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## ORIGINAL ARTICLE

# Seasonal development of a tidal mixing front drives shifts in community structure and diversity of bacterioplankton

Nathan G. King<sup>1,2</sup>  | Sophie-B Wilmes<sup>2</sup> | Samuel S. Browett<sup>3,4</sup> | Amy Healey<sup>5</sup> | Allan D. McDavitt<sup>6</sup> | Niall J. McKeown<sup>5</sup> | Ronan Roche<sup>2</sup> | Ilze Skujina<sup>5,7</sup> | Dan A. Smale<sup>1</sup> | Jamie M. Thorpe<sup>2</sup> | Shelagh Malham<sup>2</sup>

<sup>1</sup>Marine Biological Association of the United Kingdom, The Laboratory, Plymouth, UK

<sup>2</sup>Centre of Applied Marine Sciences, School of Ocean Sciences, Bangor University, Menai Bridge, UK

<sup>3</sup>Environment and Ecosystem Research Centre, School of Science, Engineering and Environment, University of Salford, Salford, UK

<sup>4</sup>Molecular Ecology Research Group, Eco-Innovation Research Centre, School of Science and Computing, South East Technological University, Waterford, Ireland

<sup>5</sup>Department of Life Sciences, Aberystwyth University, Aberystwyth, UK

<sup>6</sup>Department of Natural Resources and Environment, Atlantic Technological University, Galway, Ireland

<sup>7</sup>School of Biology and Environmental Science, University College Dublin, Dublin, Ireland

## Correspondence

Nathan G. King, Marine Biological Association of the United Kingdom, The Laboratory, Plymouth PL1 2PB, UK.  
Email: [natkin@mba.ac.uk](mailto:natkin@mba.ac.uk)

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## Abstract

Bacterioplankton underpin biogeochemical cycles and an improved understanding of the patterns and drivers of variability in their distribution is needed to determine their wider functioning and importance. Sharp environmental gradients and dispersal barriers associated with ocean fronts are emerging as key determinants of bacterioplankton biodiversity patterns. We examined how the development of the Celtic Sea Front (CF), a tidal mixing front on the Northwest European Shelf affects bacterioplankton communities. We performed 16S-rRNA metabarcoding on 60 seawater samples collected from three depths (surface, 20m and seafloor), across two research cruises (May and September 2018), encompassing the intra-annual range of the CF intensity. Communities above the thermocline of stratified frontal waters were clearly differentiated and less diverse than those below the thermocline and communities in the well-mixed waters of the Irish Sea. This effect was much more pronounced in September, when the CF was at its peak intensity. The stratified zone likely represents a stressful environment for bacterioplankton due to a combination of high temperatures and low nutrients, which fewer taxa can tolerate. Much of the observed variation was driven by *Synechococcus* spp. (cyanobacteria), which were more abundant within the stratified zone and are known to thrive in warm oligotrophic waters. *Synechococcus* spp. are key contributors to global primary productivity and carbon cycling and, as such, variability driven by the CF is likely to influence regional biogeochemical processes. However, further studies are required to explicitly link shifts in community structure to function and quantify their wider importance to pelagic ecosystems.

## KEYWORDS

16S sequencing, Celtic Sea front, microbial biogeography, microbiome, Shallow Sea front

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## 1 | INTRODUCTION

Bacterioplankton are the largest component of biomass in the ocean where they underpin marine food webs and influence biogeochemical cycles (Arrigo, 2005; Falkowski et al., 2008; Meng et al., 2022; Passow & Carlson, 2012; Pomeroy et al., 2007; Teeling et al., 2012; Zehr & Kudela, 2011). Therefore, understanding drivers of spatial and temporal variability in their distribution and abundance is a critical step in understanding wider biogeochemical processes (Bunse & Pinhassi, 2017; Fuhrman et al., 2015). Due to the interconnected, highly dynamic nature of the marine environment, and the prevalence of large-scale ocean currents, shifts in bacterioplankton communities were traditionally assumed to occur over large spatial scales (Hewson et al., 2006). However, recent studies have demonstrated the importance of smaller-scale localized oceanographic features in shaping ecological patterns. In particular, steep environmental gradients and soft dispersal barriers associated with mesoscale oceanographic features are emerging as key determinants of bacterioplankton community structure and diversity (Baltar et al., 2016; Fadeev et al., 2021; Hernando-Morales et al., 2017; Raes et al., 2018; Venkatachalam et al., 2017).

Frontal zones (i.e. physical interfaces between distinct water bodies) represent some of the worlds' most well-studied oceanographic features (Belkin et al., 2009; Sournia, 1994). Their formation is influenced by a range of processes, which in turn mediate their biophysical characteristics and ecological effects (Olson et al., 1994). They are generally characterized by elevated primary and secondary productivity, driven by processes such as nutrient retention (Franks, 1992a; Traganza et al., 1987) and entrainment of zooplankton (Franks, 1992b; Le Fevre, 1987), which permeates through the food web (Polovina et al., 2001; Waggitt et al., 2018). Our understanding of processes occurring at the microbial level is comparatively lacking, but emerging research has shown that permanent shelf-break fronts, caused by differences in pressure between offshore and onshore waters, are clear horizontal "ecotones" for bacterioplankton communities (Allen et al., 2020; Baltar et al., 2010; Baltar et al., 2016; Djurhuus et al., 2017; Hernando-Morales et al., 2017). Current understanding is particularly poor for coastal frontal zones that exhibit greater spatiotemporal variability (but see Lemonnier et al., 2020). This is important as successional patterns of bacterioplankton communities have the ability to regulate the temporal variability in biogeochemical processes (Bunse & Pinhassi, 2017).

Tidal mixing fronts develop at the boundary between mixed and stratified waters and are an interplay between depth, tidal mixing and solar radiation (Pingree & Griffiths, 1978). In deep waters during warmer months, solar radiation overpowers tidal forces resulting in surface stratification, whereas in shallower waters tidal forces are often strong enough to maintain a mixed water column. The Celtic Sea Front (CF) is a tidal mixing front located at the transition between Atlantic waters of the European Shelf and coastal waters of the Irish Sea (Simpson & Hunter, 1974). It is a typical tidal mixing front that develops in spring, intensifies over summer and dissipates in early autumn, with profound effects on coastal marine ecosystems across the region (Scales et al., 2014; Waggitt et al., 2018).

Here, we examined how the development of the CF drives changes in bacterioplankton communities during periods of minimum and maximum front intensity. In doing so, we aimed to comprehensively assess how a tidal mixing front affects bacterioplankton community structure and diversity both spatially (horizontally and vertically) and temporally through a period of front intensification.

## 2 | METHODS

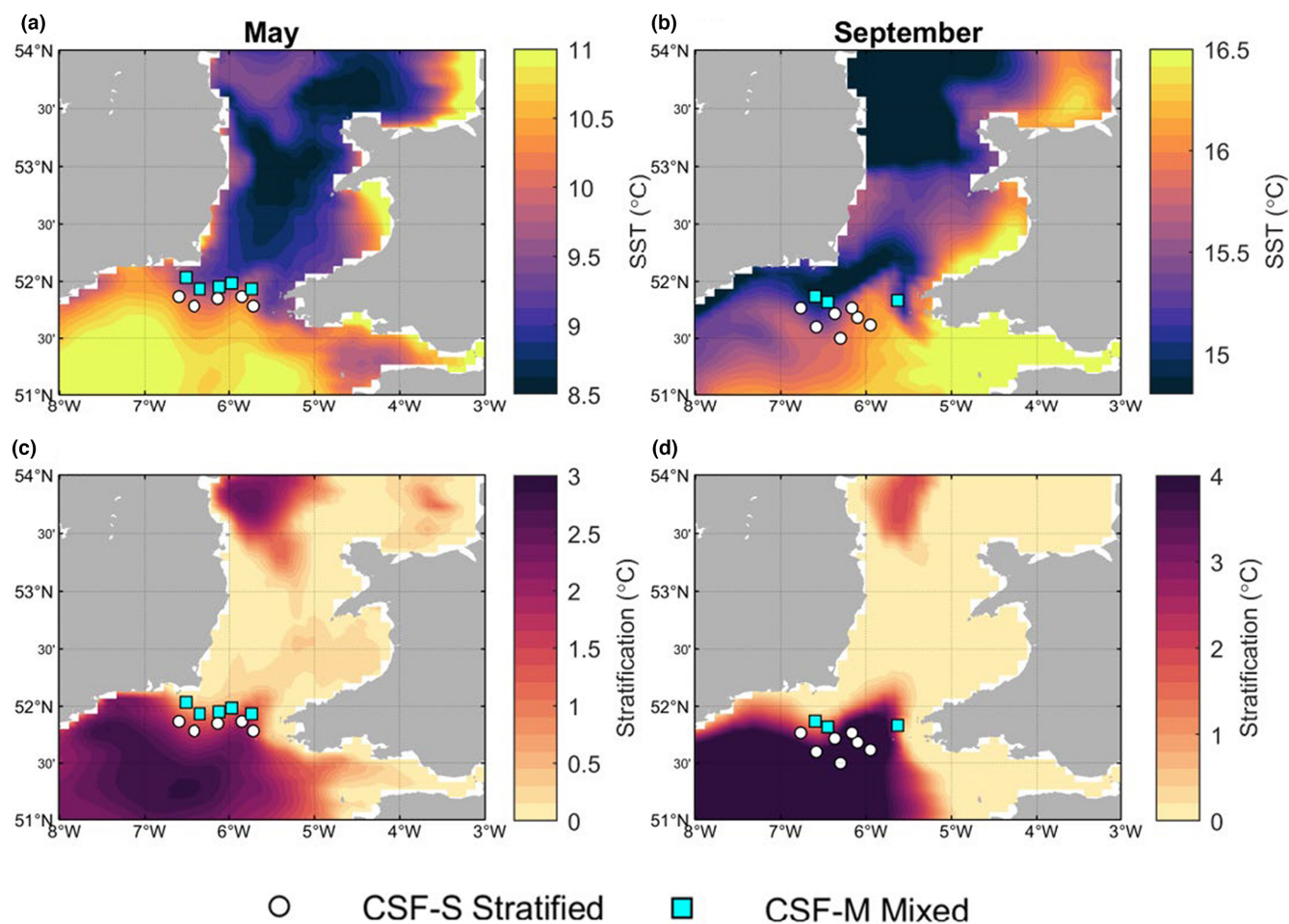
### 2.1 | Sampling

Sampling of the CF area was conducted aboard the RV Prince Madog on two independent research cruises in 2018. Cruises took place in May when the CF first became established and again in September when it reached its maximum intensity (Figure 1). The CF is dynamic, moves with the tide and is affected by weather, meaning its position and vertical thermal profile are difficult to determine exactly, a priori. Therefore, we identified its position in situ where a rapid (within 100m) increase of  $>1^{\circ}\text{C}$  Sea Surface Temperature (SST) was observed, using the ships onboard thermal profiler. Five stations were sampled on either side (one hour steam  $\sim 10$  nautical miles) of this transition, and seawater was sampled from the surface (S), 20 metres (M) and seafloor (B). The stratification of each station was then determined using the difference in temperature between surface (S) and bottom (B) seawater. Where this difference was  $<1^{\circ}\text{C}$ , stations were categorized as mixed (Celtic Front Mixed – CFM) and  $>1^{\circ}\text{C}$  they were categorized as Stratified (Celtic Front Stratified – CFS) (Figure S1). In May, this resulted in five CFM and five CFS stations and in September there were seven CFS stations and three CFM stations (Figure 1).

At each station, a conductivity-temperature-depth profile (CTD; Seabird, SBE 911) was taken and 10L of water sampled at S, M and B depths using a rosette with Niskin bottles. A number of oceanographic parameters were measured with each CTD profile. These were oxygen (mg/L), salinity (s/m), transmissometer beam attenuation (proxy for total suspended matter), chlorophyll a (chl-a) concentration (mg/m<sup>3</sup>) (via chl-a fluorescence), and irradiance (PAR – Photosynthetic Active Radiation). 500 mL of seawater was concentrated by filtering through a 0.22  $\mu\text{m}$  nitrocellulose filter. Filters were then stored at  $-20^{\circ}\text{C}$  until DNA extraction.

### 2.2 | DNA extraction and PCR amplification

DNA extraction and PCR amplification were both performed in a dedicated eDNA laboratory at the University of Salford. Genomic DNA was extracted from whole filters using Powersoil kits (Qiagen). DNA was quantified using a Qubit (Invitrogen) and re-suspended to 2 ng/ $\mu\text{L}$  following the manufacturer's instructions. We amplified DNA for the 16S rRNA V4 region using dual indexed forward and reverse primers according to (Kozich et al., 2013) and (Griffiths et al., 2018). A blank PCR control was included on each PCR plate as a negative



**FIGURE 1** Map of sea surface temperature (a + b) and stratification (surface minus bottom temperature) (c + d) for May (left) and September (right) 2018, in the Irish Sea.

control. PCRs were run in 25  $\mu$ L volumes using Solis BioDyne 5x HOT FIREPol® Blend Master Mix, 2  $\mu$ M primers and 1  $\mu$ L of sample DNA. Cycling parameters were 15 min at 95°C, followed by 28 cycles of 95°C for 20s, 50°C for 60s and 72°C for 60s and a final extension at 72°C for 10min. All PCRs were run in duplicate. Duplicates were combined into a single PCR and cleaned using HighPrep™ PCR clean up beads (MagBio). Products were quality checked using an Agilent 2200 TapeStation and quantified using a Qubit (Invitrogen). Samples were pooled in equimolar concentrations in order to minimize PCR and sequencing bias. Paired-end (2 $\times$ 250bp) amplicon sequencing was conducted using V2 chemistry on an Illumina MiSeq platform at the University of Salford, UK.

### 2.3 | Sequence processing

Sequence processing and analysis was conducted in R. Paired-end reads were processed according to the BIOCONDUCTOR workflow for microbiome data analysis (Callahan et al., 2016). Sequences were trimmed and truncated using the “filterAndTrim” function in DADA2 (truncLen,  $f=240$ ,  $r=160$ ; truncQ=2; and trimLeft,  $f=20$ ,  $r=19$ ), to remove primers and low-quality reads. Amplicon Sequence Variants

(ASVs) were resolved using DADA2. Chimeras were removed using the “removeBimeraDenovo” function in DADA2. Sequence taxonomy was assigned using the RDP naïve Bayesian classifier against the SILVA release 132 database using the “assignTaxonomy” function in DADA2. Sequence read counts, taxonomic assignments and metadata were assembled as a phyloseq object using the R package “PHYLOSEQ” (McMurdie & Holmes, 2013) and was used in downstream analysis. Samples containing <1000 reads and taxa contributing <0.01% of the reads in the dataset were removed. ASVs identified from PCR blanks along with those identified as mitochondria, chloroplasts or Archaea were also removed. Rarefaction curves of the processed reads were saturated, indicating good coverage of bacterial diversity (Figure S2). Sequences are accessible through the EMBL database (accession no. PRJEB61283). ASV table and metadata are available at (<https://figshare.com/s/94f8a742684070a1e7a4>).

### 2.4 | Statistical analysis

The whole dataset was split into two, based on sampling month (May vs September) and all analysis conducted on these separate

sampling periods. Alpha diversity for each sample was estimated through the Chao1 index (Chao, 1984) implemented through the “estimate\_richness” function in the R package “PHYLOSEQ” (McMurdie & Holmes, 2013). The Chao1 index estimates ASV richness, and the standard error surrounding this estimate, based on the observed number of ASVs, the observed number of ASVs occurring only once, and the observed number of ASVs occurring only twice (Chao, 1984). To account for differences in sequence depth between samples in alpha diversity estimates, the dataset was rarefied to the minimum sample depth (3500 reads), using the “rarefy\_even\_depth” function in PHYLOSEQ. Alpha diversity and environmental parameters were each compared using a two-way Analysis of Variance (ANOVA). Model factors consisted of Station Stratification (fixed factor; two levels: Celtic Front Mixed – CFM, Celtic Front Stratified – CFS) and Depth (fixed factor; three levels: S, M and B).

Multivariate community analysis was conducted on a relative abundance dataset. Whole pattern differences in community structure were visualized through non-Metric Multidimensional Scaling (nMDS) using the “metaMDS” function in the R package “VEGAN”. Permutational Multivariate Analysis of Variance (PERMANOVA) was used to test differences in community structure between Depth categories and Station Stratification and followed the same design as alpha diversity. PERMANOVA was implemented by the function “adonis2” in VEGAN. A Similarity of Percentage (SIMPER) procedure was conducted in VEGAN to determine which taxa contributed the most to observed dissimilarities.

To identify the environmental factors that drive bacterioplankton community structure, a distance-based redundancy analysis was performed using the function “capscale” in VEGAN. Here, models were constructed through forward selection using the “ordiR2step” function in VEGAN based on z-score transformed environmental variables. Multicollinearity between environmental variables was examined by the Variation Inflation Factor (VIF) using the function “vif.cca” (O'Brien, 2007). The significance of each constraint was confirmed with ANOVA for dbRDA using the function “anova.cca”. An ANOVA-like permutational test (function “permutest”) for dbRDA was used to assess the significance of the full model. Significant environmental variables were plotted on the dbRDA ordination using the “envfit” function.

### 3 | RESULTS

#### 3.1 | Environmental variation across the Celtic Sea front

##### 3.1.1 | Temperature

The CF was easily identified through its thermal profile in both May and September. In both months, surface waters (S) were ~1°C greater at CFS stations (i.e., in stratified waters) compared to the CFM stations (i.e., in mixed waters) (Figure S3). In both May and September, we observed a significant interaction between Station Stratification and Depth (Table S1). In May, there was no temperature difference

between surface (S) and seafloor (B) waters at CFM stations compared to ~1.5°C difference at CFS stations. In September, this pattern intensified and surface waters were 4.3°C warmer than the seafloor (B) at CFS stations, whereas the CFM stations the mean vertical temperature difference was 0.4°C (Figures S1 and S3). The depth of the thermocline shifted between sampling months. This meant that our 20m (M) sampling depth was below the thermocline (<tc) in May but above the thermocline (>tc) in September.

##### 3.1.2 | Oxygen

In May, there was no significant effect of Station Stratification or Depth on oxygen levels (Table S1). At CFS stations, oxygen levels ranged from  $2.42 \pm 0.13$  mg/L at the surface to  $2.26 \pm 0.025$  13 mg/L at M and B depths. At CFM stations, oxygen levels were generally lower and ranged from  $2.30 \pm 0.1$  at the surface to  $2.44 \pm 0.05$  at M and B depths (Figure S3). In September, we observed a significant interaction between Station Stratification and Depth. Oxygen levels were  $2.26 \pm 0.02$  mg/L above the thermocline (S and M) at CFS stations and dropped to  $1.94 \pm 0.17$  at the seafloor (B). A less dramatic decrease in oxygen levels was observed at CFM stations where S and M samples were  $2.17 \pm 0.006$  compared to  $2.11 \pm 0.008$  at the seafloor.

##### 3.1.3 | Salinity

In May, we observed a significant interaction between Station Stratification and Depth (Table S1). Salinity above the thermocline of CFS stations (S) was  $34.54 \pm 0.06$  and was lower than all depths at CFM stations. At CFS stations salinity increased to  $34.85 \pm 0.035$  at M and seafloor (B) depths whereas no increase with depth was observed at CFM stations, where salinity was  $34.7 \pm 0.04$ . In September, we observed significant effects of both Station Stratification and Depth but no interaction between the two (Table S1). Salinity was greater at CFS compared to CFM stations and significantly higher at the seafloor than at S and M depths. At CFS stations, salinity was  $35.12 \pm 0.006$  at the seafloor compared to  $35.06 \pm 0.001$  at S and M depths. At CFM stations, salinity at the seafloor was  $35.03 \pm 0.007$  at the seafloor compared to  $35.07 \pm 0.001$  at S and M depths.

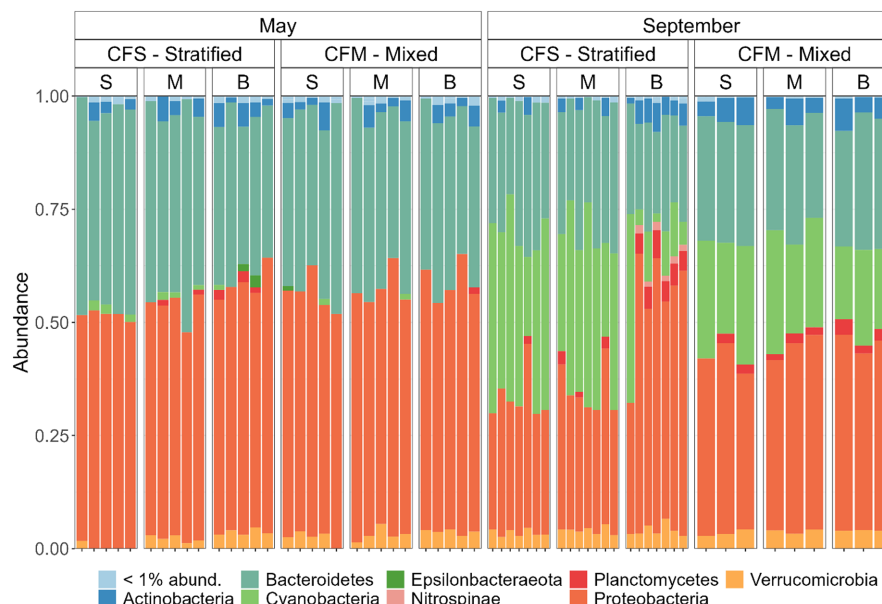
Fluorescence was variable, with no differences between Station Stratification and Depth (Table S1). Irradiance significantly decreased with depth in both May and September (Table S1). In May, irradiance fell to zero at 20m (M) and seafloor depths (B). In September, this was only seen on the seafloor (B). Beam attenuation was variable but generally consistent between months and transects. However, it was considerably lower at the seafloor (B) at CFS stations in September.

#### 3.2 | Overall biodiversity patterns

In total, we analysed 60 seawater samples, which resulted in 926,464 paired-end reads with an average coverage of 15,441 reads



**FIGURE 2** Relative abundance of bacterial phyla in seawater across the Celtic Sea Front from two research cruises in May and September 2018. S=Surface, M=mid-water (20m) and B=Seafloor. CFM, Celtic Front Mixed; CFS, within the Celtic Front Stratified.



**TABLE 1** Results of univariate (alpha diversity) ANOVAs and multivariate PERMANOVA's (community structure/ beta diversity) to test for effects of Depth and Stratification across the Celtic Sea Front.

	May			September		
	df	F	p	df	F	p
Univariate – alpha diversity						
Stratification	1	0.98	.33	1	0.40	.53
Depth	2	5.95	<b>.01</b>	2	8.16	<b>.001</b>
Stratification × Depth	2	1.47	.15	2	1.653	.15
Multivariate – beta diversity						
Stratification	1	1.91	.1	1	4.61	<b>.01</b>
Depth	2	1.91	.07	2	8.69	<b>.001</b>
Stratification × Depth	2	0.75	.45	2	2.54	<b>.04</b>

Note: Values in bold are significant at  $p < .05$ .

per sample. We identified 1170 ASVs spanning 28 phyla, 41 classes, 111 orders, 230 families and 520 genera (Figure 2; Figures S4–S6). Proteobacteria (635 ASVs, 27.5% relative abundance) and Bacteroidetes (301 ASVs, 19.7% relative abundance) were the most abundant and diverse phyla (Figure 2). Verrucomicrobia (51 ASVs, 1.97% relative abundance) and Actinobacteria (43 ASVs, 1.73% relative abundance) made notable contributions to sample diversity and abundance. In September, 7 Cyanobacteria ASVs made up ~8% of sample abundance. The remaining 140 ASVs were generally found in low abundances and transient between samples.

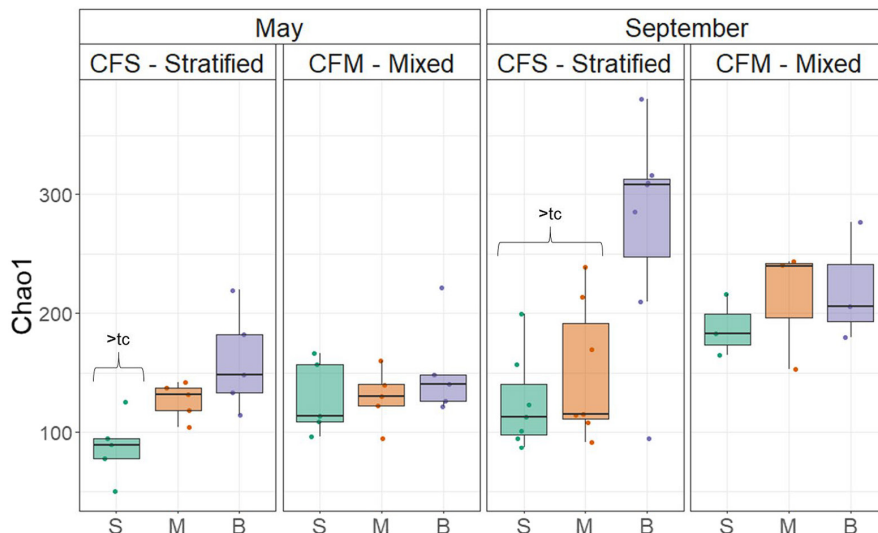
### 3.3 | Alpha diversity

In May, bacteria ASV richness (Chao1 index) did not differ between CFM and CFS stations, but there was a significant Depth effect (Table 1). Here, the surface communities (S) were significantly lower in diversity ( $107.6 \pm 10.4$ ) than those at the seafloor (B) ( $155.4 \pm 12.7$ ) (Figure 3). Similarly, in September, there was no significant difference between CFM and CFS stations, but there was a Depth effect with the

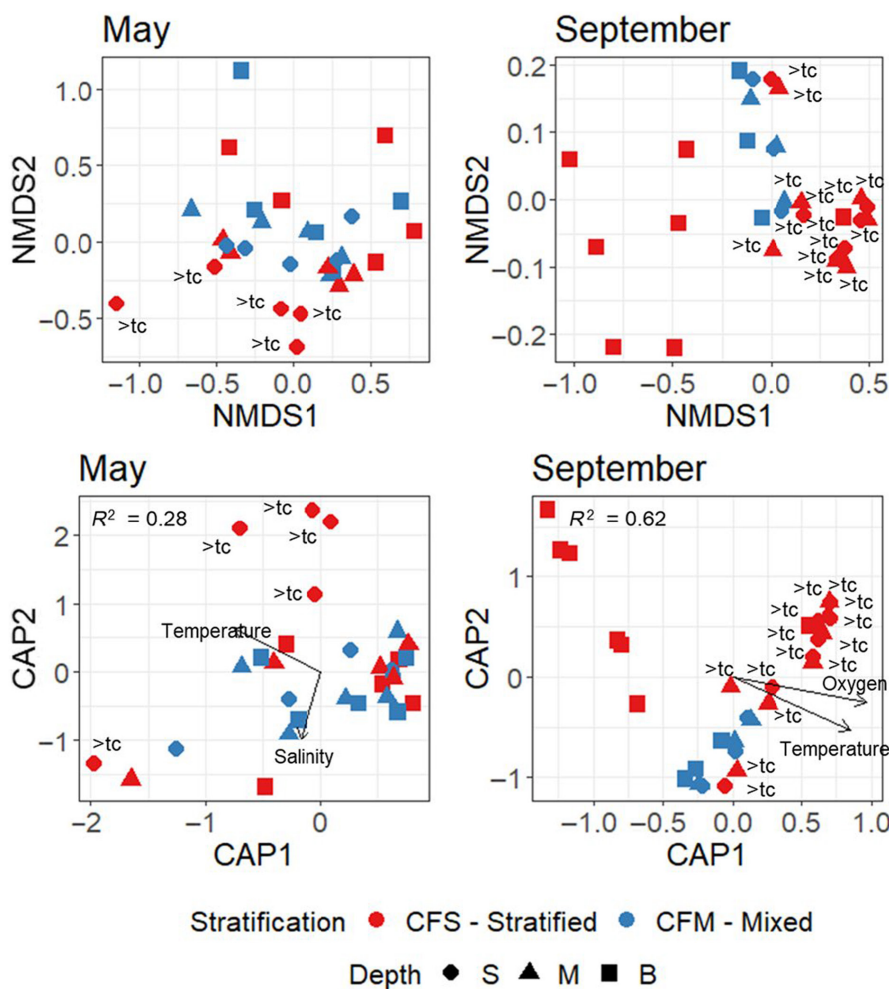
surface communities exhibiting significantly lower diversity ( $143 \pm 13.6$ ) compared to those at the seafloor ( $225.8 \pm 24.8$ ) (Figure 3). There was a clear difference in richness between communities that were above the thermocline (>tc) at CFS stations. In May, richness in communities above the thermocline (CFS-S) was  $87 \pm 11.3$  compared to  $138 \pm 6.7$  at all other stations. In September, these differences were starker, with a richness of  $140 \pm 8$  above the thermocline (CFS-S+CFS-B), compared to  $231 \pm 17.9$  at all other stations (CFS-B and CFM) (Figure 3).

### 3.4 | Community structure (Beta diversity)

Initial comparisons between sampling months showed bacterioplankton communities to be clearly differentiated (PERMANOVA,  $F(1, 58) = 90.2$ ,  $p < .001$ ) (Figure S7), and further analysis focussed on May and September datasets independently. In May, PERMANOVA revealed that bacterioplankton communities were similar between CFM (mixed) and CFS (stratified) stations and were also similar across depths (S, M and B). At higher taxonomic ranks (Class and Order), Depth was a significant main effect (Table S2; Figure S8). The



**FIGURE 3** Box plots representing alpha diversity (Chao1 index) for bacterial communities from seawater samples across the Celtic Sea Front. S=Surface, M=mid-water (20m) and B=bottom. CFM, Celtic Front Mixed; CFS, within the Celtic Front Stratified. > tc=above the thermocline of stratified stations. In May, this consisted of CFS-S communities, and in September, it consisted of CFS-S and CFS-M communities.



**FIGURE 4** nMDS (top) and dbRDA (bottom) plots depicting the structure of bacterial communities from seawater samples across the Celtic Sea Front in May (left) and September (right). CFM, Celtic Front Mixed; CFS, Celtic Front Stratified; S, Surface, M=20m and B=seafloor. > tc=above the thermocline of stratified stations. In May, this consisted of CFS-S communities, and in September, it consisted of CFS-S and CFS-M communities. Environmental variables which constrain significant variation ( $p < .05$ ) are displayed on dbRDA plots.

nMDS ordination suggested communities above the thermocline (CFS-S) are distinctive and do not overlap with other communities in multivariate space (Figure 4).

In September, there was a Transect $\times$ Depth interaction indicating community structure shifted with depth at either CFM or CFS stations (Table 1). This pattern was consistent when the data were

analysed at higher taxonomic ranks (Table S2; Figure S8). Post hoc analysis showed communities differed by depth at CFS stations. Here, communities above thermocline (S and M) were distinct from those below it (B) and all depths at CFM stations (Table S3). Communities directly below the thermocline (CFS-B) were also distinct from all depths of the CFM stations. At CFM stations community structure

was similar between depths. SIMPER analysis revealed ASV3 from the genus *Synechococcus* drove most of this variation between groups. For example, 31% of reads were from ASV3 in CFS-S compared to 17% in CFM-S (Table S4).

We used dbRDA to model the relationship between environmental variables and bacterial community structure, following forward selection. In both months, global tests were significant and forward selection was performed. For May, the final model consisted of temperature and salinity and explained 28% of overall variation. For September, the model consisted of temperature and oxygen and explained 62% of total variation in community composition (Table S5; Figure 4). However, VIF of both (21.5 and 18.9 respectively) indicated there was a high degree of multicollinearity between these two variables.

## 4 | DISCUSSION

Our study demonstrates the clear role a tidal mixing front has in driving shifts in the structure and diversity of bacterioplankton communities in the NW European Shelf. Specifically, we show the development of the CF has clear impacts on bacterial richness (alpha diversity) and community structure both horizontally across the front and vertically through the water column. Horizontally, we observed differentiation between the upper depths (S and M) of CFM and CFS stations, which follows similar observations between different water bodies at sea mounts (Djurhuus et al., 2017), eddies (Nelson et al., 2014), filaments (Fadeev et al., 2021), transient currents (Lévy et al., 2018) and shelf-break fronts (Baltar et al., 2016; Hernando-Morales et al., 2017). Vertically, we observed pronounced effects of stratification on bacterioplankton communities at CFS stations, with communities below the thermocline markedly different to those above it.

Temperature is the key determinant of bacterioplankton community structure at a global scale (Sunagawa et al., 2015). In our dataset a large proportion of community variation could be explained by a combination of temperature and oxygen, which was predominantly driven by greater temperature variability of CFS-B communities. However, temperature co-varies with a number of other environmental drivers in frontal systems, which makes its specific role here difficult to conclude. Perhaps most profound is the overlying impact of seawater stratification on phytoplankton succession. In stratified waters, phytoplankton are locked above the thermocline and rapidly proliferate due to elevated temperatures and increased light availability. This elevated productivity depletes nutrients resulting in phytoplankton mortality, decay and a high transfer of organic matter below the thermocline (Bunse & Pinhassi, 2017). The subsequent high-variability in organic matter availability below the thermocline combined with a more thermally variable environment, and less wind mixing at depth (Wu et al., 2018) may also be responsible for greater variability between our CFS-B communities.

A notable proportion of community variation remained unexplained and the underlying drivers horizontally between CFS and

CFM communities were less clear. Thermal metabolic scaling and easier diffusion of nutrients in smaller cells result in a shift in phytoplankton size toward smaller picoplankton, especially cyanobacteria, in warm oligotrophic stratified waters (Alderkamp et al., 2006; Bunse & Pinhassi, 2017; Cadier et al., 2017; Flombaum et al., 2013; Morán et al., 2010; Partensky & Garczarek, 2010). Indeed, communities above the thermocline, at CFS stations, were consistently dominated by cyanobacteria, from the genera *Synechococcus*, and drove a large proportion of observed differentiation between depths and stations. This shift in phytoplankton community structure can also shift that of the underlying bacterioplankton (Camarena-Gómez et al., 2018), largely due to differing quality and bioavailability of Dissolved Organic Matter (DOM) produced by different phytoplankton (Bolaños et al., 2021; Mühlenbruch et al., 2018). Whilst we did not directly measure nutrient profiles, organic matter or phytoplankton communities at the CF, such successional dynamics have recently been shown to be a fundamental driver of community structure of bacterioplankton at the Ushant Front, another tidal mixing front in the Iroise Sea (Lemonnier et al., 2020). Given (i) the thermocline of the wider Celtic Sea is known to act as a boundary between nutrient depleted surface waters and nutrient rich bottom waters (Pingree et al., 1977) and (ii) phytoplankton communities at the CF are similarly structured between stratified and mixed areas across the CF in a similar manner to the Ushant Front (Birrien et al., 1991; Grepma, 1988; Lemonnier et al., 2020; Pemberton et al., 2004; Videau, 1987), it is likely such dynamics are also fundamental at the CF. Moreover, the soft dispersal barrier imposed between stratified and mixed waters may result in differences arising from ecological drift (Hanson et al., 2012). Clearly, future studies encompassing a greater range of environmental drivers, overlying phytoplankton dynamics and examining underlying community assembly mechanisms are needed to fully understand the underlying drivers of bacterioplankton succession.

We observed a considerable decrease (~half) in alpha diversity, in the communities above the thermocline of CFS stations, when compared to surface waters of CFM stations. This is in contrast to shelf-break fronts, where alpha diversity has been to be similar, or increase, across frontal waters (Allen et al., 2020; Morales et al., 2019). Differences in the processes underpinning different frontal development likely account for this, with shelf-break fronts formed from pressure gradients and often associate with upwelling rather than the balance between tidal mixing and atmospheric heating in tidal mixing fronts. This heating combined with a lack of replenishment of nutrients likely create a stressful environment or need for specialism. This in turn may act as a selective filter, limiting the number of species that can tolerate local conditions, or create intense competition for limited resources (Chase, 2007; Menge et al., 2002). Moreover, in September, conditions above the thermocline were characterized by elevated dissolved oxygen and a number of studies have observed a negative relationship between oxygen concentration and alpha diversity (Spitz et al., 2015; Walsh et al., 2016; Wang et al., 2015) due to a dominance of a few opportunistic taxa in highly productive areas (Salter et al., 2015; Wemheuer et al., 2014). Indeed,



communities above the thermocline were dominated by a few (~30% of sample abundance) opportunistic *Synechococcus* sp.

The structure of marine bacterioplankton communities are closely coupled to their function (Galand et al., 2018). Therefore, the differences between water bodies observed here, and the mechanisms driving these shifts, may have implications for wider ecosystem processes and biogeochemical cycles. In our study, the overrepresentation of the genus *Synechococcus* within stratified surface waters may be important as this genus is a large contributor to primary production in marine surface water (Li, 1994; Richardson & Jackson, 2007). Whilst our findings are a critical first step toward linking pattern to regional process, future studies need to move beyond description and explicitly utilize a range of techniques to characterize function. For example, the quantification of functional genes in the bacterioplankton of mangroves has identified key roles of these communities in carbon degradation (Meng et al., 2022). On top of this, future research needs to move toward quantification of both bacterial abundance and ecological processes. This is important as bacterioplankton abundances can vary hugely over small spatial scales. Indeed, in the Celtic Sea abundances of *Synechococcus* can vary by orders of magnitude over 10s of kilometres (Martin et al., 2005, 2008). Coupled with high-resolution biogeochemical context, this will us to accurately quantify the contribution that bacterioplankton communities associated with oceanographic features, such as the CF, make toward regional processes and biogeochemical cycles.

## AUTHOR CONTRIBUTIONS

NGK and SKM conceived the designed the study. NGK, FM, and JMT conducted all fieldwork work. SSB, AH, ADMD, NJM, IS and JMT conducted all lab work. NGK, SBW and RR analysed the data. NGK lead the manuscript preparation, and all authors contributed equally to subsequent edits. All authors read and approved the final manuscript.

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## CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

## DATA AVAILABILITY STATEMENT

Sequences are accessible through the EMBL database (accession no. PRJEB61283). Associated metadata are available from Fig share (<https://figshare.com/s/94f8a742684070a1e7a4>).

## ORCID

Nathan G. King  <https://orcid.org/0000-0003-0907-544X>

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