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Defining marine bacterioplankton community assembly rules by contrasting the importance of environmental determinants and biotic interactions

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Abstract

Bacterioplankton communities govern marine productivity and biogeochemical cycling, yet drivers of bacterioplankton assembly remain unclear. Here, we contrast the relative contribution of deterministic processes (environmental factors and biotic interactions) in driving temporal dynamics of bacterioplankton diversity at three different oceanographic time series locations, spanning 15° of latitude, which are each characterized by different environmental conditions and varying degrees of seasonality. Monthly surface samples (5.5 years) were analysed using *16S* rRNA amplicon sequencing. The high- and mid-latitude sites of Maria Island and Port Hacking were characterized by high and intermediate levels of environmental heterogeneity, respectively, with both alpha diversity (72%; 24% of total variation) and beta diversity (32%; 30%) patterns within bacterioplankton assemblages explained by day length, ammonium, and mixed layer depth. In contrast, North Stradbroke Island, a sub-tropical location where environmental conditions are less variable, interspecific interactions were of increased importance in structuring bacterioplankton diversity (alpha: 33%; beta: 26%) with environment only contributing 11% and 13% to predicting diversity, respectively. Our results demonstrate that bacterioplankton diversity is the result of both deterministic environmental and biotic processes and that the importance of these different deterministic processes varies, potential in response to environmental heterogeneity.

INTRODUCTION

Bacterial community structure influences ecosystem function in fundamental ways across all natural environments (Awasthi et al., 2014; Delgado-Baquerizo et al., 2016; Loreau et al., 2001), including the ocean (Galand et al., 2018), where microorganisms represent the base of the food web and are the principal

mediators of biogeochemical cycles (Azam et al., 1983). Ecological diversity underscores community structure; therefore, elucidating the processes that govern bacterioplankton diversity is critical for predicting marine ecosystem productivity and function. There are two alternative perspectives for how bacterial diversity assembles (Huber et al., 2020; Stegen et al., 2013). One view is of determinism, where species are regulated by niche processes such as environmental filtering (Fuhrman et al., 2015a) stemming from physico-chemical factors such as inorganic nutrients availability and temperature (Hernando-Morales et al., 2018; Ward et al., 2017), as well as biotic interactions including competition, predator–prey or facultative interactions (Friedman et al., 2017; Ho et al., 2016; Rohwer & Thurber, 2009). The other view is one of neutrality, where species are considered ecologically equivalent and therefore diversity consequently arise from stochastic birth, death, colonization, immigration and speciation (Evans et al., 2017; Hanson et al., 2012; Hao et al., 2016; Hubbell, 2001; Lindström & Östman, 2011; Zhou & Ning, 2017). The relative contribution of environmental, biotic interactions and stochastic processes, and how their importance changes over space and time is currently unresolved (Langenheder & Lindström, 2019), meaning that the ability to interpret and predict marine bacterioplankton diversity is restricted.

In the ocean, both environmental factors and trophic interactions fundamentally govern bacterioplankton diversity (Gralka et al., 2020; Nguyen et al., 2021), in terms of both the number of co-occurring species (alpha diversity) and the commonality of species among environments or sampling points (beta diversity) (Fuhrman et al., 2015b; Teeling et al., 2012). For instance, bacterioplankton community richness in the English channel, was highest during the winter months and strongly predicted by day length (Gilbert et al., 2012). Ladau et al. (2013) similarly found day length to strongly associate with marine bacterioplankton richness from temperate regions. In contrast, bacterioplankton community richness from the Antarctic region was negatively correlated with seasonal increases in chlorophyll-*a* (Chl-*a*), signalling potential interactions with algal blooms (Luria et al., 2016). Community beta-diversity patterns have also been shown to have environmental and biotic links. For instance, global sampling of surface bacterioplankton from the TARA dataset showed the strong effect of temperature and oxygen in driving community composition (Sunagawa et al., 2015). Surface layer (0–5 m) bacterioplankton beta diversity across 10 years in the San Pedro oceanographic time series (SPOTS) was best predicted by abiotic factors including nitrate and day length change as well as Chl-*a* (Chow et al., 2013). From a high-resolution coastal time series, Needham et al. (2018) demonstrated that bacterial abundance patterns were more strongly coupled to phytoplankton dynamics than other environmental factors, highlighting the role of biotic processes in structuring bacterioplankton community patterns. This study also noted that bacterial abundance patterns were correlated with other groups of bacteria, indicating biotic interactions are perhaps not limited to vertical trophic interactions, but can occur horizontally through cross-feeding and antagonism. Cross-feeding within bacterial communities has been hypothesized to fundamentally governing bacterioplankton diversity (Fiegna et al., 2015; Gralka et al., 2020; Little et al., 2008). Similarly, bacteria–bacteria interactions were shown to be important for the maintenance of bacterioplankton diversity in the English Channel evidenced by bacterial OTUs having stronger correlation with other bacterial OTUs than with phytoplankton OTU's and environmental factors (Gilbert et al., 2012). Similarly, at SPOTS, network analysis demonstrated that bacteria, archaea and eukaryotes had stronger correlation with one another than with any physico-chemical factors (Steele et al., 2011). More recently, Lima-Mendez (2015), incorporated the abundance of eukaryotic and viral groups alongside environmental factors to demonstrate that abiotic factors explained a limited amount of direct variation in marine bacterioplankton diversity and that trophic and symbiotic interactions were significant contributors to overall diversity (Lima-Mendez et al., 2015). Collectively, these results underscore that while environmental factors are important regulators of bacterioplankton diversity, biotic interaction are apparent and potentially influence bacterioplankton more strongly at times, but the relative contribution of each deterministic type is yet to be resolved.

Marine environments are inherently dynamic in their environmental characteristics, fluctuating across scales of space (i.e., micrometres to kilometres), and time (i.e., microseconds to months) (Nguyen et al., 2021). Therefore, distilling out specific factors responsible for diversity is particularly challenging, and may not accurately reflect the contemporary processes responsible for observed patterns (Chesson, 2000). Considering then the cumulative impacts of a set of environmental factors (or species interactions), and their relative contribution to diversity patterns is important because the impact magnitude of the ecological process is expected to vary in response to different ecological attributes (i.e., environmental heterogeneity) (Horner-Devine et al., 2004; Langenheder & Lindström, 2019; Veech & Crist, 2007). In one example, Langenheder et al. (2012) (Langenheder et al., 2012), showed that when environmental heterogeneity among rockpools was highest, beta diversity of bacterioplankton among the same rockpools was also highest, with deterministic processes emerging as the prevailing mechanism driving beta diversity; however, when environmental heterogeneity among rockpools was low, and beta-diversity

among rockpools was also relatively low, dispersal mechanisms became increasingly more important in driving beta-diversity patterns. Fluctuations in environmental heterogeneity have been demonstrated as important drivers of spatial beta-diversity patterns in disparate ecosystems, including the Amazon river system (Huber et al., 2020) and soil bacteria communities (Ferrari et al., 2016; Ranjard et al., 2013). These results reveal that the relative contributions of deterministic processes in shaping spatial bacterioplankton diversity can change through time in accordance with spatially distributed environmental heterogeneity (Langenheder & Lindström, 2019; Stegen et al., 2012); it remains to be shown however how deterministic influence is partitioned among environmental factors versus species interactions. In addition, marine bacterioplankton studies have focused primarily on understanding the influence of spatial environmental heterogeneity on bacterial diversity or temporal variability examined at a single location (Langenheder & Lindström, 2019). Therefore, current understanding of shifts in the relative importance of differing ecological processes on the temporal dynamics of bacterioplankton diversity at different locations is critically needed to understand if processes structuring diversity patterns are universal across distinct environment or rather idiosyncratic to an environment.

Distinguishing abiotic from biotic processes in structuring community diversity requires an effective means of identifying potential species interactions (Carrara et al., 2015). Herren and McMahon (2017) developed a community complexity metric for phytoplankton microbial communities based on the product of the median correlation value of each organism in the dataset to its relative abundance value. The authors argue this value provided an index for quantifying the importance of potential interactions within the community. Indeed, their results demonstrated that characterizing the complexity of a community can improve the proportion of explained variation, but it remained unclear whether the explained variance was due to environmental and/or species interactions because the metric was based on correlations, which could arise due to multiple organisms independently tracking similar environmental factors. One potential means to overcome this limitation is by incorporating metrics that are applied to individual samples, derived from the number and strength of correlative interactions with other species in the community relative to environmental factors (Lima-Mendez et al., 2015; Ritchie et al., 2009). A similar approach has been used to partition potential species interactions from environmental drivers in freshwater macroorganism communities, which highlighted the importance of species interactions, relative to environmental drivers in determining community structure (Musters et al., 2019).

Here, we use a 5.5-year time series, including physico-chemical and *16S* microbial community data to investigate the relative importance of environmental filtering versus inter-organismal co-occurrence in influencing marine bacterioplankton structure. Three oceanographic time series spanning 15° of latitude along the east Australian coastline allowed us to determine the relative contribution of environmental factors relative to potential biotic interactions. Bacterioplankton community structure was inferred by identifying the relative contribution of deterministic processes to shaping patterns of alpha and beta diversity. Our analysis involved the integration of a novel metric for inferring potential species interactions, defined as bacteria–bacteria and phytoplankton–bacterial interactions (biotic interactions), to discriminate among the relative importance of different deterministic processes in shaping bacterioplankton structure.

EXPERIMENTAL PROCEDURE

Reference station description and environmental data collection

Monthly surface water samples were collected from three oceanographic time series stations located on the eastern continental shelf of Australia, as part of the Integrated Marine Observing System (IMOS) National Reference Station (NRS) monitoring program. These stations span latitudes of 27 to 42°S and include Maria Island (MAI: 42°35.8 S, 148°14.0 E), Port Hacking (PHB: 34°05.0 S 151°15.0 E), and North Stradbroke Island (NSI: 27°20.5 S 153°33.75 E) (Figure 1). The MAI station is situated 7.4 km off Maria Island, on the Tasmania east coast (depth 90 m) and is seasonally impacted by the southerly extent of the East Australian Current (EAC), which is a strong western boundary current (Brown et al., 2018). PHB is located at the southern extent of the EAC separation zone (Figure 1) and 5.5 km offshore (depth 100 m). NSI is located north of Brisbane (depth 50 m), and is strongly influenced by EAC waters that originate in the Coral Sea (Brown et al., 2018). Sampling at each time series station comprised collection of bulk seawater samples for microbial analyses from mooring sites at near-monthly intervals (median days between sampling events; MAI: 34; PHB: 33; NSI: 32), with physico-chemical and Chl-*a* data (collectively termed environmental from here on) collected simultaneously for approximately 5.5 years (2012–2017), totalling 157 samples (MAI: 58; PHB: 47; NSI: 52; Table 1; Table S1). Environmental variables measured at each site included temperature (°C), day length (hours), salinity (PSU), turbidity (NTU), Secchi disk depth (m),

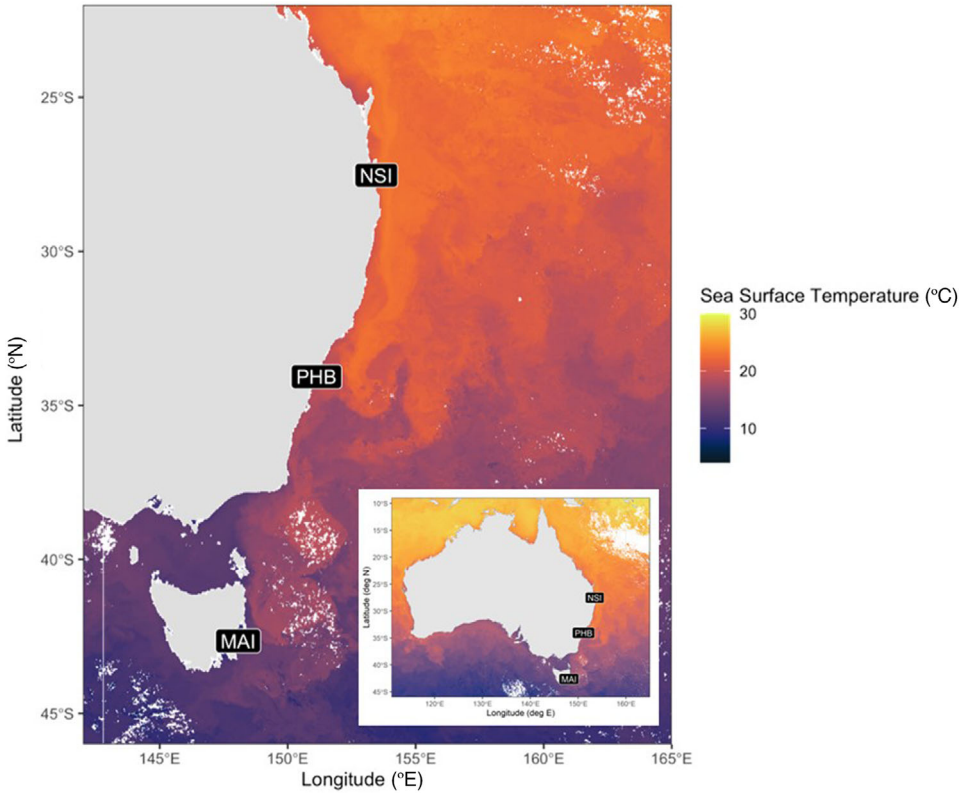

FIGURE 1 Map of sampling location. Inset figure shows the relative location of each reference station to the Australian continent. Mean sea surface temperature data from May 25, 2019 - June 15, 2019.

thermocline depth (m), dissolved silicate (μmol/L), NO_x (μmol/L), phosphate (μmol/L), ammonium (μmol/L), and Chl-*a* concentration (mg/m^3). Data were collected and analysed by IMOS (Davies & Sommerville, 2017; Lynch et al., 2014) (Table 1; Table S1). Mixed layer depth (MLD) was estimated from temperature-depth profiles (Australian National Mooring Network temperature and salinity data product at htt://aodn.com) based on Condie and Dunn (2006) and defined as the depth at which temperature decreased by 0.4°C from the surface temperature (0–2 m depth).

Sample collection and DNA extraction

Two litres of surface seawater were collected using Niskin bottles and transported on ice back to the lab. Samples were filtered through a 0.22 μm pore Sterivex GP filter (Millipore, Massachusetts. Cat. # SVGPL10RC), which were then stored at −80°C until processing. Filters were sent (on dry ice or in liquid nitrogen dewars) to the Commonwealth Scientific and Industrial Research Organization Oceans & Atmosphere (CSIRO O&A) laboratories in Hobart, Tasmania for DNA extractions. Microbial DNA was extracted using standardized procedures as part of the Marine Microbes Program (https://data.bioplatforms.com/organization/pages/bpa-marine-microbes/methods) using a modified PowerWater Sterivex DNA Isolation Kit (MOBIO Laboratories) protocol. DNA isolation included incubating Sterivex filters for 1 h on a horizontal vortex with 1.875 mL lysis buffer followed by a phenol:chloroform extraction.

Amplicon sequencing

The V1–V3 regions of the *16S* rRNA gene were PCR amplified using the bacteria-specific primers 27F (5′-AGRGTTTGATCMTGGCTCAG-3′) and 519R (5′-GWATTACCGCGGCKGCTG-3′) (Caporaso et al., 2012) with the following cycling conditions: 1-step using KAPA HiFi HotStart ReadyMix (Roche) comprised steps including 95°C initial denaturation (3 min), with 35 cycles of 95°C (30 s), 5°C (10 s) and 72°C 45 s) and a final elongation step at 72°C (5 min). Amplicons were then purified using Ampure XP beads (Agencourt Bioscience Corporation) and sequenced on the Illumina MiSeq platform (Illumina, Inc., San Diego, USA) at the Ramaciotti Center for Genomics (UNSW, Sydney, Australia), with 300 bp paired reads.

TABLE 1 Summary of imputed environmental variables.

	Maria Island ($N = 58$)	Port Hacking ($N = 52$)	North Stradbroke Island ($N = 47$)
Temperature (°C)			
Mean (SD)	15.1 (2.14)	20.2 (2.07)	23.6 (2.03)
Median [min, max]	14.5 [11.9, 20.3]	20.1 [16.8, 24.5]	23.4 [20.4, 27.6]
Missing	9 (15.5%)	3 (5.8%)	6 (12.8%)
Day length (h)			
Mean (SD)	11.7 (2.06)	11.9 (1.53)	11.8 (1.14)
Median [min, max]	11.5 [9.04, 15.3]	11.8 [9.89, 14.4]	11.5 [10.4, 13.9]
Missing	0 (0%)	0 (0%)	0 (0%)
Salinity (PSU)			
Mean (SD)	35.2 (0.801)	35.5 (0.193)	35.5 (0.234)
Median [min, max]	35.3 [29.3, 35.7]	35.5 [34.7, 35.7]	35.5 [34.3, 35.8]
Missing	9 (15.5%)	3 (5.8%)	6 (12.8%)
Turbidity (NTU)			
Mean (SD)	0.388 (0.214)	0.115 (0.0640)	0.131 (0.139)
Median [min, max]	0.285 [0.146, 1.10]	0.102 [0.0582, 0.450]	0.0878 [0.0108, 0.705]
Missing	9 (15.5%)	8 (15.4%)	8 (17.0%)
Secchi disk depth (m)			
Mean (SD)	16.5 (3.40)	15.2 (3.44)	20.0 (5.33)
Median [min, max]	16.5 [9.00, 24.0]	16.0 [9.00, 24.0]	19.0 [9.00, 34.0]
Missing	1 (1.7%)	3 (5.8%)	0 (0%
Silicate (μmol/L)			
Mean (SD)	0.660 (0.487)	0.865 (0.749)	0.586 (0.414)
Median [min, max]	0.600 [0, 2.00]	0.800 [0, 3.90]	0.500 [0, 2.10]
Missing	7 (12.1%)	7 (13.5%)	5(10.6%
NO_x (μmol/L)			
Mean (SD)	1.52 (1.42)	1.00 (1.33)	0.0679 (0.125)
Median [min, max]	1.85 [0, 5.20]	0.500 [0, 7.00]	0 [0, 0.500]
Missing	7 (12.1%)	8 (15.4%)	5 (10.6%)
Phosphate (μmol/L)			
Mean (SD)	0.216 (0.109)	0.174 (0.0975)	0.0934 (0.0365)
Median [min, max]	0.210 [0.0200, 0.480]	0.158 [0.0300, 0.650]	0.0903 [0, 0.190]
Missing	7 (12.1%)	7 (13.5%)	5 (10.6%)
Ammonium (μmol/L)			
Mean (SD)	0.164 (0.336)	0.350 (0.412)	0.293 (0.468)
Median [min, max]	0.0772 [0, 2.40]	0.231 [0.0300, 2.24]	0.125 [0, 2.60]
Missing	7 (12.1%)	10 (19.2%)	6 (12.8%)
Chl-*a* (mg/m^3)			
Mean (SD)	0.572 (0.333)	0.669 (0.298)	0.300 (0.111)
Median [min, max]	0.526 [0, 1.62]	0.617 [0.201, 1.41]	0.293 [0.0810, 0.637]
Missing	6 (10.3%)	14 (26.9%)	9 (19.1%)
Mixed-layer depth (m)			
Mean (SD)	63.3 (20.6)	32.0 (17.0)	31.4 (9.92)
Median [min, max]	74.0 [21.0, 86.0]	27.3 [11.0, 81.0]	31.0 [13.0, 57.0]
Missing	8 (13.8%)	4 (7.7%)	3 (6.4%)

Note: *N* is the number of samples in each time series or the whole entire dataset. Min and max are the minimum and maximum observed values in the dataset. Mixed layer depth is the estimated thermocline based on Condie and Dunn (2006).

Bioinformatic processing

Raw fastq files were downloaded from BioPlatforms Australia (https://data.bioplatforms.com). Amplicon quality control and analysis was performed using DaDa2 (Callahan et al., 2016). In brief, primers were truncated using cutadapt (Martin, 2011) and reads were trimmed, denoised, merged, and chimeras removed using function remove BimeraDenovo (minFoldParentOverAbundance = 4) (full code provided https://github.com/martinostrowski/marinemicrobes/tree/master/dada2). Taxonomic classification of bacterial *16S* rRNA ASVs was performed using a naïve Bayes classifier based on SILVA 138.1 and a bootstrap cut-off > 50% (Yilmaz et al., 2014). All ASVs which had a DaDa2 bootstrapped value < 50% at the taxonomic level were assigned to Kingdom unclassified.

The final bacterioplankton dataset analysed in this study resulted from removing all sequences assigned to Kingdom unclassified, Archaea, Eukaryota, Chloroplast and Mitochondria. The final step included filtering out low abundant ASVs with a total abundance across the entire dataset of less than 0.005%. The plastid dataset containing all the Chloroplast sequences was used to assess the potential importance of phytoplankton on bacteria community assembly, and taxonomic assignment was made in a similar way as bacterioplankton ASVs with bootstrapped value < 50% trimmed and taxonomic identity called with naïve Bayes classifier using PhytoRef database (Decelle et al., 2015).

Statistical analysis

Datasets used in the analysis included (1) environmental variables, (2) bacterial amplicon relative abundance, and (3) plastid amplicon relative abundance to represent the eukaryotic phytoplankton. In cases of missing environmental observations, values were imputed with rfImpute () from the randomForest package (version 4.6.14) (Breiman, 2001). Imputation was performed for each time series independently. Environmental variables were mean-centred unit variance standardized to reduce outlier influence. All analyses were performed using R version 3.6.1.

Temporal variability in environmental conditions was estimated by determining the mean dissimilarity within each time series (Ranjard et al., 2013) based on the 11 environmental variables described above. Dissimilarity among samples based on environmental variables was calculated on Euclidian distance. Heterogeneity (*Ed*) was derived for each pair of samples within a time series as follows:

$$Ed = \begin{pmatrix} Euc \\ Euc_{max} \end{pmatrix} + 0.001$$

where *Euc* is the Euclidian distance between two samples within a time series, Euc_{max} is the maximum distance observed across the entire dataset, and 0.001 is added to account for zero similarity among two samples. Mean *Ed* was then calculated within each time series and heterogeneity compared using a Kruskal–Walls χ^2 and Dunn-post hoc pairwise tests across the three time series.

To determine the importance of biological interactions, we then calculated the biological interaction indices. Building on a framework introduced by Musters et al. (2019), we developed a metric to quantify the relative contribution of potential interactions among bacteria and phytoplankton in structuring patterns of bacterioplankton diversity. Our approach regresses biological predictors (bacterial and phytoplankton ASVs) against individual bacterial ASVs (response ASV). In the case of the bacterial interaction metric, the response ASV is removed from the predictor ASV dataset. There is no reason to expect species interactions will be linear or that ASV patterns are the result of a single predictor, therefore, we extend the co-occurrence definition beyond simple pairwise co-occurrence to include more than a single bacterial ASV (or phytoplankton ASV) using a machine learning approach. In this way, we can identify nonlinear abundance relationships of an individual ASV which may be due to the abundance of multiple organisms. We additionally identified the relationship of each bacterial ASV to a combination of environmental data (e.g., temperature). Therefore, we generated three datasets including bacteria-environment, phytoplankton-environment and environment only in a series of steps (Figure S1) described below. The gradient forest (Ellis et al., 2012) method was used to regress the large number of predictors to individual bacterial ASVs. Gradient forest is a modification of regression forest, which calculates an explained variation (R^2_f) for each response ASV (Figure S1a,b). The approach then uses an out-of-bag prediction (OOB) similar to regression forest, but differs by defining the explained variation of each response and is calculated as follows:

$$R^2_f = 1 - \sum_i \left(Y_{fi} - \widehat{Y_{fi}}\right)^2 / \sum_i \left(Y_{fi} - \overline{Y_{fi}}\right)^2$$

Y_{fi} is the abundance of the *i*th occurrence of ASV *f*, $\widehat{Y_{fi}}$ is the OOB prediction for the abundance of ASV *f* at the *i*th position, and $\overline{Y_{fi}}$ is the mean abundance of ASV *f*. Calculations were conducted using the gradientForest package (version 0.1.17) in R (Pitcher et al., 2011). For each response variable, we removed all predictor variables that did not significantly explain the abundance of the response ASV (Figure S1c). Significance of predictor variables in explaining proportional distribution of the response was calculated with rfpermute (version 2.1.81). Regression forest produces partial R^2 values for each predictor of a response ASV. All partial R^2

values for a response variable are summed to produce the total R^2 of that response variable; therefore, we could sum the remaining significant predictor partial R^2 values to determine the response R^2 value (Figure S1d).

Next, we removed all bacterial response ASVs with an R^2 value less than 0.3 (thus retaining ≥ 0.3) and summed the relative abundance of each retained ASV for each sample (i.e., sample 1 from NSI, sample 2 from MAI) (Figure S1e). Finally, the bacteria–bacteria metric was calculated as the abundance difference between the bacteria-environment and environment only relative abundance for each sample. The bacteria-phytoplankton metric was calculated as the difference between the phytoplankton-environment and environment only relative abundance (Figure S1f). The resulting value is the bacterial or phytoplankton interaction metric (Figure S1g). Our approach is similar to that described by Muster et al. (2019) with the addition of the significance calculation for partial R^2 values and the identification of the relative abundance of ASVs from the dataset which are described by other bacterial or phytoplankton ASVs. Before the calculation of the metric, we performed a Hellinger transformation on predictors to reduce potential bias from highly abundant predictor ASVs. Also, to reduce computational time, we limited our analysis to include only ASVs which occurred in >25% of samples within a time series.

RESULTS AND DISCUSSION

Environmental characteristics of the three oceanographic time-series sites

Environmental heterogeneity exhibited a latitudinally defined gradient (Kruskal–Wallis $\chi^2_{df = 2} = 701.4$, $p < 0.01$; Figure 2A, B, Figure S2A–C) across the three time-series stations, whereby Maria Island (MAI) had

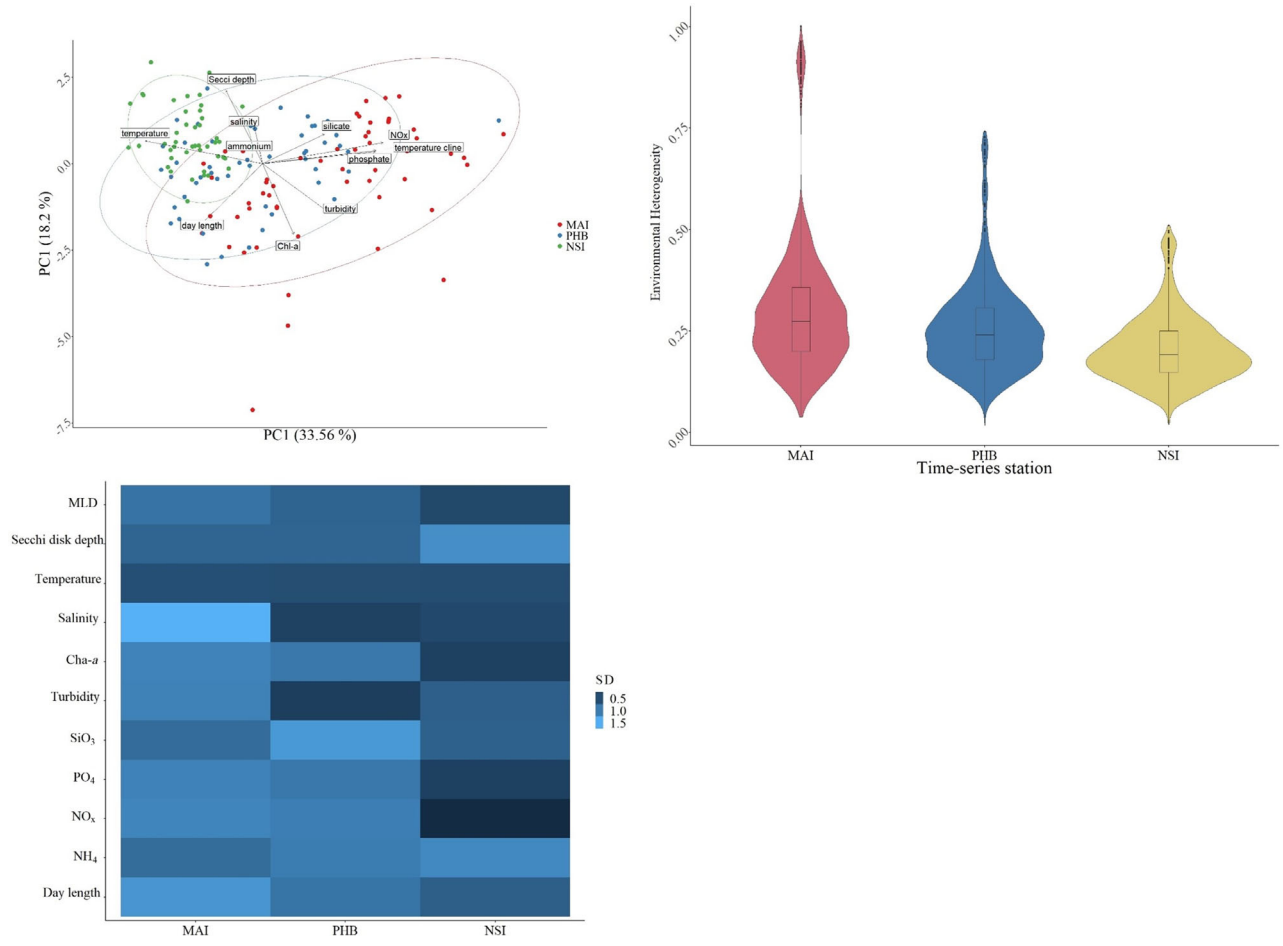

FIGURE 2 Environmental variability of each time series. (A) PCA biplot of first two dimensions discriminating samples to demonstrate environmental heterogeneity and is based on measured environmental variables. (B) Distribution of environmental heterogeneity across time series. (C) Heatmap visualizing the relative heterogeneity, measured as standard deviation of environmental variables across time series.

greater environmental heterogeneity than Pt Hacking (PHB) (Figure 2A; Dunn-test: $p < 0.01$) and PHB greater than North Stradbroke Is (NSI) (Figure 2A; Dunn-test: $p < 0.01$). At MAI (Figure 1; Lat 42°35.8 S; Lon 148°14.0 E) autumn and winter were characterized by a greater mixed layer depth (MLD; mean ± SD; 63.3 m ± 20.6), higher inorganic nutrient concentrations (Table S1; Figure S2b; NO_x: 1.52 μmol/L ± 1.42; phosphate: 0.216 μmol/L ± 0/109) and lower temperature (Figure S2a; 15.1°C ± 2.14), while spring and summer samples had higher Chl-*a* concentrations (Figure S2c; 0.572 mg/m^3 ± 0.333). At PHB (Figure 1; Lat 34°05.0 S; Lon°151 15.0 E) spring and summer temperatures (Table S1; 20.2°C ± 2.07) more closely track that of NSI than MAI (Figure S2a), while MLD (32.0 m ± 17.0), inorganic nutrient concentrations (Figure S2b; NO_x: 1.00 μmol/L ± 1.33; phosphate: 0.174 μmol/L ± 0.10; silicate: 0.864 μmol/L ± 0.749), and Chl-*a* concentration (Figure S2c; 0.67 mg/m^3 ± 0.30) during winter were more similar to MAI. NSI (Figure 1; Lat 27°20.5 S; Lon 153°33.75 E) was distinguished by relatively high-water temperatures (Figure S2a; Table S1; 23.6°C ± 2.03), and relatively low concentrations of inorganic nutrients including phosphate (Table S1; 0.09 μmol/L ± 0.04) and NO_x (Figure S2a; 0.07 μmol/L ± 0.13) concentrations. Winter samples at NSI were characterized by relatively high water clarity, measured by Secchi disk depth (20.0 m ± 5.33) while samples from the other three seasons were most distinguished by temperature. Thus, the three time series exhibited distinct environmental conditions that range from MAI having the greatest environmental heterogeneity compared to other stations, PHB with relatively intermediate nutrient concentrations and high physical environmental heterogeneity and NSI having the least environmental heterogeneity.

Contrasting drivers of bacterioplankton alpha diversity patterns across time series

The bacterioplankton datasets from the three reference stations had a varying number of observed ASVs (richness). Maria Island had the greatest number of total ASVs with 7608 (mean per sample 490.8 ± SE 26.0), then Port Hacking with 7020 (414.1 ± 20.7) and North Stradbroke Island with 4843 (431.8 ± 19.7). Richness of dataset ASVs corresponded with the total diversity of ASVs at each site where MI had the greatest alpha diversity (mean 122.14 ± SE 7. 64), followed by PHB (116.68 ± 6.36) then NSI with the least (107.27 ± 4.07). The distribution of alpha diversity was, however, not significantly different among time series (Figure S3; Kruskal–Wallis $\chi^2 = 2.10$, df = 2, p = NS). Temporal patterns in bacterioplankton diversity across the time series sites provided evidence; however, for varying degrees of seasonality among locations. Consistent yearly diversity patterns were observed at MAI, and this was less apparent or absent at PHB and NSI (Figure 3A). At MAI, bacterioplankton alpha diversity consistently peaked in the winter months and was lowest during spring, while at PHB, diversity peaked inconsistently across years. For instance, in 2012, the highest observed diversity at PHB was in winter, whereas in 2013, diversity peaked in autumn. At NSI, diversity peaks were not consistent across years. Collectively, these patterns infer that at MAI the principal factors regulating bacterioplankton alpha diversity are repeatable at seasonal scales, while at PHB and NSI, factors regulating bacterioplankton alpha diversity lack seasonal influences.

Environmental heterogeneity has been shown in other systems to be an important driver of bacterial

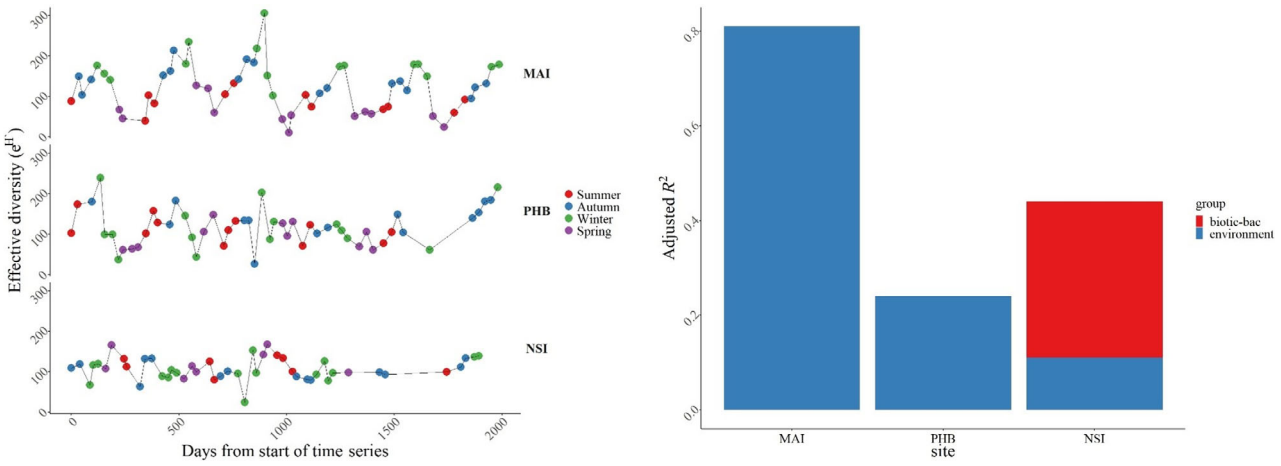

FIGURE 3 Patterns and drivers of alpha diversity across time series. (A) Scatterplot of alpha diversity through time for each time series. X-axis is time from the start of the time series and y-axis is effective diversity. Colours represent seasonal classification based on astronomical calendar. (B) Contribution of environmental variables and the biological metric to explaining variation of alpha diversity through time at each time series. The x-axis displays the three-time series, and the y-axis is the adjusted R^2 score. Blue is the adjusted R^2 from a multiple regression model of environmental variables only while red is the improved R^2 when the biological metric is included in the model.

TABLE 2 Linear model results for predictor variables that showed the best relationship with patterns of bacteria alpha diversity through time.

	Full model		Step regression						
Comparison			**Variables**	**df**	**Sum Sq**	**Rel. Imp**	**Mean Sq**	***F* value**	**Pr (>F)**
Maria Island	Adj. R^2	0.8	Day length (−)	1	30020.9	0.61	30020.89	117.871	**0.00**
	F-stat.	56.8	Ammonium (−)	1	12203.7	0.10	12203.75	47.9157	**0.00**
	p-value	<0.001	Chl-*a* (−)	1	15120.8	0.09	15120.81	59.369	**0.00**
	DF1	4	Turbidity (−)	1	546.075	0.01	546.07	2.14406	0.15
	DF2	53	Residuals	53	13498.7	0.81	254.69		
Port Hacking	Adj. R^2	0.22	Day length (−)	1	4874.45	0.12	4874.45	10.7948	**0.00**
	F-stat	4.67552886	Mixed-layer depth (+)	1	1825.19	0.10	1825.19	4.04201	**0.05**
	p-value	0.003	Silicate (+)	1	1742.86	0.04	1742.86	3.85967	0.06
	DF1	4	Temperature (+)	1	2.54951	0.03	2.55	0.00565	0.94
	DF2	47	Residuals	47	21223.1	0.28	451.56		
North Stradbroke Island	Adj. R^2	0.4	Biotic - bac (+)	1	4800.89	0.33	4800.89	23.6068	**0.00**
	F-stat	11.3996033	Ammonium (−)	1	730.21	0.06	730.21	3.59056	**0.01**
	p-value	<0.001	Chl-*a* (−)	1	1423.87	0.05	1423.87	7.00143	0.08
	DF1	3	Residuals	39	8744.87	0.44	203.36899		
	DF2	43							

Note: The full model is the results of all variables. Individual variables are the result of step regression. The (+) and (−) indicate the direction of relationship between variables and alpha diversity. The biological metric is the interspecific interaction metric. The explained variation (Exp.var) is the result of partitioning the variable sums of squares. Adj. adjusted; df = degrees of freedom; Rel. Imp = Relative importance based on CAR variance partitioning; Mean Sq = Means of the square; Sum Sq = Sums of square. Significance represented by bold font if $p < 0.05$.

diversity patterns (Curd et al., 2018; Huber et al., 2020), therefore, given the different levels of environmental heterogeneity observed between locations, we predicted that the influence of environmental factors would become less apparent with decreasing environmental heterogeneity. At MAI, diversity patterns were predominately predicted by environmental factors (Figure 3B; Table 2; $F_{df\ =\ 4,53} = 57.06$, $p < 0.001$, adjusted (Adj.) $R^2 = 0.80$). Day length had a strong inverse relationship with alpha diversity (relative importance [RI] = 0.62). Similar results have been reported for bacterioplankton richness patterns in the English Channel time series (Gilbert et al., 2012), which was also sampled at near-monthly intervals. Therefore, day length may generally be an important predictor of high latitude bacterioplankton diversity. In addition, Chl-*a* was weakly associated with bacterioplankton alpha diversity suggesting a potential trophic mediation by phytoplankton (RI = 0.09). Similar trends were observed in the Antarctic where bacterioplankton alpha diversity was inversely related to Chl-*a* (Luria et al., 2016).

At PHB, where there were lower levels of seasonal heterogeneity in environmental conditions (Figure 2A), bacterioplankton diversity was influenced by mixed layer depth, but total explained variation for alpha diversity was quite low (Figure 3B; Table 2; $F_{df\ =\ 5,46} = 4.29$, $p = 0.003$, Adj. $R^2 = 0.24$). The dominate environmental factors included day length (RI = 0.12) which was inversely correlated with alpha diversity patterns while MLD depth (RI = 0.10) was positively correlated. The high amount of unexplained variation may suggest other unmeasured environmental factors (e.g., dissolved organic carbon) more strongly influence alpha diversity. Alternatively, EAC-driven dispersal processes, which have been shown to influence bacterioplankton occurrences at PHB (Messer et al., 2020), may also be a dominant contributor to alpha diversity at the monthly time-scale interval. Dispersal is a fundamental ecological process (Vellend, 2010) and can become important in structuring bacterioplankton diversity when environmental heterogeneity is low or when dispersal rates are high enough to overshadow the effects of other ecological processes (Huber et al., 2020).

For NSI temporal bacterioplankton alpha diversity was not consistent with astronomical seasons, but total variation could be explained to a relatively high level (Figure 3B; Table 2; $F_{df\ =\ 3,43} = 11.18$, $p < 0.001$, Adj. $R^2 = 0.40$). Interestingly, and in contrast to the other two locations, biotic interactions were the main predictors of alpha diversity at this location. Bacteria–bacteria interactions specifically, were positively correlated with alpha diversity patterns and contributed a large portion of the total predicted variation (Figure 3B; partial $R^2 = 0.33$). Ammonium was also important in predicting alpha diversity and was inversely correlated

TABLE 3 Distance-based linear model results for predictor variables that showed the strongest relationship to patterns of beta diversity through time.

	Full model with selected variables					Step model					
		df	SS	*F*	*p* (>*F*)	Terms	R^2. adj.	df	AIC	*F*	Pr..F.
Maria Island	Model	6	3.346	11.345	0.001	Day length	0.20	1	140.11	15.20	**<0.001**
	Residual	51	2.507			Temperature	0.33	1	130.44	12.25	**<0.001**
						Biotic-bacteria	0.40	1	125.50	6.87	**<0.001**
						Biotic-phyto	0.44	1	122.40	4.87	**<0.001**
						Turbidity	0.46	1	121.44	2.72	**<0.001**
						Secchi depth	0.47	1	121.30	1.92	**< 0.001**
						All variables	0.48				
Port Hacking	Model	7	2.93	6.8	0.001	Day length	0.18	1	129.59	12.19	**<0.001**
	Residual	44	2.7			Temperature	0.32	1	121.10	10.95	**<0.001**
						Biotic-phyto	0.35	1	119.37	3.57	**<0.001**
						Biotic-bacteria	0.37	1	118.36	2.80	**0.002**
						Secchi depth	0.39	1	117.61	2.50	**0.002**
						Salinity	0.40	1	117.46	1.90	**0.020**
						NO_x	0.42	1	117.34	1.83	**0.030**
						All variables	0.42				
North Stradbroke Island	Model	5	1.47	7.32	0.001	Biotic-plankton	0.21	1	90.62	13.03	**<0.001**
	Residual	41	1.65			Day length	0.29	1	86.69	5.92	**<0.001**
						Biotic-bacteria	0.35	1	83.34	5.19	**<0.001**
						Temperature	0.38	1	81.75	3.33	**<0.001**
						Silicate	0.40	1	81.36	2.14	**<0.001**
						All variables	0.42				

Note: The biological metric is the interspecific interaction metric. Phyto is in reference to the phytoplankton biological metric. The full model informs on the global test for all selected variance and the step model shows the results for individual chosen variables. All variables are the model results when all variables are included.
AIC, Aikaike information criteria; df, degrees of freedom; SS, sums of squares.

(RI = 0.06) with diversity patterns. Therefore, potential interspecific interactions may be important drivers of alpha diversity patterns at this sub-tropical time series (Chesson, 2000).

Across the three time series the amount of variance that could be explained by environmental factors corresponded with trends in environmental heterogeneity. The largest contribution of environmental variables to explaining alpha diversity distribution was at MAI (80%), while an intermediate amount could be explained at PHB (22%) and the least at NSI (10%) (Figure 3B). However, the total explained variation did not correspond with trends in environmental heterogeneity. The lowest latitude site NSI which had the lowest environmental heterogeneity, had the second largest total explained variance, driven by a large contribution of biotic predictors (24%). This location had the warmest temperatures and the lowest inorganic nutrient concentrations of our study locations (Figure 2B), and under these conditions, trophic mediation, such as facilitation by Prochlorococcus and Synechococcus groups can drive bacterioplankton succession through primary productivity (Armengol et al., 2019). Biotic interactions at MAI or PHB were not important predictors of alpha diversity across the temporal scale analysed here (median 34 days), however is likely an important contributor when higher resolution time series are considered (Needham & Fuhrman, 2016). For instance, Luria et al. (2016) monitored bacterioplankton diversity in Antarctic waters across 1–2-week intervals and found richness was driven phytoplankton blooms; therefore, potentially demonstrating importance of scales in distinguishing among dominate ecological drivers of diversity patterns.

Differential drivers of beta-diversity patterns across time series

Bacterioplankton beta diversity (ratio of regional: local diversity) at each of the three reference stations exhibited seasonal trends, where intra-seasonal samples (samples from the same season) had greater observed similarity (i.e., lower beta diversity; 0: dissimilar; 1: highly similar) than inter-seasonal samples (Figure 4A, Figure S4). At MAI, the mean intra-seasonal Bray-Curtis (BC) score of 0.50 (± 0.005 SE) was significantly greater than the inter-seasonal score (0.59 ± 0.13; *t*-

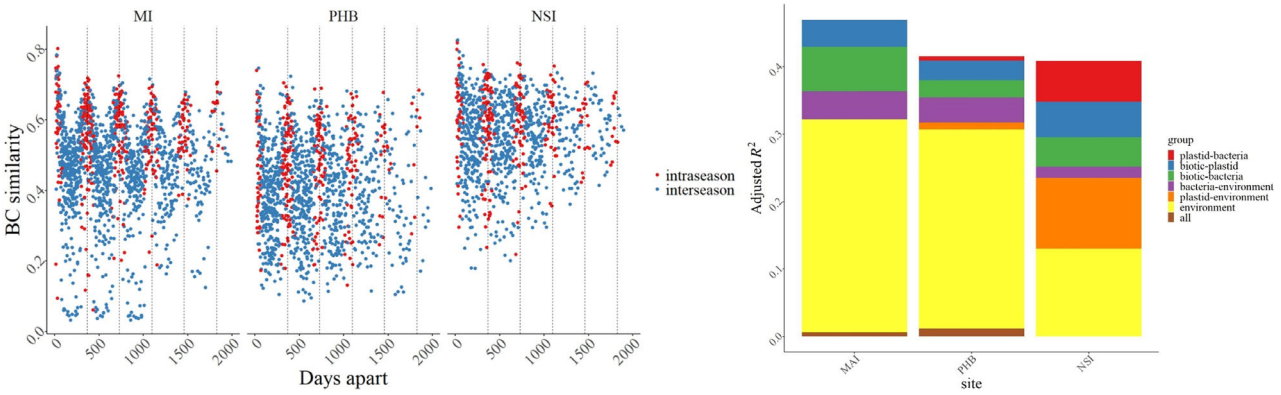

FIGURE 4 Beta-diversity patterns and contribution of deterministic drivers. (A) Time (in days) between sampling points along x-axis and Bray–Curtis dissimilarity (BC) scores along the y-axis. BC = 0, entirely the same; BC = 1 entirely different. Colours represent the category of sample; blue = inter-seasonal (two samples from different seasons), red = intra-seasonal (two samples from the same season). Dotted vertical line breaks spaced at 365 days to show length of time series. (B) Contribution of environmental and the biological metric to explaining variation of beta diversity through time across each time series. Colours correspond to the amount of variation attributed to several ecological processes derived from variance partitioning procedure.

$\text{test}_{\text{df}\,=\,749.45} = 16.00$; $p < 0.001$). Similarly, at PHB the intra-seasonal similarity (0.56 ± 0.006) was significantly greater than the inter-seasonal similarity (BC = 0.63 ± 0.003; Figure S4; $t\text{-test}_{\text{df}\,=\,528.48} = 10.32$; $p < 0001$). NSI had the lowest intra-seasonal mean BC among the time series at 0.47 ± 0.005 which was also significantly different than the inter-seasonal mean BC of 0.51 ± 0.003 (Figure S4a; $t\text{-test}_{\text{df}\,=\,473.94} = 6.00$; $p < 0.001$). Therefore, at all locations bacterioplankton communities from a given season were more similar to those from the same season in different years, than to those that were closer in time, but different in season. These results suggest ecological processes that structure bacterioplankton communities are recurrent at a given time of across years, and this occurs across despite.

Like alpha diversity, beta diversity is also expected to increase with increasing environmental heterogeneity (Chase & Leibold, 2003; Heino et al., 2015) under the assumption that greater variability in environmental factors will result in an increased number of niches for organisms to occupy (Leibold et al., 2004). We therefore predicted beta diversity would be greatest at MAI and lowest at NSI. This pattern, however, was not observed and rather the greatest mean beta diversity was observed at PHB (Figure S4b; mean ± SD; 0.61 ± 0.11), followed by MAI (0.57 ± 0.12) and NSI (0.50 ± 0.09; Kruskal–Wallis $\chi^2_{\text{df}\,=\,2} = 690.3$, $p < 0.05$). These results suggest that environmental variability is not entirely responsible for bacterioplankton composition, suggesting other ecological processes, such as biotic processes are important for structuring beta diversity.

Therefore, we investigated the key variables driving beta-diversity patterns and determined their relative contributions to these patterns. Our results indicate that different deterministic processes govern patterns of beta diversity across the three locations (Figure 4B). Variables that best-modelled beta diversity at MAI included day length, temperature, bacterial abundance, phytoplankton abundance, turbidity, and Secchi disk depth (Adj. $R^2 = 0.20, 0.13, 0.06, 0.04, 0.02, <0.01$, respectively; Table 3; $F = 11.35$, df = 6, 51, $p = 0.001$). Variance partitioning showed environmental variables had the greatest effect (32% of partitioned variation; Figure 4B) influencing beta-diversity patterns at MAI followed by bacterial interactions (7%), while phytoplankton contributed 4%. Together, the biotic interactions explained approximately 11% of the total partitioned variation. There was 4% of variance contributed by bacteria-environment overlap, suggesting a potential role of environmentally mediated bacterial influence. These results match with alpha diversity patterns where environment was the key drivers, demonstrating the importance of environment fluctuation in structuring bacterioplankton diversity. Beta diversity however had some influence by biotic factors, while alpha diversity was only predicted by environmental factors, potentially suggesting that biotic processes may facilitate the presence or absence of particular bacterioplankton groups, rather than diversity at a particular time point.

At PHB, environmental factors also had the largest contribution to beta diversity. Important variables included day length and temperature (Adj. $R^2 = 0.18$, 0.14, respectively; Table 3; $F = 6.8$, $p = 0.001$). Collectively, the environmental factors accounted for 30% of the total partitioned variation (Figure 4B) while biotic interaction (bacteria and phytoplankton) only accounted for 5% of the total variation. Environmental overlap with bacteria (4%) and phytoplankton (1%) accounted for 5% of the variation. These results are similar to alpha diversity in that environment was a key contributor to observed patterns. Interestingly, environmental contribution was similar to the amount contributed at MAI; however, total explained variance was lower due to the lower contribution of biotic influence at PHB.

A key finding in this study was that at NSI, biotic predictors played a much greater role in defining beta diversity relative to the other two locations. Phytoplankton abundance was found to be the most important factor contributing to bacterioplankton beta-diversity variation ($R^2 = 0.21$; Table 3; $F = 7.32$, df = 5, 41, $p = 0.001$). Biotic factors accounted for the largest amount of partitioned variation at 15% (Figure 4B; phytoplankton–bacteria: 6%; phytoplankton only: 5%; bacteria only: 4%) while environmental factors only accounted for 13% of the variation. There was a large amount of variation accounted for due to overlapping components, including phytoplankton-environment (11%) and bacteria-environment (2%) (Figure 4B; green segment). Based on the high observed influence of phytoplankton abundance at NSI and high overlapping variance between phytoplankton and the environment, we posit that the environment may indirectly drive bacterioplankton beta diversity through influencing the phytoplankton. These results are similar to those observed for alpha diversity patterns, where biotic predictors were also the most important contributor. Interestingly, the main biotic contributor varied across the two diversity measures, where phytoplankton was the most importance for beta diversity while for alpha diversity, bacteria were the predominate drivers. Thus, trophic links are important to structuring bacterioplankton diversity in a dynamic manor at NSI.

Together these results show that patterns of beta diversity are not shaped by environment alone, but rather a combination of environment and potential biotic interactions and that the relative importance of these can vary across locations. Interestingly, the importance of biotic interactions negatively corresponded with beta-diversity, such that the total contribution by biotic factors was greatest at NSI where beta-diversity was lowest, while PHB had the highest beta diversity and was least influenced by biotic predictors. These results potentially signal a stabilizing effect on the community against environmental fluctuation that biotic interactions can promote (Tilman & Downing, 1994). Also, in contrast to predictions, the relative contribution of deterministic processes did not entirely correspond with changes with environmental heterogeneity, as the relative contribution of environmental factors were similar at MAI and PHB, however at NSI where the lowest level of environmental heterogeneity occurred, biotic processes were the predominate deterministic driver. Interestingly, biotic influence on bacterioplankton diversity was found at all locations, suggesting previously overlooked factors driving temporal succession of bacterioplankton.

CONCLUDING REMARKS

Here, we demonstrate that temporal patterns in marine bacterioplankton diversity are structured by different inherent deterministic processes according to location, which tracks latitudinal differences that may be the result of variation in environmental heterogeneity. The most 'environmentally stable' site, which was characterized by the least seasonality displayed patterns in bacterioplankton alpha and beta diversity which were in contrasted to the site with highest levels of seasonality in environmental conditions. Bacterioplankton diversity is the consequence of multiple interacting processes including filtering by environmental factors and biotic interactions (Fuhrman et al., 2015; Needham et al., 2018). Partitioning the effects of environmental versus potential biotic influence is an important distinction as ecological theory predicts ecosystem function is linked to the processes that structure community diversity patterns (Cardinale et al., 2002; Loreau et al., 2001; Weis et al., 2007). Therefore, to accurately forecast ecosystem function, it is necessary to (1) distinguish among processes that give rise to bacterioplankton diversity and (2) identify how these processes change through space and time. This is heightened as climatic conditions are changing rapidly which can alter the balance between biotic and environmental deterministic processes (Kordas et al., 2011). However, until now no framework has been applied to bacterioplankton to identify the importance of potential biotic interactions relative to environmental factors driving total diversity patterns. Patterns of seasonality for both alpha and beta diversity observed in our study are consistent with diversity patterns from three well-studied time series, where the high latitude English Channel exhibited the highest degree of seasonality in diversity patterns, the mid-latitude SPOTS with intermediate diversity patterns and the low latitude HOTS with absent seasonal diversity patterns (Fuhrman et al., 2015). Therefore, processes driving diversity patterns along the latitudinal gradient may be general, and this study provides insight on potential drivers of this trend. Importantly, results shed insight on why some studies have identified environmental factors as having significant influence over bacterioplankton diversity (Luria et al., 2016), while others have concluded biotic processes play a stronger role in driving bacterioplankton diversity patterns (Gilbert et al., 2012; Needham et al., 2018). Predicting how biogeochemical processes will respond under future climate change scenarios requires insight to the microbial composition present, and therefore microbial diversity patterns.

AUTHOR CONTRIBUTIONS

Michael Doane: Conceptualization (equal); formal analysis (equal); software (equal); writing – original draft (equal); writing – review and editing (equal). **Martin Ostrowski:** Conceptualization (equal); formal analysis (equal). **Mark Brown:** Methodology (equal). **Anna Bramucci:** Data curation (equal). **Levente Bodrossy:** Methodology (equal). **Jodie van de Kamp:** Methodology

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CONFLICT OF INTEREST STATEMENT

The authors declare they have no conflict of interest.

DATA AVAILABILITY STATEMENT

All datasets were downloaded from https://data.bioplatforms.com. Code used in analysis is freely available and can be found at https://github.com/mpdoane2/eac_reference_station.

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