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Ecology, 0(0), 2019, e02956

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Citation: Rocca, J. D., M. Simonin, E. S. Bernhardt, A. D. Washburne, and J. P. Wright. 2020. Rare microbial taxa emerge when communities collide: freshwater and marine microbiome responses to experimental mixing. Ecology 00(00):e02956. 10.1002/ecy.2956

Abstract. Whole microbial communities regularly merge with one another, often in tandem with their environments, in a process called community coalescence. Such events impose substantial changes: abiotic perturbation from environmental blending and biotic perturbation of community merging. We used an aquatic mixing experiment to unravel the effects of these perturbations on the whole microbiome response and on the success of individual taxa when distinct freshwater and marine communities coalesce. We found that an equal mix of freshwater and marine habitats and blended microbiomes resulted in strong convergence of the community structure toward that of the marine microbiome. The enzymatic potential of these blended microbiomes in mixed media also converged toward that of the marine, with strong correlations between the multivariate response patterns of the enzymes and of community structure. Exposing each endmember inocula to an axenic equal mix of their freshwater and marine source waters led to a 96% loss of taxa from our freshwater microbiomes and a 66% loss from our marine microbiomes. When both inocula were added together to this mixed environment, interactions amongst the communities led to a further loss of 29% and 49% of freshwater and marine taxa, respectively. Under both the axenic and competitive scenarios, the diversity lost was somewhat counterbalanced by increased abundance of microbial taxa that were too rare to detect in the initial inocula. Our study emphasizes the importance of the rare biosphere as a critical component of microbial community responses to community coalescence.

Key words: 16S rRNA; bacteria; biotic interactions; environmental perturbation; mutualism; phylogenetic factorization; rare biosphere.

Introduction

When previously discrete communities combine, the assembly of the unified novel community is termed "community coalescence," first documented in A. R. Wallace's "Great American Biotic Interchange": the wholesale exchange of terrestrial fauna between the North and South American continents enabled by dispersal across the newly formed Isthmus of Panama (Wallace 1876, Webb 1976). This ongoing immense biotic exchange started 2.6 Ma, yet in the microbial realm, biotic interchanges like these occur rapidly and often. With every deposit of a fecal microbiome into the environment and every time a leaf falls to the ground, microbial communities from distinct environments encounter one another and coalesce. Despite the ubiquity of microbial community coalescence, the formal recognition of this concept is fairly recent in microbial ecology (Rillig et al. 2015). In fact, Mansour et al. (2018) suggest that many experimental studies of microbial communities are unacknowledged community coalescence experiments, and most, if not all, microbial communities are the culmination of previous coalescence events. Unlike macroorganismal movement (i.e., seed dispersal, migration) in which organisms disperse alone to a new environment, many microbial communities disperse in the aggregate and in tandem with their environment (Livingston et al. 2013, Rillig et al. 2015, Rillig and Mansour 2017). For example, every second of every day the rivers of the world drain into the sea and rising tides bring seawater in contact with bodies of freshwater. At the confluence of these water bodies, the process of community coalescence is at work.

When freshwaters blend with seawater, we observe dramatic decreases in organic carbon content because of complexation with salts, increased pH, and and decreased nutrient availability, along with increases in salinity (Craft et al. 2009, Barlow and Reichard 2010,

Manuscript received 15 June 2019; revised 1 October 2019; accepted 12 November 2019. Corresponding Editor: Matthias C. Rillig.
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Neubauer et al. 2013, Ardón et al. 2016), novel to either endpoint microbial community. The multivariate chemical transition to brackish water is well studied, and salinity is a well-documented environmental stressor (Lozupone and Knight 2017), imposing strong evolutionary selection on organisms (Logares et al. 2009, Paver et al. 2018) with deeply conserved traits for salinity tolerance (Martiny et al. 2015). Habitat specificity is fairly conserved within freshwater and marine specialists, as evolution of marine life in freshwater clades, and vice versa, is rare (Logares et al. 2009, Comte et al. 2017). Water column freshwater microbial taxa are particularly sensitive to the salt stress imposed by brackish conditions (Burke and Baird 1931, Ewert and Deming 2013), whereas marine microbial taxa are more robust to brackish waters, because of wider salinity tolerance (Herlemann et al. 2011). Compositional turnover is high for water (Kisand et al. 2005, Shen et al. 2018) and soil (Dang et al. 2019) microbial communities during brackish exposure, and we expect similar turnover of the water column community with this strong environmental filter. However, the impact of novel biotic interactions from a community blending freshwater and marine microbiomes is unknown. The copiotrophic life strategy of most freshwater microbial taxa (Zeder et al. 2009) may result in superior competition over nutrients in the brackish waters, though some marine microbes are also copiotrophs (Cottrell and Kirchman 2016), so the overall outcome of community mixing is unknown. Marine microorganisms also likely have lower minimum resource requirements, or R^* values (Tilman 1982), from their oligotrophic conditions, and therefore may be better competitors in terms of resource competition (Lauro et al. 2009). Are there rules of thumb regarding which microbes tend to be more robust to the environmental and biotic changes of seawater intrusion? Are abundant taxa more resilient to these changes? Which lineages of microbes thrive in brackish waters?

To address these questions, we combined microbial inocula from freshwater and marine endmember habitats into a corresponding mixed brackish environment to simulate seawater intrusion and compared the responses in bacterial community structure and microbial enzyme potential to the corresponding responses under home conditions or environmental perturbation alone (Fig. 1). This experimental design allowed us to ask: (Q1) what are the whole community-level structural and functional responses to the abiotic and biotic perturbations imposed through community coalescence? and (Q2) can we classify individual microbial "species" (delineated as sequence variants) based on their specific responses to the abiotic and biotic components of simulated seawater intrusion?

We can also differentiate amongst four potential outcomes of microbial community coalescence with our experimental design (Fig. 1, top panel). Beginning with the assumption that our starting endmember communities are distinct (Bouvier and del Giorgio 2002; Logares et al. 2009; Herlemann et al. 2011; 2016; Paver et al. 2018), we expect that the community resulting from their exposure to the mixed environment and to the community merger would follow one of four possible trajectories of the final community composition (Fig. 1, top panel): (A) intermediate between the two home conditions because of species sorting depending on the environment; (B) stratified, yet similar to their initial inocula because of symmetric community resistance; or (C) convergence toward the assemblage observed in only one of the two environments because of strong asymmetric environmental filtering *or* asymmetric community resistance. Alternatively, a final outcome (D) is that the coalescence conditions result in highly variable emergent communities via stochastic responses. In the scenario where the communities converge toward a single endmember (C), we introduce a methodological framework (Fig. 1, bottom panel) enabling the independent assessment of the influence of the abiotic and biotic perturbations imposed by coalescence.

Our integrative research approach allows us to disentangle the mechanisms driving changes in microbial community structure and function following community coalescence in a model system. Habitat transplants apply an environmental perturbation to identify generalist vs. specialist microbial taxa. Community coalescence allows us to identify strong vs. weak competitors and common associations amongst taxa. Microbial community coalescence provides unique opportunities for community ecological experiments at an unprecedented scale, allowing us to learn more about the niches and competitive abilities of individual microbial taxa in response to the substantial change in conditions when communities and environments collide.

Materials and Methods

Field sample collection and aquatic endmember characterization

The two endmember sources for this microcosm experiment are located in coastal North Carolina, USA (Table 1). The "Freshwater" site is a blackwater wetland ecosystem located within the Timberlake Wetland Restoration Project in Tyrrell County, North Carolina (Ardón et al. 2016), exposed to episodic or storm-triggered flows following rain or coastal storm systems. The "Marine" coastal site is located at the northern end of the Cape Hatteras National Seashore with persistent water turbulence. The Freshwater and Marine endmember sites were selected for their close proximity (64 km) and even latitude, and this choice maximizes the range of habitats involved in seawater intrusion. We acknowledge that these endmember sites do not represent locations of frequent direct blending, as mixing occurs more gradually across the gradient, but the sites are true endmembers, with minimal past exposure to seawater intrusion, and collected at the end of a seasonal period when

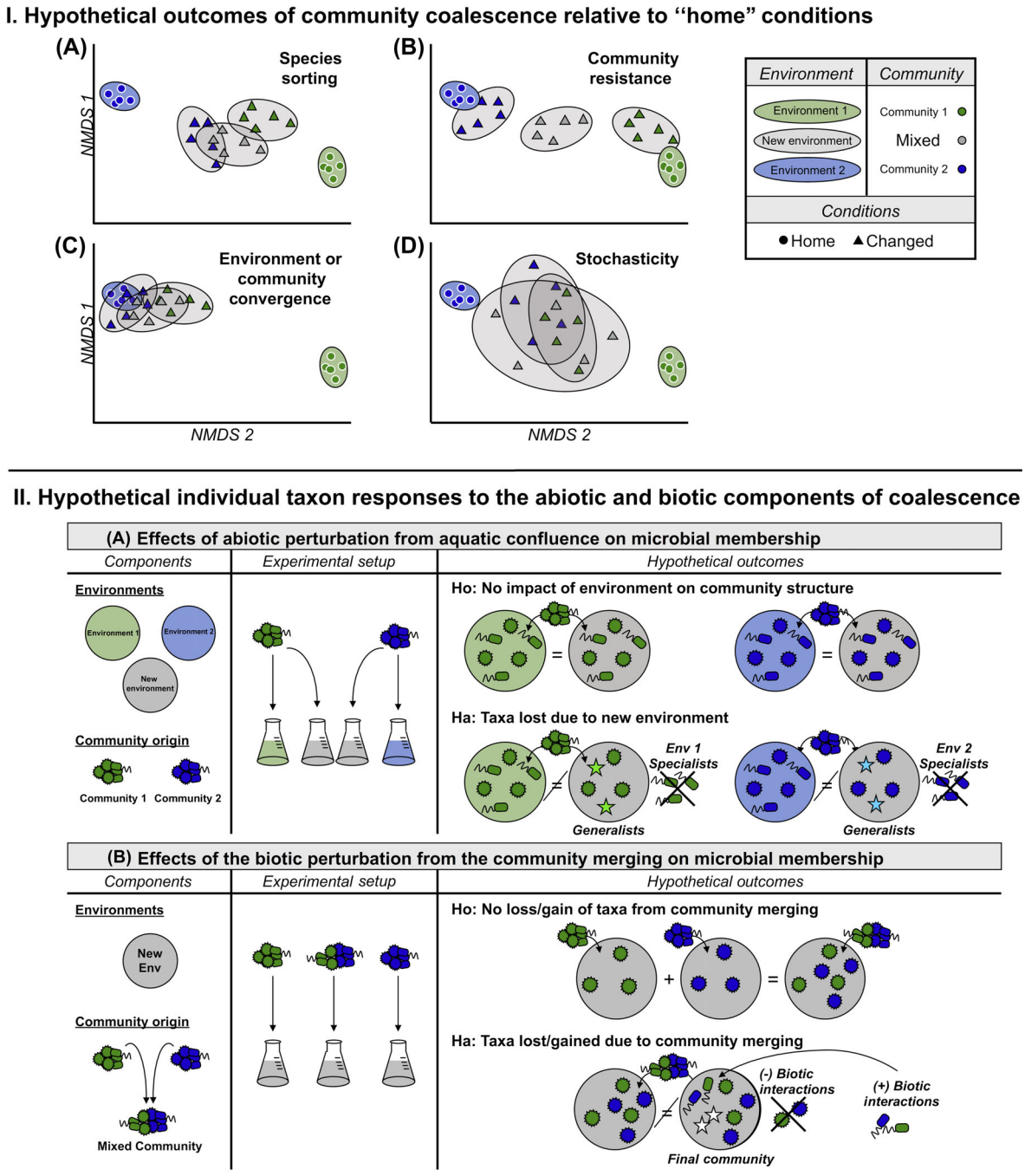

FIG. 1. I. Potential outcomes of coalescing communities relative to "home" conditions. Ellipsoids represent the theoretical variation in community structure or enzyme potential among replicates. Communities may be symmetrically impacted by coalescence (A, B), constrained primarily by environment (A), or resistant to environmental change (B); (C) asymmetrical impact may be driven by either environment or community; or (D) coalescence may result in stochastic shifts in community structure or function. II. Conceptual framework to examine the impacts of (A) abiotic and (B) biotic perturbation: components needed to impose each perturbation (left), experimental setup (center), and hypothetical outcomes (right). (A) Abiotic perturbation—assesses the impact of environmental blending, where no impact of altered environment (H_o), versus a shift in community structure (H_a), reveals environmentally sensitive/tolerant taxa, or potentially the emergence of rare microbial taxa (represented by five-pointed stars). In (B) Biotic perturbation—Determine the impact of combining the two endmember communities, with no impact (H_o) where the sum of Part 1 communities equals that of the coalesced community, versus new biotic interactions, where taxa are lost due to competition or gained due to mutualistic interactions (H_a).

TABLE 1. Location, chemical, and microbial bulk characterization of the two distinct aquatic endmember habitats in coastal North Carolina, USA: Freshwater and Marine environments and the chemical characterization of the experimentally mixed (1:1) Brackish environment and blended inoculum.

Environment	Freshwater	Brackish	Marine
Endpoint location	Timberlake Restoration	50:50 lab mixture	Cape Hatteras National Seashore
Latitude	35°53'46.4" N	–	35°49′57.4" N
Longitude	76°09'51.4" W	–	75°33'25.7" W
Mixing	Episodic/storm	–	Turbulent
Temp (June 2016)	23–25°C	–	23°C
Chemistry of environments			
pH	4.4 (±0.05)	7.8 (±0.01)	8.2 (±0.01)
Salinity (Practical Salinity Unit)	0.07 (±0.01)	26.7 (±0.9)	51.8 (±0.4)
Dissolved organic carbon (mg/L)	33.9 (±1.2)	21.7 (±3.2)	3.6 (±0.4)
Dissolved inorganic nitrogen (mg/L)	0.92 (±0.01)	0.61 (±0.05)	0.12 (±0.03)
Microbial inoculum characterization			
Biomass (ng DNA/μL)	749.7	584.8	218.3

recent seawater intrusion was least likely. However, strong storm events and/or seasonal droughts make contact between these endpoints probable, especially under forecasted climate change scenarios.

In June 2016, we collected 80 L of surface water from each site into sterile carboys, stored at the average site temperature (23°C) at the time of collection. Within 12 h, each sample was filtered through axenic 1-mm mesh (acid-washed in 3M HCl; mesh sterility confirmed by filtering sterile water and plating the filtrate onto low- and high-nutrient agar with zero colonies) to remove macro-organisms and debris. Each sample was subset for salinity, pH, and dissolved organic carbon. The two filtered endmember water samples were halved to generate (1) the microbe-free environments and (2) microbial inocula (Fig. 1).

Microcosm incubation setup

We set up a 1-week laboratory incubation, exposing three inocula isolated from each endmember sample (Freshwater [I_F], Marine [I_M], and a 1:1 mixture of Freshwater–Marine, hereafter "Coalescence" [I_C]), into three axenic aquatic environments (Freshwater [E_F], Marine [E_M], and a 1:1 mixture of the endmember environments, hereafter "Brackish" [E_B]). We generated axenic environments by autoclaving at 121°C/20 PSI for 30 min in covered acid-washed glassware. The autoclaving was repeated twice more after cooling to ensure that any microorganisms exiting dormancy were killed. Sterility was confirmed by streaking subsamples onto low- and high-nutrient agar. The heat and pressure of autoclaving can impose substantial changes in organic matter and in the redox state of chemicals in the environments, so we included unautoclaved positive controls in the incubation to account for autoclaved environmental impacts on microbiome structure (Appendix S1: Figs. S1, S3).

Intact microbial communities were isolated by concentrating cells off the remainder of the Freshwater and Marine water samples. Two complementary concentrating methods were implemented to minimize biases imposed by either method (Peterson et al. 2012). Half of each environment was gently centrifuged at 5,000 RCF in 50-mL batches in round-bottom tubes to ensure maximal viability of the microbial assemblages. In parallel, the remaining water samples were filtered over gamma-irradiated Pall Supor 0.2 μm nitrocellulose membranes (Millipore, New York, New York, USA) in small batches to minimize fouling on the filter. For each inoculum, the filter-collected and centrifuged-collected microbiomes were combined by resuspending in sterilized home.

The experiment design here consisted of 25 independent microcosms (850 mL inoculated with 550 μL), comprising five treatment conditions with an experimental replication of five (Appendix S1: Fig. S1): (1) Freshwater–Home (I_FE_F): Freshwater microbiome in Freshwater environment; (2) Marine–Home (I_ME_M): Marine microbiome in Marine environment; (3) Freshwater–Brackish (I_FE_B): Freshwater microbiome in Brackish environment; (4) Marine–Brackish (I_ME_B): Marine microbiome in Brackish environment; and (5) Brackish–Coalescence (I_CE_B): a 1:1 blended microbiome in a 1:1 mixture of the endmember environments. We set up the microcosms under UV-treated PCR-hood conditions, into sterile glass Mason jars, sealed with gas-tight lids. We also included 27 control microcosms ($n = 3$): the positive control microcosms were used to correct for any experimental artifacts from inoculum and environment preparations, which did not vary significantly from the "home" microcosms (Appendix S1: Fig. S3), and microcosms with sterile environments or sterile water with no added inocula were included as negative controls; these had no detectable microbial growth or genetic material and will not be discussed further. The incubation ran for 7 d in a growth chamber (23°C, 13.5 h diurnal light regime, and PAR: 250–450 $\mu mols \cdot m^{-2} \cdot s^{-1}$) reflecting field conditions at the time of collection. We acknowledge that despite their short lifespan, some community assembly of microbiomes can take longer than a week to

shift (Hawkes et al. 2017), but aquatic community coalescence events occur on rapid time scales. Seven days allowed enough time for community reassembly processes to occur while minimizing resource depletion within the microcosms. Twice daily, the microcosms were mixed by inverting and were rerandomized to minimize potential biases from differential light and temperature across the chamber.

Microbial enzyme profile: community property of function

Microbial extracellular enzyme potential activity (hereafter enzyme activity) was measured in each microcosm at the end of the incubation. Eight enzymes were measured from a 91-mL subsample of each microcosm following a protocol developed by Bell et al. (2013), modified to handle water. The following enzymes were targeted with fluorescently labeled substrates to capture potential N, P, C, and S degradation activity: α-1,4-glucosidase (AG), aryl-sulfatase (AS), β-1,4-glucosidase (BG), β-D-1,4-cellobiosidase (CB), L-leucine aminopeptidase (LAP), β-1,4-N-acetylglucosaminidase (NAG), alkaline phosphatase (PHOS), and β-D-xylosidase (XYL). After 3 h at room temperature, the centrifuged supernatant for each sample was read (340/460 nm) on a FLUOstar Optima spectrophotometer (BMG Labtech, Cary, North Carolina, USA) in black optical plates.

DNA extraction and bacterial community analysis

Subsamples (250 mL) of each microcosm were filtered over gamma-irradiated Pall Supor 0.2 μm nitrocellulose membranes, and the filtrate was pelleted at 10,000 *g* for 1 h to get ultrasmall microorganisms (Luef et al. 2015). We combined the pellet and filter and extracted genomic DNA with the MoBio PowerWater DNA Isolation Kit (Carlsbad, California, USA) modified with a heating (30 min at 65°C) lysis step prior to physical lysis of the standard bead beating, and fluorometrically measured resultant total DNA (Quant-iT dsDNA Assay Kit, Thermo Scientific, Waltham, Massachusetts, USA). We targeted the V4 hypervariable region of the bacterial 16S rRNA gene (515-F/806-R, Caporaso et al. 2011), and sequenced with Illumina MiSeq (PE 150bp; V2 chemistry) at the Environmental Sample Preparation and Sequencing Facility (ESPSF). Raw sequences are available at NCBI Sequence Read Archive: PRJNA589904.

ESPSF returned 25 million raw sequences, which we processed through the Quantitative Insights Into Microbial Ecology 2 (Qiime2) pipeline (Bolyen et al. 2019) to remove low-quality reads and putative chimera, and to denoise the sequences into exact sequence variants (SVs) with Dada2 (Callahan et al. 2017). We aligned the representative sequences and assigned taxonomy using the Silva V132 (99%) reference alignment (Quast et al. 2013), and a phylogeny and improved alignment were generated using the Practical Alignment using SATé and TrAnsitivity (PASTA) software (Mirarab et al. 2014). Eukaryotic, mitochondrial, and chloroplast sequences were removed, representing 3, 12, and 8% of total sequences. We rarefied the SV table to the lowest sequence depth (17,500), and the final data set contained 3389 unique SVs with 12,293,437 total reads.

Bacterial community structure and enzyme profile

We calculated alpha-diversity (Chao1) and Pielou's evenness on each sample to bulk characterize the bacterial communities, and a Bray-Curtis dissimilarity matrix of the community data set (and Euclidean for the enzyme data set) to examine differences in community structure and in the functional profile among treatments, visualized using nonmetric multidimensional scaling (NMDS). We tested significant overall and between-group shifts in community structure (Fig. 1A) using nonparametric multivariate analysis of variance (perMANOVA, Anderson 2001) to estimate correlation coefficients and p-values (permutations = 999) for the effect of each treatment with the "adonis" function in the Vegan R package (Version 3.3.1, Oksanen et al. 2017; R Development Core Team, 2017). We used "procrustes" in Vegan to perform least-squares orthogonal mapping to determine correlations among the three multivariate datasets: bacterial community structure, microbial enzyme activity profiles, and the PICRUSt-inferred metagenomes. We visualized the responses of the most abundant microbial SVs (>2%) using unweighted pair group method with arithmetic mean (UPGMA) clustering (Sokal and Michener 1958) to the environmental and microbiome mixing treatments. Univariate data that violated assumptions of normal distribution and homoscedasticity were transformed, and assumptions were subsequently reverified with Shapiro-Wilk and Levene tests and examination of Q-Q plots. Finally, we used one-way ANOVAs to test the significance of the treatments on microbiome diversity and enzyme activity and post hoc pairwise comparisons among the treatment combinations using Tukey's Honestly Significant Difference multiple means comparison.

Separate assessment of the two perturbations of coalescence

To assess the impact of environmental mixing and community blending independently, we compared specific experimental treatments (Fig. 1, bottom panel). To examine the impact of the environmental perturbation imposed by aquatic merging (Part I), we compared "home" microbiomes to the corresponding Brackish microbiomes (Freshwater–Home [I_FE_F] vs. Freshwater–Brackish [I_FE_B] or Marine–Home [I_ME_M] vs. Marine–Brackish [I_ME_B]). To examine the impact of community blending (Part II), the Freshwater–Brackish (I_FE_B) and Marine–Brackish (I_ME_B) microbiomes were compared to the Brackish–Coalescence (I_CE_B) microbiomes. We identified surviving taxa as those present in all

microcosm of a given treatment, and those that were not present in all were lost to the perturbation. Finally, we used Phylofactor to identify phylogenetic clades (factors) with changes in relative abundance (Washburne et al 2017, 2019), using Holm's sequentially rejective 5% cut-off for the family-wise error rate (Holm 1979) (Appendix S1: Fig. S6).

Results

Starting conditions: bacterial community and chemical characterization

Our two environments were distinct among the Freshwater (E_F) and Marine (E_M) endmembers (Table 1). Other than water temperature at the time of field collection, all other water chemistry properties varied substantially between our endmembers. Electrical conductivity was ~740-fold greater and pH was 3.8 units higher in the Marine (E_M) sample, and the Freshwater (E_F) environment had higher concentrations of both nutrients and dissolved organic matter.

The Freshwater (I_F) and Marine (I_M) microbial communities were also distinct from one another (Fig. 2; Appendix S1: Fig. S2). Alpha diversity (328 vs. 253 observed SVs/microcosm) and evenness (0.1 vs. 0.02) were significantly higher in the Freshwater (I_F) microbial community, and the two endmember microbiomes had minimal overlap in their community composition, with only 21 SVs (<1.4% of total SVs) representing 22% of the summed sequence count of the Fresh and Marine microbiomes (Appendix S1: Fig. S2). The Freshwater (I_F) communities were dominated by the families *Acetobacteraceae*, *Paracaedibacteraceae*, *Beijerinckiaceae*, and *Burkholderiaceae*; and the Marine (I_M) microbiomes were dominated by *Alteromonadaceae*, *Rhodobacteraceae*, *Saprospiraceae*, *Spirosomaceae*, and *Vibrionaceae* (Fig. 3; Appendix S1: Fig. S4), with a single genus—*Alteromonas*—comprising 9.7% of the Marine (I_M) microbiome. The two communities differed in their functional potential as well, exhibiting distinct enzyme profiles (Fig. 2C), with significantly higher activity in three of the eight enzymes in the Freshwater microbiomes (Appendix S1: Fig. S5).

Convergence toward marine bacterial community during coalescence

The two axenic endmember water samples combined to create our Brackish (E_B) media, with intermediate chemical properties of the two endmembers. Substantial buffering by marine salts led to the Brackish (E_B) media with a pH closer to the marine endmember, but other chemical components were essentially the average of the two contributing media (Table 1).

Environmental perturbation.—Despite their higher diversity and biomass (Fig. 2A), the Freshwater (I_F) microbial taxa did not fare well when added to the Brackish (E_B) media in the absence of Marine community blending. Only 36 of the 967 total taxa initially sequenced from our Freshwater–Home (I_FE_F) microbiomes persisted following this environmental perturbation into Brackish (E_B) media, although 169 taxa that were below our detection (i.e., rare taxa) in the initial inoculum were detected in the Brackish (E_B) media (Fig. 4). We are confident that these taxa represent increases in abundance from the rare biosphere contained in the initial inoculum, as we failed to detect any genomic DNA in our negative controls. Marine microbial taxa were more tolerant of the Brackish treatment (I_ME_B), with 199 of the original 588 taxa surviving. A large number of rare biosphere taxa (393) emerged from the Marine inoculum under Brackish conditions. Consequently, the diversity of the Marine–Brackish (I_ME_B) microbiomes was equal to the starting inoculum (592 vs. 588), and there was a substantial loss of total richness for the Freshwater–Brackish (I_FE_B) microbiomes compared to the diversity of its initial inocula (I_F; 205 vs. 967; Fig. 4). The community composition of the Marine–Brackish (I_ME_B) replicates was very similar to the Marine–Home (I_ME_M), and the Freshwater–Brackish (I_FE_B) community shifted significantly in composition toward the Marine–Home (I_ME_M) relative to its initial Freshwater–Home (I_FE_F) composition (Fig. 2B). We detected more shared taxa among these environmentally filtered communities, with 65 overlapping taxa between Freshwater–Brackish (I_FE_B) and Marine–Brackish (I_ME_B), versus the 21 overlapping taxa found among the Freshwater (I_FE_F) and Marine (I_ME_M) Home microbiomes. For the low-diversity Freshwater–Brackish (I_FE_B) replicates, these shared taxa represent more than 25% of the total diversity, including the following families, which were below detection limit in Freshwater–Home (I_FE_F): *Alteromonadaceae*, Oceanospirillaceae, *Rhodobacteraceae*, and *Vibrionaceae* (Fig. 4). The enzymatic profile of each community followed similar trends, with the Marine–Home (I_ME_M), Marine–Brackish (I_ME_B) and Freshwater–Brackish (I_FE_B) replicates having reduced enzyme activity (Appendix S1: Fig. S5) and more similar enzyme profiles relative to the Freshwater–Home (I_FE_F) enzymes (Fig. 2C).

Biotic perturbation.—In contrast to the extreme loss of abundant taxa caused by abiotic perturbation, endmember community blending into our Brackish media (Brackish–Coalescence [I_CE_B]) had a more limited effect on microbial community structure (Figs. 2 and 4). Of the 967 Freshwater (I_F) taxa found in the initial Freshwater–Home (I_FE_F) treatment, 37 were detected in the Brackish–Coalescence (I_CE_B) treatment. This set overlapped entirely with the set of taxa that survived through the environmental filter, with the exception of a single taxon that disappeared in the Freshwater–Brackish (I_FE_B) treatment, but increased in abundance in response to the addition of an interacting community assemblage. There were 145 Freshwater (I_F) taxa that

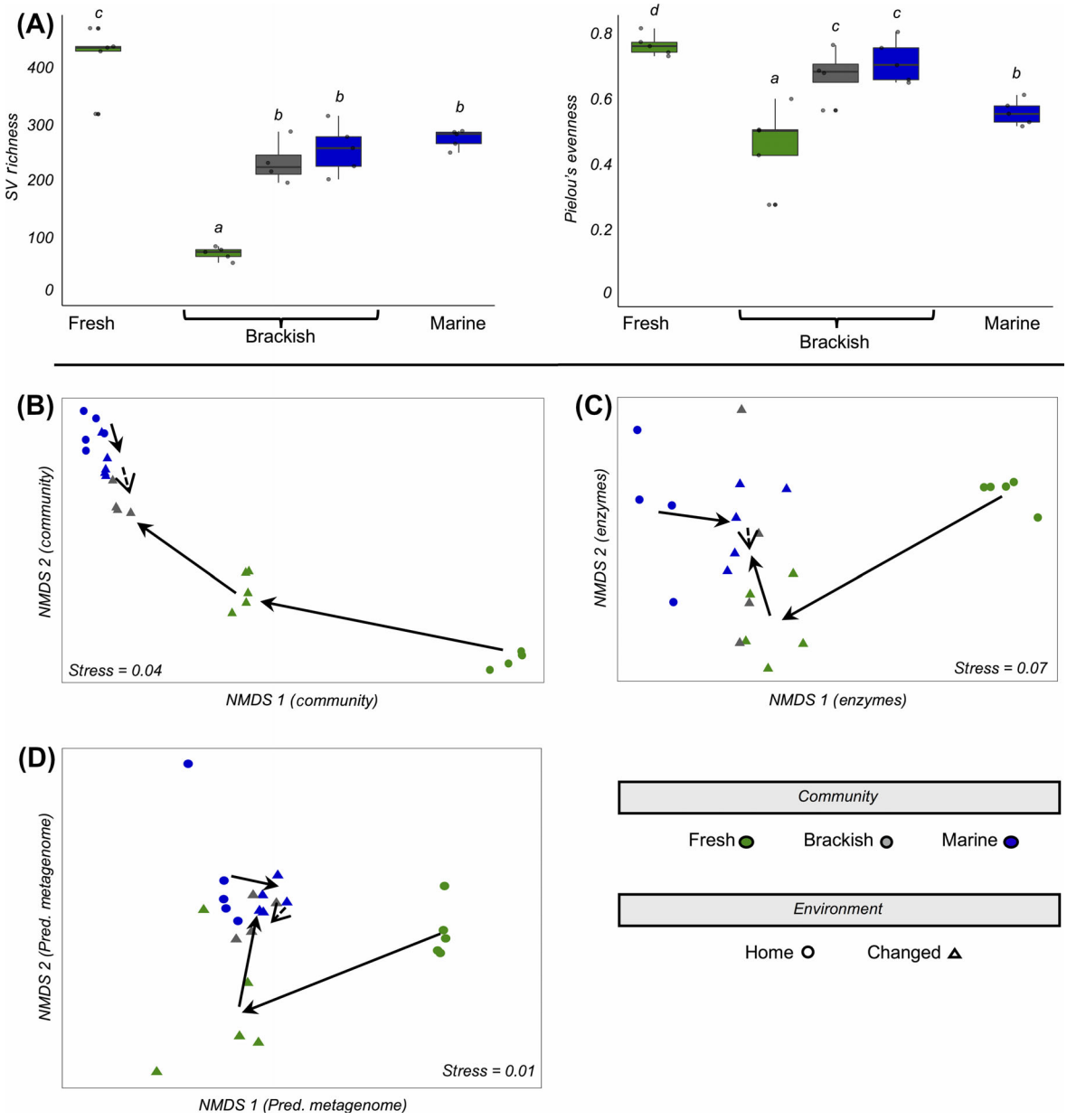

FIG. 2. Impact of coalescence on microbial community diversity, structure, and functional property: (A) alpha diversity of each treatment for sequence variant (SV) richness (left) and Pielou's evenness (right), and (B, C) nonmetric multidimensional scaling (NMDS) of (B) bacterial community structure; (C) microbial extracellular enzyme potential; and (D) predicted unstratified metagenomic profiles inferred from PICRUSt2. The NMDS arrows indicate movement through the filters relative to endmember control conditions, weighted by significance from Adonis (solid = significant change; dotted = NS). No community or functional data are available for one Brackish–Coalescent (I_CE_B) sample that was damaged during the incubation ($n = 4$, instead of experiment wide $n = 5$).

survived the Brackish (E_B) treatment but did not persist when in the presence of the new interacting microbiome (lost in the Brackish–Coalescence [I_CE_B] treatment). Of the 588 Marine taxa detected in the original Marine–Home (I_ME_M) treatment, 171 were detected in the Brackish–Coalescence (I_CE_B) treatment. This set of Marine survivors overlapped considerably with the list of taxa that were tolerant of the environmental filter (with 143 taxa found in both taxa lists). There were 28 Marine taxa (I_M) who only persisted in Brackish (E_B) media when also combined with the interacting microbiome (Brackish–Coalescence [I_CE_B]), and there were 302 Marine taxa that could survive the environmental filter (found in Marine–Brackish [I_ME_B]) but could not persist in the presence of the new blended microbiome (lost in Brackish–Coalescence [I_CE_B]).

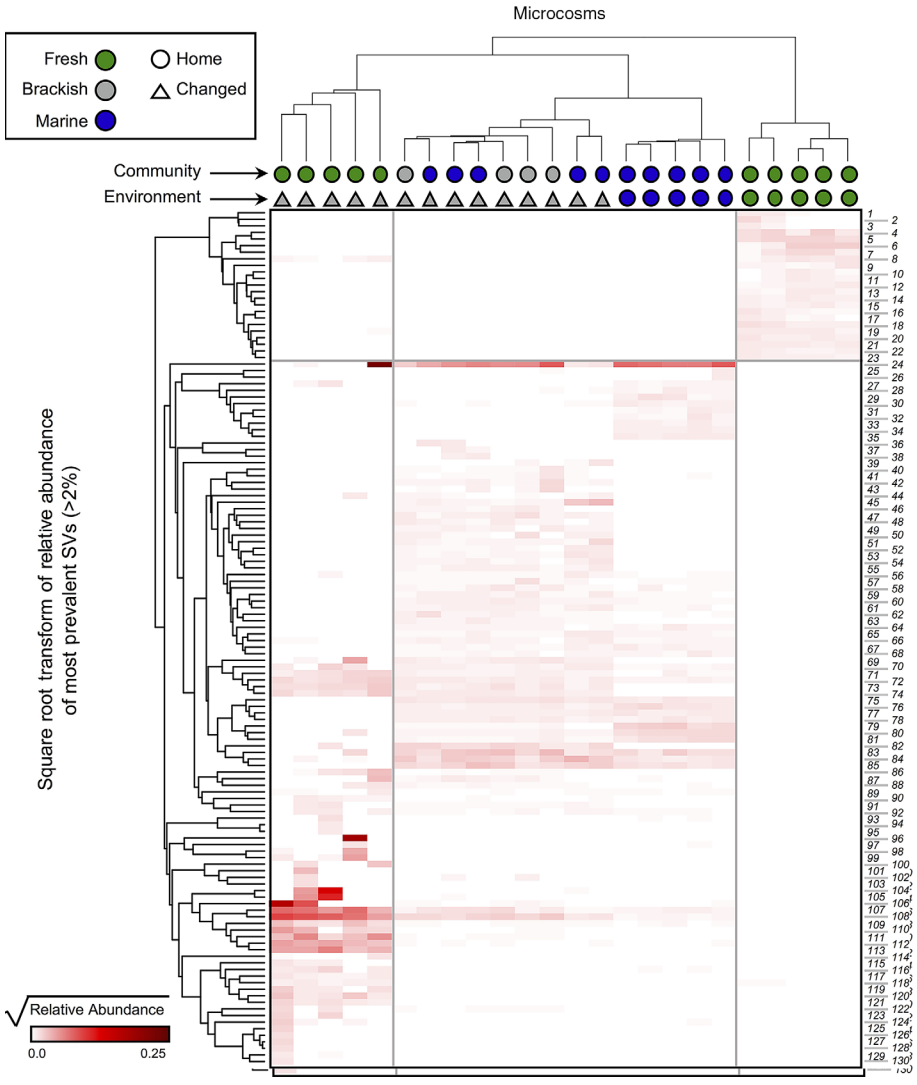

FIG. 3. Responses of the most abundant bacteria to community coalescence. Heat map of the most prevalent bacterial sequence variants (SVs; >2% relative abundance among all microcosms; square-root transformed), vertically clustered by SV (specific taxonomy in Appendix S1: Fig. S4), and horizontally by microcosm, identified by Environment (top row symbols) and Inoculum (bottom row). Gray-flanked regions identify the Freshwater (I_F) from the Marine (I_M) and Brackish–Coalescence (I_CE_B) conditions. Ranking estimated using standard UPGMA methods based on Bray-Curtis pairwise dissimilarities in Vegan R package.

We introduced at least 1,528 taxa from both endmember inocula into the Brackish–Coalescence (I_CE_B) treatments (this is the sum of the distinct taxa detected among the two endmember communities). Given the rare biosphere constituents detected in our environmentally filtered treatments, we likely added a further 495 taxa, for a total taxa pool of >2,000 microbial taxa. After coalescence, we detected only 472 taxa in the Brackish–Coalescence (I_CE_B) treatment. Although richness declined roughly to the level of the Marine–Home (I_ME_M), evenness was intermediate between the two endmembers, reflecting a shift in the shape of the dominance diversity curve (Fig. 4B). The loss of taxa was not symmetric: only 37 taxa from the original Freshwater (I_FE_F) microbiomes were detected after brackish exposure (I_FE_B), whereas 171 Marine microbial taxa survived. Ten of these "surviving" taxa were in common, all of which were members of the set of 21 taxa found in both original endmember inocula. One quarter ($n = 126$) of the taxa detected in the Brackish–Coalescence (I_CE_B) treatments were not observed in the Marine–Brackish (I_ME_B) or Freshwater–Brackish (I_FE_B) treatments, and thus we do not know from which endmember community they were derived. These rare biosphere constituents increased in relative abundance as a result of

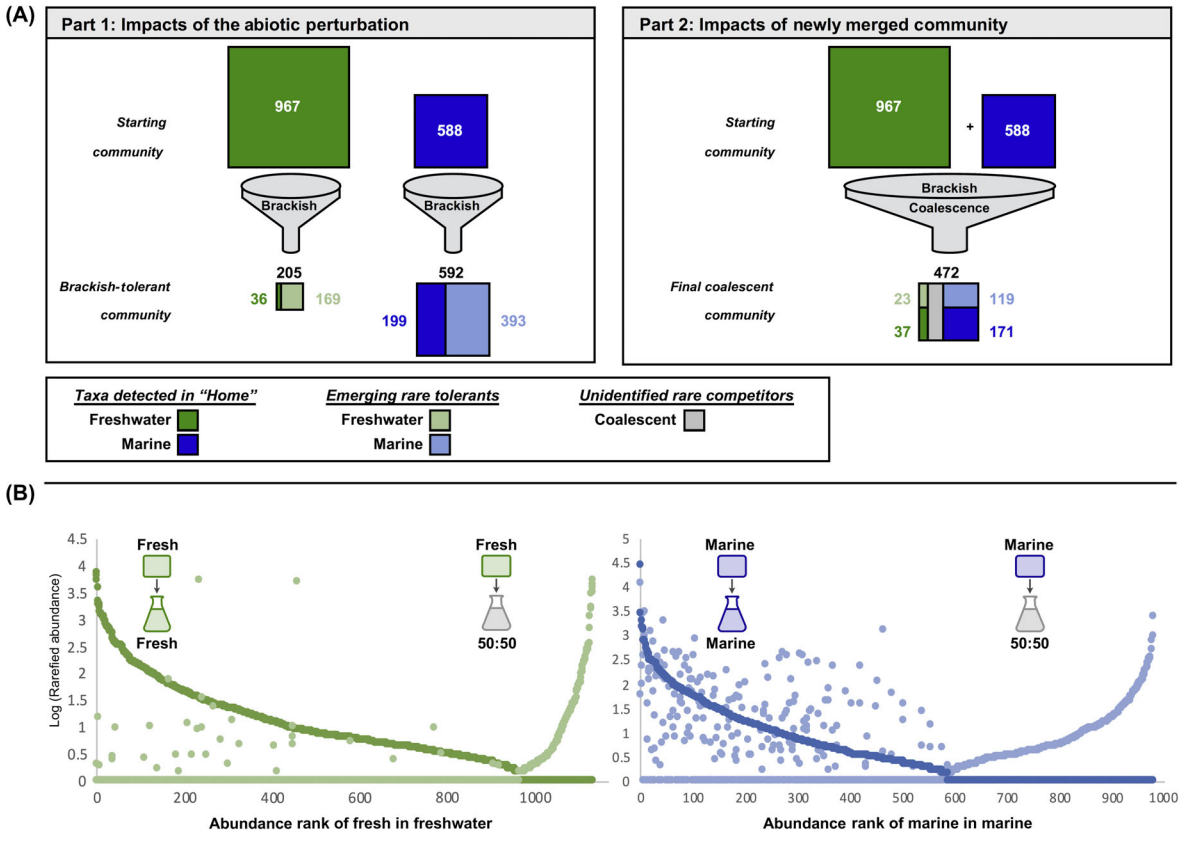

FIG. 4. Emergence of rare microbial taxa during experimental seawater intrusion. (A) Distribution of community origin due to each perturbation: Part 1: microbial taxa lost and gained due to Brackish exposure; and Part 2: microbiome response to merging with the Brackish–Coalescence (I_CE_B) treatments. The numbers inside or above each box represent the number of taxa (SVs) found among the microcosms of that treatment ($n = 5$). The numbers adjacent to the lower boxes represent the number of taxa detected in the original communities (solid) or detected as rare taxa emergence (striped), and the gray box represents rare emergence of unknown endmember origin. (B) Rank abundance curves of the natural log-transform of rarefied abundance of each taxa, ordered by "home" rank.

interactions between the endmember microbiomes under Brackish conditions.

The structure of the Brackish–Coalescence (I_CE_B) community overlapped almost entirely with the Marine endmember community (Fig. 2B). The Marine–Home (I_ME_M), Marine–Brackish (I_ME_B) and the Brackish–Coalescence (I_CE_B) communities were all dominated by *Alteromonas* (~50% of relative abundance; Fig. 3). Taxa that dominated the Freshwater microbiome were lost or dropped in abundance to below our detection limit (Figs. 35).

Enzyme profile and inferred metagenome: convergence on marine during coalescence

The enzymatic response followed a trend similar to that of the community structure. In fact, we found that the bacterial community structure and the extracellular enzyme profiles were strongly correlated (Procrustes, $R^2 = 0.69$, $P < 0.01$). The enzymatic profile for the Brackish–Coalescence (I_CE_B) microcosms was indistinguishable from the Marine–Brackish (I_ME_B) and slightly different from the Marine–Home (I_ME_M) enzyme profile, and significantly different from the enzymes of both the Freshwater–Home (I_FE_F) and Freshwater–Brackish (I_FE_B) treatments (Fig. 2C). Additionally, the inferred metagenome showed significantly similar patterns to the community structure (Procrustes, $R^2 = 0.64$, $P < 0.01$) and enzyme potential (Procrustes, $R^2 = 0.77$, $P < 0.01$) as well (Fig. 2D), with convergence toward the inferred metagenome of the marine home microbiomes.

When examining the response of each extracellular enzyme activity under the different treatments, we found that most converged on an endmember community function (Freshwater [I_FE_F] or Marine [I_ME_M] controls) and one exhibited stimulated activity relative to endmember community function. Four various enzymes involved in C, N, P, and S cycling (AS, BG, NAG, and PHOS) had the highest activity in the Freshwater conditions and were significantly suppressed (i.e., lower than average of the two end-point communities) under the coalescence

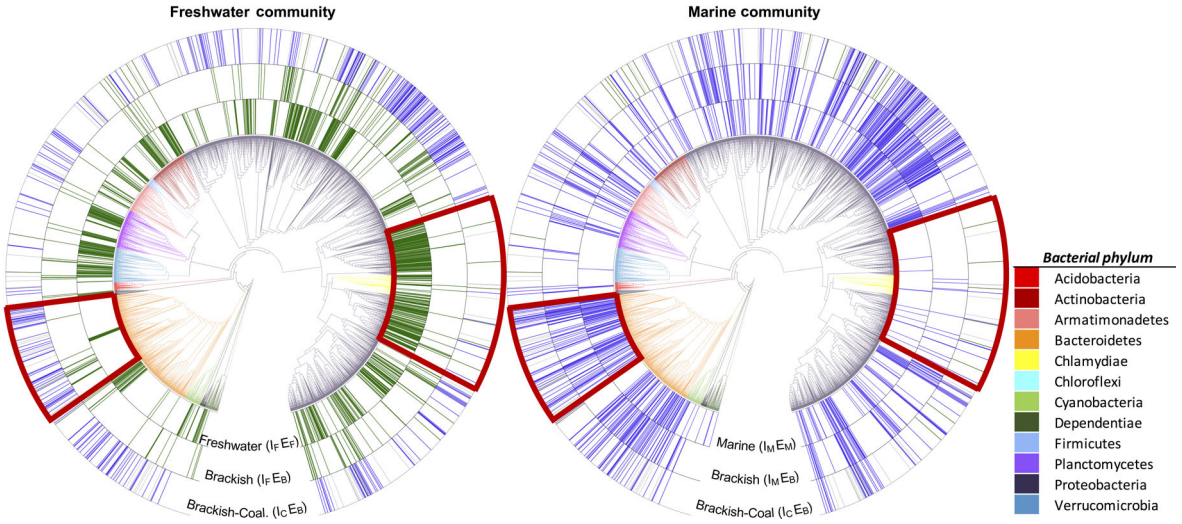

FIG. 5. Community phylogenetic response to environmental filtering and to novel biotic interactions for Freshwater (left) and Marine (right) microbiomes of individual taxa The inner ring shows the distribution of microbial taxa present in "home" conditions, the middle ring represents the distribution of microbial taxa surviving the Brackish environment, and the outer ring displays all taxa surviving to Brackish–Coalescence (I_C E_B). Taxon presence is represented by colored ticks: green (Fresh-derived taxa), blue (Marine-derived taxa), or gray (unknown origin in outer coalescence rings). The red boxes highlight example regions where the Fresh and Marine community diversity is initially distinct and converges toward Marine under Brackish environmental conditions.

treatments with levels that were not significantly different from the Marine conditions (Appendix S1: Fig. S5B, C, F, G). However, the activity of two carbon-degrading enzymes (AG and CB) in the coalescence treatments were more comparable to those of the Freshwater enzyme activity (Appendix S1: Fig. S5A, D), and XYL activity (Appendix S1: Fig. S5H) was not significantly impacted by environment treatment. Finally, the LAP enzyme (Appendix S1: Fig. S5E) exhibited similar activities with Freshwater and Marine end-point conditions and tended to be stimulated under the coalescence conditions.

Phylogenetic response to coalescence varies by endmember community

Taxa that were lost and gained from the Marine microbiome in response to environmental filter and to the biotic filter were more phylogenetically similar (Fig. 5, Appendix S1: Fig. S6). A sensitive taxon lost because of filtering was typically replaced by an increase in abundance of a tolerant sister taxa in the Marine microbiomes. In contrast, whole classes and orders of the Freshwater microbial community were lost and gained as a result of these two filters. The phylofactorization of the Freshwater (I_F) and the Marine (I_M) microbiomes in home vs. Brackish (E_B) media exposure confirmed these community shifts. For Freshwater (I_F) microbiomes, phylofactor identified six large, nonoverlapping bacterial clades (from nine phylogenetic factors) exhibiting significant changes in relative abundance with Brackish (E_B) exposure (Appendix S1: Fig. S6), with an average clade size of 91 species and 548 total species among all six clades. All but one of these phylogenetic factors showed an increase in the relative abundance of a bacterial clade relative to those exposed to Brackish (E_B) media (Appendix S1: Fig. S6). In contrast, there were four smaller Marine clades (from five phylogenetic factors) with an average size of 64 species and 255 total species that showed significant changes in the relative abundance in response to Brackish (E_B) conditions (Appendix S1: Fig. S6).

DISCUSSION

Where Freshwater meets Marine water and their microbial communities collide, both abiotic and biotic factors appear to be important drivers of which microorganisms survive in the new Brackish environment. When transplanted from their home environments into the Brackish (E_B) media, both communities lost >70% of all detectable taxa in the initial inoculum and home conditions. This was somewhat counterbalanced by the emergence of a rare biosphere from each microbiome, which accounted for 66% of the Marine microbiome and 82% of the Freshwater microbiome in the Brackish environment. The rare biosphere emergence stabilized Marine microbial richness to just over 100% of original richness. In contrast, Freshwater microbiome richness in Brackish conditions was only 21% of original richness, despite rare biosphere emergence. This emergence of the rare biosphere during Brackish exposure supports Paver et al. (2018), Sjöstedt et al. (2012), and Logares et al. (2009), who show that certain rare microbial taxa that possess wider salinity tolerance ("crossing the salty divide") may also be uniquely adapted to proliferating in

these intermediate environmental conditions. The biotic filter also imposed taxonomic shifts for both communities when added in combination to the Brackish arena. Under this Brackish–Coalescence (I_CE_B) treatment, a further 70.7% of the initial Freshwater inocula and 51% of the Marine inocula were not detected. Additionally, nearly 25% of the taxa present in the Coalescence conditions were below detection limit in either Brackish conditions or the home communities. This 25% emergence represents taxa benefiting from the combination of conditions imparted by the coalescence of Fresh (I_F) and Marine (I_M) microbiomes into Brackish (E_B) conditions. Taken together, these patterns of substantial loss of specialists and counterbalancing of emergent rare taxa explain the community convergence on the Marine microbiome.

Our study also demonstrates a clear link between microbial community structure and functional potential, with strong overlap in patterns of the bacterial community structure and the enzyme profiles. The enzymatic profiles of all three Brackish-exposed communities converged toward that of the Marine enzyme profile. The eight microbial enzyme activities measured spanned a large variety of processes involved in the mineralization of C, N, P, and S compounds, all significantly affected (stimulated or suppressed) by the coalescence perturbations relative to home. Half of the activities were strongly suppressed in the Brackish (E_B) media, in part because of the overall lower activity of most of the enzymes in the Marine compared to Freshwater and to the dominance of the Marine microbiome in these merged communities. The most severe dampened activity was observed for the alkaline phosphatase (PHOS), which is a curious result, given the alkaline pH of the Brackish (E_B) media and high efficiency of alkaline phosphatase under high pH. However, there may be enough bioavailable mineralized phosphorus in the Brackish (E_B) media to fulfill microbial phosphorus requirements without requiring additional enzymatic degradation of phosphorus. Interestingly, leucine aminopeptidase activity (LAP) showed increased activity in the Brackish treatments compared to the levels predicted by a simple average of the Marine and Freshwater communities' activities. LAP is a common protein degrading extracellular enzyme, which was twofold higher in the 50:50 Brackish (E_B) environment than in the two endpoint treatments. This activity is known to be extremely variable and highly susceptible to shift in species composition of bacterial assemblages, with single bacterial taxa potentially explaining the seasonal dynamic of this activity (Caruso and Zaccone 2000), which may explain the pattern observed here. Despite complex enzyme responses in our study, one largely consistent pattern was that individual enzymes showed equivalent activities within the Brackish (E_B) environment. When we exposed the distinct Freshwater (I_F) and Marine (I_M) microbiomes to the Brackish (E_B) environment, each community shifted in response to the perturbation and enzyme activities converged to the same level regardless of community, which supports functional redundancy.

The responses of individual taxa were also significantly different among the two endmember communities in our coalescence experiment. Although both endmember communities were significantly distinct from their coalescent counterparts, only the Freshwater community had a significant reduction in taxa richness as a result of environmental filtering (Fig. 2C). The Marine microbiome is, on the whole, more capable of dealing with the intermediate Brackish conditions for two main reasons: resistance of at least a third of the community to a range of salinity and nutrients, and resilience because of the high community buffering capacity of the Marine rare biosphere. However, despite the substantial loss to the Freshwater (I_F) community under Brackish (E_B) conditions, the surviving Freshwater-derived taxa still managed to maintain equivalent enzyme activity to the other Brackish-exposed microcosms. The link between community structure and function may hinge on the dominant taxa in more stable environments, but functional stability at aquatic confluences with varying environmental conditions may be determined by conditionally rare members of the community (Jia et al. 2018). The prevalence of Marine rare Brackish tolerant taxa and consequential convergence toward the Marine microbiome is perhaps expected, given the high physiological threshold of marine microbial taxa to a wide range of salinities (del Giorgio and Bouvier 2002, Wu et al. 2006, Herlemann et al. 2011). As we lose sensitive dominant taxa, we sample the rare biosphere more deeply. The rich "microbial seed bank" buffers fluctuations in species richness (Lennon and Jones 2011, Jousset et al. 2017), which may help explain the limited functional change in this common garden experiment.

These two unique microbial communities show distinct phylogenetic responses to the environmental filters imposed by being transplanted into Brackish (E_B) media. We saw that several large, sensitive Freshwater clades were lost, and multiple tolerant Freshwater clades become abundant enough to detect. In contrast, in our Marine community, the turnover was at a finer taxonomic resolution, with a loss and gain of sister taxa restricted to more terminal nodes. The replacement with sister taxa may implicate niche partitioning along a salinity gradient as a driver of the evolutionary divergence of these saltwater sister taxa, though more careful examination is needed to test or corroborate this hypothesis. The composition of experimental replicates was remarkably similar, indicating that there are real differences in the ability of microbial taxa to survive transplant into altered salinities and that community composition responses are predictable. Community composition converged under Brackish conditions because both endmember microbiomes contained reservoirs of tolerant rare taxa that increased in abundance when exposed to the intermediate environmental condition. The shift in composition toward the Marine microbiome

resulted from a greater reservoir of tolerant taxa within that community. The evidence for this is both the compositional shift revealed through ordination and also the fact that the loss of initially detected taxa is not accompanied by a decline in species richness for the Marine microbiome.

From an applied microbiology perspective and the natural history of microorganisms, learning which taxa are lost to environmental and biotic filtering will be instructive and useful for microbial engineering of "wild" unculturable microbial taxa (Libby and Silver 2019). Furthermore, the consistency of how microbial community composition responds to experimental treatments suggests that exposure of microbial communities to perturbations may prove quite useful in strategically identifying sensitive and tolerant taxa along many different real environmental gradients (Rocca et al. 2019). Over time, such information could lead to the development of "microbial sensors," in which microbial community composition could be used to draw inferences about environmental conditions. By better understanding the resultant microbial community structure and function when microbial worlds collide, we can modulate microbial communities more effectively in applications such as agricultural efficiency by microbial consortia (Busby et al 2017), bioremediation (Baez-Rogelio et al 2016), industrial application (Sierocinski et al. 2017), or biomedical microbial transplants (Gibbons et al. 2017).

Our experiment also raises many new fundamental questions about the environmental and biotic processes that structure the microbiome. Because we observed compositional shifts at very fine levels of taxonomic resolution (~sister taxa) for our Marine microbiome when exposed to Brackish conditions, we speculate that biotic interactions are far more important within this community. Why this might be is an interesting question for microbial ecology. Our endmember microbiomes are nearly nonoverlapping and derived from environments with very distinct biogeochemistry. Consequently, the relative importance of the environmental perturbation is not unexpected, as the intermediate environment is substantially different from either "home," and strong biotic interactions should be minimal among communities with no prior blending. We acknowledge that our study does not examine the biotic influence of viruses and microbial eukaryotes, even though our inocula included such groups, and presents an intriguing opportunity for understanding to what extent these cross-domain biotic interactions impact the outcome of community coalescence. Finally, if our conceptual framework (Fig. 1) is applied to more similar endmembers (e.g., freshwater–brackish coalescence), the biotic component may become more important during community coalescence, as the intermediate environment is chemically closer to each "home" condition, and community members have more niche overlap, resulting in stronger biotic interactions. The impact of cross-domain interactions and the influence of starting-community similarity are exciting avenues for future research.

Conclusions

Our experimental approach to the study of microbial coalescence provides one of the first demonstrations to compare the relative strength of the environmental and biotic components during community coalescence directly and separately. In the case of mixing Fresh and Marine waters, the Brackish intermediate condition proved to be a very strong environmental filter that had a greater effect on the Freshwater microbiome than on its Marine counterpart. Applying this technique to other gradients may reveal cases in which biotic interactions dominate. Collectively, the use of this approach to detect the phylogenetic distribution of sensitivity and tolerance to various environmental gradients is likely to help us rapidly advance our ecological understanding of why microbial taxa live where they do.

Acknowledgments

We thank the Timberlake Restoration Project and Cape Hatteras National Seashore for access to protected areas for obtaining our water samples. We appreciate the generous assistance of Norman Hill, Todd Smith, and Greg Piotrowski of the Duke Phytotron for help with our incubation setup; and several anonymous reviewers for their helpful feedback on earlier drafts. This material is based on research funded through a Duke University startup fund awarded to Professor Diana Nemergut, and by a grant from the National Science Foundation (DEB-1754512).

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Supporting Information

Additional supporting information may be found in the online version of this article at http://onlinelibrary.wiley.com/doi/10.1002/ecy.2956/suppinfo

Data Availability

Data are available from the NCBI Sequence Read Archive under PRJNA589904.