Title: Rapid changes in coastal ocean microbiomes uncoupled with shifts in environmental variables

Running Title: Disturbance in the coastal ocean

Jessica L. Gronniger[1], Zhao Wang[1], Genevieve R. Brandt[2], Christopher S. Ward[1], Despina Tsementzi[2], Han Mu[1], Junyao Gu[1], Zackary I. Johnson[1, 3], Konstantinos T. Konstantinidis[2] Dana E. Hunt [1, 3*]

[1] Marine Laboratory, Duke University, Beaufort NC USA
[2] Georgia Tech Atlanta GA USA
[3] Biology and Civil & Environmental Engineering, Duke University, Durham NC USA

*To whom correspondence should be directed:

Dana Hunt
135 Duke Marine Lab Rd
Beaufort NC 28516
(252) 504-7542

Originality-Significance Statement

As the impacts of global change accelerate, ecological disturbances will increasingly impact ecosystems due to extremes in temperature, precipitation and storms, among others. However, research into the microbiome impacts of disturbance has been limited; in particular, few studies include long-term pre and post disturbance measurements to quantify the persistence of impacts on microbiome composition and function, relative to normal variation in the ecosystem. Here we show how environmental disturbances differ in their microbiome responses which can be linked to both disturbance type and prior ecological history. Thus, this paper contributes unique insights into disturbance in the coastal oceans and more broadly to the field of disturbance ecology.

Summary

Disturbances, here defined as events that directly alter the 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 three 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. Further, 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 impacts and persistence on microbiomes 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). Further, communities whose composition changes can be functional redundant, where an altered community composition retains the original functional capacity. In environmental systems, disturbances have been show 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 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 (1) the type, extent and duration of the disturbance (2) the ecological history of the impacted community and (3) 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, likely 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 practiced *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 a long-term observations. While this method is limited to the detection of disturbances that (1) alter microbial community composition, and (2) 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. Further, 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).

156

157 **Results and Discussion**

158

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

179 disturbances were not followed by a second event, suggesting that either disturbance effects
180 persisted for more than a single week or that the microbiome returned to the seasonal normal
181 gradually over time.
182
183 In order to better understand potential drivers of community changes, we examined the
184 relationship of disturbance events with environmental parameters. Overall, we found no clear
185 correlation between microbial community change (Bray-Curtis dissimilarity) and environmental
186 variables (e.g. temperature, pH, chlorophyll *a*, dissolved oxygen, salinity, nutrients; Table S1;
187 Figure S3A). However, we can speculate about the linkages between individual disturbances and
188 potential environmental drivers. For example, based on environmental and microbiome context,
189 we could re-categorized disturbance 1 as a resilience event where the microbial community
190 recovered from a cold period (water temperature <10 °C, lower than the average winter
191 temperature (range ~10-15 °C), for about a month prior to disturbance 1; Figure S4A). However,
192 most disturbances had no obvious distinguishing environmental characteristics, which could be
193 due to unmeasured environmental factors, stochastic processes or changes in biological
194 interactions (e.g. mortality). Other disturbances were enigmatic: although disturbance 2 occurred
195 three days prior to the landfall of a category 1 hurricane (Irene), we found no linkages between
196 this disturbance and the environmental impacts from the hurricane (e.g. no change in wind or
197 precipitation was evident at the study site). Further, while the hurricane altered a number of
198 environmental factors (Figures S3, S4), it did not induce a disturbance response based on our
199 microbiome turnover metric (Figure 1).
200

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 (Figure 2), we found that no OTU responded in 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 (Figure 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 (Figure S5), suggesting they may be sensitive biomarkers of environmental change (Yeo et al., 2013; Giovannoni, 2017). The greatest similarities among the disturbances were observed for spring events 5 & 8 (May 2012 and 2013) which (1) clustered based on responsive taxa (Figure 2), (2) occurred in the same season and (3) exhibited similar large community shifts on the NMDS (Figure 1B). Unlike most other disturbances, both spring events' microbiomes contain a large fraction of conditionally rare taxa (CRT; Figure 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 (Figure S6). CRT were also abundant prior to disturbance 1 (a presumed resilience event), when the system experienced colder than normal temperatures (<

10 ˚C; Figure 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) (Figure 1C). While metagenomes also clustered by season (Figure 1C), OTU-level community and metagenome changes did show a positive correlation (Figure 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 (Figure 1B & 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 are 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 (Figures S3, 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 storm water microbes, a first flush of nutrients, etc. (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_x observed over the full three years of the dataset (NO_x: 1.53 µM; Figure S4F) and a substantial increase in NH_4, $SiOH_4$, and chlorophyll *a* relative to pre-hurricane conditions (reported as pre-hurricane -> post-hurricane values; NH_4: 319.94 -> 1507.72 µM; $SiOH_4$: 4.36 -> 25.27 µM, chlorophyll *a*: 5.81 -> 10.85 µg/L; Figure 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 (~7 units over five weeks; Figure S4C) and elevated levels of nutrients, including $SiOH_4$, NO_2 and PO_4 (Figure S4E-G). The continued high levels (and sometimes delayed peaks) in these environmental variables points 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 (Figures 3, 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 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-change 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 in observed for other disturbances (Figure 2, 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 (Figure S7; Table S2), which could represent taxa which were either washed in by floodwaters/ sediment resuspension, 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 four weeks after the hurricane, accounting for ~10% (OTU 8) and 6% (OTU 10129) of the libraries, compared to with maximum relative abundances of ~3% in 2012 and 2013 (Figure 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 modeling (GJAM) in conjunction with select environmental variables (temperature, NH_4, chlorophyll *a,* salinity and Mean Lower Low Water, a metric of tidal height) (Clark et al., 2017). The two diatoms (OTUs 8 & 10129) both had significant positive associations with NH_4 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 (Figure 3), suggesting that phage-mediated community selection could have altered the community before the hurricane. In contrast, the post-hurricane

316 sample exhibited increases in two dimethylsulfoniopropionate (DMSP)-mineralization genes,
317 suggesting the diatom bloom has increased degradation potential for this algal osmolite (Figure
318 3). In future work, we may consider alternate metrics for disturbance (rather than week-over-
319 week changes) as the hurricane did not result in an immediate or dramatic change in the
320 microbiome, but rather a sustained shift in specific populations. However, we note that this event
321 did not impact water temperature, which is predicted to be the major environmental driver at this
322 site, and occurred in late summer when the microbiome is relatively stable and potentially more
323 resistant to disturbance (Ward et al., 2017). Thus, when taxa are relatively well acclimated and
324 presumably adapted to environmental conditions, even relatively large and sustained changes in
325 environmental parameters primarily generated blooms in abundant organisms that were poised to
326 make use of these resources.
327
328 **Spring disturbance events**
329
330 In contrast with relatively stable summer conditions, PICO community turnover is highest during
331 the spring and fall (Ward et al., 2017). Therefore, we speculated that these transitional
332 microbiomes may be more vulnerable to invasion by rare taxa and less resistant to disturbance
333 since community members may not be adapted to the rapidly-changing environmental conditions
334 (Gibbons et al., 2016). To better understand this process, we examined two spring disturbances
335 (5 & 8), which occurred in May (2012, 2013) and exhibited similar responsive taxa (Figure 2).
336 We postulate that an equivalent spring disturbance was not observed in 2011 either due to prior
337 disruption of the microbial community due to the cold event in January 2011 (disturbance 1),
338 interannual variability and/or missing observations in April 2011. In contrast to most of the

disturbances where conditionally rare taxa (CRT) constitute <0.5% of the community, here, CRT comprised 10-30% of the community in both spring disturbances (Figure 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 (Figure 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 (Figures 2, S4L; Tables S3, S4). While many aquatic environments exhibit spring phytoplankton blooms, these events did not correspond to an increase in chlorophyll *a* (Figure 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 & 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 (Figure S4K) likely release organic carbon that favors the emergence of opportunistic copiotrophs, including an *Alteromonadaceae* phylotype (OTU181) that increased ~10^3 fold during disturbance 5 (Figure S10) (Mühlenbruch et al., 2018). We examined whether metagenomes support this shift towards copiotrophy and found that 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 reflects 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 favorable conditions for the transient invasion by conditionally rare taxa 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 (Figure 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 Piver's Island Coastal Observatory, 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. This data suggests 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 numbers of environmental extremes (e.g. marine heat waves, 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 Piver's Island Coastal Observatory (PICO) site (34.7181°N 76.6707°W) at the mouth of the Newport River Estuary weekly over a three-year period from January 2011 to December 2013. Seawater was collected at 10:30 AM local time and processed within one hour. 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 2x 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% similarity 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 conditionally rare taxa (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 x 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 highest 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 (βMNTD) 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 βMNTD values (a measure of abundance-weighted mean phylogenetic distance between closely related taxa between communities) were compared to a mean null distribution of βMNTD values obtained by randomizing OTUs across the phylogeny 999 times. The number of standard deviations between the observed and null βMNTD yielded Beta Nearest Taxon Index (βNTI) values. Mean |βNTI| > 2 indicate 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 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 2x150 nt reads on the Illumina HiSeq 2500 1T platform at either the Joint Genome Institute or Duke's Genome Sequencing and Analysis Center. In analyzing 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 4 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 17.8Gbp with an average read length above 125 base pairs for all samples. We assembled the reads with MEGAHIT, which is a de Brujin 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 to 64%).

We used MiGA (Rodriguez-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 (Rodriguez-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), 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'.

Acknowledgements: We acknowledge the contribution of the entire PICO sampling team to field work and sample processing. We specifically acknowledge funding from the National Science Foundation: to DEH and ZIJ (OCE 1416665), to DEH (ICER 2033934) and to KTK (OCE 1416673). The work conducted by the U.S. Department of Energy Joint Genome Institute, a DOE Office of Science User Facility, is supported by the Office of Science of the U.S. Department of Energy under Contract No. DE-AC02-05CH11231.

Figures

Figure 1. Microbiome disturbance identification ordination plots (A) Community dissimilarity over three years (Jan 2011 – Dec 2013) of weekly samples from the PICO (Piver's Island Coastal Observatory) time series. Bray-Curtis dissimilarity (black line) was calculated over one-week intervals, 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 ten 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 Piver's Island Coastal Observatory over three years (2011-2013). Disturbance events are labeled 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).

Figure 2. Heatmap showing OTU abundance changes pre- and post- disturbances. Plotted is the log2 of (post-disturbance absolute abundance+1)/(pre-disturbance absolute disturbance+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 (colored 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 labeled and *Thermus aquaticus* strain YF-1 as the outgroup. Conditionally rare taxa (CRT) are identified with black on the right.

Figure 3. Heatmap of metagenome changes Log_2-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 |Log2FC| 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.

Works Cited

Allison, S.D., and Martiny, J.B.H. (2008) Resistance, resilience, and redundancy in microbial communities. *Proceedings of the National Academy of Sciences* **105**: 11512-11519.

Amaral-Zettler, L.A., Rocca, J.D., Lamontagne, M.G., Dennett, M.R., and Gast, R.J. (2008) Changes in microbial community structure in the wake of Hurricanes Katrina and Rita. *Environmental science & technology* **42**: 9072-9078.

Ares, Á., Brisbin, M.M., Sato, K.N., Martín, J.P., Iinuma, Y., and Mitarai, S. (2020) Extreme storms cause rapid but short-lived shifts in nearshore subtropical bacterial communities. *Environmental Microbiology* **22**: 4571-4588.

Asmala, E., Osburn, C.L., Paerl, R.W., and Paerl, H.W. (2021) Elevated organic carbon pulses persist in estuarine environment after major storm events. *Limnology and oceanography letters* **6**: 43-50.

Atlas, R.M., Horowitz, A., Krichevsky, M., and Bej, A.K. (1991) Response of microbial populations to environmental disturbance. *Microbial Ecology* **22**: 249-256.

Balmonte, J.P., Arnosti, C., Underwood, S., McKee, B.A., and Teske, A. (2016) Riverine bacterial communities reveal environmental disturbance signatures within the Betaproteobacteria and Verrucomicrobia. *Frontiers in Microbiology* **7**: 1441.

Bolger, A.M., Lohse, M., and Usadel, B. (2014) Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics*: btu170.

Campbell, B.J., Yu, L., Heidelberg, J.F., and Kirchman, D.L. (2011) Activity of abundant and rare bacteria in a coastal ocean. *Proceedings of the National Academy of Sciences* **108**: 12776-12781

Caporaso, J.G., Paszkiewicz, K., Field, D., Knight, R., and Gilbert, J.A. (2011) The Western English Channel contains a persistent microbial seed bank. *The ISME Journal* **6**: 1089-1093.

Clark, J.S., Nemergut, D., Seyednasrollah, B., Turner, P.J., and Zhang, S. (2017) Generalized joint attribute modeling for biodiversity analysis: median-zero, multivariate, multifarious data. *Ecological Monographs* **87**: 34-56.

Coles, V., Stukel, M., Brooks, M., Burd, A., Crump, B., Moran, M. et al. (2017) Ocean biogeochemistry modeled with emergent trait-based genomics. *Science* **358**: 1149-1154.

Edgar, R.C. (2010) Search and clustering orders of magnitude faster than BLAST. *Bioinformatics* **26**: 2460-2461.

Edgar, R.C. (2013) UPARSE: highly accurate OTU sequences from microbial amplicon reads. *Nature Methods* **10**: 996-998.

Ferrenberg, S., O'Neill, S.P., Knelman, J.E., Todd, B., Duggan, S., Bradley, D. et al. (2013) Changes in assembly processes in soil bacterial communities following a wildfire disturbance. *The ISME Journal* **7**: 1102-1111.

Gibbons, S.M., Scholz, M., Hutchison, A.L., Dinner, A.R., Gilbert, J.A., and Coleman, M.L. (2016) Disturbance regimes predictably alter diversity in an ecologically complex bacterial system. *mBio* **7**: e01372-01316.

Giovannoni, S.J. (2017) SAR11 Bacteria: The Most Abundant Plankton in the Oceans. *Annual Review of Marine Science* **9**: 231-255.

Huang, K., Feng, Q., Zhang, Y., Ou, L., Cen, J., Lu, S., and Qi, Y. (2020) Comparative uptake and assimilation of nitrate, ammonium, and urea by dinoflagellate *Karenia mikimotoi* and diatom *Skeletonema costatum* sl in the coastal waters of the East China Sea. *Marine Pollution Bulletin* **155**: 111200.

Hunt, D.E. (2016). Seasonal and disturbance-related alterations in the biogeochemical cycling of estuarine carbon

Hyatt, D., Chen, G.-L., LoCascio, P.F., Land, M.L., Larimer, F.W., and Hauser, L.J. (2010) Prodigal: prokaryotic gene recognition and translation initiation site identification. *BMC Bioinformatics* **11**: 119.

Johnson, Z.I., Wheeler, B.J., Blinebry, S.K., Carlson, C.M., Ward, C.S., and Hunt, D.E. (2013) Dramatic variability of the carbonate system at a temperate coastal ocean site (Beaufort, North Carolina, USA) Is regulated by physical and biogeochemical processes on multiple timescales. *PLoS ONE* **8**: e85117.

Jones, S.E., and Lennon, J.T. (2010) Dormancy contributes to the maintenance of microbial diversity. *Proceedings of the National Academy of Sciences* **107**: 5881-5886.

Jones, S.E., Chiu, C.-Y., Kratz, T.K., Wu, J.-T., Shade, A., and McMahon, K.D. (2008) Typhoons initiate predictable change in aquatic bacterial communities. *Limnology and Oceanography* **53**: 1319-1326.

Kozich, J.J., Westcott, S.L., Baxter, N.T., Highlander, S.K., and Schloss, P.D. (2013) Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the MiSeq Illumina sequencing platform. *Applied and Environmental Microbiology* **79**: 5112-5120.

Lauro, F.M., McDougald, D., Thomas, T., Williams, T.J., Egan, S., Rice, S. et al. (2009) The genomic basis of trophic strategy in marine bacteria. *Proceedings of the National Academy of Sciences of the United States of America* **106**: 15527-15533.

Li, D., Luo, R., Liu, C.-M., Leung, C.-M., Ting, H.-F., Sadakane, K. et al. (2016) MEGAHIT v1. 0: a fast and scalable metagenome assembler driven by advanced methodologies and community practices. *Methods* **102**: 3-11.

Mühlenbruch, M., Grossart, H.P., Eigemann, F., and Voss, M. (2018) Mini-review: Phytoplankton-derived polysaccharides in the marine environment and their interactions with heterotrophic bacteria. *Environmental Microbiology* **20**: 2671-2685.

Peierls, B.L., Christian, R.R., and Paerl, H.W. (2003) Water quality and phytoplankton as indicators of hurricane impacts on a large estuarine ecosystem. *Estuaries* **26**: 1329-1343.

Polz, M.F., Hunt, D.E., Preheim, S.P., and Weinreich, D.M. (2006) Patterns and mechanisms of genetic and phenotypic differentiation in marine microbes. *Philosophical Transactions of the Royal Society B-Biological Sciences* **361**: 2009-2021.

Renes, S.E., Sjöstedt, J., Fetzer, I., and Langenheder, S. (2020) Disturbance history can increase functional stability in the face of both repeated disturbances of the same type and novel disturbances. *Scientific reports* **10**: 1-13.

Rii, Y.M., Peoples, L.M., Karl, D.M., and Church, M.J. (2022) Seasonality and episodic variation in picoeukaryote diversity and structure reveal community resilience to disturbances in the North Pacific Subtropical Gyre. *Limnology and Oceanography* **67**: S331–S351.

Rodriguez-R, L.M., and Konstantinidis, K.T. (2016) The enveomics collection: a toolbox for specialized analyses of microbial genomes and metagenomes. In: PeerJ Preprints.

Rodriguez-R, L.M., Gunturu, S., Harvey, W.T., Rosselló-Mora, R., Tiedje, J.M., Cole, J.R., and Konstantinidis, K.T. (2018) The Microbial Genomes Atlas (MiGA) webserver: taxonomic and gene diversity analysis of Archaea and Bacteria at the whole genome level. *Nucleic Acids Research* **46**: W282-W288.

Shade, A., Read, J.S., Welkie, D.G., Kratz, T.K., Wu, C.H., and McMahon, K.D. (2011) Resistance, resilience and recovery: aquatic bacterial dynamics after water column disturbance. *Environmental Microbiology* **13**: 2752-2767.

Shade, A., Jones, S.E., Caporaso, J.G., Handelsman, J., Knight, R., Fierer, N., and Gilbert, J.A. (2014) Conditionally Rare Taxa Disproportionately Contribute to Temporal Changes in Microbial Diversity. *mBio* **5**.

Shade, A., Peter, H., Allison, S.D., Baho, D.L., Berga, M., Bürgmann, H. et al. (2012) Fundamentals of microbial community resistance and resilience. *Frontiers in Microbiology* **3**: 417.

Shen, D., Langenheder, S., and Jürgens, K. (2018) Dispersal modifies the diversity and composition of active bacterial communities in response to a salinity disturbance. *Frontiers in microbiology* **9**: 2188.

Sjöstedt, J., Langenheder, S., Kritzberg, E., Karlsson, C.M., and Lindström, E.S. (2018) Repeated disturbances affect functional but not compositional resistance and resilience in an aquatic bacterioplankton community. *Environmental microbiology reports* **10**: 493-500.

Stegen, J.C., Bottos, E.M., and Jansson, J.K. (2018) A unified conceptual framework for prediction and control of microbiomes. *Current Opinion in Microbiology* **44**: 20-27.

Stegen, J.C., Lin, X., Konopka, A.E., and Fredrickson, J.K. (2012) Stochastic and deterministic assembly processes in subsurface microbial communities. *The ISME Journal* **6**: 1653-1664.

Steichen, J.L., Labonté, J.M., Windham, R., Hala, D., Kaiser, K., Setta, S. et al. (2020) Microbial, physical, and chemical changes in Galveston Bay following an extreme flooding event, Hurricane Harvey. *Frontiers in Marine Science* **7**: 186.

Stoddard, S.F., Smith, B.J., Hein, R., Roller, B.R., and Schmidt, T.M. (2014) rrnDB: improved tools for interpreting rRNA gene abundance in bacteria and archaea and a new foundation for future development. *Nucleic Acids Research*: gku1201.

Tamura, K., and Nei, M. (1993) Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular biology and evolution* **10**: 512-526.

Wang, Z., Tsementzi, D., Williams, T., Juarez, D., Garcia, N., Johnson, Z. et al. (2021) Environmental stability impacts the differential sensitivity of marine microbiomes to increases in temperature and acidity. *ISME J* **15**: 19-28.

Ward, C.S., Yung, C.-M., Davis, K.M., Blinebry, S.K., Williams, T.C., Johnson, Z.I., and Hunt, D.E. (2017) Annual community patterns are driven by seasonal switching between closely related marine bacteria. *The ISME Journal* **11**: 1412-1422.

Westergaard, K., Müller, A., Christensen, S., Bloem, J., and Sørensen, S. (2001) Effects of tylosin as a disturbance on the soil microbial community. *Soil Biology and Biochemistry* **33**: 2061-2071.

Wetz, M.S., and Paerl, H.W. (2008) Estuarine phytoplankton responses to hurricanes and tropical storms with different characteristics (trajectory, rainfall, winds). *Estuaries and Coasts* **31**: 419-429.

Yan, G., Labonté, J.M., Quigg, A., and Kaiser, K. (2020) Hurricanes accelerate dissolved organic carbon cycling in coastal ecosystems. *Frontiers in Marine Science* **7**: 248.

Yeo, S.K., Huggett, M.J., Eiler, A., and Rappé, M.S. (2013) Coastal bacterioplankton community dynamics in response to a natural disturbance. *PLOS ONE* **e56207**.

706 Yung, C.-M., Ward, C.S., Davis, K.M., Johnson, Z.I., and Hunt, D.E. (2016) Insensitivity of
707 diverse and temporally variable particle-associated microbial communities to bulk seawater
708 environmental parameters. *Applied and Environmental Microbiology* **82**: 3431-3437.
709
710 Yung, C.-M., Vereen, M.K., Herbert, A., Davis, K.M., Yang, J., Kantorowska, A. et al. (2015)
711 Thermally adaptive tradeoffs in closely-related marine bacterial strains. *Environmental*
712 *Microbiology* **17**: 2421–2429.
713
714 Zhou, J., Deng, Y., Zhang, P., Xue, K., Liang, Y., Van Nostrand, J.D. et al. (2014) Stochasticity,
715 succession, and environmental perturbations in a fluidic ecosystem. *Proceedings of the National*
716 *Academy of Sciences* **111**: E836-E845.
717

718

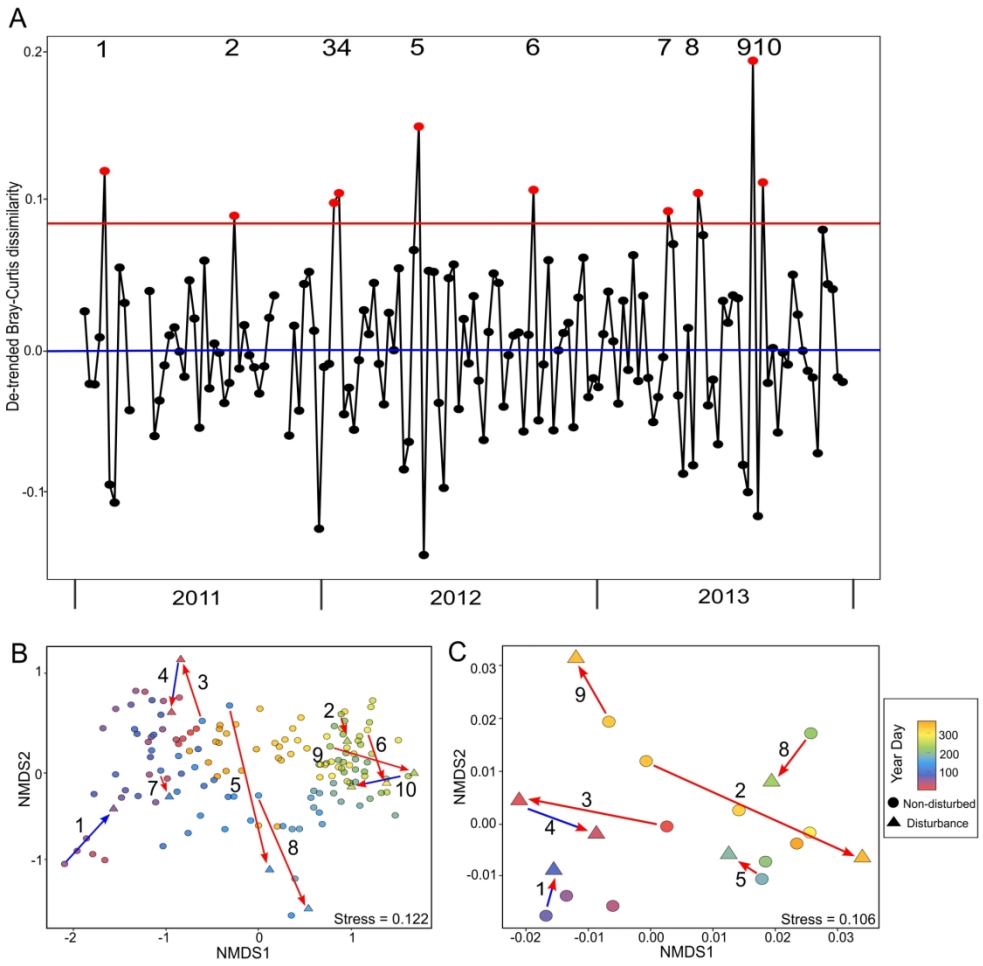

Figure 1. Microbiome disturbance identification ordination plots (A) Community dissimilarity over three years (Jan 2011 – Dec 2013) of weekly samples from the PICO (Piver's Island Coastal Observatory) time series. Bray-Curtis dissimilarity (black line) was calculated over one-week intervals, excluding 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 ten 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 Piver's Island Coastal Observatory over three years (2011-2013). Disturbance events are labeled 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).

180x201mm (300 x 300 DPI)

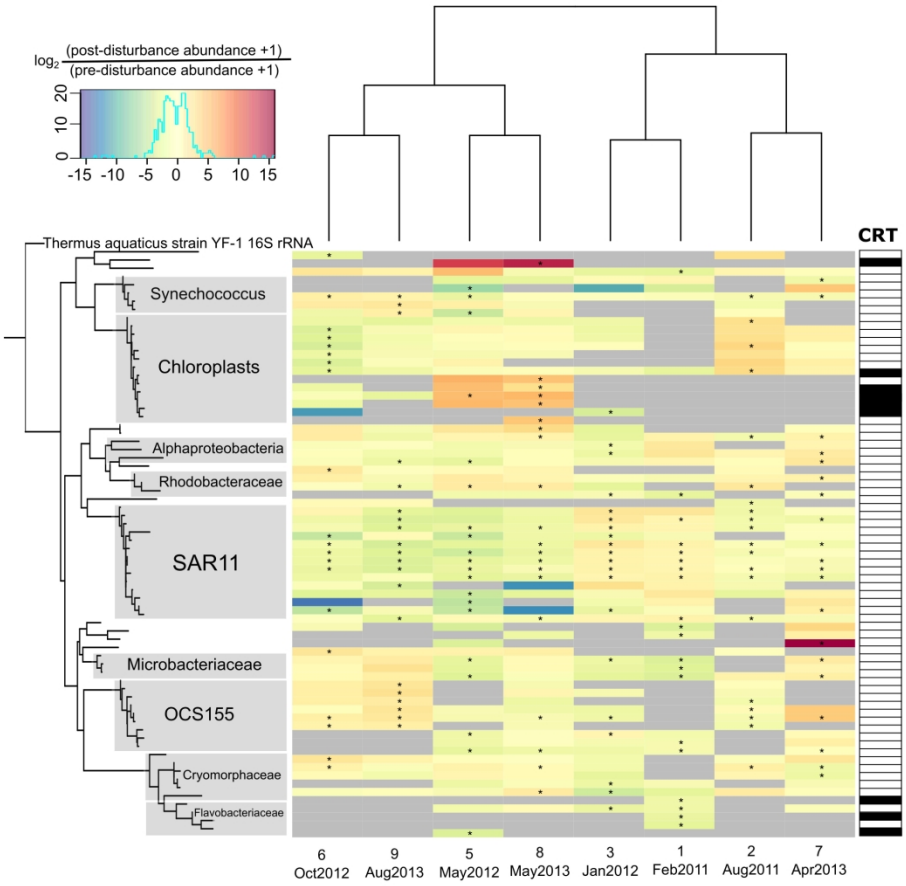

Figure 2. Heatmap showing OTU abundance changes pre- and post- disturbances. Plotted is the log2 of (post-disturbance absolute abundance+1)/(pre-disturbance absolute disturbance+1) of the 20 taxa contributing the most to the community change in each disturbance event, excluding presumed resilience events (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 (colored 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 labeled and Methanosarcina strain MSH10X4 as the outgroup. Conditionally rare taxa (CRTs) are identified in black on the right.

174x230mm (600 x 600 DPI)

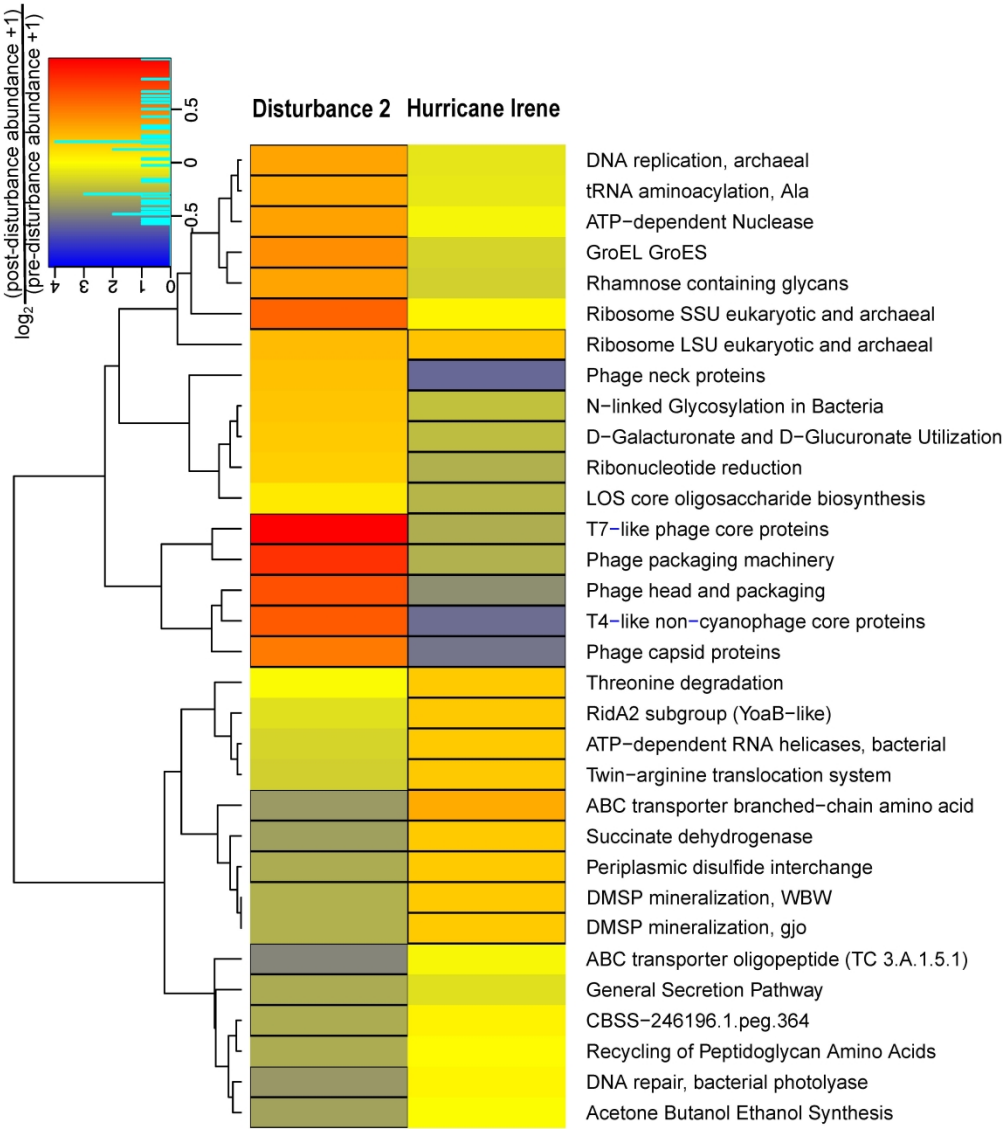

Figure 3. Heatmap of metagenome changes Log2-fold-change between pre- and post-disturbance 2 and 3 days pre-Irene (disturbance 2) and 9 days post-Irene. Includes 20 SEED categories with the highest |Log2FC| for each pair, indicated by black outlines. Only SEED categories with a minimum average relative abundance across the three samples of >0.05% are included.

179x201mm (600 x 600 DPI)