bacterial community showed the dominance of autotrophic forms in the monsoon season and the heterotrophic activity was found to be higher in the non-monsoon season. However, the shotgun metagenomic whole genome sequencing results have shown that there was an increased heterotrophic activity during the monsoon season, largely correlating with the increased primary productivity in this region. These shifts were evident from various CAZymes, peptidases and lipid utilization pathways; this paves the way to elucidate the microbial role in the marine biogeochemical processes in the recycling of the organic matter. These results were not evident from the cultivation-based and predictive functional profile analysis. However, shotgun-based functional diversity has helped to improve the understanding of the seasonal changes in microbial community structure and along the WCI which could be from the undefined and candidatus taxa. A critical component of this study involves comprehensive research work involving various methodologies for analysing bacterial diversity and its function role. The culturable methods are fundamental, they offer a limited perspective, often missing non-culturable yet functionally significant bacteria. In contrast, metagenomics provides a more comprehensive overview of the microbial community, capturing both culturable and non-culturable species, thereby broadening our understanding of microbial diversity. Metagenomics, while providing a comprehensive perspective on microbial functions, has certain limitations. These include biases inherent in the method, the possible omission of low-abundance taxa, and difficulties in accurately identifying species. To gain a fuller understanding, it's crucial to employ additional methods. RNA-based transcriptomic studies are particularly valuable in this context. They offer insights into the genes that are actively being transcribed, thus giving a real-time glimpse into microbial activities and their immediate responses to changes in the environment. Incorporating studies that focus on both metabolomics and transcriptomics will deepen our understanding of how these processes impact organic matter recycling and storage, as well as the overall microbial ecology along WCI.

Appendix

Appendix 1.1: Bacterial studies in the different areas within the Northern Indian Ocean region using conventional tools

S.No.	Study area*	Bacterial parameters studied	Bacterial activities reported	References
1.	Nearshore, coastal stations along the west and southeast coast of India from Dhabol, Maharashtra to Tuticorin, Tamil Nadu	Bacterial CFU (ZMA), biochemical tests for bacterial enzyme activity from sediment	Dehydrogenase, carbohydrase, proteolytic and lipolytic activity	Nair et al. 1978
2.	Along the 1000m depth contour off Karnataka and Kerala coast	Bacterial CFU (ZMA), biochemical tests for bacterial enzyme activity from water samples collected at different depths	Nitrate reducers, ammonia oxidisers, gelatin liquifiers, hydrogen sulphide producers, fat hydrolysers and Thiosulphate oxidisers	Nair et al. 1979
3.	Nearshore regions off the Mangalore coast	Bacterial CFU (ZMA and NA), culturable diversity through biochemical tests	Halotolerent and limnotolerent groups	Nair and Loka Bharathi 1982
4.	Coral reef lagoons near the Agatti Island of Lakshadweep	Culturable diversity of bacteria associated with sediments	Green photosynthetic bacteria	Loka Bharathi and Chandramohan,1986
5.	Ferromanganese nodules from the Equatorial Indian Ocean	Bacterial CFU (NA), biochemical tests for diversity and enzyme activity	Carbohydrase, nitrate reducers, lipolytic, gelatinolytic, amylolytic and phospholytic	Chandramohan et al. 1987
6.	Nearshore, lagoons and mudbanks off Mangalore, Calicut, Cochin and Lakshadweep Islands	Bacterial CFU (NA), biochemical tests for diversity and enzyme activity	Sulphate reducers	Loka Bharathi and Chandramohan,1990

7.	Intertidal regions off Goa at Anjuna and Baga beach and coral reef lagoons of Lakshadweep Islands	Bacterial abundance, bacterial CFU (SWC), biochemical tests for diversity and enzyme activity	Carbohydrase activity based on cellulase, alginate and pectin lyase activity	Ramaiah and Chandramohan,1992a
8.	Estuarine, coastal, and oceanic samples from the Arabian Sea region	Luminous bacterial CFU (SWC), biochemical tests for diversity and enzyme activity	L-asparaginase activity	Ramaiah & Chandramohan,1992b
9.	Offshore regions in the Arabian Sea and off Madras in the Bay of Bengal region	Bacterial CFU (ZMA), biochemical tests for activity	Nitrate and sulphate-reducing bacteria	Loka Bharathi et al.1992
10.	Central Arabian Sea and Equatorial Indian Ocean	Bacterial abundance (AO), bacterial productivity (3H-thymidine)	-	Ducklow 1993
11.	Coastal and offshore stations from 14 to 8°N, 70 to 77°E in the Arabian Sea	Bacterial abundance (DAPI), bacterial CFU (ZMA), biochemical tests for diversity and enzyme activity	Sugar utilization, carbohydrase activity, nitrate reduction and ammonium production	Nair et al. 1994
12.	Coastal and offshore stations from 22 to 10°N, 62 to 75°E in the central and eastern Arabian Sea	Bacterial abundance (AO), bacterial productivity (3H-thymidine)	-	Ramaiah et al. 1996a
13.	Coastal stations off Madras, Cuddalore, Nagapattinam, and Tuticorin off the Tamil Nadu coast	Bacterial abundance (AO), bacterial CFU (NA), biochemical tests for diversity and enzyme activity	Amylase, catalase, lipase, gelatinase and oxidase	Ramaiah et al. 1996b
14.	Offshore stations along 64°E in the Arabian Sea and 90°E in the Bay of Bengal	Bacterial abundance (AO), biochemical tests for bacterial activity and transparent exopolymeric particle quantification	-	Dileep Kumar et al. 1998

15.	Offshore stations from 22 to 10°N, 50 to 70°E in the Arabian Sea	Bacterial abundance (Flow cytometry)	-	Campbell et al.1998
16.	Offshore stations in the central Arabian Sea and coastal stations along Oman's coast	Bacterial abundance (AO), productivity (3H- leucine) and biochemical tests for enzyme activity	Phosphatase, glucosidase and aminopeptidase	Hoppe and Ullrich 1999
17.	Offshore stations in the central Arabian Sea and coastal stations along Oman's coast	Bacterial abundance (DAPI) and productivity (3H-thymidine, 3H-leucin)	-	Pomroy and Joint 1999
18.	Offshore stations in the Arabian Sea	Bacterial abundance (AO), productivity (3H-thymidine), and biochemical tests for bacterial activity	Glucosidase, chitobiose, lipase, sulfatase, phosphatase, butyrate, aminopeptidase and sulphate reduction	Boetius et al. 2000
19.	Offshore stations along 64 °E, 13 to 21°N in the Arabian Sea region	Bacterial abundance (AO), bacterial productivity (3H-thymidine), biochemical tests for bacterial activity (TEPS)	-	Ramaiah et al. 2000
20.	Offshore Arabian Sea region	Bacterial abundance (AO) and productivity (3H-thymidine) (PP 14C)	-	Prasanna Kumar et al. 2001
21.	Offshore stations in the central and western Arabian Sea	Bacterial abundance (Flow cytometry) and productivity (3H-thymidine, 3H-leucin)	-	Ducklow et al. 2001
22.	Northern and Central Arabian Sea	Bacterial abundance (AO) and Productivity (3H-thymidine)	-	Ramaiah et al. 2005
23.	Arabian Sea and the Bay of Bengal	Bacterial abundance and productivity (3H-thymidine) (PP 14C)	-	Gauns et al. 2005
24.	Western and Central Arabian Sea	Bacterial abundance (DAPI)	-	Koppelmann et al. 2005

25.	Continental slope in the western Bay of Bengal	Bacterial CFU (ZMA)	-	Das et al. 2007
26.	Coastal and Central Bay of Bengal	Bacterial abundance (AO) and productivity (3H-thymidine) (PP 14C)	-	Fernandes et al. 2008a
27.	Equatorial Indian Ocean along 83°E from 5 to 9°N	Bacterial abundance (AO) and productivity (3H-thymidine) (PP 14C)	-	Fernandes et al. 2008b
28.	Equatorial Indian Ocean along 77 and 83°E from 2 N to 2 °S	Bacterial abundance (AO), biochemical tests for bacterial activity (TEPS)	-	Damare and Raghukumar 2008
29.	Mandovi estuary off Goa along the west coast	Bacterial abundance (AO), CFU (NA), biochemical tests for bacterial activity	Nitrate reduction	Divya et al. 2009
30.	Mandovi estuary off Goa along the west coast of India	Bacterial abundance (AO) and productivity (3H-thymidine) (PP 14C)	-	Gonsalves et al. 2009
31.	Oxygen Minimum zone of the Arabian Sea	Bacterial abundance (AO), CFU and productivity (3H-thymidine), biochemical tests for bacterial activity	Nitrate reducer, Sulphate reducer	Gonsalves et al. 2011
32.	Coastal stations in the mangrove regions of Goa	Bacterial abundance (AO), CFU (NMS media) and biochemical tests for bacterial activity	Sulfur and Methane-oxidation	Kamaleson et al. 2019
33.	Coastal station in Port Blair	Bacterial CFU (Luminescent Agar), biochemical tests for diversity and bacterial enzyme activity	Protease, lipase, chitinase	Zari et al. 2020

*Studies from different regions the AS, BoB and EIO are marked in green, blue and yellow respectively, those addressing more than one region are marked white.

Appendix 1.2: Bacterial studies in the different areas within the Northern Indian Ocean region using molecular tools.

S.No.	Study area*	Bacterial parameters studied	Bacterial activities reported	References
1.	Offshore stations in the central Arabian Sea and coastal stations along Oman's coast	Metagenomic diversity based on DGGE	-	Riemann et al. 1999
2.	Central Arabian Sea and EIO	Bacterial abundance (Flow cytometry), metagenomic diversity through cloning and CARD-FISH	-	Fuchs et al. 2005
3.	Oxygen Minimum zone of the Arabian Sea	Bacterial abundance (DAPI, Flow cytometry), metagenomic diversity based on RFLP	Anammox, nirS and nirK	Jayakumar et al. 2009
4.	Oxygen Minimum zone of the Arabian Sea	16SrRNA-based culturable diversity biochemical tests for bacterial activity	Amylase, lipase, esterase, gelatinase, DNAse, urease, phosphatase and citrate synthase	Divya et al. 2010
5.	Oxygen Minimum zone of the Arabian Sea	16SrRNA-based metagenomic diversity	-	Divya et al. 2011
6.	Mandovi and Zuari estuary off Goa along the west coast of India	16SrRNA-based culturable diversity, biochemical tests for bacterial activity	Amylase, xylanase, and cellulase	Khandeparkar et al. 2011
7.	Oxygen Minimum zone of the Arabian Sea	16SrRNA-based metagenomic diversity and RNA-based qPCR analysis	PC-monoether ladderane, anammox 16S rRNA and hzo genes	Pitcher et al. 2011

8.	Coastal stations off Ratnagiri, Mormugao, Karwar and Bhatkal	Bacterial abundance (DAPI), metagenomic diversity through DGGE	-	Singh and Ramaiah 2011
9.	Oxygen Minimum zone of the Arabian Sea	16SrRNA-based metagenomic diversity and RNA-based qPCR analysis	nirS, nirK, hzo and amx	Jayakumar et al. 2013
10.	Coastal and open ocean Arabian Sea and Equatorial Indian Ocean	16SrRNA-based culturable diversity, biochemical tests for bacterial activity	Siderophore production	Rajyasabhapathi et al. 2013
11.	Northeastern Arabian Sea	Bacterial CFUs (TYE medium), culturable diversity through 16S rRNA (PP 14C)	-	Basu et al. 2013
12.	Equatorial Indian Ocean	Metagenomic diversity based on cloning	-	Khandeparkar et al. 2014
13.	Oxygen Minimum zone of the Arabian Sea	Bacterial abundance (DAPI), metagenomic diversity based on DGGE	-	Jain et al. 2014
14.	Coastal stations off Cochin	Bacterial abundance (FISH), metagenomic diversity based on DGGE, biochemical tests for bacterial activity	Ammonia oxidation	Veettil et al. 2015
15.	Coastal stations in the Gulf of Mannar region	16S rRNA-based metagenomic diversity	-	Jasmine et al. 2015
16.	Offshore stations off Cochin	16S rRNA-based metagenomic diversity	-	Nair et al. 2016
17.	Oxygen Minimum zone of the Arabian Sea	16S rRNA-based metagenomic diversity	-	Bandekar et al. 2016
18.	Coastal stations off Cochin	16S rRNA-based culturable diversity		Anas et al.2016

19.	Oxygen Minimum zone of the Arabian Sea	16S rRNA-based metagenomic diversity	-	Bandekar et al. 2017
20.	Oxygen Minimum zone of the Arabian Sea	16S rRNA-based metagenomic diversity	-	Jasmin et al. 2017
21.	Coastal station along the west coast of India, off Goa, Mangalore and Kochi	Bacterial abundance(DAPI), metagenomic diversity based on qPCR	narG, nirS and nosZ	Gomes et al. 2017
22.	Oxygen Minimum zone of the Arabian Sea	Metagenomic diversity based on DGGE	-	Divya et al. 2017
23.	Equatorial Indian Ocean and the Bay of Bengal	Metagenomic diversity based on qPCR	amoA	Wang et al. 2017
24.	Coastal stations in the mudbank region of Alapuzha, Cochin	Bacterial abundance (FISH), metagenomic diversity based on DGGE	-	Anas et al. 2018
25.	Equatorial Indian Ocean and the Bay of Bengal	Metagenomic diversity based on qPCR	Brod541F and Amx820R	Qian et al. 2018
26.	Oxygen Minimum zone of the Arabian Sea	16SrRNA-based culturable diversity, biochemical tests for bacterial activity	Nitrate reduction	Amberkar et al. 2018
27.	Oxygen Minimum zone of the Arabian Sea	Metagenomic diversity based on qPCR	nirS and hzo	Bandekar et al. 2018
28.	Oxygen Minimum zone of the Arabian Sea	16SrRNA-based culturable diversity, biochemical tests for bacterial activity	Nitrate reduction	Mulla et al. 2018

29.	Coastal stations off Kunkeshwar, Malvan, Vagator and Cabo de Ram	16SrRNA-based culturable diversity	-	Pawar et al. 2018
30.	Oxygen Minimum zone of the Arabian Sea and the Bay of Bengal	16SrRNA-based culturable diversity	Thiosulphate oxidation	Menezes et al. 2020
31.	Offshore station off Cochin	Metagenomic diversity based on qPCR	hzsA, hzo	Lincy & Manohar 2021
32.	Open ocean stations off Cochin	Bacterial abundance (FISH), 16S rRNA-based metagenomics diversity	-	Anas et al. 2021a
33.	Coastal stations off Cochin	16SrRNA-based culturable diversity	-	Anas et al.2021b
34.	Open ocean stations off Goa, Mangalore and Trivandrum	16SrRNA and FAME-based culturable diversity	-	Isaacs et al. 2021
35.	The west coast of India	Bacterial abundance, productivity, respiration and culturable diversity	Carbohydrase, Protease and Lipase activities	Parab et al. 2022

*Studies from different regions the AS, BoB and EIO are marked in green, blue and yellow respectively, those addressing more than one region are marked white.

Appendix 1.3: Bacterial studies in the different areas within the Northern Indian Ocean region through Next Generation sequencing approach.

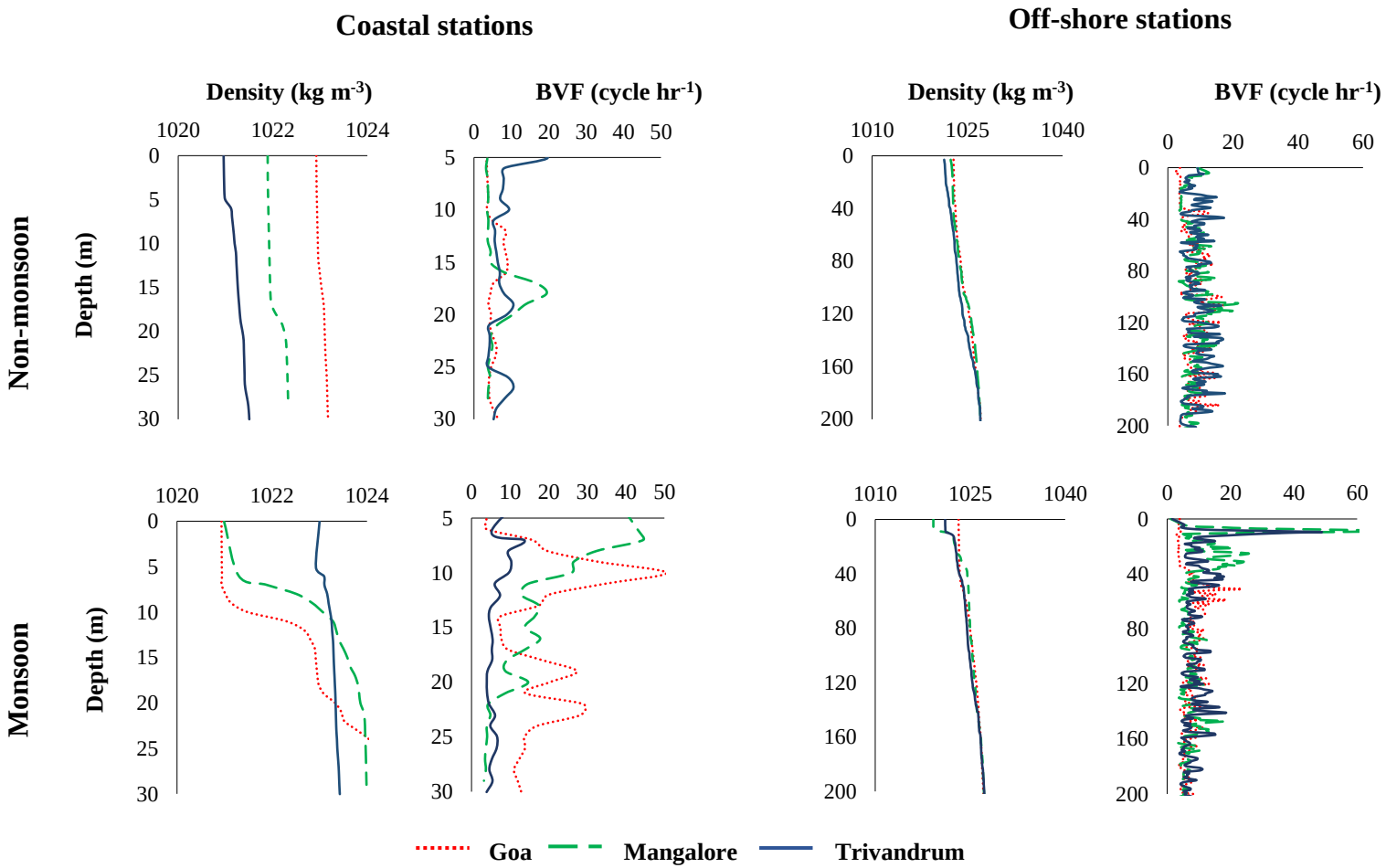
S.No.	Study area*	Bacterial parameters studied	Bacterial activities reported	References
1.	Oxygen Minimum zone	OMZ: Metagenomic diversity of anammox bacteria through NGS	-	Villanueva et al. 2014
2.	The Bay of Bengal and the Equatorial Indian Ocean	Metagenomic diversity based on DGGE and NGS	-	Jing, 2016
3.	Oxygen Minimum zone	Metagenomic diversity based on NGS and functional diversity	-	Lüke et al. 2016
4.	Mandovi and Zuari estuary off Goa	Metagenomic diversity based on NGS	-	Khandeparker et al. 2017
5.	Bay of Bengal	DGGE, Metagenomics diversity based on NGS	Carbohydrates, Amino acid metabolism	Verma et.al 2017
6.	Along the 85°E transect	Metagenomic diversity based on NGS	-	Sujith et al. 2017
7.	Oxygen Minimum zone	Metagenomic diversity based on NGS and functional diversity	Complex organic matter degraders, fermentative and exoelectrogenic bacteria, and sulfate-reducers	Fernandes et al. 2018
8.	OMZ and Non-OMZ	Metagenomic diversity based on NGS and functional diversity	Assimilatory sulphur reduction, Dissimilatory sulphate reduction	Rajpathak et al. 2018
9.	Mudbanks of Alapuzha, Cochin	Bacterial abundance (AO), metagenomic diversity based on NGS and functional diversity	Ammonia oxidation, Nitrite reduction, Sulphate reduction, Xylan degradation,	Parvathi et al. 2019

			Dehalogenation, Chitin degradation	
10.	Coastal stations off Goa	Metagenomic diversity based on NGS and functional diversity	Hydrocarbon degradation	Fernandes et al. 2019
11.	Oxygen Minimum Zone of the Bay of Bengal	16SrRNA-based culturable diversity, metagenomic diversity based on NGS and biochemical tests for bacterial activity	Nitrate reduction	Fernandes et al. 2019
12.	Equatorial Indian Ocean and the Bay of Bengal	Metagenomic diversity based on qPCR and NGS	nifH	Wu et al. 2019
13.	Oxygen Minimum zone of the Arabian Sea	Metagenomic diversity based on NGS and functional diversity	-	Paingankar et al. 2020
14.	Oxygen Minimum zone of the Arabian Sea and the Bay of Bengal	Metagenomic diversity based on NGS and functional diversity	Nitrogen fixation and carbon recycling	Lincy & Manohar 2020
15.	Coastal stations off Okha, Goa, Kasargod and Valapad	Metagenomic diversity based on NGS	-	Sachithanandam et al. 2020
16.	Oxygen Minimum zone of the Arabian Sea	16SrRNA-based culturable diversity, metagenomic diversity based on NGS and metatranscriptomics and functional diversity	-	Bhattacharya et al. 2020
17.	Coastal stations in the mangrove regions of Cochin	Metagenomic diversity based on NGS and functional diversity	Amino acid transport and metabolism, carbohydrate transport and metabolism, translation	Nathan et al. 2020

18.	Coastal and offshore stations in the Krishna and Godavari Basin	Bacterial abundance (AO), metagenomic diversity based on NGS	Sulfate reduction, Oxalic acid degradation, Dehalogenation, Nitrite reduction, Ammonia oxidation, Nitrogen fixation, Xylan degradation, and Aromatic hydrocarbons degradation.	Jasna et al. 2020
19.	Oxygen Minimum zone of the Arabian Sea and Bay of Bengal	Metagenomic diversity based on NGS and functional diversity	Nitrogen and Sulfur metabolism pathways	Fernandes et al. 2020
20.	Coastal and offshore stations in the Krishna and Godavari Basin	Metagenomic diversity based on NGS and functional diversity	Dehalogenation and Sulphide oxidation.	Parvathi et al. 2020
21.	South Eastern Arabian Sea	Metagenomics diversity based on NGS	-	Vipindas et al. 2020
22.	Coastal station off Malvan	Metagenomic diversity based on NGS and functional diversity	Photo-autotrophy, Aerobic nitrification, Sulphate reduction and Sulphide oxidization	Mote et al. 2021
23.	Oxygen Minimum zone of the Arabian Sea	Metagenomic diversity based on NGS and metatranscriptomic and functional diversity	Sulphate reducers, methanogens, acetogens, sulphur oxidation	Bhattacharya et al. 2021
24.	Equatorial Indian Ocean	Metagenomic diversity based on NGS and functional diversity	Carbon fixation and nitrogen cycling and metabolism	Ding et al. 2021
25.	Coastal stations off Mangalore and Haldia port	Bacterial abundance (Flow cytometry) and CFU (ZMA), metagenomic diversity based on NGS	-	Kuchi et al. 2021

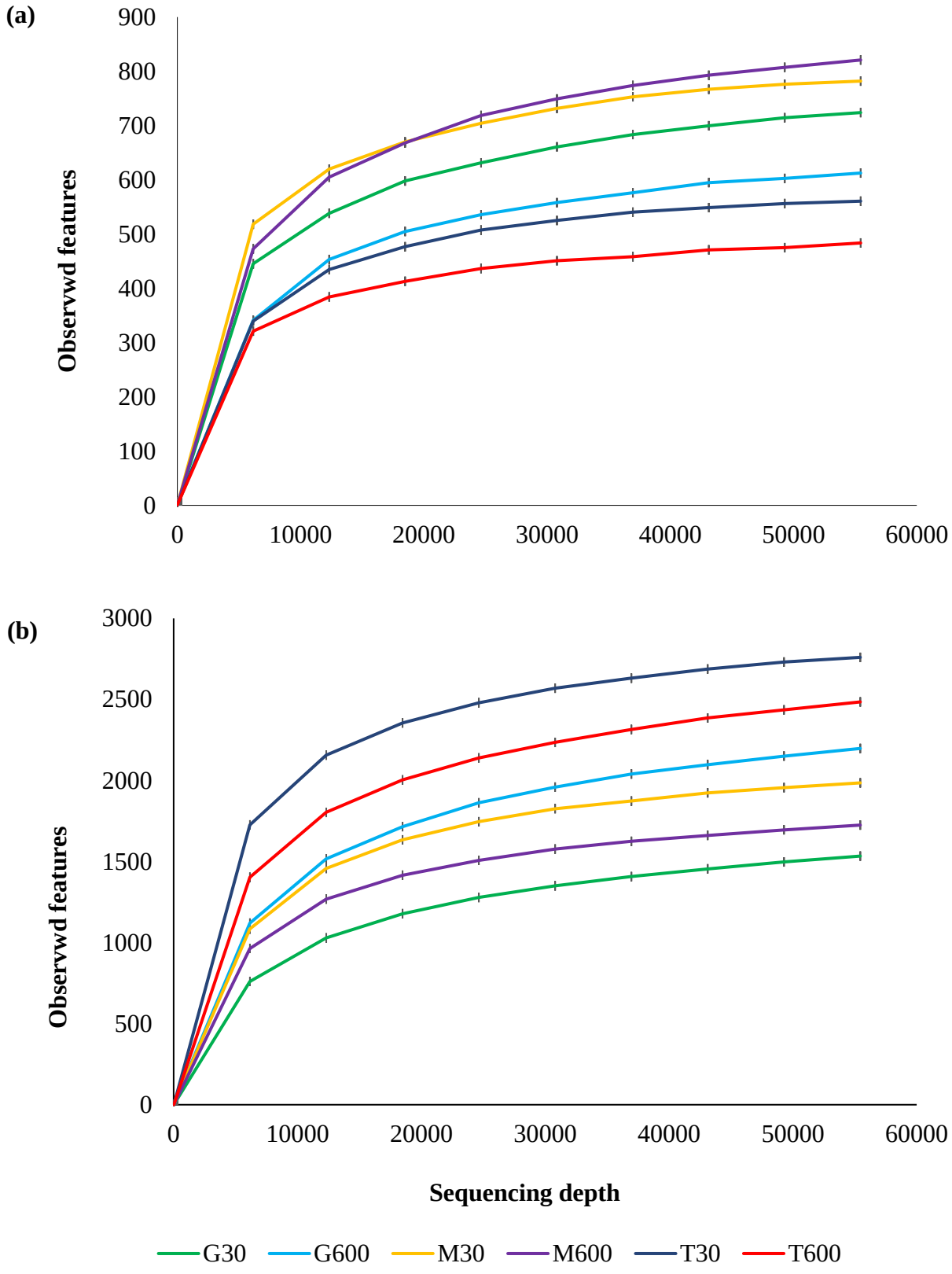
26.	Arabian Sea, Bay of Bengal and Equatorial Indian Ocean region	Metagenomic diversity based on NGS and metaproteomics analysis for functional diversity	Amino acid transport and metabolism	Xia et al. 2021
27.	Equatorial Indian Ocean along 88°E from 2 to 10°S	Metagenomic diversity based on NGS	-	Gao et al. 2021
28.	Intertidal zone of coastal regions of the Arabian Sea	Culturable and metagenomics diversity	Biofilm – forming, Hydrocarbon-degrading	Kumar et al. 2022
29.	Bay Of Bengal	Metagenomics based on NGS	Carbon dioxide fixation, sulfur, nitrogen metabolism, Bioremediation	Marimuthu et al. 2022
30.	Bay of Bengal and Equatorial Indian ocean	Metagenomics based on NGS	-	Zhu et.al 202

*Studies from different regions the AS, BoB and EIO are marked in green, blue and yellow respectively, those addressing more than one region are marked white.

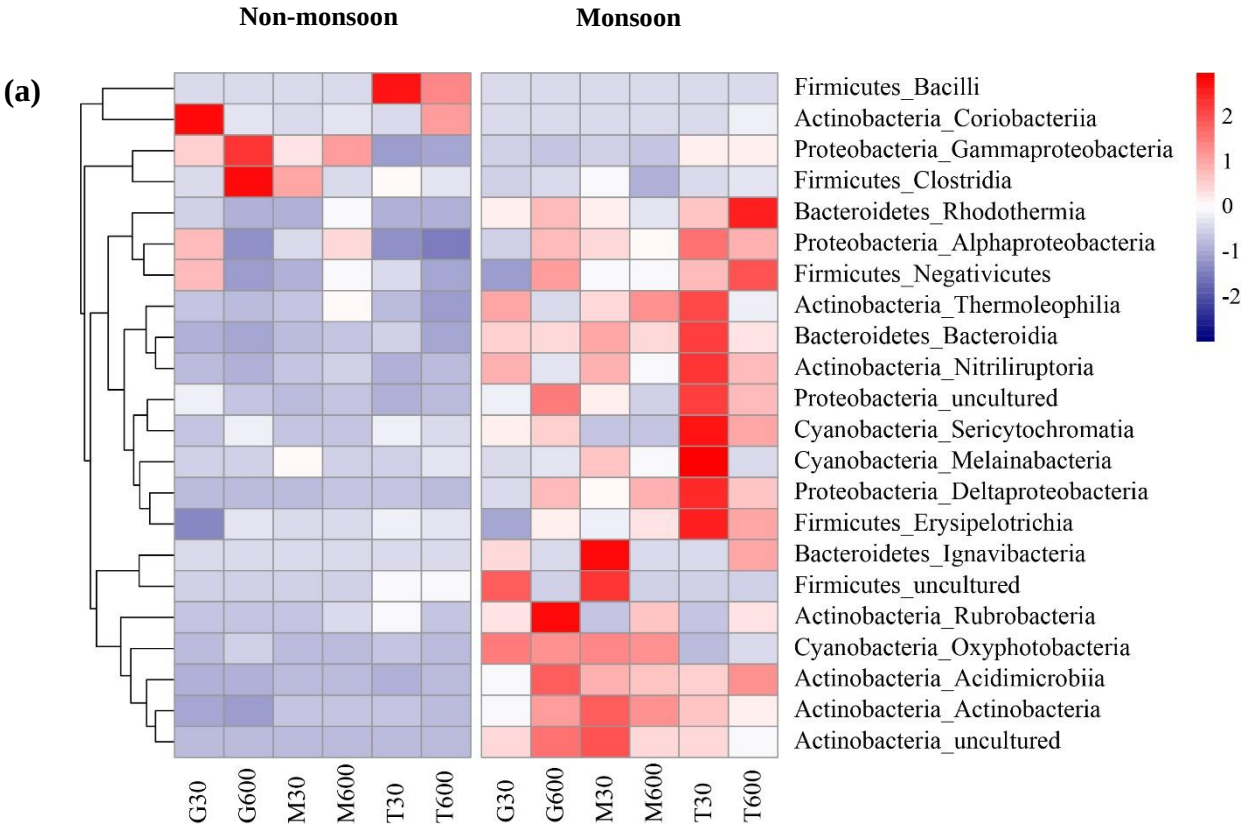


Appendix 3.1: Depth profiles of density and Brunt-Vaisala frequency[1] (BVF) in the water column of the coastal and off-shore stations during non-monsoon and monsoon season.

[1] The Brunt–Väisälä frequency (N) was determined using the formula: $\mathbf{N} = \sqrt{(-\mathbf{g}/\boldsymbol{\rho} * \mathbf{d}\boldsymbol{\rho}/\mathbf{dz})}$, where ρ represents the potential density influenced by both temperature and salinity, g signifies the gravitational acceleration and z denotes the geometric depth (Nampoothiri et al., 2020).



Appendix 3.2: Rarefactions curve at various sequencing depths for (a) non-monsoon and (b) monsoon seasons.



Appendix 3.3: Hierarchal clustering heatmap based on the Euclidian similarity index, showing the relative abundance of bacteria at class level (a) from top five phyla and (b) from other than top five phyla during non-monsoon and monsoon season

(b)


Non-monsoon
Monsoon
Chloroflexi_Chloroflexia
Chloroflexi_KD4-96
Planctomycetes_BD7-11
Acidobacteria_Subgroup 6
Acidobacteria_Subgroup 9
Planctomycetes;_
Chlamydiae_Chlamydiae
Planctomycetes_Phycisphaerae
WPS-2_Ambiguous_taxa
Dependentiae_Babeliae
Patescibacteria_Saccharimonadia
Spirochaetes_Leptospirae
Acidobacteria_Acidobacteriia
Fibrobacteres_Chitinivibrionia
Lentisphaerae_
Fibrobacteres_Fibrobacteria
Kiritimatiellaeota_Kiritimatiellae
Planctomycetes_Planctomycetacia
Planctomycetes_OM190
Tenericutes_Mollicutes
Patescibacteria_Parcubacteria
Acidobacteria_Thermoanaerobaculia
Patescibacteria_WWE3
Epsilonbacteraeota_Campylobacteria
Spirochaetes_Spirochaetia
Margulisbacteria_
Marinimicrobia (SAR406 clade)_Alteromonas macleodii
Bacteria_
Acidobacteria_Subgroup 21
Acidobacteria_Subgroup 22
Patescibacteria_ABY1
Patescibacteria_Microgenomatia
Chloroflexi_Anaerolineae
Hydrogenedentes_Hydrogenedentia
Chloroflexi_JG30-KF-CM66
Acidobacteria_Subgroup 17
Zixibacteria_uncultured bacterium
WPS-2_uncultured bacterium
Schekmanbacteria_uncultured delta proteobacterium
Nitrospirae_Thermodesulfovibrionia
Marinimicrobia (SAR406 clade)_uncultured gamma proteobacterium
Lentisphaerae_Oligosphaeria
BRC1_uncultured bacterium
Lentisphaerae_Lentisphaeria
Latescibacteria_
Latescibacteria_uncultured bacterium
Gemmatimonadetes_PAUC43f marine benthic group
Latescibacteria_Latescibacteria
Deinococcus-Thermus_Deinococci
Deferribacteres_Deferribacteres
Halanaerobiaeota_Halanaerobiia
Nitrospinae_P9X2b3D02
Schekmanbacteria_uncultured bacterium
Latescibacteria_uncultured Gemmatimonadetes bacterium
Planctomycetes_Pla4 lineage
Marinimicrobia (SAR406 clade)_Ambiguous taxa
Marinimicrobia (SAR406 clade)_uncultured bacterium
Margulisbacteria_marine metagenome
Margulisbacteria_metagenome
Marinimicrobia (SAR406 clade)_marine metagenome
Margulisbacteria_uncultured marine cyanobacterium
Marinimicrobia (SAR406 clade)_Marinimicrobia bacterium JGI 0000113-D11
Margulisbacteria_uncultured marine bacterium
Planctomycetes_SGST604
Planctomycetes_vadinHA49
Acidobacteria_Blastocatellia (Subgroup 4)
PAUC34f_uncultured marine bacterium
Marinimicrobia (SAR406 clade)_
Marinimicrobia (SAR406 clade)_uncultured marine bacterium
Marinimicrobia (SAR406 clade)_unidentified marine bacterioplankton
Marinimicrobia (SAR406 clade)_uncultured bacterium FPPU_33B15
Verrucomicrobia_Verrucomicrobiae
Margulisbacteria_uncultured bacterium
Dadabacteria_Dadabacteriia
Marinimicrobia (SAR406 clade)_Marinimicrobia bacterium SCGC AAA003-L08
Marinimicrobia (SAR406 clade)_uncultured marine microorganism
Nitrospinae_Nitrospinia
Patescibacteria_Gracilibacteria
Gemmatimonadetes_BD2-11 terrestrial group
Chloroflexi_Dehalococcoidia
Marinimicrobia (SAR406 clade)_uncultured Candidatus Marinimicrobia bacterium
Marinimicrobia (SAR406 clade)_uncultured Fibrobacteres/Acidobacteria group bacterium
Marinimicrobia (SAR406 clade)_metagenome
Fusobacteria_Fusobacteriia
Patescibacteria_WS6 (Dojkabacteria)
Marinimicrobia (SAR406 clade)_hydrothermal vent metagenome
Planctomycetes_Pla3 lineage
3
2
1
0
-1
-2
-3
G30
G600
M30
M600
T30
T600
G30
G600
M30
M600
T30
T600

Appendix 3.4: Depth-wise values of chlorophyll-a (Chl-a), primary productivity (PP), bacterial productivity (BP), bacterial abundance (BA), bacterial carbon (BC), and total viable count (TVC) in the water column of the coastal and off-shore stations during non-monsoon (NMS) and monsoon season (MS).

Stations	**Depth**	**Chl-a (mg m^{-3})**		**PP (mg C m^{-3} d^{-1})**		**BP (mg C m^{-3} d^{-1})**		**BA (Cells × 10^9 L^{-1})**		**BC (mg C m^{-3})**		**TVC (CFU mL^{-1})**	
		NMS	MS	NMS	MS	NMS	MS	NMS	MS	NMS	MS	NMS	MS
G30	Surface	0.12	0.53	18.70	266.24	0.66	13.10	8.90	59.89	178.09	1197.85	152	5200
	C-Max	0.11	1.05	10.77	83.01	2.01	13.88	13.73	62.39	274.51	1247.76	85	90000
	30	0.08	0.07	8.08	29.62	10.25	14.68	9.30	36.53	186.03	730.51	1090	7200
G600	Surface	0.07	0.06	11.82	73.44	3.56	11.38	13.78	129.54	275.64	671.52	500	6850
	C-Max	0.09	0.07	8.68	59.68	2.77	7.35	17.92	39.70	358.45	2590.8	2240	1600
	100	0.02	0.004	6.58	36.20	4.15	5.48	11.80	57.40	235.94	794.03	80	13400
	200	ND	ND	5.38	59.83	4.37	5.62	11.23	68.97	224.6	1147.94	1770	6500
	400	ND	ND	ND	ND	1.00	5.95	6.92	17.70	138.39	1379.34	580	36900
	600	ND	ND	ND	ND	1.87	8.25	6.18	29.27	123.64	353.91	3560	15300

M30	Surface	0.14	0.12	145.38	75.98	0.82	6.80	13.95	55.13	279.04	585.31	3600	7550
	C-Max	0.14	0.35	48.01	50.11	6.15	6.96	12.76	66.02	255.22	353.91	4480	2300
	30	0.12	0.36	18.85	33.06	4.41	7.63	14.24	45.37	284.72	1102.56	4020	17400
M600	Surface	0.02	0.05	5.24	65.51	3.74	10.76	7.37	52.86	147.46	907.46	170	750
	C-Max	0.16	0.23	8.53	31.56	29.93	5.28	8.96	50.14	179.22	744.12	3080	35000
	100	0.01	0.02	3.14	30.66	32.42	6.38	7.15	51.27	142.92	694.21	3335	25000
	200	ND	ND	7.48	29.76	31.39	9.61	6.81	31.99	136.12	1057.19	7640	11500
	400	ND	ND	ND	ND	27.55	8.20	4.59	31.08	91.88	1002.74	1055	3550
	600	ND	ND	ND	ND	30.48	7.21	3.29	30.17	65.79	1025.43	6080	15000
T30	Surface	0.05	0.87	8.23	94.83	6.73	16.93	11.06	84.85	221.19	639.76	6640	1500
	C-Max	0.18	0.40	11.37	55.64	7.69	14.38	10.89	43.78	217.79	621.61	475	250
	30	0.29	0.29	10.47	37.99	5.83	12.23	10.95	34.71	218.92	603.46	17840	100
T600	Surface	0.03	0.03	7.63	76.58	2.09	11.07	11.23	73.50	224.6	1329.43	18080	1280
	C-Max	0.82	0.05	14.21	37.39	15.00	8.41	16.79	80.08	335.76	766.8	9320	830
	100	0.06	0.04	1.79	29.91	20.00	9.65	9.70	42.65	193.97	650.35	3080	430
	200	ND	ND	1.20	32.61	15.00	12.99	10.10	27.22	201.91	635.22	5080	860

400	ND	ND	ND	ND	25.90	9.26	8.17	22.69	163.34	281.31	3900	680
600	ND	ND	ND	ND	28.82	14.19	4.25	18.15	85.07	1470.09	4450	670

ND: Not determined

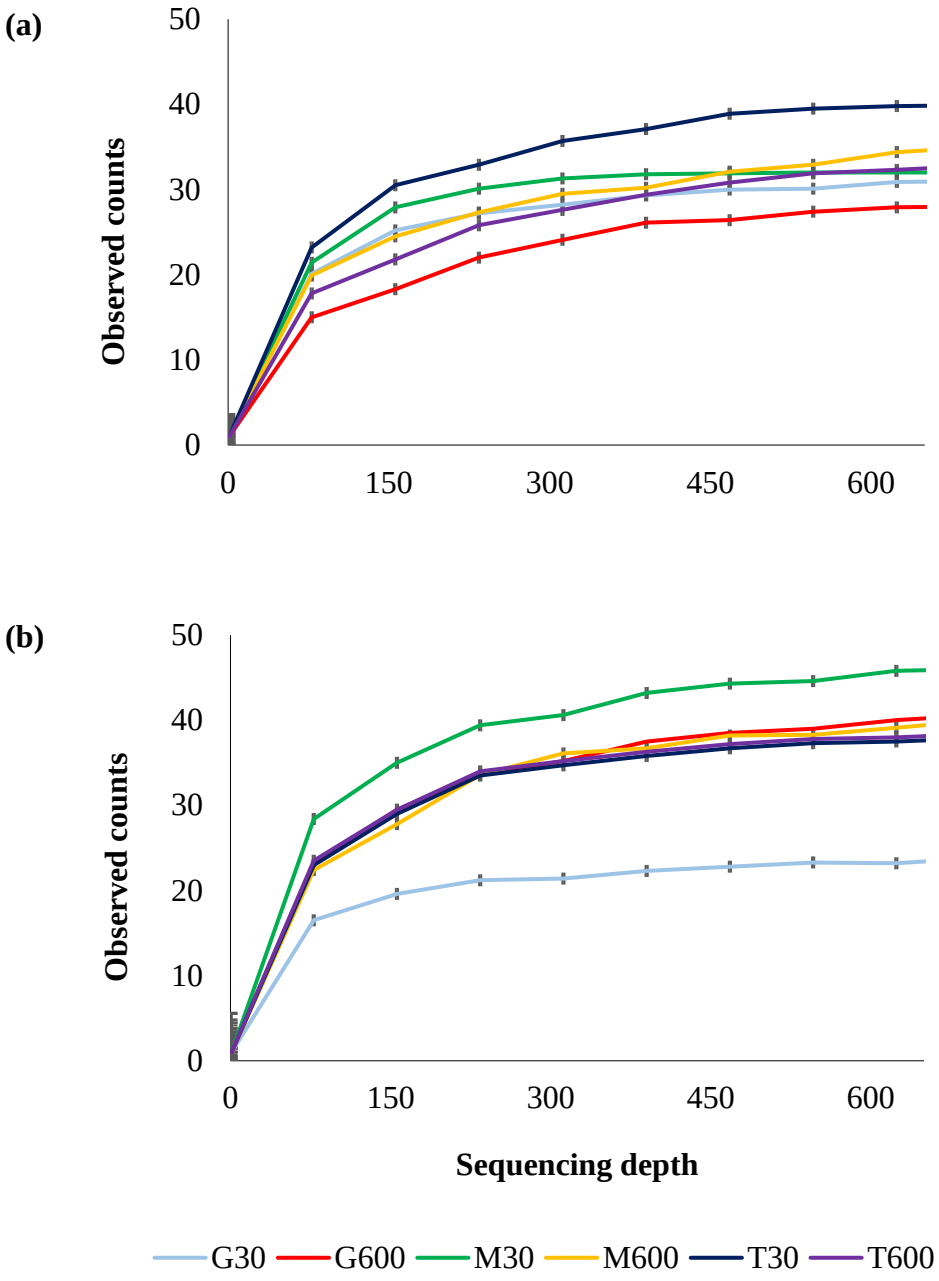
Appendix 3.5: Read statistics of the amplicon sequence variants (ASVs) and operational taxonomic units (OTUs) generated.

Station	**ASVs**			**OTUs**
	Input	**Merged**	**Non-chimeric**	
Non-monsoon season				
G30	1160519	842896	69470	740
G600	1043626	795324	108281	657
M30	871125	633751	55446	740
M600	1056229	786561	82877	657
T30	1211616	913814	107047	590
T600	854647	510738	66070	492
Monsoon season				
G30	1179010	857178	138751	1737
G600	1204483	850357	148939	2444
M30	1255518	914848	151014	2172
M600	1033245	736013	135516	1869
T30	1120905	790040	100403	2878
T600	1121972	758076	96196	2619

Appendix 3.6: Analysis of variance (ANOVA: single factor) assessing the seasonal difference in the bacterial OTUs generated and diversity indices between non-monsoon and monsoon season.

	df	**MS**	**F**	**F crit**	**P-value**
OTUs	1	7691204	72.45	4.96	< 0.001**
Simpson	1	0.005	5.83	4.96	0.036 *
Shannon	1	8.240	15.22	4.96	0.003**
Chao-1	1	7680800	72.75	4.96	< 0.001**
ACE	1	7830975	73.05	4.96	< 0.001**

Df: degree of freedom, F value greater than F critical value indicates statistical significance. **: significant at 0.1%, *: significant at 1%.



Appendix 4.1: Rarefaction analysis curves of species richness based on observed counts from the sampling locations during (a) non-monsoon and (b) monsoon season.

Appendix 4.2: Comparative heatmap of relative abundances of EggNOG pathways across all stations during non-monsoon and monsoon seasons. The symbols * and ** represent significance levels of $p < 0.05$ and $P < 0.001$, respectively.

Appendix 4.3: Station details and read statistics of metagenomics sequences during non-monsoon (NMS) and monsoon season (MS).

Station	**Latitude (◦N)**	**Longitude (◦E)**	**Chl-M depth (m)**		**Raw reads (×10^6)**		**Filtered reads (×10^5)**		**Counts**		**ORFs (×10^5)**	
			NMS	MS	NMS	MS	NMS	MS	NMS	MS	NMS	MS
G30	15.2454	73.3633	3	10	14.69	8.41	9.03	4.22	722	778	2.21	2.68
G600	15.2301	72.4677	49	47	26.04	10.29	12.62	5.12	710	775	2.81	3.68
M30	12.5062	74.4087	19	15	13.21	10.12	6.47	4.85	719	767	2.19	3.36
M600	12.5076	73.5683	57	27	14.31	8.27	8.10	3.75	702	756	1.95	3.02
T30	8.2889	76.5144	26	9	20.08	9.78	7.95	4.46	791	777	6.20	3.27
T600	8.3011	76.1384	55	9	16.98	9.47	6.78	5.07	767	787	4.92	3.25

Appendix 4.4: Variations in KEGG pathway abundance between non-monsoon and monsoon seasons.

Categories	Pathways	Relative frequency (%) Non-monsoon	Relative frequency (%) Monsoon	p-values
Metabolism	Amino acid metabolism	9.68	10.26	0.016
	Energy metabolism	7.76	8.85	< 0.001
	Metabolism of cofactors and vitamins	5.82	6.33	0.0088
Genetic Information Processing	Folding, sorting and degradation	1.91	2.14	0.035
	Information processing in viruses	0.17	0.28	0.004
	Transcription	0.64	0.51	0.024
	Translation	2.90	3.50	< 0.001
Environmental Information Processing	Membrane transport	5.49	4.48	< 0.001
	Signal transduction	5.55	4.42	< 0.001
Cellular Processes	Cellular community - prokaryotes	4.17	3.36	< 0.001
Organismal Systems	Sensory system	0.05	0.01	0.0052
Human Diseases	Infectious disease: bacterial	1.39	1.16	0.012
	Infectious disease: viral	1.41	1.64	0.017

Appendix 4.5: Variations in EggNOG pathway abundance between non-monsoon and monsoon seasons.

EggNOG pathways	Relative frequency (%) Non-monsoon	Relative frequency (%) Monsoon	p-values
Amino acid transport and metabolism	8.08	8.74	< 0.001
Carbohydrate transport and metabolism	3.90	4.16	< 0.001
Cell motility	0.73	0.52	< 0.001
Cell wall/membrane/envelope biogenesis	6.22	5.87	< 0.001
Coenzyme transport and metabolism	4.50	4.69	0.011
Cytoskeleton	0.08	0.05	0.002
Defence mechanisms	1.51	1.27	< 0.001
Energy production and conversion	7.78	8.87	< 0.001
Function unknown	23.56	24.32	< 0.001
Inorganic ion transport and metabolism	5.50	5.04	< 0.001
Intracellular trafficking, secretion, and vesicular transport	1.81	1.65	< 0.001
Nuclear structure	0.00	0.01	0.010
Nucleotide transport and metabolism	3.29	3.57	< 0.001
Replication, recombination and repair	5.89	5.02	< 0.001
RNA processing and modification	0.13	0.08	< 0.001
Secondary metabolites biosynthesis, transport, and catabolism	2.00	2.18	< 0.001
Signal transduction mechanisms	3.19	2.17	< 0.001
Transcription	5.07	4.24	< 0.001
Translation, ribosomal structure and biogenesis	6.96	7.60	< 0.001

Bibliography

Al Azhar M, Lachkar Z, Lévy M, Smith S. (2017). Oxygen Minimum Zone Contrasts Between the Arabian Sea and the Bay of Bengal Implied by Differences in Remineralization Depth. Geophysical Research Letters 44(21): 106-11.

Albin, K. J., Jyothibabu, R., Alok, K. T., Santhikrishnan, S., Sarath, S., Sudheesh, V., Sherin, C. K., Balachandran, K. K., Asha Devi, C. R., & Gupta, G. V. M. (2022). Distinctive phytoplankton size responses to the nutrient enrichment of coastal upwelling and winter convection in the eastern Arabian Sea. Progress in Oceanography, 203, 102779.

AldeSrkamp AC, Van Rijssel M, Bolhuis H (2007) Characterization of marine bacteria and the activity of their enzyme systems involved in the degradation of the algal storage glucan laminarin. FEMS Microbiol Ecol 59:108-117.

Aldunate, M., De La Iglesia, R., Bertagnolli, A.D., Ulloa, O., (2018). Oxygen modulates bacterial community composition in the coastal upwelling waters off central Chile. Deep Sea Research Part II: Topical Studies in Oceanography 156, 68–79.

Alonso, C., Pernthaler, J., (2006). Roseobacter and SAR11 dominate microbial glucose uptake in coastal North Sea waters. Environ Microbiol 8, 2022–2030.

Alonso-Gutiérrez, J., Lekunberri, I., Teira, E., Gasol, J.M., Figueras, A., Novoa, B., (2009). Bacterioplankton composition of the coastal upwelling system of 'Ría de Vigo', NW Spain. FEMS Microbiology Ecology 70, 493–505.

Amberkar U, Khandeparker R, D Menezes L, Meena RM (2021) Phylogenetic diversity of culturable marine bacteria from sediments underlying the oxygen minimum zone of the Arabian Sea and their role in nitrate reduction. Mar Ecol 42:e12646.

Amin SA, Hmelo LR, Van Tol HM, Durham BP, Carlson LT, Heal KR, Morales RL, Berthiaume CT, Parker MS, Djunaedi B, Ingalls AE (2015) Interaction and signalling between a cosmopolitan phytoplankton and associated bacteria. Nature 522:98-101.

Anas A, Chekidhenkuzhiyil J, Nilayangod C, Krishna K, Raju GT. (2021). Prevalence of obligate and facultative anaerobic bacteria in the mudbank along the southwest coast of India. Regional Studies in Marine Science 42:101660.

Anas A, Nilayangod C, Jasmin C, Vinothkumar S, Parameswaran PS, Nair S. (2016). Diversity and bioactive potentials of culturable heterotrophic bacteria from the surficial sediments of the Arabian Sea. 3 Biotech 6(2):238.

Anas A, V.A. S, C. J, T.R. G, Mathew D, Krishna K, Nair S, K.R. M, K.V. J. (2018). Upwelling induced changes in the abundance and community structure of archaea and bacteria in a recurring mud bank along the southwest coast of India. Regional Studies in Marine Science 18:113–121.

Anas, A., E.M., B. T., C., J., Chandran, C., P.V., V., Narayanan, S., & K.U., A. J. (2021). Microbial community shifts along an estuarine to open ocean continuum. Regional Studies in Marine Science, 41, 101587.

Arandia-Gorostidi N, Krabberød AK, Logares R, Deutschmann IM, Scharek R, Morán XAG, González F, Alonso-Sáez L (2022) Novel Interactions Between Phytoplankton and Bacteria Shape Microbial Seasonal Dynamics in Coastal Ocean Waters. Front Mar Sci 9, 901201.

Arnosti C. Microbial extracellular enzymes and the marine carbon cycle (2011) Annu Rev Mar Sci 3:401-25.

Arnosti, C., Wietz, M., Brinkhoff, T., Hehemann, J.-H., Probandt, D., Zeugner, L., & Amann, R. (2021). The biogeochemistry of marine polysaccharides: Sources, inventories and bacterial drivers of the carbohydrate cycle. Annual Review of Marine Science, 13(1), 81–108.

Aytan U, Feyzioglu AM, Valente A, Agirbas E, Fileman ES (2018) Microbial plankton communities in the coastal southeastern Black Sea: biomass, composition and trophic interactions. Oceanologia 60, 139–152.

Azam, F., Malfatti, F., (2007). Microbial structuring of marine ecosystems. Nat Rev Microbiol 5, 782–791.

Bachmann, J., Heimbach, T., Hassenrück, C., Kopprio, G. A., Iversen, M. H., Grossart, H. P., & Gärdes, A. (2018). Environmental drivers of free-living vs. Particle-attached bacterial community composition in the Mauritania upwelling system. Frontiers in Microbiology, 9.

Bachmann, J., Heimbach, T., Hassenrück, C., Kopprio, G.A., Iversen, M.H., Grossart, H.P., Gärdes, A., (2018). Environmental Drivers of Free-Living vs. Particle-Attached Bacterial Community Composition in the Mauritania Upwelling System. Front. Microbiol. 9, 2836.

Bandekar M, Ramaiah N, Jain A, Meena RM. (2018). Seasonal and depth-wise variations in bacterial and archaeal groups in the Arabian Sea oxygen minimum zone. Deep Sea Research Part II: Topical Studies in Oceanography 156:4–18.

Bandekar M, Ramaiah N, Meena RM. (2018). Diversity and abundance of denitrifying and anammox bacteria from the Arabian Sea oxygen minimum zone. Deep Sea Research Part II: Topical Studies in Oceanography 156:19–26.

Banse K. (1959). On upwelling and bottom-trawling off the southwest coast of India. Journal of the Marine Biological Association of India 1(1):33–49.

Banse K. (1968). Hydrography of the Arabian Sea Shelf of India and Pakistan and effects on demersal fishes. Deep Sea Research and Oceanographic Abstracts 15(1):45–79.

Basu S, Deobagkar DD, Matondkar SP, Furtado I (2013) Culturable bacterial flora associated with the dinoflagellate green Noctiluca miliaris during active and declining bloom phases in the Northern Arabian Sea. Microb Ecol 65:934-954.

Basu S, Matondkar SGP, Furtado I. (2013). Retrieved bacteria from Noctiluca miliaris (green) bloom of the northeastern Arabian Sea. Chin J Ocean Limnol 31(1):10–20.

Bergen, B., Herlemann, D.P.R., Jürgens, K., (2015). Zonation of bacterioplankton communities along ageing upwelled water in the northern Benguela upwelling. Front. Microbiol. 6.

Bhaskar, P. V., & Bhosle, N. B. (2006). Dynamics of transparent exopolymeric particles (TEP) and particle-associated carbohydrates in the Dona Paula Bay, west coast of India. Journal of Earth System Science, 115(4), 403–413.

Bhaskar, P. V., & Bhosle, N. B. (2008). Bacterial production, glucosidase activity and particle-associated carbohydrates in Dona Paula Bay, west coast of India. Estuarine, Coastal and Shelf Science, 80(3), 413–424.

Bhattacharya S, Mapder T, Fernandes S, Roy C, Sarkar J, Rameez MJ, Mandal S, Sar A, Chakraborty AK, Mondal N, et al. (2021). Sedimentation rate and organic matter dynamics shape microbiomes across a continental margin. Biogeosciences 18(18):5203–5222.

Bhattacharya S, Roy C, Mandal S, Sarkar J, Rameez MJ, Mondal N, Mapder T, Chatterjee S, Pyne P, Alam M, et al. (2020). Aerobic microbial communities in the sediments of a marine oxygen minimum zone. FEMS Microbiology Letters 367(19): fnaa157.

Boetius A, Ferdelman T, Lochte K. (2000). Bacterial activity in sediments of the deep Arabian Sea in relation to vertical flux. Deep Sea Research Part II: Topical Studies in Oceanography 47(14):2835–2875.

Bolch CJ, Bejoy TA, Green DH (2017) Bacterial associates modify growth dynamics of the dinoflagellate Gymnodinium catenatum. Front Microbiol 8:670.

Bolger, A. M., Lohse, M., & Usadel, B. (2014). Trimmomatic: A flexible trimmer for Illumina sequence data. Bioinformatics, 30(15), 2114–2120.

Bolyen, E., Rideout, J.R., Dillon, M.R., Bokulich, N.A., Abnet, C.C., Al-Ghalith, G.A., et al. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat Biotechnol 37, 852–857.

Bora, S.S., Dullah, S., Dey, K.K., Hazarika, D.J., Sarmah, U., Sharma, D., Goswami, G., Singh, N.R., Barooah, M., (2022). Additive-induced pH determines bacterial community composition and metabolome in traditional mustard seed fermented products. Front. Sustain. Food Syst. 6, 1006573.

Bourgoin L-H, Tremblay L. (2010). Bacterial reworking of terrigenous and marine organic matter in estuarine water columns and sediments. Geochimica et Cosmochimica Acta 74(19):5593–5609.

Broman, E., Bonaglia, S., Norkko, A., Creer, S., & Nascimento, F. J. A. (2021). High throughput shotgun sequencing of eRNA reveals taxonomic and derived functional shifts across a benthic productivity gradient. Molecular Ecology, 30(13), 3023–3039.

Buchan A, LeCleir GR, Gulvik CA, González JM. (2014). Master recyclers: features and functions of bacteria associated with phytoplankton blooms. Nat Rev Microbiol 12(10):686–698.

Bunse, C., Bertos-Fortis, M., Sassenhagen, I., Sildever, S., Sjöqvist, C., Godhe, A., Gross, S., Kremp, A., Lips, I., Lundholm, N., Rengefors, K., Sefbom, J., Pinhassi, J., Legrand, C., (2016). Spatio-Temporal Interdependence of Bacteria and Phytoplankton during a Baltic Sea Spring Bloom. Front. Microbiol. 7.

Cabello-Yeves, P. J., Callieri, C., Picazo, A., Mehrshad, M., Haro-Moreno, J. M., Roda-Garcia, J. J., Dzhembekova, N., Slabakova, V., Slabakova, N., Moncheva, S., & Rodriguez-Valera, F. (2021). The microbiome of the Black Sea water column was analyzed by shotgun and genome centric metagenomics. Environmental Microbiome, 16(1), 5.

Cai, Y.-M., Ning, X.-R., Liu, C.-G., Hao, Q., (2007). Distribution Pattern of Photosynthetic Picoplankton and Heterotrophic Bacteria in the Northern South China Sea. J Integrative Plant Biology 49, 282–298.

Campbell L, Landry MR, Constantinou J, Nolla HA, Brown SL, Liu H, Caron DA. (1998). Response of microbial community structure to environmental forcing in the Arabian Sea. Deep Sea Research Part II: Topical Studies in Oceanography 45(10–11):2301–2325.

Cantalapiedra, C. P., Hernández-Plaza, A., Letunic, I., Bork, P., & Huerta-Cepas, J. (2021). Eggnog-mapper v2: Functional annotation, orthology assignments, and domain prediction at the metagenomic scale. Molecular Biology and Evolution, 38(12), 5825–5829.

Caroppo, C., Azzaro, F., Bergamasco, A., Caruso, G., Decembrini, F., (2022). Phytoplankton and Bacterial Communities' Patterns in a Highly Dynamic Ecosystem (Central Mediterranean Sea). Water 14, 2057.

Carpenter JH (1965) The Chesapeake Bay Institute technique for the Winkler dissolved oxygen method. Limnol Oceanogr 10:141-143.

Castillo DJ, Dithugoe CD, Bezuidt OK, Makhalanyane TP. (2022). Microbial ecology of the Southern Ocean. FEMS Microbiology Ecology 98(11): fiac123.

Chandramohan D, Loka bharathi PA, Nair S, Matondkar SGP. (1987). Bacteriology of ferromanganese nodules from the Indian Ocean. Geomicrobiology Journal 5(1):17–31. https://doi.org/10.1080/01490458709385954

Chen H, Jiang J, JIang F, Li S, Hu Z (2021) Temporal variability of free-living microbial culturability and community composition after an Akashiwo sanguinea bloom in Shenzhen, China. Ecotoxicology 30:975-985.

Chen P, Zhang L, Guo X, Dai X, Liu L, Xi L, Wang J, Song L, Wang Y, Zhu Y (2016) Diversity, biogeography and biodegradation potential of actinobacteria in the deep-sea sediments along the southwest Indian ridge. Front Microbiol 7:1340.

Chen, S., He, Y.-B., Xie, Z.-X., Kong, L.-F., Yan, K.-Q., Li, D.-X., Wu, P.-F., Zheng, R.-W., Peng, L., Chen, J.-W., Lin, L., Liu, S.-Q., Fan, G.-Y., Wang, D.-Z., (2021). Metaproteomics reveals nutrient availability shaping distinct microbial community and metabolic niche in the nutrient-depleted and replete layers of an oligotrophic euphotic zone. Science of The Total Environment 774, 145123.

Cho, J.-C., Giovannoni, S.J., (2004). Cultivation and Growth Characteristics of a Diverse Group of Oligotrophic Marine Gammaproteobacteria. Appl Environ Microbiol 70, 432–440.

Cho, J.-C., Stapels, M.D., Morris, R.M., Vergin, K.L., Schwalbach, M.S., Givan, S.A., Barofsky, D.F., Giovannoni, S.J., (2007). Polyphyletic photosynthetic reaction centre genes in oligotrophic marine Gammaproteobacteria. Environ Microbiol 9, 1456–1463.

Cole JJ, Findlay S, Pace ML. (1988). Bacterial production in fresh and saltwater ecosystems: a cross-system overview. Marine Ecology Progress Series 43(1/2):1–10.

Cullen, J.J., (2015). Subsurface Chlorophyll Maximum Layers: Enduring Enigma or Mystery Solved? Annu. Rev. Mar. Sci. 7, 207–239. https://doi.org/10.1146/annurev-marine-010213-135111

Dalabehara HB, Sarma VV. Physical forcing controls spatial variability in primary production in the Indian Ocean (2021) Deep Sea Res Part II Top Stud Oceanogr 183:104906.

Dalpadado P, Roxy MK, Arrigo KR, Van Dijken GL, Chierici M, Ostrowski M, Skern-Mauritzen R, Bakke G, Richardson AJ, Sperfeld E. (2023). Rapid climate change alters the environment and biological production of the Indian Ocean. Science of The Total Environment:167342.

Damare V, Raghukumar S. (2008). Abundance of thraustochytrids and bacteria in the equatorial Indian Ocean, in relation to transparent exopolymeric particles (TEPs): Thraustochytrids, bacteria and TEPs in the Indian Ocean. FEMS Microbiology Ecology 65(1):40–49.

Dang, H., & Lovell, C. R. (2016). Microbial surface colonization and biofilm development in marine environments. Microbiology and Molecular Biology Reviews, 80(1), 91–138.

Danovaro, R., Corinaldesi, C., Dell'Anno, A., Rastelli, E., (2017). Potential impact of global climate change on benthic deep-sea microbes. FEMS Microbiology Letters 364.

Das S, Lyla PS, Khan SA. (2007). Spatial variation of aerobic culturable heterotrophic bacterial population in sediments of the continental slope of western Bay of Bengal. IJMS Vol36(1).

Decho, A. W., & Gutierrez, T. (2017). Microbial extracellular polymeric substances (EPSS) in ocean systems. Frontiers in Microbiology, 8, 922.

Delong EF, Franks DG, Yayanos AA. (1997). Evolutionary relationships of cultivated psychrophilic and barophilic deep-sea bacteria. Appl Environ Microbiol 63(5):2105–2108.

DeVries, T. (2022). The ocean carbon cycle. Annual Review of Environment and Resources, 47(1), 317–341.

Ding C, Wu C, Guo C, Gui J, Wei Y, Sun J. (2021). The Composition and Primary Metabolic Potential of Microbial Communities Inhabiting the Surface Water in the Equatorial Eastern Indian Ocean. Biology 10(3):248.

Dithugoe, C. D., Bezuidt, O. K. I., Cavan, E. L., Froneman, W. P., Thomalla, S. J., & Makhalanyane, T. P. (2023). Bacteria and archaea regulate particulate organic matter export in suspended and sinking marine particle fractions. mSphere, 8(3), e00420-22.

Dittmar, T., Lennartz, S. T., Buck-Wiese, H., Hansell, D. A., Santinelli, C., Vanni, C., Blasius, B., & Hehemann, J.-H. (2021). Enigmatic persistence of dissolved organic matter in the ocean. Nature Reviews Earth & Environment, 2(8), 570–583.

Divya B, Fernandes SO, Sheelu G, Nair S, Loka Bharathi PA, Chandramohan D. (2009). Limno-tolerant bacteria govern nitrate concentration in Mandovi Estuary, India. Estuarine, Coastal and Shelf Science 82(1):29–34.

Divya B, Parvathi A, Loka Bharathi PA, Nair S. (2011). 16S rRNA-based bacterial diversity in the organic-rich sediments underlying oxygen-deficient waters of the eastern Arabian Sea. World J Microbiol Biotechnol 27(12):2821–2833.

Divya B, Soumya K, Nair S (2010) 16SrRNA and enzymatic diversity of culturable bacteria from the sediments of oxygen minimum zone in the Arabian Sea. Antonie Leeuwenhoek 98:9-18.

Divya B. (2017). Bacterial Community Profiling of the Arabian Sea Oxygen Minimum Zone Sediments using Cultivation Independent Approach. EIMBO 1(1).

Doré, H., Leconte, J., Guyet, U., Breton, S., Farrant, G.K., Demory, D., Ratin, M., Hoebeke, M., Corre, E., Pitt, F.D., Ostrowski, M., Scanlan, D.J., Partensky, F., Six, C., Garczarek, L., (2022). Global Phylogeography of Marine Synechococcus in Coastal Areas Reveals Strong Community Shifts. mSystems 7, e00656-22.

Douglas, G.M., Maffei, V.J., Zaneveld, J.R., Yurgel, S.N., Brown, J.R., Taylor, C.M., Huttenhower, C., Langille, M.G.I., (2020). PICRUSt2 for prediction of metagenome functions. Nat Biotechnol 38, 685–688.

Drula, E., Garron, M.-L., Dogan, S., Lombard, V., Henrissat, B., & Terrapon, N. (2022). The carbohydrate-active enzyme database: Functions and literature. Nucleic Acids Research, 50(D1), D571–D577.

Du, J., Xiao, K., Li, L., Ding, X., Liu, H., Lu, Y., Zhou, S., (2013). Temporal and Spatial Diversity of Bacterial Communities in Coastal Waters of the South China Sea. PLoS ONE 8, e66968.

Ducklow HW, Smith DC, Campbell L, Landry MR, Quinby HL, Steward GF, Azam F. (2001). Heterotrophic bacterioplankton in the Arabian Sea: Basinwide response to year-round high primary productivity. Deep Sea Research Part II: Topical Studies in Oceanography 48(6):1303–1323.

Ducklow HW, Steinberg DK, Buesseler KO (2001) Upper ocean carbon export and the biological pump. Oceanography 14:50–58.

Ducklow HW. (1993). Bacterioplankton distributions and production in the northwestern Indian Ocean and Gulf of Oman, September 1986. Deep Sea Research Part II: Topical Studies in Oceanography 40(3):753–771.

Ducklow, H.W., Quinby, H.L., Carlson, C.A., (1995). Bacterioplankton dynamics in the equatorial Pacific during the 1992 El Niño. Deep Sea Research Part II: Topical Studies in Oceanography 42, 621–638.

Eigemann, F., Rahav, E., Grossart, H.-P., Aharonovich, D., Voss, M., & Sher, D. (2023). Phytoplankton producer species and transformation of released compounds over time define bacterial communities following phytoplankton dissolved organic matter pulses. Applied and Environmental Microbiology, 89(7), e00539-23.

Engel A, Händel N (2011) A novel protocol for determining the concentration and composition of sugars in particulate and in high molecular weight dissolved organic matter (HMW-DOM) in seawater. Mar Chem 127:180-191. https://doi.org/10.1016/j.marchem.2011.09.004

Enke, T. N., Leventhal, G. E., Metzger, M., Saavedra, J. T., & Cordero, O. X. (2018). Microscale ecology regulates particulate organic matter turnover in model marine microbial communities. Nature Communications, 9(1), 2743.

Fang J, Zhang L, Li J, Kato C, Tamburini C, Zhang Y, Dang H, Wang G, Wang F (2015) The POM-DOM piezophilic microorganism continuum (PDPMC)-The role of

piezophilic microorganisms in the global ocean carbon cycle. Sci China Earth Sci 58:106-115.

Fenice M, Gallo AM, Juarez-Jimenez B, Gonzalez-Lopez J (2007) Screening for extracellular enzyme activities by bacteria isolated from samples collected in the Tyrrhenian Sea. Ann Microbiol 57:93-9.

Fernandes C, Kankonkar H, Meena RM, Menezes G, Shenoy BD, Khandeparker R. (2019). Metagenomic analysis of tarball-associated bacteria from Goa, India. Marine Pollution Bulletin 141:398–403.

Fernandes GL, Shenoy BD, Damare SR. (2020). Diversity of Bacterial Community in the Oxygen Minimum Zones of Arabian Sea and Bay of Bengal as Deduced by Illumina Sequencing. Front Microbiol 10:3153.

Fernandes GL, Shenoy BD, Menezes LD, Meena RM, Damare SR. (2019). Prokaryotic Diversity in Oxygen Depleted Waters of the Bay of Bengal Inferred Using Culture-Dependent and -Independent Methods. Indian J Microbiol 59(2):193–199.

Fernandes V, Ramaiah N, Paul JT, Sardessai S, Jyoti Babu R, Gauns M. (2008). Strong variability in bacterioplankton abundance and production in central and western Bay of Bengal. Mar Biol 153(5):975–985.

Fernandes V, Rodrigues V, Ramaiah N, Paul JT. (2008). Relevance of bacterioplankton abundance and production in the oligotrophic equatorial Indian Ocean. Aquat Ecol 42(4):511–519.

Fernandes, V., Bogati, K., (2022). Analysis of Bacteria–Phytoplankton relationships at three discrete locations in the Eastern Arabian Sea during winter. Continental Shelf Research 243, 104751.

Frank, A.H., Garcia, J.A.L., Herndl, G.J., Reinthaler, T., (2016). Connectivity between surface and deep waters determines prokaryotic diversity in the North Atlantic Deep Water. Environmental Microbiology 18, 2052–2063.

Fuchs B, Woebken D, Zubkov M, Burkill P, Amann R. (2005). Molecular identification of picoplankton populations in contrasting waters of the Arabian Sea. Aquat Microb Ecol 39:145–157.

Fuhrman, J. A., Cram, J. A., & Needham, D. M. (2015). Marine microbial community dynamics and their ecological interpretation. Nature Reviews Microbiology, 13(3), 133–146.

Galloway-Peña, J., & Hanson, B. (2020). Tools for analysis of the microbiome. Digestive Diseases and Sciences, 65(3), 674–685.

Galperin, M. Y., Makarova, K. S., Wolf, Y. I., & Koonin, E. V. (2015). Expanded microbial genome coverage and improved protein family annotation in the COG database. Nucleic Acids Research, 43(D1), D261–D269.

Gao P, Qu L, Du G, Wei Q, Zhang X, Yang G. (2021). Bacterial and archaeal communities in deep sea waters near the Ninetyeast Ridge in Indian Ocean. J Ocean Limnol 39(2):582–597.

Gauns M, Madhupratap M, Ramaiah N, Jyothibabu R, Fernandes V, Paul JT, Prasanna Kumar SP. (2005). Comparative accounts of biological productivity characteristics and estimates of carbon fluxes in the Arabian Sea and the Bay of Bengal. Deep Sea Research Part II: Topical Studies in Oceanography 52(14–15):2003–2017.

Ghai R, Mizuno CM, Picazo A, Camacho A, Rodriguez-Valera F (2013) Metagenomics uncovers a new group of low GC and ultra-small marine Actinobacteria. Sci Rep 3:1-8. https://doi.org/10.1038/srep02471

Gifford, S. M., Zhao, L., Stemple, B., DeLong, K., Medeiros, P. M., Seim, H., & Marchetti, A. (2020). Microbial niche diversification in the Galapagos archipelago and its response to el niño. Frontiers in Microbiology, 11.

Giovannoni S, Stingl U (2007) The importance of culturing bacterioplankton in the 'omics' age. Nat Rev Microbiol 5, 820–826. https://doi.org/10.1038/nrmicro1752

Giovannoni SJ, Stingl U. 2005. Molecular diversity and ecology of microbial plankton. Nature 437(7057):343–348.

Girão M, Ribeiro I, Ribeiro T, Azevedo IC, Pereira F, Urbatzka R, Leão PN, Carvalho MF (2019) Actinobacteria isolated from Laminaria ochroleuca: a source of new bioactive compounds. Front Microbiol 10:683.

Gomes J, Khandeparker R, Bandekar M, Meena RM, Ramaiah N. (2018). Quantitative analyses of denitrifying bacterial diversity from a seasonally hypoxic monsoon governed tropical coastal region. Deep Sea Research Part II: Topical Studies in Oceanography 156:34–43.

Gomes J, Khandeparker R, Meena RM, Ramaiah N (2019) Bacterial community composition markedly altered by coastal hypoxia. Indian J Microbiol 59:200-208.

Gomes, J., Khandeparker, R., Meena, R. M., & Ramaiah, N. (2019). Bacterial community composition markedly altered by coastal hypoxia. Indian Journal of Microbiology, 59(2), 200–208.

Gómez-Consarnau L, Needham DM, Weber PK, Fuhrman JA, Mayali X (2019) Influence of light on particulate organic matter utilization by attached and free-living marine bacteria. Front Microbiol 10:1204.

Gong, F., Ji, Q., Li, G., Yin, K., Gong, J., (2022). Diversity and distribution of bacterioplankton in the coastal upwelling waters off Hainan Island, China. Acta Oceanol. Sin. 41, 76–85.

Gonsalves M, Nair S, Loka Bharathi P, Chandramohan D. (2009). Abundance and production of particle-associated bacteria and their role in a mangrove-dominated estuary. Aquat Microb Ecol 57:151–159.

Gonsalves MJ, Paropkari AL, Fernandes CE, Bharathi PL, Krishnakumari L, Fernando V, Nampoothiri GE (2011) Predominance of anaerobic bacterial community over aerobic community contribute to intensify 'oxygen minimum zone'in the eastern Arabian Sea. Cont Shelf Res 31:1224-1235.

Gupta, G.V.M., Jyothibabu, R., Ramu, C.V., Yudhistir Reddy, A., Balachandran, K.K., Sudheesh, V., Kumar, S., Chari, N.V.H.K., Bepari, K.F., Marathe, P.H., Bikram Reddy, B., Vijayan, A.K., (2021). The world's largest coastal deoxygenation zone is not anthropogenically driven. Environ. Res. Lett. 16, 054009.

Gupta, G.V.M., Sudheesh, V., Sudharma, K.V., Saravanane, N., Dhanya, V., Dhanya, K.R., Lakshmi, G., Sudhakar, M., Naqvi, S.W.A., (2016). Evolution to decay of upwelling and associated biogeochemistry over the southeastern Arabian Sea shelf. J. Geophys. Res. Biogeosci. 121, 159–175.

Gupta, M., Williams, R. G., Lauderdale, J. M., Jahn, O., Hill, C., Dutkiewicz, S., & Follows, M. J. (2022). A nutrient relay sustains subtropical ocean productivity. Proceedings of the National Academy of Sciences, 119(41), e2206504119.

Gurevich, A., Saveliev, V., Vyahhi, N., & Tesler, G. (2013). QUAST: Quality assessment tool for genome assemblies. Bioinformatics, 29(8), 1072–1075.

Gutiérrez-Barral, A., Teira, E., Hernández-Ruiz, M., Fernández, E., (2021). Response of prokaryote community composition to riverine and atmospheric nutrients in a coastal embayment: Role of organic matter on Vibrionales. Estuarine, Coastal and Shelf Science 251, 107196.

Habeebrehman, H., Prabhakaran, M.P., Jacob, J., Sabu, P., Jayalakshmi, K.J., Achuthankutty, C.T., Revichandran, C., (2008). Variability in biological responses influenced by upwelling events in the Eastern Arabian Sea. Journal of Marine Systems 74, 545–560.

Hach, P. F., Marchant, H. K., Krupke, A., Riedel, T., Meier, D. V., Lavik, G., Holtappels, M., Dittmar, T., & Kuypers, M. M. M. (2020). Rapid microbial diversification of dissolved organic matter in oceanic surface waters leads to carbon sequestration. Scientific Reports, 10(1), 13025. h

Hammer, Ø., & Harper, D. A. T. (2006). Paleontological data analysis. Blackwell Pub.

Han W, Webster PJ. 2002. Forcing Mechanisms of Sea Level Interannual Variability in the Bay of Bengal. J Phys Oceanogr 32(1):216–239.

Hansell DA, Carlson CA (2001) Marine dissolved organic matter and the carbon cycle. Oceanography 14:41-49.

Hansell DA, Carlson CA (2013) Localized refractory dissolved organic carbon sinks in the deep ocean. Global Biogeochem Cycles 27:705-710.

Hede, N., & Khandeparker, L. (2020). Extracellular polymeric substances mediate the coaggregation of aquatic biofilm-forming bacteria. Hydrobiologia, 847(20), 4249–4272.

Heinrichs, M. E., Mori, C., & Dlugosch, L. (2020). Complex interactions between aquatic organisms and their chemical environment elucidated from different perspectives. In S. Jungblut, V. Liebich, & M. Bode-Dalby (Eds.), YOUMARES 9—The Oceans: Our Research, Our Future: Proceedings of the 2018 conference for YOUng MArine RESearcher in Oldenburg, Germany (pp. 279–297). Springer International Publishing.

Ho, A., Lonardo, D.P.D., Bodelier, P.L.E., (2017). Revisiting life strategy concepts in environmental microbial ecology. Microbiology Ecology fix006.

Hoppe H, Ullrich S. (1999). Profiles of ectoenzymes in the Indian Ocean: phenomena of phosphatase activity in the mesopelagic zone. Aquat Microb Ecol 19:139–148.

Hugenholtz P, Goebel BM, Pace NR. (1998). Impact of Culture-Independent Studies on the Emerging Phylogenetic View of Bacterial Diversity. J Bacteriol 180(18):4765–4774.

Huisman, J., Codd, G. A., Paerl, H. W., Ibelings, B. W., Verspagen, J. M. H., & Visser, P. M. (2018). Cyanobacterial blooms. Nature Reviews Microbiology, 16(8), 471–483.

Hutchins, D. A., & Fu, F. (2017). Microorganisms and ocean global change. Nature Microbiology, 2(6), 1–11. https://doi.org/10.1038/nmicrobiol.2017.58

Inomura, K., Deutsch, C., Jahn, O., Dutkiewicz, S., & Follows, M. J. (2022). Global patterns in marine organic matter stoichiometry driven by phytoplankton ecophysiology. Nature Geoscience, 15(12), 1034–1040.

Isaacs MJ, Ramadoss D, Parab AS, Manohar CS (2021) Evaluating the bacterial diversity from the southwest coast of India using fatty acid methyl ester profiles. Curr Microbiol 78:649-658.

Izabel-Shen, D., Albert, S., Winder, M., Farnelid, H., & Nascimento, F. J. A. (2021). Quality of phytoplankton deposition structures bacterial communities at the water-sediment interface. Molecular Ecology, 30(14), 3515–3529.

Jacob J, Jayaraj K, Rehman HH, Chandramohanakumar N, Balachandran K, Raveendran T, Joseph T, Nair M, Achuthankutty C (2009) Biogeochemical characteristics of the surface sediments along the western continental shelf of India. Chem Ecol 25:135-149.

Jain A, Bandekar M, Gomes J, Shenoy D, Meena R, Naik H, Khandeparkar R, Ramaiah N. (2014). Temporally invariable bacterial community structure in the Arabian Sea oxygen minimum zone. Aquat Microb Ecol 73(1):51–67.

James, A. K., Kelly, L. W., Nelson, C. E., Wilbanks, E. G., & Carlson, C. A. (2019). Elevated p CO 2 alters marine heterotrophic bacterial community composition and metabolic potential in response to a pulse of phytoplankton organic matter. Environmental Microbiology, 21(2), 541–556.

James, C. C., Barton, A. D., Allen, L. Z., Lampe, R. H., Rabines, A., Schulberg, A., Zheng, H., Goericke, R., Goodwin, K. D., & Allen, A. E. (2022). Influence of nutrient supply on plankton microbiome biodiversity and distribution in a coastal upwelling region. Nature Communications, 13(1), 2448.

Jasmin C, Anas A, Nair S. (2015). Bacterial Diversity Associated with Cinachyra cavernosa and Haliclona pigmentifera, Cohabiting Sponges in the Coral Reef Ecosystem of Gulf of Mannar, Southeast Coast of India. Hong Y, editor. PLoS ONE 10(5): e0123222.

Jasmin C, Anas A, Tharakan B, Jaleel A, Puthiyaveettil V, Narayanane S, Lincy J, Nair S. 2017. Diversity of sediment-associated Planctomycetes in the Arabian Sea oxygen minimum zone. J Basic Microbiol 57(12):1010–1017.

Jasna V, Nathan VK, Parvathi A. (2020). Comparison of bacterial community structure in coastal and offshore waters of the Bay of Bengal, India. Regional Studies in Marine Science 39:101414.

Jayakumar A, O'Mullan GD, Naqvi SWA, Ward BB. (2009). Denitrifying Bacterial Community Composition Changes Associated with Stages of Denitrification in Oxygen Minimum Zones. Microb Ecol 58(2):350–362.

Jayakumar A, Peng X, Ward B. (2013). Community composition of bacteria involved in fixed nitrogen loss in the water column of two major oxygen minimum zones in the ocean. Aquat Microb Ecol 70(3):245–259.

Jenkinson, I. R. (2023). Plankton genes and extracellular organic substances in the ocean. Journal of Marine Science and Engineering, 11(4), 783.

Jiao N, Azam F, Sanders S, editors. (2011). Microbial Carbon Pump in the Ocean. Science/AAAS.

Jiao N, Herndl GJ, Hansell DA, Benner R, Kattner G, Wilhelm SW, Kirchman DL, Weinbauer MG, Luo T, Chen F, Azam F. (2010). Microbial production of recalcitrant dissolved organic matter: long-term carbon storage in the global ocean. Nat Rev Microbiol 8(8):593–599.

Jiao N, Herndl GJ, Hansell DA, Benner R, Kattner G, Wilhelm SW, Kirchman DL, Weinbauer MG, Luo T, Chen F (2011) The microbial carbon pump and the oceanic recalcitrant dissolved organic matter pool. Nat Rev Microbiol 9:555-555.

Jiao N, Robinson C, Azam F, Thomas H, Baltar F, Dang H, Hardman-Mountford N, Johnson M, Kirchman D, Koch B (2014) Mechanisms of microbial carbon sequestration in the ocean–future research directions. Biogeosciences 11:5285-5306.

Jiao, N., & Zheng, Q. (2011). The microbial carbon pump: From genes to ecosystems. Applied and Environmental Microbiology, 77(21), 7439–7444.

Jing, H., Xia, X., Suzuki, K., Liu, H., (2013). Vertical Profiles of Bacteria in the Tropical and Subarctic Oceans Revealed by Pyrosequencing. PLoS ONE 8, e79423.

Jurgensen, S. K., Roux, S., Schwenck, S. M., Stewart, F. J., Sullivan, M. B., & Brum, J. R. (2022). Viral community analysis in a marine oxygen minimum zone indicates increased potential for viral manipulation of microbial physiological state. The ISME Journal, 16(4), 972–982.

Kadnikov, V.V., Mardanov, A.V., Beletsky, A.V., Karnachuk, O.V., Ravin, N.V., (2020). Microbial Life in the Deep Subsurface Aquifer Illuminated by Metagenomics. Front. Microbiol. 11, 572252.

Kamaleson, A. S., Gonsalves, M.-J., Kumar, S., Jineesh, V. K., & LokaBharathi, P. A. (2019). Spatio-temporal variations in sulfur-oxidizing and sulfate-reducing

bacterial activities during upwelling, off south-west coast of India. Oceanologia, 61(4), 427–444.

Kamjam M, Sivalingam P, Deng Z, Hong K (2017) Deep Sea actinomycetes and their secondary metabolites. Front Microbiol 8:760.

Kämpf, J., & Chapman, P. (2016). The functioning of coastal upwelling systems. In J. Kämpf & P. Chapman (Eds.), Upwelling Systems of the World: A Scientific Journey to the Most Productive Marine Ecosystems (pp. 31–65). Springer International Publishing.

Karl DM, Hebel DV, Bjorkman K, Letelier RM. (1998) The role of dissolved organic matter release in the productivity of the oligotrophic North Pacific Ocean. Limnol Oceanogr 43: 1270–1286.

Khalifa A, Aldayel M (2019) Isolation and characterisation of the agarolytic bacterium Pseudoalteromonas ruthenica. Open Life Sci 14(1):588-94.

Khandeparker L, Kuchi N, Kale D, Anil AC (2017) Microbial community structure of surface sediments from a tropical estuarine environment using next generation sequencing. Ecol Indic 74:172-181.

Khandeparker R, Meena RM, Deobagkar D. (2014). Bacterial Diversity in Deep-Sea Sediments from Afanasy Nikitin Seamount, Equatorial Indian Ocean. Geomicrobiology Journal 31(10):942–949.

Khandeparker R, Verma P, Meena RM, Deobagkar DD. (2011). Phylogenetic diversity of carbohydrate degrading culturable bacteria from Mandovi and Zuari estuaries, Goa, west coast of India. Estuarine, Coastal and Shelf Science 95(4):359–366.

Khodse VB, Bhosle NB (2013) Distribution, origin and transformation of amino sugars and bacterial contribution to estuarine particulate organic matter. Cont Shelf Res 68:33-42.

Khodse, V. B., & Bhosle, N. B. (2011). Bacterial utilization of size-fractionated dissolved organic matter. Aquatic Microbial Ecology, 64(3), 299–309.

Kieft, B., Li, Z., Bryson, S., Hettich, R. L., Pan, C., Mayali, X., & Mueller, R. S. (2021). Phytoplankton exudates and lysates support distinct microbial consortia with specialized metabolic and ecophysiological traits. Proceedings of the National Academy of Sciences, 118(41), e2101178118.

Kilgour, D. B., Novak, G. A., Sauer, J. S., Moore, A. N., Dinasquet, J., Amiri, S., Franklin, E. B., Mayer, K., Winter, M., Morris, C. K., Price, T., Malfatti, F., Crocker, D. R., Lee, C., Cappa, C. D., Goldstein, A. H., Prather, K. A., & Bertram,

T. H. (2022). Marine gas-phase sulfur emissions during an induced phytoplankton bloom. Atmospheric Chemistry and Physics, 22(2), 1601–1613.

Kim B, Kim SH, Kwak JH, Kang CK, Lee SH, Hyun JH (2017) Heterotrophic bacterial production, respiration, and growth efficiency associated with upwelling intensity in the Ulleung Basin, East Sea. Deep Sea Res Part II Top Stud Oceanogr 143:24-35.

Kindt, R., & Coe, R. (2005). Tree diversity analysis: a manual and software for common statistical methods for ecological and biodiversity studies. World Agroforestry Centre. ISBN 92-9059-179-X

Kirchman, D.L., (2002). The ecology of Cytophaga–Flavobacteria in aquatic environments. FEMS Microbiology Ecology 39, 91–100.

Koppelmann R, Zimmermann-Timm H, Weikert H. (2005). Bacterial and zooplankton distribution in deep waters of the Arabian Sea. Deep Sea Research Part I: Oceanographic Research Papers 52(11):2184–2192.

Kottayil A, Xavier A, Xavier P, Koovekkallu P, Mohanakumar K (2022) Evolution of l arge-scale factors influencing extreme rainfall over south western coast of India. Int J Climatol 42:4164-78.

Krüger K, Chafee M, Francis TB, Del Rio TG, Becher D, Schweder T, Amann RI, Teeling H (2019) In marine Bacteroidetes the bulk of glycan degradation during algae blooms is mediated by few clades using a restricted set of genes. ISME J 13:2800-2816.

Kuchi N, Khandeparker L, Anil AC. (2021). Response of the bacterial metagenome in port environments to changing environmental conditions. Marine Pollution Bulletin 172:112869.

Kumar M, Kumar R, Chaudhary DR, Jha B. 2022. An appraisal of early stage biofilm-forming bacterial community assemblage and diversity in the Arabian Sea, India. Marine Pollution Bulletin 180:113732.

Kumar MD, Sarma VVSS, Ramaiah N, Gauns M, De Sousa SN. (1998). Biogeochemical significance of transport exopolymer particles in the Indian Ocean. Geophys Res Lett 25(1):81–84.

Kumar SP, Madhupratap M, Kumar MD, Muraleedharan P, De Souza S, Gauns M, Sarma V (2001) High biological productivity in the central Arabian Sea during the summer monsoon driven by Ekman pumping and lateral advection. Curr Sci 81: 1633-1638.

Kumar SP, Nuncio M, Ramaiah N, Sardesai S, Narvekar J, Fernandes V, Paul JT (2007) Eddy-mediated biological productivity in the Bay of Bengal during fall and spring inter monsoons. Deep Sea Res Part I Oceanogr Res Pap 54:1619-1640.

Kumar, S.P., Madhupratap, M., Kumar, M.D., Gauns, M., Muraleedharan, P.M., Sarma, V.V.S.S., De Souza, S.N., (2000). Physical control of primary productivity on a seasonal scale in central and eastern Arabian Sea. J Earth Syst Sci 109, 433–441.

Kumar, S.P., Ramaiah, N., Gauns, M., Sarma, V.V.S.S., Muraleedharan, P.M., Raghukumar, S., Dileep Kumar, M., Madhupratap, M., (2001). Physical forcing of biological productivity in the Northern Arabian Sea during the Northeast Monsoon. Deep Sea Research Part II: Topical Studies in Oceanography 48, 1115–1126.

Kuypers, M.M.M., Lavik, G., Woebken, D., Schmid, M., Fuchs, B.M., Amann, R., Jørgensen, B.B., Jetten, M.S.M., (2005). Massive nitrogen loss from the Benguela upwelling system through anaerobic ammonium oxidation. Proc. Natl. Acad. Sci. U.S.A. 102, 6478–6483.

Labbé, N., Parent, S., & Villemur, R. (2004). Nitratireductor aquibiodomus gen. Nov., sp. Nov., a novel alpha-proteobacterium from the marine denitrification system of the Montreal Biodome (Canada). International Journal of Systematic and Evolutionary Microbiology, 54(Pt 1), 269–273.

Lamont, T., Barlow, R.G., Kyewalyanga, M.S., (2014). Physical drivers of phytoplankton production in the southern Benguela upwelling system. Deep Sea Research Part I: Oceanographic Research Papers 90, 1–16.

Landa M, Blain S, Christaki U, Monchy S, Obernosterer I (2016) Shifts in bacterial community composition associated with increased carbon cycling in a mosaic of phytoplankton blooms. ISME J 10:39-50.

Landry, Z., Swan, B. K., Herndl, G. J., Stepanauskas, R., & Giovannoni, S. J. (2017). Sar202 genomes from the dark ocean predict pathways for the oxidation of recalcitrant dissolved organic matter. mBio, 8(2), 10.1128/mbio.00413-17.

Langmead, B., & Salzberg, S. L. (2012). Fast gapped-read alignment with Bowtie 2. Nature Methods, 9(4), 357–359.

Lever, M.A., Rogers, K.L., Lloyd, K.G., Overmann, J., Schink, B., Thauer, R.K., Hoehler, T.M., Jørgensen, B.B., (2015). Life under extreme energy limitation: a synthesis of laboratory- and field-based investigations. FEMS Microbiology Reviews 39, 688–728.

Li J, Li N, Li F, Zou T, Yu S, Wang Y, Qin S, Wang G (2014) Spatial diversity of bacterioplankton communities in surface water of northern South China Sea. PLoS ONE 9:e113014.

Li, D., Luo, R., Liu, C.-M., Leung, C.-M., Ting, H.-F., Sadakane, K., Yamashita, H., & Lam, T.-W. (2016). MEGAHIT v1.0: A fast and scalable metagenome assembler driven by advanced methodologies and community practices. Methods, 102, 3–11.

Li, M., Jain, S., & Dick, G. J. (2016). Genomic and transcriptomic resolution of organic matter utilization among deep-sea bacteria in Guaymas basin hydrothermal plumes. Frontiers in Microbiology, 7.

Li, W., Fu, L., Niu, B., Wu, S., & Wooley, J. (2012). Ultrafast clustering algorithms for metagenomic sequence analysis. Briefings in Bioinformatics, 13(6), 656–668.

Li, Y., Sun, L.-L., Sun, M.-L., Su, H.-N., Zhang, X.-Y., Xie, B.-B., Chen, X.-L., Zhang, Y.-Z., Qin, Q.-L., (2018). Vertical and horizontal biogeographic patterns and major factors affecting bacterial communities in the open South China Sea. Sci Rep 8, 8800.

Li, Y.-Y., Chen, X.-H., Xie, Z.-X., Li, D.-X., Wu, P.-F., Kong, L.-F., Lin, L., Kao, S.-J., Wang, D.-Z., (2018). Bacterial Diversity and Nitrogen Utilization Strategies in the Upper Layer of the Northwestern Pacific Ocean. Frontiers in Microbiology 9.

Lian J, Zheng X, Zhuo X, Chen YL, He C, Zheng Q, Cai R (2021). Microbial transformation of distinct exogenous substrates into analogous composition of recalcitrant dissolved organic matter. Environ Microbiol 23:2389-2403.

Lincy J, Manohar CS. (2020). A comparison of bacterial communities from OMZ sediments in the Arabian Sea and the Bay of Bengal reveals major differences in nitrogen turnover and carbon recycling potential. Marine Biology Research 16(8–9):656–673.

Lincy J, Manohar CS. (2021). 16s rRNA and Hydrazine Gene-Based Profiling of the Candidatus Scalindua Community from the Arabian Sea Hypoxic Sediments. Current Science 120(4):684.

Liu, S., & Liu, Z. (2018). Free extracellular enzymes dominate initial peptide hydrolysis in coastal seawater. Marine Chemistry, 199, 37–43.

Liu, S., Liu, Z., (2020). Distinct capabilities of different Gammaproteobacterial strains on utilizing small peptides in seawater. Sci Rep 10, 464.

Liu, X., Xie, N., Li, J., Bai, M., Sen, B., & Wang, G. (2022). Potential contribution of coastal upwelling to carbon sink through interaction between cyanobacteria and microbial eukaryotes. Water, 14(19), 3097.

Liu, Y., Wang, X., & Sun, J. (2022). Bacterial transformation and processing of diatom-derived organic matter: A case study for Skeletonema dohrnii. Frontiers in Microbiology, 13.

Loka Bharathi PA, Chandramohan D. (1986). Characterization of purple and green photosynthetic bacteria isolated from the lagoon of Agatti Atoll (Lakshadweep Sea).

Loka Bharathi PA, Chandramohan D. (1990). Sulfate-Reducing Bacteria from the Arabian Sea—Their Distribution in Relation to Thiosulfate-Oxidizing and Heterotrophic Bacteria. Bulletin of Marine Science. 47(3):622–630.

Loka Bharathi PA, Nair S, Chandramohan D. 1992. Nitrate and sulfate reducers-retrievable number of bacteria and their activities in Indian waters.

Lønborg, C., Baltar, F., Carreira, C., & Morán, X. A. G. (2019). Dissolved organic carbon source influences tropical coastal heterotrophic bacterioplankton response to experimental warming. Frontiers in Microbiology, 10, 2807.

Loza, A., García-Guevara, F., Segovia, L., Escobar-Zepeda, A., Sanchez-Olmos, M. D. C., Merino, E., Sanchez-Flores, A., Pardo-Lopez, L., Juarez, K., & Gutierrez-Rios, R.-M. (2022). Definition of the metagenomic profile of ocean water samples from the Gulf of Mexico based on comparison with reference samples from sites worldwide. Frontiers in Microbiology, 12, 781497.

Lucas J, Koester I, Wichels A, Niggemann J, Dittmar T, Callies U, Wiltshire KH, Gerdts G (2016) Short-term dynamics of North Sea bacterioplankton-dissolved organic matter coherence on molecular level. Front Microbiol 7:321.

Lüke C, Speth DR, Kox MAR, Villanueva L, Jetten MSM. (2016). Metagenomic analysis of nitrogen and methane cycling in the Arabian Sea oxygen minimum zone. PeerJ. 4:e1924.

Madhupratap M, Gauns M, Ramaiah N, Prasanna Kumar S, Muraleedharan PM, De Sousa SN, Sardessai S, Muraleedharan U. (2003). Biogeochemistry of the Bay of Bengal: physical, chemical and primary productivity characteristics of the central and western Bay of Bengal during summer monsoon 2001. Deep Sea Research Part II: Topical Studies in Oceanography 50(5):881–896.

Madhupratap M, Prasanna Kumar S, Bhattathiri PMA, Kumar MD, Raghukumar S, Nair KKC, Ramaiah N. (1996). Mechanism of the biological response to winter cooling in the northeastern Arabian Sea. Nature 384(6609):549–552.

Mahmoudi N, Enke TN, Beaupré SR, Teske AP, Cordero OX, Pearson A (2019) Illuminating microbial species-specific effects on organic matter remineralization in marine sediments. Environ Microbiol 22:1734-47.

Mahmoudi N, Hagen SM, Hazen TC, Steen AD (2020) Patterns in extracellular enzyme activity and microbial diversity in deep-sea Mediterranean sediments. Deep Sea Res Part I Oceanogr Res Pap 158:103231.

Manni, M., Berkeley, M. R., Seppey, M., Simão, F. A., & Zdobnov, E. M. (2021). Busco update: Novel and streamlined workflows along with broader and deeper phylogenetic coverage for scoring of eukaryotic, prokaryotic, and viral genomes. Molecular Biology and Evolution, 38(10), 4647–4654.

Manucharova N (2009) The microbial destruction of chitin, pectin, and cellulose in soils. Eurasian Soil Sci 42:1526-1532.

Margesin R, Schinner F (2001) Biodegradation and bioremediation of hydrocarbons in extreme environments. Appl Microbiol Biotechnol 56:650-663.

Mari, X., Passow, U., Migon, C., Burd, A. B., & Legendre, L. (2017). Transparent exopolymer particles: Effects on carbon cycling in the ocean. Progress in Oceanography, 151, 13–37.

Marimuthu J, Rangamaran VR, Subramanian SHS, Balachandran KRS, Thenmozhi Kulasekaran N, Vasudevan D, Lee J-K, Ramalingam K, Gopal D. (2022). Deep-sea sediment metagenome from Bay of Bengal reveals distinct microbial diversity and functional significance. Genomics 114(6):110524.

Maßmig M, Lüdke J, Krahmann G, Engel A (2020) Bacterial degradation activity in the eastern tropical South Pacific oxygen minimum zone. Biogeosciences 17:215-230.

Matsumoto, K., Sakami, T., Watanabe, T., Taniuchi, Y., Kuwata, A., Kakehi, S., Engkong, T., Igarashi, Y., Kinoshita, S., Asakawa, S., Hattori, M., Watabe, S., Ishino, Y., Kobayashi, T., Gojobori, T., Ikeo, K., (2021). Metagenomic analysis provides functional insights into seasonal change of a non-cyanobacterial prokaryotic community in temperate coastal waters. PLoS ONE 16, e0257862.

Maya, M. V., Karapurkar, S. G., Naik, H., Roy, R., Shenoy, D. M., & Naqvi, S. W. A. (2011). Intra-annual variability of carbon and nitrogen stable isotopes in suspended

organic matter in waters of the western continental shelf of India. Biogeosciences, 8(11), 3441–3456.

McKee LS, La Rosa SL, Westereng B, Eijsink VG, Pope PB, Larsbrink J (2021) Polysaccharide degradation by the Bacteroidetes-mechanisms and nomenclature. Environ Microbiol Rep 13:599-581.

McMurdie, P.J., Holmes, S., (2013). phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. PLoS ONE 8, e61217.

Meddeb-Mouelhi F, Moisan JK, Beauregard M (2014) A comparison of plate assay methods for detecting extracellular cellulase and xylanase activity. Enzyme Microb Technol 66:16-9.

Mena, C., Reglero, P., Balbín, R., Martín, M., Santiago, R., Sintes, E., (2022). Dynamics of actively dividing prokaryotes in the western Mediterranean Sea. Sci Rep 12, 2064.

Menezes LD, Fernandes GL, Mulla AB, Meena RM, Damare SR. (2020). Diversity of culturable Sulphur-oxidising bacteria in the oxygen minimum zones of the northern Indian Ocean. Journal of Marine Systems 209:103085.

Mestre, M., Ruiz-González, C., Logares, R., Duarte, C.M., Gasol, J.M., Sala, M.M., (2018). Sinking particles promote vertical connectivity in the ocean microbiome. Proc. Natl. Acad. Sci. U.S.A. 115.

Molina V, Belmar L, Levipan HA, Ramírez-Flandes S, Anguita C, Galán A, Montes I, Ulloa O (2020) Spatiotemporal Distribution of Key Pelagic Microbes in a Seasonal Oxygen-Deficient Coastal Upwelling System of the Eastern South Pacific Ocean. Front Mar Sci 7, 561597.

Molina, V., Fernández, C., 2020. Bacterioplankton response to nitrogen and dissolved organic matter produced from salmon mucus. MicrobiologyOpen 9.

Montes, T., Guerrero-Feijóo, E., Moreira-Coello, V., Bode, A., Ruiz-Villarreal, M., Mouriño-Carballido, B., Varela, M.M., (2020). Vertical zonation of bacterial assemblages attributed to physical stratification during the summer relaxation of the coastal upwelling off Galicia (NW Spain). Estuarine, Coastal and Shelf Science 245, 106791.

Moran MA, Kujawinski EB, Schroer WF, Amin SA, Bates NR, Bertrand EM, Braakman R, Brown CT, Covert MW, Doney SC, Dyhrman ST (2022) Microbial metabolites in the marine carbon cycle. Nat microbiol 7:508-23.

Moran, M. A., Kujawinski, E. B., Schroer, W. F., Amin, S. A., Bates, N. R., Bertrand, E. M., Braakman, R., Brown, C. T., Covert, M. W., Doney, S. C., Dyhrman, S. T., Edison, A. S., Eren, A. M., Levine, N. M., Li, L., Ross, A. C., Saito, M. A., Santoro, A. E., Segrè, D., Vardi, A. (2022). Microbial metabolites in the marine carbon cycle. Nature Microbiology, 7(4), 508–523.

Moran, M. A., Kujawinski, E. B., Stubbins, A., Fatland, R., Aluwihare, L. I., Buchan, A., Crump, B. C., Dorrestein, P. C., Dyhrman, S. T., Hess, N. J., Howe, B., Longnecker, K., Medeiros, P. M., Niggemann, J., Obernosterer, I., Repeta, D. J., & Waldbauer, J. R. (2016). Deciphering ocean carbon in a changing world. Proceedings of the National Academy of Sciences, 113(12), 3143–3151.

Morris, R.M., Frazar, C.D., Carlson, C.A., (2012). Basin-scale patterns in the abundance of SAR11 subclades, marine Actinobacteria (OM1), members of the Roseobacter clade and OCS116 in the South Atlantic: Bacterioplankton communities in the South Atlantic. Environmental Microbiology 14, 1133–1144.

Mote S, Gupta V, De K, Nanajkar M, Damare SR, Ingole B. (2021). Bacterial diversity associated with a newly described bioeroding sponge, Cliona thomasi, from the coral reefs on the West Coast of India. Folia Microbiol 66(2):203–211.

Mühlenbruch M, Grossart HP, Eigemann F, Voss M (2018) Mini-review: Phytoplankton-derived polysaccharides in the marine environment and their interactions with heterotrophic bacteria. Environ Microbiol 20:2671-2685.

Mukherjee R, Dutta MK, Sanyal P, Bhadury P, Mukhopadhyay SK (2020) Bacterioplankton abundance and community structure during post-monsoon in mangrove dominated estuaries of the Indian Sundarbans; An insight to biogeochemical processes. Estuar Coast Shelf Sci 243:106895.

Mulla A, Fernandes G, Menezes L, Meena RM, Naik H, Gauns M, Damare S. (2018). Diversity of culturable nitrate-reducing bacteria from the Arabian Sea oxygen minimum zone. Deep Sea Research Part II: Topical Studies in Oceanography 156:27–33.

Nageswar Rao, M., Ram, A., Pradhan, U. K., & Siddaiah, V. (2019). Factors controlling organic matter composition and trophic state in seven tropical estuaries along the west coast of India. Environmental Geochemistry and Health, 41(2), 545–562.

Nagura M, McPhaden MJ. 2018. The Shallow Overturning Circulation in the Indian Ocean. Journal of Physical Oceanography 48(2):413–434.

Nair HP, Puthusseri RM, Vincent H, Bhat SG. (2017). 16S rDNA-based bacterial diversity analysis of Arabian Sea sediments: A metagenomic approach. Ecological Genetics and Genomics 3–5:47–51.

Nair S, Loka Bharathi PA, Achuthankutty CT. (1978). Distribution of Heterotrophic Bacteria in Marine Sediments. IJMS Vol07(1).

Nair S, Loka Bharathi PA, Achuthankutty CT. (1979). Heterotrophic bacteria and biochemical activities off the west coast of India. Mahasagar 12:75-81.

Nair S, Loka Bharathi PA. 1982. Bacteriological studies off Mangalore coast.

Nair SH, Loka Bharathi PA, Chandramohan DO. (1994). Culturable heterotrophic bacteria from the euphotic zone of the Indian-Ocean during the summer monsoon. Oceanologica acta. 17:63-8.

Nair SH, Loka Bharathi PA. Bacteriological studies off Mangalore coast. (1982). Mahasagar 15:215-22.

Nandakumar, K., Shetye, S. S., Kurian, S., Aparna, S. G., Gauns, M., & Dora, S. (2023). Role of physical processes in regulating the biological responses in the Eastern Arabian Sea during the late southwest monsoon. Regional Studies in Marine Science, 62, 102977.

Naqvi S, Bange HW, Farías L, Monteiro P, Scranton M, Zhang J (2010) Marine hypoxia/anoxia as a source of CH 4 and N 2 O. Biogeosciences 7:2159-2190.

Naqvi SWA, Jayakumar DA, Narvekar PV, Naik H, Sarma VVSS, D'Souza W, Joseph S, George MD. (2000). Increased marine production of N2O due to intensifying anoxia on the Indian continental shelf. Nature 408(6810):346–349.

Paingankar MS, Ahire K, Mishra P, Rajpathak S, Deobagkar DD. (2020). Microbial diversity analysis in the oxygen minimum zones of the Arabian Sea using metagenomics approach. Curr Sci 118(7):1042-1051.

Parab AS, Ghose MP, Manohar CS. 2023. Variations in bacterial community at Chlorophyll Maximum (C-Max) depths along the west coast of India due to seasonal changes in the primary productivity.

Parab AS, Jagtap AS, Meena RM, Manohar CS. (2022). Bacterial dynamics along the west coast of India during the non-monsoon and monsoon season. Continental Shelf Research 251:104876.

Parab SG, Matondkar SP, Gomes HD, Goes JI (2006) Monsoon driven changes in phytoplankton populations in the eastern Arabian Sea as revealed by microscopy and HPLC pigment analysis. Cont Shelf Res 26:2538-2558.

Parks, D. H., Tyson, G. W., Hugenholtz, P., & Beiko, R. G. (2014). STAMP: Statistical analysis of taxonomic and functional profiles. Bioinformatics, 30(21), 3123–3124. https://doi.org/10.1093/bioinformatics/btu494

Parulekar NN, Kolekar P, Jenkins A, Kleiven S, Utkilen H, Johansen A, Sawant S, Kulkarni-Kale U, Kale M, Sæbø M (2017) Characterization of bacterial community associated with phytoplankton bloom in a eutrophic lake in South Norway using 16S rRNA gene amplicon sequence analysis. PLoS ONE 12:e0173408.

Parvathi A, Jasna V, Aswathy VK, Aparna S, Nathan VK, Jyothibabu R. (2020). Dominance of Wolbachia sp. in the deep-sea sediment bacterial metataxonomic sequencing analysis in the Bay of Bengal, Indian Ocean. Genomics 112(1):1030–1041.

Parvathi A, Jasna V, Aswathy VK, Nathan VK, Aparna S, Balachandran KK. (2019). Microbial diversity in a coastal environment with co-existing upwelling and mud-banks along the southwest coast of India. Mol Biol Rep 46(3):3113–3127.

Passow U, Christina L, Arnosti C, Grossart H-P, Murray AE, Engel A (2007) Microbial dynamics in autotrophic and heterotrophic seawater mesocosms. I. Effect of phytoplankton on the microbial loop. Aquat Microb Ecol 49:109-121.

Pawar R, Mohandass C, Rajasabapathy R, Meena RM. (2018). Molecular diversity of marine pigmented bacteria in the central Arabian Sea with special reference to antioxidant properties.

Pawar RT, Mohandass C, Rajasabapathy R, Meena RM. (2018). Molecular diversity of marine pigmented bacteria in the central Arabian Sea with special reference to antioxidant properties. Cah Biol Mar 59(5):409-420.

Paysan-Lafosse, T., Blum, M., Chuguransky, S., Grego, T., Pinto, B. L., Salazar, G. A., Bileschi, M. L., Bork, P., Bridge, A., Colwell, L., Gough, J., Haft, D. H., Letunić, I., Marchler-Bauer, A., Mi, H., Natale, D. A., Orengo, C. A., Pandurangan, A. P., Rivoire, C., Bateman, A. (2023). InterPro in 2022. Nucleic Acids Research, 51(D1), D418–D427.

Pinhassi, J., Sala, M.M., Havskum, H., Peters, F., Guadayol, Ò., Malits, A., Marrasé, C., (2004). Changes in Bacterioplankton Composition under Different Phytoplankton Regimens. Appl Environ Microbiol 70, 6753–6766.

Pitcher A, Villanueva L, Hopmans EC, Schouten S, Reichart G-J, Sinninghe Damsté JS. (2011). Niche segregation of ammonia-oxidizing archaea and anammox bacteria in the Arabian Sea oxygen minimum zone. ISME J 5(12):1896–1904.

Pomroy A, Joint I. (1999). Bacterioplankton activity in the surface waters of the Arabian Sea during and after the 1994 SW monsoon. Deep Sea Research Part II: Topical Studies in Oceanography 46(3–4):767–794.

Porter KG, Feig YS (1980) The use of DAPI for identifying and counting aquatic microflora. Limnol Oceanogr 25:943-948.

Pradhan, U. K., Wu, Y., Shirodkar, P. V., Zhang, J., & Zhang, G. (2014). Sources and distribution of organic matter in thirty five tropical estuaries along the west coast of India-a preliminary assessment. Estuarine, Coastal and Shelf Science, 151, 21–33.

Prasanna Kumar S, Muraleedharan PM, Prasad TG, Gauns M, Ramaiah N, De Souza SN, Sardesai S, Madhupratap M. (2002). Why is the Bay of Bengal less productive during summer monsoon compared to the Arabian Sea?: The bay of Bengal less productive during summer monsoon. Geophys Res Lett 29(24):88-1-88–4.

Prasanna Kumar S, Ramaiah N, Gauns M, Sarma VVSS, Muraleedharan PM, Raghukumar S, Dileep Kumar M, Madhupratap M. (2001). Physical forcing of biological productivity in the Northern Arabian Sea during the Northeast Monsoon. Deep Sea Research Part II: Topical Studies in Oceanography 48(6–7):1115–1126.

Prüß, B.M., (2017). Involvement of Two-Component Signaling on Bacterial Motility and Biofilm Development. J Bacteriol 199.

Puthiya Veettil V, Abdulaziz A, Chekidhenkuzhiyil J, Kalanthingal Ramkollath L, Karayadi Hamza F, Kizhakkepat Kalam B, Kallungal Ravunnikutty M, Nair S. (2015). Bacterial Domination Over Archaea in Ammonia Oxidation in a Monsoon-Driven Tropical Estuary. Microb Ecol 69(3):544–553.

Qian G, Wang J, Kan J, Zhang Xiaodong, Xia Z, Zhang Xuecheng, Miao Y, Sun J. (2018). Diversity and distribution of anammox bacteria in water column and sediments of the Eastern Indian Ocean. International Biodeterioration & Biodegradation 133:52–62.

Qin, Q.-L., Zhang, X.-Y., Wang, X.-M., Liu, G.-M., Chen, X.-L., Xie, B.-B., Dang, H.-Y., Zhou, B.-C., Yu, J., & Zhang, Y.-Z. (2010). The complete genome of Zunongwangia profunda SM-A87 reveals its adaptation to the deep-sea environment and ecological role in sedimentary organic nitrogen degradation. BMC Genomics, 11, 247.

Qu, W., Lin, D., Zhang, Z., Di, W., Gao, B., Zeng, R., (2018). Metagenomics Investigation of Agarlytic Genes and Genomes in Mangrove Sediments in China:

A Potential Repertory for Carbohydrate-Active Enzymes. Front. Microbiol. 9, 1864.

Quince, C., Walker, A. W., Simpson, J. T., Loman, N. J., & Segata, N. (2017). Shotgun metagenomics, from sampling to analysis. Nature Biotechnology, 35(9), 833–844.

Rajasabapathy R, Mohandass C, Vijayaraj AS, Madival VV, Meena RM. (2013). Iron bacterial phylogeny and their execution towards iron availability in Equatorial Indian Ocean and coastal Arabian Sea. Pol J Microbiol. 62(4):391–400.

Rajpathak SN, Banerjee R, Mishra PG, Khedkar AM, Patil YM, Joshi SR, Deobagkar DD. (2018). An exploration of microbial and associated functional diversity in the OMZ and non-OMZ areas in the Bay of Bengal. J Biosci 43(4):635–648.

Ram ASP, Nair S, Chandramohan D (2003) Seasonal shift in net ecosystem production in a tropical estuary. Limnol Oceanogr 48: 1601-1607.

Ramaiah N, Chandramohan D. (1992). Densities, cellulases, alginate and pectin lyases of luminous and other heterotrophic bacteria associated with marine algae. Aquatic Botany 44(1):71–81.

Ramaiah N, Fernandes V, Rodrigues VV, Paul JT, Gauns M (2009) Bacterioplankton abundance and production in Indian Ocean regions, in: Wiggert, J.D., Hood, R.R., Naqvi, S.W.A., Brink, K.H., Smith, S.L. (Eds.), Geophysical Monograph Series. American Geophysical Union, Washington, D. C., pp. 119–132.

Ramaiah N, Raghukumar C, Sheelu G, Chandramohan D. (1996). Bacterial abundance, communities and heterotrophic activities in the coastal waters off Tamil Nadu. Indian J Mar Sci 25(3):234-239.

Ramaiah N, Raghukumar S, Gauns M. 1996. Bacterial abundance and production in the central and eastern Arabian Sea. Current Science 71(11):878–882.

Ramaiah N, Raghukumar S, Mangesh G, Madhupratap M (2005) Seasonal variations in carbon biomass of bacteria, thraustochytrids and microzooplankton in the Northern Arabian Sea. Deep Sea Res Part II Top Stud Oceanogr 52:1910-1921.

Ramaiah N, Sarma VVSS, Gauns M, Kumar MD, Madhupratap M. (2000). Abundance and relationship of bacteria with transparent exopolymer particles during the 1996 summer monsoon in the Arabian Sea. J Earth Syst Sci 109(4):443–451.

Ramaiah, N., Raghukumar, S., Gauns, M., (1996). Bacterial abundance and production in the central and eastern Arabian Sea. Current Science 71, 878–882.

Rawlings, N. D., Barrett, A. J., Thomas, P. D., Huang, X., Bateman, A., & Finn, R. D. (2018). The MEROPS database of proteolytic enzymes, their substrates and

inhibitors in 2017 and a comparison with peptidases in the PANTHER database. Nucleic Acids Research, 46(D1), D624–D632.

Reiner K (2012) Carbohydrate Fermentation Protocol. Washington, DC: Am Soc Microbiol 11:12

Reji, L., Tolar, B.B., Chavez, F.P., Francis, C.A., (2020). Depth-Differentiation and Seasonality of Planktonic Microbial Assemblages in the Monterey Bay Upwelling System. Front. Microbiol. 11, 1075.

Riemann L, F. Steward G, Fandino LB, Campbell L, Landry MR, Azam F. (1999). Bacterial community composition during two consecutive NE Monsoon periods in the Arabian Sea studied by denaturing gradient gel electrophoresis (DGGE) of rRNA genes. Deep Sea Research Part II: Topical Studies in Oceanography 46(8–9):1791–1811.

Robinson C, Ramaiah N (2011) Microbial heterotrophic metabolic rates constrain the microbial carbon pump. In: Jiao N, Azam F, Sanders S (eds) Microbial carbon pump in the ocean, 1st edn. Science/AAAS, Washington DC, pp 52-53

Rocke E, Cheung S, Gebe Z, Dames NR, Liu H, Moloney CL (2020) Marine Microbial Community Composition During the Upwelling Season in the Southern Benguela. Front Mar Sci 7:255.

Rodriguez-Mora MJ, Scranton MI, Taylor GT, Chistoserdov AY (2013) Bacterial community composition in a large marine anoxic basin: a Cariaco Basin time-series survey. FEMS Microbiol Ecol 84:625-639.

Sachithanandam V, Saravanane N, Chandrasekar K, Karthick P, Lalitha P, Sai Elangovan S, Sudhakar M. (2020). Microbial diversity from the continental shelf regions of the Eastern Arabian Sea: A metagenomic approach. Saudi Journal of Biological Sciences 27(8):2065–2075.

Santhikrishnan S, Jyothibabu R, Albin KJ, Alok KT, Karnan C, Arunpandi N, Camey MF, Kumar TG (2021) Biophysical implications of the freshwater influx over small spatial scale in the coastal waters along the southwest coast of India during the Southwest Monsoon. Cont Shelf Res 214:104337.

Sarma V, Swathi P, Kumar MD, Prasannakumar S, Bhattathiri P, Madhupratap M, Ramaswamy V, Sarin M, Gauns M, Ramaiah N (2003) Carbon budget in the eastern and central Arabian Sea: An Indian JGOFS synthesis. Global Biogeochem Cycles 17.

Sarma, V. V. S. S., Krishna, M. S., Prasad, V. R., Kumar, B. S. K., Naidu, S. A., Rao, G. D., Viswanadham, R., Sridevi, T., Kumar, P. P., & Reddy, N. P. C. (2014). Distribution and sources of particulate organic matter in the Indian monsoonal estuaries during monsoon. Journal of Geophysical Research: Biogeosciences, 119(11), 2095–2111.

Saunders, J. K., McIlvin, M. R., Dupont, C. L., Kaul, D., Moran, D. M., Horner, T., Laperriere, S. M., Webb, E. A., Bosak, T., Santoro, A. E., & Saito, M. A. (2022). Microbial functional diversity across biogeochemical provinces in the central Pacific Ocean. Proceedings of the National Academy of Sciences, 119(37), e2200014119.

Saw, J. H. W., Nunoura, T., Hirai, M., Takaki, Y., Parsons, R., Michelsen, M., Longnecker, K., Kujawinski, E. B., Stepanauskas, R., Landry, Z., Carlson, C. A., & Giovannoni, S. J. (2020). Pangenomics analysis reveals diversification of enzyme families and niche specialization in globally abundant SAR202 bacteria. mBio, 11(1), e02975-19.

Sawant SS, Salunke BK, Kim BS (2015) A rapid, sensitive, simple plate assay for detection of microbial alginate lyase activity. Enzyme Microb Technol 77:8-13.

Schattenhofer, M., Fuchs, B.M., Amann, R., Zubkov, M.V., Tarran, G.A., Pernthaler, J., (2009). Latitudinal distribution of prokaryotic picoplankton populations in the Atlantic Ocean. Environmental Microbiology 11, 2078–2093.

Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB, Lesniewski RA, Oakley BB, Parks DH, Robinson CJ (2009) Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl Environ Microbiol 75:7537-7541.

Seemann, T. (2014). Prokka: Rapid prokaryotic genome annotation. Bioinformatics, 30(14), 2068–2069.

Shannon E, Abu-Ghannam N (2016) Antibacterial derivatives of marine algae: An overview of pharmacological mechanisms and applications. Mar Drugs 14:81.

Sheik, C.S., Jain, S., Dick, G.J., (2014). Metabolic flexibility of enigmatic SAR 324 revealed through metagenomics and metatranscriptomics. Environmental Microbiology 16, 304–317.

Shetye S, Shenoi S, Gouveia A, Michael G, Sundar D, Nampoothiri G (1991) Wind-driven coastal upwelling along the western boundary of the Bay of Bengal during the southwest monsoon. Cont Shelf Res 11:1397-1408.

Shetye, S. S., Kurian, S., Vidya, P. J., Gauns, M., Shenoy, D. M., Aparna, S. G., Nandakumar, K., & Karapurkar, S. G. (2021). Total organic carbon and its role in oxygen utilization in the eastern Arabian Sea. Marine Pollution Bulletin, 163, 111939.

Shetye, S. S., Nandakumar, K., Kurian, S., Gauns, M., Shenoy, D. M., Naik, H., Vidya, P. J., & Karapurkar, S. G. (2022). Organic carbon dynamics in the continental shelf waters of the eastern Arabian Sea. Environmental Monitoring and Assessment, 194(10), 716.

Shindoh, S., Obayashi, Y., & Suzuki, S. (2021). Induction of extracellular aminopeptidase production by peptides in some marine bacterial species. Microbes and Environments, 36(1), n/a.

Showalter G, Deming J (2021) Extracellular enzyme activity of model cold adapted bacteria and Arctic sea-ice microbial communities under sub-zero hypersaline conditions. Aquat Microb Ecol 87:99-111.

Siegel DA, DeVries T, Doney SC , Bell T (2021) Assessing the sequestration time scales of some ocean-based carbon dioxide reduction strategies. Environ Res Lett 16:104003.

Siezen, R.J., Van Hylckama Vlieg, J.E., (2011). Genomic diversity and versatility of Lactobacillus plantarum, a natural metabolic engineer. Microb Cell Fact 10, S3.

Silori, S., Biswas, H., Chowdhury, M., Sharma, D., Magloire, M.-Y., & Cardinal, D. (2022). Interannual variability in particulate organic matter distribution and its carbon stable isotope signatures from the western Indian shelf waters. Science of The Total Environment, 844, 157044.

Silori, S., Sharma, D., Chowdhury, M., Biswas, H., Bandyopadhyay, D., Shaik, A. U. R., Cardinal, D., Mandeng-Yogo, M., & Narvekar, J. (2021). Contrasting phytoplankton and biogeochemical functioning in the eastern Arabian Sea shelf waters recorded by carbon isotopes (SW monsoon). Marine Chemistry, 232, 103962.

Singh S, Ramaiah N (2011) Denaturing gradient gel electrophoresis profiling of bacterial communities composition in Arabian Sea. J Environ Biol 32:339-46.

Smitha B, Sanjeevan V, Vimalkumar K, Revichandran C (2008) On the upwelling off the southern tip and along the west coast of India. J Coast Res 24:95-102.

Smitha BR, Sanjeevan VN, Vimalkumar KG, Revichandran C. (2008). On the Upwelling off the Southern Tip and along the West Coast of India. Journal of Coastal Research 24(4C):95–102.

Smitha, B.R., Sanjeevan, V.N., Vimalkumar, K.G., Revichandran, C., (2008). On the Upwelling off the Southern Tip and along the West Coast of India. Journal of Coastal Research 24, 95–102.

Smriga S, Fernandez VI, Mitchell JG, Stocker R (2016) Chemotaxis toward phytoplankton drives organic matter partitioning among marine bacteria. Proc Natl Acad Sci USA 113:1576-1581.

Sosa, O. A., Repeta, D. J., Ferrón, S., Bryant, J. A., Mende, D. R., Karl, David. M., & DeLong, E. F. (2017). Isolation and characterization of bacteria that degrade phosphonates in marine dissolved organic matter. Frontiers in Microbiology, 8, 1786.

Spring, S., Scheuner, C., Göker, M., Klenk, H.-P., (2015). A taxonomic framework for emerging groups of ecologically important marine gammaproteobacteria based on the reconstruction of evolutionary relationships using genome-scale data. Front. Microbiol. 6.

Sridevi B, Sabira Sk, Sarma VVSS. (2023). Impact of ocean warming on net primary production in the northern Indian Ocean: role of aerosols and freshening of surface ocean. Environ Sci Pollut Res 30(18):53616–53634.

Sripan D, Wilantho A, Khitmoh K, Wongsawaeng D, Ouazzani J, Chavanich S, Tongsima S, Somboonna N (2021) Marine Bacteria Community in a 150-m Depth Tachai Island, the Southeast Andaman Sea of Thailand. Front Mar Sci 8:124.

Stoecker D K, Hansen PJ, Caron DA, Mitra A (2017) Mixotrophy in the marine plankton. Ann Rev Mar Sci 9:311–335.

Suh, S.-S., Park, M., Hwang, J., Kil, E.-J., Jung, S. W., Lee, S., & Lee, T.-K. (2015). Seasonal dynamics of marine microbial community in the south sea of Korea. PLOS ONE, 10(6), e0131633.

Sujith PP, Gonsalves MJBD, Bhonsle S, Shaikh S, Loka Bharathi PA. (2017). Bacterial activity in hydrogenetic ferromanganese crust from the Indian Ocean: a combined geochemical, experimental and pyrosequencing study. Environ Earth Sci 76(5):191.

Sun, F., Wu, M., Wang, Y., Sun, C., & Xu, Z. (2020). Diversity and potential function of bacterial communities in different upwelling systems. Estuarine, Coastal and Shelf Science, 237, 106698.

Sun, P., Wang, Y., Huang, X., Huang, B., Wang, L., (2022). Water masses and their associated temperature and cross-domain biotic factors co-shape upwelling microbial communities. Water Research 215, 118274.

Teeling H, Fuchs BM, Bennke CM, Krueger K, Chafee M, Kappelmann L, Reintjes G, Waldmann J, Quast C, Gloeckner FO (2016) Recurring patterns in bacterioplankton dynamics during coastal spring algae blooms. eLife 5:e11888.

Thiele, S., Basse, A., Becker, J. W., Lipski, A., Iversen, M. H., & Mollenhauer, G. (2018). Microbial communities in the nepheloid layers and hypoxic zones of the Canary Current upwelling system. MicrobiologyOpen, 8(5), e00705.

Thomas, A. M., & Segata, N. (2019). Multiple levels of the unknown in microbiome research. BMC Biology, 17(1), 48.

Thrash, J. C., Seitz, K. W., Baker, B. J., Temperton, B., Gillies, L. E., Rabalais, N. N., Henrissat, B., & Mason, O. U. (2017). Metabolic roles of uncultivated bacterioplankton lineages in the northern Gulf of Mexico "dead zone." mBio, 8(5), e01017-17.

Traving SJ, Thygesen UH, Riemann L, Stedmon CA (2015) A model of extracellular enzymes in free-living microbes: which strategy pays off? Appl Environ Microbiol 81:7385-7393.

Traving, S. J., Rowe, O., Jakobsen, N. M., Sørensen, H., Dinasquet, J., Stedmon, C. A., Andersson, A., & Riemann, L. (2017). The effect of increased loads of dissolved organic matter on estuarine microbial community composition and function. Frontiers in Microbiology, 8.

Truong, D. T., Franzosa, E. A., Tickle, T. L., Scholz, M., Weingart, G., Pasolli, E., Tett, A., Huttenhower, C., & Segata, N. (2015). MetaPhlAn2 for enhanced metagenomic taxonomic profiling. Nature Methods, 12(10), 902–903.

Tseng, C.-H., Chiang, P.-W., Lai, H.-C., Shiah, F.-K., Hsu, T.-C., Chen, Y.-L., Wen, L.-S., Tseng, C.-M., Shieh, W.-Y., Saeed, I., Halgamuge, S., & Tang, S.-L. (2015). Prokaryotic assemblages and metagenomes in pelagic zones of the South China Sea. BMC Genomics, 16(1), 219.

Underwood GJ, Fietz S, Papadimitriou S, Thomas DN, Dieckmann GS (2010) Distribution and composition of dissolved extracellular polymeric substances (EPS) in Antarctic sea ice. Mar Ecol Prog Ser 404:1-19.

UNESCO, (1994) UNESCO Protocols for the Joint Global Ocean Flux Study (JGOFS) Core Measurements, IOC Manuals and Guides, vol. 29, UNESCO, Paris (1994), p. 170

Usman, M., Zhao, S., Jeon, B.-H., Salama, E.-S., & Li, X. (2022). Microbial β-oxidation of synthetic long-chain fatty acids to improve lipid biomethanation. Water Research, 213, 118164.

Valliappan K, Sun W, Li Z (2014) Marine actinobacteria associated with marine organisms and their potentials in producing pharmaceutical natural products. Appl Microbiol Biotechnol 98:7365-7377.

Verma P, Raghavan RV, Jeon CO, Lee HJ, Priya PV, Dharani G, Kirubagaran R. (2017). Complex bacterial communities in the deep-sea sediments of the Bay of Bengal and volcanic Barren Island in the Andaman Sea. Marine Genomics 31:33–41.

Vijayan J, Ammini P, Nathan VK (2021) Diversity pattern of marine culturable heterotrophic bacteria in a region with coexisting upwelling and mud banks in the southeastern Arabian Sea. Environ Sci Pollut Res 29:3967-3982.

Vijayan, J., Nathan, V. K., Ammini, P., & Ammanamveetil, A. M. H. (2023). Bacterial diversity in the aquatic system in India based on metagenome analysis—A critical review. Environmental Science and Pollution Research, 30(11), 28383–28406.

Villanueva L, Speth DR, Van Alen T, Hoischen A, Jetten MSM. (2014). Shotgun metagenomic data reveals significant abundance but low diversity of “Candidatus Scalindua” marine anammox bacteria in the Arabian Sea oxygen minimum zone. Front Microbiol 5.

Vinayachandran, P. N. M., Masumoto, Y., Roberts, M. J., Huggett, J. A., Halo, I., Chatterjee, A., Amol, P., Gupta, G. V. M., Singh, A., Mukherjee, A., Prakash, S., Beckley, L. E., Raes, E. J., & Hood, R. (2021). Reviews and syntheses: Physical and biogeochemical processes associated with upwelling in the Indian Ocean. Biogeosciences, 18(22), 5967–6029.

Wang J, Kan J, Zhang Xiaodong, Xia Z, Zhang Xuecheng, Qian G, Miao Y, Leng X, Sun J. (2017). Archaea Dominate the Ammonia-Oxidizing Community in Deep-Sea Sediments of the Eastern Indian Ocean—from the Equator to the Bay of Bengal. Front Microbiol 8.

Ward, C.S., Yung, C.-M., Davis, K.M., Blinebry, S.K., Williams, T.C., Johnson, Z.I., Hunt, D.E., (2017). Annual community patterns are driven by seasonal switching between closely related marine bacteria. ISME J 11, 1412–1422.

Wei, T., Zhao, C., Quareshy, M., Wu, N., Huang, S., Zhao, Y., Yang, P., Mao, D., & Chen, Y. (2021). A glycolipid glycosyltransferase with broad substrate specificity from the marine bacterium "Candidatus pelagibacter sp." Strain htcc7211. Applied and Environmental Microbiology, 87(14), e00326-21.

Wiggert, J.D., Hood, R.R., Banse, K., Kindle, J.C., (2005). Monsoon-driven biogeochemical processes in the Arabian Sea. Progress in Oceanography 65, 176–213.

Williams, T. J., Wilkins, D., Long, E., Evans, F., DeMaere, M. Z., Raftery, M. J., & Cavicchioli, R. (2013). The role of planktonic Flavobacteria in processing algal organic matter in coastal East Antarctica revealed using metagenomics and metaproteomics. Environmental Microbiology, 15(5), 1302–1317.

Wright JJ, Konwar KM, Hallam SJ (2012) Microbial ecology of expanding oxygen minimum zones. Nat Rev Microbiol 10:381-394.

Wu C, Kan J, Liu H, Pujari L, Guo C, Wang X, Sun J. (2019). Heterotrophic Bacteria Dominate the Diazotrophic Community in the Eastern Indian Ocean (EIO) during Pre-Southwest Monsoon. Microb Ecol 78(4):804–819.

Wyrtki K. (1973). An Equatorial Jet in the Indian Ocean. Science 181(4096):262–264.

Yang W, Zheng SZ, Zhou SH, Zhao L, Zhu JY, Lukwambe B, Nicholaus R, Li CH, Zheng ZM (2020) Structure and Functional Diversity of Surface Bacterioplankton Communities in an Overwintering Habitat for Large Yellow Croaker, Pseudosciaena crocea, of the Southern East China Sea. Front Mar Sci 7:472.

Yaradoddi, J. S., Kontro, M. H., Ganachari, S. V., Banapurmath, N. R., Oli, A., Katti, A. S., & Sulochana, M. B. (2021). Actinobacteria in marine environments. In J. S. Yaradoddi, M. H. Kontro, & S. V. Ganachari (Eds.), Actinobacteria: Ecology, Diversity, Classification and Extensive Applications (pp. 21–38). Springer Nature.

Yilmaz, P., Yarza, P., Rapp, J.Z., Glöckner, F.O., (2016). Expanding the World of Marine Bacterial and Archaeal Clades. Front. Microbiol. 6.

Yu, T., Wu, W., Liang, W., Wang, Y., Hou, J., Chen, Y., Elvert, M., Hinrichs, K.-U., & Wang, F. (2023). Anaerobic degradation of organic carbon supports uncultured microbial populations in estuarine sediments. Microbiome, 11(1), 81.

Zari A, Ramesh CH, Mohanraju R. (2020). Non-species specific composition of bioluminescent bacteria in non-bioluminescent squid Sepioteuthis lessoniana (Lesson, 1830). Indian J Geo Mar Sci 49(6):975-981.

Zhang Z, Chen Y, Wang R, Cai R, Fu Y, Jiao N (2015) The fate of marine bacterial exopolysaccharide in natural marine microbial communities. PLoS ONE 10:e0142690.

Zhang, D., Li, S. H.-J., King, C. G., Wingreen, N. S., Gitai, Z., & Li, Z. (2022). Global and gene-specific translational regulation in Escherichia coli across different conditions. PLOS Computational Biology, 18(10), e1010641.

Zhang, H., Yohe, T., Huang, L., Entwistle, S., Wu, P., Yang, Z., Busk, P. K., Xu, Y., & Yin, Y. (2018). Dbcan2: A meta server for automated carbohydrate-active enzyme annotation. Nucleic Acids Research, 46(W1), W95–W101.

Zhao D, Cao X, Huang R, Zeng J, Wu QL (2017) Variation of bacterial communities in water and sediments during the decomposition of Microcystis biomass. PLoS ONE 12:e0176397.

Zhou J, Richlen ML, Sehein TR, Kulis DM, Anderson DM, Cai Z (2018) Microbial community structure and associations during a marine dinoflagellate bloom. Front Microbiol 9:1201.

Zhou, L., Zhou, Y., Tang, X., Zhang, Y., Jang, K.-S., Székely, A. J., & Jeppesen, E. (2021). Resource aromaticity affects bacterial community successions in response to different sources of dissolved organic matter. Water Research, 190, 116776.

Zhu D, Sethupathy S, Gao L, Nawaz MZ, Zhang W, Jiang J, Sun J. (2022). Microbial diversity and community structure in deep-sea sediments of South Indian Ocean. Environ Sci Pollut Res 29(30):45793–45807.

Zimmerman AE, Martiny AC, Allison SD (2013) Microdiversity of extracellular enzyme genes among sequenced prokaryotic genomes. ISMEJ 7:1187-1199.

Zorz, J., Willis, C., Comeau, A. M., Langille, M. G. I., Johnson, C. L., Li, W. K. W., & LaRoche, J. (2019). Drivers of regional bacterial community structure and diversity in the northwest Atlantic Ocean. Frontiers in Microbiology, 10.

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Bacterial dynamics along the west coast of India during the non-monsoon and monsoon season

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ABSTRACT

The West Coast of India (WCI) is influenced largely by semi-annual changes in the wind pattern and monsoonal currents. This is reported to cause upwelling which increases the phytoplankton growth in this region during the summer monsoon and it is recorded to be one of the world's highest primary productivity. Increase in the phytoplankton productivity is known to influence the microbial activity which is one of the key biogeochemical processes of the marine ecosystem as it plays an important role in the biotransformation of organic matter. However, the changes in the bacterial community structure and their heterotrophic enzyme activity with increasing primary production are not clearly understood from this region. Hence in this study, the bacterial dynamics along the WCI at Goa, Mangalore and Trivandrum, were investigated from the continental shelf, slope and off-shore region during the non-monsoon and monsoon season. Seasonal changes in the bacterial parameters such as abundance, viable counts and radioactivity based *in-situ* primary and bacterial productivity were estimated, along with the physicochemical characteristics. Statistical analysis shows a significant ($p < 0.05$) correlation between primary and bacterial productivity. Significant seasonal variations could also be established for the physicochemical parameters such as dissolved oxygen, nitrate, nitrite and phosphate, fluorescence values representing the chlorophyll concentration, bacterial abundance, viable counts and productivity. Taxonomic diversity studies on the cultivable bacterial morphotypes have shown that Proteobacteria, Firmicutes and Actinobacteria were the major phyla found during both the non-monsoon and monsoon season and Bacteroidetes, was represented only in the non-monsoon season. The bacterial community structure at phylum, genus and operational taxonomic unit levels also changed with higher diversity in the monsoon season. However, the hydrolytic enzyme activity of the bacterial morphotypes was comparatively lower during the monsoon season probably due to the active primary production. Though this study has improved our understanding of the monsoonal influence on the bacterial dynamics along the WCI, further studies based on metagenomic and transcriptome analysis are required to get a better understanding of the role played by the bacterial community from this region.

1. Introduction

Nearly one-half of the global photosynthesis occurs in the ocean due to the carbon fixation by the phytoplankton community and this leads to the production of huge amounts of organic matter (OM) (Karl et al., 1998; Mühlenbruch et al., 2018). Various physical, chemical and biological processes such as viral lysis, microbial activities and protistan grazing of phytoplankton cells; modify, decompose and redistribute the OM within diverse time frames ranging from a few hours to millennia (Robinson and Ramaiah, 2011; Stoecker et al., 2017). The carbon fixed by the phytoplankton can be categorized into two distinct oceanic carbon pools: particulate organic matter and dissolved organic matter (Jiao et al., 2014). The phytoplankton biomass, zooplanktons, nektons and their degraded cells, including the microbial communities constitute the particulate organic matter, which is estimated to be approximately 30×10^{15} g C (Hansell and Carlson 2001). Most of it is immediately respired back as CO_2 by diverse biota and a small fraction is actively transported to the sea bed by higher organisms through the "biological pump" and the "microbial carbon pump" by the heterotrophic microbial communities (Ducklow et al., 2001; Jiao et al., 2011; Enke et al., 2018). The

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heterotrophic activity of the microbes, especially the enzymatic breakdown of OM is the major pathway that leads to the production of dissolved OM and drives the oceanic carbon cycle (Fang et al., 2015; Moran et al., 2022).

Understanding the importance of microbial activity has increased significantly after the role of microbial transformation of particulate OM to labile and refractory forms in the oceanic habitats was characterised (Jiao et al., 2014; Lian et al., 2021). Though most of the OM would be readily respired, a small fraction is known to be converted into refractory organic matter which has a turnover time of millennia (Hansell and Carlson 2013; Siegel et al., 2021). Recent studies indicate that specific lineages of bacterioplankton are responsible for remineralizing the OM (Landa et al., 2016). It is also evident that many of the bacterial communities are structured with unique metabolic capabilities that are important in determining the fate of the exported OM and its lability (Robinson and Ramaiah 2011). Heterotrophic bacteria utilize the OM through the hydrolytic activity of the extracellular enzymes and the ability of the bacterial groups to utilize the OM varies considerably. Reports have shown that Alpha, Beta and Gamma Proteobacteria and Bacteroidetes have the metabolic genes necessary to produce and secrete a variety of extracellular hydrolytic enzymes (Zimmerman et al., 2013; Teeling et al., 2016; Mahmoudi et al., 2019). This implies that the ability to produce these enzymes is a functionally distinct rather than a functionally redundant feature and that changes in the taxonomic composition will affect the hydrolytic activity (Mahmoudi et al., 2020). The ability of microbial extracellular hydrolytic enzymes to access and break down complex organic substances as a source of carbon and energy is the central metabolic process of the marine habitat as it can, in turn control the production of the primary producers (Arnosti, 2011; Amin et al., 2015; Bolch et al., 2017).

The West Coast of India (WCI) extends from 7°N to 25°N bordering the eastern Arabian Sea covering a total length of approximately 3010 km coastline of the Indian sub-continent. This region is influenced largely by semi-annual changes in the climate, wind pattern and monsoonal currents. The Arabian Sea and its coastal regions are known to be one of the highly productive regions of the World Ocean (Sarma et al., 2003; Dalabehara and Sarma 2021). Studies have shown that the average primary productivity (PP) along the WCI is calculated to range from about 210 mg C m^{-2} d^{-1} and it increases close to 950 mg C m^{-2} d^{-1} during the summer or southwest monsoon season (Gauns et al., 2005). During this season, the upwelling of nutrient-rich subsurface waters leads to an increase in the phytoplankton biomass and this is known to control the production of OM in these regions (Ram et al., 2003; Jacob et al., 2009, Santhikrishnan et al., 2021). Bacterial abundance and activities are also shown to have a similar changing pattern with low numbers and reduced bacterial productivity, during the winter (December–February) which increases during the summer monsoon (June–September) (Ramaiah et al., 2009). However, the bacterial activity of the communities that increase in growth during the monsoon season is not elucidated thoroughly from this region. Hence the aim of this study was to understand the relationship between the physicochemical characteristics and PP on the bacterial abundance, cultivable diversity and its activity during the non-monsoon and monsoon season along the WCI representing the continental shelf, slope and off-shore regions.

2. Study area and methods

2.1. Study area, sample collection and measurement of physicochemical parameters

Sampling was carried out during the non-monsoon season onboard RV Sindhu Sankalp (Cruise # SSK-113) in January 2018 and the productive, summer monsoon season onboard RV Sindhu Sadhana (Cruise # SSD-053) in July 2018. Samples were collected from both these seasons along the WCI at three stations, off Goa, Mangalore and Trivandrum, which lies between 8 and 16°N and 72 to 76°E (Fig. 1). The three locations at each station are (i) in the coastal, continental shelf region at ~ 30 m water depth, (ii) in the slope along the ~300 m contour and (iii) in the off-shore location at ~ 600 m water depth. The sampling stations off Goa from the continental shelf, slope and off-shore region are designated as G30, G300 and G600 respectively, similarly, sampling stations off Mangalore are designated as M30, M300 and M600 and off Trivandrum station as T30, T300 and T600 (Fig. 1).

The conductivity-temperature-depth (CTD) underwater profiler (Sea-Bird, USA, model: SBE-911 plus) was used to measure the water column physicochemical properties temperature, salinity, dissolved oxygen (DO) and fluorescence representing the chlorophyll concentration using the respective electronic probes at all the nine stations in the January and July 2018 sampling. Water samples from the shelf stations G30, M30 and T30 were collected from the surface, chlorophyll maxima depth (Chl-M), based on the CTD profile and bottom waters, at about 3 m above the seabed. Similarly, at the slope stations, G300, M300 and T300 samples were collected at the surface, Chl-M, 100 m, 200 m and bottom water depths (~300 m). In the off-shore stations, along the 600 m contour, samples were collected from the surface, Chl-M, 100, 200, 300, 400 m and bottom depth (~600 m). The Chl-M depth varied for each station and it was determined based on the values obtained from the fluorescence sensor representing the chlorophyll concentration during the downward haul of the CTD profiler and the water samples were collected at discrete depths during the upward haul, using pre-cleaned Niskin samplers connected to the underwater CTD unit (Sea-Bird, USA). Water samples collected were immediately sub-sampled and stored appropriately for further analysis. Nutrients such as nitrate, nitrite, phosphate and silicate levels in the water samples were analysed using an automated continuous flow analyser (SKALAR 5000, Netherlands).

2.2. Primary productivity measurements

The measurements in the water samples from the coastal, shelf and off-shore stations in both seasons was measured based using the ^{14}C-labelled inorganic C, $NaH^{14}CO_3$ to determine PP. For this water samples were collected from the surface, Chl-M and 30 m depth at the shelf stations and from the surface, Chl-M, 100 m and 200 m of the off-shore stations in three light and one dark, 300 ml polycarbonate bottles (Nalgene, Germany). One ampoule of $NaH^{14}CO_3$ (Board of Radiation and Isotope Technology, Mumbai; Specific activity 185 kBq) was added to each bottle and incubated *in-situ* using a mooring system for 12 h from sunrise to sunset as per JGOFS protocols (UNESCO 1994). At the end of the incubation period, water samples were filtered through GF/F 47 mm diameter, 0.7 μm pore size filters (Whatman, USA) and stored in the dark in scintillation vials. For analysis, filters were dissolved overnight in concentrated HCl, followed by incubation in a liquid scintillation cocktail (Sisco Research Laboratory, Mumbai). The radioactivity was measured in the scintillation counter (PerkinElmer Wallac 1409 DSA, USA) and expressed as mg C m^{-3} d^{-1}.

2.3. Estimation of bacterial abundance, viable counts, productivity, respiration and growth efficiency

Bacterial abundance (BA) was determined following the modified method of Porter and Feig (1980). In brief, subsamples of ~20 ml water, from each depth were preserved with buffered formaldehyde (2% final concentration) immediately after sampling and stored at room temperature in the dark for further analysis. One to five ml aliquots of the preserved samples in triplicates were incubated with 4′6′-diamidino-2-phenylindole (DAPI) at the final concertation of 1 μg ml^{-1} and filtered onto black, 0.22 μm pore size polycarbonate membrane filters (Millipore, U.S.A). Counts were made from 10 to 20 microscopic fields with an epifluorescence microscope (Olympus BX-51, Japan). The final bacterial counts were expressed as cells L^{-1} of the

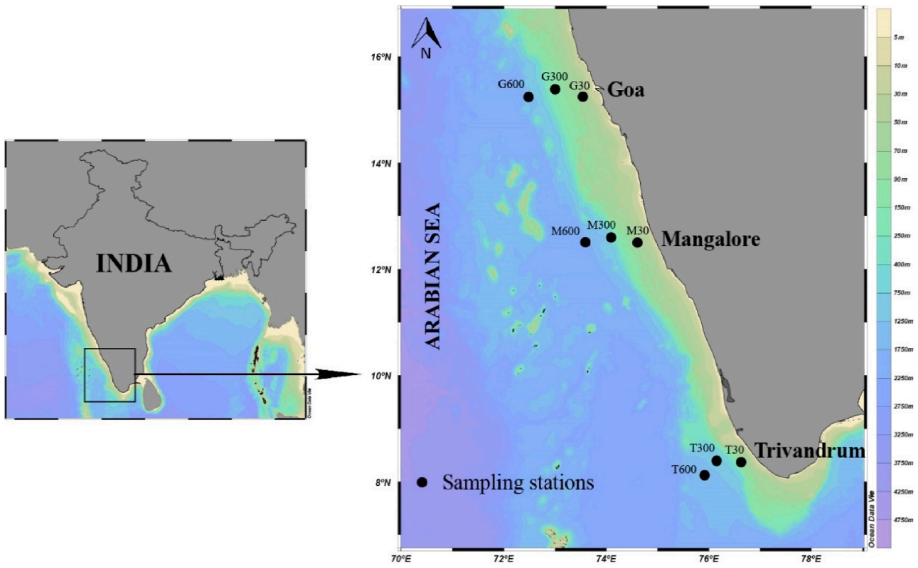

Fig. 1. Study area and sampling stations along the west coast of India at Goa, Mangalore and Trivandrum.

water sample.

Total viable counts (TVC) of bacteria were enumerated from water samples following the spread plate technique on Zobell Marine Agar (ZMA) microbiological plates. For this, seawater samples of 100 μL aliquots were plated aseptically on ZMA plates in triplicates and incubated aseptically for 24–48 h at 28 °C along with appropriate controls to minimise contamination. Bacterial colony counts were determined at the end of the incubation and expressed as colony forming units (CFUs). Bacterial colonies grown were characterized based on their colony morphology and grouped into morphotypes. One representative colony for each morphotype was streak purified on ZMA plates for further analysis.

The bacterial production (BP) rate was determined based on the ^{3}H-thymidine incorporation rates in the water samples following the method described in JGOFS Protocols (UNESCO 1994). BP was estimated at shelf, slope and off-shore stations from all the depths during both seasons. For this, 20 ml of water samples in triplicates were incubated in the dark with ^{3}H-thymidine (specific activity 18 Ci mmol^{-1}, Bhabha Atomic Research Centre, Mumbai) for 2 h at ambient temperature. The incubation was terminated by the addition of filtered formaldehyde and the samples were filtered through 0.22 μm cellulose acetate, 25 mm diameter filters (Millipore India, Bangalore). The filters were rinsed with cold trichloroacetic acid (10% w/v) followed by ethanol (70% v/v) and stored in scintillation vials for further analysis. To measure the radioactivity, the filter papers were incubated overnight in ethyl acetate, followed by the addition of a liquid scintillation cocktail (Sisco Research Laboratory, Mumbai) in recommended volume and measured in a scintillation counter (PerkinElmer Wallac 1409 DSA, USA) and expressed as mg C m^{-3} d^{-1}.

Community respiration (TR) rates were estimated from the decrease in DO concentration in water samples incubated in pre-cleaned 125 ml of BOD bottles for 24 h. For this water samples were collected in BOD bottles in two sets as triplicates, the DO in the first set was immediately fixed with Winkler reagents to estimate the initial concentration. The DO in the second set was quantified at the end of the incubation period in the dark for 24 h and the decrease in DO concentration was estimated. DO was determined using Dosimat-865 automated titrator following the Winkler titration method modified by Carpenter (1965). Bacterial respiration (BR) was calculated from the ratio of BR to TR of 0.45. Bacterial growth efficiency (BGE) was estimated as the ratio of net bacterial production (BP) to gross bacterial production (BP + BR) (Kim et al., 2017).

2.4. Molecular identification of bacterial morphotypes and phylogenetic analysis

Bacterial genomic DNA was extracted from freeze-dried pure cultures of all the bacterial morphotypes grown for 24 h in Zobell Marine Broth at 28 °C using a high salt concentration buffer followed by chloroform-phenol extraction and precipitation with isopropanol. The DNA extracted was subjected to PCR amplification of the 16S rRNA gene using the universal primers 27F (5′AGAGTTTGATCMTGGCTCAG3′) and 1492R (5′TACGGYTACCTTGTTACGACTT-3′). The 25 μL PCR reaction mixture included genomic DNA, 1X PCR buffer, 1.5 mM $MgCl_2$, 200 μM concentration of each deoxy nucleotide triphosphate, 0.5 μM concentration of each oligonucleotide primer and the final volume was adjusted to 25 μL with PCR-grade water. The amplification was performed using initial denaturation at 94 °C for 5 min, followed by 30 cycles of 94 °C for 50 s, 54 °C for 45 s and 72 °C for 1 min, with a final extension at 72 °C for 7 min with 1U Taq DNA polymerase (Sigma Aldrich) in an Eppendorf vapour protect master cycler. The confirmation of 16S rRNA amplification was done by agarose gel electrophoresis in 1% agarose gel prepared using 1X TAE buffer and results were visualized in SYGENE Gel documentation system. PCR product was purified using the HiPurA™ PCR purification kit (HiMedia). The PCR products were sequenced in Applied Biosystems (ABI) 3730 DNA Stretch Sequencer, with the XL Upgrade and the ABI Prism BigDye Terminator version 3.1 Cycle Sequencing Ready Reaction Kit. They were processed to remove primers and were also screened for quality using Chromas version 2.6.6. These sequences were analysed in the NCBI database using the bioinformatics tool BLAST (Basic local alignment search tool) to confirm their taxonomic and phylogenetic similarity. The morphotypes at the genus level were characterised and represented as a "Doughnut" chart using Excel (Microsoft) program. GenBank accession numbers of sequences deposited of the bacterial morphotypes are MW785066-MW785148 and MW795735-MW795873.

The 16S rRNA sequences of the bacterial morphotypes obtained from January and July 2018 sampling were aligned against the SILVA reference alignment in MOTHUR version 1.46.1 to obtain pairwise alignment. Based on the distance matrix the sequences were assigned into operational taxonomic units (OTUs) in MOTHUR using the cluster command and the average neighbour rule at a cut-off of 0.1 sequence similarity. A total of 222 sequences obtained from both the samplings were assigned to 111 OTUs. A representative sequence from each OTU was identified using the get.oturep command (Schloss et al., 2009) and a

phylogenetic tree of representative OTUs was built in MEGA-X using the maximum likelihood method and Kimura 2-parameter substitution model, with bootstrap values calculated using the neighbour-joining method with a resampling size of n = 1000.

2.5. Estimation of hydrolytic enzyme activity

The different morphotypes isolated were analysed for their carbohydrase, protease and lipase activity based on qualitative substrate utilization assay following standard protocols. The utilization of monosaccharides by bacterial morphotypes was studied by inoculating each bacterial morphotype in media containing pH indicator (Bromocresol purple) and supplemented with different monosaccharides such as dextrose, arabinose, rhamnose and galactose individually (Reiner, 2012). The monosaccharide utilization ability was estimated based on the colour change from purple to yellow due to a change in pH due to substrate utilization after 48 h of incubation at 28 °C. Extracellular enzyme activity for hydrolysis of polysaccharides such as starch (1%), carboxyl methylcellulose (CMC 1%), agar (2%), alginate (0.5%) and xylan (0.5%) was also carried out by using each of the substrates as a carbon source in basal salt agar plates for estimating the carbohydrase activity (Meddeb-Mouelhi et al., 2014; Sawant et al., 2015; Khalifa and Aldayel, 2019). Protease and lipase activity was tested on basal salt agar plates supplemented with Skim Milk (SM) and Tween 80, respectively (Fenice et al., 2007). Each of the bacterial morphotypes was grown on the substrate plates and incubated at 28 °C for 48 h and observed for their zones of hydrolysis around bacterial colonies. The hydrolytic activity of amylase, agarose and alginate lyase was determined by staining with Gram's iodine for 10 min at the end of the incubation period. Cellulase and xylanase activity was determined by staining with 0.5% Congo red for 15 min followed by destaining with 1N NaCl for 10 min. The protease and lipase activity was estimated by measuring the zone of hydrolysis around bacterial colonies. The bacterial morphotypes showing hydrolytic enzyme activity (%) were categorized into no activity or minimum activity for monosaccharide substrates. For the polysaccharide, protease and lipase activity (%) the morphotypes could be grouped based on the zone of hydrolysis exhibited into isolates with very good activity (21–30 mm), good activity (11–20 mm), minimum activity (1–10 mm) and no activity group. Hydrolytic enzyme activity (%) on different substrates by the bacterial morphotypes within each phylum was also estimated.

2.6. Statistical analysis

The significance of distribution between physicochemical and biological characteristics in the two seasons was determined using a single factor ANOVA. Pearson's correlation analysis was performed to interpret relationships within physicochemical and biological parameters between two seasons at the 5% ($p < 0.05$) level of significance. Statistical analyses were performed using SPSS 21.

3. Results

3.1. Physicochemical characteristics, primary productivity and bacterial dynamics

Seasonal changes were observed in the physical and chemical parameters determined from the sampling locations along the WCI. The average sea surface temperature (SST) was 28 ± 1 °C in all the stations during the non-monsoon season in January 2018 sampling and in July 2018, monsoon season the SST reduced to 27 ± 1 °C in almost all the stations, except at Trivandrum shelf station, where the lowest temperature of 22 °C was recorded. In all the shelf (30 m) stations the temperature was almost uniform throughout the water column in January sampling. However, during the monsoon period, there was a reduction in temperature by 2–4 °C from the surface to the bottom waters in the shelf stations. In the 300 m and 600 m stations, the thermocline was up to ~ 100 m during the non-monsoon sampling and in the monsoon season the thermocline depth was reduced to ~ 50 m at Goa and Mangalore and in the southern station at Trivandrum, it was shallow at ~ 25 m depth (Fig. 2). The sea surface salinity (SSS) in the January sampling, was 34 ± 2 PSU and the values were uniform throughout the water column in the shelf stations. During the July sampling in the shelf stations, the salinity varies from the surface to the bottom from ~30 to 34 PSU at Goa and ~33 to 35 PSU at Mangalore. However, there was no change at Trivandrum (30 m) station. The SSS at the 300 m (slope station) and 600 m station (off-shore station) were 34 ± 2 PSU in both seasons and did not show any seasonal changes. The halocline depth, in the slope and off-shore station, was similar to the thermocline depth at 100 m during the non-monsoon season and is reduced to 25–50 m depth in the monsoon season (Fig. 2).

The surface DO values in January 2018, during the non-monsoon season were ~ 6 mg L^{-1} at the shelf, slope and off-shore station. In the productive monsoon season, the surface DO values dropped down to ~ 4 mg L^{-1} in all the stations and at the shelf region, in Trivandrum station T30, the value was <1 mg L^{-1}. The DO values in the 30 m stations were uniform throughout the water column during the non-monsoon period, but the values at Goa and Mangalore dropped to <1 mg L^{-1} at mid-depth during the monsoon season. At the 300 m and 600 m stations the mixed layer depth (MLD) based on the DO concentration was at 100 m, where the levels reached close to anoxic conditions. During the monsoon season, the MLD was reduced to a shallower depth (25–50 m) like the other hydrographic features observed based on the temperature and salinity profiles (Fig. 2).

The chlorophyll concentration was determined using the fluorescence sensor mounted on the underwater CTD unit in all the locations sampled along the WCI. High surface fluorescence values of 10 mg m^{-3} were observed at the G30 station off Goa and 6 mg m^{-3} at the offshore station, T600 during the southwest monsoon season sampling in July 2018. Average Chlorophyll maxima observed during the monsoon season was at 15 m depth and in the non-monsoon season, it was at 25 m on the shelf and 50 m on the slope and off-shore stations. The chlorophyll maxima were observed to be shallower during the monsoon season than during the non-monsoon season. Average fluorescence values recorded during the productive monsoon season in the water column were ~0.92 mg m^{-3} which was comparatively higher than the non-monsoon season where the average water column value was ~0.24 mg m^{-3} (Fig. 2). Nitrate concentration, in all the stations in the WCI during the January 2018 sampling was an average of ~13 μM and it reduced to ~0.2 μM in the monsoon season (Fig. 3). The average nitrite concentration along all the stations was only ~0.1 μM in the non-monsoon season and it increased to ~6.6 μM in the monsoon season. Average phosphate and silicate concentration in the water column during the non-monsoon season and monsoon season was ~2 and ~10 μM respectively. The concentration of phosphate and silicate did not vary much between both seasons (Fig. 3).

The average PP in the water column during January 2018 was ~48 mg C m^{-3} d^{-1} and the values increased during July 2018 in the monsoon to ~63 mg C m^{-3} d^{-1} (Table 1). A similar observation was recorded in the BP measured during both seasons. BP was lower during the non-monsoon season in the range of 1.2–6.4 mg C m^{-3} d^{-1}, as compared to the monsoon season which was in the range of 7.2–10.9 mg C m^{-3} d^{-1}. Average TR during the non-monsoon season was ~73 mg C m^{-3} d^{-1} across the sampling stations and in the monsoon season it was ~125 mg C m^{-3} d^{-1}. A similar trend was observed in the BR and BGE calculated. During the non-monsoon season and monsoon season, the BR was 33 and 57 mg C m^{-3} d^{-1} respectively. The average BGE was 46% during the sampling in the non-monsoon season and increased to 57% during the monsoon season (Table 1).

The average BA, which is the count of free-living bacteria in the water samples during the non-monsoon season was 13.6 × 10^9 cells L^{-1} and in the monsoon season, the average water column bacterial counts

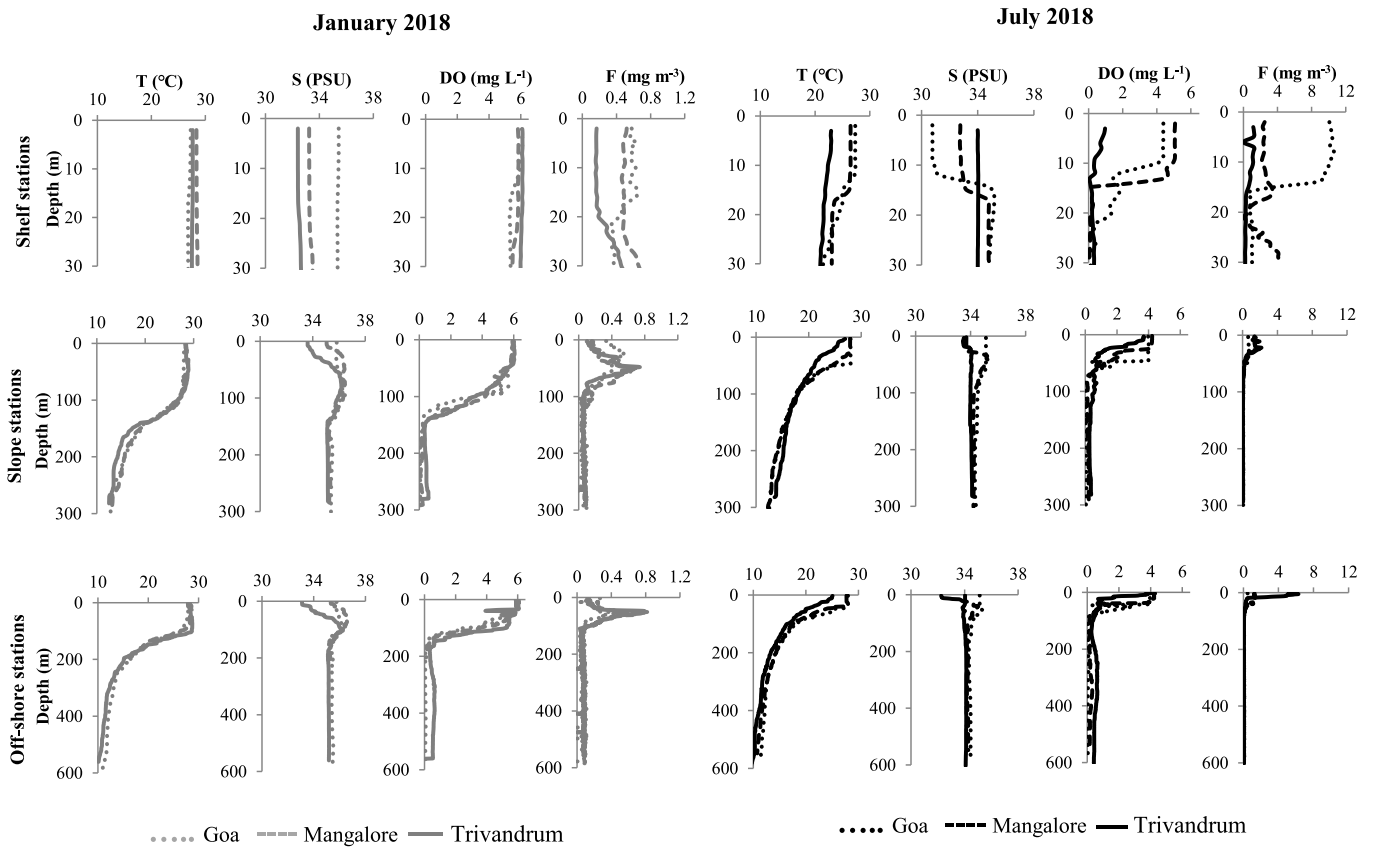

Fig. 2. Profiles of physiochemical variables temperature (T, °C), salinity (S, PSU), dissolved oxygen (DO, mg L^{-1}) and fluorescence (F, mg m^{-3}) in the water column of the shelf, slope and off-shore stations during January 2018 and July 2018 sampling.

were 4.9 × 10^9 cells L^{-1}. BA in the water column was observed to be uniform in the order of 10^9 -cells L^{-1} in all the stations during both seasons (Fig. 4). Total viable counts of cultivable bacteria determined based on the CFU, estimated during the non-monsoon season were in the range of 10–1910 CFU ml^{-1} and during July 2018 monsoon sampling, it was in the range of 75–3425 CFU ml^{-1}. Average viable counts during the non-monsoon season were 224 CFU ml^{-1} and 928 CFU ml^{-1} in the monsoon season (Fig. 4).

Statistical correlation of the physical, chemical, autotrophic and heterotrophic, bacterial parameters in both seasons was studied. DO and Temperature shows a very good correlation with a significance level of 0.01 (Table 2). Temperature influences Fluorescence, PP, TR and BR significant correlation at 0.05 level. DO also showed a significant correlation with the PP, TR and BR. PP parameters, fluorescence and PP show a correlation with very good significance (Table 2). A negative correlation with good significance between DO and bacterial parameters, BP and TVC and between nitrate concentration with BP, BR and TVC observed, could be due to the impact of the OMZ (Gonsalves et al., 2011). Denitrifying conditions are reported from these stations and the positive correlation between nitrite and BP could be because of the secondary nitrite maxima reported from these regions due to bacterial activity (Lincy and Manohar 2021). Correlation between PP and BP with good significance shows that the bacterial parameters are influenced by the changes in the PP (Table 2). ANOVA analysis of different parameters between both seasons shows variations with good significance for DO and nutrients, such as nitrate, nitrite and phosphate which proves the influence of upwelling on the autotrophic activity (Fluorescence). Significant seasonal variations are also observed for bacterial parameters BA, TVC and BP (Table 3). These results suggest that the bacteria utilize labile OM which is available in abundance during the productive period (Ramaiah et al., 2005, 2009).

3.2. Bacterial diversity and community structure

Unique morphotypes were characterized based on the 16S rRNA sequence for diversity analysis from non-monsoon and monsoon season sampling. A total number of 83 and 139 unique morphotypes were picked from the January and July 2018 sampling respectively. Proteobacteria, Firmicutes and Actinobacteria were the major phyla found during both the non-monsoon and monsoon season and Bacteroidetes were represented only in the non-monsoon season (Fig. 5). Relative abundance of Firmicutes phylum was highest (55%) in January 2018, during the non-monsoon season, followed by Proteobacteria (42%), Actinobacteria and Bacteroidetes phyla contributed to 1% each in the non-monsoon season (Fig. 5a). During the July 2018 sampling in the monsoon season, the bacterial communities included Firmicutes, which was represented with the highest relative abundance of 53% followed by Proteobacteria with 27%. The contribution of Proteobacteria was relatively lower in July (monsoon) compared to January (non-monsoon) sampling (Fig. 5). However, Actinobacterial diversity increased to 20% in the monsoon sampling and Bacteroidetes were completely absent. Phylum-wise distribution of the bacterial morphotypes in the shelf, slope and off-shore locations at each sampling station was also determined. The results show that in the non-monsoon sampling, the distribution of bacterial morphotypes from the major phyla Proteobacteria and Firmicutes were uniform in all the stations. Actinobacteria and Bacteroidetes which were represented by 1% each were identified from the Trivandrum shelf (T300) and Goa shelf location (G30) respectively (Fig. 5a). The major phyla, during the monsoon season, included morphotypes from Firmicutes, Proteobacteria and Actinobacteria. The distribution of these morphotypes was almost uniform across the shelf, slope and off-shore stations at all the locations (Fig. 5b).

Molecular characterisation of the different morphotypes at the genus

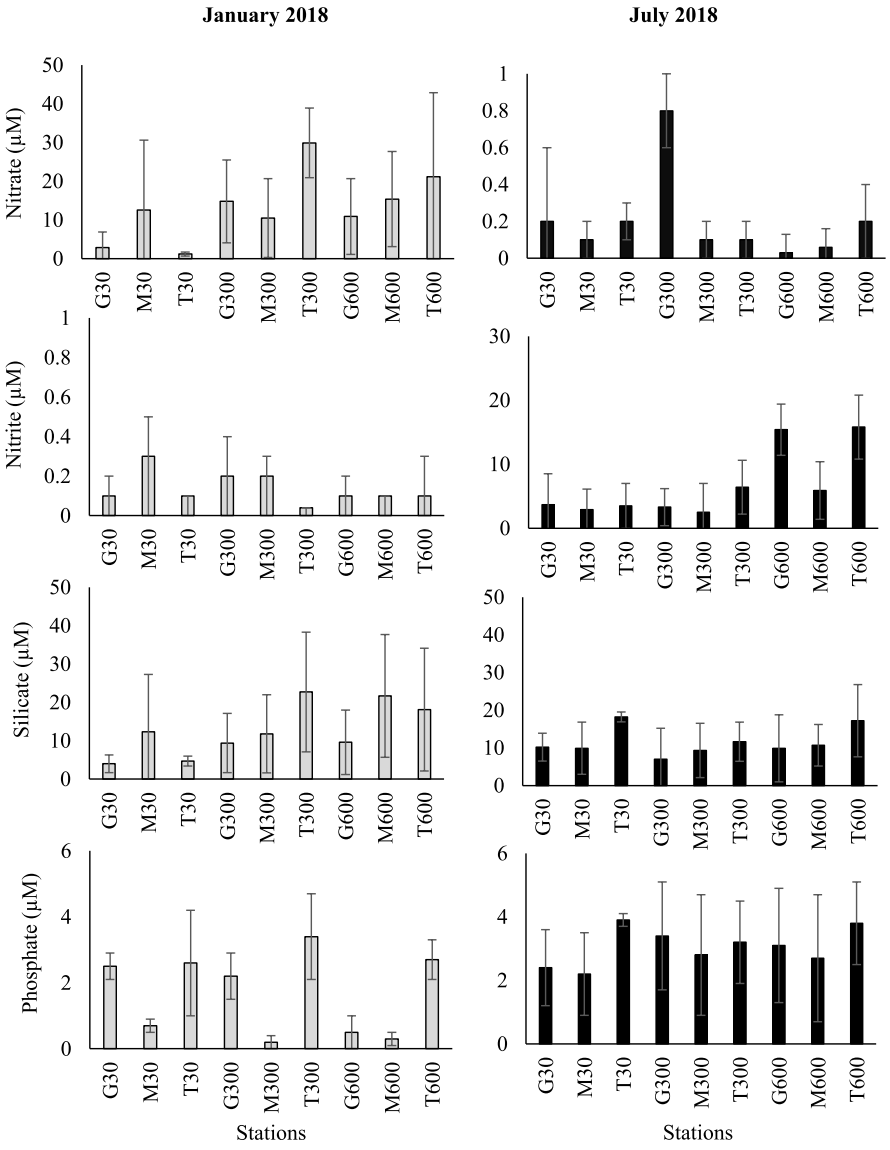

Fig. 3. Station-wise variables of the chemical parameters nitrate (NO_3^-), nitrite (NO_2^-), silicate (SiO_3^{2-}) and phosphate (PO_4^{3-}) measured during January 2018 and July 2018 in the shelf (G30, M30, T30), slope (G300, M300, T300) and off-shore stations (G600, M600, T600).

level from both seasons was carried out (Fig. 6). The total of 222 morphotypes from both the sampling seasons was grouped into 39 genera, with 22 genera represented from the non-monsoon season and 27 from the monsoon season and only 10 genera were common for both the seasons. Analysis shows that in January 2018, during non-monsoon sampling, the bacterial morphotypes could be grouped into 18 genera of Proteobacteria representing alpha (4), beta (2) and gamma (8) subphylum. Firmicutes were represented by 5 genera and the phylum Actinobacteria and Bacteroidetes were represented by only one genus each. *Pseudomonas* and *Vibrio* belonging to subphylum, Gammaproteobacteria; *Bacillus* and *Staphylococcus* of phylum Firmicutes were the dominant genera during the non-monsoon season (Fig. 6). During the monsoon sampling in July 2018, Firmicutes was the dominant phylum represented by 8 genera, followed by Proteobacteria with 9 genera belonging only to Alpha and Gammaproteobacteria subphylum and Actinobacteria with 10 genera (Fig. 6). Though the total number of genera increased from 22 to 27 in the monsoon season *Bacillus,* of phylum Firmicutes was the only major genus contributing to about 40% in the monsoon season. Contribution of genera such as *Pseudomonas* of subphylum, Gammaproteobacteria and *Staphylococcus* of Firmicutes reduced considerably and representation of *Vibrio* was completely absent during the monsoon season. However, 17 genera from all the three major phyla Proteobacteria, Firmicutes and Actinobacteria were represented only from the monsoon season. This includes *Hoeflea* and *Paracoccus* of Alphaproteobacteria, *Idiomarina* and *Marinobacter* of Gammaproteobacteria, *Lysinibacillus, Ornithibacillus* and *Marinococcus* of Firmicutes. Actinobacteria was represented by 10 genera which included *Microbacterium, Actinobacteria, Corynebacterium, Demequina* and *Gordonia* in the monsoon sampling from almost all the stations. Bacteroidetes was represented by the genus *Flavobacterium* and was only observed in the Goa shelf station during the non-monsoon season and were completely absent during the monsoon season (Fig. 6).

Distribution of the morphotypes at genus level in the epipelagic zone (EZ) representing the upper 200 m water column and mesopelagic zone (MZ) extending from below the 200 m water column was also studied. Molecular analysis of the 16S rRNA sequences showed that a total number of 22 genera were represented in the water column during January 2018 sampling during the non-monsoon season. Among that 16, were from the upper 200 m column representing the epipelagic zone (EZ) and 12 genera were from below 200 m water column i.e., the

Table 1
Station-wise profiles of primary productivity (PP), bacterial productivity (BP), total respiration (TR), bacterial respiration (BR) and bacterial growth efficiency (BGE) during the non-monsoon and monsoon season.

Station Number	PP (mg C m^{-3} d^{-1})	BP (mg C m^{-3} d^{-1})	TR (mg C m^{-3} d^{-1})	BR (mg C m^{-3} d^{-1})	BGE (%)
January 2018 (non-monsoon)					
G30	36.8 ± 61	5.1 ± 1.4	60.5 ± 88	27.2 ± 40	46 ± 48
G300	ND	1.2 ± 1.3	9.4 ± 15	4.2 ± 7	68 ± 45
G600	20.7 ± 36	1.8 ± 2.0	298.2 ± 421	134.2 ± 190	41 ± 51
M30	111.1 ± 165	2.5 ± 2.1	2.6 ± 4	1.2 ± 2	78 ± 39
M300	ND	3.1 ± 4.9	29.5 ± 23	13.3 ± 10	13 ± 13
M600	37.0 ± 48	6.4 ± 5.2	18.2 ± 26	8.2 ± 12	60 ± 44
T30	60.3 ± 41	1.2 ± 0.9	153.5 ± 263	69.1 ± 118	52 ± 50
T300	ND	4 ± 3.5	57.8 ± 76	26 ± 34	35 ± 24
T600	24.0 ± 27	1.2 ± 1.8	27.1 ± 25	12.2 ± 11	25 ± 38
July 2018 (monsoon)					
G30	76.6 ± 95	10.9 ± 0.8	55.4 ± 80	24.9 ± 36	56 ± 41
G300	ND	7.9 ± 1.6	241.7 ± 333	108.8 ± 150	29 ± 25
G600	22.9 ± 35	8.5 ± 1.1	166.7 ± 243	75 ± 109	308 ± 662
M30	93.2 ± 91	9.1 ± 0.4	1.5 ± 3	0.7 ± 1	28 ± 48
M300	ND	8 ± 0.6	168.2 ± 133	75.7 ± 60	13 ± 7
M600	58.2 ± 65	8.9 ± 1.8	91.9 ± 124	44.4 ± 57	23 ± 22
T30	89.1 ± 76	10.5 ± 2.3	130.8 ± 6	58.9 ± 3	15 ± 2
T300	ND	7.2 ± 2.1	75.3 ± 55	33.9 ± 25	26 ± 18
T600	38.3 ± 40	8.4 ± 2.3	193.6 ± 240	90.7 ± 107	14 ± 8

ND- No data.

mesopelagic zone (MZ) and 6 genera were shared in both the zones (Fig. 7). During the monsoon season, in July 2018, 27 genera were observed, among that 22 were from EZ and 18 were from MZ. The number of shared genera during the monsoon season was 13 which were present in both zones. Morphotypes belonging to *Pseudomonas,* of phylum Proteobacteria and genus *Bacillus* belonging to Firmicutes were represented with the highest percentage during both the seasons in the EZ and MZ. *Alteromonas,* of phylum Proteobacteria and genus *Halobacillus* belonging to Firmicutes were represented only in the EZ from both seasons. Bacteria from the genus *Escherichia, Photobacterium* and *Acidovorax* belonging to Proteobacteria, *Oceanobacillus* of Firmicutes and *Flavobacterium* (Bacteroidetes) were observed only in the EZ during the non-monsoon period. Similarly, bacteria from the genus *Idiomarina, Paracoccus* (Proteobacteria), *Lysinibacillus* of Firmicutes and two Actinobacteria genera *Actinobacteria* and *Aeromicrobium* were observed only in the EZ during the monsoon season. Bacterial genera *Stenotrophomonas, Spingomonas* and *Comamonas* belonging to Proteobacteria were represented only in the MZ during the non-monsoon season, while *Pseudoclavibacter, Brachybacterium* and *Marinobacter* were observed only in MZ during monsoon season. The overall diversity in the EZ was higher in both seasons with 16 and 22 genera and the MZ was represented by 12 and 18 genera (Fig. 7).

The 16S rRNA sequences of 83 and 139 morphotypes from January and July 2018 sampling were analysed together using DOTUR and clustered into 111 OTUs which are sequentially represented from BWCI1 to BWCI111. Phylogenetic analysis of the OTUs shows that 49 OTUs belonged to Proteobacteria which was clustered within Alpha, Beta and Gamma Proteobacteria groups. Firmicutes were represented by 41 OTUs, Actinobacteria with 20 OTUs and Bacteroidetes by only a single OTU (Fig. 8 a-d). Bacteroidetes were represented by one OTU (BWCI91) only from the non-monsoon season and clustered with strong bootstrap support with *Flavobacterium* sp. (Fig. 8a). Actinobacteria was represented by 1% only during the January 2018 sampling and it increased (20%) during the monsoon sampling. Within, Actinobacteria, OTU, BWCI81 *Micrococcus luteus* was the only representative in the non-monsoon season and during the monsoon season, OTUs BWCI83 and BWCI22 clustered with *Micrococcus* sp. sequences. Other Actinobacterial OTUs belonged to major genera such as *Aeromicrobium* sp., *Corynebacterium* sp., *Brachybacterium* sp., *Pseudoclavibacter* sp. and *Actinobacteria bacterium*. *Brevibacterium* spp. was represented by 2 OTUs, *Gordonia* spp. and *Demequina* spp., with 3 OTUs and *Microbacterium* spp. with 4 OTUs with a total representation of 19 OTUs belonging to the monsoon season and there was no shared OTUs within this cluster (Fig. 8a).

The 49 OTUs of phyla Proteobacteria grouped within the alpha, beta and gamma sub-phylum clusters (Fig. 8b and c). No representatives were observed belonging to the delta and epsilon Proteobacteria cluster from both the samplings. The OTUs belonging to Proteobacteria clustered within Alphaproteobacteria with 13 OTUs (Fig. 8b), the Betaproteobacteria cluster had only 2 OTUs and Gamma cluster had the maximum representation with 34 OTUs belonging to well classified bacterial genera (Fig. 8c). Alphaproteobacteria and Gammaproteobacteria had representative OTUs from both seasons which included major representatives grouping with *Erythrobacter* spp., from the former and *Alteromonas* spp., *Alcanivorax* spp. and *Pseudomonas* spp. from the latter. Betaproteobacteria OTUs clustering with *Acidovorax* sp. and *Comamonas* sp. were represented only from the non-monsoon season and none of the OTUs were common to both the sampling seasons (Fig. 8b and c). OTUs belonging to Firmicutes are represented uniformly in both the seasons and of the 41 OTUs clustered within this group, 6 OTUs were shared between both seasons. Majority of the OTUs clustered with the *Bacillus* spp. followed by *Staphylococcus* spp. within the Firmicutes cluster (Fig. 8d).

3.3. Hydrolytic enzyme activity of culturable bacterial morphotypes

Characterization of the hydrolytic enzyme activity was determined in all the representative 83 and 139 morphotypes. The results showed that the bacterial morphotypes were able to utilize all three major groups of substrates as they exhibited carbohydrase, protease and lipase enzyme activity. It was observed that monosaccharide glucose was the most utilized substrate in both seasons (Fig. 9).

The bacterial morphotypes from January 2018 sampling showed higher hydrolytic enzyme activity with only an average of 50% which showed no activity and during the monsoon season from July 2018 sampling an average of 78% of the bacterial morphotypes did not show activity in any of the substrates tested (Fig. 9). According to the zone of hydrolysis, the percentage of morphotypes from the January 2018 sampling showed activity in alginate > agar > starch > CMC > Tween 80 > SM > xylan (Fig. 9a). The activity of the morphotypes was relatively lower in the morphotypes from the monsoon season (July 2018) sampling and the hydrolytic enzyme activity was characteristically distinct, especially the protease activity which was relatively the highest. The morphotypes showed activity in SM > alginate > agar > starch > CMC > xylan > Tween 80 (Fig. 9b). A small percentage of morphotypes were largely *Bacillus* spp. or *Pseudomonas* spp. exhibited very good activity in the non-monsoon and monsoon seasons (Fig. 9). This shows that the isolates from the non-monsoon season exhibited better hydrolytic enzyme activity.

The hydrolytic enzyme activity of the morphotypes belonging to each phylum was studied and the results show that bacterial isolates from Phylum Firmicutes followed by Proteobacteria from both the sampling season exhibited good hydrolytic activity and were able to

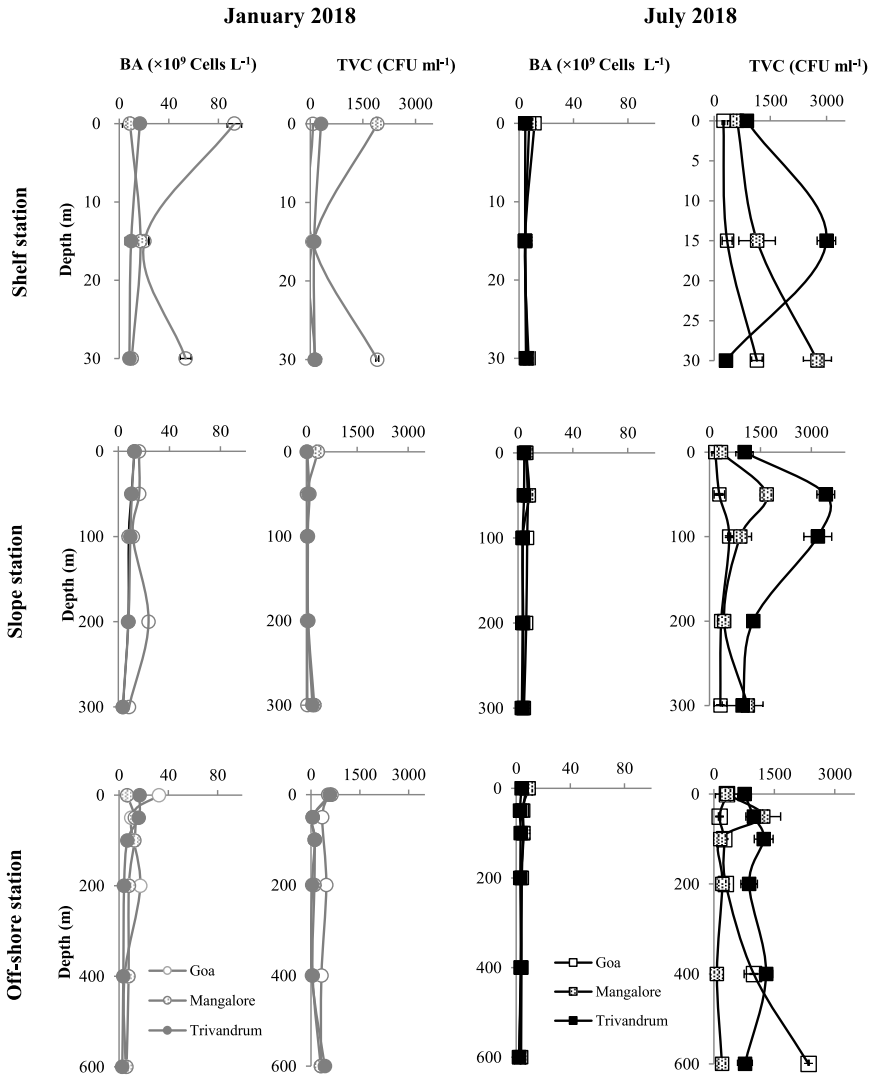

Fig. 4. Profiles of bacterial abundance (BA) and total viable counts (TVC) in the shelf, slope and off-shore stations in January 2018 and July 2018 sampling.

Table 2
Pearson's correlation matrix of physical, chemical and biological variables, Temperature (T), dissolved oxygen (DO), nitrate (NO_3^-), nitrite (NO_2^-), Fluorescence (F), primary production (PP), bacterial production (BP), Total respiration (TR), bacterial respiration (BR), Bacterial growth efficiency (BGE) and total viable counts (TVC).

	T	DO	NO_3^-	NO_2^-	F	PP	BP	TR	BR	BGE	TVC
T	1										
DO	0.87[a]	1									
NO_3^-	−0.20[b]	−0.09	1								
NO_2^-	−0.12	−0.11	−0.20[b]	1							
F	0.29[b]	0.15	−0.17	0.04	1						
PP	0.39[b]	0.24[b]	−0.07	0.09	0.46[a]	1					
BP	−0.15	−0.32[b]	−0.37[b]	0.29[b]	0.24[b]	0.23[b]	1				
TR	0.31[b]	0.27[b]	−0.02[b]	0.14	0.18	0.12	0.01	1			
BR	0.30[b]	0.27[b]	−0.22[b]	0.14	0.18	0.12	0.02	0.99[a]	1		
BGE	−0.18	−0.11	−0.02	0.00	−0.07	−0.16	0.11	−0.15	−0.15	1	
TVC	−0.12	−0.31[b]	−.32[b]	0.14	0.17	0.22[b]	0.42[a]	0.05	0.05	0.21[b]	1

[a] Correlation is significant at the 0.01 level (2-tailed).
[b] correlation is significant at the 0.05 level (2-tailed).

utilize all the 11 substrates tested. Actinobacteria was represented by 1% only at phylum and genus level during the non-monsoon period, which increased up to 20% during the monsoon period (Fig. 6). However, its activity increased marginally from 1 to 8% during the monsoon season (Fig. 10). Bacteroidetes which were represented only by 1% in the non-monsoon season showed minimum activity in major carbohydrate substrates along with protease and lipase activity. Results show that Firmicutes exhibited good hydrolytic enzyme activity in both seasons. Distribution and activity of morphotypes from phylum Proteobacteria and Bacteroidetes reduced or were absent and Actinobacteria increased during the monsoon season (Figs. 6 and 10).

Table 3
ANOVA: single factor to show the significance of distribution between different parameters, Temperature (T), dissolved oxygen (DO), nitrate (NO_3^-), nitrite (NO_2^-), Fluorescence (F), primary production (PP), bacterial production (BP), Total respiration (TR), bacterial respiration (BR), Bacterial growth efficiency (BGE) and total viable counts (TVC) during two seasons.

Variables	Df	F value	F critical	p-value
Temperature	1	2.22	3.96	0.14
DO	1	19.08	3.96	<0.001[a]
Salinity	1	0.36	3.96	0.55
Fluorescence	1	6.19	3.96	0.01[b]
NO_3-	1	34.74	3.96	<0.001[a]
NO_2-	1	12.27	3.96	<0.001[a]
PO_4^{3-}	1	22.47	3.96	<0.001[a]
SiO_3^{2-}	1	0.84	3.96	0.36
BA	1	12.88	3.96	<0.001[a]
TVC	1	34.92	3.96	<0.001[a]
PP	1	0.49	4.08	0.49
BP	1	91.24	3.96	<0.001[a]
TR	1	2.14	3.96	0.15
BR	1	2.30	3.96	0.13
BGE	1	0.28	3.96	0.60

Df: degree of freedom, F value greater than F critical value indicates statistical significance.
[a] significant at 0.1%.
[b] significant at 1%.

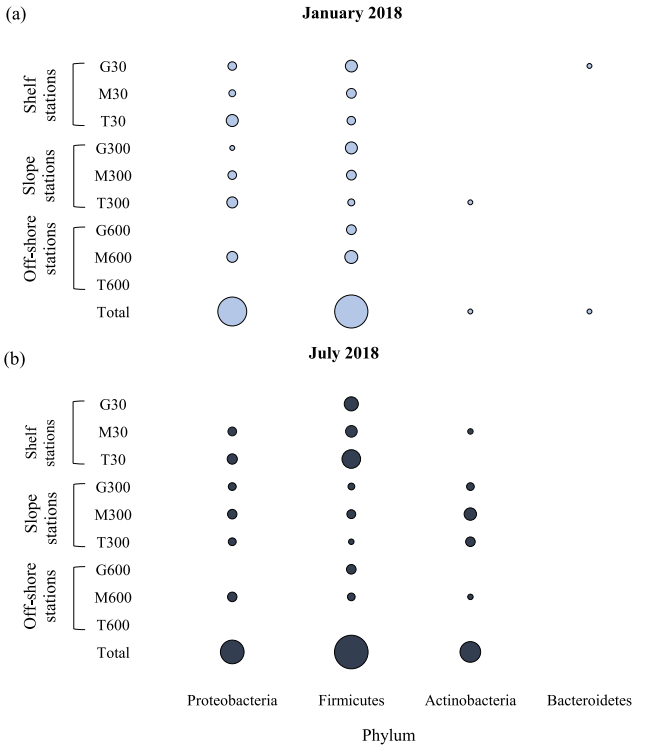

Fig. 5. Phylum-wise distribution of the bacterial morphotypes (%) along the shelf, slope and off-shore stations during (a) January 2018 and (b) July 2018 sampling season.

4. Discussion

4.1. Relationship between physicochemical characteristics, primary productivity and bacterial dynamics

The northern Indian Ocean is shown to experience seasonally changing monsoonal circulation which leads to seasonal differences in PP. The north western region of the Indian Ocean, the Arabian Sea, especially the WCI has higher biological productivity than the eastern, Bay of Bengal or the central, Equatorial Indian Ocean regions. Studies have affirmed that the semi-annual reversal of monsoonal winds annually from June to September induces open ocean upwelling and divergence in the Arabian Sea. These seasonal features regulate near-surface currents, influence mixed layer development and affect the nutrient supply to this region (Shetye et al., 1991; Sarma et al., 2003; Kumar et al., 2007). In addition, winter cooling during the northeast monsoon (December–February) also adds up to the variability of physical features (Kumar et al., 2001). The predominant characteristics which include strong winds, cooler waters, heavy rainfall and a significant increase in phytoplankton production during the monsoon and warm waters, weak winds, low rainfall and low phytoplankton production from the non-monsoon season were reported from the coastal, southwest region in 2018 (Santhikrishnan et al., 2021; Kottayil et al., 2022). In this study, the consequence of the seasonal differences which influence primary productivity and their impact on bacterial productivity, abundance, diversity and community structure was investigated.

The water column characteristics assessed during the monsoon and non-monsoon seasons revealed distinct variations during both seasons. The temperature and salinity values characteristically influenced the MLD in the continental slope and off-shore station, which was observed to be at ~100 m depth in the non-monsoon season and it was shallower at 25–50 m depth in the monsoon season. A similar trend was seen in the oxycline depths in both seasons (Fig. 2), shallow MLD which is an upwelling phenomenon was observed during the monsoon season (July 2018) as previously recorded (Smitha et al., 2008). A significant increase in fluorescence value was recorded during the monsoon season (Fig. 2). Earlier studies from the WCI have shown that the average PP increases during the monsoon season (June–September) due to upwelling (Ramaiah et al., 2005). The concentration of essential nutrient, nitrate reduced significantly during the monsoon season (Fig. 3), which could be due to the active PP which could have depleted the nitrates. An increase in nitrite concentration in the July 2018 sampling season (Fig. 3) is possibly due to the nitrite build-up from denitrification activity in the coastal stations reported occurring from seasonal hypoxia along the WCI (Naqvi et al., 2010; Gonsalves et al., 2011; Lincy and Manohar 2021). TR representing the community respiration, PP measuring the autotrophic activity and heterotrophic activity based on BP, BR and BGE all show a significant increase in the monsoon season during the July 2018 sampling in comparison with January 2018, non-monsoon sampling (Table 1). BA estimated during this study was in the order of 10^9 cells L^{-1} during both seasons. However, the bacterial CFUs during the monsoon increased in comparison to the non-monsoon season (Fig. 4). Increased bacterial abundance during the monsoon season highlights the bacterial dependence on phytoplankton-derived organic matter (Molina et al., 2020). These results are consistent with those of the previous studies from the Arabian Sea suggesting that increases in primary productivity during the monsoon influence bacterial numbers (Ramaiah et al., 2005; Fernandes and Bogati 2022). However, there are no reports on the seasonal variations of the bacterial community and its activity which is characterised in this study to understand their ecological role during the monsoon season with increasing productivity.

4.2. Seasonal variations in the bacterial community structure

Bacterial diversity from water samples assessed by 16S rRNA sequencing revealed that the morphotypes from the study area belonged to four major phyla, Proteobacteria, Firmicutes, Actinobacteria and Bacteroidetes. During the non-monsoon season, the morphotypes retrieved were majorly grouped within Proteobacteria and Firmicutes phyla which were represented from shelf, slope and off-shore locations (Fig. 7). The contribution of Actinobacteria and Bacteroidetes was very minimal (1% each) in this season during January 2018 sampling. A change in the bacterial community at the phylum level was observed

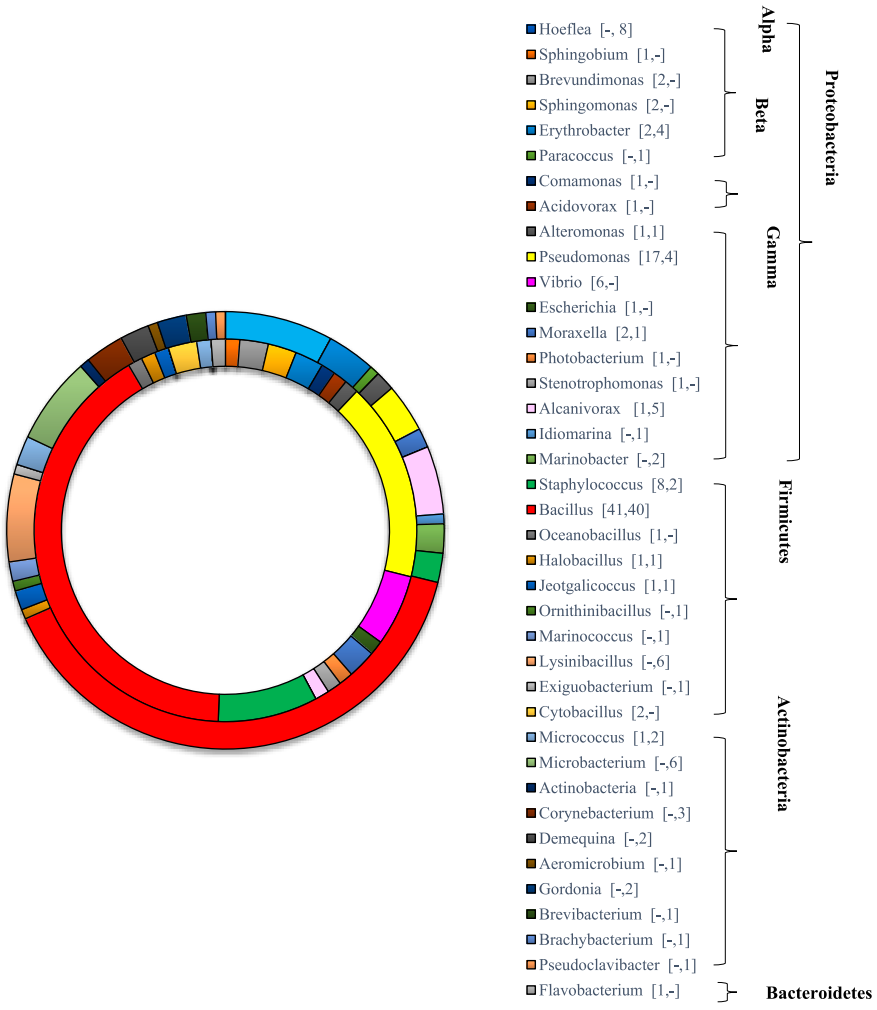

Fig. 6. Distribution of bacterial genera (% frequency) in the study area during January (inner ring) and July 2018 (outer ring), values from both the sampling are provided in the parenthesis of the legend.

during the monsoon season, with no representation of Bacteroidetes and nearly half of the morphotypes retrieved from this season belonged to the Firmicutes (53%) followed by Proteobacteria and Actinobacteria, which was represented significantly only from the monsoon season (Fig. 7). Analysis of the bacterial community structure at the genus level shows that the morphotypes grouped into 22 genera within the four phyla during the non-monsoon season. During the monsoon season, the diversity of the morphotypes at the genus level increased which was represented by 27 genera and only 10 genera were common to both the seasons which included representatives from Proteobacteria, Firmicutes and Actinobacteria (Fig. 8), which are identified as the predominant culturable bacterial groups from marine habitats (Amberkar et al., 2021).

Proteobacteria contributed about 42% of the bacterial diversity during the non-monsoon period and is reduced to 27% during the monsoon season. During the non-monsoon season, the Proteobacteria had representative morphotypes belonging to Alpha, Beta and Gammaproteobacteria, however, in the monsoon season, Betaproteobacteria was completely absent. Gammaproteobacteria was the dominant class found within Proteobacteria phylum during both seasons (Fig. 8). Proteobacteria is the major phylum represented in many marine habitats such as the coastal regions (Khandeparker et al., 2017), upwelling zones (Li et al., 2014; Vijayan et al., 2021) and OMZ (Bandekar et al., 2018a; Mulla et al., 2018). Bacterial genera such as *Sphinogbium*, *Brevundimonas*, *Spingomonas* of Alphaproteobacater, representatives of the subphylum Betaproteobacteria such as *Comamaonas* and *Acidovorax*, *Vibrio*, *Escherichia*, *Photobacterium* of Gammaproteobacteria was represented only from the non-monsoon season and *Erythrobacter, Alteromonas, Pseudomonas* and *Strenotrophpomonas* were represented in both the seasons. Though a few genera such as *Hoeflea*, *Paracoccus*, *Idiomarina* and *Marinobacter* were represented only in the monsoon season, there was an overall reduction in the percentage contribution of Proteobacteria during the monsoon season due to the reduction in the contribution of *Pseudomonas*, a major bacterial genus belonging to Gammaproteobacteria from 17 to 4% in the monsoon season (Figs. 8 and 9). Bacterial diversity at the OTU level showed that Proteobacteria had maximum representation in this study with 49 OTUs from alpha, beta and gamma subphyla. Seven of the OTUs BWCI80, BWCI62 and BWCI3 within alpha and BWCI72, BWCI92 and BWCI68 and BWCI13 of Gammaproteobacteria clustered as unclassified bacterial groups with good bootstrap support (Fig. 8b and c). There were no shared OTUs within the Proteobacteria cluster and their numbers reduced from 29 to 20 OTUs in the monsoon season. OTUs clustering with *Pseudomonas* spp., *Vibrio* spp., *Alteromonas* spp. and *Alcanivorax* spp. were the major Gammaproteobacter identified. Recent studies have shown that Gammaproteobacteria of phylum Proteobacteria are the dominant groups (80% of the total bacterial communities) during algal blooms. Within Gammaproteobacteria *vibrio* spp. and *Psudoalteromonas* spp. were the major genera contributing to the bacterial communities associated with algal bloom (Chen et al., 2021). Gammaproteobacteria *Pseudomonas* and *Vibrio*

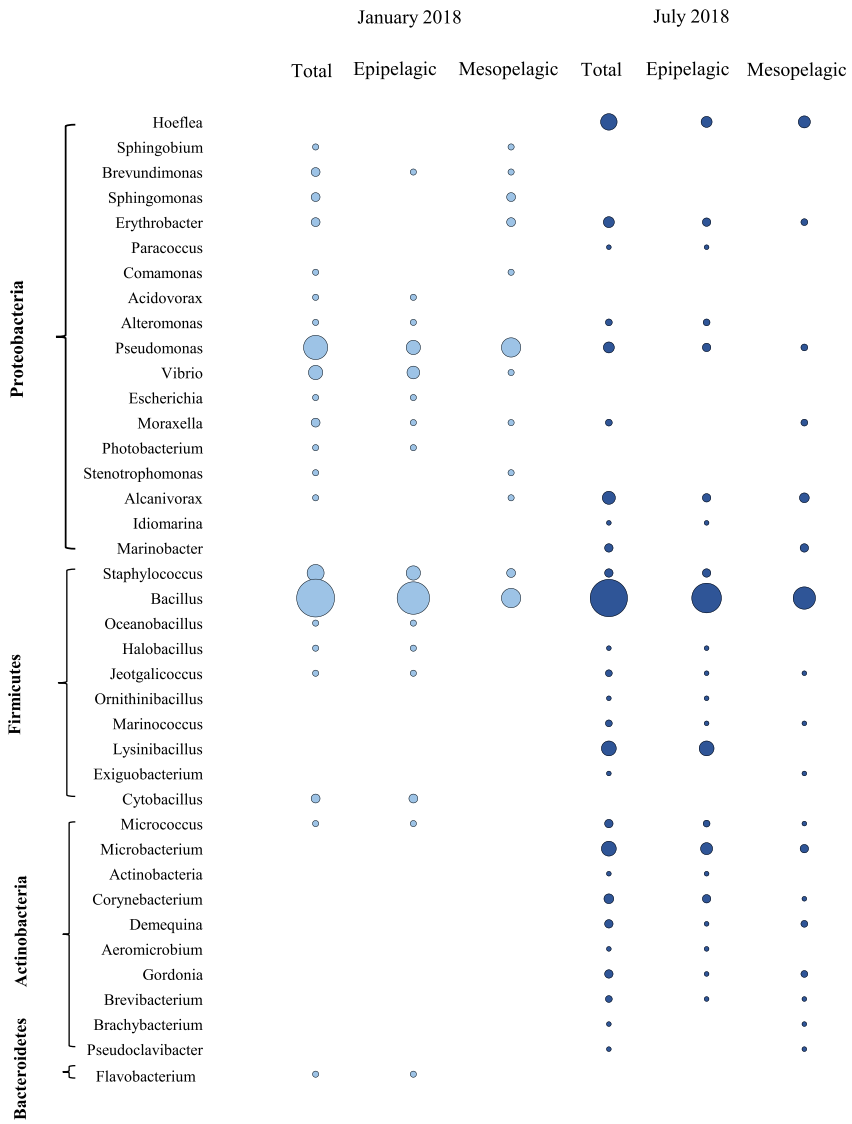

Fig. 7. Distribution of bacterial genera (%) in total during the January 2018 and July 2018 sampling season and their distribution in the epipelagic and mesopelagic zone.

representation reduced from the Proteobacterial community during the monsoon season (Figs. 7 and 6).

Firmicutes contributed almost equally in both seasons, with a representation of four genera *Staphylococcus* spp., *Bacillus* spp., *Halobacillus* spp. and *Jeotgalicoccus* sp. from both seasons and a few genera which was exclusively isolated from the non-monsoon and monsoon season (Fig. 6). *Bacillus* was the major genera that were represented during both seasons and were present in considerable numbers in both the EZ and MZ (Fig. 7). Diversity of bacterial morphotypes at the genus level shows that *Bacillus* and other *Bacillus* groups belonging to Firmicutes exhibited equal contribution in both seasons. One of the OTU BWCI43 clustered as an unclassified bacteria within the *Bacillus* cluster (Fig. 8d). Major bacterial genera such as *Staphylococcus* which had a major contribution during the non-monsoon season reduced considerably during the monsoon season, which is one of the major pathogenic groups of bacteria (Isaacs et al., 2021). They could not proliferate during the monsoon season due to the presence of active phytoplankton communities which are known to produce anti-bacterial compounds to prevent infection (Shannon and Abu-Ghannam 2016; Mühlenbruch et al., 2018). This prevents the growth of pathogenic bacteria and in turn pave way for the proliferation of native bacterial community such as *Marinococcus* spp. which are represented in higher numbers during the productive monsoon season (Figs. 6 and 7). Studies have reported that *Bacillus* spp. and *Staphylococcus* spp. are the major culturable bacteria associated with the phytoplankton bloom (Basu et al., 2013). Their dominance was also reported during the phytoplankton bloom decay period (Zhao et al., 2017; Gómez-Consarnau et al., 2019). Many of the Firmicutes bacteria are also known to be euryhaline, which can degrade OM efficiently in marine habitats (Margesin and Schinner 2001).

Genus *Micrococcus* was the only representative of phylum Actinobacteria in the non-monsoon season. It increased considerably in the monsoon season and was represented by genera such as *Microbacterium*, *Corynebacterium*, *Aeromicrobium* and *Gordonia* (Figs. 6 and 7). The presence of Actinobacteria in the coastal waters has been previously characterised for its bioactivity (Singh and Ramaiah 2011). They are also reported in the OMZ, mesopelagic and deep-sea regions (Wright

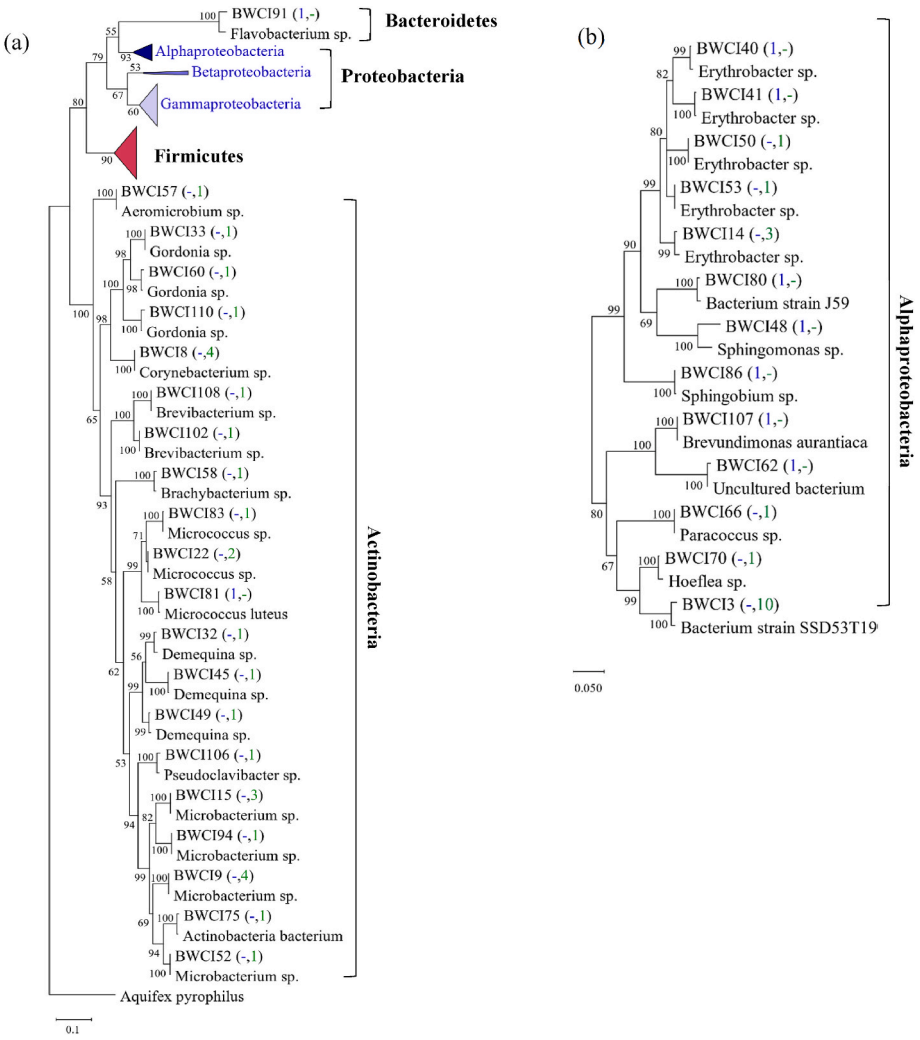

Fig. 8. Maximum-likelihood-based phylogenetic tree of 16 rRNA gene sequences of representative OTUs belonging to (a) Actinobacteria and Bacteroidetes, (b) Alphaproteobacteria, (c) Gammaproteobacteria and Betaproteobacteria and (d) Firmicutes. The number in parentheses following OTU names indicates the number of sequences obtained from the January 2018 and July 2018 sampling season.

et al., 2012; Rodriguez-Mora et al., 2013; Kamjam et al., 2017) from culturable and metagenomics studies (Ghai et al., 2013). Marine Actinobacteria are also found in association with marine benthic communities such as the seaweeds, sponges and coral reefs as mutualists and produce different bioactive compounds (Valliappan et al., 2014; Girão et al., 2019). The seasonal appearance of Actinobacteria in many marine habitats is often attributed to the presence of phytoplankton blooms (Basu et al., 2013; Parulekar et al., 2017; Zhou et al., 2018). Recent research also suggests that changes in the phytoplankton communities have a significant impact on the dynamics of heterotrophic bacterial diversity in the coastal and oceanic waters. Which is reported to be influenced by the autochthonous organic matter generated by the changing phytoplankton composition (Landa et al., 2016; Aytan et al., 2018; Arandia-Gorostidi et al., 2022).

Marine Bacteroidetes are known to express the polysaccharide utilization loci which facilitate the degradation of algal polysaccharides (Krüger et al., 2019; McKee et al., 2021). Genus *Flavobacterium* sp. was the only Bacteroidetes isolated from the non-monsoon season and this group was not represented in the monsoon season (Figs. 6 and 7). They are reported to be one of the major contributors to the remineralisation of carbohydrates leading to refractory carbon storage in the ocean (Zhang et al., 2015). Bacterial diversity studied from phylum, genus and OTU levels from the WCI shows that there are seasonal differences with higher diversity in the monsoon season at genus and OTU levels (Figs. 6–8). Firmicutes, Alpha and Gammaproteobacteria were the major phylum represented in both seasons. The diversity of Betaproteobacteria and Bacteroidetes was completely absent and Actinobacterial representation increased considerably in the monsoon season. Many marine bacterial genera such as *Idiomarina*, *Marinococcus* and *Marinobacter* were exclusively obtained only from the monsoon season which is majorly known for the degradation of OM (Lucas et al., 2016; Gomes et al., 2019; Mukherjee et al., 2020). Studies have suggested that the bacterial metabolic processes and products of remineralisation can also influence the phytoplankton community structure (Bolch et al., 2017). More detailed studies on this could help us to understand the complex bacteria phytoplankton interactions.

Studies on the bacterial diversity and community structure in the oxygen minimum zone (OMZ), along the WCI have described the denitrifying and anammox bacterial communities along with their functional characteristics (Jain et al., 2014; Bandekar et al., 2018b; Mulla et al., 2018; Lincy and Manohar 2021; Amberkar et al., 2021). Hydrolytic enzyme activities of bacteria and their enzymatic potential are studied in various coastal regions (Khandeparker et al., 2011; Singh and Ramaiah 2011). However, comparative studies on the hydrolytic enzyme activities of bacteria from the coastal/shelf, slope and open ocean water column are not addressed to understand the seasonal difference in the

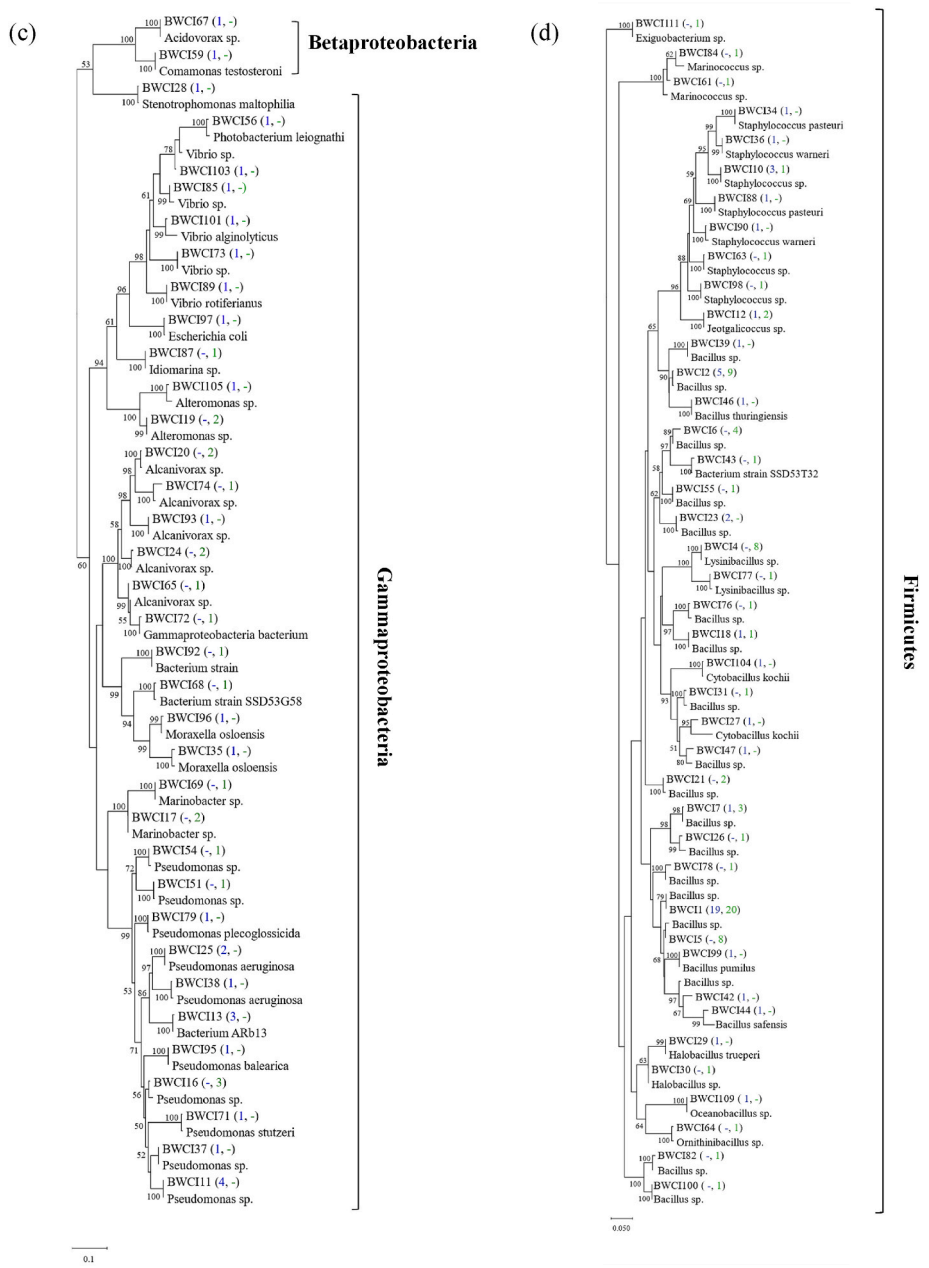

Fig. 8. (*continued*).

BA, community structure and hydrolytic enzyme activities. Such studies can help in establishing the importance of phylogenetic diversity to its ecological functioning.

4.3. Seasonal variations in the bacterial hydrolytic enzyme activity

Bacterial communities play an important role by synthesising extracellular enzymes for remineralisation of oceanic OM which is composed of carbohydrate polysaccharides, monosaccharides, lipids and protein polymers (Traving et al., 2015; Bolch et al., 2017). Different types of carbohydrates, lipid and protein substrates were tested in this study to characterize the hydrolytic enzyme activity of bacteria during the non-monsoon season and productive monsoon season (Fig. 9). Carbohydrates make up a 90% share of phytoplankton-derived OM including various polysaccharides and monosaccharides (Underwood et al., 2010), neutral monosaccharides like glucose, arabinose, rhamnose and galactose are the major contributors to the phytoplankton derived OM (Passow et al., 2007; Engel and Händel 2011). Results from this study show that 50% of bacterial morphotypes exhibit enzyme activity during the non-monsoon season in January 2018 and from July 2018 sampling an average of only 20% of morphotypes showed activity. This result suggests the metabolic potential of microbes was higher during the non-monsoon season, which could be due to the availability of DOM which is sustained for an extended period in the water column and could be available for bacterial utilization (Smriga et al., 2016). The low hydrolytic activity during the monsoon season suggests that culturable bacteria isolated during the monsoon season did not express enzymes to utilize fresh and complex OM generated from phytoplankton blooms. Bacterial morphotypes were able to utilize a majority of substrates tested during both seasons. Polysaccharide alginate lyase and protease were the highest detected activity during January and July 2018 respectively. The survival of microbes aided by extracellular hydrolytic enzymes allowed them to breakdown, complex organic molecules in marine habitats. Studies have also found that the enzyme

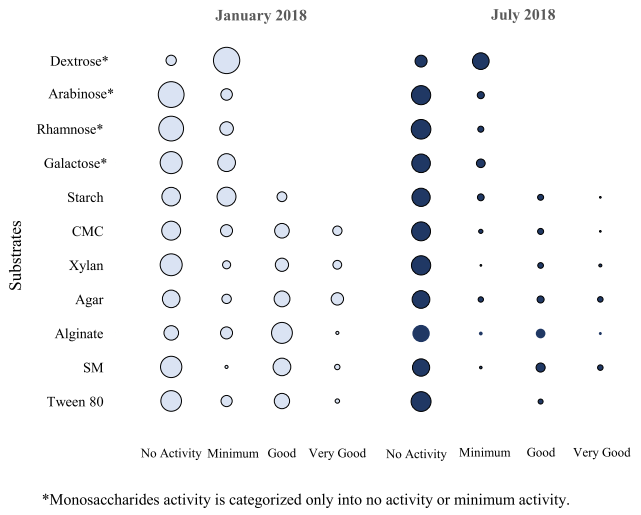

Fig. 9. Hydrolytic enzyme activity of bacterial morphotypes (%) isolated during the January 2018 and July 2018 sampling season.

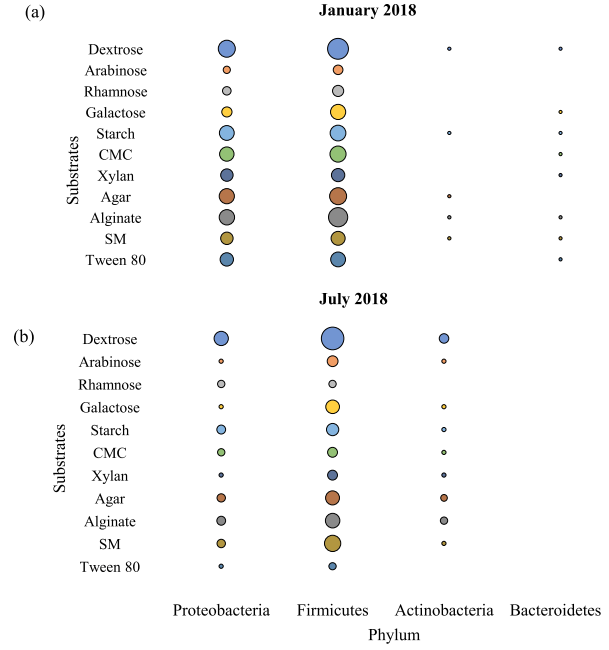

Fig. 10. Hydrolytic enzyme activity of bacterial morphotypes (%) of different phyla isolated during the January 2018 (a) and July 2018 (b) sampling season.

activity of bacteria exhibited variation based on the availability and nature of OM, implying an unequal distribution of enzyme activity in different seasons and zones (Basu et al., 2013; Khodse and Bhosle 2013; Mahmoudi et al., 2020).

Proteobacteria, such as *Stenotrophomonas* sp. and *Alcanivorax* spp., that degrade organic substances are shown to have made a significant contribution to bacterial communities; a similar finding was obtained in recent research in the Southern East China Sea, where the existence of these groups was recognized (Yang et al., 2020). Recent research also shows that the production of extracellular enzymes in bacteria increases in harsh environments to recycle available organic materials (Bolch et al., 2017; Showalter and Deming 2021). Bacterial numbers and extracellular enzyme activity were found to be sustained even at low oxygen concentrations in recent investigations in the eastern tropical South Pacific OMZ, implying that bacteria can recycle OM in the OMZ (Maβmig et al., 2020). Our results accordance with, previous studies which have also found that Gammaproteobacteria synthesize enzymes for the hydrolysis of diverse types of polysaccharides enzymes (Alderkamp et al., 2007; Teeling et al., 2016). The shift in the bacterial community suggests that the availability of OM is the major factor that controls the expression of extracellular enzymes in bacterial groups. It is also reported that among Firmicutes, *Bacillus* spp. plays an important role in OM degradation in marine sediments (Divya et al., 2010). Actinobacteria can produce extracellular enzymes like cellulase, chitinase, as well as Actinobacteria, which are also known for degrading recalcitrant OM (Manucharova 2009). Recent studies have documented that Actinobacteria from marine sediment possess enzymes to degrade refractory OM as well as different polysaccharide compounds (Chen et al., 2016). Significant seasonal fluctuations in bacterial communities reported in the southern Benguela upwelling region imply that bacterial communities alter depending on the nature of the OM present in the water column (Rocke et al., 2020). Recent metagenomic studies have revealed that microbial communities and metabolism potentials differ between the epipelagic and mesopelagic zones of the water column, with microbial communities in the mesopelagic zone having a higher metabolic potential to degrade amino acids, lipids, nucleotides and xenobiotics (Sripan et al., 2021). Though metagenomics approaches have greatly enhanced our understanding of bacterial diversity, there is still a large diversity that is still unexplored. It is also very vital to cultivate native, bacteria from marine habitats using media to mimic the native habitat and to understand the metabolic processes (Giovannoni and Stingl 2007).

5. Conclusion

The purpose of the present research was to estimate the changes in bacterial diversity and its activity along the WCI during the non-monsoon and monsoon seasons. This study has shown that primary productivity positively influences bacterial productivity, viable counts and diversity in the monsoon. The difference in the bacterial diversity characterised shows distinct phyla, genera and OTUs during both seasons with a very low percentage of overlap. Bacterial abundance and diversity were higher during the monsoon season. However, the substrate utilization was lower in the monsoon season and the morphotypes from the non-monsoon season showed higher hydrolytic enzyme activity. The decrease in bacterial activity in the monsoon could be due to the active primary production in this season. Though, we still lack an understanding of the factors that influence the extracellular enzyme activity in marine habitats. It is interesting to note that monsoon influences the abundance and culturable diversity of heterotrophic bacteria positively and decreases the bacterial activity along the WCI.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Alderkamp, A.C., Van Rijssel, M., Bolhuis, H., 2007. Characterization of marine bacteria and the activity of their enzyme systems involved in degradation of the algal storage glucan laminarin. FEMS Microbiol. Ecol. 59, 108–117. https://doi.org/10.1111/j.1574-6941.2006.00219.x.

Amberkar, U., Khandeparker, R., D Menezes, L., Meena, R.M., 2021. Phylogenetic diversity of culturable marine bacteria from sediments underlying the oxygen minimum zone of the Arabian Sea and their role in nitrate reduction. Mar. Ecol. 42, e12646 https://doi.org/10.1111/maec.12646.

Amin, S.A., Hmelo, L.R., Van Tol, H.M., Durham, B.P., Carlson, L.T., Heal, K.R., Morales, R.L., Berthiaume, C.T., Parker, M.S., Djunaedi, B., Ingalls, A.E., 2015. Interaction and signalling between a cosmopolitan phytoplankton and associated bacteria. Nature 522, 98–101.

Arandia-Gorostidi, N., Krabberød, A.K., Logares, R., Deutschmann, I.M., Scharek, R., Morán, X.A.G., González, F., Alonso-Sáez, L., 2022. Novel interactions between phytoplankton and bacteria shape microbial seasonal dynamics in coastal ocean waters. Front. Mar. Sci. 9, 901201 https://doi.org/10.3389/fmars.2022.901201.

Arnosti, C., 2011. Microbial extracellular enzymes and the marine carbon cycle. Ann. Rev. Mar. Sci 3, 401–425.

Aytan, U., Feyzioglu, A.M., Valente, A., Agirbas, E., Fileman, E.S., 2018. Microbial plankton communities in the coastal southeastern Black Sea: biomass, composition and trophic interactions. Oceanologia 60, 139–152. https://doi.org/10.1016/j.oceano.2017.09.002.

Bandekar, M., Ramaiah, N., Jain, A., Meena, R.M., 2018a. Seasonal and depth-wise variations in bacterial and archaeal groups in the Arabian Sea oxygen minimum zone. Deep Sea Res. Part II Top. Stud. Oceanogr. 156, 4–18. https://doi.org/10.1016/j.dsr2.2017.12.015.

Bandekar, M., Ramaiah, N., Meena, R.M., 2018b. Diversity and abundance of denitrifying and anammox bacteria from the Arabian Sea oxygen minimum zone. Deep Sea Res. Part II Top. Stud. Oceanogr. 156, 19–26. https://doi.org/10.1016/j.dsr2.2018.08.008.

Basu, S., Deobagkar, D.D., Matondkar, S.P., Furtado, I., 2013. Culturable bacterial flora associated with the dinoflagellate green Noctiluca miliaris during active and declining bloom phases in the Northern Arabian Sea. Microb. Ecol. 65, 934–954. https://doi.org/10.1007/s00248-012-0148-1.

Bolch, C.J., Bejoy, T.A., Green, D.H., 2017. Bacterial associates modify growth dynamics of the dinoflagellate Gymnodinium catenatum. Front. Microbiol. 8, 670. https://doi.org/10.3389/fmicb.2017.00670.

Carpenter, J.H., 1965. The Chesapeake Bay Institute technique for the Winkler dissolved oxygen method. Limnol. Oceanogr. 10, 141–143. https://doi.org/10.1016/j.gca.2010.06.037.

Chen, H., Jiang, J., Jiang, F., Li, S., Hu, Z., 2021. Temporal variability of free-living microbial culturability and community composition after an Akashiwo sanguinea bloom in Shenzhen, China. Ecotoxicology 30, 975–985. https://doi.org/10.1007/s10646-021-02407-4.

Chen, P., Zhang, L., Guo, X., Dai, X., Liu, L., Xi, L., Wang, J., Song, L., Wang, Y., Zhu, Y., 2016. Diversity, biogeography and biodegradation potential of actinobacteria in the deep-sea sediments along the southwest Indian ridge. Front. Microbiol. 7, 1340. https://doi.org/10.3389/fmicb.2016.01340.

Dalabehara, H.B., Sarma, V.V., 2021. Physical forcing controls spatial variability in primary production in the Indian Ocean. Deep Sea Res. Part II Top. Stud. Oceanogr. 183, 104906 https://doi.org/10.1016/j.dsr2.2020.104906.

Divya, B., Soumya, K., Nair, S., 2010. 16SrRNA and enzymatic diversity of culturable bacteria from the sediments of oxygen minimum zone in the Arabian Sea. Antonie Leeuwenhoek 98, 9–18. https://doi.org/10.1007/s10482-010-9423-7.

Ducklow, H.W., Steinberg, D.K., Buesseler, K.O., 2001. Upper ocean carbon export and the biological pump. Oceanography 14, 50–58.

Engel, A., Händel, N., 2011. A novel protocol for determining the concentration and composition of sugars in particulate and in high molecular weight dissolved organic matter (HMW-DOM) in seawater. Mar. Chem. 127, 180–191. https://doi.org/10.1016/j.marchem.2011.09.004.

Enke, T.N., Leventhal, G.E., Metzger, M., Saavedra, J.T., Cordero, O.X., 2018. Microscale ecology regulates particulate organic matter turnover in model marine microbial communities. Nat. Commun. 9, 2743.

Fang, J., Zhang, L., Li, J., Kato, C., Tamburini, C., Zhang, Y., Dang, H., Wang, G., Wang, F., 2015. The POM-DOM piezophilic microorganism continuum (PDPMC)-The role of piezophilic microorganisms in the global ocean carbon cycle. Sci. China Earth Sci. 58, 106–115. https://doi.org/10.1007/s11430-014-4985-2.

Fenice, M., Gallo, A.M., Juarez-Jimenez, B., Gonzalez-Lopez, J., 2007. Screening for extracellular enzyme activities by bacteria isolated from samples collected in the Tyrrhenian Sea. Ann. Microbiol. 57, 93–99. https://doi.org/10.1007/BF03175056.

Fernandes, V., Bogati, K., 2022. Analysis of bacteria–phytoplankton relationships at three discrete locations in the eastern Arabian Sea during winter. Continent. Shelf Res. 243, 104751 https://doi.org/10.1016/j.csr.2022.104751.

Gauns, M., Madhupratap, M., Ramaiah, N., Jyothibabu, R., Fernandes, V., Paul, J.T., Kumar, S.P., 2005. Comparative accounts of biological productivity characteristics and estimates of carbon fluxes in the Arabian Sea and the Bay of Bengal. Deep Sea Res. Part II Top. Stud. Oceanogr. 52, 2003–2017. https://doi.org/10.1016/j.dsr2.2005.05.009.

Ghai, R., Mizuno, C.M., Picazo, A., Camacho, A., Rodriguez-Valera, F., 2013. Metagenomics uncovers a new group of low GC and ultra-small marine Actinobacteria. Sci. Rep. 3, 1–8. https://doi.org/10.1038/srep02471.

Giovannoni, S., Stingl, U., 2007. The importance of culturing bacterioplankton in the 'omics' age. Nat. Rev. Microbiol. 5, 820–826. https://doi.org/10.1038/nrmicro1752.

Girão, M., Ribeiro, I., Ribeiro, T., Azevedo, I.C., Pereira, F., Urbatzka, R., Leão, P.N., Carvalho, M.F., 2019. Actinobacteria isolated from Laminaria ochroleuca: a source of new bioactive compounds. Front. Microbiol. 10, 683. https://doi.org/10.3389/fmicb.2019.00683.

Gomes, J., Khandeparker, R., Meena, R.M., Ramaiah, N., 2019. Bacterial community composition markedly altered by coastal hypoxia. Indian J. Microbiol. 59, 200–208. https://doi.org/10.1007/s12088-019-00790-5.

Gómez-Consarnau, L., Needham, D.M., Weber, P.K., Fuhrman, J.A., Mayali, X., 2019. Influence of light on particulate organic matter utilization by attached and free-living marine bacteria. Front. Microbiol. 10, 1204. https://doi.org/10.3389/fmicb.2019.01204.

Gonsalves, M.J., Paropkari, A.L., Fernandes, C.E., Bharathi, P.L., Krishnakumari, L., Fernando, V., Nampoothiri, G.E., 2011. Predominance of anaerobic bacterial community over aerobic community contribute to intensify 'oxygen minimum zone'in the eastern Arabian Sea. Continent. Shelf Res. 31, 1224–1235. https://doi.org/10.1016/j.csr.2011.04.011.

Hansell, D.A., Carlson, C.A., 2001. Marine dissolved organic matter and the carbon cycle. Oceanography 14, 41–49. https://doi.org/10.5670/oceanog.2001.05.

Hansell, D.A., Carlson, C.A., 2013. Localized refractory dissolved organic carbon sinks in the deep ocean. Global Biogeochem. Cycles 27, 705–710. https://doi.org/10.1002/gbc.20067.

Isaacs, M.J., Ramadoss, D., Parab, A.S., Manohar, C.S., 2021. Evaluating the bacterial diversity from the southwest coast of India using fatty acid methyl ester profiles. Curr. Microbiol. 78, 649–658. https://doi.org/10.1007/s00284-020-02315-6.

Jacob, J., Jayaraj, K., Rehman, H.H., Chandramohanakumar, N., Balachandran, K., Raveendran, T., Joseph, T., Nair, M., Achuthankutty, C., 2009. Biogeochemical characteristics of the surface sediments along the western continental shelf of India. Chem. Ecol. 25, 135–149. https://doi.org/10.1080/02757540902758750.

Jain, A., Bandekar, M., Gomes, J., Shenoy, D., Meena, R.M., Naik, H., Khandeparkar, R., Ramaiah, N., 2014. Temporally invariable bacterial community structure in the Arabian Sea oxygen minimum zone. Aquat. Microb. Ecol. 73, 51–67. https://doi.org/10.3354/ame01704.

Jiao, N., Herndl, G.J., Hansell, D.A., Benner, R., Kattner, G., Wilhelm, S.W., Kirchman, D.L., Weinbauer, M.G., Luo, T., Chen, F., 2011. The microbial carbon pump and the oceanic recalcitrant dissolved organic matter pool. Nat. Rev. Microbiol. 9 https://doi.org/10.1038/nrmicro2386-c5, 555-555.

Jiao, N., Robinson, C., Azam, F., Thomas, H., Baltar, F., Dang, H., Hardman-Mountford, N., Johnson, M., Kirchman, D., Koch, B., 2014. Mechanisms of microbial carbon sequestration in the ocean–future research directions. Biogeosciences 11, 5285–5306. https://doi.org/10.5194/bg-11-5285-2014.

Kamjam, M., Sivalingam, P., Deng, Z., Hong, K., 2017. Deep sea actinomycetes and their secondary metabolites. Front. Microbiol. 8, 760. https://doi.org/10.3389/fmicb.2017.00760.

Karl, D.M., Hebel, D.V., Bjorkman, K., Letelier, R.M., 1998. The role of dissolved organic matter release in the productivity of the oligotrophic North Pacific Ocean. Limnol. Oceanogr. 43, 1270–1286.

Khalifa, A., Aldayel, M., 2019. Isolation and characterisation of the agarolytic bacterium Pseudoalteromonas ruthenica. Open Life Sci. 14 (1), 588–594. https://doi.org/10.1515/biol-2019-0066.

Khandeparker, L., Kuchi, N., Kale, D., Anil, A.C., 2017. Microbial community structure of surface sediments from a tropical estuarine environment using next generation sequencing. Ecol. Indicat. 74, 172–181. https://doi.org/10.1016/j.ecolind.2016.11.023.

Khandeparker, R., Verma, P., Meena, R.M., Deobagkar, D.D., 2011. Phylogenetic diversity of carbohydrate degrading culturable bacteria from Mandovi and Zuari estuaries, Goa, west coast of India. Estuar. Coast Shelf Sci. 95, 359–366. https://doi.org/10.1016/j.ecss.2011.09.004.

Khodse, V.B., Bhosle, N.B., 2013. Distribution, origin and transformation of amino sugars and bacterial contribution to estuarine particulate organic matter. Continent. Shelf Res. 68, 33–42. https://doi.org/10.1016/j.csr.2013.08.004.

Kim, B., Kim, S.H., Kwak, J.H., Kang, C.K., Lee, S.H., Hyun, J.H., 2017. Heterotrophic bacterial production, respiration, and growth efficiency associated with upwelling intensity in the Ulleung Basin, East Sea. Deep Sea Res. Part II Top. Stud. Oceanogr. 143, 24–35. https://doi.org/10.1016/j.dsr2.2017.07.002.

Kottayil, A., Xavier, A., Xavier, P., Koovekkallu, P., Mohanakumar, K., 2022. Evolution of l arge-scale factors influencing extreme rainfall over south western coast of India. Int. J. Climatol. 42, 4164–4178. https://doi.org/10.1002/joc.7455.

Krüger, K., Chafee, M., Francis, T.B., Del Rio, T.G., Becher, D., Schweder, T., Amann, R.I., Teeling, H., 2019. In marine Bacteroidetes the bulk of glycan degradation during algae blooms is mediated by few clades using a restricted set of genes. ISME J. 13, 2800–2816. https://doi.org/10.1038/s41396-019-0476-y.

Kumar, S.P., Madhupratap, M., Kumar, M.D., Muraleedharan, P., De Souza, S., Gauns, M., Sarma, V., 2001. High biological productivity in the central Arabian Sea during the summer monsoon driven by Ekman pumping and lateral advection. Curr. Sci. 81, 1633–1638.

Kumar, S.P., Nuncio, M., Ramaiah, N., Sardesai, S., Narvekar, J., Fernandes, V., Paul, J.T., 2007. Eddy-mediated biological productivity in the Bay of Bengal during fall and spring inter monsoons. Deep-Sea Res. Part I Oceanogr. Res. Pap. 54, 1619–1640. https://doi.org/10.1016/j.dsr.2007.06.002.

Landa, M., Blain, S., Christaki, U., Monchy, S., Obernosterer, I., 2016. Shifts in bacterial community composition associated with increased carbon cycling in a mosaic of phytoplankton blooms. ISME J. 10, 39–50. https://doi.org/10.1038/ismej.2015.105.

Li, J., Li, N., Li, F., Zou, T., Yu, S., Wang, Y., Qin, S., Wang, G., 2014. Spatial diversity of bacterioplankton communities in surface water of northern South China Sea. PLoS One 9, e113014. https://doi.org/10.1371/journal.pone.0113014.

Lian, J., Zheng, X., Zhuo, X., Chen, Y.L., He, C., Zheng, Q., Cai, R., 2021. Microbial transformation of distinct exogenous substrates into analogous composition of recalcitrant dissolved organic matter. Environ. Microbiol. 23, 2389–2403.
Lincy, J., Manohar, C.S., 2021. 16S rRNA and hydrazine gene-based profiling of the CandidatusScalindua community from the Arabian Sea hypoxic sediments. Curr. Sci. 120, 684–693. https://doi.org/10.18520/cs/v120/i4/684-693.
Lucas, J., Koester, I., Wichels, A., Niggemann, J., Dittmar, T., Callies, U., Wiltshire, K.H., Gerdts, G., 2016. Short-term dynamics of North Sea bacterioplankton-dissolved organic matter coherence on molecular level. Front. Microbiol. 7, 321. https://doi.org/10.3389/fmicb.2016.00321.
Mahmoudi, N., Enke, T.N., Beaupré, S.R., Teske, A.P., Cordero, O.X., Pearson, A., 2019. Illuminating microbial species-specific effects on organic matter remineralization in marine sediments. Environ. Microbiol. 22, 1734–1747. https://doi.org/10.1111/1462-2920.14871.
Mahmoudi, N., Hagen, S.M., Hazen, T.C., Steen, A.D., 2020. Patterns in extracellular enzyme activity and microbial diversity in deep-sea Mediterranean sediments. Deep-Sea Res. Part I Oceanogr. Res. Pap. 158, 103231 https://doi.org/10.1016/j.dsr.2020.103231.
Manucharova, N., 2009. The microbial destruction of chitin, pectin, and cellulose in soils. Eurasian Soil Sci. 42, 1526–1532. https://doi.org/10.1134/S1064229309130146.
Margesin, R., Schinner, F., 2001. Biodegradation and bioremediation of hydrocarbons in extreme environments. Appl. Microbiol. Biotechnol. 56, 650–663. https://doi.org/10.1007/s002530100701.
Maßmig, M., Lüdke, J., Krahmann, G., Engel, A., 2020. Bacterial degradation activity in the eastern tropical South Pacific oxygen minimum zone. Biogeosciences 17, 215–230. https://doi.org/10.5194/bg-17-215-2020.
McKee, L.S., La Rosa, S.L., Westereng, B., Eijsink, V.G., Pope, P.B., Larsbrink, J., 2021. Polysaccharide degradation by the Bacteroidetes-mechanisms and nomenclature. Environ Microbiol Rep 13. https://doi.org/10.1111/1758-2229.12980, 599-581.
Meddeb-Mouelhi, F., Moisan, J.K., Beauregard, M., 2014. A comparison of plate assay methods for detecting extracellular cellulase and xylanase activity. Enzym. Microb. Technol. 66, 16–19. https://doi.org/10.1016/j.enzmictec.2014.07.004.
Molina, V., Belmar, L., Levipan, H.A., Ramírez-Flandes, S., Anguita, C., Galán, A., Montes, I., Ulloa, O., 2020. Spatiotemporal distribution of key pelagic microbes in a seasonal oxygen-deficient coastal upwelling system of the eastern South Pacific ocean. Front. Mar. Sci. 7, 561597 https://doi.org/10.3389/fmars.2020.561597.
Moran, M.A., Kujawinski, E.B., Schroer, W.F., Amin, S.A., Bates, N.R., Bertrand, E.M., Braakman, R., Brown, C.T., Covert, M.W., Doney, S.C., Dyhrman, S.T., 2022. Microbial metabolites in the marine carbon cycle. Nat microbiol 7, 508–523.
Mühlenbruch, M., Grossart, H.P., Eigemann, F., Voss, M., 2018. Mini-review: phytoplankton-derived polysaccharides in the marine environment and their interactions with heterotrophic bacteria. Environ. Microbiol. 20, 2671–2685. https://doi.org/10.1111/1462-2920.14302.
Mukherjee, R., Dutta, M.K., Sanyal, P., Bhadury, P., Mukhopadhyay, S.K., 2020. Bacterioplankton abundance and community structure during post-monsoon in mangrove dominated estuaries of the Indian Sundarbans; an insight to biogeochemical processes. Estuar. Coast Shelf Sci. 243, 106895 https://doi.org/10.1016/j.ecss.2020.106895.
Mulla, A., Fernandes, G., Menezes, L., Meena, R.M., Naik, H., Gauns, M., Damare, S., 2018. Diversity of culturable nitrate-reducing bacteria from the Arabian Sea oxygen minimum zone. Deep Sea Res. Part II Top. Stud. Oceanogr. 156, 27–33. https://doi.org/10.1016/j.dsr2.2017.12.014.
Naqvi, S., Bange, H.W., Farías, L., Monteiro, P., Scranton, M., Zhang, J., 2010. Marine hypoxia/anoxia as a source of CH 4 and N 2 O. Biogeosciences 7, 2159–2190. https://doi.org/10.5194/bg-7-2159-2010.
Parulekar, N.N., Kolekar, P., Jenkins, A., Kleiven, S., Utkilen, H., Johansen, A., Sawant, S., Kulkarni-Kale, U., Kale, M., Sæbø, M., 2017. Characterization of bacterial community associated with phytoplankton bloom in a eutrophic lake in South Norway using 16S rRNA gene amplicon sequence analysis. PLoS One 12, e0173408. https://doi.org/10.1371/journal.pone.0173408.
Passow, U., Christina, L., Arnosti, C., Grossart, H.-P., Murray, A.E., Engel, A., 2007. Microbial dynamics in autotrophic and heterotrophic seawater mesocosms. I. Effect of phytoplankton on the microbial loop. Aquat. Microb. Ecol. 49, 109–121. https://doi.org/10.3354/ame01138.
Porter, K.G., Feig, Y.S., 1980. The use of DAPI for identifying and counting aquatic microflora. Limnol. Oceanogr. 25, 943–948. https://doi.org/10.4319/lo.1980.25.5.0943.
Ram, A.S.P., Nair, S., Chandramohan, D., 2003. Seasonal shift in net ecosystem production in a tropical estuary. Limnol. Oceanogr. 48, 1601–1607. https://doi.org/10.4319/lo.2003.48.4.1601.
Ramaiah, N., Fernandes, V., Rodrigues, V.V., Paul, J.T., Gauns, M., 2009. Bacterioplankton abundance and production in Indian Ocean regions. In: Wiggert, J. D., Hood, R.R., Naqvi, S.W.A., Brink, K.H., Smith, S.L. (Eds.), Geophysical Monograph Series. American Geophysical Union, Washington, D. C., pp. 119–132. https://doi.org/10.1029/2008GM000711
Ramaiah, N., Raghukumar, S., Mangesh, G., Madhupratap, M., 2005. Seasonal variations in carbon biomass of bacteria, thraustochytrids and microzooplankton in the Northern Arabian Sea. Deep Sea Res. Part II Top. Stud. Oceanogr. 52, 1910–1921. https://doi.org/10.1016/j.dsr2.2005.05.004.
Reiner, K., 2012. Carbohydrate Fermentation Protocol, vol. 11. Am Soc Microbiol, Washington, DC, p. 12.
Robinson, C., Ramaiah, N., 2011. Microbial heterotrophic metabolic rates constrain the microbial carbon pump. In: Jiao, N., Azam, F., Sanders, S. (Eds.), Microbial Carbon Pump in the Ocean, first ed. Science/AAAS, Washington DC, pp. 52–53.
Rocke, E., Cheung, S., Gebe, Z., Dames, N.R., Liu, H., Moloney, C.L., 2020. Marine microbial community composition during the upwelling season in the southern Benguela. Front. Mar. Sci. 7, 255. https://doi.org/10.3389/fmars.2020.00255.
Rodriguez-Mora, M.J., Scranton, M.I., Taylor, G.T., Chistoserdov, A.Y., 2013. Bacterial community composition in a large marine anoxic basin: a Cariaco Basin time-series survey. FEMS Microbiol. Ecol. 84, 625–639. https://doi.org/10.1111/1574-6941.12094.
Santhikrishnan, S., Jyothibabu, R., Albin, K.J., Alok, K.T., Karnan, C., Arunpandi, N., Camey, M.F., Kumar, T.G., 2021. Biophysical implications of the freshwater influx over small spatial scale in the coastal waters along the southwest coast of India during the Southwest Monsoon. Continent. Shelf Res. 214, 104337 https://doi.org/10.1016/j.csr.2020.104337.
Sarma, V., Swathi, P., Kumar, M.D., Prasannakumar, S., Bhattathiri, P., Madhupratap, M., Ramaswamy, V., Sarin, M., Gauns, M., Ramaiah, N., 2003. Carbon budget in the eastern and central Arabian Sea: an Indian JGOFS synthesis. Global Biogeochem. Cycles 17. https://doi.org/10.1029/2002gb001978.
Sawant, S.S., Salunke, B.K., Kim, B.S., 2015. A rapid, sensitive, simple plate assay for detection of microbial alginate lyase activity. Enzym. Microb. Technol. 77, 8–13. https://doi.org/10.1016/j.enzmictec.2015.05.003.
Schloss, P.D., Westcott, S.L., Ryabin, T., Hall, J.R., Hartmann, M., Hollister, E.B., Lesniewski, R.A., Oakley, B.B., Parks, D.H., Robinson, C.J., 2009. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl. Environ. Microbiol. 75, 7537–7541. https://doi.org/10.1128/AEM.01541-09.
Shannon, E., Abu-Ghannam, N., 2016. Antibacterial derivatives of marine algae: an overview of pharmacological mechanisms and applications. Mar. Drugs 14, 81. https://doi.org/10.3390/md14040081.
Shetye, S., Shenoi, S., Gouveia, A., Michael, G., Sundar, D., Nampoothiri, G., 1991. Wind-driven coastal upwelling along the western boundary of the Bay of Bengal during the southwest monsoon. Continent. Shelf Res. 11, 1397–1408. https://doi.org/10.1016/0278-4343(91)90042-5.
Showalter, G., Deming, J., 2021. Extracellular enzyme activity of model cold adapted bacteria and Arctic sea-ice microbial communities under sub-zero hypersaline conditions. Aquat. Microb. Ecol. 87, 99–111. https://doi.org/10.3354/AME01974.
Siegel, D.A., DeVries, T., Doney, S.C., Bell, T., 2021. Assessing the sequestration time scales of some ocean-based carbon dioxide reduction strategies. Environ. Res. Lett. 16, 104003.
Singh, S., Ramaiah, N., 2011. Denaturing gradient gel electrophoresis profiling of bacterial communities composition in Arabian Sea. J. Environ. Biol. 32, 339–346.
Smitha, B., Sanjeevan, V., Vimalkumar, K., Revichandran, C., 2008. On the upwelling off the southern tip and along the west coast of India. J. Coast Res. 24, 95–102. https://doi.org/10.2112/06-0779.1.
Smriga, S., Fernandez, V.I., Mitchell, J.G., Stocker, R., 2016. Chemotaxis toward phytoplankton drives organic matter partitioning among marine bacteria. Proc. Natl. Acad. Sci. U.S.A. 113, 1576–1581. https://doi.org/10.1073/pnas.1512307113.
Sripan, D., Wilantho, A., Khitmoh, K., Wongsawaeng, D., Ouazzani, J., Chavanich, S., Tongsima, S., Somboonna, N., 2021. Marine bacteria community in a 150-m depth tachai island, the southeast andaman sea of Thailand. Front. Mar. Sci. 8, 124. https://doi.org/10.3389/fmars.2021.624624.
Stoecker, D.K., Hansen, P.J., Caron, D.A., Mitra, A., 2017. Mixotrophy in the marine plankton. Ann. Rev. Mar. Sci 9, 311–335.
Teeling, H., Fuchs, B.M., Bennke, C.M., Krueger, K., Chafee, M., Kappelmann, L., Reintjes, G., Waldmann, J., Quast, C., Gloeckner, F.O., 2016. Recurring patterns in bacterioplankton dynamics during coastal spring algae blooms. Elife 5, e11888. https://doi.org/10.7554/eLife.11888.
Traving, S.J., Thygesen, U.H., Riemann, L., Stedmon, C.A., 2015. A model of extracellular enzymes in free-living microbes: which strategy pays off? Appl. Environ. Microbiol. 81, 7385–7393. https://doi.org/10.1128/AEM.02070-15.
Underwood, G.J., Fietz, S., Papadimitriou, S., Thomas, D.N., Dieckmann, G.S., 2010. Distribution and composition of dissolved extracellular polymeric substances (EPS) in Antarctic sea ice. Mar. Ecol. Prog. Ser. 404, 1–19. https://doi.org/10.3354/meps08557.
UNESCO, 1994. Protocols for the joint global ocean flux study (JGOFS). Manual. Guides 29, 170.
Valliappan, K., Sun, W., Li, Z., 2014. Marine actinobacteria associated with marine organisms and their potentials in producing pharmaceutical natural products. Appl. Microbiol. Biotechnol. 98, 7365–7377. https://doi.org/10.1007/s00253-014-5954-6.
Vijayan, J., Ammini, P., Nathan, V.K., 2021. Diversity pattern of marine culturable heterotrophic bacteria in a region with coexisting upwelling and mud banks in the south eastern Arabian Sea. Environ. Sci. Pollut. Res. 29, 3967–3982. https://doi.org/10.1007/s11356-021-15772-8.
Wright, J.J., Konwar, K.M., Hallam, S.J., 2012. Microbial ecology of expanding oxygen minimum zones. Nat. Rev. Microbiol. 10, 381–394. https://doi.org/10.1038/nrmicro2778.
Yang, W., Zheng, S.Z., Zhou, S.H., Zhao, L., Zhu, J.Y., Lukwambe, B., Nicholaus, R., Li, C. H., Zheng, Z.M., 2020. Structure and functional diversity of surface bacterioplankton communities in an overwintering habitat for large yellow croaker, pseudosciaena crocea, of the Southern East China sea. Front. Mar. Sci. 7, 472. https://doi.org/10.3389/fmars.2020.00472.
Zhang, Z., Chen, Y., Wang, R., Cai, R., Fu, Y., Jiao, N., 2015. The fate of marine bacterial exopolysaccharide in natural marine microbial communities. PLoS One 10, e0142690. https://doi.org/10.1371/journal.pone.0142690.

Zhao, D., Cao, X., Huang, R., Zeng, J., Wu, Q.L., 2017. Variation of bacterial communities in water and sediments during the decomposition of Microcystis biomass. PLoS One 12, e0176397. https://doi.org/10.1371/journal.pone.0142690.

Zhou, J., Richlen, M.L., Sehein, T.R., Kulis, D.M., Anderson, D.M., Cai, Z., 2018. Microbial community structure and associations during a marine dinoflagellate bloom. Front. Microbiol. 9, 1201. https://doi.org/10.3389/fmicb.2018.01201.

Zimmerman, A.E., Martiny, A.C., Allison, S.D., 2013. Microdiversity of extracellular enzyme genes among sequenced prokaryotic genomes. ISME J. 7, 1187–1199. https://doi.org/10.1038/ismej.2012.176.

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Insights into the seasonal changes in the taxonomic and functional diversity of bacteria in the eastern Arabian Sea: Shotgun metagenomics approach

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ABSTRACT

The eastern Arabian Sea (EAS) is known for its unique oceanographic features such as the seasonal monsoonal winds, upwelling of nutrient-rich waters and a significant increase in primary productivity during the monsoon season. In this study, we utilised the shotgun metagenomics approach to determine the seasonal variations in bacterial taxonomic and functional profiles during the non-monsoon and monsoon seasons in the EAS. Significant seasonal variations in the bacterial community structure were observed at the phylum and genera levels. These findings also correspond with seasonal shifts in the functional profiles of the bacterial communities based on the variations of genes encoding enzymes associated with different metabolic pathways. Pronounced seasonal variation of bacterial taxa was evident with an increased abundance of *Idiomarina, Marinobacter, Psychrobacter* and *Alteromonas* of Proteobacteria, *Bacillus* and *Staphylococcus* of Firmicutes during the non-monsoon season. These taxa were linked to elevated nucleotide and amino acid biosynthesis, amino acid and lipid degradation. Conversely, during the monsoon, the taxa composition changed with *Alteromonas*, Candidatus *Pelagibacter* of Proteobacteria and Cyanobacteria *Synechococcus*; contributing largely to the amino acid and lipid biosynthesis, fermentation and inorganic nutrient metabolism which was evident from functional analysis. Regression analysis confirmed that increased seasonal primary productivity significantly influenced the abundance of genes associated with carbohydrate, protein and lipid metabolism. These highlight the pivotal role of seasonal changes in primary productivity in shaping the bacterial communities, their functional profiles and driving the biogeochemical cycling in the EAS.

1. Introduction

Marine bacteria are fundamental players in the biogeochemical cycles, decomposing organic matter and recycling the nutrients essential for sustaining the ecosystem. This is facilitated by the bacterial extracellular enzymes, such as carbohydrases, proteases and lipases, which act on organic matter (Brown et al., 2022; Lloyd et al., 2022; Dithugoe et al., 2023). In addition to supporting the marine food web, the recycling of organic matter by bacterial communities also plays a key role in regulating the global carbon cycle by facilitating the long-term storage of carbon in recalcitrant forms (Moran et al., 2016; DeVries, 2022). In marine ecosystems, the taxonomic composition and functional abilities of bacterial communities are influenced directly and indirectly by various environmental factors such as water temperature, salinity and nutrients that control the primary productivity and subsequently organic matter availability (Vijayan et al., 2021; Gao et al., 2021; Eigemann et al., 2023). Bacterial activity in marine environments demonstrates a marked variability, which appears to be influenced by the availability and characteristics of organic matter, suggesting a distinct distribution of enzymatic activities across seasons and regions (Balmonte et al., 2019; Arnosti et al., 2021; Lloyd et al., 2024). The diversity and composition of these bacterial communities based on metagenomics and metatranscriptomics studies have shown that they are crucial in determining the rate and efficiency of organic matter recycling (Franzosa et al., 2018; Pontiller et al., 2021; Delgadillo-Nuño et al., 2024). Hence, understanding the bacterial community structure and their heterotrophic functional activities is of global importance in the present climate change scenario.

The study region, along the eastern Arabian Sea (EAS), is characterized by semi-annual changes in the climate, wind pattern, monsoonal

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currents and upwelling, which plays a significant role in shaping the phytoplankton productivity dynamics in this region (Vinayachandran et al., 2021; Gupta et al., 2022). Increased productivity during the southwest monsoon in the EAS leads to the generation and accumulation of substantial amounts of organic matter (Vijayan et al., 2021; Shetye et al., 2022). During the monsoon season, intense rainfall and terrestrial inputs further contribute to the organic matter dynamics (Nageswar Rao et al., 2019; Silori et al., 2022). However, during the non-monsoon season, the EAS experiences a relatively low-productive environment (Santhikrishnan et al., 2021). Understanding the intricate seasonal dynamics of productivity, organic matter generation and its relation to bacterial functional activities is crucial to comprehend the complex ecological processes in the EAS.

Despite the importance of bacterial communities in biogeochemical cycling in marine ecosystems, little is known about their composition and diversity in the EAS. Previous studies have primarily focused on the physicochemical factors influencing bacterial diversity and community structure in specific regions such as oxygen minimum zones, mud banks regions and sediment cores (Vijayan et al., 2023 and reference therein), however, the specific bacterial communities and their functional diversity involved in the biogeochemical processes have not been studied thoroughly from this region. Furthermore, there is limited knowledge about how bacterial communities vary during different seasons, which is important for understanding the temporal dynamics of bacterial functional activities recycling organic matter. In our previous study examining culturable bacterial diversity in the EAS, we observed a pronounced increase in bacterial abundance during the monsoon season, especially in the chlorophyll maxima depths (C-Max) depths which consistently reported the highest viable bacterial counts (Parab et al., 2022). The C-Max depths, located between 20 and 100 m in marine water columns, are known for high phytoplankton productivity due to the favourable conditions for growth, leading to the increased availability of organic matter (Cullen, 2015; Garg et al., 2024). Despite its importance, there is limited research in the EAS on the impact of seasonal changes in primary productivity on the bacterial functions in C-Max zones, especially using advanced metagenomics sequencing methods.

To address these knowledge gaps, the present study used high-throughput shotgun sequencing to investigate the bacterial taxonomy and functional genes at C-Max depths in the EAS during non-monsoon and monsoon seasons. Shotgun metagenomics represents a robust and superior approach for functional analysis in microbial ecology and environmental studies (Quince et al., 2017), unlike targeted methods like 16S rRNA sequencing that focus on specific conserved regions. By sequencing the total genetic material in environmental samples, shotgun metagenomics enables a comprehensive exploration of microbial diversity and the identification of functional genes and pathways (Galloway-Peña and Hanson, 2020). Thus, this study provides a detailed report of bacterial functional diversity based on shotgun metagenomics, offering a comprehensive view of the diverse bacterial communities and their functional potential during non-monsoon and monsoon seasons from the EAS. This research contributes to a better understanding of the ecological significance of bacterial communities in this region and it will provide valuable insights into seasonal variations in bacterial heterotrophic activities.

2. Materials and methods

2.1. Sample collection

Sampling was conducted on board RV Sindhu Sankalp in the study region in the EAS, during the non-monsoon season in February 2019 (Cruise # SSK-125) and southwest monsoon season in September 2019 (Cruise # SSK-131). Samples were collected from coastal (30 m) and offshore (600 m) locations off Goa (G30 and G600), Mangalore (M30 and M600) and Trivandrum (T30 and T600) in the EAS (Table 1). At each of the six sampling stations, the physicochemical characteristics, including water column temperature, salinity, dissolved oxygen (DO) and fluorescence, were measured using the conductivity-temperature-depth (CTD) underwater profiler (Sea-Bird, SBE-911 plus, USA) in both the seasons. The depth at which the highest reading from the fluorescence sensor was recorded from the photic zone, was defined as the C-Max depth (Parab et al., 2023). The *in-situ* radiotracer-based primary productivity was determined in the water column following the JGOFS protocol (UNESCO, 1994) which is described in Parab et al. (2023). For this study, water samples collected at the C-Max depths in all the locations were immediately sub-sampled and stored for shotgun metagenomic analysis.

2.2. DNA extraction, shotgun sequencing and assembly

Water samples collected at C-Max depths from all 6 stations during both cruises were filtered using a peristaltic pump on board the research vessel for metagenomic DNA extraction. Approximately 20 L of water sample in triplicates was filtered through 0.22 μm pore size Durapore filters having a diameter of 47 mm (Millipore, USA) and stored in cryovials containing DNA storage buffer, consisting of 0.75M sucrose, 40 mM EDTA and 50 mM Tris (pH 8.3) and was maintained at −80 °C. Subsequently, DNA extraction was performed using the FastDNA™ SPIN kit (MP Biomedical, USA), from the triplicate samples following the manufacturer's instructions and the total DNA was pooled together for further analysis. The extracted metagenomic DNA was assessed for quality and an adequate amount of DNA was obtained for subsequent library preparation and sequencing. DNA samples were sequenced using the shotgun metagenomics technique at the National Institute of Biomedical Genomics (NIBG, West Bengal, India). For this, library preparation was carried out using TruSeq DNA PCR-Free Library Prep Kit (Illumina, USA) and the library was sequenced on the NovaSeq 6000 system (v1.5, Illumina, USA) according to Illumina's recommended protocols with the sequencing depth of 10 million reads per samples (Illumina, USA).

Quality control and assembly of the shotgun sequencing reads were performed using bioinformatics tools. Raw sequencing reads were initially assessed for quality using FastQC v0.12.0. Low-quality bases and adapter sequences were removed from the reads using Trimmomatic v0.39 (Bolger et al., 2014).

A sliding window trimming approach was applied with a window size of 4 bases and a quality threshold of Q20. Sequences with average

Table 1
Physicochemical variables during non-monsoon (NMS) and monsoon (MS) seasons in the eastern Arabian Sea.

Station	Location	Chl-M depth (m)		Temperature (°C)		Salinity (PSU)		DO (mg L^{-1})		Fluorescence (mg m^3)		PP (mg C m^3 d^{-1})	
		NMS	MS	NMS	MS	NMS	MS	NMS	MS	NMS	MS	NMS	MS
G30	15.2454°N, 73.3633°E	3	10	27.29	27.46	35.37	35.59	3.96	1.95	1.21	2.15	18.7	83.01
G600	15.2301°N, 72.4677°E	49	47	27.74	28.11	35.69	36.13	3.88	3.6	0.57	0.22	8.7	59.68
M30	12.5062°N, 74.4087°E	19	15	28.81	26.31	34.80	35.84	3.75	2.17	0.68	0.8	48.0	75.98
M600	12.5076°N, 73.5683°E	57	27	28.89	27.34	36.04	35.84	3.71	2.6	1.20	0.67	8.5	31.56
T30	8.2889°N, 76.5144°E	26	9	27.99	26.82	33.55	35.33	5.68	3.09	0.33	3.49	10.5	94.83
T600	76.1384°E, 76.1384°E	55	9	29.22	28.88	35.64	33.6	5.37	6.35	0.82	3.84	14.2	76.58

quality of bases below a Phred score of 20, in the read window were trimmed at that point. Additionally, any adapter sequences and reads shorter than 50 bases were also discarded. To ensure the exclusive use of bacterial DNA in the analyses, KneadData v0.11.0 was employed in conjunction with Bowtie2 to align the reads against reference databases. This process effectively removed non-bacterial DNA, including human, plant, viral and other eukaryotic sequences, from the dataset. The resulting high-quality reads were then subjected to de novo assembly using MEGAHIT v1.2.8 (Li et al., 2016), resulting in the generation of contigs. Assembly quality was evaluated using QUAST v5.1.0 (Gurevich et al., 2013) and BUSCO v5.4.7 was used to assess the completeness of the bacterial contig assembly (Manni et al., 2021). Post-assembly processing involved read mapping using Bowtie2 v2.5.1 (Langmead and Salzberg, 2012).

2.3. Taxonomic and functional annotation

For taxonomic annotation, MetaPhlAn2 was utilised to profile the metagenomic content of the assembly, enabling the identification and quantification of bacterial taxa within the dataset (Truong et al., 2015). For functional diversity assessment, open reading frames (ORF) were identified using Prokka v1.14.6 for comprehensive genome annotation (Seemann, 2014). For each of the samples, gene clustering based on sequence identity, CD-HIT v 4.8.1 was employed (Li et al., 2012). For evaluation of the overall functional diversity of bacterial metabolic, cellular and carbohydrate utilisation processes; the ORFs were annotated against reference databases like KEGG (Kyoto Encyclopedia of Genes and Genomes), EggNOG (Evolutionary Genealogy of Genes: Non-supervised Orthologous Groups) and CAZymes (carbohydrate-active enzymes). KEGG annotation was conducted utilizing kofamscan v1.3.0 to assign KEGG ortholog terms to the predicted ORFs. Functional classification based on orthologous groups from the EggNOG database was performed using eggnog-mapper v2.1.9 (Cantalapiedra et al., 2021). Identification of CAZymes within the ORFs was achieved by mapping the data set against the CAZymes database using run_dbcan v2.0.0 (Zhang et al., 2018).

To further investigate the organic matter degradation potential, information on the various genes encoding enzymes involved in carbohydrate, protein and lipid metabolism was extracted from the annotated data. Carbohydrate-degrading enzymes were assessed by estimating the gene abundance of various sub-families from the CAZymes annotated data. Protein breakdown was studied by extracting peptidase families, with a particular emphasis on bacterial extracellular peptidases. The InterPro database (Paysan-Lafosse et al., 2023), which provides a classification of protein families and the MEROPS database, dedicated to peptidase classification, were utilised for this purpose (Rawlings et al., 2018). Subsequently, these peptidases were queried against the EggNOG annotated data in R studio v3.5.1 and the abundance of genes encoding peptidases of interest was extracted. For lipid degradation analysis, ORFs were annotated against the KEGG database to extract the genes associated with pathways responsible for fatty acid degradation processes. Functional profiles from the KEGG, EggNOG and CAZymes annotation output files were imported into R Studio v3.5.1 to identify specific genes and pathways associated with carbohydrate, protein and lipid metabolism. Custom searches, filtering and sorting operations were performed on the functional annotation data to extract the relevant information (Tseng et al., 2015). To complement the functional annotation with associated bacterial taxa and provide a comprehensive analysis of metabolic pathways, HUMAnN2 was employed. It utilizes the taxonomic profiles generated from MetaPhlAn2 and leverages this information to infer the genes associated with different metabolic pathways based on the MetaCyc database in the bacterial taxa (Franzosa et al., 2018).

2.4. Data analysis

The data was analysed to statistically evaluate the significance level of the differences in the bacterial community and functional profiles between non-monsoon and monsoon seasons. The assessment of bacterial alpha and beta diversity indices was conducted on OTU-based taxonomic abundance data extracted from MetaPHlAn2. Alpha diversity indices were estimated using PAST v4.03 software (Hammer and Harper, 2006). To assess the normal distribution of each alpha diversity index, the Shapiro-Wilk test was conducted in R v4.0.2, confirming normal distribution and homogeneity of variance to ensure the validity of the *t*-test. Upon confirmation, a *t*-test was conducted to evaluate significant differences in bacterial alpha diversity indices between the non-monsoon and monsoon seasons. For beta diversity assessment, non-metric multidimensional scaling (NMDS) analysis, was conducted utilizing the R package BiodiversityR (Kindt and Coe, 2005). This analysis explored the impact of physicochemical parameters including fluorescence and primary productivity data characterized in the earlier work (Parab et al., 2023) on the bacterial communities from different locations and seasons. Additionally, the permutational multivariate analysis of variance (PERMANOVA) was performed, to evaluate the statistical significance of diversity distribution across different sampling locations and seasons. A comprehensive heatmap of all identified bacterial communities was generated using the R package Pheatmap v1.0.12.

Statistical significance between taxonomic and functional gene abundance was assessed using STAMP (statistical analysis of taxonomic and functional profiles) bioinformatics software v2.1.3 (Parks et al., 2014). The linear regression analysis was performed in R studio to evaluate the relationship between *in-situ* primary productivity (mg C m^{-3} d^{-1}) estimated from the study region (Parab et al., 2023) and gene abundance of various CAZymes families, peptidases and lipid metabolism were estimated. Following the linear regression analysis, multiple linear regression analysis was also carried out to evaluate the importance of the significant variable within each metabolic group. Data visualization for various statistical tests was performed using the R package ggplot2 (R Core Team, 2015).

3. Results

3.1. Bacterial distribution and taxonomic composition

The physicochemical parameters analysed in the water column of the study region clearly showed distinct variations and increased primary productivity in the monsoon than in the non-monsoon season. The C-Max depth had the maximum fluorescence and primary productivity along with increased bacterial abundance in the monsoon season (Parab et al., 2023). Shotgun sequences obtained from the C-Max depths of the water samples collected from each station in both seasons were in the range of about $\sim$ 10 to 26 $\times$ 10^6 raw reads. These sequences were denoised and filtered to generate $\sim$ 2 to 23 $\times$ 10^6 trimmed reads, which were used for further analysis. Taxonomic profiling of these reads yielded an average of $\sim$ 5 to 9 $\times$ 10^5 targeted bacterial reads which grouped into an average of about 750 unique OTUs (Table A1). A rarefaction curve was plotted to assess the sequencing depth and its efficacy in capturing the bacterial diversity, which resulted in a plateauing curve, suggesting that the sequencing depth was optimal in both seasons (Fig. A1). Based on the diversity indices estimated, the mean Simpson's index was 0.36 and 0.42 during the non-monsoon and monsoon seasons respectively and the mean Shannon diversity index was about 0.81 in the samples analysed (Table 2). During the non-monsoon, the evenness values ranged from 0.12 to 0.24 and in the monsoon, a wider range of values from 0.22 to 1.00 was estimated, which showed that there was a significant seasonal difference. The Chao-1 index exhibited significant seasonal variations, with higher values (12.50) in the non-monsoon; than in the monsoon season (6.33). The *t*-test analysis performed to

Table 2
Diversity indices estimated during the non-monsoon (NMS) and monsoon season (MS) in the eastern Arabian Sea.

Stations	Diversity indices							
	Simpson		Shannon		Evenness[a]		Chao-1[a]	
	NMS	MS	NMS	MS	NMS	MS	NMS	MS
G30	0.58	0.00	1.26	0.00	0.24	1.00	15.00	1.00
G600	0.27	0.79	0.67	1.60	0.12	0.99	16.00	5.00
M30	0.53	0.48	1.22	0.90	0.24	0.31	14.00	8.00
M600	0.40	0.58	0.94	1.01	0.16	0.55	16.00	5.00
T30	0.17	0.31	0.36	0.71	0.18	0.25	8.00	8.00
T600	0.19	0.37	0.38	0.88	0.24	0.22	6.00	11.00
Mean	0.36	0.42	0.81	0.85	0.20	0.55	12.50	6.33

[a] Significant at 0.05 level (*t*-test).

evaluate the differences in diversity indices showed significant seasonal variations in the Evenness and Chao-1 index ($p < 0.05$) (Table 2).

Based on the NMDS analysis of the physicochemical, primary productivity parameters and bacterial taxonomic profiles, a distinct seasonal variation in the bacterial communities from the sampling locations, off Goa, Mangalore and Trivandrum in the EAS was observed. The non-monsoon samples from coastal (30 m) and offshore (600 m) stations of Goa and Mangalore, displayed a closer clustering pattern, suggesting a higher degree of similarity in comparison to the Trivandrum samples (Fig. 1). During monsoon season, the bacterial communities from the sampling locations exhibited wider variations in the diversity patterns. The influence of DO, primary productivity and fluorescence was evident with its close association with the monsoon samples. The two-way PERMANOVA analysis validated the NMDS results, showing significant seasonal variation ($p < 0.001$) in the bacterial community structure.

The bacterial taxonomic composition in the water column at C-Max depths revealed the presence of bacterial phyla including Proteobacteria, Bacteroidetes, Firmicutes and Actinobacteria during the non-monsoon season and in the monsoon season; Cyanobacteria was exclusively reported during monsoon season (Fig. 2). The relative abundance of bacterial genera exhibited distinct differences in the non-monsoon and monsoon seasons. In the Proteobacteria phylum, within the Gammaproteobacteria class, pronounced seasonal variation was observed which includes genera *Idiomarina,* whose relative abundance significantly decreased from 53.06 % in the non-monsoon season to 2.96 % in the monsoon season ($p < 0.001$, Fig. 2). In contrast, *Alteromonas* significantly increased from 28.17 % in the non-monsoon season to 58.49 % in the monsoon season ($p < 0.001$). Proteobacterial genera *Nitratireductor* and *Pelagibaca* also displayed an increase in their relative abundance during the monsoon season.

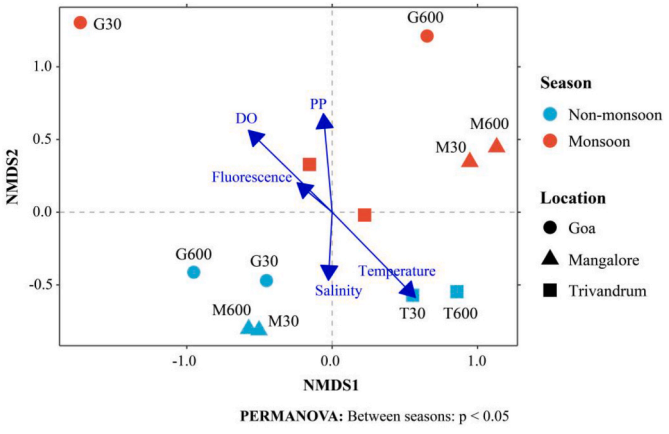

Fig. 1. NMDS analysis illustrating seasonal differences in bacterial community composition between non-monsoon and monsoon seasons.

Significant differences were also observed in the Actinobacteria phylum, with *Nocardioides* genera increasing from a relative abundance of 0.48 % in the non-monsoon season to 5.76 % in the monsoon season ($p < 0.001$). Within the Bacteroidetes and Firmicutes phylum, several genera present during the non-monsoon season were not detected in the monsoon season (Fig. 2). Within the Cyanobacteria phylum, significant variation was seen in the genus *Synechococcus,* which was not detected during the non-monsoon season but contributed to a relative abundance of 21.65 % during the monsoon season (Fig. 2).

3.2.1. Cellular processes

The functional profiles of the bacterial community from the different samples were categorized and annotated from the trimmed reads which generated approximately 3–17 $\times$ 10^5 contigs and were subsequently assembled into 2 to 6 $\times$ 10^5 ORFs during both seasons (Table 2). Downstream processing of these ORFs for functional annotation using databases like KEGG, EggNOG and CAZymes provided valuable insights into the functional profiles of bacterial communities in the EAS. Among the six major KEGG pathways, metabolism emerged as the predominant functional category, constituting ~55 % during both the non-monsoon and monsoon seasons (Fig. 3a). A relative abundance of genetic information processing pathways and cellular processes was represented around 8.5 % during both seasons. Environmental information processing pathways contributed about 11 % during the non-monsoon season and marginally reduced in the monsoon season (9 %). Pathways associated with organismal systems and human diseases exhibited a proportion of ~4 % and 12.5 % during the non-monsoon season and monsoon season respectively (Fig. 3a). Within the metabolic pathways, the relative abundance of energy metabolism increased significantly during the monsoon season ($p < 0.001$). A similar significant increase in relative abundance was also observed in pathways for amino acid metabolism and metabolism of cofactors and vitamins during monsoon season (Fig. 3b and Table A2). There was an increase in the pathways for folding, sorting and degradation under the genetic information processing pathways, such as viral information processing ($p = 0.004$) and translation process ($p < 0.001$) in the monsoon season (Fig. 3b and Table A2). Seasonal variations were also observed in the environmental information processing category pathways, with a significant increase in membrane transport and signal transduction processes ($p < 0.001$) during non-monsoon season. Within the cellular processes pathways, the non-monsoon season exhibited an increase in relative abundance in the cellular community of prokaryotes ($p < 0.001$) and sensory system process, within organismal systems pathways ($p < 0.05$, Fig. 3b). The relative abundance of various metabolism and cellular process pathways annotated against the EggNOG database was also analysed.

Results obtained were similar to the analysis based on KEGG annotations with a higher relative abundance of several pathways including those involving energy and amino acid metabolism in the monsoon season (Fig. A2 and Table A3).

3.2. Insights into functional profiles of the bacterial community

3.2.2. Carbohydrate metabolism

The comparative analysis of the ORFs belonging to the CAZymes families exhibited distinct variations in the non-monsoon and monsoon seasons. Within the CAZymes, Glycosyltransferases (GTs), responsible for synthesizing the glycosidic bond in carbohydrates, emerged as the predominant group during both seasons with a relative abundance of ~48 %. Following it, the Glycoside Hydrolases (GHs), were the dominant family which included the key enzymes in hydrolysing the glycosidic bond between two or more carbohydrates (Fig. 4a). Carbohydrate Esterases (CEs), that cleave linkages of carbohydrate esters, observed a significant increase in their relative abundance during the monsoon ($p < 0.001$). In the monsoon season, a slight decrease was observed in the presence of genes associated with Auxiliary Activities (AAs) enzymes, which are essential for lignin degradation and modification, as well as

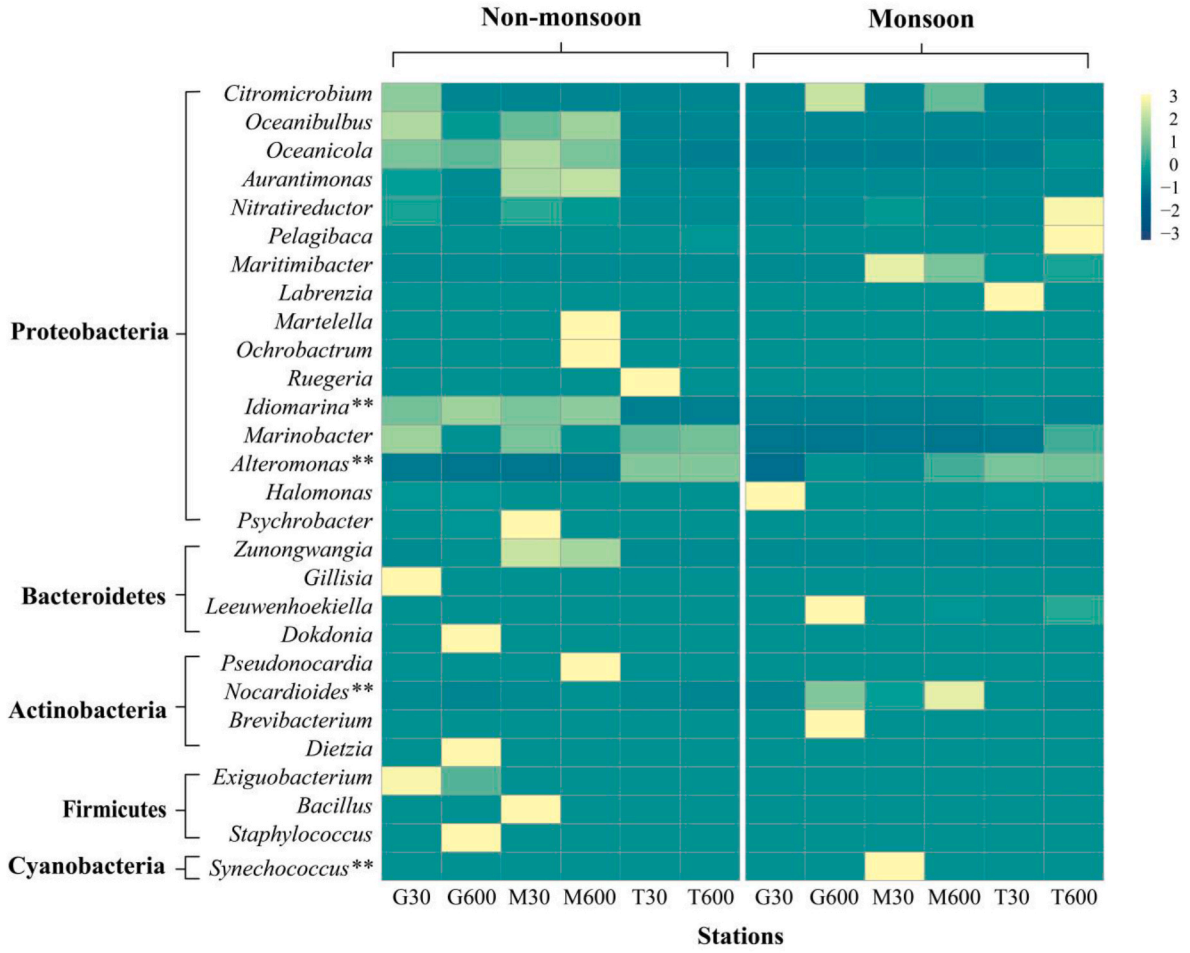

Fig. 2. Heatmap displaying the relative abundance of bacterial taxa during non-monsoon and monsoon seasons in the eastern Arabian Sea, the significance of seasonal variations at $p < 0.001$** is also represented for specific bacterial genera.

Carbohydrate-Binding Modules (CBMs), which are important for directing enzymes to their complex carbohydrate substrates. The relative abundance of Polysaccharide Lyases (PLs), involved in the non-hydrolytic cleavage of polysaccharides, remained steady at about 1 % throughout both seasons (Fig. 4a).

To further understand carbohydrate degradation, the glycoside hydrolase subfamily was examined in depth, revealing distinct distribution patterns across sampling seasons (Fig. 4b). During the non-monsoon season, GH0, a non-classified glycoside hydrolase, exhibited a relative abundance of 4.07 %, which significantly increased to 6.74 % in the monsoon season ($p < 0.001$). Genes for enzyme subfamilies, GH92 and GH38, both associated with mannose hydrolysis, presented contrasting dynamics.

Relative abundance of GH92 was higher in the non-monsoon season, however, GH38 was increased during the monsoon season. Genes for enzymes catalysing xylose degradation, such as GH3 and GH39 varied in their seasonal trends with higher relative abundance during non-monsoon. Similarly, genes for GH2 enzymes, which are associated with lactose hydrolysis were only detectable during the non-monsoon season. Seasonal variations were also observed in peptidoglycan hydrolases such as GH23, GH73 and GH103 (Fig. 4b). During monsoon season, genes for maltose hydrolysing enzymes such as GH65 and starch-degrading enzyme GH13 also increased significantly ($p < 0.001$). Similarly, genes for GH1 associated with cellulolytic activities and GH28 enzyme, associated with pectin hydrolysis increased significantly during the monsoon season. Furthermore, genes of β-1, 3-glucan targeting enzymes such as GH17 and GH33, associated with glycoprotein and glycolipid degradation were only detected during monsoon season (Fig. 4b).

In the CAZymes subfamily, genes for various enzymes from AAs, CBMs and CEs exhibited significant presence during both seasons. Subfamily CBM47, associated with fucose degradation and CBM48+GH13_11, known for sorbitol degradation, were significantly increased in the monsoon season (Fig. A3). Similarly, genes associated with CE11, which degrades N-acetylglucosamine; CE14, associated with chitin degradation and CE20, linked to xyloglucan degradation, all demonstrated significantly higher abundance during the monsoon season. Genes for unclassified CAZymes subfamily AA0 also showed increased abundance during monsoon season. During the non-monsoon season, genes for the enzyme subfamily AA3_2 associated with the degradation of β-D-glucose were significantly higher. Similarly, genes for CBM50, involved in the degradation of chitin or peptidoglycan; CE8, responsible for pectin degradation was present in the non-monsoon season (Fig. A3).

3.2.3. *Protein metabolism*

The analysis of genes associated with peptidase enzymes revealed the occurrence of various peptidases across sampling seasons. Aminopeptidases, which are associated with the cleaving of amino acids from the N-terminal end of proteins showed significant seasonal variation. Aminopeptidase N (Peptidase M1), aminopeptidase Y (Peptidase M3) and glycyl aminopeptidase (Peptidase M61) were significantly higher during the non-monsoon season ($p < 0.001$, Fig. A4). Carboxypeptidases, essential for cleaving amino acids from the C-terminal end, such as

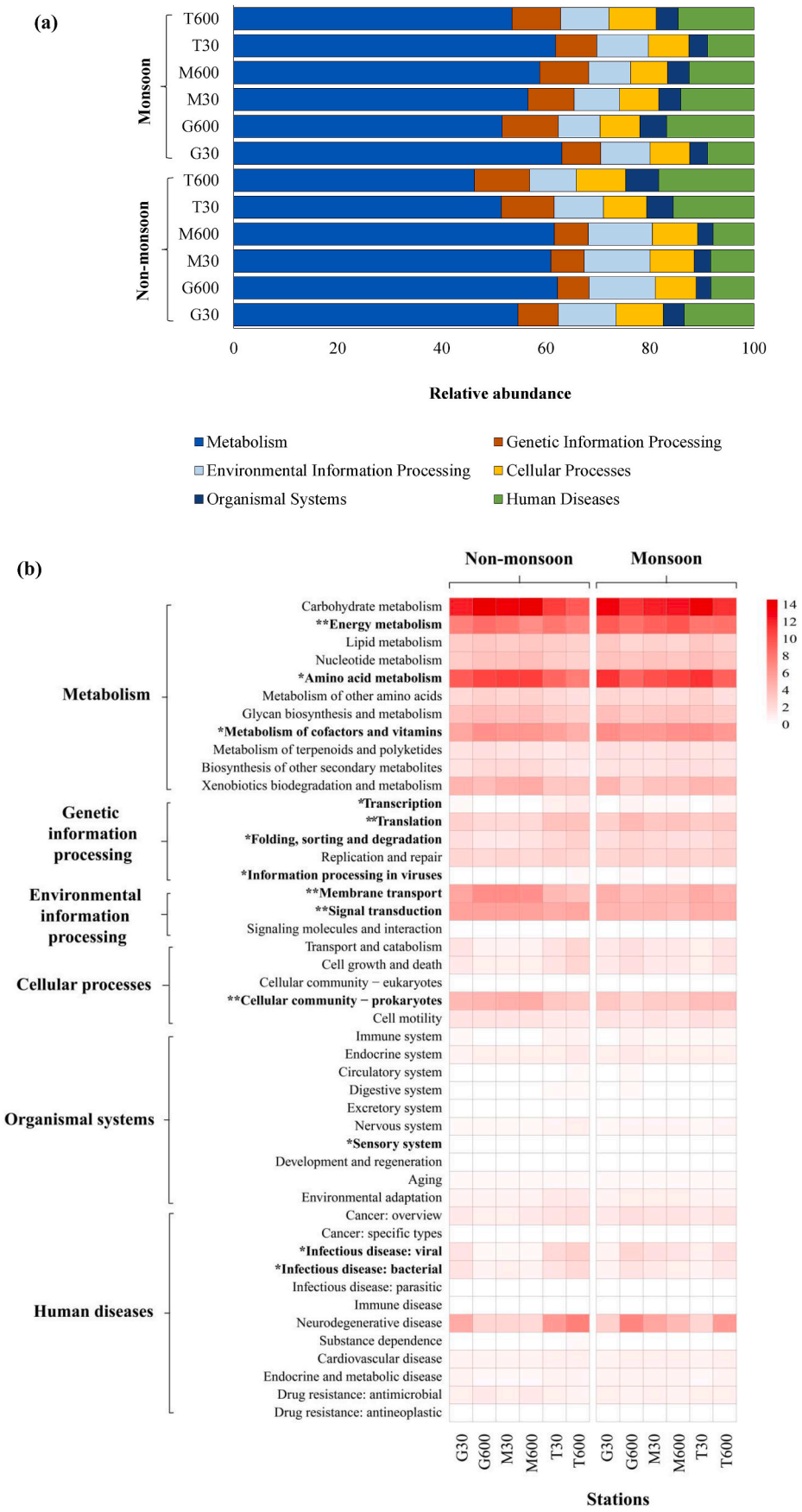

Fig. 3. (a) Relative abundance of major KEGG pathways and (b) comparative heatmap of all major pathways across all stations during the non-monsoon and monsoon seasons, the significance of seasonal variations at $p < 0.05$* and $p < 0.001$** is also represented for specific pathways.

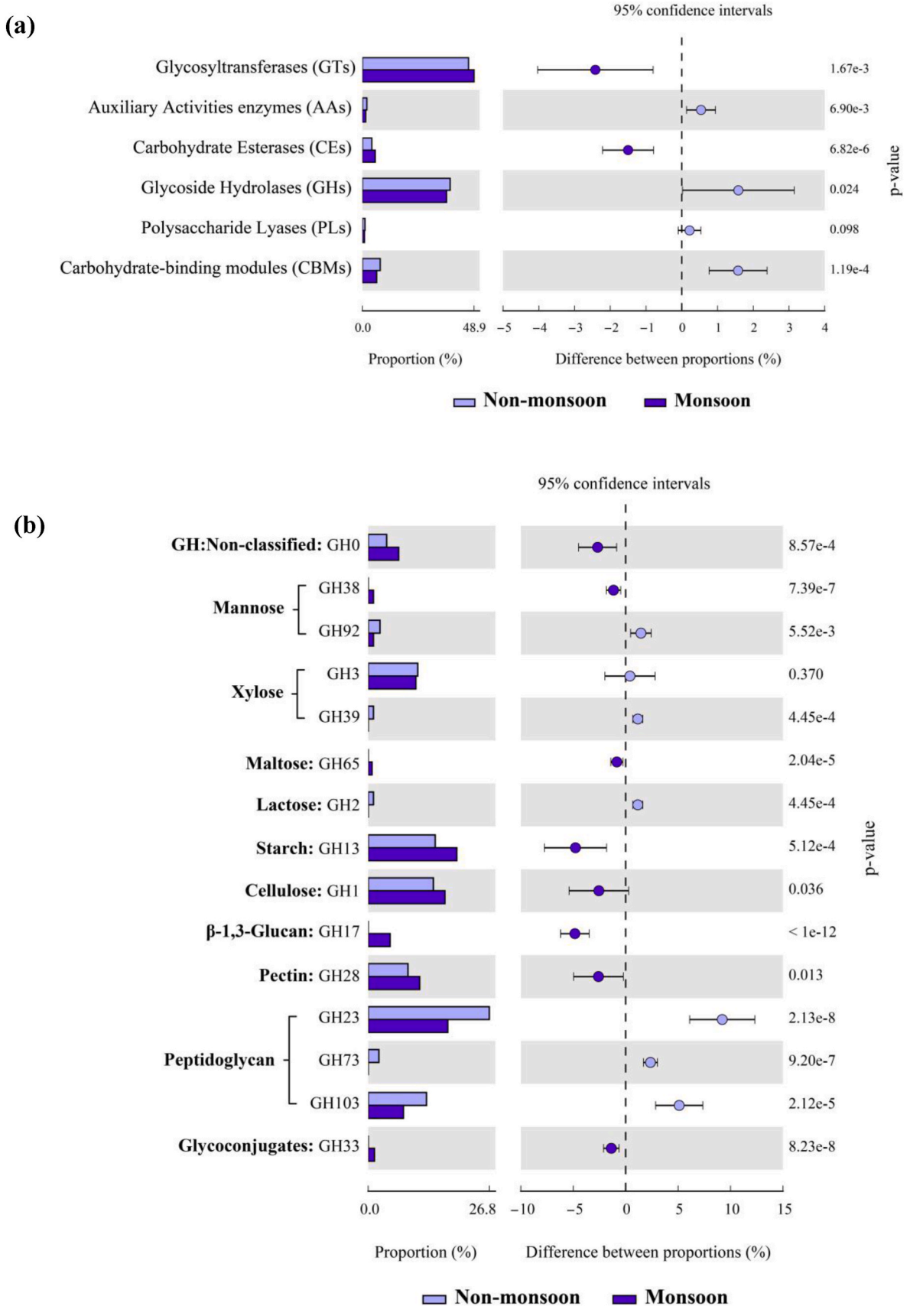

Fig. 4. Extended error bar plot of (a) major CAZymes families and (b) Glycoside Hydrolases (GHs) during non-monsoon and monsoon season.

carboxypeptidase A (Peptidase M14) and LD-carboxypeptidase (Peptidase S66) detected during the non-monsoon season, but their relative abundance significantly decreased in the monsoon season ($p < 0.001$). The relative abundance of endopeptidase pitrilysin (Peptidase M16) was also significantly higher during non-monsoon season. The genes associated with several enzymes with specialized functions such as imelysin (Peptidase M75) and prepilin peptidase (Peptidase A24), were only detected during the non-monsoon season (Fig. A4). During monsoon season, aminopeptidases such as methionine aminopeptidase 1 (Peptidase M24) marked a significant increase in the relative abundance of 22.73 % ($p < 0.001$). Along with it, the relative abundance of glutamate carboxypeptidase (Peptidase M20) also increased significantly during the monsoon season ($p = 0.003$). Within the endopeptidase category, which acts on internal peptide bonds, the relative abundance of betalytic metallopeptidase (Peptidase M23) and prolyl oligopeptides (Peptidase S9) significantly increased during the monsoon season ($p < 0.001$, Fig. A4).

3.2.4. Lipid metabolism

The analysis of genes responsible for lipid degradation pathways revealed significant differences in the abundance of specific enzymes during the non-monsoon and monsoon seasons (Fig. A5). During the non-monsoon season, genes of enzymes responsible for the initial activation pathways of fatty acid degradation such as long-chain fatty acid ligase had a significantly higher relative abundance of 9.59 % ($P < 0.001$). Similarly, genes for the β-oxidation cycle enzymes presented significant activity with a marginally higher abundance of 3-hydroxybutyryl-CoA epimerase enzyme ($p = 0.01$). Genes for enzymes associated with the aldehyde and alcohol metabolism, such as acetaldehyde dehydrogenase and alcohol dehydrogenase exhibited a higher relative abundance during the non-monsoon season ($p < 0.001$). The monsoon season witnessed a shift in functional profiles, with an evident surge in the genes related to β-oxidation cycle enzymes during the monsoon season (Fig. A5). Notably, acyl-CoA dehydrogenase, enoyl-CoA hydratase and Delta3-delta2-enoyl-CoA isomerase exhibited a significantly higher relative abundance of 15.82 %, 5.95 % and 18.45 %, respectively ($P < 0.001$). Genes for other β-oxidation enzymes such as Enoyl-CoA isomerase and Butyryl-CoA dehydrogenase also showed a significant increase in their relative abundance during monsoon season (Fig. A5). Relative abundance of Long-chain acyl-CoA synthetase was also detected to be higher during monsoon season. Similarly, genes for CoA transfer and shuttle enzymes such as Glutaryl-CoA dehydrogenase ($p = 0.002$), acetyl-CoA acyltransferase, acetyl-CoA C-acetyltransferase and 3-phenylpropionate dioxygenase exhibited a significant increase in relative abundance during monsoon season ($P < 0.001$, Fig. A5).

3.3. Bacterial taxa and their associated metabolic pathways

Analysis of the major bacterial taxa contributing to the functional activity based on HUMAnN2 analysis revealed distinct microbial activity and metabolic pathways patterns across both seasons in the EAS. Results from this analysis showed that in non-monsoon season Proteobacterial genera including *Idiomarina, Marinobacter, Psychrobacter, Alteromonas* and Bacteroidetes *Zunogngwangia* were the major contributors associated with most of the metabolic pathways (Fig. 5).

Firmicutes genera *Bacillus* and *Staphylococcus* were exclusively observed as dominant contributors in metabolic pathways during the non-monsoon. In the monsoon season, Candidatus *Pelagibacter*, *Alteromonas* and unknown bacterial genera from, Alpha, Gamma and Betaproteobacteria were dominant contributors in metabolic pathways.

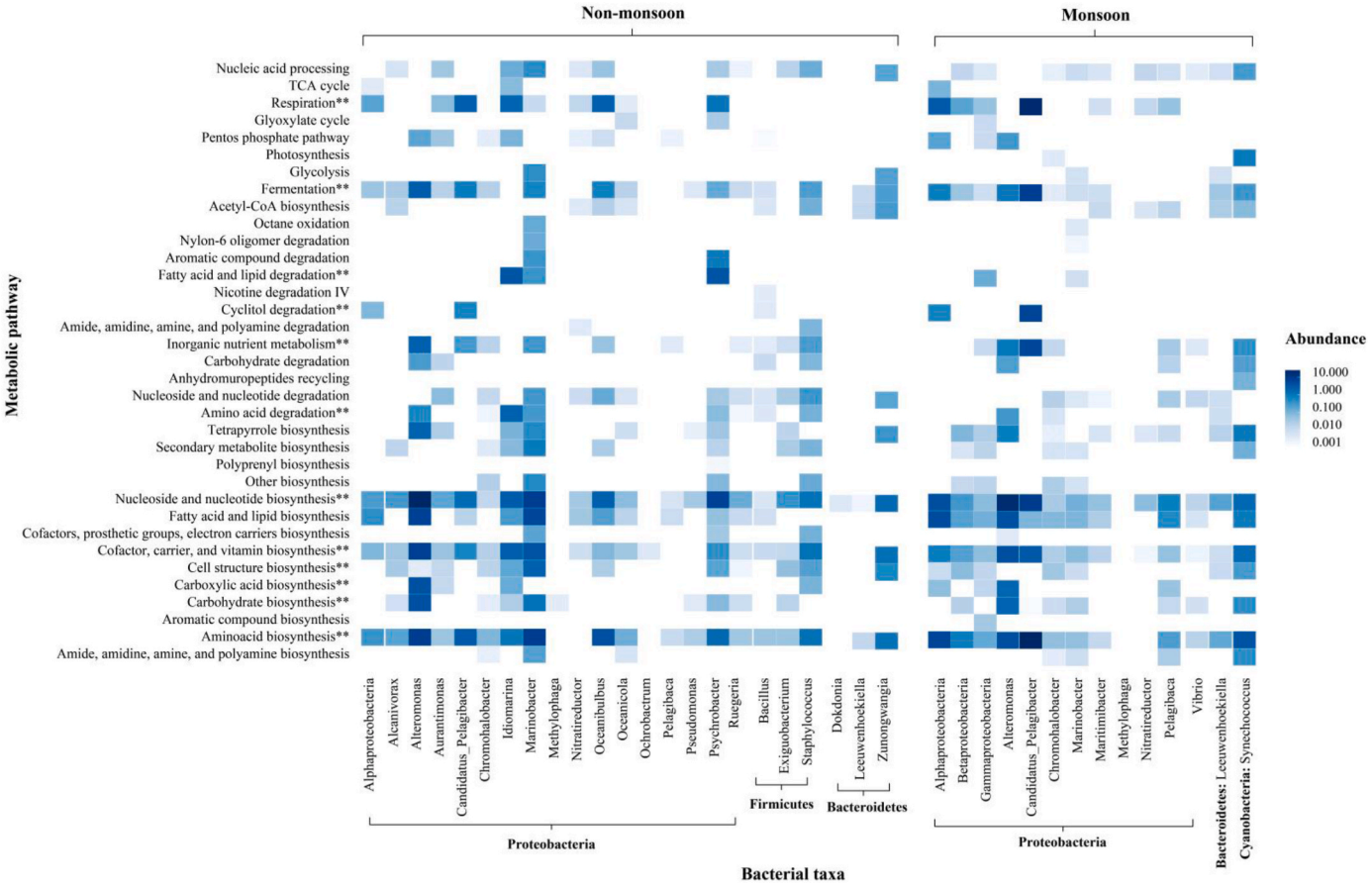

Fig. 5. Comparative heatmap of major bacterial taxa and its associated metabolic pathways during the non-monsoon and monsoon seasons, the significance of seasonal variations at $p < 0.05$* and $p < 0.001$** is also represented.

Significant contributions of Bacteroidetes genera *Leeuwenhoekiella* and Cyanobacteria *Synechococcus* were also observed.

In the non-monsoon season, *Marinobacter* and *Alteromonas* were the primary contributing organisms, predominantly associated with nucleoside and nucleotide biosynthesis (31.79%) and amino acid biosynthesis (18.12%) pathways. These were also common contributors to fatty acid and lipid biosynthesis and cofactor, carrier and vitamin biosynthesis in the non-monsoon season, which showed considerable activity with a slight decrease during the monsoon season (Fig. 5). In degradation pathways, *Idiomarina* and *Alteromonas* were the key players in amino acid degradation during the non-monsoon season. Similarly, *Idiomarina* and *Psychrobacter* primarily drive fatty acid and lipid degradation. The degradation of aromatic compounds, present in the non-monsoon season due to *Psychrobacter* and *Marinobacter*, was not observed during the monsoon. *Marinobacter* and *Zunongwangia* were the predominant bacteria in nucleic acid processing during the non-monsoon season (Fig. 5).

In the monsoon season, Candidatus *Pelagibacter*, Alphaproteobacteria unknown and *Alteromonas* were primarily responsible for a significant increase in amino acid biosynthesis ($P < 0.001$), which increased to 24.45% and nucleoside and nucleotide biosynthesis (21.84 %). These bacteria also dominantly contributed to lipid, cofactor and vitamin biosynthesis. The bacterial taxa Candidatus *Pelagibacter* and an unidentified Alphaproteobacteria were associated with the cyclitol degradation pathway during the monsoon season, which exhibited a marked increase with levels from 0.43 to 4.52, indicating a significant monsoon influence ($p < 0.001$). *Alteromonas* and Candidatus *Pelagibacter* were the main contributors to the increase in inorganic nutrient metabolism and carbohydrate degradation during the monsoon season ($p < 0.001$). Candidatus *Pelagibacter*, *Alteromonas*, Alphaproteobacteria unknown and Betaproteobacteria unknown were also associated with a notable increase in fermentation ($p < 0.001$) and heightened respiratory activities during the monsoon season, as indicated by a significant increase in the respiration pathway from 4.62 to 14.25 ($p < 0.001$). *Synechococcus* emerged as the main contributor during the monsoon season in nucleic acid processing, reflecting a shift in microbial activity and community composition between seasons (Fig. 5).

3.4. Impact of seasonal variations on bacterial taxonomic and functional potential

The primary productivity in the water column of the study area varied between 1.20 and 145.38 mg C m^{-3} d^{-1} during the non-monsoon season, while during the monsoon season, it ranged from 29.62 to 266.24 mg C m^{-3} d^{-1} (Parab et al., 2023). To understand the influence of these seasonal variations on the functional profiles of the bacterial communities regression analysis was performed. The analysis showed that there was no significant correlation between primary productivity and various enzymes including CAZymes families, peptidases and lipid metabolism during the non-monsoon season (Table 3 and Fig. A6). However, during the monsoon season, all three major groups of metabolic enzyme groups exhibited a significant correlation with primary productivity. In CAZymes, a significant correlation between primary productivity with AAs and GHs was observed. Significant correlations were also observed between primary productivity and several peptidase categories such as Aminopeptidase, Carboxypeptidase, Endopeptidase and specialise peptidases (Table 3). During the monsoon season, genes associated with lipid metabolism exhibited a significant correlation with primary productivity (Table 3 and Fig. A6). Multiple regression analysis of the carbohydrate ($p = 0.01$; $R^2 = 0.93$) and protein ($p = 0.05$; $R2 = 0.96$) metabolism genes also showed a statistically significant relationship with primary productivity (Table A4).

From these analyses, it is evident that the seasonal increase in productivity during the monsoon season has a positive influence on the functional profiles of the bacterial communities (Table 3).

Table 3
Regression analysis between primary productivity and genes of enzymes associated with different metabolic pathways across seasons.

Genes of different metabolic pathways	Non-monsoon	Monsoon
Linear regression analysis with genes associated with carbohydrate metabolism		
Auxiliary Activities enzymes	$p = 0.609$ ($R^2 = 0.071$)	**$p = 0.005$ ($R^2 = 0.891$)**
Carbohydrate-binding modules	$p = 0.599$ ($R^2 = 0.075$)	$p = 0.1$ ($R^2 = 0.532$)
Carbohydrate Esterases	$p = 0.066$ ($R^2 = 0.612$)	$p = 0.089$ ($R^2 = 0.556$)
Glycoside Hydrolases	$p = 0.417$ ($R^2 = 0.17$)	**$p = 0.01$ ($R^2 = 0.845$)**
Glycosyltransferases	$p = 0.564$ ($R^2 = 0.09$)	$p = 0.848$ ($R^2 = 0.01$)
Polysaccharide Lyases	$p = 0.833$ ($R^2 = 0.012$)	$p = 0.542$ ($R^2 = 0.1$)
Multiple regression analysis[a]		**$p = 0.01$ ($R^2 = 0.933$)**
Linear regression analysis with genes associated with protein metabolism		
Aminopeptidase	$p = 0.765$ ($R^2 = 0.025$)	**$p = 0.032$ ($R^2 = 0.721$)**
Carboxypeptidase	$p = 0.525$ ($R^2 = 0.109$)	**$p = 0.011$ ($R^2 = 0.833$)**
Endopeptidase	$p = 0.782$ ($R^2 = 0.021$)	**$p = 0.016$ ($R^2 = 0.801$)**
Specialise peptidases	$p = 0.772$ ($R^2 = 0.023$)	**$p = 0.045$ ($R^2 = 0.674$)**
Multiple regression analysis[a]		**$p = 0.050$ ($R^2 = 0.962$)**
Linear regression analysis with genes associated with lipid metabolism		
Initial Activation and Modification	$p = 0.978$ ($R^2 = 0.00$)	$p = 0.796$ ($R^2 = 0.019$)
β-Oxidation Cycle	$p = 0.173$ ($R^2 = 0.407$)	**$p = 0.003$ ($R^2 = 0.909$)**
Aldehyde and Alcohol Metabolism	$p = 0.563$ ($R^2 = 0.09$)	**$p = 0.025$ ($R^2 = 0.753$)**
CoA Transfer and Shuttle Enzymes	$p = 0.599$ ($R^2 = 0.075$)	**$p = 0.038$ ($R^2 = 0.699$)**
Oxidative Enzymes	$p = 0.416$ ($R^2 = 0.17$)	$p = 0.416$ ($R^2 = 0.17$)
Reductive Processes	$p = 0.146$ ($R^2 = 0.448$)	Not represented
Multiple regression analysis[a]		$p = 0.10$ ($R^2 = 0.922$)

Note: significant p-values and R^2 values are highlighted as bold fonts.
[a] : Multiple regression analyses with PP and the gene abundance exhibiting significance in the linear regression analysis.

4. Discussion

4.1. Influence of seasonal variation in primary productivity on bacterial diversity and composition

The EAS is characterized by monsoonal variations which are reported to influence primary productivity, leading to the generation of a higher amount of organic matter in EAS during monsoon season, compared to non-monsoon season (Shetye et al., 2022). Bacterial diversity studies in the EAS, based on cultivable bacterial assessments (Anas et al., 2021a; a; Anas et al., 2021b; Parab et al., 2022, 2024) as well as through 16S rRNA amplicon sequencing-based metagenomics studies (Parvathi et al., 2019; Paingankar et al., 2020; Parab et al., 2023), have reported the bacterial diversity in this region (Table A5). The studies based on cultivable diversity and phylogenetic analysis from coastal regions along the EAS largely reported the presence of major phyla such as Proteobacteria, Bacteroidetes, Firmicutes and Actinobacteria. Bacterial taxonomy studied from this region to understand the seasonal variation using 16S rRNA amplicon sequencing approach reported the presence of taxa such as Proteobacteria, Bacteroidetes, Firmicutes, Cyanobacteria, Actinobacteria from both seasons. These studies also revealed the presence of candidatus phyla and an increased number of phyla during the monsoon season. Analysis of the functional profiles,

carried out using predictive metabolic pathway analyses, revealed the prevalence of heterotrophic activities associated with organic matter utilisation, xenobiotic degradation and nitrogen cycle. However, the functional profiles of bacterial communities and the influence of seasonal changes in productivity are limited in this region. Hence, in this study we used shotgun metagenomics, to explore the functional profile of bacterial communities at the C-Max depths along the EAS.

Inferences from this study, during the non-monsoon and monsoon seasons revealed a significant influence of DO, primary productivity and fluorescence on the bacterial diversity, community structure and associated functional profiles (Figs. 1 and 4). During both seasons, three dominant bacterial phyla were observed including Proteobacteria, Bacteroidetes and Actinobacteria. These are largely reported from various marine regions and are known to play pivotal roles in various biogeochemical recycling processes (Fuhrman et al., 2015; Sun et al., 2020; Castillo et al., 2022; Arandia-Gorostidi et al., 2022). The Proteobacteria phylum, specifically the Gammaproteobacteria class, displayed the most substantial seasonal variation and higher metabolic potential. The Gammaproteobacteria play a crucial role as primary decomposers during phytoplankton blooms, where they are responsible for breaking down complex organic materials, including polysaccharides and proteins (Francis et al., 2021). Genera like *Idiomarina* and *Marinobacter* showed significant reductions in their relative abundance from the non-monsoon to the monsoon season. Such declines in specific taxa could be attributed to various factors, including competition for resources, predation, or changes in water parameters brought about by the monsoon. Conversely, certain genera within the Alphaproteobacteria class, like *Nitratireductor* and *Pelagibaca*, exhibited an increased abundance during the monsoon season, suggesting that these taxa might have ecological or metabolic advantages under increased organic load during monsoonal conditions (Labbé et al., 2004; Tseng et al., 2015; Thiele et al., 2018). An increase in *Alteromonas* genera, which is known for its utilisation of algal polysaccharides (Neumann et al., 2015; Jain et al., 2020; Balmonte et al., 2024) was also observed in the monsoon season. Seasonal shifts were also observed in Bacteroidetes genera *Zunongwangia*, whose abundance increased during the non-monsoon season, which highlights its ability to break down complex organic matter (Williams et al., 2013; Reintjes et al., 2017). On the other hand, Bacteroidetes genera *Leeuwenhoekiella* increased during the monsoon season, which shows its resilience or adaptability to changing conditions (Gifford et al., 2020). Within the Actinobacteria phylum, the increase in *Nocardioides'* relative abundance from the non-monsoon to the monsoon season could imply a role for this genus in decomposing complex organic matter, a function often associated with Actinobacteria in marine environments (Yaradoddi et al., 2021).

Bacterial genera from phylum Firmicutes were only detected during the non-monsoon season, possibly due to environmental stresses or competitive interactions (Hede and Khandeparker, 2020). Interestingly, the Cyanobacteria phylum, represented by the genus *Synechococcus*, increases during the monsoon season (Fig. 2). This observed increase in Cyanobacterial genera was also evident in previous studies based on 16S rRNA amplicon sequencing (Anas et al., 2021a; Parab et al., 2023). As primary producers, Cyanobacteria play a crucial role in marine carbon cycling (Huisman et al., 2018; Liu et al., 2022). Their surge in the monsoon season suggests that the autotrophic bacterial community proliferated due to increased nutrient availability and conditions favourable for photosynthesis during the monsoon (Bachmann et al., 2018; Liu et al., 2022; De La Iglesia-Vélez et al., 2024). However, the nondetection of phyla such as cyanobacteria during the non-monsoon season and Firmicutes in the monsoon season may be due to the methodological constrictions associated with the MetaPhlAn2 taxonomic classification used in this study (Miossec et al., 2020). As these bacterial taxa were reported in the earlier study based on targeted 16S rRNA gene sequencing from this study region (Parab et al., 2023). MetaPhlAn2 is a popular taxonomic profiling tool for the analysis of shotgun metagenomic data, which is efficient and robust for detecting taxa present in higher abundance. However, it is not equipped to perform de novo variant calling and hence cannot identify sub-specific level diversity. These predicted taxonomic profiles also depend on the pre-defined marker gene databases, are less sensitive and are not efficient in identifying taxa present in low abundance. Utilizing sensitive pipelines that rely on whole-genome alignments, can provide a nearly complete understanding of the microbial community structure (Miossec et al., 2020). However, the observed seasonal variations in bacterial taxa, distribution and each of their functional diversity underscore the dynamic nature of microbial communities in marine ecosystems (Figs. 2 and 5). These shifts can offer valuable insights into the ecological and biogeochemical processes that govern the distribution of microbial communities in these environments.

4.2. *Influence of seasonal variation in primary productivity on bacterial functional profiles*

Marine phytoplankton are a significant source of abundant organic carbon in the ocean. The increased dissolved organic matter and its implications for the biological pump and microbial loop largely rest on the degradation processes carried out by heterotrophic bacteria and the specific nature of the organic matter (Mühlenbruch et al., 2018; Kieft et al., 2021; Moran et al., 2022). This relationship and impact have been explored and discussed in lab-based studies (Khodse and Bhosle, 2011, 2013; Basu et al., 2013) and in productive marine ecosystems (Balmonte et al., 2021; Balmonte et al., 2024). Studies have also assessed the heterotrophic enzyme activities to establish links between organic matter degradation and bacterial interactions along the estuarine and coastal regions based on culturable diversity in the EAS (Divya et al., 2010; Parab et al., 2022). Building upon this, our shotgun metagenomics analyses have facilitated in-depth functional assessments, elucidating the bacterial taxa and their genes associated with enzymes and pathways involved in organic matter recycling, thereby enhancing our understanding of the functional importance of bacterial communities in the EAS region.

Shotgun metagenomics based on KEGG and EggNOG pathway analyses has shown that the presence of genes associated with metabolic pathways had the highest representation in both seasons, emphasizing the central role bacteria play in marine biogeochemical cycles. A significant increase in energy metabolism pathways could reflect the increased availability of organic substrates (Traving et al., 2017), possibly due to increased productivity as well as organic matter deposition from terrestrial runoff in the EAS during monsoon season (Maya et al., 2011; Pradhan et al., 2014). This surge in metabolic activity might facilitate the microbial communities' ability to rapidly exploit available resources during the monsoon. A significant increase in amino acid transport and metabolism pathways during the monsoon season possibly indicates an increased demand for protein synthesis, aligning with the observed increase in genes associated with translation processes (Fig. 3). This reiterates the notion that features of the monsoon season, such as increased organic matter concentrations due to increased primary productivity, influence the microbial succession and function, driving the microbial communities towards an intensified metabolic state and optimizing the growth and proliferation (Teeling et al., 2012; Teeling et al., 2016; Smriga et al., 2016; Traving et al., 2017). The representation of genes associated with genetic information processing during the monsoon season suggests increased genomic activity, possibly due to higher bacterial replication and repair processes during the dynamic monsoonal conditions (Smriga et al., 2016; Jurgensen et al., 2022). The increase in genes associated with translation activity (Fig. 3 and Fig. A2) emphasizes an upscaling of protein synthesis, which is a key process to meet metabolic demands during the monsoon season (Zhang et al., 2022).

CAZymes serve as the primary biochemical tool harnessed by marine microbial communities to decompose and break complex polysaccharides (Arnosti et al., 2021; Drula et al., 2022; Eigemann et al.,

2023). The seasonal variations in genes associated with CAZymes profiles within the sampling regions in the EAS offered valuable insights into the carbohydrate metabolism of the bacterial communities, which have not been previously investigated through metagenomics approaches. The consistent presence of Glycosyltransferases, which increase during the monsoon season (Fig. 4 and Fig. A3), underscores their pivotal role in marine carbohydrate dynamics, possibly facilitating the synthesis of complex polysaccharides as a carbon storage strategy (Thrash et al., 2017; Wei et al., 2021). This surge might be linked to a higher production of extracellular polymeric substances observed due to phytoplankton blooms, a phenomenon prevalent in the southwest monsoon season in the EAS (Ramaiah et al., 2000; Bhaskar and Bhosle, 2006). Glycoside hydrolases showed considerable fluctuation between non-monsoon and monsoon seasons, highlighting a likely increase in phytoplankton-derived organic matter degradation during the latter (Fig. 4b). This can be attributed to the enhanced presence of genes associated with enzymes GH13 and GH1, which are responsible for targeting starch and cellulose, respectively. This phenomenon could be attributed to the increased primary productivity and terrestrial influences which results in a carbohydrate-rich environment leading to an increased representation of associated metabolic processes (Sarma et al., 2014; Nageswar Rao et al., 2019; Paczkowska et al., 2019), demanding more robust enzymatic breakdown processes (Balmonte et al., 2019; Arnosti et al., 2021; Lloyd et al., 2024). The abundance of genes related to enzymes like GH38 and GH65, which target mannose and maltose respectively, could be indicative of the degradation of specific phytoplankton-derived monosaccharides in the water column during productive monsoon season (Mühlenbruch et al., 2018; James et al., 2019; Lloyd et al., 2024). Furthermore, terrestrial run-offs during monsoon due to increased rainfall introduce a range of organic compounds into the marine system in the EAS (Sarma et al., 2014; Nageswar Rao et al., 2019). The presence of genes associated with enzymes like GH17, targeting β-1, 3-Glucan and GH28, acting on pectin (Fig. 4), can be linked to the decomposition of terrestrial organic matter introduced to the marine system from the increased run-off (Krüger et al., 2019; Sidhu et al., 2023). The consistent occurrence of Polysaccharide lyases in both seasons highlights their persistent role in breaking down algal cell walls rich in polysaccharides such as pectin and alginate (Thrash et al., 2017; Balmonte et al., 2024).

The analysis of genes associated with peptidase enzymes has shown significant variations among peptidase types across monsoon and non-monsoon seasons (Fig. A4). The surge in the genes related to methionine aminopeptidase 1 in monsoon season could suggest that microbes are involved in the targeted cleavage of prevalent amino acids (Li et al., 2016; Cabello-Yeves et al., 2021; Balmonte et al., 2021; Kilgour et al., 2022; Shindoh et al., 2021). Genes related to glutamate carboxypeptidase increased during the monsoon season, potentially reflecting the bacterial adaptation to changes in the composition of organic matter. Carboxypeptidases were known to associate with sinking marine particles, playing a substantial role in the degradation of labile organic matter and facilitating carbon and nitrogen cycling in the ocean (Liu and Liu, 2018; Dithugoe et al., 2023). The significant presence observed of genes related to lipid metabolism enzymes, such as acetaldehyde dehydrogenase and alcohol dehydrogenase during the non-monsoon season (Fig. A5). This underscores their role in the conversion of recalcitrant organic compounds to more labile forms, such as carboxylic acid (Landry et al., 2017; Thrash et al., 2017; Saw et al., 2020). The pronounced increase in the genes related to β-oxidation cycle enzymes during monsoon season underscores the microbial community's transition towards fatty acid degradation due to increased primary productivity rich in lipids and fatty acids (Liu and Liu, 2018; Usman et al., 2022; Loza et al., 2022). These findings underscore the complex interaction between seasonal shifts in the primary productivity with the bacterial taxa and its genes associated with various enzymes for the biogeochemical cycling of organic matter (James et al., 2019; Inomura et al., 2022; Jenkinson, 2023). The observed functional shifts with increased genes associated with carbohydrase, peptidase and lipase in the monsoon season, highlight the increased microbial activity, ensuring ecological stability in marine biogeochemical processes. The impact of primary productivity on bacterial functional profiles was confirmed to be statistically significant through linear and multiple regression analyses. This shows a significant influence of primary productivity on gene abundance of various CAZymes, protein and lipid metabolism enzymes (Table 3 and Fig. A6). This suggests that the seasonal variations in primary productivity not only alter the quantity but also the quality of available organic substrates, it drives the shifts in microbial processes thus controlling the biogeochemical processes in these ecosystems.

The HUMAnN2 analysis was performed to identify the major bacterial taxa associated with the seasonal variations in the functional profiles. In the non-monsoon season, *Marinobacter* and *Alteromonas* were the primary organisms contributing to the major metabolic pathways such as the nucleic acid and amino acid biosynthesis pathways. *Marinobacteria* and *Alteromonas* are associated with remineralising organic matter, playing significant roles in organic matter recycling (Landa et al., 2018; Stephens et al., 2020). These were also common contributors to fatty acid and lipid biosynthesis and cofactor, carrier and vitamin biosynthesis in the non-monsoon season. These major metabolic pathways were also predominant during the monsoon season, however, the bacterial taxa such as Candidatus *Pelagibacter* and an undefined Alphaproteobacteria were the major taxa involved in these metabolic pathways (Fig. 5). Metabolic processes associated with organic matter degradation were the second most dominant pathways in both the seasons with a relatively lower representation in the monsoon season. The bacterial taxa *Idiomarina* and *Psychrobacter* were exclusively responsible for fatty acid and lipid degradation pathways during the non-monsoon season, while *Alteromonas* contributed to these pathways in both seasons. This suggests that *Idiomarina* and *Psychrobacter* are particularly adapted to the less productive conditions of the non-monsoon season (Rizzo and Lo Giudice, 2020; Anas et al., 2021b), whereas *Alteromonas* exhibits metabolic versatility, allowing them to thrive and participate in lipid degradation regardless of seasonal changes. During the monsoon season, undefined Gammaproteobacteria was observed to be the key player in the degradation pathways. In the monsoon season, the metabolic pathways such as inorganic nutrient metabolism and carbohydrate degradation, fermentation and respiratory process were also dominant. Candidatus *Pelagibacter*, *Alteromonas*, Alphaproteobacteria unknown and Betaproteobacteria and *Synechococcus* were largely responsible for these functional activities reflecting a shift in microbial activity and community composition between seasons (Fig. 5). The marked increase in Candidatus *Pelagibacter* and an unidentified Alphaproteobacteria associated with the cyclitol degradation pathway during the monsoon season underscores the significant impact of monsoonal nutrient enrichment and organic matter input on microbial metabolic activities. Cyclitols, Algae and plant-derived sugar alcohols become more abundant during the monsoon, providing a rich substrate for these bacteria (Talbot et al., 2008; Bauersachs et al., 2017; Lehmann et al., 2020). This seasonal shift highlights their ecological role in sugar alcohol degradation and carbon cycling (Lehmann et al., 2020), demonstrating the dynamic response of microbial communities to environmental changes. Statistical analysis also showed that seasonal increase in productivity during the monsoon season significantly positively influences the functional profiles of the bacterial communities (Table 3). Further studies to determine the metagenome-assembled genomes and direct functional information based on transcriptome, protein or metabolite expression would enhance our understanding of the microbial metabolic pathways and provide an in-depth understanding of their role in organic matter utilisation and long-term storage along the EAS.

5. Conclusion

In this study, we employed shotgun metagenomics to uncover the bacterial diversity and their functional dynamics in EAS. Seasonal shifts,

especially from non-monsoon to monsoon season were observed in bacterial taxonomic composition and the genes associated with their metabolic pathways. Significant seasonal variations in the bacterial community structure were observed at the phylum and genera levels. Among the major phylum reported from the study there was a seasonal change in the taxanomic compostion at genera level in Proteobacteria, Acrinobacteria and Bacterodites. The Firmicutes taxa such as *Bacillus* and *Staphylococcus* which were observed to play an important role in the non-monsson season were not detected in the monsoon season. The prominent bacterial taxa such as *Idiomarina*, *Marinobacter* and *Alteromonas* during the non-monsoon season were associated with nucleotide and amino acid biosynthesis and degradation pathways. During the monsoon, the dominance of *Alteromonas*, Candidatus *Pelagibacter* and *Synechococcus* underscores a shift towards amino acid and lipid biosynthesis, fermentation and inorganic nutrient metabolism, reflecting that these bacterial taxa proliferate in these highly productive environmental conditions. Furthermore, notable differences observed in the gene abundance of CAZymes, peptidases and lipid utilisation pathways in the study underscore the integral roles these bacterial communities play in marine biogeochemical cycles in the EAS. These seasonal variations are shown to be largely associated with seasonal changes in primary productivity, which underscores its complex interaction and influence on the bacterial communities and its functional profiles in the EAS.

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CRediT authorship contribution statement

Ashutosh Shankar Parab: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation. **Cathrine Sumathi Manohar:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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References

Anas, A., Chekidhenkuzhiyil, J., Nilayangod, C., Krishna, K., Raju, G.T., 2021a. Prevalence of obligate and facultative anaerobic bacteria in the mudbank along the southwest coast of India. Regional Studies in Marine Science 42, 101660. https://doi.org/10.1016/j.rsma.2021.101660.

Anas, A., B T, E.M., C, J., Chandran, C., V, P.V., Narayanan, S., U, A.J.K., 2021b. Microbial community shifts along an estuarine to open ocean continuum. Regional Studies in Marine Science 41, 101587. https://doi.org/10.1016/j.rsma.2020.101587.

Arandia-Gorostidi, N., Krabberød, A.K., Logares, R., Deutschmann, I.M., Scharek, R., Morán, X.A.G., González, F., Alonso-Sáez, L., 2022. Novel interactions between phytoplankton and bacteria shape microbial seasonal dynamics in coastal ocean waters. Front. Mar. Sci. 9, 901201 https://doi.org/10.3389/fmars.2022.901201.

Arnosti, C., Wietz, M., Brinkhoff, T., Hehemann, J.-H., Probandt, D., Zeugner, L., Amann, R., 2021. The biogeochemistry of marine polysaccharides: sources, inventories and bacterial drivers of the carbohydrate cycle. Ann. Rev. Mar. Sci 13 (1), 81–108. https://doi.org/10.1146/annurev-marine-032020-012810.

Bachmann, J., Heimbach, T., Hassenrück, C., Kopprio, G.A., Iversen, M.H., Grossart, H. P., Gärdes, A., 2018. Environmental drivers of free-living vs. Particle-attached bacterial community composition in the Mauritania upwelling system. Front. Microbiol. 9. https://www.frontiersin.org/articles/10.3389/fmicb.2018.02836.

Balmonte, J.P., Buckley, A., Hoarfrost, A., Ghobrial, S., Ziervogel, K., Teske, A., Arnosti, C., 2019. Community structural differences shape microbial responses to high molecular weight organic matter. Environ. Microbiol. 21 (2), 557–571. https://doi.org/10.1111/1462-2920.14485.

Balmonte, J.P., Giebel, H., Arnosti, C., Simon, M., Wietz, M., 2024. Distinct bacterial succession and functional response to alginate in the South, Equatorial, and North Pacific ocean. Environ. Microbiol. 26 (3), e16594 https://doi.org/10.1111/1462-2920.16594.

Balmonte, J.P., Simon, M., Giebel, H., Arnosti, C., 2021. A sea change in microbial enzymes: heterogeneous latitudinal and depth-related gradients in bulk water and particle-associated enzymatic activities from 30°S to 59°N in the Pacific Ocean. Limnol. Oceanogr. 66 (9), 3489–3507. https://doi.org/10.1002/lno.11894.

Basu, S., Deobagkar, D.D., Matondkar, S.P., Furtado, I., 2013. Culturable bacterial flora associated with the dinoflagellate green Noctiluca miliaris during active and declining bloom phases in the northern Arabian Sea. Microb. Ecol. 65 (4), 934–954. https://doi.org/10.1007/s00248-012-0148-1.

Bauersachs, T., Talbot, H.M., Sidgwick, F., Sivonen, K., Schwark, L., 2017. Lipid biomarker signatures as tracers for harmful cyanobacterial blooms in the Baltic Sea. PLoS One 12 (10), e0186360. https://doi.org/10.1371/journal.pone.0186360.

Bhaskar, P.V., Bhosle, N.B., 2006. Dynamics of transparent exopolymeric particles (TEP) and particle-associated carbohydrates in the Dona Paula Bay, west coast of India. J. Earth Syst. Sci. 115 (4), 403–413. https://doi.org/10.1007/BF02702869.

Bolger, A.M., Lohse, M., Usadel, B., 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30 (15), 2114–2120. https://doi.org/10.1093/bioinformatics/btu170.

Brown, S.A., Balmonte, J.P., Hoarfrost, A., Ghobrial, S., Arnosti, C., 2022. Depth-related patterns in microbial community responses to complex organic matter in the western North Atlantic Ocean. Biogeosciences 19 (24), 5617–5631. https://doi.org/10.5194/bg-19-5617-2022.

Cabello-Yeves, P.J., Callieri, C., Picazo, A., Mehrshad, M., Haro-Moreno, J.M., Roda-Garcia, J.J., Dzhembekova, N., Slabakova, V., Slabakova, N., Moncheva, S., Rodriguez-Valera, F., 2021. The microbiome of the Black Sea water column analyzed by shotgun and genome centric metagenomics. Environmental Microbiome 16 (1), 5. https://doi.org/10.1186/s40793-021-00374-1.

Cantalapiedra, C.P., Hernández-Plaza, A., Letunic, I., Bork, P., Huerta-Cepas, J., 2021. Eggnog-mapper v2: functional annotation, orthology assignments, and domain prediction at the metagenomic scale. Mol. Biol. Evol. 38 (12), 5825–5829. https://doi.org/10.1093/molbev/msab293.

Castillo, D.J., Dithugoe, C.D., Bezuidt, O.K., Makhalanyane, T.P., 2022. Microbial ecology of the southern ocean. FEMS (Fed. Eur. Microbiol. Soc.) Microbiol. Ecol. 98 (11), fiac123 https://doi.org/10.1093/femsec/fiac123.

Cullen, J.J., 2015. Subsurface chlorophyll maximum layers: enduring enigma or mystery solved? Annu. Rev. Mar. Sci. 7, 207–239. https://doi.org/10.1146/annurev-marine-010213-135111.

De La Iglesia-Vélez, B., Díaz-Pérez, L., Acuña, J.L., Morán, X.A.G., 2024. Spatial and seasonal variability of picoplankton abundance and growth rates in the southern Bay of Biscay. Mar. Environ. Res. 194, 106331 https://doi.org/10.1016/j.marenvres.2023.106331.

Delgadillo-Nuño, E., Teira, E., Pontiller, B., Lundin, D., Joglar, V., Pedrós-Alió, C., Fernández, E., Pinhassi, J., Martínez-García, S., 2024. Coastal upwelling systems as dynamic mosaics of bacterioplankton functional specialization. Front. Mar. Sci. 10, 1259783 https://doi.org/10.3389/fmars.2023.1259783.

DeVries, T., 2022. The ocean carbon cycle. Annu. Rev. Environ. Resour. 47 (1), 317–341. https://doi.org/10.1146/annurev-environ-120920-111307.

Dithugoe, C.D., Bezuidt, O.K.I., Cavan, E.L., Froneman, W.P., Thomalla, S.J., Makhalanyane, T.P., 2023. Bacteria and archaea regulate particulate organic matter export in suspended and sinking marine particle fractions. mSphere 8 (3), e00420–e00422. https://doi.org/10.1128/msphere.00420-22.

Divya, B., Soumya, K.V., Nair, S., 2010. 16SrRNA and enzymatic diversity of culturable bacteria from the sediments of oxygen minimum zone in the Arabian Sea. Antonie Leeuwenhoek 98 (1), 9–18. https://doi.org/10.1007/s10482-010-9423-7.

Drula, E., Garron, M.-L., Dogan, S., Lombard, V., Henrissat, B., Terrapon, N., 2022. The carbohydrate-active enzyme database: functions and literature. Nucleic Acids Res. 50 (D1), D571–D577. https://doi.org/10.1093/nar/gkab1045.

Eigemann, F., Rahav, E., Grossart, H.-P., Aharonovich, D., Voss, M., Sher, D., 2023. Phytoplankton producer species and transformation of released compounds over time define bacterial communities following phytoplankton dissolved organic matter pulses. Appl. Environ. Microbiol. 89 (7), e00539 https://doi.org/10.1128/aem.00539-23, 23.

Francis, B., Urich, T., Mikolasch, A., Teeling, H., Amann, R., 2021. North Sea spring bloom-associated Gammaproteobacteria fill diverse heterotrophic niches. Environmental Microbiome 16 (1), 15. https://doi.org/10.1186/s40793-021-00385-y.

Franzosa, E.A., McIver, L.J., Rahnavard, G., Thompson, L.R., Schirmer, M., Weingart, G., Lipson, K.S., Knight, R., Caporaso, J.G., Segata, N., Huttenhower, C., 2018. Species-level functional profiling of metagenomes and metatranscriptomes. Nat. Methods 15 (11), 962–968. https://doi.org/10.1038/s41592-018-0176-y.

Fuhrman, J.A., Cram, J.A., Needham, D.M., 2015. Marine microbial community dynamics and their ecological interpretation. Nat. Rev. Microbiol. 13 (3), 133–146. https://doi.org/10.1038/nrmicro3417.

Galloway-Peña, J., Hanson, B., 2020. Tools for analysis of the microbiome. Dig. Dis. Sci. 65 (3), 674–685. https://doi.org/10.1007/s10620-020-06091-y.

Gao, P., Du, G., Zhao, D., Wei, Q., Zhang, X., Qu, L., Gong, X., 2021. Influences of seasonal monsoons on the taxonomic composition and diversity of bacterial community in the eastern tropical indian ocean. Front. Microbiol. 11 https://doi.org/10.3389/fmicb.2020.615221.

Garg, S., Gauns, M., Pratihary, A.K., 2024. Response of oceanic subsurface chlorophyll maxima to environmental drivers in the Northern Indian Ocean. Environ. Res. 240, 117528 https://doi.org/10.1016/j.envres.2023.117528.

Gifford, S.M., Zhao, L., Stemple, B., DeLong, K., Medeiros, P.M., Seim, H., Marchetti, A., 2020. Microbial niche diversification in the Galapagos archipelago and its response to el niño. Front. Microbiol. 11. https://www.frontiersin.org/articles/10.3389/fmicb.2020.575194.

Gupta, M., Williams, R.G., Lauderdale, J.M., Jahn, O., Hill, C., Dutkiewicz, S., Follows, M.J., 2022. A nutrient relay sustains subtropical ocean productivity. Proc. Natl. Acad. Sci. USA 119 (41), e2206504119. https://doi.org/10.1073/pnas.2206504119.

Gurevich, A., Saveliev, V., Vyahhi, N., Tesler, G., 2013. QUAST: quality assessment tool for genome assemblies. Bioinformatics 29 (8), 1072–1075. https://doi.org/10.1093/bioinformatics/btt086.

Hammer, Ø., Harper, D.A.T., 2006. Paleontological Data Analysis. Blackwell Pub.

Hede, N., Khandeparker, L., 2020. Extracellular polymeric substances mediate the coaggregation of aquatic biofilm-forming bacteria. Hydrobiologia 847 (20), 4249–4272. https://doi.org/10.1007/s10750-020-04411-x.

Huisman, J., Codd, G.A., Paerl, H.W., Ibelings, B.W., Verspagen, J.M.H., Visser, P.M., 2018. Cyanobacterial blooms. Nat. Rev. Microbiol. 16 (8), 471–483. https://doi.org/10.1038/s41579-018-0040-1.

Inomura, K., Deutsch, C., Jahn, O., Dutkiewicz, S., Follows, M.J., 2022. Global patterns in marine organic matter stoichiometry driven by phytoplankton ecophysiology. Nat. Geosci. 15 (12), 1034–1040. https://doi.org/10.1038/s41561-022-01066-2.

Jain, A., Krishnan, K.P., Begum, N., Singh, A., Thomas, F.A., Gopinath, A., 2020. Response of bacterial communities from Kongsfjorden (Svalbard, arctic ocean) to macroalgal polysaccharide amendments. Mar. Environ. Res. 155, 104874 https://doi.org/10.1016/j.marenvres.2020.104874.

James, A.K., Kelly, L.W., Nelson, C.E., Wilbanks, E.G., Carlson, C.A., 2019. Elevated *p* CO_2 alters marine heterotrophic bacterial community composition and metabolic potential in response to a pulse of phytoplankton organic matter. Environ. Microbiol. 21 (2), 541–556. https://doi.org/10.1111/1462-2920.14484.

Jenkinson, I.R., 2023. Plankton genes and extracellular organic substances in the ocean. J. Mar. Sci. Eng. 11 (4), 783. https://doi.org/10.3390/jmse11040783.

Jurgensen, S.K., Roux, S., Schwenck, S.M., Stewart, F.J., Sullivan, M.B., Brum, J.R., 2022. Viral community analysis in a marine oxygen minimum zone indicates increased potential for viral manipulation of microbial physiological state. ISME J. 16 (4), 972–982. https://doi.org/10.1038/s41396-021-01143-1.

Khodse, V.B., Bhosle, N.B., 2011. Bacterial utilization of size-fractionated dissolved organic matter. Aquat. Microb. Ecol. 64 (3), 299–309. https://doi.org/10.3354/ame01529.

Khodse, V.B., Bhosle, N.B., 2013. Distribution, origin and transformation of amino sugars and bacterial contribution to estuarine particulate organic matter. Continent. Shelf Res. 68, 33–42. https://doi.org/10.1016/j.csr.2013.08.004.

Kieft, B., Li, Z., Bryson, S., Hettich, R.L., Pan, C., Mayali, X., Mueller, R.S., 2021. Phytoplankton exudates and lysates support distinct microbial consortia with specialized metabolic and ecophysiological traits. Proc. Natl. Acad. Sci. USA 118 (41), e2101178118. https://doi.org/10.1073/pnas.2101178118.

Kilgour, D.B., Novak, G.A., Sauer, J.S., Moore, A.N., Dinasquet, J., Amiri, S., Franklin, E. B., Mayer, K., Winter, M., Morris, C.K., Price, T., Malfatti, F., Crocker, D.R., Lee, C., Cappa, C.D., Goldstein, A.H., Prather, K.A., Bertram, T.H., 2022. Marine gas-phase sulfur emissions during an induced phytoplankton bloom. Atmos. Chem. Phys. 22 (2), 1601–1613. https://doi.org/10.5194/acp-22-1601-2022.

Kindt, R., Coe, R., 2005. Tree Diversity Analysis: a Manual and Software for Common Statistical Methods for Ecological and Biodiversity Studies. World Agroforestry Centre. ISBN 92-9059-179-X.

Krüger, K., Chafee, M., Ben Francis, T., Glavina Del Rio, T., Becher, D., Schweder, T., Amann, R.I., Teeling, H., 2019. In marine *Bacteroidetes* the bulk of glycan degradation during algae blooms is mediated by few clades using a restricted set of genes. ISME J. 13 (11), 2800–2816. https://doi.org/10.1038/s41396-019-0476-y.

Labbé, N., Parent, S., Villemur, R., 2004. *Nitratireductor aquibiodomus* gen. Nov., sp. Nov., a novel alpha-proteobacterium from the marine denitrification system of the Montreal Biodome (Canada). Int. J. Syst. Evol. Microbiol. 54 (Pt 1), 269–273. https://doi.org/10.1099/ijs.0.02793-0.

Landa, M., Blain, S., Harmand, J., Monchy, S., Rapaport, A., Obernosterer, I., 2018. Major changes in the composition of a Southern Ocean bacterial community in response to diatom-derived dissolved organic matter. FEMS (Fed. Eur. Microbiol. Soc.) Microbiol. Ecol. 94 (4) https://doi.org/10.1093/femsec/fiy034.

Landry, Z., Swan, B.K., Herndl, G.J., Stepanauskas, R., Giovannoni, S.J., 2017. Sar202 genomes from the dark ocean predict pathways for the oxidation of recalcitrant dissolved organic matter. mBio 8 (2). https://doi.org/10.1128/mbio.00413-17.

Langmead, B., Salzberg, S.L., 2012. Fast gapped-read alignment with Bowtie 2. Nat. Methods 9 (4), 357–359. https://doi.org/10.1038/nmeth.1923.

Lehmann, M.F., Carstens, D., Deek, A., McCarthy, M., Schubert, C.J., Zopfi, J., 2020. Amino acid and amino sugar compositional changes during *in vitro* degradation of algal organic matter indicate rapid bacterial re-synthesis. Geochem. Cosmochim. Acta 283, 67–84. https://doi.org/10.1016/j.gca.2020.05.025.

Li, D., Luo, R., Liu, C.-M., Leung, C.-M., Ting, H.-F., Sadakane, K., Yamashita, H., Lam, T.-W., 2016. MEGAHIT v1.0: a fast and scalable metagenome assembler driven by advanced methodologies and community practices. Methods 102, 3–11. https://doi.org/10.1016/j.ymeth.2016.02.020.

Li, M., Jain, S., Dick, G.J., 2016. Genomic and transcriptomic resolution of organic matter utilization among deep-sea bacteria in Guaymas basin hydrothermal plumes. Front. Microbiol. 7 https://doi.org/10.3389/fmicb.2016.01125.

Li, W., Fu, L., Niu, B., Wu, S., Wooley, J., 2012. Ultrafast clustering algorithms for metagenomic sequence analysis. Briefings Bioinf. 13 (6), 656–668. https://doi.org/10.1093/bib/bbs035.

Liu, S., Liu, Z., 2018. Free extracellular enzymes dominate initial peptide hydrolysis in coastal seawater. Mar. Chem. 199, 37–43. https://doi.org/10.1016/j.marchem.2018.01.005.

Liu, X., Xie, N., Li, J., Bai, M., Sen, B., Wang, G., 2022. Potential contribution of coastal upwelling to carbon sink through interaction between cyanobacteria and microbial eukaryotes. Water 14 (19), 3097. https://doi.org/10.3390/w14193097.

Lloyd, C.C., Brown, S., Balmonte, J.P., Hoarfrost, A., Ghobrial, S., Arnosti, C., 2022. Particles act as 'specialty centers' with expanded enzymatic function throughout the water column in the western North Atlantic. Front. Microbiol. 13, 882333 https://doi.org/10.3389/fmicb.2022.882333.

Lloyd, C.C., Brown, S., Giljan, G., Ghobrial, S., Vidal-Melgosa, S., Steinke, N., Hehemann, J.-H., Amann, R., Arnosti, C., 2024. Correlations among carbohydrate inventories, enzyme activities, and microbial communities in the western North Atlantic Ocean. https://doi.org/10.5194/egusphere-2024-615.

Loza, A., García-Guevara, F., Segovia, L., Escobar-Zepeda, A., Sanchez-Olmos, M.D.C., Merino, E., Sanchez-Flores, A., Pardo-Lopez, L., Juarez, K., Gutierrez-Rios, R.-M., 2022. Definition of the metagenomic profile of ocean water samples from the Gulf of Mexico based on comparison with reference samples from sites worldwide. Front. Microbiol. 12, 781497 https://doi.org/10.3389/fmicb.2021.781497.

Manni, M., Berkeley, M.R., Seppey, M., Simão, F.A., Zdobnov, E.M., 2021. Busco update: novel and streamlined workflows along with broader and deeper phylogenetic coverage for scoring of eukaryotic, prokaryotic, and viral genomes. Mol. Biol. Evol. 38 (10), 4647–4654. https://doi.org/10.1093/molbev/msab199.

Maya, M.V., Karapurkar, S.G., Naik, H., Roy, R., Shenoy, D.M., Naqvi, S.W.A., 2011. Intra-annual variability of carbon and nitrogen stable isotopes in suspended organic matter in waters of the western continental shelf of India. Biogeosciences 8 (11), 3441–3456. https://doi.org/10.5194/bg-8-3441-2011.

Moran, M.A., Kujawinski, E.B., Schroer, W.F., Amin, S.A., Bates, N.R., Bertrand, E.M., Braakman, R., Brown, C.T., Covert, M.W., Doney, S.C., Dyhrman, S.T., Edison, A.S., Eren, A.M., Levine, N.M., Li, L., Ross, A.C., Saito, M.A., Santoro, A.E., Segrè, D., Vardi, A., 2022. Microbial metabolites in the marine carbon cycle. Nature Microbiology 7 (4), 508–523. https://doi.org/10.1038/s41564-022-01090-3.

Moran, M.A., Kujawinski, E.B., Stubbins, A., Fatland, R., Aluwihare, L.I., Buchan, A., Crump, B.C., Dorrestein, P.C., Dyhrman, S.T., Hess, N.J., Howe, B., Longnecker, K., Medeiros, P.M., Niggemann, J., Obernosterer, I., Repeta, D.J., Waldbauer, J.R., 2016. Deciphering ocean carbon in a changing world. Proc. Natl. Acad. Sci. USA 113 (12), 3143–3151. https://doi.org/10.1073/pnas.1514645113.

Mühlenbruch, M., Grossart, H., Eigemann, F., Voss, M., 2018. Mini-review: phytoplankton-derived polysaccharides in the marine environment and their interactions with heterotrophic bacteria. Environ. Microbiol. 20 (8), 2671–2685. https://doi.org/10.1111/1462-2920.14302.

Miossec, M.J., Valenzuela, S.L., Pérez-Losada, M., Johnson, W.E., Crandall, K.A., Castro-Nallar, E., 2020. Evaluation of computational methods for human microbiome analysis using simulated data. PeerJ 8, e9688. https://doi.org/10.7717/peerj.9688.

Nageswar Rao, M., Ram, A., Pradhan, U.K., Siddaiah, V., 2019. Factors controlling organic matter composition and trophic state in seven tropical estuaries along the west coast of India. Environ. Geochem. Health 41 (2), 545–562. https://doi.org/10.1007/s10653-018-0150-8.

Neumann, A.M., Balmonte, J.P., Berger, M., Giebel, H., Arnosti, C., Voget, S., Simon, M., Brinkhoff, T., Wietz, M., 2015. Different utilization of alginate and other algal polysaccharides by marine *A lteromonas macleodii* ecotypes. Environ. Microbiol. 17 (10), 3857–3868. https://doi.org/10.1111/1462-2920.12862.

Paczkowska, J., Rowe, O.F., Figueroa, D., Andersson, A., 2019. Drivers of phytoplankton production and community structure in nutrient-poor estuaries receiving terrestrial organic inflow. Mar. Environ. Res. 151, 104778 https://doi.org/10.1016/j.marenvres.2019.104778.

Paingankar, M.S., Ahire, K., Mishra, P., Rajpathak, S., Deobagkar, D.D., 2020. Microbial diversity analysis in the oxygen minimum zones of the Arabian Sea using metagenomics approach. Current Sci. 118, 1042–1051.

Parab, A.S., Ghose, M.P., Manohar, C.S., 2023. Variations in bacterial community at Chlorophyll Maximum (C-max) depths along the west coast of India due to seasonal changes in the primary productivity [Preprint]. Microbiology. https://doi.org/10.1101/2023.07.05.547789.

Parab, A.S., Jagtap, A.S., Meena, R.M., Manohar, C.S., 2022. Bacterial dynamics along the west coast of India during the non-monsoon and monsoon season. Continent. Shelf Res. 251, 104876 https://doi.org/10.1016/j.csr.2022.104876.
Parks, D.H., Tyson, G.W., Hugenholtz, P., Beiko, R.G., 2014. STAMP: statistical analysis of taxonomic and functional profiles. Bioinformatics 30 (21), 3123–3124. https://doi.org/10.1093/bioinformatics/btu494.
Parvathi, A., Jasna, V., Aswathy, V.K., Nathan, V.K., Aparna, S., Balachandran, K.K., 2019. Microbial diversity in a coastal environment with co-existing upwelling and mud-banks along the south west coast of India. Mol. Biol. Rep. 46, 3113–3127. https://doi.org/10.1007/s11033-019-04766-y.
Paysan-Lafosse, T., Blum, M., Chuguransky, S., Grego, T., Pinto, B.L., Salazar, G.A., Bileschi, M.L., Bork, P., Bridge, A., Colwell, L., Gough, J., Haft, D.H., Letunić, I., Marchler-Bauer, A., Mi, H., Natale, D.A., Orengo, C.A., Pandurangan, A.P., Rivoire, C., Bateman, A., 2023. InterPro in 2022. Nucleic Acids Res. 51 (D1), D418–D427. https://doi.org/10.1093/nar/gkac993.
Pontiller, B., Pérez-Martínez, C., Bunse, C., Osbeck, C.M.G., González, J.M., Lundin, D., Pinhassi, J., 2021. Taxon-specific shifts in bacterial and archaeal transcription of dissolved organic matter cycling genes in a stratified fjord. mSystems 6 (6), e00575. https://doi.org/10.1128/mSystems.00575-21, 21.
Pradhan, U.K., Wu, Y., Shirodkar, P.V., Zhang, J., Zhang, G., 2014. Sources and distribution of organic matter in thirty five tropical estuaries along the west coast of India-a preliminary assessment. Estuar. Coast Shelf Sci. 151, 21–33. https://doi.org/10.1016/j.ecss.2014.09.010.
Quince, C., Walker, A.W., Simpson, J.T., Loman, N.J., Segata, N., 2017. Shotgun metagenomics, from sampling to analysis. Nat. Biotechnol. 35 (9), 833–844. https://doi.org/10.1038/nbt.3935.
Ramaiah, N., Sarma, V.V.S.S., Gauns, M., Kumar, M.D., Madhupratap, M., 2000. Abundance and relationship of bacteria with transparent exopolymer particles during the 1996 summer monsoon in the Arabian Sea. J. Earth Syst. Sci. 109 (4), 443–451. https://doi.org/10.1007/BF02708332.
Rawlings, N.D., Barrett, A.J., Thomas, P.D., Huang, X., Bateman, A., Finn, R.D., 2018. The MEROPS database of proteolytic enzymes, their substrates and inhibitors in 2017 and a comparison with peptidases in the PANTHER database. Nucleic Acids Res. 46 (D1), D624–D632. https://doi.org/10.1093/nar/gkx1134.
Reintjes, G., Arnosti, C., Fuchs, B.M., Amann, R., 2017. An alternative polysaccharide uptake mechanism of marine bacteria. ISME J. 11 (7), 1640–1650. https://doi.org/10.1038/ismej.2017.26.
Rizzo, C., Lo Giudice, A., 2020. The variety and inscrutability of polar environments as a resource of biotechnologically relevant molecules. Microorganisms 8 (9), 1422. https://doi.org/10.3390/microorganisms8091422.
Santhikrishnan, S., Jyothibabu, R., Albin, K.J., Alok, K.T., Karnan, C., Arunpandi, N., Camey, M.F., Gireesh Kumar, T.R., 2021. Biophysical implications of the freshwater influx over small spatial scale in the coastal waters along the southwest coast of India during the Southwest Monsoon. Continent. Shelf Res. 214, 104337 https://doi.org/10.1016/j.csr.2020.104337.
Sarma, V.V.S.S., Krishna, M.S., Prasad, V.R., Kumar, B.S.K., Naidu, S.A., Rao, G.D., Viswanadham, R., Sridevi, T., Kumar, P.P., Reddy, N.P.C., 2014. Distribution and sources of particulate organic matter in the Indian monsoonal estuaries during monsoon. J. Geophys. Res.: Biogeosciences 119 (11), 2095–2111. https://doi.org/10.1002/2014JG002721.
Saw, J.H.W., Nunoura, T., Hirai, M., Takaki, Y., Parsons, R., Michelsen, M., Longnecker, K., Kujawinski, E.B., Stepanauskas, R., Landry, Z., Carlson, C.A., Giovannoni, S.J., 2020. Pangenomics analysis reveals diversification of enzyme families and niche specialization in globally abundant SAR202 bacteria. mBio 11 (1), e02975. https://doi.org/10.1128/mBio.02975-19, 19.
Seemann, T., 2014. Prokka: rapid prokaryotic genome annotation. Bioinformatics 30 (14), 2068–2069. https://doi.org/10.1093/bioinformatics/btu153.
Shetye, S.S., Nandakumar, K., Kurian, S., Gauns, M., Shenoy, D.M., Naik, H., Vidya, P.J., Karapurkar, S.G., 2022. Organic carbon dynamics in the continental shelf waters of the eastern Arabian Sea. Environ. Monit. Assess. 194 (10), 716. https://doi.org/10.1007/s10661-022-10390-4.
Shindoh, S., Obayashi, Y., Suzuki, S., 2021. Induction of extracellular aminopeptidase production by peptides in some marine bacterial species. Microb. Environ. 36 (1), n/a. https://doi.org/10.1264/jsme2.ME20150.
Sidhu, C., Kirstein, I.V., Meunier, C.L., Rick, J., Fofonova, V., Wiltshire, K.H., Steinke, N., Vidal-Melgosa, S., Hehemann, J.-H., Huettel, B., Schweder, T., Fuchs, B.M., Amann, R.I., Teeling, H., 2023. Dissolved storage glycans shaped the community composition of abundant bacterioplankton clades during a North Sea spring phytoplankton bloom. Microbiome 11 (1), 77. https://doi.org/10.1186/s40168-023-01517-x.
Silori, S., Biswas, H., Chowdhury, M., Sharma, D., Magloire, M.-Y., Cardinal, D., 2022. Interannual variability in particulate organic matter distribution and its carbon stable isotope signatures from the western Indian shelf waters. Sci. Total Environ. 844, 157044 https://doi.org/10.1016/j.scitotenv.2022.157044.
Smriga, S., Fernandez, V.I., Mitchell, J.G., Stocker, R., 2016. Chemotaxis toward phytoplankton drives organic matter partitioning among marine bacteria. Proc. Natl. Acad. Sci. USA 113 (6), 1576–1581. https://doi.org/10.1073/pnas.1512307113.
Stephens, B.M., Opalk, K., Petras, D., Liu, S., Comstock, J., Aluwihare, L.I., Hansell, D.A., Carlson, C.A., 2020. Organic matter composition at ocean station papa affects its bioavailability, bacterioplankton growth efficiency and the responding taxa. Front. Mar. Sci. 7, 590273 https://doi.org/10.3389/fmars.2020.590273.
Sun, F., Wu, M., Wang, Y., Sun, C., Xu, Z., 2020. Diversity and potential function of bacterial communities in different upwelling systems. Estuar. Coast Shelf Sci. 237, 106698 https://doi.org/10.1016/j.ecss.2020.106698.
Talbot, H.M., Summons, R.E., Jahnke, L.L., Cockell, C.S., Rohmer, M., Farrimond, P., 2008. Cyanobacterial bacteriohopanepolyol signatures from cultures and natural environmental settings. Org. Geochem. 39 (2), 232–263. https://doi.org/10.1016/j.orggeochem.2007.08.006.
Teeling, H., Fuchs, B.M., Becher, D., Klockow, C., Gardebrecht, A., Bennke, C.M., Kassabgy, M., Huang, S., Mann, A.J., Waldmann, J., Weber, M., Klindworth, A., Otto, A., Lange, J., Bernhardt, J., Reinsch, C., Hecker, M., Peplies, J., Bockelmann, F. D., et al., 2012. Substrate-controlled succession of marine bacterioplankton populations induced by a phytoplankton bloom. Science 336 (6081), 608–611. https://doi.org/10.1126/science.1218344.
Teeling, H., Fuchs, B.M., Bennke, C.M., Krüger, K., Chafee, M., Kappelmann, L., Reintjes, G., Waldmann, J., Quast, C., Glöckner, F.O., Lucas, J., Wichels, A., Gerdts, G., Wiltshire, K.H., Amann, R.I., 2016. Recurring patterns in bacterioplankton dynamics during coastal spring algae blooms. Elife 5, e11888. https://doi.org/10.7554/eLife.11888.
Thiele, S., Basse, A., Becker, J.W., Lipski, A., Iversen, M.H., Mollenhauer, G., 2018. Microbial communities in the nepheloid layers and hypoxic zones of the Canary Current upwelling system. MicrobiologyOpen 8 (5), e00705. https://doi.org/10.1002/mbo3.705.
Thrash, J.C., Seitz, K.W., Baker, B.J., Temperton, B., Gillies, L.E., Rabalais, N.N., Henrissat, B., Mason, O.U., 2017. Metabolic roles of uncultivated bacterioplankton lineages in the northern Gulf of Mexico "dead zone.". mBio 8 (5), e01017. https://doi.org/10.1128/mBio.01017-17, 17.
Traving, S.J., Rowe, O., Jakobsen, N.M., Sørensen, H., Dinasquet, J., Stedmon, C.A., Andersson, A., Riemann, L., 2017. The effect of increased loads of dissolved organic matter on estuarine microbial community composition and function. Front. Microbiol. 8 https://doi.org/10.3389/fmicb.2017.00351.
Truong, D.T., Franzosa, E.A., Tickle, T.L., Scholz, M., Weingart, G., Pasolli, E., Tett, A., Huttenhower, C., Segata, N., 2015. MetaPhlAn2 for enhanced metagenomic taxonomic profiling. Nat. Methods 12 (10), 902–903. https://doi.org/10.1038/nmeth.3589.
Tseng, C.-H., Chiang, P.-W., Lai, H.-C., Shiah, F.-K., Hsu, T.-C., Chen, Y.-L., Wen, L.-S., Tseng, C.-M., Shieh, W.-Y., Saeed, I., Halgamuge, S., Tang, S.-L., 2015. Prokaryotic assemblages and metagenomes in pelagic zones of the South China Sea. BMC Genom. 16 (1), 219. https://doi.org/10.1186/s12864-015-1434-3.
UNESCO, 1994. Protocols for the Joint Global Ocean Flux Study (JGOFS), 29. Core Measurements, Scientific committee on Oceanic Research Manual and Guides, p. 170, 1994.
Usman, M., Zhao, S., Jeon, B.-H., Salama, E.-S., Li, X., 2022. Microbial β-oxidation of synthetic long-chain fatty acids to improve lipid biomethanation. Water Res. 213, 118164 https://doi.org/10.1016/j.watres.2022.118164.
Vijayan, A.K., Reddy, B.B., Sudheesh, V., Marathe, P.H., Nampoothiri, V.N., Harikrishnachari, N.V., Kavya, P., Gupta, G.V.M., Ramanamurthy, M.V., 2021. Phytoplankton community structure in a contrasting physico-chemical regime along the eastern Arabian Sea during the winter monsoon. J. Mar. Syst. 215, 103501 https://doi.org/10.1016/j.jmarsys.2020.103501.
Vijayan, J., Nathan, V.K., Ammini, P., Ammanamveetil, A.M.H., 2023. Bacterial diversity in the aquatic system in India based on metagenome analysis—a critical review. Environ. Sci. Pollut. Control Ser. 30 (11), 28383–28406. https://doi.org/10.1007/s11356-023-25195-2.
Vinayachandran, P.N.M., Masumoto, Y., Roberts, M.J., Huggett, J.A., Halo, I., Chatterjee, A., Amol, P., Gupta, G.V.M., Singh, A., Mukherjee, A., Prakash, S., Beckley, L.E., Raes, E.J., Hood, R., 2021. Reviews and syntheses: physical and biogeochemical processes associated with upwelling in the Indian Ocean. Biogeosciences 18 (22), 5967–6029. https://doi.org/10.5194/bg-18-5967-2021.
Wei, T., Zhao, C., Quareshy, M., Wu, N., Huang, S., Zhao, Y., Yang, P., Mao, D., Chen, Y., 2021. A glycolipid glycosyltransferase with broad substrate specificity from the marine bacterium "*Candidatus pelagibacter* sp." Strain htcc7211. Appl. Environ. Microbiol. 87 (14), e00326 https://doi.org/10.1128/AEM.00326-21, 21.
Williams, T.J., Wilkins, D., Long, E., Evans, F., DeMaere, M.Z., Raftery, M.J., Cavicchioli, R., 2013. The role of planktonic *Flavobacteria* in processing algal organic matter in coastal East Antarctica revealed using metagenomics and metaproteomics. Environ. Microbiol. 15 (5), 1302–1317. https://doi.org/10.1111/1462-2920.12017.
Yaradoddi, J.S., Kontro, M.H., Ganachari, S.V., Banapurmath, N.R., Oli, A., Katti, A.S., Sulochana, M.B., 2021. Actinobacteria in marine environments. In: Yaradoddi, J.S., Kontro, M.H., Ganachari, S.V. (Eds.), Actinobacteria: Ecology, Diversity, Classification and Extensive Applications. Springer Nature, pp. 21–38. https://doi.org/10.1007/978-981-16-3353-9_2.
Zhang, D., Li, S.H.-J., King, C.G., Wingreen, N.S., Gitai, Z., Li, Z., 2022. Global and gene-specific translational regulation in Escherichia coli across different conditions. PLoS Comput. Biol. 18 (10), e1010641 https://doi.org/10.1371/journal.pcbi.1010641.
Zhang, H., Yohe, T., Huang, L., Entwistle, S., Wu, P., Yang, Z., Busk, P.K., Xu, Y., Yin, Y., 2018. Dbcan2: a meta server for automated carbohydrate-active enzyme annotation. Nucleic Acids Res. 46 (W1), W95–W101. https://doi.org/10.1093/nar/gky418.

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