Title: A Gulf Stream cold-core eddy harbors a distinct microbiome compared to environmentally-similar adjacent waters

Running Title: Gulf Stream frontal eddy microbiome

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Originality-Significance Statement

Oceanic eddies frequently form along every major global current and have been found to trap, transport and alter microbial communities, often resulting in eddy-specific microbiomes. However, research into the effect of oceanic eddies on the microbial community have primarily focused on phytoplankton communities, often failing to investigate bacterioplankton all together. Furthermore, of the studies focusing on bacterioplankton, few have been undertaken along the Gulf Stream, a major current with global implications. We found a unique eddy-associated microbiome that was distinct from surrounding waters and, although it contained some taxa predominantly associated with nearshore samples suggesting some legacy effect from the microbiome entrapped during eddy formation, the eddy community appeared to be primarily shaped by environmental filtering. Thus, this paper not only expands our understanding of eddy effects to an understudied yet consequential region, but also provides unique insights into the processes shaping microbiomes in these frequently occurring eddies.

Summary

Marine microbiome community assembly and consequently biogeochemical cycling are shaped by past and current environments, as well as stochasticity, dispersal etc. To better understand the processes that structure marine microbial communities, we examine a cyclonic, Gulf Stream frontal eddy, whose microbiome potentially contains signatures of both the shelf community entrained during formation as well as current environmental conditions. In spite of the potential for eddy-driven upwelling to introduce nutrients and stimulate primary production, the eddy

surface waters exhibit lower chlorophyll *a* along with a distinct and less even microbial community relative to the neighboring Gulf Stream waters. Although the eddy microbiome is distinct from the Gulf Stream's, especially in cyanobacteria (e.g. lower *Trichodesmium* and higher *Prochlorococcus*), it is most similar to the Gulf Stream, suggesting eddy microbiome assembly favors environmental filtering over historical contingencies. However, better delineation of the relative roles of ecological processes will likely require Lagrangian sampling throughout the photic zone to clarify the contribution of these mesoscale features to primary production and export as well as identifying the key processes driving microbiome assembly in the oceans.

Introduction

Marine microorganisms are a crucial mediator of biogeochemical cycles, supporting critical ecosystem functions on a global scale; yet these biogeochemical processes depend on the composition, function, and activity of microbial communities (P. Falkowski, 2012; P. G. Falkowski et al., 2008). Marine microbiology has historically emphasized environmental filtering in dictating community assembly: factors such as water temperature (Ibarbalz et al., 2019; Thomas et al., 2012), salinity and nutrient availability are thought to shape microbial communities (Martin et al., 2021; Sunagawa et al., 2015). However, microbiome community assembly includes processes such as biological interactions and stochasticity (Zhou et al., 2014), as well as the microbiome's ecological history (Stegen et al., 2013). These historical contingencies, the effects of prior conditions on current microbiome assemblages, have emerged

as a significant component shaping perturbed communities (Hawkes & Keitt, 2015; Stegen et al., 2018). For example, prior environment changes cause microbiomes to be more resistant (composition remains unchanged after the disturbance) or resilient (composition is altered but eventually returns to pre-disturbance state) to future perturbations (Jurburg et al., 2017; Waring & Hawkes, 2015), while microbiomes from stable environments may be more sensitive to perturbation (Z. Wang et al., 2020; Waring & Hawkes, 2015). Investigating historical contingencies in aquatic systems is particularly challenging due to factors including water movement and the seasonality of environmental parameters (Evans et al., 2017; Shen et al., 2018). Although aquatic dispersal limitation is reduced relative to other systems (e.g. soils), oceanographic features such as steep frontal gradients between water masses can limit dispersal (Agogué et al., 2011; Baltar et al., 2016; Condie & Condie, 2016). Here, we examine a frontal eddy, which can isolate and transport microbial seed populations (Condie & Condie, 2016), to investigate the relative roles of environmental filtering and historical contingencies in an aquatic system.

Frontal eddies occur along western boundary currents where instabilities in the current, often stemming from interactions with bathymetry, cause meanders to form in the current and evolve into a distinct rotating water mass. This process partially isolates water with distinct microbiomes and environmental parameters from surrounding waters. However, eddy variability in size, location, lifespan, atmospheric conditions, and ecological history, complicates our ability to predict how they may impact microbiomes. For example, cyclonic eddies, which typically upwell cold, nutrient-rich waters into the core of the eddy, generally stimulate phytoplankton blooms (Chen et al., 2007; Lochte & Pfannkuche, 1987; Vaillancourt et al., 2003; L. Wang et al.,

2016), although not universally (Sweeney et al., 2003). However, depending on conditions, the bloom-forming phytoplankton taxa within eddies can include diatoms (Barlow et al., 2017; Chen et al., 2007; L. Wang et al., 2016), dinoflagellates (Coria-Monter et al., 2014; Lochte & Pfannkuche, 1987) and coccolithophores (Gonzále et al., 2001), with associated consequences for heterotrophic composition and organic matter export (Doblin et al., 2016; Lochte & Pfannkuche, 1987; McGillicuddy & Robinson, 1997). These differences in microbiome responses are attributed to eddy characteristics, such as the initial microbiome and nutrient status (Doblin et al., 2016) Y.-L. L. Chen et al., 2007; Harke et al., 2021) as well as environmental filtering (temperature, nutrient fluxes, mixed layer depth, etc.). For frontal eddies in the Gulf Stream a more limited range of ecological responses has been documented, though less work exists on this specific system. Hydrographic surveys describe substantial upwelling (Lee et al., 1981) and in turn phytoplankton concentrations increase substantially (Lee et al., 1991) with enhanced diatoms, dinoflagellates, though this study was limited to light microscopy for identification (Paffenhöfer et al., 1987). Though secondary consumer responses are even more poorly studied, previous work on ecosystem implications of frontal eddies shows that the upwelled nutrients are consumed within two weeks and this may drive a zooplankton bloom of copepods and doliolids (Paffenhofer 1987).

In order to better understand the implications of coherent structures broadly and frontal eddies specifically on phyto- and bacterioplankton composition and distribution, we compare the eddy microbiomes with surrounding waters. We conducted shipboard transects that encompassed continental shelf, continental slope, Gulf Stream and eddy waters off the coast of Cape Hatteras, where the Gulf Stream separates from the continental shelf and travels northeast across the

Atlantic Ocean. The Gulf Stream has widespread implications for the transport of heat and macronutrients (Palter, 2015; Whitt, 2019), and is thought to contribute to the multi-trophic biodiversity hot spot north of Cape Hatteras where it meets the Slope Sea and Mid-Atlantic Bight (MAB) shelf water (Cerame-Vivas & Gray, 1966; Lee et al., 1991). Frontal eddies form frequently (~every 3-7 days)(Glenn & Ebbesmeyer, 1994) and are projected to play a significant role in nutrient transport into the euphotic zone (Baltar et al., 2010; Belkin et al., 2022; Benitez-Nelson et al., 2007) which may enhance primary and possibly secondary production substantially at this location (e.g. in other regions primary production is enhanced by cyclonic eddy ~20% (P. G. Falkowski et al., 1991). Previous eddy studies at this location have focused primarily on their implications for oceanographic parameters (Gula et al., 2015, 2016) or phytoplankton populations (Lee et al., 1981, 1991; Yoder et al., 1981), while eddy microbiomes off the US Southeastern coast remain poorly characterized. Here, we employ high-resolution sampling to compare the microbiome within a frontal eddy and in surrounding waters in the dynamic, coastal zone.

Results & Discussion

In order to identify the factors that shape eddy microbiomes, we tracked a cyclonic, frontal eddy that developed off of Charleston, South Carolina, USA on August 25-26th 2021 and traveled north along the Gulf Stream's western front (Gray et al., 2023). The eddy was formed from shelf water and then isolated by a warmer, Gulf Stream-derived streamer (Gray et al., 2023); although the eddy was visible via satellite as a cooler, chlorophyll *a* enriched region (Gray et al., 2023), there was no evidence of a surface bloom, with satellite- measured surface chlorophyll *a* levels

declining over time, likely either through nutrient depletion or mixing with Gulf Stream waters (Gray et al., 2023). Here, ~two weeks after it formed, we obtained *in situ* samples of the eddy and surrounding waters as it moved past Cape Hatteras on September 5-7th 2021, using five surface water transects (49 stations) to obtain high-resolution measurements of microbial community composition and environmental conditions (Figure 1A).

Initially, physical parameters i.e. surface water temperature and salinity (PSU) were used to group samples: Gulf Stream > 28.2 °C, eddy <28.2 °C and > 35.75 PSU, continental slope <35.75 PSU and > 34 PSU, and continental shelf <34 PSU (Figure S1)(Gray et al., 2023). However, these physical parameter-based assignments did not align with microbiome composition, particularly among the Gulf Stream and eddy samples (Figure S2A). Thus, we used self-organizing maps (SOM) (Wehrens & Buydens, 2007)to cluster the microbiome data independently of the environmental variables. Using these purely biological-clusters we investigate interactions between water masses and the physical extent of the eddy's influence on the microbiome. In spite of some incongruences, the SOM biological clusters and the physical parameter-based classifications did overlap (Figure 1B). SOM cluster 1 largely corresponds to the continental shelf (87.5%), cluster 2 the continental slope (67%), cluster 3 the Gulf Stream (77%) and cluster 4 the eddy (62.5%) stations. Satellite data suggests that disagreements between these physical and microbiome-based classifications occurred at transitions between water masses, particularly around the periphery of the eddy. As the microbiome SOM-approach classified more samples as eddy-associated, physical parameters alone may underestimate the biogeochemical influence of the eddy (Figure S3). Henceforth, for clarity, these statistically-

distinct SOM clusters (ANOSIM, $p<0.05$) will be referred to by their most common location (1 = continental shelf, 2 = continental slope, 3 = Gulf Stream, 4 = eddy).

Next, we examined how environmental factors differed among these clusters; as might be predicted, variables associated with resource availability are higher on the continental shelf and slope compared to the Gulf Stream and eddy, consistent with greater terrestrial influence on nearer-shore environments. For example, the continental shelf and slope exhibit higher cell abundances (total prokaryotes, *Synechococcus*) and higher PO_4 along with lower salinity and cooler water temperatures compared to the eddy and Gulf Stream (Figure S6, Pairwise Wilcoxon ranked sum test $p< 0.05$) (Z. Wang et al., 2019). Comparing just the Gulf Stream and eddy samples as they are similar in both environment and microbiome: the eddy is ~1 °C cooler, has lower chlorophyll *a*, lower *Synechococcus* and higher non-phycoerythrin containing picocyanobacteria (hereafter labeled *Prochlorococcus*) (Figure S6; Pairwise Wilcoxon ranked sum test $p< 0.05$). Additionally, while eddy PO_4 concentrations are statistically higher than in the Gulf Stream, eddy PO_4 levels have a larger range, suggesting potential variability in nutrient fluxes within these water parcel types (Figure S6). Overall, eddy surface waters are generally more oligotrophic than the Gulf Stream; in spite of forming via entrainment of continental shelf water and the potential for upwelling-driven nutrient additions. Examination of the microbiome in relation to select environmental variables [temperature, salinity, chlorophyll *a*, nutrients (SiO_4, NO_3, NH_4, PO_4), and flow-cytometric abundances of bacteria, *Synechococcus* and *Prochlorococcus* (Figure 1B)] identified significant associations, except for NO_3 and NH_4, (Figure S6D, I). However, within-cluster environmental regressions revealed that only the eddy and chlorophyll *a* concentrations was significant (Multiple Regression, $R^2=0.6862$, $p<0.05$),

suggesting that within the eddy, the microbiome shifts with photosynthetic biomass. Thus, although we focus here on categorical identification of water masses, there is variability within SOM clusters for both environmental variables and the microbiome (Figures 1, S6). A closer examination of the microbiome may offer additional clues to factors that shaped these communities across water masses in the coastal ocean.

Microbial Assemblages

As eddies can enhance microbial diversity (Pajares, 2021), we compared diversity and evenness indexes across categories. Here, the eddy exhibits lower richness and evenness compared to the Gulf Stream (Figure S5), reflecting the dominance of specific taxa, particularly *Prochlorococcus* (Figure 2). Although the eddy initially formed from continental shelf waters and retains comparable richness (Figure S5), the eddy is statistically less even than the other water masses ~two weeks after formation, suggesting continued development of the microbiome. If dispersal truly limited the shift in eddy composition, then we would predict that based on presence-absence data (Sorenson index), the eddy would more strongly resemble the continental shelf with shifts in relative abundances to match the current environmental conditions. However, again the eddy is more similar to the Gulf Stream than the slope or shelf microbiomes (Figure S7), as was observed for Bray-Curtis dissimilarity (Figure 1B), suggesting that environmental filtering dominates over dispersal limitation in community assembly. We attempted to quantify the different processes shaping community assembly in the eddy using the Beta Mean Nearest Taxon Distance (βMNTD) method (Stegen et al., 2012) but our data did not exhibit the required phylogenetic signal (non-significant Mantel correlogram).

Across all samples the microbiome exhibits prevalent *Synechococcaceae* (~25-40%), *Pelagibacteraceae* (~28-40%), Actinobacteria family OCS155 (~7%), the Archaeal Marine Group II clade (~2-4%) and the SAR406 clade A714017 (~2%) (Partensky et al., 1999; Z. Wang et al., 2019; Yilmaz et al., 2016) (Figure 2). However, we can identify differences between the clusters; for example, the inversion in *Synechococcaceae* genera, with *Synechococcus* dominating the shelf and slope, and *Prochlorococcus* the Gulf Stream and eddy (Figure 2). However, specific taxa may offer additional clues about the factors which shaped these microbiomes.

To identify ASV relationships with specific clusters, the 250 most abundant ASVs, representing ~73% of the microbial community, were analyzed using both linear discriminant effect size analysis (LEfSe) and a Bayesian generalized joint attribute model (GJAM)(Clark et al., 2017; Segata et al., 2011) (Table S2). These two metrics differ in approach: LEfSe identifies enrichment in a specific cluster while GJAM can identify the magnitude and directionality (positive or negative) of associations across multiple clusters. These approaches generally agree: both finding increased relative abundance of *Pelagibacter* and *Synechococcus* in continental shelf and slope waters and enrichment of *Prochlorococcus* and *OCS155* in Gulf Stream and eddy waters, further suggesting the eddy community more closely approximates the Gulf Stream microbiome than the nearshore waters. GJAM identified a single difference between the eddy and Gulf Stream clusters, a High Light clade I *Prochlorococcus* ASV (Figure 3B) that was enriched in both the eddy and slope samples, suggesting the potential for a continued legacy of nearshore waters on the eddy microbiome. Although there are similarities between the eddy and Gulf Stream samples, their differences may more clearly reveal the current (e.g. environmental

filtering) and antecedent (e.g. priority effects, historical contingencies) conditions that shape the microbiome. A comparison of just the eddy and Gulf Stream using both LEfSe and DESeq2 (Love et al., 2014) more clearly reveals these differences. A number of high-light clade *Prochlorococcus* ASVs (Figure S8) were enriched in the eddy over the Gulf Stream. Additionally, DESeq2 analysis revealed higher relative abundance of ASVs from *Flavobacteriales* NS9, *Alteromonadales* OM60, and *Balneolaceae* in the eddy (Figure S8). Although the eddy environmental variables are indicative of a low- nutrient oligotrophic system (Figure S6), the Gulf Stream samples are enriched in a putatively nitrogen-fixing *Trichodesmium* ASV, suggesting that despite similar environmental conditions, specifically bioavailable nitrogen levels, that differences between microbiomes may reveal hidden truths about the nutrient status or environmental contingencies. Overall, the eddy sustains higher concentrations of *Prochlorococcus* and total bacterioplankton; coupled with statistically higher levels of phosphate, may indicate that the eddy experiences higher nutrient fluxes, supporting higher primary and secondary production.

Conclusions

Here we show that a cyclonic frontal eddy that formed along the western boundary of the Gulf Stream harbored a unique microbial community relative to surrounding waters. Differences between the eddy microbiome and the surrounding Gulf Stream waters included the enrichment of several *Prochlorococcus* ASVs but lower abundances of the nitrogen fixing *Trichodesmium*. Thus, the distinct eddy community seems largely shaped by environmental selection, rather than dispersal limitation. While this study was limited to surface samples and a brief time range, new research technologies are enabling the Lagrangian study of eddies as they develop and at depths

where nutrient upwelling likely has a greater influence on the microbiome (Zhang et al., 2021). Although more research is needed to understand the impact of eddies globally, our study deepens our understanding of the forces shaping Gulf Stream-associated eddy microbiomes off the South Atlantic Bight and provides important directions for future studies. While there was not support for eddy contributions to the biodiversity hotspot located off Cape Hatteras, other frontal eddies may contribute to this hotspot by delivering distinct microbial communities, enhanced zooplankton populations, and upwelling of resources (Gray et al., 2023). Furthermore, the frequency of these eddies and their associated nutrient injections into the Gulf Stream may play a significant role in carbon sequestration and productivity along the South Atlantic Bight. Although we found this eddy harbored a distinct microbial community, further investigations will be needed to fully understand the role of historical contingencies and the temporal dynamics of eddy-associated communities and their potential role in global biogeochemical cycles. Microbially-defined ecological units can help us disentangle dynamics along frontal zones where the convergence and mixing of water parcels can obscure the extent to which coherent structures like eddies alter microbiomes and biogeochemical processes.

Methods

Sample collection and sea water parameter characterization

Seawater was collected aboard the *R/V Shearwater* across 5 transects from September 5-7th, 2022; transects were planned to cross the front between the shelf and eddy or shelf and Gulf Stream waters. Water samples were collected during transit from the ship's flow-through seawater system (~1 m depth). Temperature and salinity were collected with a SeaBird SBE38

Thermosalinograph. Chlorophyll *a* concentrations were measured by filtering 100 mL of seawater onto a ~0.7 µm glass fiber filter (Whatmann APFF02500) using gentle vacuum. Chlorophyll *a* pigments were extracted in 100% methanol for 48h at -20 °C and fluorescence was measured using a Turner Designs 10-AU fluorometer as previously described (Johnson et al., 2010). Duplicate whole seawater samples were collected, fixed with net 0.125% glutaraldehyde and stored at -80 °C until processing. Prokaryotic phytoplankton populations were enumerated using a Becton Dickinson FACSCalibur Flow Cytometer and categorized as previously described (Johnson et al., 2010). Bacterioplankton were quantified using SYBR Green-I on the Attune NxT acoustic flow cytometer (Life Technologies) (Marie et al., 1997). Duplicate 0.22 µm filtered seawater samples were collected for dissolved inorganic nutrient quantification and stored at -80 °C until processing. Nutrients (NO_2, NO_3, PO_4, SiO_4) were analyzed at the UC San Diego Scripps Institution of Oceanography ODF chemical laboratory using a Seal Analytical continuous-flow AutoAnalyzer 3 using the analytical methods described by (Atlas et al., 1971; Gordon et al., 1992; Hager et al., 1972).

DNA extraction and library preparation:

Microbial DNA was collected by filtering ~4 liters of whole seawater through a 0.22 micron Sterivex filter (Milipore). Filters were stored at -20 °C while on the research vessel and then at -80 °C upon return to the lab until extraction. Genomic DNA for 16S rRNA gene libraries was extracted from half of each filter using the Gentra Puregene Yeast/Bacteria kit (QIAGEN) supplemented with 60 seconds of bead beating. Extracted DNA was quantified using a Nanodrop ND-100 and 16S rRNA gene libraries were generated using gene primers targeting the V4-V5 region 515F- (5′-GTGYCA GCMGCCGCGGTAA) and 926R (5′-CCGYCAATTYM

TTTRAGTTT) (D. Needham et al., 2018; D. M. Needham et al., 2018; Parada et al., 2016). PCR reactions were performed in triplicate with 20 µL reactions containing 20 ng template DNA, 1× Taq Buffer, 0.5 µM of each primer, 200 µM of dNTPs, and 0.4 U of Q5 DNA polymerase (NEB). The thermal cycling conditions were 30 sec at 98 °C, followed by 25 cycles of 10 sec at 98 °C, 30 sec at 50 °C, 30 sec at 72 °C, and a final extension at 72 °C for 2 min. Triplicate PCR reactions were pooled and gel purified. Libraries were pooled and sequenced at the Duke Center for Genomic and Computational Biology using V2 2 × 250 bp sequencing on the Illumina MiSeq.

Sequence Processing:

Barcodes were removed and sequences were demultiplexed and assigned to samples using Sabre (https://github.com/najoshi/sabre). Sequences were cleaned and clustered using VSEARCH v2.5.1 (Rognes et al., 2016). Low quality sequence ends were trimmed at the Phred quality score (Q) of 30 using a 10 bp running window. Paired-end reads with 10 bp overlap and no mismatches were merged and sequences with expected errors >1 and/or a length < 360 bp were removed. Minimum entropy decomposition analysis (v2.1) was used to resolve amplicon sequences variants (ASVs) (Eren et al., 2015). Sequencing-error associated noise was reduced by retaining only those ASVs with unique sequences with a minimum abundance of 20 occurrences. Representative ASV sequences were assigned taxonomy using the RDP classifier in MacQIIME v1.9.1. Mitochondrial sequences were removed and libraries were subsampled to 14,187 reads per library. SSU rRNA library sequences are deposited as part of Bioproject PRJNA868376. AmpliCopyrighter (v.0.46) was used to correct for differential gene copy numbers across taxa

(Angly et al., 2014). “Absolute abundance” was calculated by multiplying relative abundances of ASVs by prokaryotic cell counts obtained from flow cytometry analysis.

Self Organizing Map

The Kohonen package (Wehrens & Buydens, 2007) in R (v. 4.0.0) was used to cluster samples based on microbial community structures. A self-organizing map was constructed using ASV relative abundances and each sample was assigned to a map unit based on similarities in their community structures. Our map was composed of 4x4 circular nodes in a hexagonal, nontoroidal configuration. Clusters were generated using k-means clustering and a reasonable value for k was chosen by estimating the point of inflection in a scree plot of within-clusters sum of squares (Figure S3).

Discriminative taxa identification

Generalized joint attribute modelling (GJAM) was applied to model the 250 most abundant ASVs and select environmental factors (temperature, salinity, chlorophyll *a*, PO_4), using the GJAM v. 2.6.2 package in R. Iteration was set at 20,000 and burning at 5,000. Results were visualized using the built-in function ‘gjamPlot’. In order to identify discriminative taxa between SOM clusters, the 250 most abundant ASVs were analyzed with LEfSe (Segata et al., 2011). The threshold for significance on the logarithmic LDA score for discriminative features was two. P-values were obtained from the Kruskall-Wallis test for differences in ASV abundances among SOM cluster assignments. ASVs exhibiting differential abundances between the Gulf Stream and eddy were further identified using DESeq2 (Love et al., 2014). Only ASVs with an average relative abundance >0.05% averaged across samples being compared (Gulf

Stream and eddy) were included. Significant differences were considered those with a Benjamin-Hochberg multiple hypothesis adjusted $p<0.05$.

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CRediT Contributions:

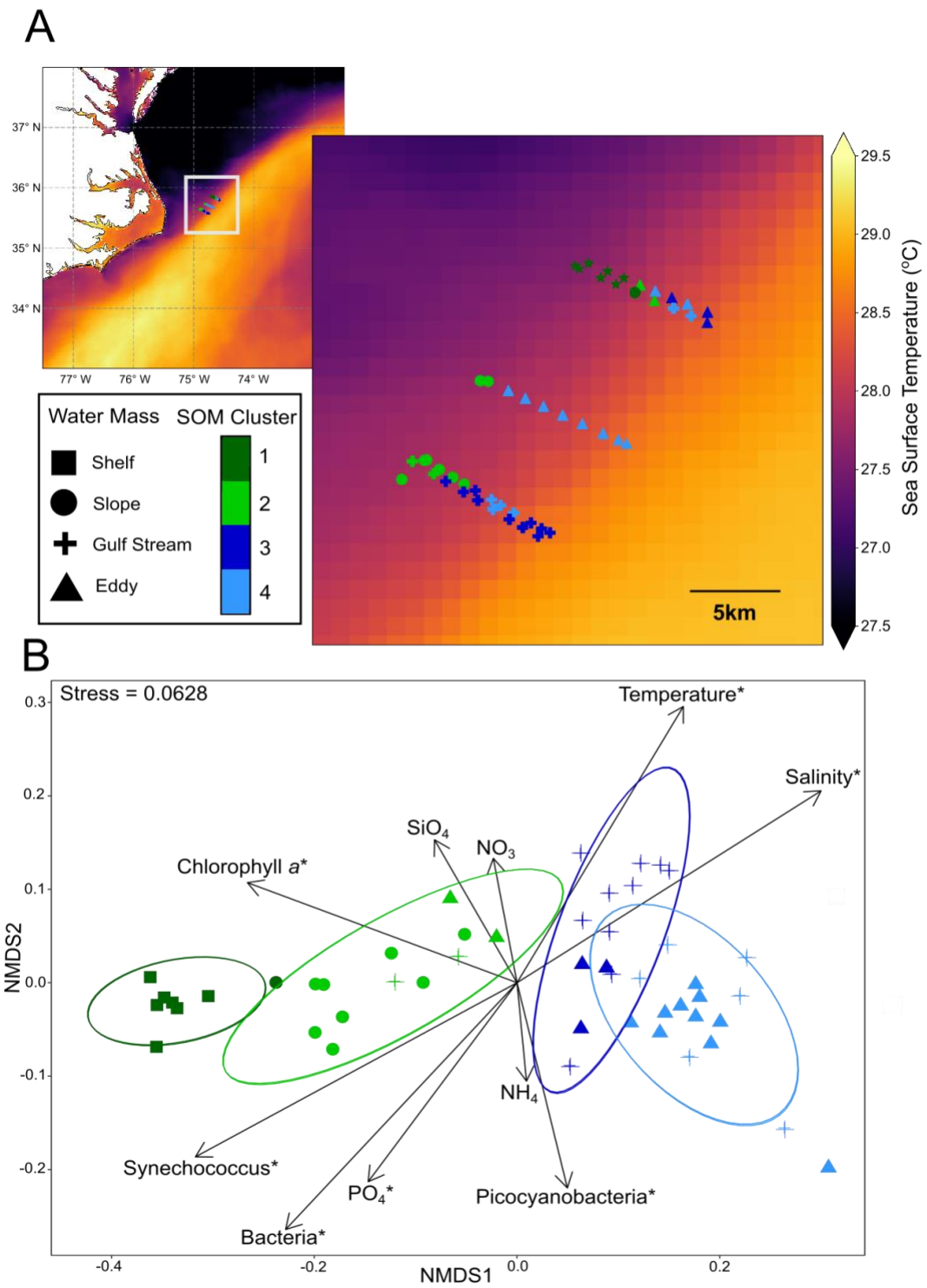
Jessica Gronniger- Conceptualization; Data Curation; Formal Analysis; Visualization; Writing-original draft;

Patrick C. Gray- Conceptualization; Data Curation; Formal Analysis; Visualization; Writing-review and editing

Alexandria K. Niebergall- Formal Analysis; Writing-review and editing

Zackary I. Johnson- Resources; Writing-review and editing

Dana E. Hunt- Conceptualization; Resources; Writing-original draft;


A
37° N
36° N
35° N
34° N
77° W
76° W
75° W
74° W
Water Mass
SOM Cluster
Shelf
Slope
Gulf Stream
Eddy
1
2
3
4
5km
29.5
29.0
28.5
28.0
27.5
27.0
27.5
Sea Surface Temperature (°C)
B
Stress = 0.0628
Temperature*
Salinity*
SiO4
NO3
Chlorophyll a*
NH4
Synechococcus*
PO4*
Picocyanobacteria*
Bacteria*
NMDS2
NMDS1
0.3
0.2
0.1
0.0
-0.1
-0.2
-0.4
-0.2
0.0
0.2

364

Figure 1 Locations and microbiome characteristics of field samples (A) Map of sampling sites for five ~10-15 km long oceanographic transects off the coast of Cape Hatteras in North Carolina, USA from September 5-7, 2021. Background color represents average sea surface temperatures from August 20th to September 10th obtained from the Geostationary Operational Environmental Satellite 16 (GOES-16). Each sampling point is shown along the transects and categorized by physical water mass (shapes), defined by temperature and salinity, and microbial-community based k-means clusters (colors), generated using the self-organizing map (SOM) R package 'kohonen'. (B) Non-metric multidimensional scaling (NMDS) ordination computed based on Bray-Curtis dissimilarity for 16S rRNA gene libraries. Ellipses show the multivariate t-distribution 95% confidence interval for the mean of each SOM cluster. Environmental factors found to be statistically significant (Multiple regression permutation test, $p<0.05$) are indicated with an '*'.

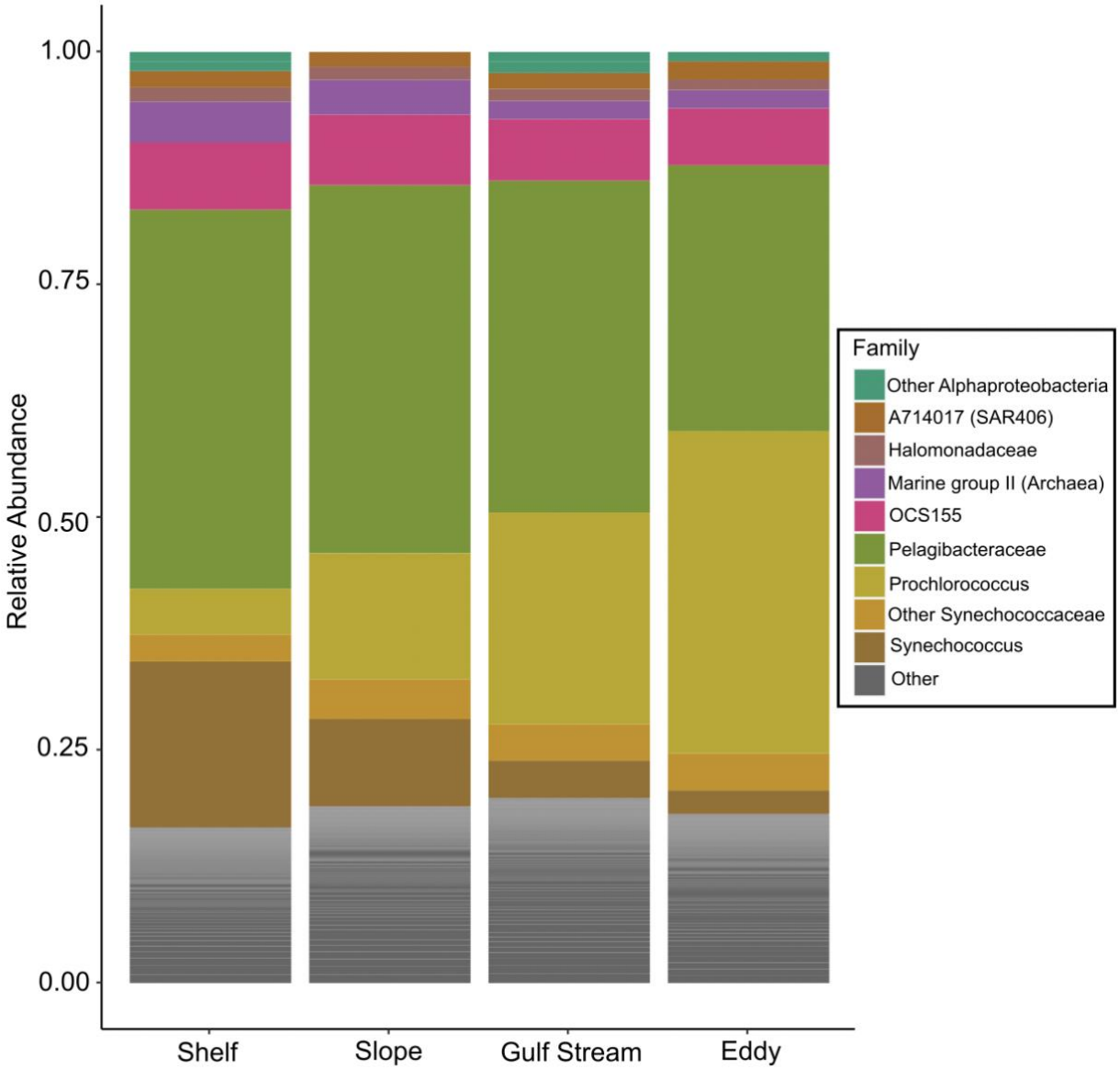


Figure 2 Average family-level community for each Self-Organizing Map (SOM) cluster. The average community was calculated by taking the mean relative abundance of each taxon across all samples within that cluster, and average relative abundances were normalized to 100%. ASVs with a relative abundance of less than 1% are grouped as "Other." ASVs were generally grouped at the family level, but when family is undefined they are labeled at the phylum level.

Additionally, *Synechococcaceae* was resolved at the genus level to illustrate the *Synechococcus*-*Prochlorococcus* inversion between clusters.

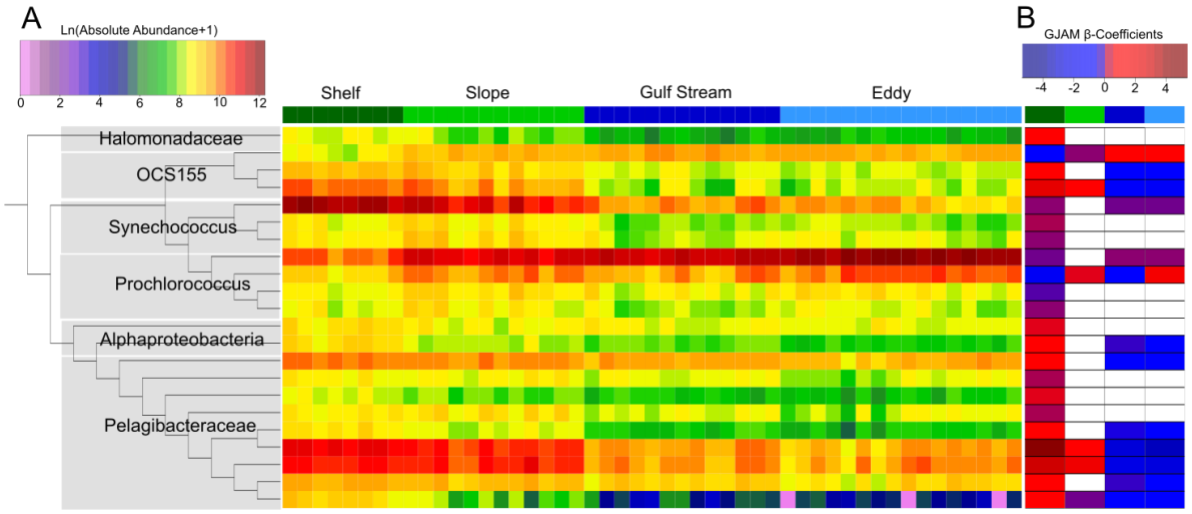


Figure 3 ASV associations with specific SOM groupings (A) Heatmap of ln(absolute abundance+1) of the 22 ASVs identified with at least one significant SOM cluster beta coefficient obtained from generalized joint attribute modeling (GJAM) analysis of the 250 most abundant ASVs across all samples. (B) Heatmap of SOM cluster-associated beta coefficients. Color bars at the top of each column identifies the SOM cluster. Y-axis dendrogram represents maximum likelihood phylogenetic tree constructed using Smart Model Selection in PhyML 3.0 with taxonomic assignments based on the RDP naïve Bayesian classifier using Greengenes version 13.5 database.

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