

# Rapid changes in coastal ocean microbiomes uncoupled with shifts in environmental variables

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## Summary

**Disturbances, here defined as events that directly alter microbial community composition, are commonly studied in host-associated and engineered systems. In spite of global change both altering environmental averages and increasing extreme events, there has been relatively little research into the causes, persistence and population-level impacts of disturbance in the dynamic coastal ocean. Here, we utilize 3 years of observations from a coastal time series to identify disturbances based on the largest week-over-week changes in the microbiome (i.e. identifying disturbance as events that alter the community composition). In general, these microbiome disturbances were not clearly linked to specific environmental factors and responsive taxa largely differed, aside from SAR11, which generally declined. However, several disturbance metagenomes identified increased phage-associated genes, suggesting that unexplained community shifts might be caused by increased mortality. Furthermore, a category 1 hurricane, the only event that would likely be classified *a priori* as an environmental disturbance, was not an outlier in microbiome composition, but did enhance a bloom in seasonally abundant phytoplankton. Thus, as extreme environmental changes intensify, assumptions of what constitutes a disturbance should be re-examined in**

**the context of ecological history and microbiome responses.**

## Introduction

As global change continues to increase the frequency and intensity of environmental disturbances, understanding their microbiome impacts and persistence is critical to assess potential changes in microbial-mediated ecosystem processes. Shade *et al.* (2012) define disturbances as causal events that either alter microbiome-relevant environmental parameters or directly alter the microbial community. However, even this definition has limitations; for example, critical environmental parameters are undefined in many systems, limiting our ability to accurately identify disturbed versus ‘normal’ conditions or to characterize potential proximal drivers of disturbance responses. Additionally, metrics that assess the microbial community as a whole may miss disturbance events that disproportionately affect biogeochemically important taxa that constitute a small fraction of the total community. Despite these limitations, microbial ecology has developed a general framework to describe disturbance responses. Microbiome responses to disturbance include sensitivity, where the community composition changes and does not return immediately to its prior state; resilience, where the community is initially altered but returns to its original composition; and resistance, where community composition remains the same (Allison and Martiny, 2008). Furthermore, communities whose composition changes can be functionally redundant, where an altered community composition retains the original functional capacity. In environmental systems, disturbances have been shown to alter microbial diversity, biogeochemical rates and functional capacities (Atlas *et al.*, 1991; Allison and Martiny, 2008; Renes *et al.*, 2020). In contrast to host-associated ecosystems where disturbance often results from a disease state or drug treatment, disturbances in non-host associated ecosystems, such as soil or aquatic environments, are often complex, frequently altering multiple factors simultaneously (e.g. storms, wildfires, co-occurring contaminants). While disturbances vary in origin and specific impacts, non-resistant microbiomes generally exhibit

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reduced diversity (Atlas *et al.*, 1991; Renes *et al.*, 2020), increased stochasticity in community assembly (Ferrenberg *et al.*, 2013; Zhou *et al.*, 2014), increased physiological tolerance and metabolic versatility (Atlas *et al.*, 1991), and shifts towards disturbance-resistant taxa (Westergaard *et al.*, 2001; Renes *et al.*, 2020).

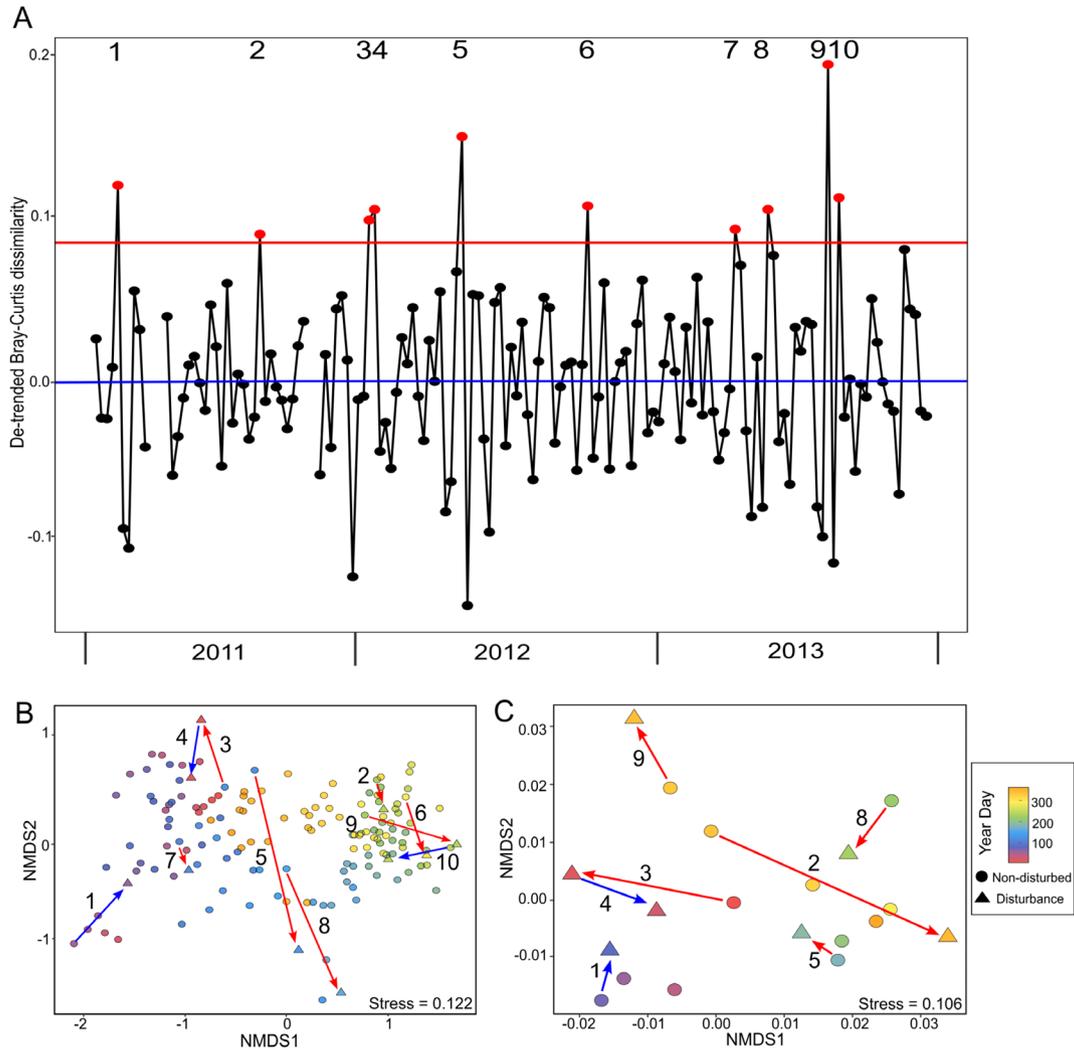
Despite some similarities in the disturbance responses, microbiomes can differ significantly in their resilience time (the length of time required for the microbial community to return to its original state), due to a combination of (i) the type, extent and duration of the disturbance, (ii) the ecological history of the impacted community and (iii) other ecosystem characteristics (e.g. dispersal or turnover rates, etc.). Disturbances can be classified as either pulse, or short-term, events (e.g. rain events, washout) or press, or long-term chronic events (e.g. climate change) and microbiome responses likely depend on the duration of altered environmental conditions with shifts in microbiomes persisting for weeks to years even in relatively dynamic aquatic environments (Westergaard *et al.*, 2001; Peierls *et al.*, 2003; Wetz and Paerl, 2008; Shade *et al.*, 2011). Ecosystems exposed to high levels of environmental variation, both over annual cycles and due to episodic events, may have microbiomes that are adapted to change and thus are more resistant or resilient to disturbance (Stegen *et al.*, 2018; Renes *et al.*, 2020; Wang *et al.*, 2021). Our ability to generalize about disturbance persistence is limited by studies that often focus on subsets of the community (e.g. phytoplankton) (Wetz and Paerl, 2008) or relatively stable soil communities (Westergaard *et al.*, 2001); thus, these findings may not broadly apply to all taxa, environments or disturbance types. For example, aquatic microbiomes generally exhibit higher dispersal rates compared to soil microbiomes; thus marine environments, in particular, are likely to exhibit faster resilience than other environments due to dispersal from adjacent undisturbed waters (Zhou *et al.*, 2014; Shen *et al.*, 2018; Rii *et al.*, 2022). While disturbance remains an active area of investigation, there are still open questions of how microbiome responses vary across ecosystems and disturbance types.

Other challenges remain: commonly practised *a priori* identification of disturbances limits conclusions to well-defined drivers and specific microbial responses. Additionally, environmental microbiomes are often characterized only after the disturbance has occurred, removing crucial context for both the microbiome and environment about the mean and variance of the non-disturbed state (Jones *et al.*, 2008; Shade *et al.*, 2011; Ferrenberg *et al.*, 2013). To address some of these challenges, here we define disturbed communities as those with larger-than-expected changes in community composition as inferred from long-term observations. While this method

is limited to the detection of disturbances that (i) alter microbial community composition, and (ii) occur on a weekly timescale, it takes a microbiome-centric approach focusing on community structure and function that could be overlooked through a *priori* determination of disturbance events. Here, we adopt a modified definition of disturbance as events that alter microbiome composition, which we use to identify disturbance-responsive populations, and additionally, for a subset of events, examine changes in metagenomes. Furthermore, as this study occurs at a well-studied, long-term coastal time series (Piver's Island Coastal Observatory: PICO), changes in both environmental factors and microbiome composition can be contextualized within a background of annual and episodic changes in environmental parameters and microbiomes (Johnson *et al.*, 2013; Ward *et al.*, 2017; Wang *et al.*, 2021).

## Results and discussion

We utilized 3 years of weekly water samples (Jan. 2011–Dec. 2013) from a time series site located at the mouth of an estuary (PICO) to examine the coastal ocean microbiome. This location exhibits annual cycles in community composition and strong seasonal population dynamics, which are correlated with light and temperature (Ward *et al.*, 2017). Here, we utilized this dataset to identify disturbance events based on changes in microbiome composition. This microbiome-centric approach has the advantage of identifying events that alter microbial communities, but will not capture resistance events when the community composition does not change (Allison and Martiny, 2008). As the highest rates of community change occur in the spring and fall (Ward *et al.*, 2017), we minimized seasonal bias by subtracting a time-averaged rate of community change from the weekly Bray–Curtis dissimilarity (Fig. S1). After removing seasonal trends, the 10 largest weekly community changes were identified as potential ‘disturbance events’ (Fig. 1A). Interestingly, this metric of disturbance did not correspond to increased  $\beta$ NTI-based turnover in the 200 most abundant taxa (Fig. S2), or increased stochasticity compared to non-disturbance conditions (Wilcoxon test  $p > 0.05$ ). We did note that some disturbances were paired, with a large community change (the ‘disturbance’) followed by a second large community change 1–2 weeks later (Fig. 1A: disturbances 3 and 4, 9 and 10), which we interpret as resilience events with the community returning to a seasonally normal state (Fig. 1B: blue arrows). A single aberrant week that was rapidly followed by a return to normal conditions (e.g. disturbances 3 and 4) could be explained as sampling a rare microenvironment such as a large particle (Yung *et al.*, 2016). Yet, most disturbances were not



**Fig. 1.** Microbiome disturbance identification ordination plots.

A. Community dissimilarity over 3 years (Jan. 2011–Dec. 2013) of weekly samples from the PICO time series. Bray–Curtis dissimilarity (black line) was calculated over 1-week interval, excluding missing samples. Values presented are relative to those expected based on a smoothed local average (LOWESS, locally weighted scatterplot smoothing) for that time period. Mean Bray–Curtis dissimilarity (blue line) is shown. Numbers indicate the 10 largest community changes (disturbance events 1–10). A category 1 hurricane that directly impacted the study site immediately following disturbance 2.

B. Non-metric multidimensional scaling (NMDS) ordination computed based on Bray–Curtis dissimilarity for 16S rRNA gene libraries of weekly samples at the PICO over 3 years (2011–2013). Disturbance events are labelled with numbers and indicated by red arrows. Three events (1, 4 and 10) believed to be resilience events are indicated by blue arrows.

C. Non-metric multidimensional scaling (NMDS) ordination computed based on Bray–Curtis dissimilarity for all metagenome samples. Disturbance events are numbered and are indicated by red arrows. Predicted post-disturbance resilience events are shown with blue arrows (1, 4).

followed by a second event, suggesting that either disturbance effects persisted for more than a single week or that the microbiome returned to the seasonal normal gradually over time.

In order to better understand potential drivers of community changes, we examined the relationship of disturbance events with environmental parameters. Overall, we found no clear correlation between microbial community change (Bray–Curtis dissimilarity) and environmental variables (e.g. temperature, pH, chlorophyll *a*, dissolved

oxygen, salinity, nutrients; Table S1; Fig. S3A). However, we can speculate about the linkages between individual disturbances and potential environmental drivers. For example, based on the environmental and microbiome context, we could re-categorized disturbance 1 as a resilience event where the microbial community recovered from a cold period [water temperature  $<10^{\circ}\text{C}$ , lower than the average winter temperature (range  $\sim 10^{\circ}\text{C}$ – $15^{\circ}\text{C}$ ), for about a month prior to disturbance 1; Fig. S4A]. However, most disturbances had no obvious distinguishing

environmental characteristics, which could be due to unmeasured environmental factors, stochastic processes or changes in biological interactions (e.g. mortality). Other disturbances were enigmatic: although disturbance 2 occurred 3 days prior to the landfall of a category 1 hurricane (Irene), we found no linkages between this disturbance and the environmental impacts from the hurricane (e.g. no change in wind or precipitation was evident at the study site). Furthermore, while the hurricane altered a number of environmental factors (Figs S3 and S4), it did not induce a disturbance response based on our microbiome turnover metric (Fig. 1).

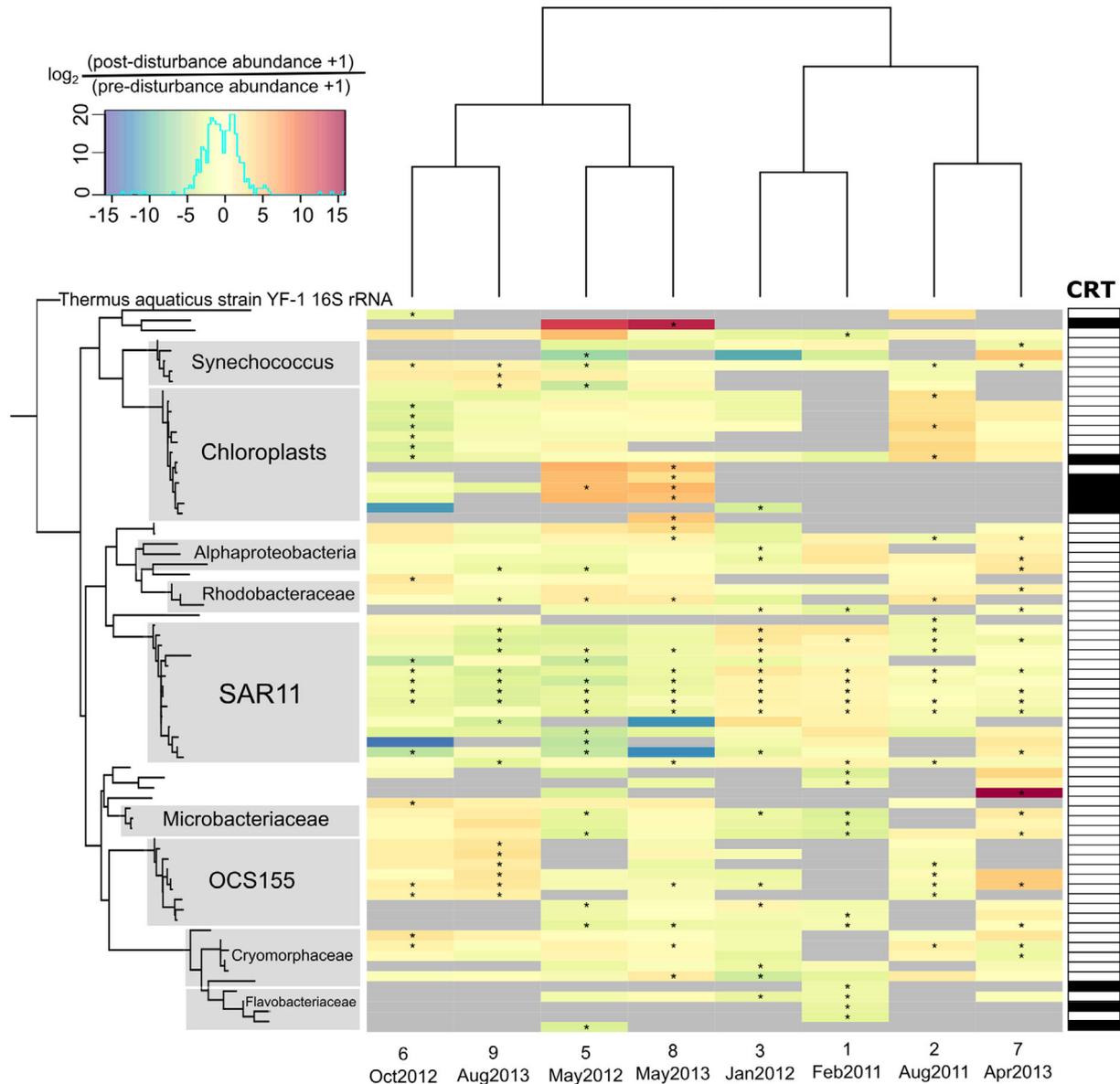
Individual populations may offer additional insights into the characteristics of disturbance-responsive taxa or, potentially, the drivers of these events. Among the 20 taxa that contributed the most to community change for each disturbance (Fig. 2), we found that no OTU responded to all disturbances. However, some clades seemed disproportionately disturbance-responsive, such as members of the streamlined marine oligotroph SAR11 clade, which predominantly decreased post-disturbance, as well as members of the OCS155 clade of Actinobacteria which both increased and decreased (Fig. 2). As SAR11 are a ubiquitous marine group, we further examined SAR11 oligotypes (unique sequence types); and identified rapid shifts across multiple populations for these 10 disturbances and in response to other events not detected in the community-wide analysis (Fig. S5), suggesting they may be sensitive biomarkers of environmental change (Yeo *et al.*, 2013; Giovannoni, 2017). The most similar disturbances were spring events 5 and 8 (May 2012 and 2013) which (i) clustered based on responsive taxa (Fig. 2), (ii) occurred in the same season and (iii) exhibited similar large community shifts on the NMDS (Fig. 1B). Unlike most other disturbances, both spring events' microbiomes contain a large fraction of conditionally rare taxa (CRT; Fig. S6), phylotypes that are generally a small fraction of the microbiome but occasionally become abundant, often as a result of altered environmental conditions or microbial community dynamics (Shade *et al.*, 2014). These rare taxa are thought to act as a 'seed bank' to replenish lost diversity and functional capacities within a disturbed community (Jones and Lennon, 2010; Campbell *et al.*, 2011; Caporaso *et al.*, 2011). However, contrary to predictions of disturbance responsiveness, CRT did not comprise a significant fraction of the microbiome after other disturbances or Hurricane Irene (Fig. S6). CRT were also abundant prior to disturbance 1 (a presumed resilience event), when the system experienced colder than normal temperatures (<10°C; Fig. S4); suggesting that these CRT may be able to grow at lower temperatures, enabling them to out-compete winter-associated taxa (Yung *et al.*, 2015). Overall, a population-level analysis of these disturbances

highlights that, while some disturbances are characterized by opportunistic taxa (disturbances 5 and 8), most disturbance-responsive taxa in fact do not all fit with the common narrative of fast-growing taxa that bloom due to an influx of new resources following a disturbance.

As disturbances did not select for canonical opportunistic taxa (Polz *et al.*, 2006), microbiome functional potential (i.e. metagenomes) may offer insights into these disturbances (Sjöstedt *et al.*, 2018) (Fig. 1C). Similar to the community data, metagenomes also clustered by season (Fig. 1C); and disturbance OTU-level community and metagenome changes showed a positive correlation (Fig. 1; Mantel test on Bray–Curtis dissimilarities,  $r = 0.5136$ ,  $p = 0.0001$ ). However, the largest community and metagenome changes were not always aligned; disturbance 2 showed the largest metagenome change despite having the smallest community change among the 10 disturbance events (Fig. 1B and C). Enriched disturbance 2 metagenome categories largely corresponded to phage-related genes, suggesting top-down controls could cause these community changes. This decoupling between community composition and functional potential could indicate that disturbances select for specific functional traits rather than taxonomic groups (Coles *et al.*, 2017), and it may provide insights into the biology that drives or responds to these events. As neither taxa nor functional potential is conserved across disturbances, we focused on several case studies, including the passage of a hurricane and repeated spring events, to more deeply understand these events.

### Hurricane Irene

We first examined Hurricane Irene, which was not identified as a disturbance based on our community-turnover metric. In spite of a hurricane likely being categorized *a priori* as a disturbance, the microbiome remained within the seasonal average, suggesting some degree of resistance, even though environmental conditions formed a distinct outlier cluster (Figs S3 and S4). Hurricane Irene made landfall about 10 miles from our study site (at Cape Lookout, NC, USA) as a category 1 hurricane on August 27, 2011, and was characterized by substantial precipitation (~350 mm), a large wind field and localized flooding. As our first post-hurricane sample was taken 3 days after landfall, we may have missed some short-term impacts, e.g. introduction of stormwater microbes, a first flush of nutrients, and so on (Ares *et al.*, 2020). Nevertheless, a comparison of samples collected pre- (August 24) and post- (August 30) hurricane landfall (August 27) reveals decreases in both pH (~0.11 units) and dissolved inorganic carbon (~230 µM), as well as the highest concentrations of NO<sub>x</sub>, observed over the full 3 years of the dataset (NO<sub>x</sub>: 1.53 µM; Fig. S4F) and a substantial



**Fig. 2.** Heatmap showing OTU abundance changes pre- and post-disturbances. Plotted is the  $\log_2$  of (post-disturbance absolute abundance + 1)/(pre-disturbance absolute abundance + 1) of the 20 taxa contributing the most to the community change in each disturbance event (identified with asterisks), excluding presumed resilience events (Disturbances 4 and 10). Responsive taxa for each disturbance included only those with a minimum average relative abundance across pre- and post-disturbance samples of 0.05%. In other disturbances, taxa below the 0.05% minimum threshold abundance were removed from analysis (coloured in grey). Absolute abundances are calculated based on relative abundances multiplied by total cell counts. Disturbance events are grouped by a cladogram based on the similarity of the heatmap. Taxa are ordered based on a maximum likelihood phylogenetic tree, with the major phyla labelled and *Thermus aquaticus* strain YF-1 as the outgroup. CRT are identified with black on the right.

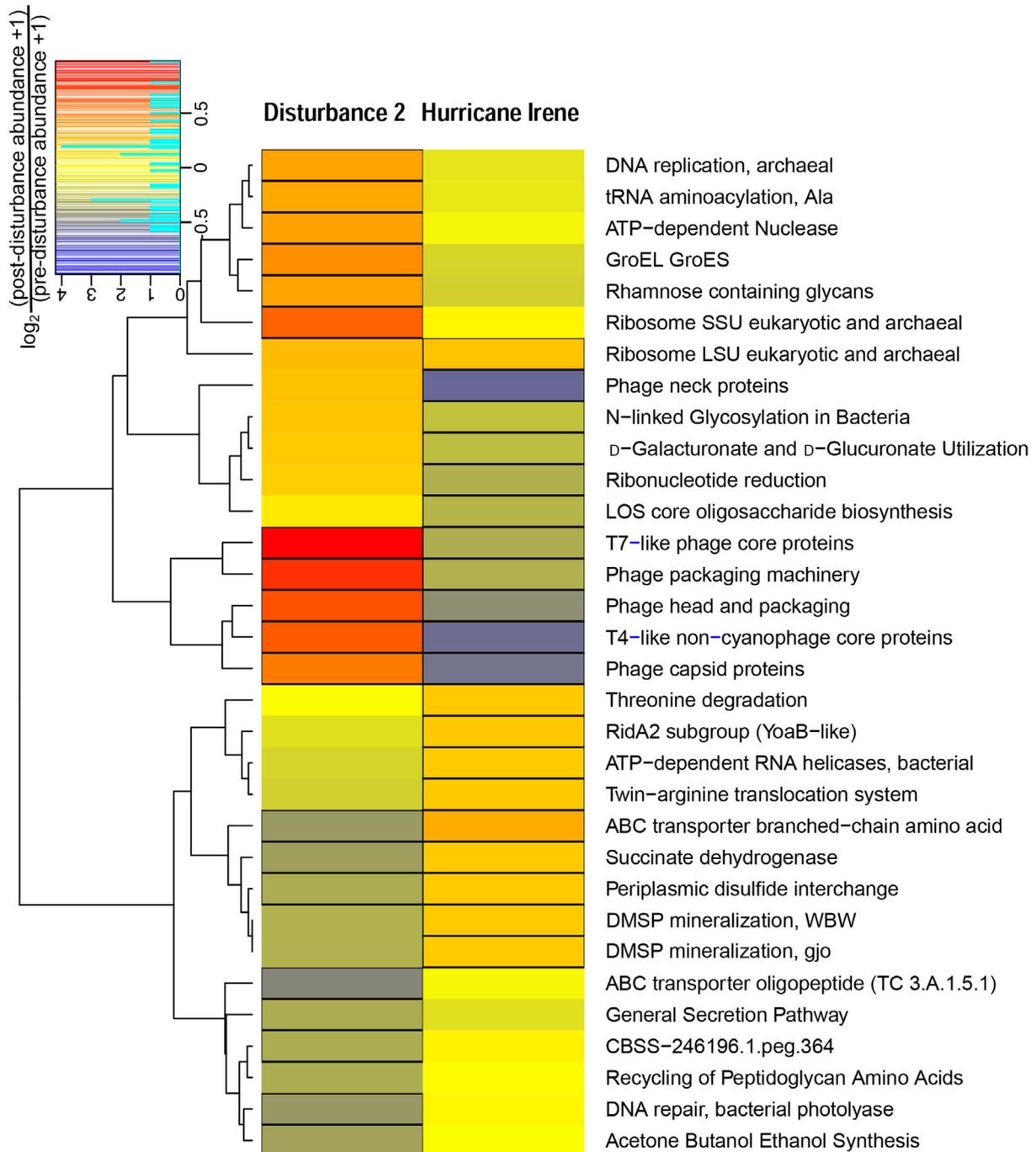
increase in  $\text{NH}_4$ ,  $\text{SiOH}_4$  and chlorophyll *a* relative to pre-hurricane conditions (reported as pre-hurricane  $\rightarrow$  post-hurricane values;  $\text{NH}_4$ : 319.94  $\rightarrow$  1507.72  $\mu\text{M}$ ;  $\text{SiOH}_4$ : 4.36  $\rightarrow$  25.27  $\mu\text{M}$ , chlorophyll *a*: 5.81  $\rightarrow$  10.85  $\mu\text{g L}^{-1}$ ; Fig. S4E, G, H).

In addition to these short-term changes in environmental parameters, some environmental factor shifts persisted for weeks following the hurricane: decreased

salinity ( $\sim 7$  units over 5 weeks; Fig. S4C) and elevated levels of nutrients, including  $\text{SiOH}_4$ ,  $\text{NO}_2$  and  $\text{PO}_4$  (Fig. S4E-G). The continued high levels (and sometimes delayed peaks) in these environmental variables point to continued nutrient fluxes and freshwater inputs from surface water movement through the watershed, groundwater discharge and long-term processing of storm-derived material in estuaries (Johnson *et al.*, 2013; Asmala

et al., 2021). Yet, in contrast to previous hurricane research that found substantial alterations to microbial composition (Peierls et al., 2003; Ares et al., 2020; Steichen et al., 2020) and function (Yan et al., 2020), we observed only minor turnover in community composition and functional potential (Fig. 3 and Fig. S7). We cannot

discount that disturbance 2 immediately prior to the hurricane may have masked potential hurricane effects (Wetz and Paerl, 2008), pre-selected for a more disturbance-resistant community (Sjöstedt et al., 2018; Renes et al., 2020) or alternately, our weekly sampling interval may not have captured rapid and/or finer scale effects



**Fig. 3.** Heatmap of metagenome changes Log<sub>2</sub>-fold-change between pre- and post-disturbance 2 and 3 days pre-Irene (disturbance 2) and 9 days post-Irene. Includes 20 SEED categories with the highest |Log<sub>2</sub>FC| for each pair, indicated by black outlines. Only SEED categories with a minimum average relative abundance across the three samples of >0.05% are included.

such as an influx of freshwater or terrestrial microbes in floodwaters (Ares *et al.*, 2020) even though other environmental changes were observed. Overall, these results suggest that our capacity to predict events that alter microbiomes may be more limited than previously thought.

Although Hurricane Irene was not categorized as a disturbance based on our microbiome-turnover metric, a number of taxa did exhibit large changes in relative abundance (Table S2): several eukaryotic phytoplankton phylotypes increased or decreased and SAR11 taxa exhibited increases, in contrast to the general declines in SAR11 observed for other disturbances (Fig. 2 and Table S2). In addition to new resources (nutrients, organic matter), hurricanes potentially introduce allochthonous bacteria (Amaral-Zettler *et al.*, 2008; Balmonte *et al.*, 2016). A number of hurricane responsive taxa were below the sequencing detection limit either before or after the hurricane (Fig. S7; Table S2), which could represent taxa that were washed in by floodwaters/sediment re-suspension, rapidly bloomed or alternately died due to the environmental conditions present. While it can be difficult to assign habitat origins to specific taxa (e.g. terrestrial vs. aquatic), the soil-associated *Sediminicola* genus (OTU 883) increased post-hurricane (Table S2). During this period, other taxa likely responded to autochthonous change – the most dramatic hurricane response occurred in two diatoms: OTU 8 (*Skeletonema pseudocostatum*) and OTU 10129 (*Eunotogramma* sp.), which, while normally abundant during the summer and fall, bloomed post-hurricane. These phylotypes increased ~10- fold (relative abundance) between pre- and post-hurricane samples and peaked 4 weeks after the hurricane, accounting for ~10% (OTU 8) and 6% (OTU 10129) of the libraries, compared to maximum relative abundances of ~3% in 2012 and 2013 (Fig. S8). As hurricanes alter multiple environmental factors, we sought to better link these population level-responses to potential environmental drivers using Bayesian generalized joint attribute modelling (GJAM) in conjunction with select environmental variables (temperature, NH<sub>4</sub>, chlorophyll *a*, salinity and Mean Lower Low Water, a metric of tidal height) (Clark *et al.*, 2017). The two diatoms (OTUs 8 and 10129) both had significant positive associations with NH<sub>4</sub> and chlorophyll *a* and a negative relationship with salinity (GJAM), even when the data from the year of Hurricane Irene (2011) was removed (data not shown), suggesting the high nutrient, low salinity post-hurricane conditions would promote growth. *Skeletonema* spp. have high growth and nitrogen-uptake rates, giving these diatoms a competitive advantage when pulses of nutrients occur (Huang *et al.*, 2020). While disturbance is typically thought to allow rare taxa to bloom, altered conditions can also

benefit dominant taxa, particularly those that quickly respond to increased resource availability and/or reduced competition (Polz *et al.*, 2006; Wetz and Paerl, 2008; Steichen *et al.*, 2020). Finally, a comparison of metagenomes the week before disturbance 2, disturbance 2 (3 days prior to hurricane Irene) and 9 days post-hurricane reveals a number of phage-related gene categories increased in disturbance 2 (Fig. 3), suggesting that phage-mediated community selection could have altered the community before the hurricane. In contrast, the post-hurricane sample exhibited increases in two dimethylsulfoniopropionate-mineralization genes, suggesting the diatom bloom has increased degradation potential for this algal osmolyte (Fig. 3). In future work, we may consider alternate metrics for disturbance (rather than week-over-week changes) as the hurricane did not result in an immediate or dramatic change in the microbiome, but rather a sustained shift in specific populations. However, we note that this event did not impact water temperature, which is predicted to be the major environmental driver at this site, and occurred in late summer when the microbiome is relatively stable and potentially more resistant to disturbance (Ward *et al.*, 2017). Thus, when taxa are relatively well acclimated and presumably adapted to environmental conditions, even relatively large and sustained changes in environmental parameters primarily generated blooms in abundant organisms that were poised to make use of these resources.

### Spring disturbance events

In contrast with relatively stable summer conditions, PICO community turnover is highest during the spring and fall (Ward *et al.*, 2017). Therefore, we speculated that these transitional microbiomes may be more vulnerable to invasion by rare taxa and less resistant to disturbance since community members may not be adapted to the rapidly changing environmental conditions (Gibbons *et al.*, 2016). To better understand this process, we examined two spring disturbances (5 and 8), which occurred in May (2012, 2013) and exhibited similar responsive taxa (Fig. 2). We postulate that an equivalent spring disturbance was not observed in 2011 either due to prior disruption of the microbial community due to the cold event in January 2011 (disturbance 1), interannual variability and/or missing observations in April 2011. In contrast to most of the disturbances where CRT constitute <0.5% of the community, here, CRT comprised 10%–30% of the community in both spring disturbances (Fig. S6). Many of these CRT were specific to these two events, including several OTUs with the best BLAST hit to the diatom *Leptocylindrus danicus*) and one OTU in the *Alteromonadaceae* family (Fig. S10).

In investigating the origin of this disturbance, we noted that many of the responsive taxa were photosynthetic, with an increase in the relative abundance of chloroplast sequences (diatoms) in both events and a decrease in the cyanobacterium *Synechococcus* in disturbance 5 (Fig. 2 and Fig. S4L; Tables S3 and S4). While many aquatic environments exhibit spring phytoplankton blooms, these events did not correspond to an increase in chlorophyll *a* (Fig. S4H) suggesting turnover in phytoplankton composition rather than an overall increase in photosynthetic biomass. Many disturbance-responsive taxa showed significant GJAM associations with temperature, salinity and chlorophyll *a* (Tables S3 and S4), which were important environmental factors for many microbiome populations, even outside of disturbance (Ward *et al.*, 2017) and therefore did not point to a specific environmental trigger. These seasonal shifts in phytoplankton communities towards larger eukaryotic taxa (Fig. S4K) likely release more organic carbon that favours the emergence of opportunistic copiotrophs, including an *Alteromonadaceae* phylotype (OTU181) that increased  $\sim 10^3$ -fold during disturbance 5 (Fig. S10) (Mühlenbruch *et al.*, 2018). Metagenomes further support this shift towards copiotrophy: in both events 5 and 8 four motility-related pathways increased (Table S4). As motility demarcates oligotrophic and copiotrophic strategies (Lauro *et al.*, 2009), increased motility-associated and also general secretion pathway genes likely reflect the increases in opportunistic copiotrophs. These consistent changes in both community composition and functional capacities during the spring microbiome disturbances suggest that rapid, seasonally associated turnover in phytoplankton composition alters resource availability, leading to favourable conditions for the transient invasion by CRT and/or fast-growing opportunistic copiotrophs. Despite their overall similarities in responsive taxa, disturbance 8 also included declines in a number of phage-related pathways, suggesting a role for density-dependent selection in rapid community changes (Fig. S9). These microbiome disturbances offer unique insights into the ecology of coastal ocean microbial communities, with large, repeating, but ephemeral microbiome alterations in the absence of detectable changes in key environmental variables.

Here we utilized a long-term coastal time series, the PICO, to identify potential disturbances in the context of annual patterns in microbiome composition and environmental variables. While these disturbances differed in origin (or remain unexplained), they frequently shared some characteristics, including decreases in SAR11. Yet this community turnover-based method surprisingly did not identify a category 1 hurricane as a disturbance, likely because the hurricane resulted (generally) in gradual shifts of existing taxa rather than measured increases in

rare or allochthonous organisms. These data suggest a critical need to understand the context and the role of microbiome stability (i.e. resistance) during changes in ecosystem parameters, and the potential consequence of increased invasibility of the system during periods of microbiome instability (Shade *et al.*, 2012; Gibbons *et al.*, 2016). As global change increases the number of environmental extremes (e.g. marine heatwaves, strong tropical cyclones, etc.), it is critical to understand how these (potential) disturbances impact microbiomes and their associated biogeochemical processes.

## Methods

### *Environmental data and microbial community analysis*

The microbial time series used in this study was previously described in Ward *et al.* (2017).

In brief, samples were collected at the PICO site (34.7181°N 76.6707°W) at the mouth of the Newport River Estuary weekly over a 3-year period from January 2011 to December 2013. Seawater was collected at 10:30 AM local time and processed within 1 h. Methods for determination of surface water temperature, pH, salinity, dissolved inorganic nutrient concentrations, chlorophyll *a* concentration, and bacterioplankton and phytoplankton abundances were described previously (Johnson *et al.*, 2013; Ward *et al.*, 2017). Nucleic acids were extracted from 0.22-micron Sterivex filters (Millipore), and libraries of prokaryotic and chloroplast 16S rRNA genes were generated as previously described (Ward *et al.*, 2017). Briefly, 16S rRNA V3–V4 libraries (Kozich *et al.*, 2013) were sequenced on the MiSeq (Illumina) with  $2 \times 250$  nt paired-end sequencing, and sequences were processed using USEARCH v7 (Edgar, 2010). Merged paired-end sequences were clustered into OTUs in which all assigned sequences are at least 97% similar using centroid-based clustering in UPARSE (Edgar, 2013) with a pairwise identity of 98.5% to the centroid. OTUs occurring less than five times in the entire dataset were removed, yielding a total of 10 357 OTUs. Libraries were rRNA copy number corrected using *rrnDB* (Stoddard *et al.*, 2014) and subsampled to 20 082 reads per library. The taxonomies of representative sequences were classified using the RDP naïve Bayesian classifier using the Greengenes version 13.5 database.

### *Community-level and population-level analyses*

To examine short-term variability in microbial community, we quantified differences in community composition over weekly intervals using Bray–Curtis after LOWESS smoothing with a span of 0.8; and the 10 largest weekly

changes in community composition were identified as disturbances. We identified CRT (Shade *et al.*, 2014) as having bimodality values (a measure of taxa that are predominantly rare with occasional periods of high abundance) greater than 0.90 and a relative abundance exceeding 0.25% at least once during the 3 years of the time series. After identification of disturbance events, relative abundances of each OTU were converted to absolute abundances using total bacterioplankton counts obtained using flow cytometry (i.e. relative abundance  $\times$  total prokaryotic cell counts). Although absolute abundances should not be interpreted as 'cell counts', they help to correct for the potential distortion in relative abundance due to changes in the abundance of other taxa, for example due to blooms. Top 20 contributors (OTUs with the largest contributions to community dissimilarity) with a minimum pre–post-disturbance average abundance of 0.05% in each disturbance event were identified as potentially disturbance-responsive taxa and plotted using 'heatmap.plus' in the R vegan package.

To employ the Beta Mean Nearest Taxon Distance ( $\beta$ MNTD) method of comparing stochastic and deterministic processes (Stegen *et al.*, 2012), we first calculated the niche value as the abundance-weighted mean of temperature for the 200 most abundant OTUs using the *dniche* function in R. A mantel correlogram using the pairwise matrix of OTU niche distances and phylogenetic distances (Tamura and Nei, 1993) with 999 permutations verified that closely related taxa have similar temperature niches. Calculated  $\beta$ MNTD values (a measure of abundance-weighted mean phylogenetic distance between taxa in communities) were compared to a mean null distribution of  $\beta$ MNTD values obtained by randomizing OTUs across the phylogeny 999 times. The number of standard deviations between the observed and null  $\beta$ MNTD yielded Beta Nearest Taxon Index ( $\beta$ NTI) values. Mean  $|\beta$ NTI|  $>2$  indicates communities with significantly higher ( $>2$ ; heterogeneous selection) or lower ( $<-2$ ; homogeneous selection) community turnover than expected under a null model.

#### Metagenome sequencing and analysis

Metagenomes were constructed from the same DNA extractions used for 16S rRNA gene libraries (Table S6), 10 ng of DNA was sheared to 300 bp using the Covaris LE220 and size was selected using SPRI beads (Beckman Coulter). The fragments were treated with end-repair, A-tailing, and ligation of Illumina compatible adapters using the KAPA-Illumina library creation kit followed by 5 cycles of PCR to enrich for the final library. These libraries were sequenced with  $2 \times 150$  nt reads on the Illumina HiSeq 2500 1T platform at either the Joint Genome Institute or Duke's Genome Sequencing and

Analysis Center. In analysing the resulting data, we first used Trimmomatic (Bolger *et al.*, 2014) to remove adapters, the first 10 bases, and low-quality regions in the first and last 20 bases. We used a sliding window of four with an average quality cutoff of 20 for the entire read and a minimum length cutoff of 50 base pairs. We retained an average sequencing depth of 17.8 Gbp with an average read length above 125 base pairs for all samples. We assembled the reads with MEGAHIT, which is a de Bruijn graph approach that is time and energy efficient, and produces high-quality metagenome assemblies (Li *et al.*, 2016). The assembly was performed using the default options and from the resulting contigs only those greater than 1000 base pairs in length were retained for further analysis. Many of the resulting assemblies had over 50% of the metagenomic reads represented (range 22%–64%).

We used MiGA (Rodríguez-R *et al.*, 2018) for downstream analysis following assembly, including gene prediction with Prodigal (Hyatt *et al.*, 2010). After gene prediction, we used BLAST to functionally annotate genes based on the UniProt database. We estimated the abundance of genes in each metagenome by read mapping using BLASTn (mapping the reads of each metagenome to the combined genes file and normalized for the number of reads in each metagenome). The abundance of each gene was estimated by the [BlastTab.seqdepth.pl](#) script of the [enve-omics](#) collection (Rodríguez-R and Konstantinidis, 2016), which normalizes the read counts by gene length and reports the X coverage of the gene. We used these normalized read counts to find the abundance in each SEED subsystem category per sample by combining the counts in each category. Metagenomic reads are deposited as NCBI bioproject PRJNA643505 and NCBI Projects 441405–441416 (Hunt, 2016) and 16S rRNA gene libraries are available as PRJNA309156.

#### GJAM analysis

Generalized joint attribute modelling (GJAM) was applied to model the 200 most abundant OTUs and environmental factors [temperature, tidal height (MLLW),  $\text{NH}_4$ , chlorophyll *a* and salinity] using the GJAM v. 2.3.2 package in R. Iteration was set at 20 000 and burning at 10 000. Results were visualized using the built-in function 'gjamPlot'.

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## Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

**Appendix S1.** Supporting Information.