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ARTICLE

Environmental stability impacts the differential sensitivity of marine microbiomes to increases in temperature and acidity

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Abstract

Ambient conditions shape microbiome responses to both short- and long-duration environment changes through processes including physiological acclimation, compositional shifts, and evolution. Thus, we predict that microbial communities inhabiting locations with larger diel, episodic, and annual variability in temperature and pH should be less sensitive to shifts in these climate-change factors. To test this hypothesis, we compared responses of surface ocean microbes from more variable (nearshore) and more constant (offshore) sites to short-term factorial warming (+3 °C) and/or acidification (pH −0.3). In all cases, warming alone significantly altered microbial community composition, while acidification had a minor influence. Compared with nearshore microbes, warmed offshore microbiomes exhibited larger changes in community composition, phylotype abundances, respiration rates, and metatranscriptomes, suggesting increased sensitivity of microbes from the less-variable environment. Moreover, while warming increased respiration rates, offshore metatranscriptomes yielded evidence of thermal stress responses in protein synthesis, heat shock proteins, and regulation. Future oceans with warmer waters may enhance overall metabolic and biogeochemical rates, but they will host altered microbial communities, especially in relatively thermally stable regions of the oceans.

Introduction

Global change is predicted to alter both the mean and the variance of environmental factors in the oceans, with near-term global pH decreases and temperature increases in ocean surface waters. As microbes play a crucial role in marine biogeochemical cycles, climate models should incorporate alterations in microbial community composition and biogeochemical functions [1]. On short time scales, microbial community responses to shifts in environmental factors include a change in composition (sensitivity), no change in composition (resistance), or a change in composition with unaltered function (functional redundancy) [2, 3]. This functional redundancy can arise through individual taxa with broad physiological breadth or coexistence of taxa with distinct physiological properties [4, 5]. As both the mean and variability of the environment shape community responses to change, a number of recent studies in soils have emphasized the importance of environmental context (i.e., historical contingencies) when examining microbial community responses to disturbances or environmental shifts [6]. Considering past exposures is likely also important, although as yet poorly explored, in marine microbes with global change [7].

Both the degree of environmental heterogeneity and current conditions relative to an organism's physiological constraints affect a community's potential to cope with warming and acidification. In ectotherms, growth rates generally increase gradually with temperature until an organism's thermal optimum is reached, followed by a dramatic decline with additional warming [4, 8]. Although tropical phytoplankton generally live near their thermal optima, microbial growth in temperate regions can be temperature limited [9]; thus, for individual taxa warming

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potentially inhibits, does not affect or increases the growth rate. Similarly, increasing pCO_2 may have negative, neutral, or positive impacts on the growth of marine microbes [10, 11]. Moreover, as microbes live in communities, changes in the abundance or physiology of one organism can influence other taxa, propagating the impacts of ecosystem changes through the community [12, 13]. Previous manipulations of temperature found significant microbial community composition and transcriptome changes with warming [14, 15], which are consistent with field observations [16–18]. Yet, the impact of acidification on microbial communities is unresolved and is likely difficult to detect [19]: some studies report no or minimal observed compositional changes [20–22], while others reported significant changes in microbial community composition [23, 24], and/or altered (meta)transcriptomes [13, 25, 26]. Moreover, apparent impacts of acidification on heterotrophic bacterial populations may be mediated by interactions with phytoplankton [26, 27], especially during induced phytoplankton blooms, making it difficult to determine the direct impacts of ocean acidification on microbial community composition, activity, and biogeochemical potential. Differences between experiments could also relate to prior and current environmental conditions; these historical contingencies likely impact both which microbes are present and how well acclimated they are to warming and acidification.

To address microbial community responses to major global change parameters, we manipulated microbial exposure to warming and acidification at a well-studied nearshore, mesotrophic site [Piver's Island Coastal Observatory (PICO)] and an oligotrophic site on the continental shelf break, 87 km offshore (adjacent to the Sargasso Sea). These two sites exhibit differences in pH and temperature mean and variability: the nearshore PICO site exhibits annual cycles of ~0.3 pH units and >20 °C (range: 6–31 °C) [28], whereas the less-variable continental shelf break (offshore) site experiences smaller fluctuations in pH and temperature and overall warmer conditions (~0.05 pH units and 7 °C, range: 23–30 °C) [29]. While the two sites host distinct microbial communities, they share some 16S rRNA gene phylotypes [29], allowing for direct comparisons of a population's response in different environmental backgrounds. As the nearshore site experiences greater daily and annual fluctuations in pH and temperature due to both biological and physical processes [28–30], we asked whether nearshore microbial communities are more resistant to warming and acidification versus their offshore counterparts. Here, microbial responses were assayed at the levels of community composition, individual taxa, and community function (biogeochemical rates and metatranscriptomes). To account for seasonal differences in both microbial populations and ambient conditions, we repeated experiments in both summer and winter. We hypothesized that offshores microbes, with bacterioplankton that have streamlined genomes and inhabit more stable environments [31], would be more sensitive to small changes in environmental parameters compared to their nearshore counterparts. Thus, we investigated short-term responses of extant microbial populations to future-climate conditions; the 5 day time period reduces the impact of evolutionary responses and nutrient limitation, both of which could complicate interpretation.

Materials and methods

Experimental manipulations

Experimental manipulations examined the impacts of warming and acidification independently as well as both warming and acidification, concurrently, on microbial communities. Here, the subscript A (e.g., $_A$) indicates ambient seawater conditions at the time of sampling. The experimental conditions included control (pH_A, T_A), acidified ($pH_A-0.3$, T_A), warmed (pH_A, $T_A + 3$ °C) and acidified and warmed ($pH_A-0.3$, $T_A + 3$ °C) to mimic the ~100 year predicted changes in surface ocean conditions [32]. Each treatment consists of two 20 L acid-washed, polycarbonate carboys. Carboys were incubated in environmental chambers set at the ambient seawater temperature and were constantly purged with air filtered through a charcoal filter at ~10 L min^{-1} and stirred at ~200 rpm. The light/dark cycle was set at 12:12 h at a light level of ~100 µE m^{-2} s^{-1}.

Carboys were filled at either the coastal PICO site (34.7181°N 76.6707°W) at the mouth of the Newport River estuary [16, 28] or 87.45 km offshore (34.0349°N, 76.1975°W) at the continental shelf break. Water from 1 m below the surface was pumped into carboys and prescreened with a 200 µm nylon mesh to avoid the presence of large zooplankton. Samples for in situ environmental variables [pH, temperature, chlorophyll *a*, dissolved inorganic carbon (DIC) etc.] were concurrently collected using a 5 L Niskin bottle as described previously [28]. Upon returning to the lab, carboys were purged overnight with ambient air to determine the pH at equilibrium with the atmosphere. The following day at ~10:30 a.m. local time, carboys were sampled (T_0) and pH and temperature manipulations were initiated. Carboys were acidified by bubbling with 5% CO_2 controlled by a Qubit pH/CO_2 controller system to decrease pH by 0.3 and in warmed samples temperature was increased by 3 °C using aquarium heaters (ViaAqua 50 W). Carboys were incubated for 5 more days under climate-change conditions and sampled daily for pH, DIC, flow cytometry (picophytoplankton and total prokaryotes), chlorophyll *a*, and primary production ($^{14}CO_2$ incorporation). In addition, O_2 respiration was measured and nucleic

acids were collected for in situ samples as well as after 3 and 5 days of manipulation.

Sample collection

Carboys were sampled every day at 10:30 a.m. local. All measurements were replicate samples from replicate carboys. Spectrophotometric pH samples were measured with m-cresol purple on a UV-Vis-NIR spectrophotometer (Cary 4000, Varian Inc.) [33]. Temperature was logged every 5 min by automatic temperature loggers (HOBO pro v2 Onset). Chlorophyll *a* pigment samples were extracted in 100% methanol and measured fluorometrically using a calibrated Turner 10-AU fluorometer. DIC was measured in triplicate on mercuric chloride poisoned samples by acidification and subsequent quantification of released CO_2 using a CO_2 detector (Li-Cor 7000). Primary production rates were calculated by incubating samples for 2 h under ambient conditions and measuring $H^{14}CO_3$ uptake with a scintillation counter (Beckman Coulter LS6500) [34]. O_2 respiration was measured using the Winkler method [35]. Phytoplankton were enumerated using a BD FACSCalibur Flow Cytometer and populations were characterized as previously described [36]. Bacterioplankton were quantified by staining the samples with SYBR Green-I as previously described [37]. Nucleic acid samples were collected by filtering 1–4 L of water through 0.22 µm Sterivex filters, which were snap frozen in liquid nitrogen, and stored at −80 °C until extraction.

Genomic DNA extraction and library preparation

Genomic DNA for 16S rRNA gene libraries was extracted from a quarter of each filter using the Gentra Puregene Yeast/Bacteria kit (QIAGEN) supplemented with bead beating (60 s). Genomic DNA was subsequently cleaned using the Zymo OneStep PCR inhibitor removal kit and quantified using a Nanodrop ND-100. 16S rRNA gene primers targeting the V4–V5 region 515F-Y (5′-GTGYCA GCMGCCGCGGTAA) and 926R (5′-CCGYCAATTYM TTTRAGTTT) with added barcodes and adapters were used to construct 16S rRNA gene libraries [38]. 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 Taq DNA polymerase (Lucigen). The thermal cycling conditions were 2 min at 95 °C for 1 cycle, followed by 25 cycles of 1 min of 95 °C, 1 min of 50 °C, 30 s of 72 °C, a final extension of 72 °C for 10 min. The 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 assigned to each sample using CASAVA (Illumina) and MacQIIME v1.9.1, sequences were then cleaned and clustered using USEARCH v.9.2 [39]. Low-quality sequence ends were trimmed at the Phred quality score (Q) of 30 using a 10 bp running window. Paired-end reads were merged if reads had greater than 10 bp overlap and no mismatches. Sequences with expected errors > 1 and/or a length < 400 bp were removed. Potential chimeras were filtered with uchime2 in USEARCH v.9.2. Sequences were then aligned using PyNast in MacQIIME, against the template "core_set_aligned.fasta.imputed" from the Greengene server. Shannon entropy analysis and oligotyping (v 2.1) was then performed using the 215 most variable base positions to resolve amplicon sequence variant (ASV) [40]. To reduce sequencing-error associated noise, only ASVs with a unique sequence with a minimum abundance of 20 were retained for analysis. 9205 ASVs were analyzed, which represent 3,389,636 sequences, or 78.4% of reads analyzed with a purity score of 0.91. The taxonomies of representative ASVs were classified using MacQIIME v1.9.1 using RDP classifier. Mitochondrial sequences were removed and the libraries were then subsampled to 22,554 reads per library. SSU rRNA library and metatranscriptome sequences are deposited as part of Bioproject PRJNA521532.

Microbial community analysis

Shannon's diversity indexes were analyzed using the vegan 2.5-6 package in R [41]. Bray–Curtis dissimilarities calculated for the bacterial communities were visualized using nonmetric multidimensional scaling ordination. For statistical analysis, beta-diversity distances (Bray–Curtis) were analyzed by permutational multivariate ANOVA (PERMANOVA) using the adonis function (two-factor design: warmed and acidified) on days 3 and 5, separately. Similarly, in order to evaluate the effect of the historical contingencies (station and season) on the effect of warming, we extracted the Bray–Curtis dissimilarity index between the warmed and the T_A samples in each experiment, and performed a single PERMANOVA using station, season, and their interaction as factors. Equivalent tests for acidification or acidification and warming together were not performed because pH manipulations did not affect the community composition in any experiment.

Absolute abundance was calculated by total prokaryotic cell counts × relative abundance of 16S rRNA gene ASVs. Absolute abundances for ASVs, with an added pseudo count of 1 to avoid excessive zeros inflating the model, were used to identify taxa with statistically significant effects of acidification, warming, or interaction terms using

DESeq2 using a multifactor design. We also applied a consistent threshold of relative abundance >0.05% in each experiment while analyzing and comparing responses of each ASV across experiments, to minimize bias induced by stochasticity. Significant differences were identified when $p < 0.05$ (adjusted for multiple hypothesis testing using the Benjamini–Hochberg method).

Metatranscriptome analysis

Samples were collected on 0.22-µm Sterivex filters (Millipore) using 3 µm in-line polycarbonate prefilters in less than 6 min in a final volume of ~1 L for the nearshore and ~3 L for the offshore samples. Total RNA was extracted using an organic extraction method as described previously [42]. Filters were incubated for 30 min at 37 °C with lysis buffer (50 mM Tris-HCl, 40 mM EDTA, 0.75 M sucrose) and subsequently in a second 2-h incubation at 55 °C after the addition of 1% SDS and 10 mg/mL proteinase K. The lysates were processed twice in organic extraction with acid phenol and chloroform and RNA was isolated using filter columns from the mirVANA RNA isolation kit (Ambion) following the kit's instruction manual. Total RNA samples were processed with the ScriptSeq RNA library Preparation Kit (Illumina) following the manufacturer's recommendations and omitting the rRNA subtraction step. Quality and quantity of nucleic acids during the cDNA preparation protocols were monitored using the Agilent RNA 6000 Pico kit (Agilent), and Qubit RNA assay kit (Invitrogen). The resulting cDNA libraries were sequenced (150-bp paired-end reads) using an Illumina MiSeq sequencer.

Metatranscriptomic sequences were trimmed using SolexaQA [43] with a PHRED score cutoff of 20 and a minimum fragment length of 50 bp. Bacterial and eukaryotic rRNA reads (16S, 18S, 23S, 28S) were identified using the Mytaxa2 [44] and 5 and 5.8S rRNA sequences were identified with SortMeRNA v2.0 [45] using the respective Rfam databases [46]. After rRNA removal, this yielded 6.6–52 million reads per sample. Metatranscriptome 16S rRNA reads (rRNA) and 16S rRNA gene libraries (rDNA) were both used in closed reference OTU picking based on the Greengenes database 13.5 in MacQIIME v1.9.1. DESeq2 was used to calculate $\log_2$ fold changes based on RNA and DNA OTU tables and to identify statistically significant changes with treatment. Finally, in order to explore the bacterial community gene expression profiles without a potential bias from highly expressed eukaryotic genes, we identified the potential bacterial encoded transcripts by mapping the metatranscriptomic datasets using BLAT [47] with 98% identify cutoff against a collection of high-quality metagenomic assembled genomes (MAGs) previously described: 7903 MAGs recovered from 1.5K metagenomes from environmental sources [48], 1.5K high-quality (>70% completeness) MAGs recovered from the TARA Oceans metagenomic collection [49], and 1108 MAGs recovered from the Chattahoochee lakes and estuaries metagenomic collection [50, 51]. Downstream analysis was performed using the total (rRNA excluded) metatranscriptomic dataset, or with the subset of potential bacterial reads as identified by mapping against the collection of MAGs.

Functional annotations

Metatranscriptomic reads were functionally annotated based on protein-level searches against the SwissProt database [52] using the Gene Ontology (GO) terms [53], and against the SEED database using the subsystems categories [54]. Query reads were assigned the functional annotation of their best-match in SwissProt containing GO terms or in SEED containing subsystem categories, based on blastx searches using diamond [55] with the "-sensitive" option. Differential abundance of functional annotation terms (subsystem categories) between groups of samples was identified using a negative binomial test as implemented in the DESeq2 package [56] with an adjusted p value of 0.05 (Benjamini–Hochberg correction). Datasets were compared in a two-factor design to capture both individual effects of acidification and warming, as well as their potential interactions.

Results and discussion

The goal of this study was to compare warming and acidification responses of microbial communities from relatively thermally and pH stable environments (offshore) to those from more variable environments (nearshore; Fig. S1). We manipulated pH (−0.3 unit) and temperature (+3 °C) both individually and in combination for seawater from nearshore and offshore locations during summer and winter to determine how the environmental context alters microbial community responses at the levels of community composition, individual phylotype abundances, and community function (biogeochemical rates and metatranscriptomes). While warming increased respiration in all experiments (29–132%), consistent with temperature-limited secondary production in temperate regions [14, 57], it was only significantly higher in the winter, offshore experiment (two-way ANOVA, $p < 0.05$) (Fig. S2). In contrast, neither acidification nor warming significantly altered bacterial abundance, phytoplankton biomass (chlorophyll *a*), or primary production (^{14}C-bicarbonate incorporation rates) in any experiment (Figs. S3–S5). An absence of statistically significant impacts of acidification is consistent with previous studies that found the dominant marine cyanobacteria

at our study site (*Prochlorococcus* and *Synechococcus*) only showed minor responses to elevated pCO_2, and thus are probably not carbon limited [10, 58]. The reported effects of elevated pCO_2 on eukaryotic algae have been more variable with positive, negative and neutral responses reported [11, 59, 60], likely reflecting differences in inorganic carbon acquisition mechanisms or in experimental design [60]. However, while bulk primary production rates remained constant, growth rates of specific taxa within the community could change dramatically, thereby altering the overall community composition and potentially biogeochemical rates.

To examine microbial community composition in climate-change manipulations, we sequenced 16S rRNA gene libraries. At the end point of all experiments, warming significantly altered microbial community composition (PERMANOVA, $p < 0.05$; Fig. 1, Fig. S6, and Table S1). These results are consistent with strong relationships between temperature and marine microbial community composition [16, 61], but in contrast with recent work suggesting that major community changes only occur when temperatures exceed the historical maximum [7]. Consistent with our predictions, warming had the largest impact at the offshore station (Bray–Curtis Dissimilarity, ANOVA, $p < 0.05$; Fig. S7 and Table S2), suggesting potentially greater temperature sensitivity in this lower-variability environment. In contrast with temperature, acidification alone did not significantly alter microbial community composition in any experiment (Fig. 1, Fig. S6, and Table S1). Previous studies that observed community-level acidification responses often included nutrient-induced phytoplankton blooms [21, 62], which complicate interpretations of pH responses due to differences in bloom progression. Even though acidification did not alter microbial community composition here, we did observe a significant interaction term between acidification and warming in one experiment (summer offshore; Fig. 1, Fig. S6, and Table S1). Although multistressor interactions, where the impact of multiple factors is distinct from the sum of each individual stressor's effect, have been observed previously

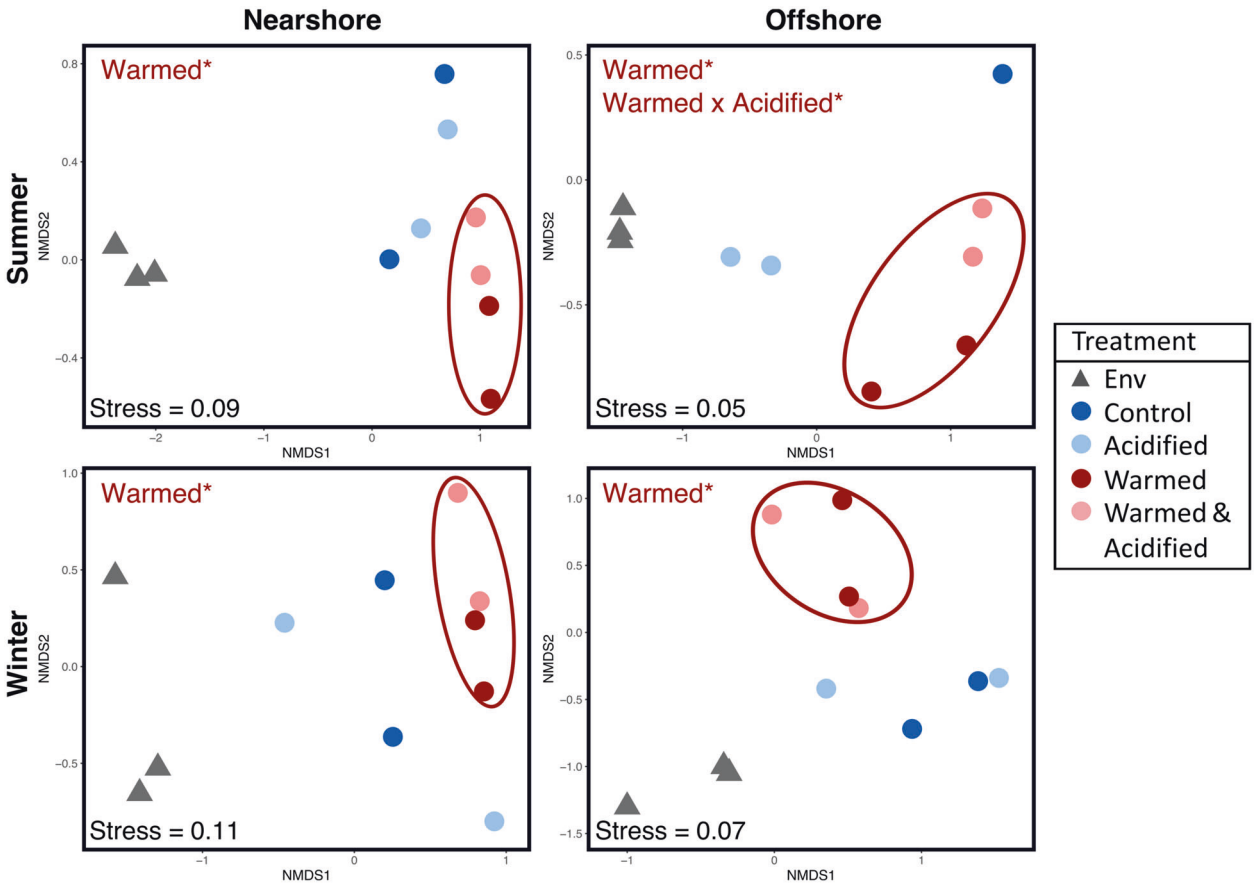

Fig. 1 Nonmetric multidimensional scaling (NMDS) ordination computed based on Bray–Curtis dissimilarity for 16S rRNA gene libraries. Samples were collected from each carboy after 5 days of experimental manipulation. "Env" indicates the environmental sample collected in field. Ellipses identify significant community changes in specific treatments as analyzed using a factorial design. Treatments labeled with asterisks on the left top corner in each plot indicate significant treatment impacts (PERMANOVA, $p < 0.05$) assessed after 5 days of treatment.

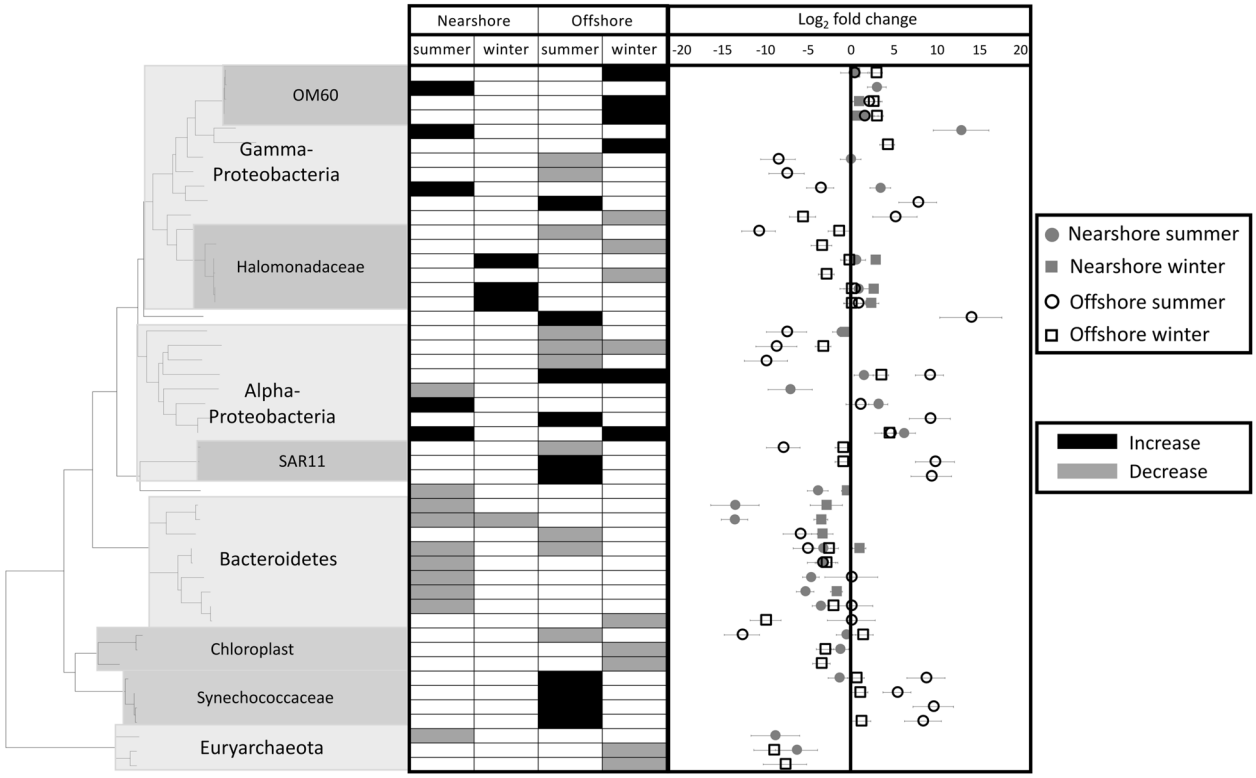

Fig. 2 Phylotypes (ASVs) that significantly respond to warming. ASVs are organized by a maximum likelihood phylogenetic tree with key clades labeled. ASVs are shown in the figure if: (1) they are identified as significantly responding to warming using DESeq2 in any of the four experiments and (2) they exceed the threshold of a relative abundance of 0.05% in the corresponding experiment. Left panel table indicates a significant increase (black) or decrease (gray) of each ASV in warmed treatments relative to ambient temperature treatments. In the right panel average Log_2 fold changes and standard deviations ($n = 4$) in warmed relative to ambient temperature treatments are plotted, if the ASV exceed the abundance threshold (0.05%) in that experiment.

[20]; here, the observed "interaction" may be an experimental artifact resulting from the dramatic decrease of cyanobacteria in all treatments likely due to the stress of incubation. This cyanobacteria decline was less severe with increased pCO_2 (Figs. S8 and S9); however, as this represents a response to an artificial environment, these results are not applicable to natural settings. Thus, elevated temperature's effects on microbial physiology and community composition appears to be the dominant signal in this temperature and pCO_2 factorial study.

In order to better understand the shifts underlying microbial community changes, we investigated population-level responses of 16S rRNA gene ASVs. We used a multifactor design to examine the impacts of both warming and acidification, as well as their potential interaction, e.g., all warmed treatments ($n = 4$) were compared against all ambient temperature treatments ($n = 4$) from the same time point. Similar to the community-level analysis, acidification only significantly altered the absolute abundance of five ASVs, including three cyanobacteria; genus *Synechococcus*, of the 48 taxa with >0.05% abundance (summer, offshore experiment; Fig. S10). Again, this minimal impact of acidification was observed previously [26, 27]. In contrast, warming significantly impacted 49 ASVs in at least one of the four experiments, across the phylogenetic breadth of the microbial community (Fig. 2), reflecting widespread direct or indirect effects of warming. Phylotypes that responded to warming represent 2.8–8.9% of the community, highlighting that not all taxa are significantly impacted by warming, in this study (Table S3). Consistent with the hypothesis that organisms from more stable environments are more sensitive to environmental changes, a total of 36 ASVs responded to warming in offshore experiments while there were only 19 responsive taxa in nearshore experiments. Moreover, in both seasons, offshore ASVs exhibited larger changes in abundance relative to those in nearshore experiments (two-way ANOVA, $p < 0.05$) (Fig. 2, Fig. S11, and Table S4). In addition, within a station, taxa declined significantly more in summer than in winter experiments (two-way ANOVA, $p < 0.05$; Fig. 2 and Fig. S11b), which may reflect that higher summer temperatures were more likely to exceed populations' thermal optima (Table S5). There were some trends across phylogenetic groups, e.g., four OM60 (Gammaproteobacteria) ASVs all increased with warming. Similarly, four members of the *Synechococcaceae* (one *Prochlorococcus* and three *Synechococcus*

ASVs) increased in offshore communities under elevated temperature as did the family *Synechococcaceae* in the summer (mean $\log_2$ fold change of 2.86 ($p < 0.05$); Fig. 2 and Table S6), consistent with cyanobacteria generally exhibiting high temperature growth optima [63]. In contrast, the phylum Bacteroidetes ($\log_2$ fold change: −0.5, $p < 0.05$) and Marine Group II Archaea ($\log_2$ fold change: −5.8, $p < 0.05$) both decreased with warming, suggesting their potential direct or indirect sensitivity to warming (Fig. 2 and Table S7). However, phylum-level trends generally reflect the responses of only a few dominant taxa rather than a consistent response to warming across all ASVs in a phylum. While not all taxa were present in all experiments due to strong seasonal and spatial partitioning of phylotypes [16, 29], five ASVs significantly responded to warming in multiple experiments (Fig. 2): three *Alphaproteobacteria* (two unclassified and one *Rhodobacteraceae*), and two Bacteroidetes (one *Cryomorphaceae* and one *Flavobacteriaceae*). In all cases, phylotype abundance increased or decreased consistently across experiments despite ambient temperatures ranging from 11.7 to 29.2 °C, suggesting that these clades are either acclimated to ambient temperatures or that closely related strains (sub-ASV level) exhibit temperature specialization [64]. However, the majority of temperature-responsive taxa significantly changed in only a single experiment, highlighting the importance of environmental context in microbial populations' responses to climate-change parameters (Fig. 2). Taken together, our study indicates that warming could alter the abundance of some microbial populations, especially in thermally stable environments.

In addition to compositional changes, climate-change factors may alter the metatranscriptome, potentially indicating changes in biogeochemical cycles or identifying physiological responses to environmental shifts [15, 25]. Thus, we examined winter metatranscriptomes by mapping mRNA to high-quality metagenome assembled genomes and annotating functional groups using SEED subsystems [54]. In contrast with a previous mesocosm study of Mediterranean Sea bacterioplankton where acidification elevated gene transcripts involved in respiration, proteorhodopsin, and membrane transporters [25], no SEED subsystems significantly responded to acidification in this study. Therefore, we again applied a factorial design to focus on the effects of warming; and we again observed more responsive subsystems in metatranscriptomes from the offshore site (Fig. 3 and Table S8). In the nearshore samples, there were only two responsive subsystems ("glutamine

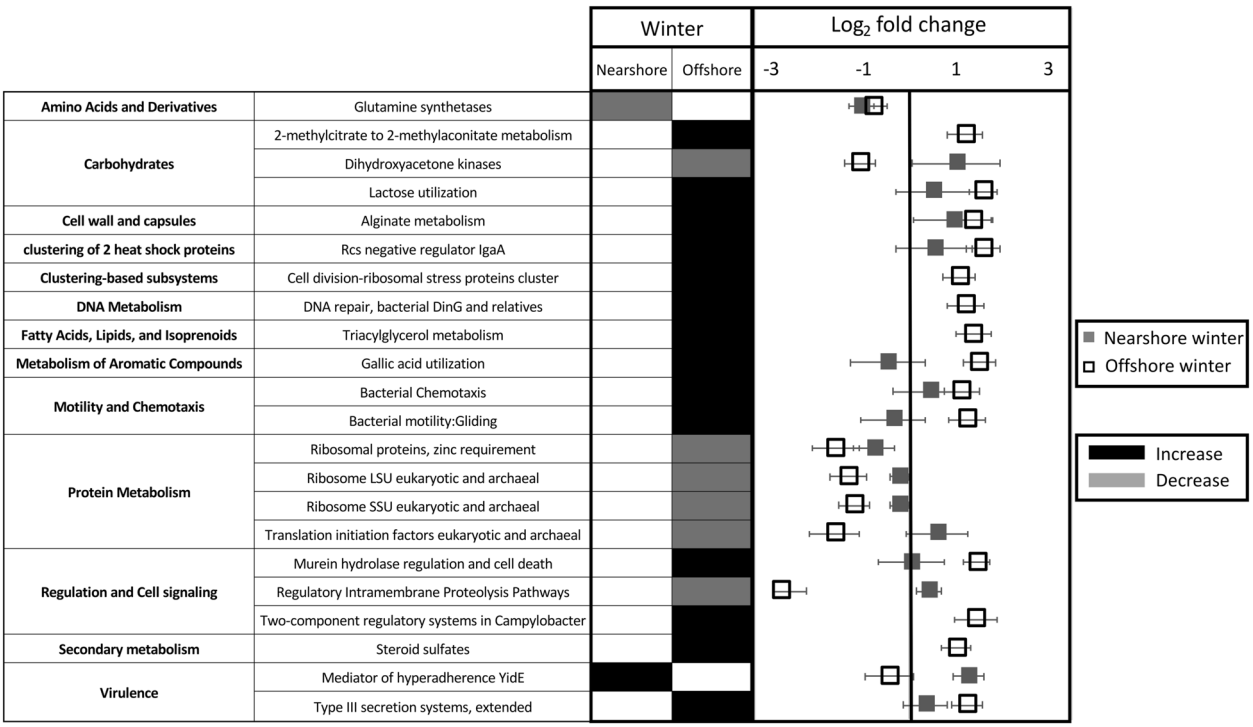

Fig. 3 $\log_2$ fold changes of SEED subsystems in response to warming in two experiments (nearshore winter and offshore winter). SEED Subsystems are shown in the plot: they are (1) identified as significantly responding to warming using DESeq2 in either of the experiments and (2) exceed the threshold of a relative abundance of 0.003% of the metatranscriptome in that experiment. Left panel table indicates significant increases (black) or decreases (gray) of one SEED subsystem in warmed treatment relative to ambient temperature treatments. In the right hand panel, the average $\log_2$ fold changes and standard deviations ($n = 4$) in warmed relative to ambient temperature treatments are plotted for subsystems that exceed the abundance threshold (0.003%) in that experiment.

synthetases" and "mediator of hyperadherence YidE") (Fig. 3). As neither of these subsystems is related to canonical microbial temperature or stress responses, e.g., upregulation of heat shock proteins, transcriptional regulators, signal transduction, and downregulation of translation [65, 66], these metatranscriptome differences likely reflect subtle changes in the genomic composition of the warmed community or spurious results rather than a specific temperature-related transcriptional response. In contrast, in the offshore, winter experiment, 20 SEED subsystems significantly differed in abundance between warmed and ambient temperature treatments (Fig. 3), suggesting that offshore microbial community is more sensitive to warming, as we predicted due to the limited temperature range at this site. The altered subsystems included indicators of cell stress responses (DNA repair, cell regulation and signaling), membrane fluidity, and protein-synthesis potential (ribosome-associated transcripts) as well as changes in cell signaling and motility. Several of these pathways correspond to common thermal responses such as changes in cell membrane composition [67], others such as carbohydrate metabolism may reflect shifting pools of labile organic matter produced through compositional or physiological changes in primary producers. In addition, the cell motility and chemotaxis-associated transcripts that were elevated with warming are typically associated with copiotrophs, which may reflect increased abundances of *Rhodobacteraceae* (*Alphaproteobacteria*) and OM60 (*Gammaproteobacteria*) (Fig. 2 and Table S6) [68]. Although offshore microbes are thought to have streamlined genomes and are less able to regulate gene expression with environmental conditions [31]; here, we observe major metatranscriptome changes with warming. Moreover, the dominant signal in the offshore metatranscriptome is a stress-associated response, including alterations in environmental sensing and cell signaling (e.g., IgaA) and declines in protein-synthesis-associated transcripts. Thus, while warming significantly increased respiration rates (Fig. S2) and the warmed temperature was within the range commonly experienced at the site (26 °C), the offshore warmed community exhibits hallmarks of a stress response.

To further investigate one aspect of the warming-induced stress response, we focus on an apparent decline in the ribosomal machinery; unlike other pathways, the ribosomal gene content is fairly well conserved across lineages [69], thus decreases in ribosomal transcript abundance is generally assumed to reveal decreases in protein-synthesis potential (and often growth rate) or alternately temperature-associated increases in ribosomal efficiency [15] or other physiological changes [70]. As 3 °C of warming did not significantly decrease ribosome-associated transcript levels in the nearshore metatranscriptome, the decline the offshore community (Fig. 3) likely represents lower protein-synthesis potential, consistent with the other stress-related changes in the metatranscriptome. As our metatrascriptome analysis is not taxonomically resolved, we investigated phylotype rRNA/rDNA ratios as a potential indicator of differential protein-synthesis potential within the bacterioplankton community [71, 72]. However, rRNA/rDNA ratios were generally similar under ambient and warmed conditions and did not identify specific taxa that might be more responsive to warmed conditions than the community average, suggesting a generally consistent response in the rRNA relative to abundance (rDNA) at both sites.

Overall these results support the hypothesis that environmental stability magnifies warming's impacts on the marine microbial community composition, individual taxa abundances, and metatranscriptomes. Warming shows contrasting impacts at these two sites: the nearshore microbiome exhibits potential functional redundancy (a change in composition, without an apparent functional change), while the offshore community is sensitive to warming with altered composition and function [3]. This study differs from prior single-strain or single-site studies of microbial responses to temperature and/or acidification that largely do not consider prior environmental exposures and their influence on microbial phenotypic plasticity, acclimation, and evolutionary adaptation. Moreover, the majority of prior climate-change manipulation studies have been carried out in temperate, nearshore environments, likely due to logistics. Yet if environmental context shapes microbial responses to environmental perturbations, temporally dynamic coastal environments may be more resistant or resilient to environment changes than more stable environments such as the open ocean gyres. As coastal climate manipulations are not representative of the global oceans, in future experiments, researchers should consider environmental context including the range of normal parameter distributions [7] as well as use caution in extrapolating perturbation results from one site to water masses with distinct histories and variability regimes [5, 6].

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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