Oxygen Levels Differentially Attenuate the Structure and Diversity of Microbial Communities in the Oceanic Oxygen Minimal Zones

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Abstract

Global change mediated shifts in ocean temperature and circulation patterns, compounded by human activities, are leading to the expansion of marine oxygen minimum zones (OMZs) with concomitant alterations in nutrient and climate-active trace gas cycling. While many studies have reported distinct bacterial communities within OMZs, much of this research compares across depths rather with oxygen status and does not include eukayrotic microbes. Here, we investigated the Bay of Bengal (BoB) OMZ, where low oxygen conditions are persistent, but trace levels of oxygen remain (< 20 μM from 200 to 500m). As other environmental variables are similar between OMZ and non-OMZ (NOZ) stations, we compared the abundance, diversity, and community composition of several microbial groups (bacterioplankton, Labyrinthulomycetes, and fungi) across oxygen levels. While prokaryote abundance decreased with depth, no significant differences existed across oxygen groups. In contrast, Labyrinthulomycetes abundance was significantly higher in non-OMZ stations but did not change significantly with depth, while fungal abundance was patchy without clear depth or oxygen-related trends. Bacterial and fungal diversity was lower in OMZ stations at 500 meters, while Labyrinthulomycetes diversity only showed a depth-related profile, decreasing below the euphotic zone. Surprisingly, previously reported OMZ-associated bacterial taxa were not significantly more abundant at OMZ stations. Furthermore, compared to the bacterioplankton, fewer Labyrinthulomycetes and fungi taxa showed responses to oxygen status. Thus, this research identifies stronger oxygen-level linkages within the bacterioplankton than in the examined microeukaryotes.

Introduction

Oceanic deoxygenation mediated by global change has decreased oxygen levels by 2% in the last 50 years [1-5]; with oxygen declines below critical thresholds predicted to impact multiple marine biogeochemical cycles [6, 7]. This deoxygenation is contributing to the expansion of Oxygen Minimum Zones (OMZs), generally defined as midwater regions with oxygen concentrations less than 20 μM, which occur in upper bathyal depths (200-1200 m) where limited mixing is combined with oxygen drawdown. OMZs support complex microbial communities with distinct biogeochemical processes and rates compared to oxygenated waters [8-11]. OMZs are widespread, including in the eastern tropical North Pacific, the eastern tropical South Pacific, the Arabian Sea, and the Bay of Bengal, among others [8, 12]. However, anthropocentric alterations of the marine environment (e.g., ocean warming and eutrophication) are driving the vertical and horizontal expansion of OMZs, which are predicted to expand by 7.0 ± 5.6% by 2100 (RCP8.5 scenario, relative to 1850-1900) [13-16]. In most OMZs, oxygen concentrations are low enough to allow denitrification and/or anammox [17], transforming biologically available nitrogen (NH_4, NO_3^- and NO_2^-) to N_2, which is lost to the atmosphere and explaining 30-50% of fixed-nitrogen losses in OMZs [18-21]. Recent studies also suggest the presence of cryptic sulfur cycling in OMZs, where the production and consumption of reduced sulfur occurs at nearly equivalent rates [22-24]. Drawdown of oxygen allows these processes to happen in the water column; moreover, even low levels of oxygen may shape microbial communities and their functions in the mesopelagic zone.

A recent analysis of TARA Oceans data found that vertical oxygen gradients altered protist communities while exerting a modest effect on prokaryotes [25, 26]. However, these global-scale comparisons of microbial prokaryotes and eukaryotes cannot disentangle the impacts of oxygen from other spatial and depth-related changes in environmental variables, including temperature, pressure, nutrients, etc. [7, 12, 18, 25, 27-30]. Yet, determining oxygen's effect on microbiomes is critical to predicting the impacts of deoxygenation on future ocean microbiomes and biogeochemical rates

[31]. Furthermore, while prokaryotes have long been considered to be the major engines of marine biogeochemical cycles [32], their eukaryotic counterparts can be critical ecological players through their metabolic activities and their influence on prokaryotes [33]. Although eukaryotes exhibit less metabolic flexibility [34, 35] and potentially less resilience with environmental change [36, 37], their distinct metabolic abilities may complement those of the prokaryotes [34]. While most metazoans are absent from anoxic waters, diverse microeukaryotes, including protists, fungi, and zooplankton, inhabit OMZs; thus, oxygen levels can potentially shape their community composition [25, 38, 39]. These microeukaryotes are vital components of aquatic food webs in the surface and deep-ocean ecosystems and are proposed to play critical ecological roles in coastal OMZs through predatory or symbiotic interactions with prokaryotes [25, 40]. Additionally, prior studies on OMZ microeukaryotes revealed significantly different communities in oxic and anoxic/hypoxic waters [16, 25, 28-30, 40-47]. Some eukaryotes are also likely facultative anaerobes [7], with fungal sequences representing a substantial fraction of 18S rRNA gene libraries from anaerobic environments, and protozoa, including some ciliates and foraminifera can use nitrate as a terminal electron acceptor [48]. Compared with OMZ prokaryotes, the population structure and composition of pelagic microeukaryotes in these oxygen-depleted environments remain largely unknown.

Most OMZ studies currently focus on comparisons between depths within a given station, which may conflate oxygen status with other depth-related environmental factors. Considering the critical importance of oxygen for microbially-mediated biogeochemical cycling, the responses of microbial groups to oxygen levels are an understudied research area. To that end, we focus on the microbiome of the Bay of Bengal (BoB) OMZ, where the mesopelagic (200 to 1000 m) exhibits consistent low-oxygen (< 20 μM). The BoB generally retains low oxygen levels despite riverine inputs of freshwater, which carry large fertilizer loads, enhancing primary productivity in surface waters and increasing oxygen demand in the water column [49]. However, this OMZ is comparatively weaker than other permanent OMZs due to the deeper

remineralization depth of mineral-rich particulate organic matter [50, 51]. Consequently, in comparison with other OMZs, the BoB exhibits lower levels of denitrification and anammox, which can occur at low levels of oxygen (< 20 μM) but become significant only with functional anoxia (< 1 μM). These characteristics make the BoB a good case study of global-change mediated deoxygenation, yielding insights into how microbial communities respond to localized oxygen depletion, specifically in restructuring microbial eukaryotic communities.

2. Materials and methods

2.1 Sample sites and collection

A research cruise on the *R/V Shiyan 3* sampled the Eastern Indian Ocean from March 25 to April 30, 2018. A conductivity-temperature-depth (CTD, Seabird SBE-911) rosette equipped with 8 L Niskin bottles was used to collect water samples as previously described [52]. Samples from 8 stations at up to 10 depths ranging from 5 to 2,000 m were selected for the current study (Table S1, Fig. S1). Environmental factors, including temperature, pH, salinity, nutrients, CTD-based chlorophyll *a* fluorescence, and dissolved oxygen, were measured using previously described methods [9, 52, 53]. In addition, nucleic acid samples were collected by filtering 2 L of water through 0.22 μm polycarbonate Isopore membranes, which were then flash-frozen in liquid nitrogen onboard and stored at -80 °C until DNA extraction.

2.2 DNA extraction, library preparation, and pyrosequencing

Total DNA was extracted using the E.Z.N.A water DNA kit (OMEGA, USA). The extracted DNA was suspended in 100 μL of sterile water and stored at -20 °C until subsequent processing. A total of 59 DNA samples were selected for bacterial and fungal community analysis, while 56 samples were processed for Labyrinthulomycetes community analysis (Table S1).

Labyrinthulomycetes-targeted 18S rRNA gene amplification employed the barcoded primers LABY-A (5'-GGGATCGAAGATGATTAG-3') and LABY-Y (5'-CWCRAACTTCCTTCCGGT-3'), following previously established protocols [52, 54]

The PCR mixture contained 0.2 U of KOD FX Neo DNA Taq polymerase (TOYOBO, Osaka, Japan), 50 ng of template DNA, and a final concentration of 0.3 μM of each primer, 1× KOD FX Neo Buffer, and 400 μM of each dNTP. Thermocycling consisted of an initial denaturation at 95 °C for 5 min, followed by 25 cycles of 95 °C for 30 s, 50 °C for 30 s, 72 °C for 40 s, and a final extension at 72 °C for 7 min. The samples were pooled and then cleaned using the E.Z.N.A.^{TM} Cycle-Pure Kit (Omega) column, followed by gel purification using the Monarch DNA Gel Extraction Kit.

Bacterial 16S rRNA gene amplicons were constructed using the barcoded primer pair 338F (5'-ACTCCTACGGGAGGCAGCA-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3'), targeting the V3-V4 region of the 16S rRNA gene as described previously [55]. Fungal ITS amplicons were generated using the barcoded primer pair ITS1-F/ITS2 (ITS1-F: 5'-CTTGGTCATTTAGAGGAAGTAA-3' and ITS2: 5'-GCTGCGTTCTTCATCGATGC-3') [56]. PCR amplification and thermocycling were performed as previously described [57]. The bacterial and fungal PCR samples were purified following gel excision using the same kit as above. All amplicons were paired-end sequenced using 2 × 250 nt on an Illumina HiSeq 2500 platform by a commercial sequencing facility (Biomarker Technology Corporation, Beijing, China). The raw sequences were deposited in NCBI under BioProject PRJNA794046.

2.3 Quantification of prokaryote, Labyrinthulomycetes, and fungal abundances.

The cell abundances of prokaryotes and Labyrinthulomycetes were quantified using a BD FACS Calibur Flow Cytometer per established methodologies [52, 54, 58]. Specifically, prokaryotes were identified using the DNA dye SYBR-I green (Molecular Probes, USA) while Labyrinthulomycetes were quantified using the Labyrinthulomycetes-specific dual fluorescent dye acriflavine-HCl (Sigma, Germany). Green-fluorescent polystyrene latex beads (Molecular Probes, USA) were used as an internal standard to normalize the cell counts. The total abundance of fungal ITS gene copies per liter of seawater was determined by quantitative PCR following a previously established protocol [57].

2.4 Sequence processing

Bacterioplankton, Labyrinthulomycetes, and fungi sequences were imported into the QIIME 2 (v. qiime2-2020.6) bioinformatics platform [59] using SampleData[PairedEndSequencesWithQuality] for bacterioplankton and Labyrinthulomycetes, while the single-end forward sequences of fungi were imported in the SampleData[SequencesWithQuality] using "qiime tools import" method. The primers for bacterioplankton and Labyrinthulomycetes were removed using the Cutadapt plugin [60]. Due to limitations in the ability of Cutadapt to entirely remove fungal primers, the fungal primers were concurrently trimmed by using the "trim" parameter to remove 22 nt during the denoising process. Paired-end reads were merged if they exhibited an overlap > 10 bp with a maximum of 1 bp mismatch. Different plugins were employed to perform quality filtering, denoising, and chimera removal. Specifically, Deblur was applied to denoise the bacterial sequences [61], while DADA2 was used to denoise the Labyrinthulomycetes and fungal datasets, based on prior research [58, 62].

The taxonomies of amplicon sequence variants (ASVs) were identified using classifiers trained with the "q2-feature-classifier" method [63]. The taxonomic annotations of bacterial, Labyrinthulomycetes, and fungal ASVs were based on the SILVA rRNA (16S/18S) and UNITE (ITS) databases, respectively [64, 65]. ASVs with a total abundance below 10 were removed from the Labyrinthulomycetes and those below 30 were removed from the bacterioplankton and fungi ASV tables. Furthermore, bacterial ASVs occurring in less than 2 samples or annotated as mitochondria were excluded from the ASV table. Using sequence annotations of Labyrinthulomyctes, ASVs belonging to other groups were removed before rarefaction and analysis. As all ITS sequences were assigned to fungi, no additional filtering was performed for fungal ASVs. The filtered sequences were then rarefied based on the lowest sequence count observed across all samples. Specifically, rarefaction was performed to the following sequence depths: bacterioplankton (5,536), Labyrinthulomycetes (1,565), and fungi (33,464).

204

205 **2.5 Microbial community analysis**

206 Alpha diversity indices, including Shannon diversity, Pielou's evenness, and
207 richness (observed ASVs), were calculated using the q2-diversity plugin in QIIME 2.
208 The differences in microbial community abundance and diversity between depths and
209 stations were compared using the Kruskal-Wallis test and ANOVA following a
210 normality check, respectively, using SPSS (version 26 for Windows; IBM). Nonmetric
211 multidimensional scaling (NMDS) ordination was used to compare the microbial
212 composition from different depths and oxygen regions based on Bray-Curtis
213 community dissimilarity, calculated using the core-metrics-phylogenetic pipeline in
214 QIIME 2. For statistical analysis, beta-diversity distances (Bray-Curtis) were analyzed
215 by single permutational multivariate ANOVA (PERMANOVA) with depth as a factor,
216 using the *adonis* function in R. To identify discriminative taxa based on oxygen status,
217 we analyzed the 100 most abundant bacterioplankton ASVs and the 50 most abundant
218 ASVs for eukaryotes (Labyrinthulomycetes and fungi) at 200m and 500m using
219 DESeq2. Significance was defined by log2 fold changes exceeding 2 and p-values less
220 than 0.05 [66]. For the DESeq2 analysis of photoautotrophs in surface waters, we
221 additionally applied a consistent relative abundance threshold of > 0.05% to minimize
222 bias induced by low sequence counts.

223

224 **3. Results and discussion**

225 **3.1 Environmental parameters**

226 To investigate vertical and horizontal oxygen gradients, we compared dissolved
227 oxygen (DO) concentrations across 8 stations in the Bay of Bengal. In general, the
228 vertical profiles of DO concentrations displayed two distinct patterns (Fig. 1); the first
229 group (stations I107, I202, I205, and I208) consistently displayed low DO
230 concentrations (< 20 μM, mean ~10.51 μM) below the oxycline (predicted oxygen
231 minimum zone, 200-500 m), while the second set of stations (I105, I302, I313, and I501)
232 showed statistically higher DO concentrations (>20 μM, mean ~ 53.01 μM) for the same
233 depths (One-way ANOVA, $p < 0.05$, Table S2) [67]. Based on these results, we

classified stations using data from 200 m and 500 m depths where the oxygen divergence occurred, into the oxygen minimum zone (OMZ, < 20 µM DO) or non-oxygen minimum zone (NOZ, >20 µM DO). This 20 µM threshold corresponds to 'microbial hypoxia' where oxygen levels influence microbial biogeochemical cycles, and inhibit anaerobic processes (e.g. denitrification) [67-70]. Although it contains a large and permanent marine OMZ, the Bay of Bengal generally exhibits detectable oxygen, in contrast with other OMZs (*i.e.*, Eastern Tropical North Pacific, Eastern Tropical South Pacific, and Arabian Sea) where levels reach functional anoxia, e.g., < 1 µM DO [71, 72]. Although denitrifiers and bacteria that perform anammox are observed in the Bay of Bengal, mediating low but significant fixed nitrogen loss, these rates are inhibited by the persistent water column oxygen [49].

In this system, several environmental factors exhibited depth-dependent patterns (Fig. S1), which could potentially influence the microbiome [8, 73]; temperature, salinity, pH, density, chlorophyll, and nutrients (silicate, phosphate, nitrate, total phosphate) were significantly correlated with depth (Spearman, $p < 0.05$, Table S3), likely along with unmeasured factors such as organic matter flux and lability. To separate oxygen from these other depth-related environmental variables (Spearman, $p < 0.05$, Table S3), we compared oxygen minimum zone stations to nearby stations outside the OMZ. While the dissolved oxygen (DO) concentrations at 100, 200, 500, and 1,000 m were significantly lower in OMZ compared to NOZ stations (One-way ANOVA, $p < 0.01$, Table S2), other environmental factors (e.g., temperature, salinity, deep chlorophyll maximum, pH, and nutrients) were not significantly different at these depths (Table S2). Although OMZs are generally considered sites for the dissimilatory reduction of oxidized nitrogen compounds [74], our findings suggest a similar environmental context aside from oxygen levels.

3.2 Abundance of prokaryotes, Labyrinthulomycetes, and fungi

To better understand microbial community responses to oxygen minimum zones in the Bay of Bengal, we first quantified the abundance of prokaryotes,

Labyrinthulomycetes, and fungi with depth and across oxygen levels (Fig. S2). The observed decrease in prokaryote cell abundance with depth (5-25 m average = 1.41×10^6 cells mL^{-1}, 75-1,000 m average = 2.79×10^5 cells mL^{-1}) is consistent with previous reports in multiple ocean regions [75]. Yet, we did not identify a significant difference in prokaryote abundance with oxygen status (OMZ vs NOZ, Kruskal Wallis test, Fig. S2A at 200 and 500 m), suggesting a limited impact of localized oxygen depletion. In contrast with the prokaryotes, Labyrinthulomycetes abundance was significantly higher in the NOZ stations (857.78 cells mL^{-1} vs 116.46 cells mL^{-1}, Kruskal Wallis test, $p < 0.05$, Fig. S2B averaged at 200 and 500 m). In contrast with a previous study that observed a five-fold enrichment of Labyrinthulomycetes in OMZ depths compared to well-oxygenated surface samples in the North Atlantic Ocean [76]. Additionally, there is no clear Labyrinthulomycetes increase with depth (Figure S2B), as has been reported previously [52]. Similarly, we observed no significant differences in fungal abundance with depth or oxygen status (Fig. S2C, Kruskal Wallis, $p > 0.05$); however, high variability in fungal abundance was observed between depths and stations, which may reflect the distribution of fungi's preferred resources, including particles and phytoplankton [77] (Fig. S2C). While fungi are generally considered obligate aerobes, prior research suggests at least a tolerance of reduced oxygen and anaerobic environments [78-84]. The observed high microeukaryote between-station variability (Fig 2B, C) is consistent with prior research in aerobic coastal and pelagic regions [76, 77]. In general, microeukaryotic communities are less strongly associated with commonly measured environmental factors than bacterioplankton [85], and their distributions may more closely reflect the availability of specific resources, for example, particulate organic matter [77, 86].

3.3 Community characteristics of bacterioplankton, Labyrinthulomycetes, and fungi

To gain greater insight into the structure and composition of microbial communities in the Bay of Bengal, we compared the diversity metrics of these three microbial groups across depths and oxygen categories (Fig. S3). These microbial

groups exhibited distinct vertical diversity patterns, with the bacterioplankton exhibiting both a clear depth-related pattern in beta diversity (Fig. 2A) and the highest alpha diversity in the deep euphotic zone (100 and 200 m) (Fig S3A-C). Although depth also significantly impacted the community structure of Labyrinthulomycetes and fungi (PERMANOVA, $p < 0.001$), we observed clusters within a given depth rather than a consistent trend with depth (Fig. 2B, C). For example, the Labyrinthulomycetes exhibited separation of euphotic versus aphotic zone samples, while fungi communities in the surface ocean (≤ 75 m) are distinct from deeper samples (Fig. 2B, C). Consistent with previous reports, bacterial diversity indices were lower in the euphotic zone (0-200 m) than in the aphotic zone (>200 m, average Shannon diversity: 5.86 vs. 7.17, richness: 393.68 vs. 643.38, and evenness: 0.68 vs. 0.77; Fig S3A-C), whereas the trend is reversed for Labyrinthulomycetes (average Shannon diversity: 4.45 vs 2.64, richness: 74.91 vs 22.62, and evenness: 0.719 vs 0.597; Fig. S3D-E) [52]. In contrast with the other two microbial groups, only fungal richness declined slightly with increasing depth (Fig. S3H), with no depth-related trends for Shannon diversity and evenness (Fig. S3G, I). While these results suggest that microeukaryotic groups respond differently to depth-related environmental factors (e.g., temperature, pressure, nutrients), given strong correlations among environmental factors, we cannot identify the proximal driver of these microbiome patterns [87]. Our findings highlight the need to compare microbiomes across stations with distinct oxygen statuses at the same depths to minimize the confounding effects of environmental factors that co-vary with depth (as in [40, 88, 89]).

Therefore, to better understand the impacts of OMZs on the microbiome, we specifically compared stations with different oxygen levels at OMZ-relevant depths (200 and 500 m). At these depths, bacterial alpha diversity indices (*i.e.*, Shannon's diversity, richness, and evenness) were significantly lower in the OMZ versus NOZ stations at 500 m (e.g., Richness average = 669.33 vs 720, Fig. S3A-C; Table S4; One-way ANOVA, $p < 0.05$). This finding is consistent with the reported declines in prokaryotic diversity from oxygen-rich surface water to the permanent OMZ in the

Eastern Tropical North Pacific [90] but differs from recent findings that ocean deoxygenation could enhance prokaryotic diversity in regions where oxygen levels decline to 20 μM, which was based on comparison across different depths [91, 92]. At these DO concentrations, aerobic and anaerobic processes may co-occur in OMZ waters, and lead to the increase in diversity [93, 94]. Unexpectedly, bacterial alpha diversity did not differ at 200 m (Table S4) with oxygen status, even though the divergence in mean oxygen level was more significant at 200 m than at 500 m (47.02 versus 37.98 μM DO, respectively). Thus, other factors may obscure general relationships between diversity and oxygen status.

While diversity did not significantly differ with oxygen status for fungi (200 m) or Labyrinthulomycetes (200 and 500 m;Fig. S3 D-I; Table S4), fungal diversity metrics (*i.e.*, Shannon's diversity and Pielou's evenness) were lower in OMZ versus NOZ stations at 500 m (Fig. S3 G-I; Table S4; One-way ANOVA, $p < 0.05$). Overall, these observations align with negative correlations between bacterial richness and DO in seasonal OMZs [89-91, 95] but suggest that OMZ fungi are potentially less diverse [96, 97]. Taken together, our findings suggest that oxygen can potentially decrease both bacterial and fungal diversity; however, Labyrinthulomycetes diversity, while exhibiting vertical patterns, shows a minimal response to oxygen levels. Thus, these results validate our approach of within-depth comparisons due to the robust depth partitioning among the bacterioplankton and to avoid the biases introduced by comparisons between anoxic/hypoxic zone with the overlying or underlying waters.

To obtain a more nuanced understanding of how microbial phylotypes respond to OMZ conditions, we compared the 100 most abundant Amplicon Sequence Variants (ASVs) for bacterioplankton and the 50 most abundant ASVs for Labyrinthulomycetes and fungi at OMZ depths (Fig. 3, Fig. S4, S5). For bacterial taxa, samples clustered by depth (Fig. 3) as in the NMDS (Fig. 2A); but, within a given depth, the community further clusters with oxygen status, with the exception of NOZ samples from 500 m (Fig. 3), supporting a bacterioplankton response to OMZ conditions. DESeq2 identified

15 bacterial ASVs as OMZ-associated across both 200 m and 500 m samples (Fig. 3). ASVs associated with OMZ conditions (200 m) belong to the SUP05, SAR324, SAR202, SAR406, and SAR11 clade II, *Ruegeria*, *Marinobacter*, and the family Microtrichaceae. Members of the SAR324, SAR202, SAR406, and SAR11 clade II were previously reported in OMZs [12, 98-101] and play pivotal roles in global biogeochemical cycles and inhabit diverse metabolic niches [8]. For example, SAR324, SAR11, and SAR406 lineages contain coxMSL genes [102-104], encoding putative carbon monoxide dehydrogenases, which catalyze the oxidation of carbon monoxide to CO_2, allowing microbial survival in extreme habitats [102-105]. SAR202 is capable of degrading recalcitrant dissolved organic matter (DOM) [106], while SUP05 can oxidize sulfur compounds in hydrothermal sulfur-rich plumes [107]. Members of *Ruegeria* spp. are prevalent in oxygen-depleted waters and the Arabian Sea OMZ, where they actively participate in dimethylsulfoniopropionate (DMSP) metabolism [108]. Members of *Marinobacter* spp. have been identified as the dominant genus among culturable isolates in the Bay of Bengal OMZ and are assumed to degrade hydrocarbons [109]. *Nocardioides* taxa were identified as the primary methanotrophs in the Eastern Tropical North Pacific OMZ off central Mexico [110], and members of *Alcanivorax* recognized as hydrocarbon degraders in the sediments below the OMZ in the Bay of Bengal [111]. However, phylotypes previously found in OMZs, including taxa affiliated with the phylum Planctomycetes, the genus *Nitrospina*, and *Candidatus Scalindua* [12, 112], which play critical roles in anammox and nitrite oxidization [73, 89, 91, 92, 95, 112-114], were not identified as OMZ-associated as they either did not meet the abundance criteria (100 most abundant) or were not specific to OMZ stations. Interestingly, ASVs of SAR11 clade IV, clade II, SAR406 clade, *Nocardioides*, *Pseudohongiella*, and the family Microtrichaceae are significantly enriched at 200 m in NOZ stations. In contrast, at 500 meters, NOZ-associated taxa included ASVs belonging to *Prochlorococcus* MIT9313, family Micavibrionaceae, LS-NOB (Low-Salinity Nitrite-Oxidizing Bacteria, belonging to the family Nitrospinaceae), Chloroplasts, and *Candidatus* Actinomarina (Table S5). Notably, LS-NOB, presumed nitrite oxidizers [89], were detected as NOZ specialists, suggesting an association with depth and the nutricline

rather than low oxygen conditions. The NOZ-associated photoautotrophs, far below the euphotic zone (500 m), are likely export production, which is surprising as export might be assumed to be higher in OMZ stations. However, total photoautotrophs were a higher fraction of libraries in NOZ surface waters (Kruskal-Wallis test, $p < 0.05$), but no photoautotrophic taxa were identified as significantly different between surface samples of NOZ and OMZ stations, suggesting similar primary producer communities. While an ASV affiliated with low-light *Prochlorococcus* was identified as NOZ-associated (500 m), its relative abundance was higher in the photic zone (a peak of 13.49% of library abundance at 100 m versus 6.74% at 500 m), suggesting export as its source.

Compared to bacterial groups, Labyrinthulomycetes, and fungi displayed less clear clustering with either depth or oxygen level (Fig. S4, S5), and fewer taxa associated with oxygen status. At 200 m, a Labyrinthulomycetes phylotype affiliated with *Aurantiochytrium* was identified as an OMZ specialist, while 3 ASVs from unclassified Labyrinthulomycetes and Thraustochytriaceae were identified as OMZ associated at 500 m (Fig. S4, Table S6). Notably, *Aurantiochytrium* is a saprophytic clade that uses decaying phytoplankton as a carbon source [115-121], and can lead to hypoxia following phytoplankton blooms [68]. In contrast, members of Thraustochytriaceae are considered to complement bacterial decomposition and potentially degrade recalcitrant organic material in the deep ocean [52]. These findings suggest distinct carbon sources in OMZ stations or highlight other differences (e.g., ocean physics) between sites with distinct oxygen profiles. However, a different *Aurantiochytrium* ASV was identified as NOZ associated (Fig. S4), suggesting differing within-clade tolerances to OMZ/NOZ conditions. NOZ-associated Labyrinthulomycetes belong to the genus *Aplanochytrium*, which co-occurs with phytoplankton and may rely on exported organic matter [122-126]. Compared to Labyrinthulomycetes, fungi demonstrate a more limited response to OMZ conditions with only a single NOZ-associated ASV, and 2 and 3 taxa identified as OMZ-associated at 200 and 500 m, respectively (Fig. S5, Table S6). Although recent studies in permanently anoxic habitats have found novel fungal clades (e.g., LKM group, uncultured fungi), which are restricted to low-oxygen environments [127-129],

OMZ specialists were only identified at the kingdom level, providing further evidence of previously-undescribed fungal phylotypes from the deep-sea, especially in extreme niches such as OMZs [130-132]. Interestingly, denitrifying *Fusarium* (Ascomycota) fungi [129] are observed here (although they are not OMZ-associated) despite the absence of denitrification in the BoB OMZ (Figure S5). However, as microbial eukaryote communities have a high beta diversity, it may be necessary to sample more extensively to identify OMZ diagnostic taxa.

We can only speculate as to the mechanisms that might allow microeukaryote resistance to changes in oxygen levels. However, in fungi and other eukaryotic microorganisms, there are two de-nitrification pathways; one is typically localized to mitochondria and usually occurs under low O_2 conditions, while the other, often referred to as ammonia fermentation, is localized in the cytosol [133] and is activated under strict anoxic conditions. The latter pathway involves the reduction of nitrate to ammonium using a reductant generated by the catabolic oxidation of ethanol (the donor of electrons) and concomitant acetate synthesis coupled to substrate-level phosphorylation [134]. As limited information is available on the response mechanism of micro-eukaryotes in marine OMZs, there are likely other mechanisms which may be better revealed by genomes and transcriptomes of microeukaryotes. Thus, we propose further research on this question as a topic of future study. Furthermore, given the limited research on microeukaryote ecology in low-oxygen environments, extrapolating our BOB findings to other OMZs may be challenging due to between-site differences, e.g., strict anoxic conditions. Despite these limitations, this study provides a window into the complexity of the eukaryotic microbial communities in marine hypoxic environments.

CONCLUSIONS

Our study sheds light on the much stronger relationships of bacteria versus the selected microeukaryotic communities with the Bay of Bengal oxygen minimum zone (OMZ),

a low but not anoxic environment, that is relevant to predicting the impacts of ocean deoxygenation. Across these three microbial groups, we observed a sharp contrast between the bacterioplankton and the two microeukaryotic groups: the bacterioplankton exhibit the most robust patterns in terms of depth and oxygen status. This observation is consistent with previous reports, as microeukaryotes (esp. fungi) generally display weaker environmental associations than bacterioplankton [34, 52, 77]. However, they could also be less sensitive to oxygen levels than the bacterioplankton, reflecting their associations with low-oxygen particulate microenvironments even in NOZs [133]. To clarify these relationships, future work could focus on microeukaryote colonization of particulate material and assessment of these microeukaryotes' activity across different microenvironments and depths. As the BoB is distinct among OMZs in that trace oxygen levels remain, new research could compare microeukaryotes across a range of oxygen levels in the deep ocean to further clarify their potential responses to ocean deoxygenation on a changing planet.

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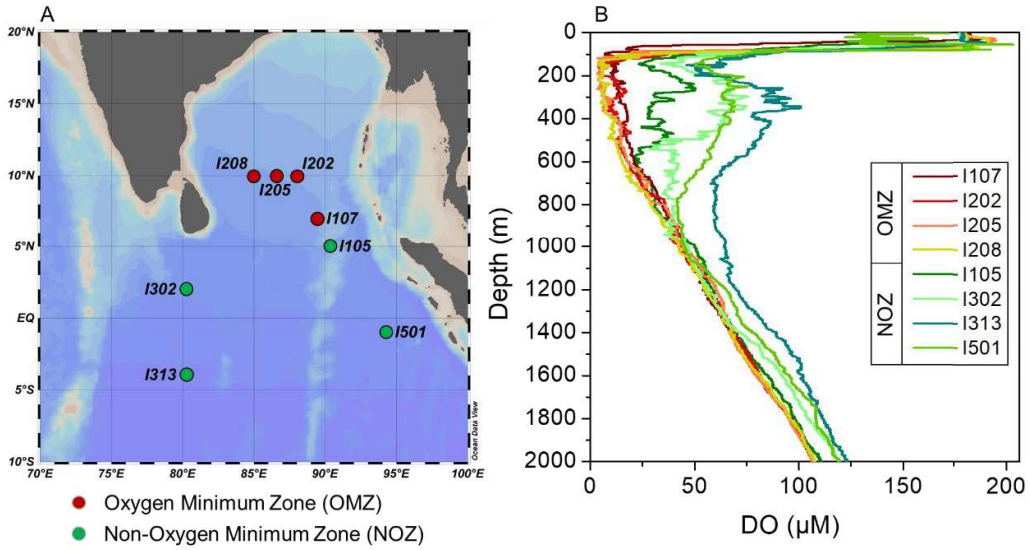

Figure 1. **Map and vertical dissolved oxygen (DO) profile of the sampling stations.** A. The map displays the distribution of sampling stations. The stations marked with red dots (I208, I205, I202, I107) were identified as the oxygen minimum zone (OMZ), characterized by persistently low oxygen levels between 200 m and 500 m. Stations indicated by green dots (I105, I302, I313, I501) were identified as non-oxygen minimum zone (NOZ). B. Dissolved oxygen (DO) profiles of the stations. OMZ refers to stations in the oxygen minimum zone, where oxygen levels remained consistently below 20 µM at 200-500 m. The NOZ group represents the profiles of stations, with oxygen levels above 20 µM in the same depth range. Sampling station positions were plotted using the Ocean Data View software (https://odv.awi.de), accessed on 3rd June 2020.

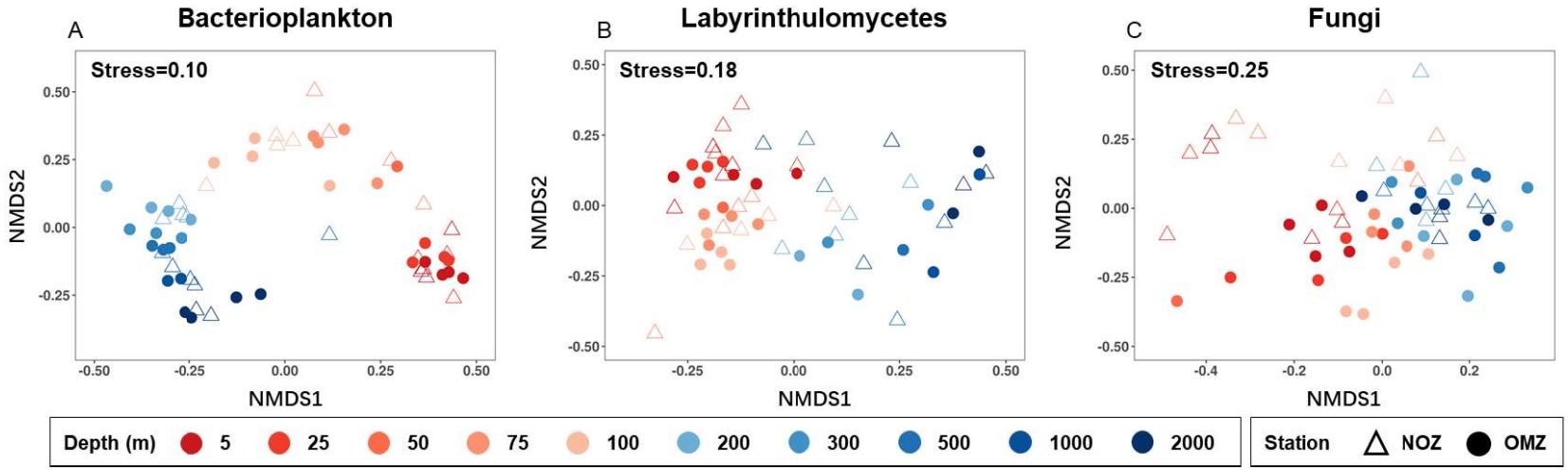

Figure 2. Nonmetric multidimensional scaling (NMDS) ordination based on Bray-Curtis dissimilarities. A. Bacterial NMDS ordination derived from 16S rRNA gene libraries. B. Labyrinthulomycetes NMDS ordination derived from 18S rRNA gene libraries. C. Fungal NMDS ordination derived from rRNA ITS1 gene libraries. Each point represents an individual sample, and the shapes indicate oxygen status (Triangle: NOZ; Circle: OMZ) and colors the depth.

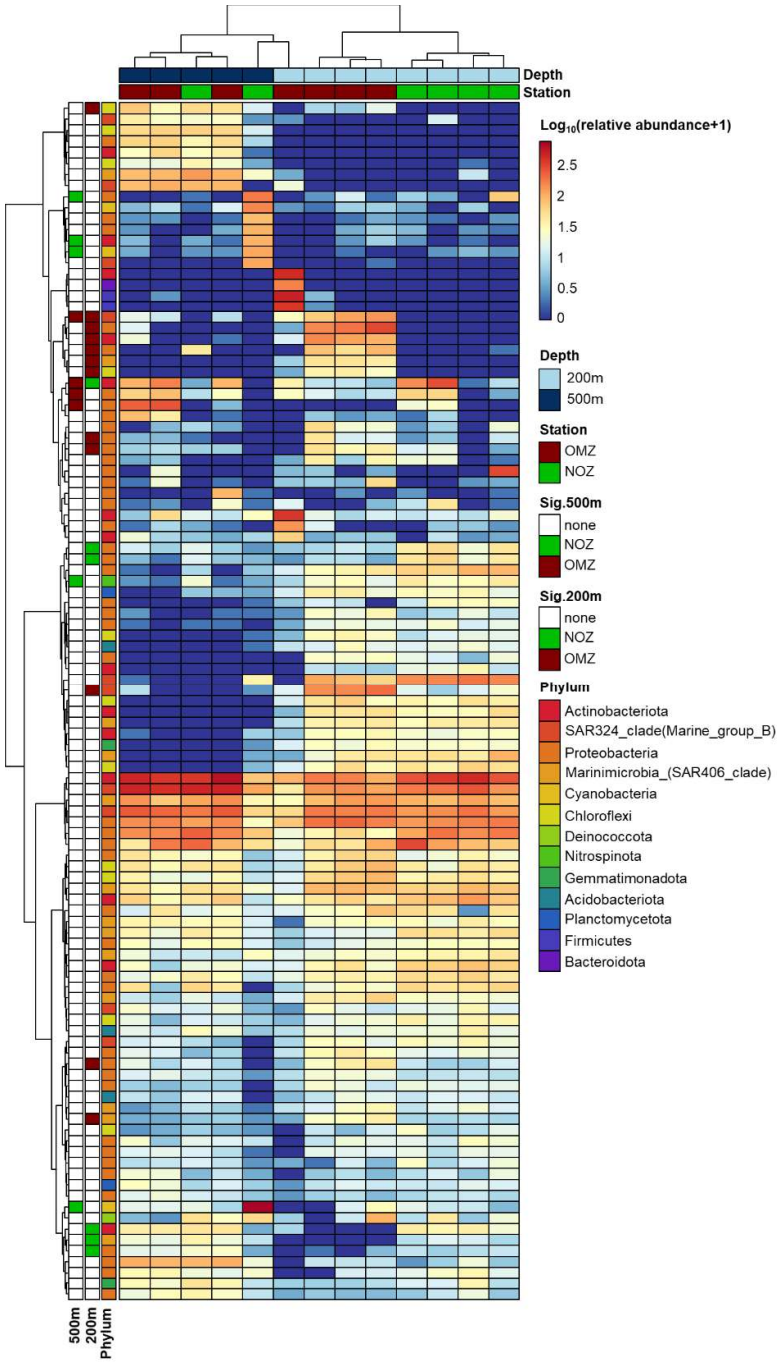

Figure 3. **Heatmap of the 100 most abundant bacterial taxa at 200 and 500 m.** The heatmap displays the 100 most abundant bacterial Amplicon Sequence Variants (ASVs). ASVs (rows) and samples (columns) are clustered using Ward's hierarchical agglomerative method based on Euclidean distance. ASVs are colored by phylum. Samples are labeled across the top by depth (Blue: 200m; Dark blue: 500m) and oxygen minimum zone status (Red: OMZ; Green: NOZ). ASVs associated with different oxygen concentrations (Red: OMZ; Green: NOZ) at each depth were assessed using DESeq2 ($p<0.05$).