# Depth-Dependent Variables Shape Community Structure and Functionality in the Prince Edward Islands

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## Abstract

Physicochemical variables limit and control the distribution of microbial communities in all environments. In the oceans, this may significantly influence functional processes such the consumption of dissolved organic material and nutrient sequestration. Yet, the relative contributions of physical factors, such as water mass variability and depth, on functional processes are underexplored. We assessed microbial community structure and functionality in the Prince Edward Islands (PEIs) using 16S rRNA gene amplicon analysis and extracellular enzymatic activity assays, respectively. We found that depth and nutrients substantially drive the structural patterns of bacteria and archaea in this region. Shifts from epipelagic to bathypelagic zones were linked to decreases in the activities of several extracellular enzymes. These extracellular enzymatic activities were positively correlated with several phyla including several Alphaproteobacteria (including members of the SAR 11 clade and order *Rhodospirillales*) and Cyanobacteria. We show that depth-dependent variables may be essential drivers of community structure and functionality in the PEIs.

## Keywords

- Bacteria
- Archaea
- Prince Edward Islands
- Community structure
- Extracellular enzymatic activity
- Correlation analyses

## Introduction

Climate change has clear impacts on both terrestrial and marine ecosystems globally [1,2,3]. Ocean acidification, calcification and warming are just some of the environmental consequences of climate change on marine environments [4,5,6]. Together, these stressors directly affect marine fauna and phytoplankton communities, with several studies suggesting direct decreases in biodiversity [7]. Macroecology studies have reported drastic decreases in the diversity of several species of corals and seaweeds [1, 8,9,10]. In several marine regions,

species are replaced by less productive and less complex species, resulting in habitats with a diminished capacity to provide key ecosystem services [8, 11].

Microbial communities (bacteria, archaea, fungi, protist and viruses) are important components of healthy marine environments [12,13,14]. Several studies have shown that ecosystem services such as nutrient cycling, nitrogen and carbon sequestration are driven by marine microorganisms [15,16,17]. Microbial ecology studies have also shown that environmental stressors also influence the diversity and function of microorganisms [18,19,20]. Yet, in contrast to macrofauna, we lack a clear understanding on the effects of environmental stressors on physiological responses, evolutionary dynamics and on the diversification of microbial communities for several reasons. The abundance and diversity of microorganisms vary substantially at different regional and global scales [13, 21]. In the oceans, the availability of light, temperature, oceanic currents, latitude and depth have all been shown to affect the biogeography, structure and metabolisms of microbial communities [22,23,24,25]. Together, these variables may determine the extent to which microbial communities catalyse crucial ecosystem services. However, few studies have examined the effects of ocean variables on microbial extracellular enzymes throughout the water column.

In the surface ocean, microorganisms metabolize half of the annual dissolved organic matter (DOM) produced via photosynthesis [26]. These communities are thought to markedly influence primary productivity rates and nutrient availability throughout the water column, ultimately effecting marine food webs [27, 28]. Below the surface, the degradation of organic matter is primarily facilitated by microbial guilds [29, 30]. Early estimates suggest that approximately 0.1% of organic matter may be buried in marine sediments, with the majority of DOM remineralized throughout the deep ocean and sediment surface [31]. Given these estimates, it is clear that microbial communities likely influence the rate and extent of organic carbon storage. However, there is a paucity of data providing quantitative estimates of DOM linking rates to microbial communities in the euphotic and aphotic zones of the ocean [32]. The lack of data is even more pronounced in understudied regions of the ocean, such as the Indian sector of the Southern Ocean, where severe environmental factors largely regulate ecosystem services. This knowledge deficit harpers our capacity to predict the effects of variability and possible feedbacks of nutrient cycling in this region.

Sub-Antarctic islands are located within the Southern Ocean between the northern and southern boundaries of the Antarctic Circumpolar Current (ACC). These remote oceanic islands are subject to cool, windy and wet climates. The Prince Edward Islands (PEIs) archipelago are situated in the south-west Indian sector of the Southern Ocean over 2000 km from South Africa [33]. The nearest landmass being the Crozet Island group approximately 950 km to the east **(**Fig. 1**)**. The PEIs are located in the path of the ACC, within the Antarctic Polar Frontal Zone (APFZ), a transitional region between warm subtropical waters to the north and cold sub-Antarctic waters towards Antarctica [34]. These islands are also between the Sub-Antarctic Front (SAF), indicating the northern boundary of the APFZ and the Antarctic Polar Front (APF) to the south, marking the southern boundary of the PFZ [34,35,36]. Previous studies have demonstrated the influence of oceanographic variability on near-shore microbiota [37]. These studies have also provided an overview of the taxonomic diversity in this environment. However, no study has examined the link between oceanographic variables, biogeography and functionality in these important marine waters. Disentangling microbial community variations with depth may provide insights regarding nutrient acquisition along the water column.

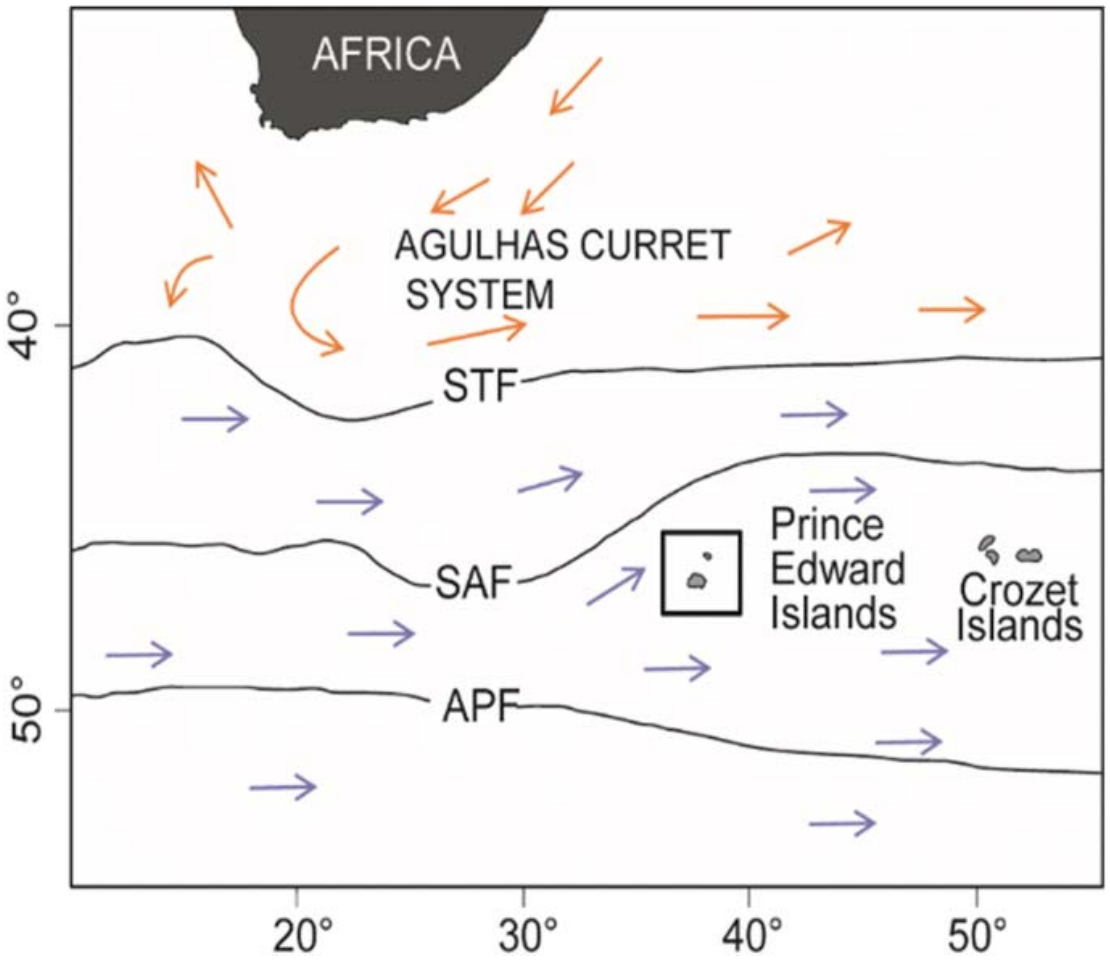


**Fig 1.** Geographic location of sub-Antarctic Prince Edward Islands in the Indian sector of the Southern Ocean. The average geographic positions of the three major frontal systems: Subtropical Front (STF), Sub-Antarctic Front (SAF) and the Antarctic Polar Front (APF) are shown (Figure adapted from Allan et al. [33]). Red arrows represent the surface ocean circulation of warm subtropical waters driven by the Agulhas current, and blue arrows illustrate cooler sub-Antarctic waters south of the STF

Recent studies in the SIO have broadened our understanding regarding the effects of ocean circulation dynamics, including eddy fluxes and frontal systems, on PEI surface microbial communities [37, 38]. Yet, the structure and functional dynamics of microbial communities in the PEI remains unclear, especially in mesopelagic (200–1000 m) and bathypelagic zones (>1000 m). Using samples collected within the vicinity of the PEIs, we provide the first insights on microbial biogeography along the water column. We used 16S rRNA gene amplicon sequencing to assess taxonomic structure, composition and depth-related variability. We used nutrient measurements and extracellular enzymatic activities to test the effect of oceanographic variables on microbial communities. We predict that higher nutrient levels, associated with proximity to the PEIs, may substantially effect bacterial and archaeal structure in these waters. We also predict that, consistent with shifts in diversity, potential enzymatic activities may vary markedly across the water column as a response to changing oceanographic conditions.

## Materials and Methods

### *Sample Collection and Processing*

Seawater was collected during austral autumn from the 15th of April to the 9th of May 2015 on-board the R/VS.A. Agulhas II (Table S1). Seawater was collected using a Conductivity-Temperature-and-Depth (CTD) Rosette sampler (Sea-Bird SBE-911 plus V2 CTD System; Sea-Bird Electronics, Inc., Bellevue, WA, USA) in 24 × 20 l Niskin bottles from several locations in the open ocean and between the PE Islands following the protocol described previously [39]. In total, 15 samples were collected from five stations (PEI1–PEI5) (Fig. 2a). For molecular analysis, 5 l of seawater were collected from each location at three predetermined depths designated as (i) Deep (>10 m above seafloor), (ii) Low dissolved oxygen zone (OMZ; oxygen minimum zone), and (iii) Fluorescence maximum (F-max) (Table S1). Each sample was pre-filtered through a 47-mm, 0.45-µm pore cellulose acetate membrane filter to limit biomass saturation (Sartorius Stedim Biotech GmbH, Göttingen, Germany) as detailed previously [41]. This was followed by filtration using a 0.2-µm pore cellulose acetate membrane filter (Sartorius Stedim Biotech, Göttingen, Germany). Biomass-containing filters were stored at − 20 °C until further use. Unfiltered seawater (~300 ml) was collected for nutrient analysis and enzyme activity assays at each depth in six sterile 50-ml Falcon tubes and stored at − 20 °C. All seawater and biomass samples were transported to CMEG laboratory on ice until analysis.

### *Nutrient and Extracellular Enzyme Activity Analysis*

Nitrate, phosphate and silicate concentrations were determined according to standard methods [42] using a Seal Autoanalyser 3 (Seal Analytical, Germany) (Table S2). Extracellular enzyme activities were measured using fluorogenic 4-methylumbelliferone (MUB) and 7-amino-4-methylcoumarin (AMC) substrates (Sigma-Aldrich, USA) [43, 44] from a 0.45-µm filtrate. The five enzymes and substrates assayed were leucine aminopeptidase (LAP-AMC), alkaline phosphatase (MUB-AP), β-glucosidase (MUB-BG), β-xylosidase (MUB-BX), and *N*-acetylglucosaminidase (MUB-NAG) (Table S3). These extracellular enzymes have been used to assess key functional activities in marine environments [28, 45,46,47,48,49]. Assays were performed as described by Hoarfrost and Arnosti [28] and Del Negro et al. [48], with modified incubation temperatures and times. Briefly, the 96-well black flat-bottom plates (Greiner Bio One, Germany) were incubated in the dark for 2.5 h at 7 °C for F-max and 4 °C for OMZ and Deep samples. Fluorescence was measured using a Spectramax® Paradigm Multi-Mode Microplate Reader (Molecular Devices, USA) at 360-nm excitation and 450-nm emission wavelengths. Extracellular enzyme activities were thereafter calculated as previously described [50].

### *DNA Extraction and Sequence Analysis*

DNA was extracted from both 45-µm and 0.2-µm filters for each sample as described in Phoma et al. [39] using the Power Soil™ DNA Isolation Kit (MO BIO Laboratories Inc., USA) following instructions detailed by the manufacturer. Briefly, the DNA from both filters (per sample) were pooled and sent to the Molecular Research DNA sequencing facility (MR DNA; USA) for sequencing on the Illumina MiSeq platform.

Barcoded universal sequencing primers, 515F (5′-GTGCCAGCMGCCGCGGTAA-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) were used to amplify the hypervariable

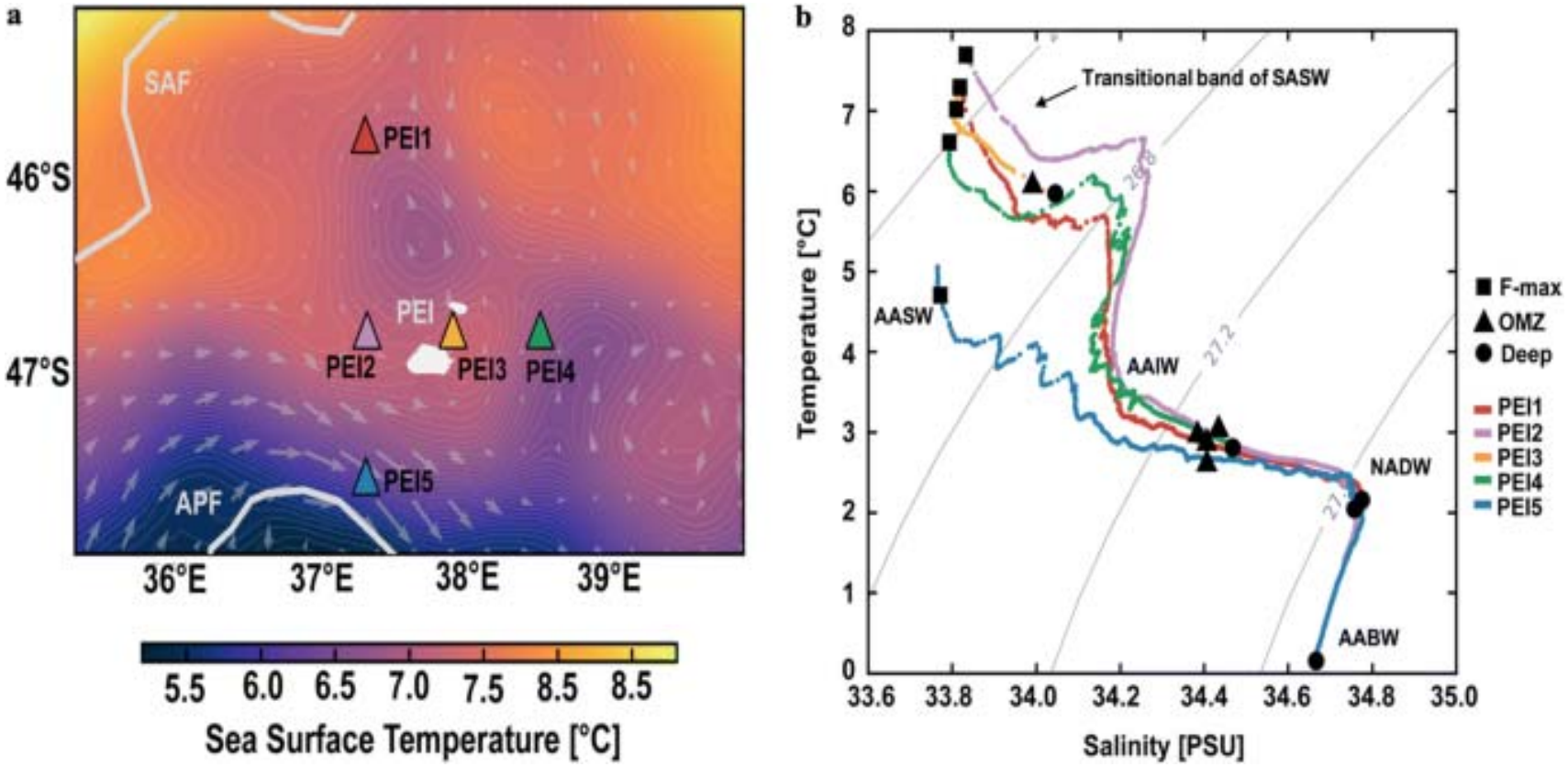


**Fig. 2.** Location, distribution and temperature/salinity profiles of samples recovered within the vicinity of the PEIs. (**a**) The distribution of hydrographic stations. The SAF and APF bands are indicated as grey lines. Arrows indicate sea surface transport. Figure was generated using satellite sea surface temperature (SST) values obtained during the cruise. (**b**) Temperature and salinity (T/S) profiles showing the different water masses from each of the PEI stations during the cruise as reported from Ansorge et al. [40]. B was constructed using Ocean Data View (Schlitzer, R., Ocean Data View, http://odv.awi.de, 2018)

bacterial and archaeal V4–V5 regions as previously described [51, 52]. The primer pair used has been previously reported to underrepresent the SAR11 clade [53]. We resolved the variation of microbial communities trends along depth, and did not necessarily focus on resolving individual taxa (e.g. members of the SAR 11 clade) [54]. Once the sequences were obtained, analysis was carried out using an open-source software pipeline, QIIME (version 1.9.1) as described previously [51, 55].

Briefly, fasta and quality files were merged to generate a fastq file for quality filtering. The metadata mapping file was corrected before separating libraries to individual samples using the *split_libraries_fastq.py* script. The detection and removal of Chimeric sequences were detected and removed using USEARCH 6.1 [56, 57], using both the de novo and reference-based (Chimera Slayer reference database) methods. The remaining high-quality sequences were clustered into operational taxonomic units (OTUs) based on 97% sequence similarity, using the SILVA reference database [58, 59]. Lastly, singleton reads were removed using the *filter_otus_from_otu_table.py* script.

The output file (biom format) was used to convert reads to an OTU table of relative abundances and associated taxa using the summarize_taxa.py script. This output was used to compare taxonomic units between PEI samples and stations. The biom file was used to compute the 'core microbiome', defined as the unique OTUs shared among the 15 samples (i.e., F-max 1–5, OMZ 1–5 and Deep 1–5) and across each sample depth (F-max, OMZ and Deep). The file was uploaded on the *MicrobiomeAnalyst* tool [60] (available at: http://www.microbiomeanalyst.ca). The output was normalised using the relative log expression (RLE; a DESeq-2-like approach). The core microbiome analysis was subsequently performed using default parameters (20% sample prevalence and 0.2% relative abundance).

The non-rarefied OTU table was also normalized to account for uneven sampling efforts by subsampling all sequences to the lowest number of reads. Rarefied sequences were then used to analyse biodiversity via the *core_diversity_analyses.py* script to estimate alpha diversity indices.

### *Statistical Analysis*

The R software (http://cran.r-project.org/; R version 3.4.3) was used for all statistical analyses. Rarefaction curves were generated for all samples using the *rarecurve* function in *vegan*. Alpha, gamma and beta-Whittaker (gamma/alpha) matrices were calculated using the rarefied matrix in *vegan* [61]. Differences in alpha diversity indices between the respective sample groups (F-max vs OMZ, OMZ vs Deep and Deep vs F-max) were tested using the *wilcox.test* function ($p < 0.05$) and two-way analysis of variance (ANOVA) analysis (ANOVA $p < 0.05$) in R. Alpha diversity indices for richness (Chao1 and ACE) and evenness (Shannon and Simpson) were depicted as box plots using the *qplot* and *grid* functions in *ggplot2* [62]. Bar plots were constructed and visualized to illustrate the overall microbial communities in all samples using *phyloseq* [63] and *ggplot2* R packages. Two-way ANOVA tests were also used to test significant (ANOVA $p < 0.05$) differences between potential extracellular enzyme levels by sampled depths.

Mantel tests (*mantel* function of the *vegan* package) were carried out to assess differences between non-rarefied and rarefied matrices using Bray-Curtis distance for beta diversity analysis. The tests were performed using Spearman's rank correlation analysis. Thereafter, the non-rarefied sequence count matrix generated from QIIME was used to assess spatial

patterns in bacterioplankton composition among the F-max, OMZ and Deep sample groups, as described previously [64]. Bray-Curtis dissimilarities were calculated using the Hellinger-transformed OTU matrix using *vegan* [65]. Thereafter, a non-metric multidimensional scaling (NMDS) plot was constructed to visualize potential spatial patterns based on community composition of all samples using the *metaMDS* and *plot* functions in *vegan*. Permutational multivariate analysis of variance, PERMANOVA (*adonis* function in *vegan* R package), was used to test whether community composition was significantly dissimilar from Bray-Curtis distances (*adonis* $p < 0.05$) using *vegan*. The *adonis p* values was corrected according to Benjamini and Hochberg [66]. This method was also used to assess the influence of the 'Island Mass Effect' on community compostion. To do this, we grouped samples that were closest to the island (Stations PEI2 and 3) versus those further from the islands (PEI1, PEI5 and PEI4).

The Shapiro-Wilk test was used to assess the normality of physicochemical variables and potential enzymatic activities data prior to log transformations of the dataset using the *stats* package. Non-parametric Spearman's rank order correlations and associated *p* values were generated to investigate relationships between (i) measured variables (nutrient concentrations and potential enzymatic activities) and in-situ seawater properties (CTD metadata) and (ii) bacterial and archaeal communities (>1% relative abundance at order level) and all physicochemical variables (measured and in-situ variables) via the *rcorr* function of the *Hmisc* package. These relationships were visualized using heat maps based on Spearman's *r* correlation coefficients and *p* values (<0.05) using *ggplot2* and *reshape2* packages. Corrected *p* values were used to account for multiple testing as previously described [66] using the *p.adjust* function of the *stats* package.

Redundancy analysis (RDA) was used to investigate the relationships between environmental variables and microbial community dissimilarity and to determine variables which explained the community structure [65]. Forward selection was applied using the *ordistep* function to select variables that contributed significantly to the RDA model [67] while removing non-significant ones. Forward selection significance was tested using Monte Carlo permutation *p* values. Significant environmental variables ($p < 0.05$) were thereafter shown on the RDA ordination plot using the *envfit* function of the *vegan* package. Variation partitioning analysis (VPA) was then used to calculate the variance explained by forward selected variables on the community structure using the *varpart* function.

### *Sequence Data Availability*

The 16S rRNA gene data were deposited to the National Centre for Biotechnology Information (NCBI) Sequence Read Archive (SRA) under the BioProject PRJNA326465 accession numbers SRR9995156 to SRR9995160.

## Results

### *Water Masses and Nutrient Characteristics*

We acquired samples from distinct water depths within the vicinity of the PEIs in the South Indian Ocean (Table S1). The location of surface expressions of the Sub-Antarctic Front (SAF) and Antarctic Polar Front (APF) (Fig. 2a) corresponded with previous published data [34, 68]. The selected hydrographic stations spanned key water masses based on their distinct temperature and salinity signatures (Fig. 2b) as reported in Ansorge, Froneman, Pakhomov,

Lutjeharms, Perissinotto and van Ballegooyen [40]. The conditions in the first four stations (PEI1–PEI4) differed substantially from those in the last station (PEI5) consistent with Sub-Antarctic and Antarctic water masses, respectively. Sub-Antarctic surface waters (SASW) were slightly warmer (6.68 to 7.29 °C) compared with the Sub-Antarctic Front (SAF) (Fig. 2b). Samples from the inter-island station (PEI3) were generally shallower (ranging from 50.57 to 199.24 m) with all samples from the SASW. SASW was characterized by low nutrient concentrations and the highest recorded levels of dissolved oxygen (Table S1). The surface sample (PEI1) has the highest fluorescence level, consistent with Agulhas current-driven subtropical waters (Table S1). In contrast, the surface sample (F-max5) was characterized by cooler (4.77 °C) and more oxygenated Antarctic surface waters consistent with the Polar Frontal (APF) zone (Fig. 2a).

With the exception of samples from station PEI5, which were from Antarctic Intermediate Waters (AAIW), all surface waters were from SASW. Samples from station PEI4 oxygen minimum zone and deep waters were characteristic of the AAIW. Bathypelagic samples were consistent with North Atlantic water (Deep1 and Deep2) and Antarctic bottom water (Deep5). NADW is transported to the South Indian Ocean via the global thermohaline circulation and often intersects the northward-flowing current (the Antarctic Bottom Water; AABW). The AABW (>4000 m) was characterised by moderately low salinity (34.66 PSU), lowest temperature (0.21 °C), and highest silicate and phosphate concentrations among all samples (ANOVA, $p = 0.002$; 135.07 μm/l and 3.9 μm/l, respectively; Table S2).

### *Extracellular Enzymatic Activity Depth Trends*

The potential activities of the five extracellular enzymes analysed were low. These activities differed substantially by depth and proximity from the PEIs (Table S2). For instance, the shallow inter-island CTD station (PEI3; depth = 50.57 m) had the highest enzymatic levels across all samples (Fig. 3a). Moreover, activities for all enzymes were higher among F-max samples. However, less variation was observed for carbon acquisition enzymes (β-glucosidase and β-xylosidase) among OMZ and Deep samples, respectively. Two-way analysis of variance (ANOVA) revealed that enzyme activities were significantly different (ANOVA $p < 0.05$) among the three depths for carbon acquisition enzymes, alkaline phosphatase and leucine aminopeptidase, respectively. No differences (ANOVA $p > 0.05$) were found for alkaline phosphatase, β-glucosidase and β-xylosidase activities from pairwise analysis of OMZ vs. Deep samples. However, we found clear differences in leucine aminopeptidase pairwise analyses. *N*-acetylglucosaminidase levels among the three sampled depths were not significant (average ANOVA $p = 0.19$) (Fig. 3a).

### *Correlations between Environmental Variables Versus Nutrient Concentrations and Potential Enzyme Activities*

Spearman correlation analyses were used to evaluate the relationship between environmental variables, nutrients and potential extracellular enzymatic activities (Fig. 3b). Our analysis revealed that depth and salinity were highly positively correlations with phosphate (Spearman's correlation coefficient rho = 0.98; $p < 0.001$ and Spearman's correlation coefficient rho = 0.91; $p < 0.001$, respectively) and with silicate (rho = 0.98; $p < 0.001$ and rho = 0.90; $p < 0.001$, respectively) levels. However, we found no correlation between depth and salinity with nitrate concentrations (both $p = 0.07$, respectively). Moreover, depth and salinity were negatively correlated with all enzyme activities. Fluorescence and oxygen showed similar trends in response to nutrients and enzyme concentrations. They both were

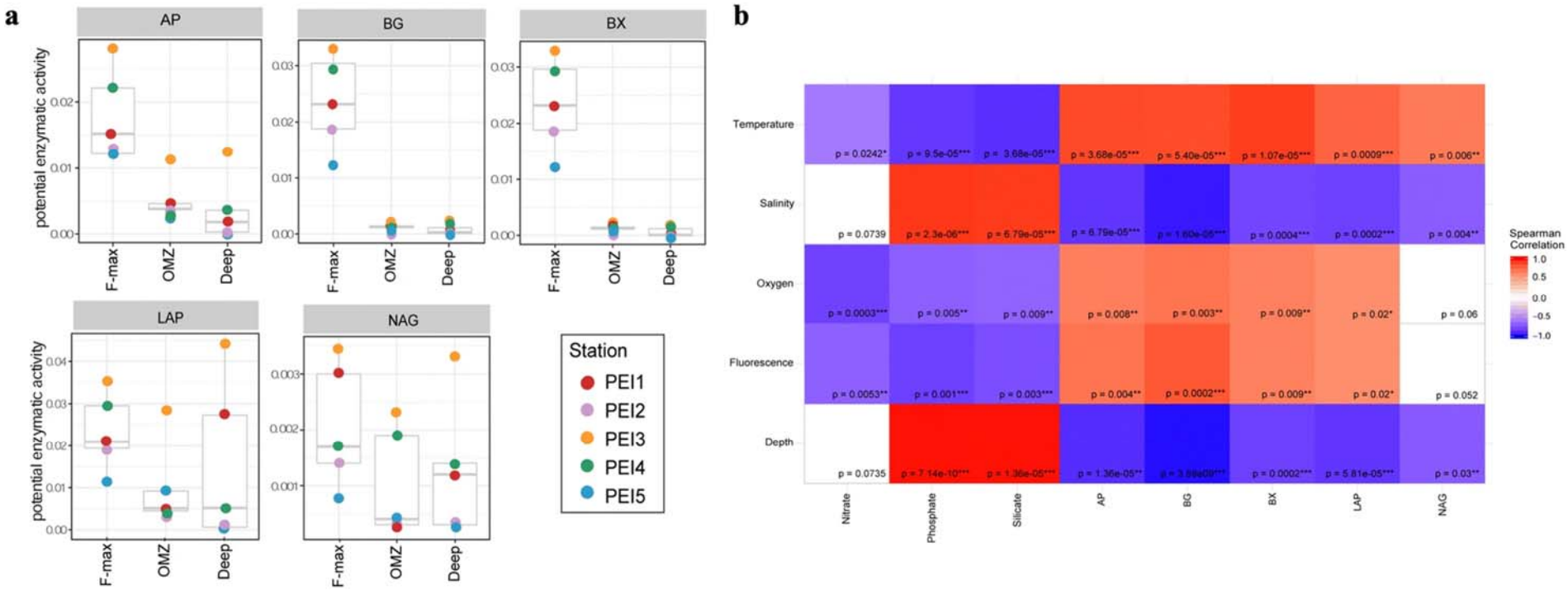


**Fig. 3.** Patterns of physicochemical variables across fluorescence maximum (F-max), oxygen minimum (OMZ) and deep (Deep) zones. (**a**) Extracellular enzymatic activities are shown as boxplots (1 to 5): alkaline phosphatase (AP), β-glucosidase (BG), β-xylosidase (BX), leucine aminopeptidase (LAP) and *N*-acetylglucosaminidase (NAG). (**b**) A heat map showing Spearman's rank correlation analysis of seawater properties with nutrients and extracellular enzyme levels. A correlation coefficient rho value of 1 (red) indicates positive correlation, a value of − 1 (blue) indicates an inverse correlation, and a value of 0 (white) indicates no correlation. Spearman *p* values are shown in each square

negatively correlated to all nutrients and had no significant correlation with *N*-acetylglucosaminidase ($p > 0.05$). Temperature concentrations were positively correlated to enzyme concentrations (average rho = 0.80; $p = 1.43e{-}03$).

***Bacterioplankton and Archaeoplankton Diversity Patterns***

In total, 847,979 raw 16S rRNA gene sequences were obtained from the 15 sampling stations. After quality filtering and chimera removal, 819,419 sequences were retained (ranging from 28,117 in OMZ2 to 56,977 in F-max3; standard deviation (SD) ± 8371. Following singleton removal, the output file was used to illustrate rarefication curves of all samples (Fig. S1a). In order to correct for variation in sequencing depth, samples were rarefied (subsampled) to the lowest number of sequences among 15 samples (28,117). Subsequently, 623,665 sequences were retained for alpha diversity analysis (Table S4).

The number of observed OTUs ranged from 381 (OMZ2) to 807 (Deep1) (Table S4). Standard alpha diversity metrics (Chao1, ACE, Simpson and Shannon) were also evaluated (Fig. S1b). Sample richness and evenness metrics demonstrated heterogeneous patterns between depth and sampling stations (ANOVA, $p$ value < 0.05). Specifically, sample richness was either (i) higher in Deep, followed by F-max and OMZ at PEI3 and PEI1 stations, (ii) higher in F-max, followed by Deep and OMZ at PEI2, (iii) increased by depth at PEI5, or (iv) decreased by depth at PEI4. ACE abundance and Chao1 richness estimates were higher than the observed OTUs. For Shannon diversity, no differences were observed based on pairwise Wilcoxon tests ($p > 0.05$). The average Shannon index for Deep samples was higher (6.81; ± 0.30), followed closely by OMZ (6.38; ± 0.38) and F-max (5.98; ± 0.41). In contrast, gamma diversity was found to be higher for Deep followed by F-max and OMZ samples, at 1530, 1183 and 1153, respectively. Notably, coverage estimates (percentage Good's coverage; average of 99.48%) indicated that the 16S rRNA gene libraries for each sample were sufficient to capture majority of the estimated OTUs at 97% similarity level (Table S3). However, rarefaction curves failed to reach saturation (Fig. S1b), signifying that the sequencing effort was not satisfactorily large to capture the complete microbial community diversity.

The non-rarefied matrix contained 612,911 total read counts (ranging from 26,573 to 56,814; average counts per sample of 40,860). This dataset was used for beta diversity analysis. A total of 1891 unique OTUs were identified across all samples at a 3% dissimilarity threshold cut-off. Bacterial lineages in the PEI were higher (91.32% of the total OTUs assigned), with lower percentage abundance of Archaea (7.35%) and unassigned sequences (1.32%). A non-metric distance scaling (NMDS) ordination plot was generated to illustrate the dissimilarities between the 16S rRNA gene dataset, using Bray-Curtis distances (Fig. S2a). The plot showed that the sampled depths were broadly dissimilar on the basis of taxonomic distance. However, OMZ3 and Deep3 communities clustered with F-max communities, likely due to the shallow sampling stations (Fig. S2b). Global PERMANOVA (*adonis*) comparisons validated the overall difference between all samples (*adonis* $R^2 = 0.34$; $p = 0.005$). F-max communities formed a clustered and differed markedly from both OMZ ($R = 0.37$; $p_{corrected} = 0.017$) and Deep communities ($R^2 = 0.36$; $p_{corrected} = 0.014$). However, no significant variability was found between OMZ and Deep ($R^2 = 0.10$, $p_{corrected} = 0.593$) (Table S5). The *adonis* pairwise analysis was also used to query the influence of the PEIs on community composition ('Island Mass Effect'). Using PERMANOVA (*adonis*), we found a significant correlation between community compositions of CTD stations that are near (PEI1, PEI2 and PEI4) and far (PEI2

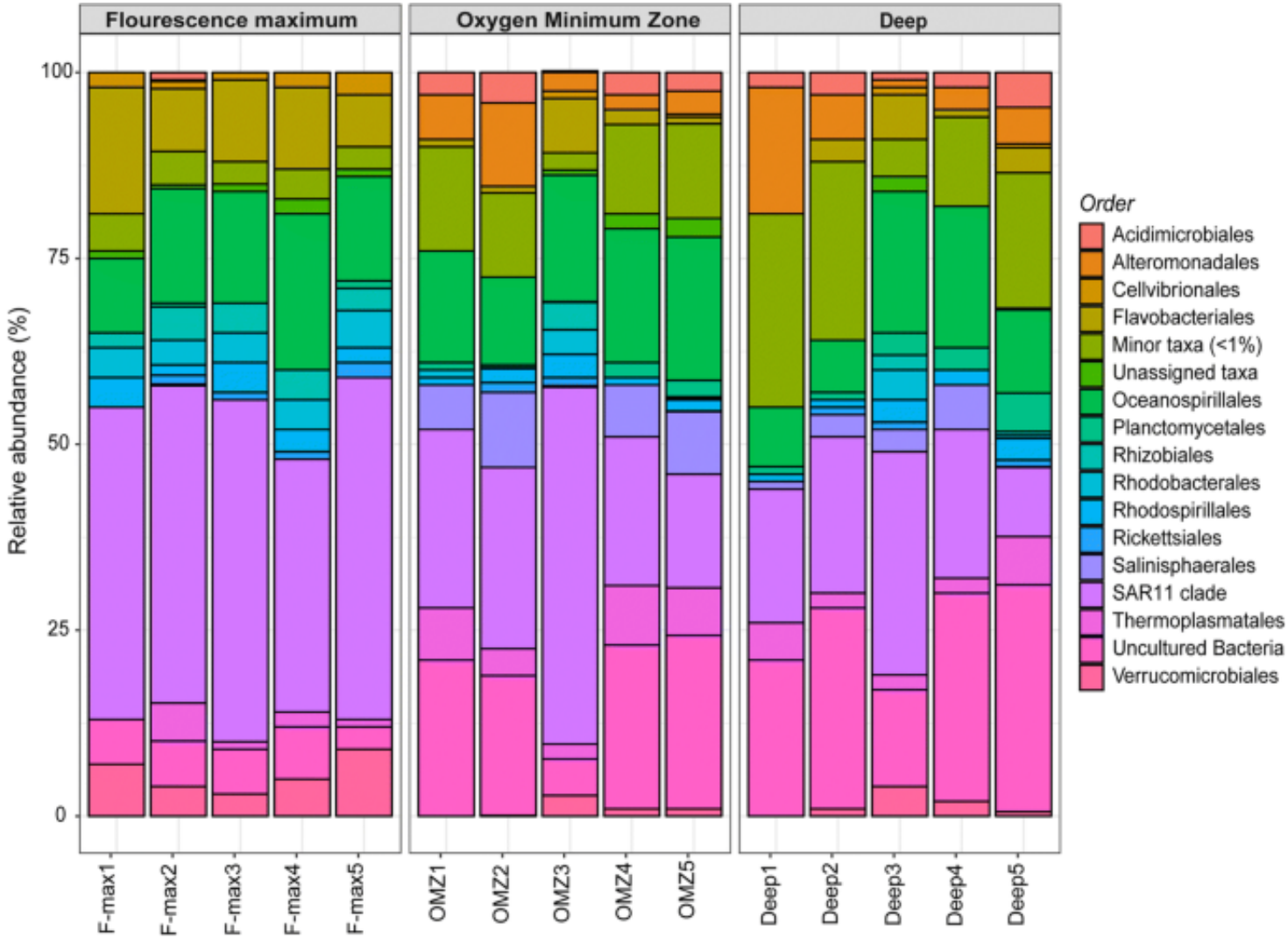


**Fig. 4.** The relative abundance of dominant (>1%) bacterial and archaeal taxa at order level across F-max, OMZ and Deep samples

and PEI3) from islands, albeit at low levels (adonis $r^2 = 0.1420$; $p_{corrected} = 0.043$), suggesting an occurrence 'Island mass effect'.

***Community Composition, Relationship with Depth and Core Taxa***

Each PEI station and sampled depth harboured diverse proportions of each of the 18 assigned phyla. Among bacterial phyla ($n = 14$; 82.5%), members of the Proteobacteria were dominant throughout the PEI transect (average = 64.85%; ± 10.68%), with the highest fraction at OMZ3 (81.81%) at 197.26 m. At class level, the most dominant Proteobacteria were Alphaproteobacteria (mostly affiliated to order SAR11 clade) (Fig. 4), and Gammaproteobacteria (mostly order Oceanospirillales; family SAR86 clade) at different proportions. For instance, the relative abundances of SAR11 generally decreased with depth (ANOVA, $p < 0.002$), while SAR86 clade increased with depth (ANOVA, $p < 0.005$) in all stations. However, SAR11 were dominant at all depths at the shallow station (PEI3). Three other ubiquitous phyla, Bacteroidetes (mostly *Flavobacteriaceae)*, Verrucomicrobia (*Roseibacillus sp.*) and Cyanobacteria, were observed at differing abundances across all stations. The relative abundances of *Flavobacteriaceae* decreased with depth (ANOVA, $p < 0.02$) and ranged from 0.62 to 14.81%, with the highest proportion found at station F-max1. Although order Verrucomicrobiales were present at lower abundances in OMZ, their proportion along the water column was higher than Bacteroidetes and Cyanobacteria at station F-max5. Cyanobacteria were copious in F-max samples (dominated by *Prochlorococcus)*, with the highest abundances at a station with highest recorded in situ fluorescence (F-max1) (Fig. S3).The ubiquitous archaeal phyla, Thaumarchaeota and Euryarchaeota, also varied considerably across stations. Thaumarchaeota (mostly comprised of *Candidatus Nitrosopelagicus*) generally increased with depth (ANOVA, $p = 0.03$), with the highest proportion of 20.45% at Deep4 and lowest at F-max2 (0.11%). Euryarchaeota (mostly Marine Group II) were dominant in OMZ samples and had the lowest proportion among F-max samples. Whereas, Woesearchaeota were only detected in deep samples (data not shown).

The PEI core microbiome comprised a total of 71 OTUs (3.75%) of all OTUs (Table S6). Taxa contributing to the total core microbiome at class level included Acidimicrobiia (Sva0996 marine group; 2.82%), Alphaproteobacteria (26.77%; mostly SAR11 clade), Cyanobacteria (3.96%; 2 *Prochlorococcus and 1 Synechococcus*), Deltaproteobacteria (2.82%; SAR324 clade), Flavobacteriia (4.23%; NS5 marine group), Gammaproteobacteria (28.18%; mostly SAR86 clade), Marine Group I (14.08%; *Candidatus Nitrosopumilus*), Planctomycetacia (1.41%; *Planctomycetaceae*), Thermoplasmata (7.04%; Marine Group II), Marinimicrobia (SAR406 clade) (7.04%) and Verrucomicrobiae (1.41%; *Roseibacillus sp.*). F-max samples (core OTUs = 34) was dominated by SAR11 clade (22.54%), followed by SAR 86 clade (21.13%) and NS5 marine group (14.71%) (Table S6). OMZ and Deep samples (both with 41 core OTUs) were both dominated by Marine Group I (*Candidatus Nitrosopumilus*) at 17.07%).

Environmental variables which correlated with community relative abundances were identified at order level (Fig. 5). Spearman's correlation coefficients and associated *p* values ($p < 0.05$) confirmed that bacterial and archaeal taxa were significantly associated to seawater nutrient concentrations, physicochemical variables and potential enzyme activities. All potential extracellular enzymatic activities were positively correlated with Flavobacteriales, Rhizobiales, Rhodobacterales, Rhodospirillales, SAR 11 clade and Verrucomicrobiales. However, Cellvibrionales were positively correlated with β-Glucosidase, β-Xylosidase,

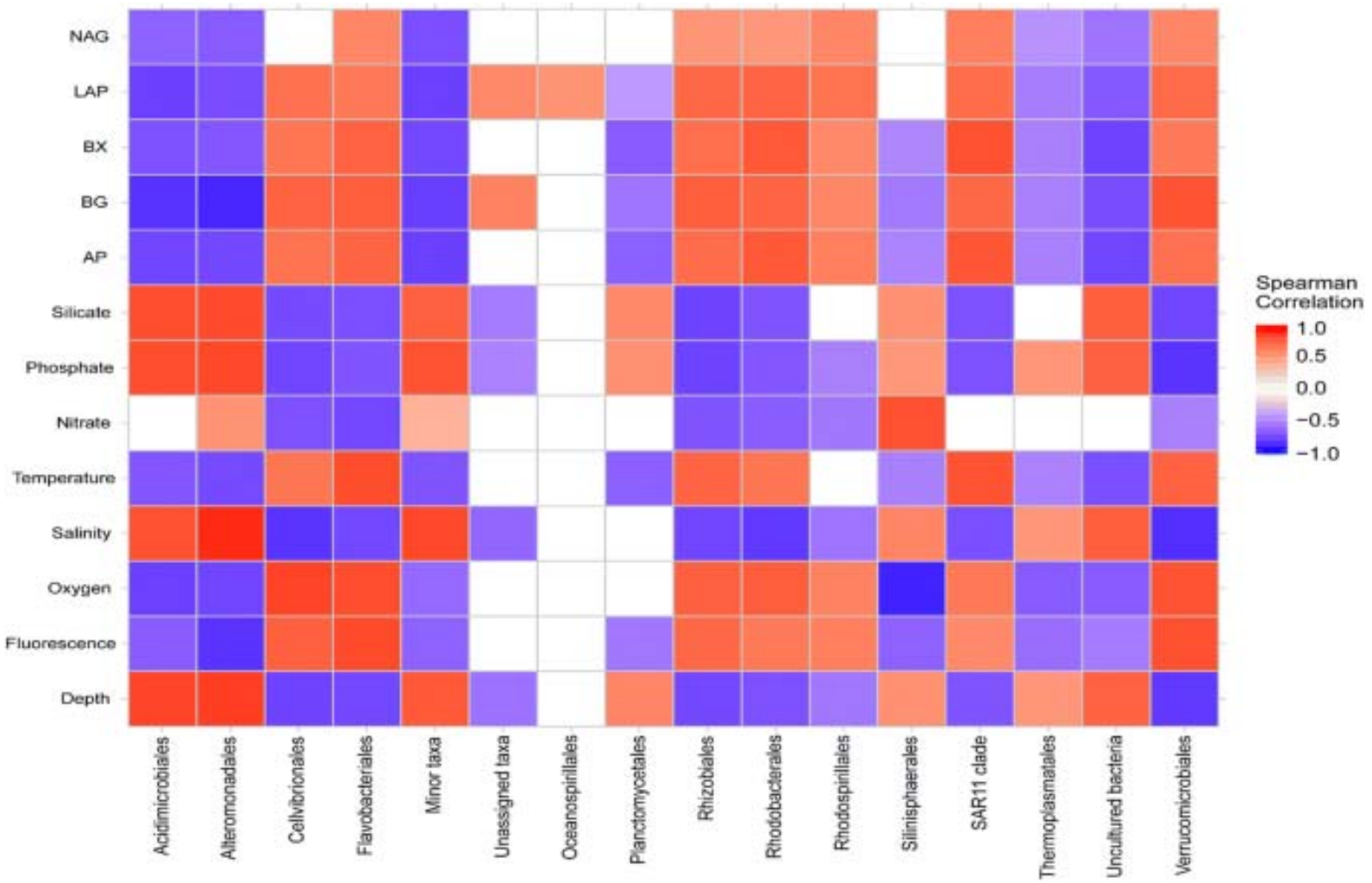


**Fig. 5.** Heat map showing Spearman's rank correlation analysis between physicochemical variables and significant taxa at order level ($p < 0.05$). A correlation coefficient rho value of 1 (red) indicates positive correlation, a value of − 1 (blue) indicates an inverse correlation, and a value of 0 (white) indicates no correlation

activity, alkaline phosphatase and leucine aminopeptidase**,** except N-acetylglucosaminidase. Oceanospirillales was only positively correlated with leucine aminopeptidase. Conversely, Acidimicrobiales, Alteromonadales, Thermoplasmatales, minor order (less than 1% relative abundance; dominated by SAR324 clade of class Deltaproteobacteria) and uncultured bacteria (dominated by uncultured Candidatus Marinimicrobia bacterium and Cyanobacteria SubsectionI bacterium) were inversely correlated to enzyme activities.

Our data analyses demonstrate that known oligotrophs, such as F-max dominating Alphaproteobacteria (SAR11 clade) and Cyanobacteria (Subsection I Cyanobacteria bacterium), were inversely correlated with nutrient concentrations (Fig. 5). However, Alteromonadales were positively correlated to all nutrients. Among all environmental variables, we found that when taxa were positively correlated with depth and salinity, they were also inversely correlated with temperature, oxygen and fluorescence, and vice versa.

### *Linking Microbial Communities to Environmental Variables and Potential Functionality*

Redundancy analysis (RDA) was performed on microbial communities and environmental variables. In total, 10 explanatory variables, including depth, phosphate, silicate, nitrate, oxygen, temperature, fluorescence, leucine aminopeptidase, β-glucosidase, β-xylosidase, salinity and were assessed **(**Fig. 6a**)**. Stepwise reduction analysis revealed that salinity, *N*-acetylglucosaminidase and alkaline phosphatase were not significant for the structuring of bacterioplankton communities ($p > 0.05$). The RDA plot showed that environmental variables shaped bacterioplankton structure along the first axis (RDA1), distinguishing epipelagic (<200 m; F-max1–5, OMZ3 and Deep 3) and aphotic zones (>1000 m) (Fig. 6a). Thus, the most crucial variables shaping shallow communities were found to be oxygen, temperature, β-glucosidase and β-xylosidase. Our analyses also revealed that depth, silicate and nitrate structured bacterioplankton occupying the aphotic waters. At order and genus levels, we found that known shallow-associated OTUs (such as SAR11 clade, Marine Group II, *Prochlorococcus*) emerged among oxygen, fluorescence and enzyme activities, indicating that synergistic effect of environment on taxa. Similarly, Deep-associated taxa, i.e. *Pseudoalteromonas* and *Candidatus nitrosopumilus* were largely driven by depth and nitrate levels, respectively. The RDA1 axis was strongly related to depth ($r = 0.66$), whereas RDA2 was best correlated with oxygen ($r = 0.51$). These axes accounted for 43.55% (adjusted $r^2$; $p = 0.004$) of the variation in the dataset.

Variation partitioning was conducted to quantify the relative contribution of significant ($p < 0.05$) predictor variables (i.e. extracellular enzymes, nutrients, oxygen and temperature, and depth) to the bacterial and archaeal community composition. Co-linear properties, temperature and depth were found to be the most influential predictor variables, followed by nutrients and potential extracellular enzyme activity (Fig.6b). Thus, the VPA showed that depth may be a primary explanatory factor shaping community composition as it accounted for 15.45% of the total variance. Altogether, the variables explained 43.42% of the variation observed for PEI microbial community with the bulk of the variance (56.58%) unexplained.

## Discussion

In the oceans, nutrient availability varies considerably along the water column [69,70,71]. These shifts may be more pronounced closer to landmasses, which may change the nutrient dynamics in surrounding marine environments by influencing currents [72]. The resultant nutrient regimes may directly affect marine microorganisms and the functions they mediate

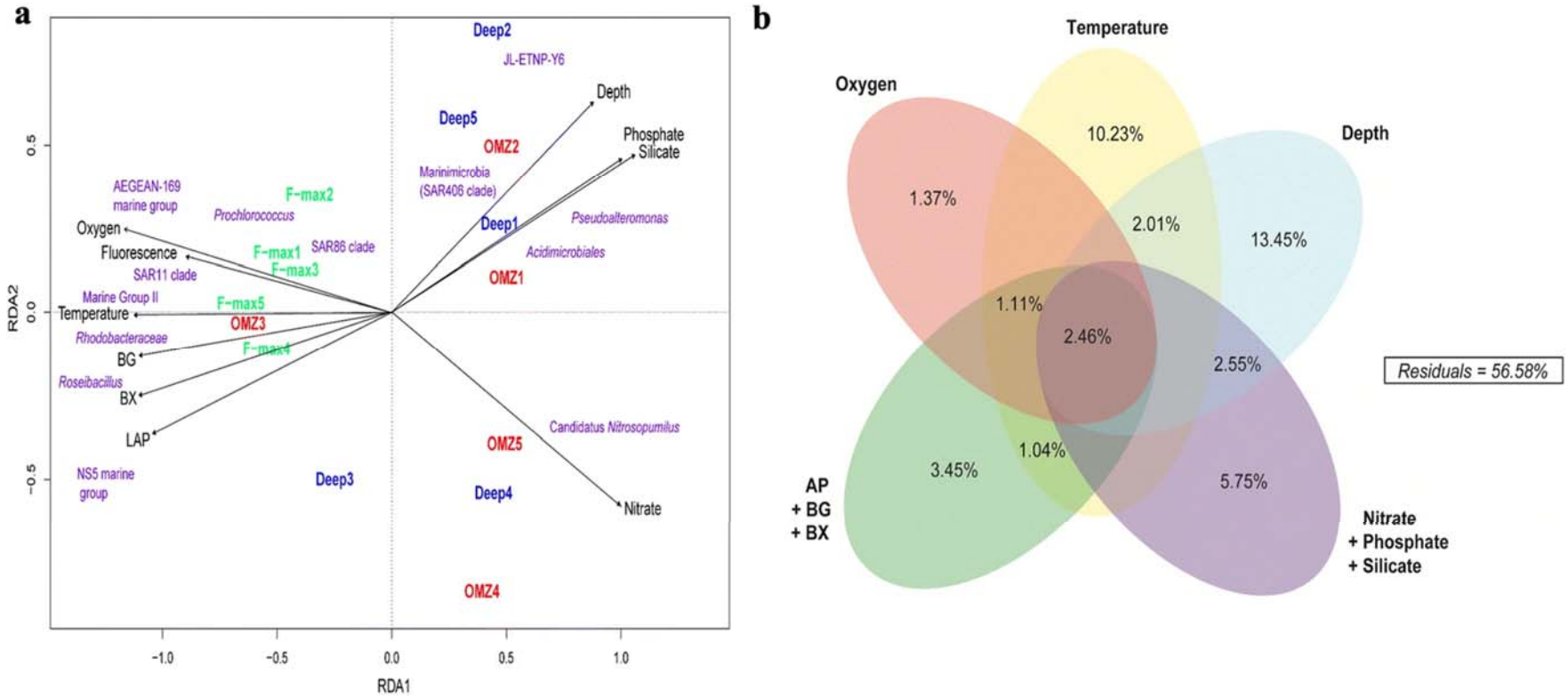


**Fig. 6.** The relationship between community composition and seawater variables. (**a**) Redundancy analysis (RDA) biplot of significant bacterial and archaeal taxa, nutrients and extracellular enzymes. Extracellular enzymes (β-glucosidase (BG), β-xylosidase (BX), and leucine aminopeptidase (LAP), in-situ seawater properties (depth, temperature, oxygen, fluorescence) and all measured nutrients significantly explained variability in community structure. These variables were fitted to the ordination (black arrows, $p_{envfit} < 0.05$). (**b**) Venn diagram of variation partitioning analysis of microbial community composition. The model represents proportions (%) of variation in community composition that are explained by significant environmental variables. Overlapping areas indicate shared variation. Unexplained proportion (%) is shown in a text box

[73,74,75]. We predicted that physicochemical and oceanographic properties will substantially structure the bacterial and archaeal communities in sub-Antarctic Indian Ocean waters. Moreover, we expected that these shifts in structure may be complemented by functional variations as measured by the activity of extracellular enzymatic activities.

***Depth-Dependent Variables Substantially Influence Community Structure***

In contrast to our prediction, our analysis suggest that water mass mixing and depth-dependent environmental variables explained community structure patterns. This finding has important implications for establishing the likely effects of climate change on microbial diversity and potential functionality. In distinct ocean regions with different water masses, nutrient transport along the water column may impact the capacity for mediating biogeochemical cycles. Water mass mixing has previously been shown to influence nutrient levels and prokaryotic community composition [76, 77]. A previous study on Northeast Atlantic deep waters showed that water mass mixing was a substantial factor regulating the distribution of inorganic nutrients supporting the activity of prokaryotes in this region [76]. We showed the importance of water masses in structuring microbial communities in the Agulhas current system and the Subtropical Front in the SIO [39]. These studies are in line with our findings showing that sub-Antarctic and Antarctic water masses differed substantially and shaped the hydrographic conditions in the respective stations. These findings extend previous studies and confirm the importance of water masses in the PEIs. Similarly, depth was shown to be an important factor in marine environments [78,79,80]. Depth-dependent variables including salinity, oxygen, nutrients and temperature are important regulators of ocean ecosystem services [71, 81, 82]. For instance, temperature is a key depth-dependent variable and regulates the kinetic rates of metabolic processes [83, 84]. Several analysis showed that depth-dependent variables influenced microbial community structure. This work extends previous studies which have shown that depth and depth-dependent variables are important drivers of picoplankton in marine environments globally [30, 80, 85,86,87,88,89]. Our results show that microbial communities in marine regions which are geographically proximal to landmasses are also driven by depth and depth-related variables. This is likely due to the differences in physicochemical properties linked to water masses [39, 45], frontal systems [54, 90], mesoscale eddies [91] and advection [90, 92].

The results from core microbiota analysis suggest a low number of specialist taxa. In other systems such as root [93] and gut [94] microbiomes, core microbiota have been ascribed to niche specificity. Fast growing specialist taxa are thought to thrive despite the competition and invasion which may pose as barriers to dispersal [95]. Similarly in surface marine environments, previous studies have suggested that ecologically rare taxa may be associated with shifts that restrict the distribution of community members [96]. Our findings suggest that both depth and depth-dependent variables may act as habitat transitions that may limit the distributional patterns of microbial communities. The lack of metabolic versatility may explain the specialist role of some taxa.

We note that a large proportion (56.58%) of community variance was not explained by variation partitioning analysis. Several other factors which were not measured in our study including predation, seasonal variability, viral lysis, organic nutrient availability and hydrologic conditions may explain community structural patterns [97,98,99,100]. Nevertheless, correlation and RDA analyses confirmed known environment-taxa relationships and the effects of physicochemical variables. For instance, several surface-associated marine communities including members of the SAR11 clade and Cyanobacteria

(predominantly *Prochlorococcus*) were negatively correlated with depth and positively correlated with fluorescence (proxy for primary productivity) and oxygen (proxy for respiration). This is entirely in line with previous studies which have shown that the miniature genomes associated with members of the SAR11 clade and Prochlorococcus are streamlined for photic waters [101, 102]. Equally, the positive correlations shown for Candidates *Nitrosopelagicus* and nitrate is consistent with their known ammonia-oxidising capacity [103]. Variation partitioning analysis and RDA analysis showed that depth, temperature and oxygen were significant for structuring microbial biogeography. However, these findings highlight the role of the influence of the oceanographic setting within the PEIs as a significant determinant of bacterial and archaeal taxa with possible effects on function.

### *Vertical and Horizontal Trends in Extracellular Enzymatic Activities*

We detected extracellular enzymatic activities linked to carbon (β-glucosidase and β-xylosidase), nitrogen (leucine aminopeptidase and *N*-acetylglucosaminidase) and phosphate (alkaline phosphatase) function, albeit at low concentrations. We collected samples in late April, where seasonally cool temperatures are likely to constrain functionality which explains the low activity levels. Nevertheless, the low activity detection levels indicate the presence of functional heterotrophic communities. These extracellular enzymatic activities were strongly linked to depth, with higher levels at fluorescence maximum samples. Surface marine environments are known to be highly productive and harbour the most primary producers [104]. Moreover, the higher levels of dissolved organic matter in the surface ocean, primarily explains the higher levels of hydrolytic activity observed. These findings are consistent with previous studies by showing decreases in enzyme activities with depth in the upper 100 m of the North Atlantic Ocean [105]. Here, we show that these patterns also extend to the aphotic zones of the ocean, and specifically waters in the vicinity of the PEIs.

In contrast, previous studies in the same region have shown higher extracellular enzymatic activities (for leucine aminopeptidase and alkaline phosphatase) in the deep ocean (>7000 m). Our results agree in part, with the study by Baltar et al. [91] as we also found higher extracellular activities for leucine aminopeptidase in deep samples. We note, however, that the measured extracellular enzymatic activities were highly variable possibly due to intra-sample variability in the sampled depth. This discrepancy suggests that more studies are required to resolve the vertical extracellular activity patterns for leucine aminopeptidase.

We observed the lowest extracellular enzymatic activity levels for *N*-acetylglucosaminidase and leucine aminopeptidase in OMZ waters. Previous studies have suggested that OMZ regions may be a focal point for oceanic nitrogen depletion [106]. The constrained nitrogen cycle may explain the low levels of these two extracellular enzymatic activities. It has been suggested that the majority of the nitrogen cycle activity may be confined to the free living fraction of marine microbial communities [107]. These extracellular enzymatic activities are possibly not appropriate for measuring microbial activities linked to large-particle–associated fractions of the ocean [108]. Previous studies also suggested that OMZ waters may impede nutrient cycling, plankton distribution and productivity [109].

The variation in enzyme activity may also be due to location [110], temporal patterns of substrate utilization [111], latitudinal distance [112], and the Island Mass Effect [113]. In our case, freshwater runoff from the PEIs has been reported to increase DOM levels and influence phytoplankton blooms (briefly referred to as the ‘Island Mass Effect’ hypothesis) [33, 114, 115], which could in turn have an effect on marine extracellular enzyme activities.

Runoff contains high macronutrient concentrations, dominated by reduced forms of nitrogen produced by top predators [115]. During these events, a large portion of organic detritus in the photic layer sinks to the seafloor as part of marine snow aggregates and constitutes the main organic nutriment for the benthic microbial community [116]. This may explain the observed high levels of enzyme activities in seawater from the shallow inter-island deep station (Deep3) (Fig. 3a). This observation may be explained by the presence of different ecological niches supported by marine snow with increasing depth [117, 118].

The potential organic matter hydrolysis also correlated significantly with the relative abundance of bacteria, suggesting that extracellular enzyme activity may be attributed to heterotrophic cells [26]. All enzyme activities were positively correlated with Flavobacteriales, Rhizobiales, Rhodobacterales, Rhodospirillales, SAR 11 clade and Verrucomicrobiales. This is likely due to phytoplankton-derived organic matter, suggesting that these taxa may largely benefit from organic matter mineralisation to circumvent environmental stressors in this region. For instance, SAR11 clade has been reported to be an unlikely candidate for extracellular enzyme production, although they consume labile dissolved organic matter [119]. Order Flavobacteriales (predominantly, the uncultured NS5 marine group) encompasses copiotroph members that are associated with marine blooms and able to decompose high molecular weight substances [120]. Previous studies have also demonstrated that Flavobacteriales can mediate phytoplankton organic matter degradation. Using network analysis, Li et al. [121] showed a positive correlation between order Flavobacteriales with Alphaproteobacteria, Cyanobacteria and Gammaproteobacteria, suggesting the influence of Flavobacteriia-derived organic products on the growth of marine heterotrophic microbial communities. Another metabolically versatile copiotroph, the Gammaproteobacterial SAR86 clade, has been implicated in the hydrolysis of various substrates at all depths. Several studies have identified this group as prime degraders of polymeric matter throughout the water column [122,123,124].

## Conclusions

In summary, the environmental and oceanographic variables, the varying responses of microbial assemblages and the extracellular enzymatic activities together provide the first insights on SIO microbiomes. Despite the augmented nutrient regimes proximal to the islands, we show that microbial community structure was comparable to other sub-Antarctic and Antarctic water masses. Depth-dependent variables were the major factors explaining the spatial heterogeneity of microbial communities and their extracellular enzymatic activity. These data hint at the potential role played by microbial communities in oceanographic regions with disparate nutrient regimes. However, we acknowledge that our findings based on amplicon relative abundances, may not entirely explain microbial dynamics along the water column. These data may require additional analysis based on quantifying absolute transcript abundances (i.e. metatranscriptomics) along the water column. Linkages to proteomes (metaproteomes) and additional measurements of POM or DOM, these data will clarify the role played by heterotrophic and chemolithotroph guilds throughout the water column. Taken together, these data will provide a basis for developing models to predict the likely impacts of climate change on microbe-mediated functional processes in marine environments such as the PEIs.

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