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Journal

PNAS Nexus, 2(9)

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Publication Date

2023-09-01

DOI

10.1093/pnasnexus/pgad287

Peer reviewed

Microorganisms and dissolved metabolites distinguish Florida's Coral Reef habitats

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Edited By: Stephen Palumbi

Abstract

As coral reef ecosystems experience unprecedented change, effective monitoring of reef features supports management, conservation, and intervention efforts. Omic techniques show promise in quantifying key components of reef ecosystems including dissolved metabolites and microorganisms that may serve as invisible sensors for reef ecosystem dynamics. Dissolved metabolites are released by reef organisms and transferred among microorganisms, acting as chemical currencies and contributing to nutrient cycling and signaling on reefs. Here, we applied four omic techniques (taxonomic microbiome via amplicon sequencing, functional microbiome via shotgun metagenomics, targeted metabolomics, and untargeted metabolomics) to waters overlying Florida's Coral Reef, as well as microbiome profiling on individual coral colonies from these reefs to understand how microbes and dissolved metabolites reflect biogeographical, benthic, and nutrient properties of this 500-km barrier reef. We show that the microbial and metabolite omic approaches each differentiated reef habitats based on geographic zone. Further, seawater microbiome profiling and targeted metabolomics were significantly related to more reef habitat characteristics, such as amount of hard and soft coral, compared to metagenomic sequencing and untargeted metabolomics. Across five coral species, microbiomes were also significantly related to reef zone, followed by species and disease status, suggesting that the geographic water circulation patterns in Florida also impact the microbiomes of reef builders. A combination of differential abundance and indicator species analyses revealed metabolite and microbial signatures of specific reef zones, which demonstrates the utility of these techniques to provide new insights into reef microbial and metabolite features that reflect broader ecosystem processes.

Keywords: coral reefs, metagenomics, metabolomics, Florida's Coral Reef

Significance Statement

Microorganisms and the dissolved metabolites they process are central to the functioning of ocean ecosystems. These “invisible” ocean features are poorly understood in biodiverse and productive coral reef ecosystems, where they contribute to nutrient cycling and signaling cues among reef organisms. This study demonstrates that reef water microbes and metabolites successfully distinguish reef habitats, including geographical, reef compositional, and environmental features, and together they provide novel insights into reef ecosystem characteristics. Microbes and dissolved metabolites offer a new means to examine reef features and have applications for conservation, monitoring, and restoration efforts in these changing ecosystems.

Introduction

Coral reefs, one of the most biodiverse and economically valuable ocean ecosystems, are dynamic habitats that are impacted worldwide by climate change and other human pressures. Coral reef ecosystems have responded positively and negatively to disturbances through time (1). In the past 50 years, however, hard coral cover, a metric that represents the percentage of benthic habitat

these keystone species encompass, has been declining worldwide. This includes 20–30% losses in Caribbean reefs (2, 3), 50% in the Great Barrier Reef (4), and as high as 92% losses in Florida's Coral Reef (FCR) (5–7). Although reef environments are declining, we are only beginning to understand the value of the innumerable microbial and chemical components of coral reefs that interact to support the most biodiverse ocean ecosystem.

Competing Interest: The authors declare no competing interest.

Received: March 20, 2023. **Accepted:** August 24, 2023

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Central to the success of reef ecosystems are the invisible microbial and chemical dynamics that play a major role in reef processes, such as settlement (8–10) and coral growth and health (11–15). Detrimental microbial and chemical dynamics may even be central to reef decline, as exemplified by the microbialization hypothesis, in which algal-exuded dissolved organic carbon promotes growth of pathogenic microorganisms, which in turn threaten corals and other reef organisms (16). Although microorganisms and chemicals are emerging as critical components for the success or failure of reefs, monitoring programs generally do not incorporate these parameters, due to the lack of scientific evidence documenting their utility as well as the high levels of cost and expertise needed to evaluate them. Therefore, integrative studies that employ a suite of chemical and microbial comparisons are critical for understanding how these parameters may differentiate reef habitats and reflect ecological dynamics.

Reef microorganisms and dissolved chemicals, or metabolites, are well-poised to serve as sensors of reef ecosystem dynamics (17, 18). The production of dissolved metabolites within reef seawater is species-specific, with corals producing diverse nitrogen and phosphorus-based compounds and macroalgae yielding prenol lipids, steroids (19), and organic compounds enriched in neutral sugars (20). Additionally, coral host metabolomes are further influenced by metabolites derived from algal symbionts (21). Collectively, these organism-specific signals make up the metabolome signature of a reef, which differs from open-ocean waters (22). In seawater overlying a reef, microorganisms are a primary consumer of metabolites, and while certain lineages of microorganisms are enriched in response to species-specific benthic exudates (20, 23, 24), we are only beginning to understand which individual metabolites may be responsible for such enrichment (25). Studies have documented relationships between reef microbial composition and reef parameters, including hard coral cover (26, 27), temperature (28, 29), chlorophyll (28), organic carbon (29), biogeography (27, 30), and reef protection (31). To date, most investigations study either metabolites or microbes in isolation (16, 19, 22), though studies are increasingly combining techniques (32, 33). A direct comparison of reef water metabolites and microorganisms is needed to inform the individual or collective value of these factors within reef habitats.

FCR is an ideal environment for examining reef microbial and metabolic characteristics within the context of broader reef processes. Decades of research have demonstrated biogeographic reef zones partitioning the system, largely driven by reef hydrography, with the Gulf of Mexico Loop Current and meandering Florida Current producing many gyres and eddies (34–36). These currents interact with tidal mixing from Florida Bay, especially in the Lower and Middle Keys, but less so in the Upper Keys, which are more isolated from Florida Bay water, contributing to zonal patterns of nearshore nutrients and organic matter (37). For example, the Upper, Middle, and Lower Keys zones vary and are distinct from more offshore reef zones, such as the Marquesas and Dry Tortugas, in terms of, but not limited to, total organic carbon (TOC), nutrients, and turbidity (38). Geological evidence also reflects these regional patterns, with historical reef accretion and present-day coral calcification rates higher in the remote Dry Tortugas zone compared to other zones within the Florida Keys (39, 40). Presently, FCR coral communities are facing the highly contagious stony coral tissue loss disease (SCTLD), a devastating disease that has already led to the regional extinction of the pillar coral, *Dendrogyra cylindrus*, in Florida (41, 42). This years-long disease outbreak is likely caused by a bacterial pathogen(s) (43–45), and although microbial and metabolomic indicators have been

identified (21, 46–50), the causative agent remains elusive. This disease, along with climate pressures causing unprecedented levels of warming leading to bleaching and mortality (51), is leading to increased homogenization on the reefs (52), though some zonation within the benthic community persists (53, 54). Considering the recent stressors to the FCR, especially SCTLD, examinations of microorganisms and metabolites have the potential to add significant value to the breadth of historic and present research on FCR reef ecology.

To better understand the metabolite and microbial landscape of FCR and the potential utility of these invisible sensors to reef ecosystem dynamics, we leveraged a cruise of opportunity on OceanX's M/V *Alucia* during June 2019 to study 85 reefs dispersed along the 500-km reef tract. We examined if and how reef water microbes and metabolites differentiate each reef within the context of geography, reef benthos, disease, and environmental parameters, using four omic approaches (taxonomic microbiome via amplicon sequencing, functional microbiome via shotgun metagenomics, targeted metabolomics, and untargeted metabolomics). We also conducted microbiome profiling via amplicon sequencing of near-coral seawater and coral hosts. Our results demonstrate that microbes and metabolites successfully distinguish between individual reefs according to biogeochemical zones and that their combined use provides novel insights into reef ecosystem parameters that may benefit monitoring, conservation, and restoration activities in these important ecosystems.

Results

This comparative omic study was conducted over 15 days in June 2019 along FCR. At 13 geographically dispersed reefs, we collected seawater (1.7 L in biological triplicates) from within 0.5 m of the reef benthos for both targeted and untargeted metabolome analyses (Fig. 1a). At 27 reefs, including the 13 examined for metabolomics, we collected benthic seawater (2 L in duplicate) for taxonomic (bacteria and archaea 16S ribosomal RNA gene) and functional (shotgun metagenome) microbiome analyses and chlorophyll (Fig. 1a). Given the active SCTLD outbreak, we also targeted apparently healthy and diseased coral tissue and near-coral seawater within 1–3 cm from the coral for taxonomic microbiome (16S rRNA gene) analysis (11 reefs). To comprehensively assess the reef habitat, at 85 reefs (Fig. 2a), we measured prevalence of SCTLD, nutrients (TOC, total organic nitrogen [TON], and inorganic nutrients), and abundances of microbial functional groups (*Prochlorococcus*, *Synechococcus*, picoeukaryotes, and heterotrophic microbes [unpigmented bacteria and archaea]), from reef depth waters (Fig. 2a). Of the 85 reefs, high-resolution photomosaics were taken at 45 reefs to examine the composition of benthic organisms (Fig. S1). Detailed methods and information on these data sets can be found in the Methods S02.

Four high-resolution omic data sets were produced from the benthic seawater samples. The reef water taxonomic microbiome (27 reefs) approach yielded $43,118 \pm 6,834$ sequences per sample and 468 total amplicon sequence variants (ASVs, similar to microbial species-level designations (55)), with 110–191 ASVs per sample (see Methods S02). The functional microbiome (metagenomics, 27 reefs) effort produced 13.6 ± 3.4 M paired-end reads per sample, which were assembled into contigs and annotated and mapped to 592,912 bacterial gene annotations. Targeted metabolomics (13 reefs) was applied to the reef water, with samples analyzed via ultrahigh-performance liquid chromatography coupled to a heated electrospray ionization source (H-ESI) and triple stage quadrupole mass spectrometer. This approach

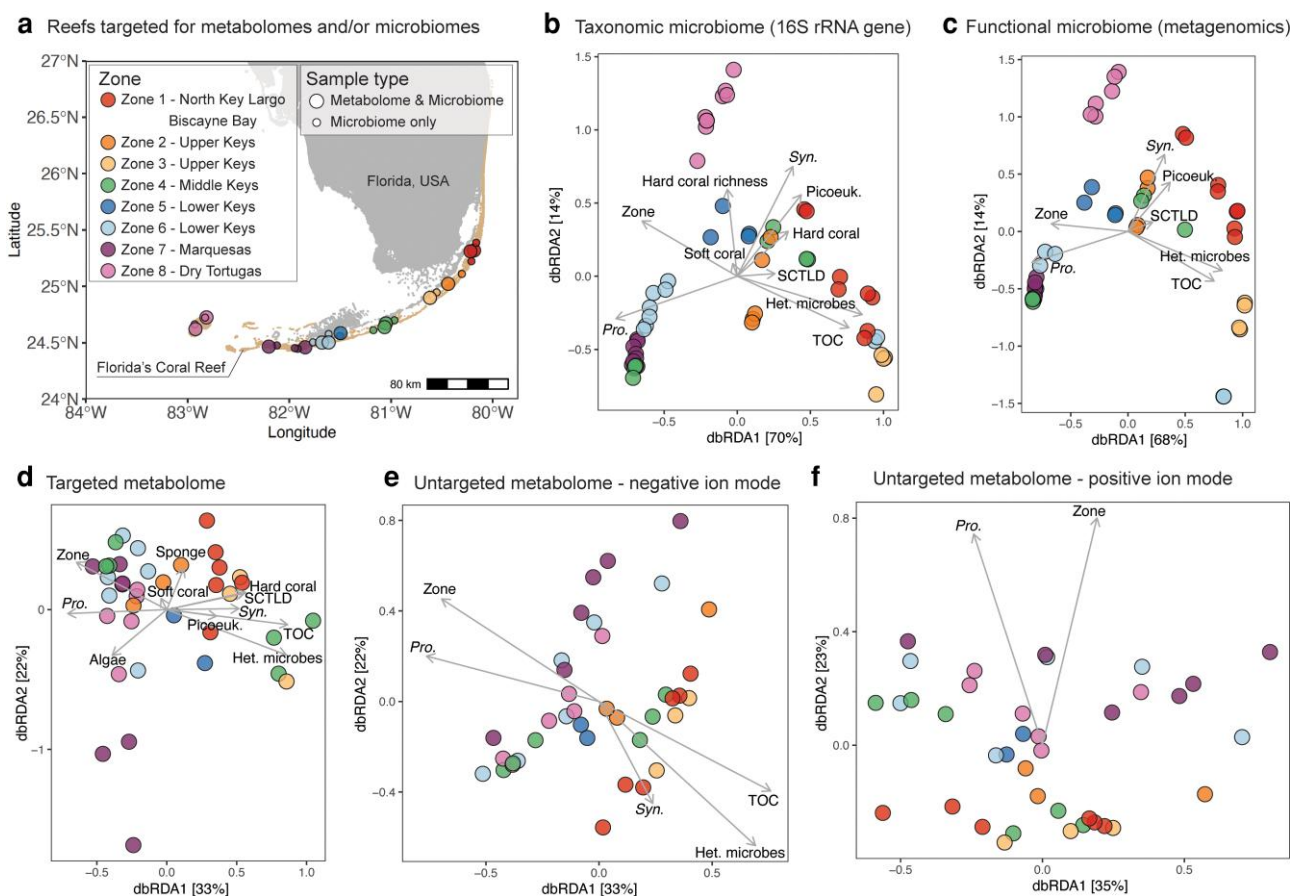


Fig. 1. dbRDA reveals seawater microbiomes and metabolomes are significantly explained by Florida's Coral Reef biogeography and measured reef microbial and environmental parameters (ANOVA on dbRDA model, $P < 0.05$). (a) Map of 27 coral reefs (out of 85 reefs visited in total) sampled across eight zones for either both seawater metabolomes and microbiomes (13 reefs) or only seawater microbiomes (27 reefs total) during June 2019. The dbRDA include (b) reef water taxonomic microbiome via 16S rRNA gene sequencing of bacteria and archaea, (c) functional microbiome via shotgun metagenomics, (d) targeted metabolomes, (e) untargeted metabolomes that ionized in negative mode, and (f) untargeted metabolomes that ionized in positive mode. Syn., *Synechococcus*; Pro., *Prochlorococcus*; Picoeuk., picoeukaryotes; Het. Microbes, heterotrophic microbes.

quantified 39 environmentally relevant dissolved metabolites in the samples. The untargeted metabolomic approach analyzed extracts via ultrahigh-performance liquid chromatography system coupled to an Orbitrap Fusion Lumos Tribrid mass spectrometer and ionized chemical features in two modes, positive and negative (see [Methods S02](#)). After removing features associated with blanks and with low variance across samples, 2,759 features remained in the positive ion mode data set, and 1,428 features remained in the negative ion mode data set.

Microorganism and metabolite compositions reflect zonal habitat patterns

Analysis of seawater omic data sets (taxonomic and functional microbiome and targeted and untargeted metabolome) via ordinations revealed significant clustering of all data types according to biogeographic reef zone (depicted via colors in [Fig. 1](#)). A distance-based redundancy analysis (dbRDA) followed by an ANOVA revealed that biogeographic reef zone was the most significant parameter in structuring the composition of seawater omic data sets ([Table 1](#); Pseudo-F values indicate significance). For untargeted metabolomes with triplicate biological replication per reef, reef structured the chemical composition to a greater extent than zone, which contained multiple reefs, though both were significant ([Fig. S2](#); permutational multivariate ANOVA [PERMANOVA] results in [Table S1](#)).

We further demonstrated that benthic, geographic, and environmental parameters significantly structured the seawater microbiome and metabolome compositions (PERMANOVA for dbRDA with Bray-Curtis dissimilarity, $P < 0.05$; [Fig. 1](#) and [Table 1](#)). Reef zone and *Prochlorococcus* abundance structured all omic data types. In the microbiome analyses, zone and *Prochlorococcus* explained between 12.6–16.7% and 9.5–12% of the variance, respectively, and the variance explained was lower in the metabolome analyses ([Table 1](#), adjusted R^2). TOC, heterotrophic microbes, and *Synechococcus* explained changes in the microbiome as well as in the targeted and negative mode untargeted metabolome, with heterotrophic microbial abundances explaining the most variation (15.6–18.7% in microbiome analyses and 6.0–7.3% in metabolome analyses) ([Fig. 1](#) and [Table 1](#)). Significant structuring by other parameters varied by omic data type, with the targeted metabolome significantly structured by all tested parameters ([Fig. 1](#) and [Table 1](#)).

Dry Tortugas National Park (zone 8) was distinctly clustered from zone 1 to zone 7 in seawater taxonomic and functional microbiomes ([Fig. 1b](#) and [c](#)). Environmental parameters were also different at Dry Tortugas, which harbored significantly lower prevalence of SCTL, seawater TOC, heterotrophic microbial abundances, ratio of heterotrophic microbes:photosynthetic microbes, and cover of turf algae compared to zones 1–7. In contrast, *Synechococcus* abundances were higher at zone 8 compared to

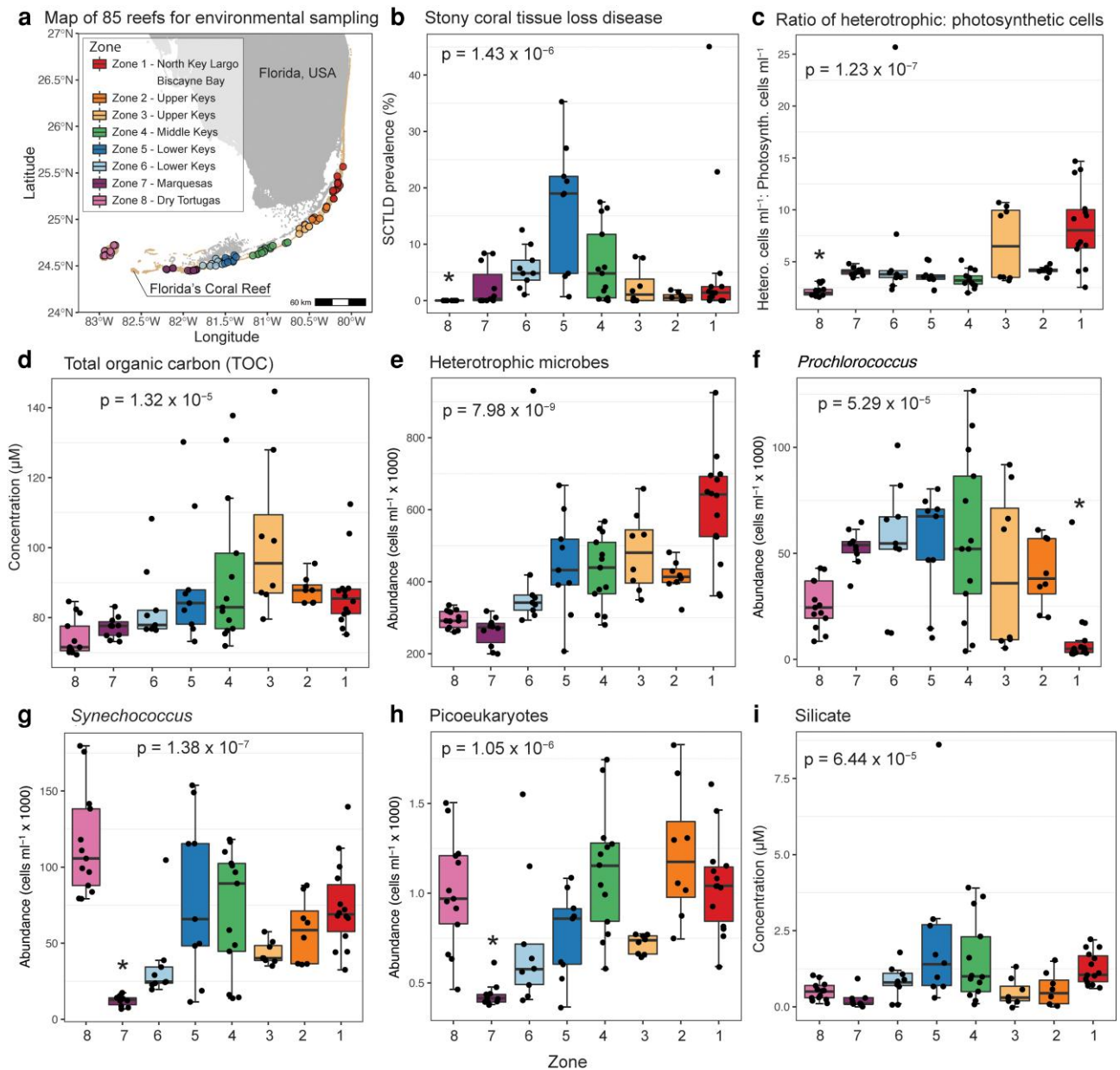


Fig. 2. Zones in FCR have significantly different disease prevalence, microbial abundances, and organic carbon and silicate concentrations. (a) Map of all 85 reefs sampled for environmental variables are oriented by zone. Environmental variables that changed significantly by zone include (b) SCTLD prevalence (percent of live coral), (c) ratio of heterotrophic to photosynthetic microbial cells, (d) total organic carbon concentration, (e) heterotrophic microbial abundances (unpigmented bacteria and archaea), (f) *Prochlorococcus* abundances, (g) *Synechococcus* abundances, (h) picoeukaryote abundances, and (i) silicate concentration that was significantly different between FCR zones as a result of a Kruskal–Wallis test and less than the Bonferroni-corrected P -value of 0.00192. Asterisks denote the zone was significantly different than all other zones tested in the pairwise Wilcoxon rank sum test (Benjamini–Hochberg adjusted $P < 0.05$).

other zones (Wilcoxon rank sum test with Bonferroni-corrected $P < 0.001667$; Fig. S3).

Benthic components significantly explained differences in both the targeted metabolomes (soft coral, hard coral, sponge, and algae cover) and taxonomic microbiomes (soft and hard coral cover and hard coral richness) (Fig. 1b and d and Table 1). Unlike reef seawater omic data, benthic reef composition was not significantly structured by zone (PERMANOVA on principal components analysis; Fig. S1b). Individual tests of zonal changes in hard coral and algal cover, as well as hard coral species richness, common metrics used to assess reef quality and coral diversity, changed slightly but were not significantly different (Kruskal–Wallis rank sum test with Bonferroni-corrected $P > 0.00192$; Figs. S4 and S5). In

contrast, hard coral species composition alone was significantly different by zone, with Dry Tortugas National Park (zone 8) clustered separately from other reefs (PERMANOVA, $P < 0.05$; Fig. S5). Distance to shore and percent cover of algae were both significantly negatively correlated with coral cover (linear regression, $P < 0.05$; Figs. S6 and S7).

Zonal changes were evident in many environmental parameters measured at 85 reefs across eight zones (Kruskal–Wallis test with Bonferroni-corrected $P < 0.00192$; Fig. 2). The ratio of heterotrophic microbes:photosynthetic microbes, TOC concentrations, and heterotrophic microbial abundances all increased north-eastward across reefs from zone 8 to zone 1 (Fig. 2c–e). SCTLD prevalence generally peaked in zones 4–6 (Fig. 2b), zones that

Table 1. Reef benthic and environmental characteristics significantly explained reef seawater omics.

PERMANOVA ^a term	Microbiome						Metabolome								
	Taxonomic (16S rRNA gene)			Functional (metagenomics)			Targeted			Untargeted negative			Untargeted positive		
	F ^b	R ^{2c}	P-value	F ^b	R ^{2c}	P-value	F ^b	R ^{2c}	P-value	F ^b	R ^{2c}	P-value	F ^b	R ^{2c}	P-value
Zone	97.0	0.126	0.001	62.5	0.167	0.001	6.21	0.018	0.001	6.02	0.021	0.002	3.38	0.020	0.012
<i>Prochlorococcus</i>	49.2	0.095	0.001	33.6	0.120	0.001	3.10	0.009	0.001	4.31	0.054	0.002	4.06	0.054	0.008
Total organic carbon	60.1	0.132	0.001	39.4	0.128	0.001	2.90	0.035	0.002	2.25	0.020	0.03	1.36	0.006	0.185
Heterotrophic microbes	34.3	0.187	0.001	19.0	0.156	0.001	3.23	0.073	0.002	2.24	0.060	0.044	1.79	0.062	0.099
<i>Synechococcus</i>	21.0	0.040	0.001	12.2	0.039	0.001	1.99	0.006	0.024	2.40	-0.006	0.028	2.04	-0.008	0.07
SCTLD prevalence	5.50	0.003	0.007	3.28	0.005	0.034	3.53	0.014	0.001	1.57	0.000	0.126	0.95	-0.010	0.402
Picoeukaryotes	4.57	0.011	0.007	3.76	0.010	0.018	2.30	0.005	0.014	1.20	0.002	0.262	0.97	0.001	0.379
Soft coral cover	3.17	0.010	0.043	2.76	0.001	0.07	2.73	0.016	0.002	1.37	0.007	0.174	1.11	-0.007	0.275
Hard coral cover	4.26	0.012	0.017	2.37	-0.001	0.091	1.95	0.044	0.031	1.35	0.017	0.205	0.91	-0.003	0.434
Sponge cover	2.89	0.090	0.052	2.49	0.089	0.075	1.98	-0.001	0.034	0.69	-0.009	0.68	0.81	-0.013	0.512
Algae (fleshy and turf) cover	1.18	0.024	0.292	1.53	0.003	0.176	2.16	0.035	0.02	1.60	0.000	0.112	1.25	-0.010	0.217
Hard coral richness	2.87	0.075	0.047	1.69	0.092	0.174	1.51	0.025	0.121	1.65	0.027	0.11	1.11	0.009	0.264

Bold indicates significant at $P < 0.05$.

^aPERMANOVA is a permutational ANOVA for distance-based redundancy analysis (dbRDA) using 999 permutations.

^bPseudo-F value indicates overall significance of the term (higher is more significant).

^cAdjusted R^2 was calculated using the varpart function and represents the amount of variance explained by the individual fraction (variable), and this can be negative because the underlying dbRDA can yield negative eigenvalues.

also had high silicate concentrations (Fig. 2i). Across zones, abundances of *Prochlorococcus* had a parabolic distribution, while *Synechococcus* and picoeukaryote abundances were higher in Dry Tortugas and then increased from the Marquesas zone 7 to zone 1 (Fig. 2f–h). Chlorophyll *a* and inorganic nitrogen and phosphorus concentrations shifted with zone, but these trends were not significant (Kruskal–Wallis test with Bonferroni-corrected $P > 0.00192$; Fig. S8).

Reef seawater metabolites driven by FCR zone

To evaluate which metabolites may be signatures of reef zones, we conducted ANOVA or Kruskal–Wallis tests on targeted and untargeted (positive and negative ion modes) metabolites. Two of the 39 targeted metabolites in reef seawater changed across reef zones: 5'-methylthioadenosine (MTA) and taurocholic acid (Kruskal–Wallis test with Bonferroni-corrected $P < 0.00128$; Fig. 3a and b). Taurocholic acid median concentrations were variable across zones, while MTA concentrations generally increased as sampling moved northeast (Fig. 3a and b). Additionally, the extracellular seawater concentration of MTA was positively related to the relative abundance of microbial genes found in seawater metagenomes that use MTA (sinks—MTA phosphorylase, MTA/S-adenosylhomocysteine [SAM] nucleosidase, and MTA/SAM deaminase) and produce MTA (sources—polyamine aminopropyltransferase and isovaleryl-homoserine lactone synthase) (model II ordinary least squares linear regression $P < 0.001$, 999 permutations; Fig. S9). Within the untargeted metabolome data sets, 33 of the 2,759 features in negative ion mode and 23 of the 1,428 features in positive ion mode differed across zones (Kruskal–Wallis test with Bonferroni-corrected $P < 7.246 \times 10^{-5}$ and $P < 3.378 \times 10^{-5}$ for negative and positive metabolites, respectively) (Fig. 3c and d).

Reef seawater microbiome indicators of FCR zone

We identified microbial ASVs and functional genes diagnostic of individual reef zones via indicator value analysis (indicspecies R package, specificity ≥ 0.6 , sensitivity ≥ 0.6 , $P < 0.05$; see Methods S02) (Fig. 4). Of the 468 seawater ASVs, 24 were diagnostic of one

of the reef zones (summarized in File S04), with most representing Dry Tortugas (zone 8), including *Alcanivorax*, *Coxiella*, *Synechococcus*, *Fluviicola*, and the families Cryomorphaceae and PS1 clade of Parvibaculales (Fig. 4a; File S04). Zones 1 and 3 also harbored several indicator taxa, including SAR11 and SAR116 clades; the NS5 marine group and the PS1 clade of Parvibaculales in zone 1 and the SAR116 clade; and Pirellulaceae, *Propionigenium*, *Coxiella*, and *Turneriella* (Fig. 4a) in zone 3. Zone 5, which harbored the greatest prevalence of SCTLD, had three indicator taxa including *Oleispira* and the families Cryomorphaceae and Bacteriovoraceae.

Functional microbial genes were abundance-filtered to 103,665 genes (see Methods S02), and of these, 324 were diagnostic of a reef zone (Fig. 4b). All genes and their respective pathways are summarized in File S04. Like the taxonomic microbiome, the highest number of indicator genes were for Dry Tortugas National Park (zone 8, $n = 127$). Some of these genes were involved in biosynthetic pathways unique to zone 8, including arginine, cysteine, folate, glutamine, isoprenoid, serine, and ubiquinone biosynthesis. Additionally, genes from two types of ATP synthases (FoF1 and A/V) and 66 genes with unknown pathways were zone 8 indicators (Fig. 4b; File S04). Zone 3 in the Upper Keys also had many functional indicators (119), including some involved in asparagine, biotin, heme, lipoate, lysine, menaquinone, and nicotinamide adenine dinucleotide (NAD) biosynthesis pathways. Additionally, 75 of the 119 zone 3 indicator genes were from unknown pathways encoded for proteins such as a flagellar basal body protein, nitrate reductase, phosphate import protein, zinc metalloprotease, and a type II secretion system protein, among others (Fig. 4b; File S04). Zone 1 had 55 indicator genes, including a lipid A biosynthesis gene, as well as 30 indicator genes of unknown pathways (Fig. 4b; File S04). The other zones (2, 5, 6, and 7) harbored fewer indicator functional genes overall, and of these, zone 5 contained 7 indicators, including a protein in a pyrimidine salvage pathway (Fig. 4b; File S04).

Coral host-associated microbiomes driven by reef habitat

To examine if reef seawater zonal patterns are reflected in the corals, we applied the most cost-effective omic technique, taxonomic

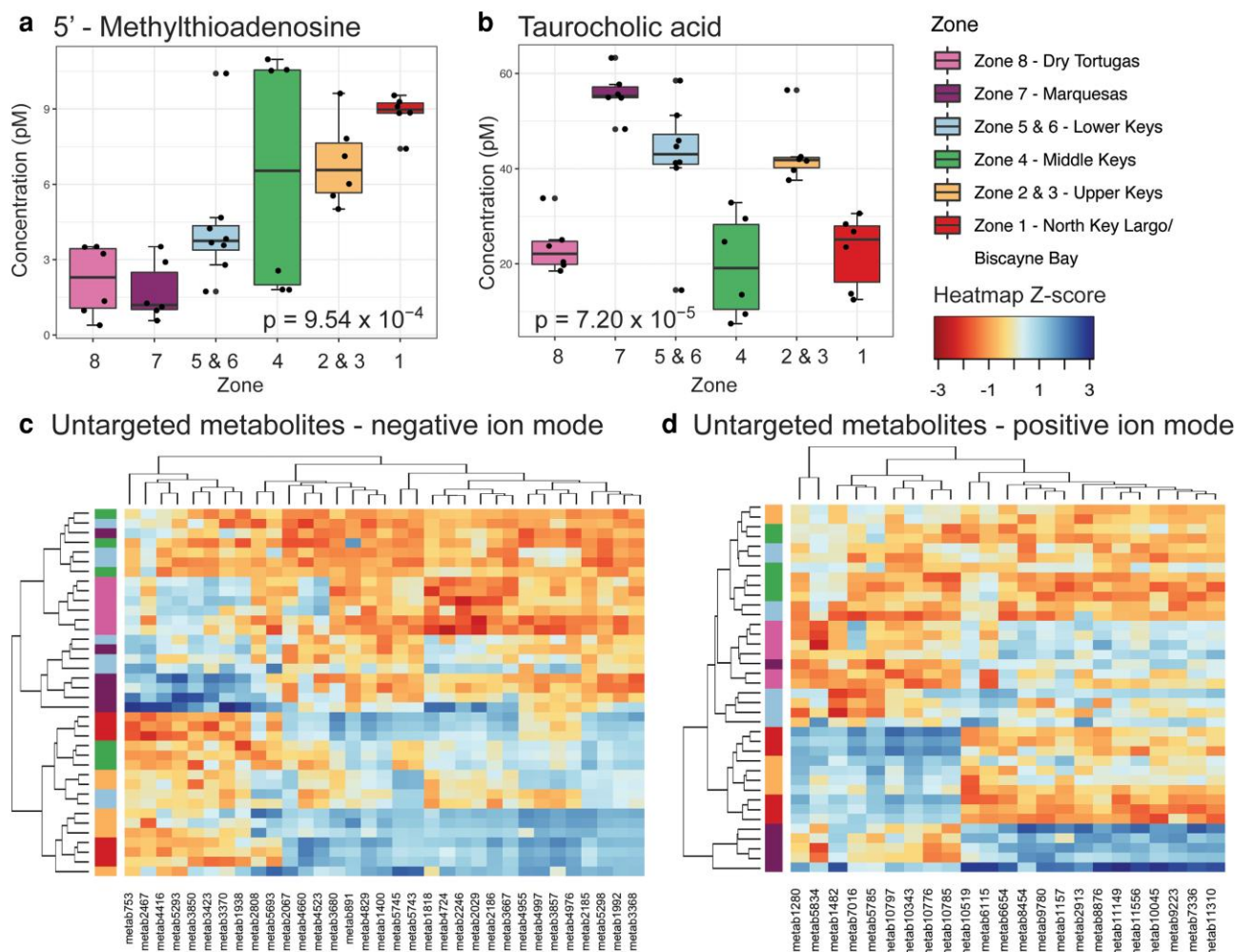


Fig. 3. Zones in FCR harbor significantly different concentrations of dissolved metabolites (a) MTA and (b) taurocholic acid. Concentrations were significantly different between FCR zones (Kruskal–Wallis test, Bonferroni-corrected $P < 0.00128$). Heat maps of z-score standardized feature peak intensities depict untargeted metabolites that ionized in (c) negative or (d) positive ion modes that were significantly different across zones (ANOVA or Kruskal–Wallis test, significant at Bonferroni-corrected P ; see [Methods S02](#)). Squares to the left of the heat map indicate reef zone. Adjacent zones 5 and 6 in the Lower Keys and zones 2 and 3 in the Upper Keys were combined to ensure at least two reefs were within each group for statistical tests.

microbiome profiling, to corals and near-coral seawater collected within five reef zones (11 reefs). We collected samples from five coral species (*Colpophyllia natans*, *Dichocoenia stokesii*, *Montastraea cavernosa*, *Orbicella faveolata*, and *Pseudodiploria strigosa*) that were either apparently healthy or showing active SCTL lesions, and this resulted in $116,942 \pm 59,302$ sequences per sample and a total of 3,020 ASVs after filtering (see [Methods S02](#)). Within coral-associated microbiomes, zone significantly structured the microbial community composition, reflective of microbial patterns in overlying seawater microbiomes (Figs. 5a and 1b). Coral species and disease state were also significantly related (PERMANOVA on Bray–Curtis dissimilarity, $P < 0.05$; Fig. 5a). Near-coral seawater taxonomic microbiomes were significantly structured by zone and coral species but not disease state (PERMANOVA; Fig. 5b).

We next identified specific microbial taxa that may be related to the biogeographic environment. We conducted a differential abundance test by zone and identified 12 ASVs that significantly differed in apparently healthy colonies (false discovery rate [FDR] adjusted $P < 0.05$; Fig. 5c). The ASVs that differed included two Clostridiaceae bacteria, which varied in abundance by zone. Several other ASVs also had variable abundances relative to zone 3 (Upper Keys, chosen as the reference zone because it had

the highest ratio of heterotrophs: autotrophs and highest organic carbon), including *Alteromonas*, *Paramoritella*, Rickettsiales S25–593, *Balneola*, and *Acinetobacter*. The Pirellulaceae family and SAR11 clade III microbes were depleted at all zones relative to zone 3, while *Rubritalea*, Desulfocapsaceae, and Sandaracinaceae were enriched in all zones relative to zone 3 (Fig. 5c).

Discussion

We present a comprehensive omic analysis of microorganisms and dissolved metabolites spanning hundreds of kilometers of FCR, which has suffered over the past 50 years from numerous natural and anthropogenic stressors. Following comparative analyses of these omic data sets, we demonstrate that reef water metabolites and microorganisms as well as coral-associated microbes distinguish between biogeographically distributed reef habitats. Further, taxonomic microbiomes and targeted metabolomic methods were related to underlying benthic organisms and environmental features such as organic carbon and abundances of planktonic microbes. These data provide novel insights into reef ecosystem parameters and may be useful for identifying non-visible changes in coral reef ecosystems.

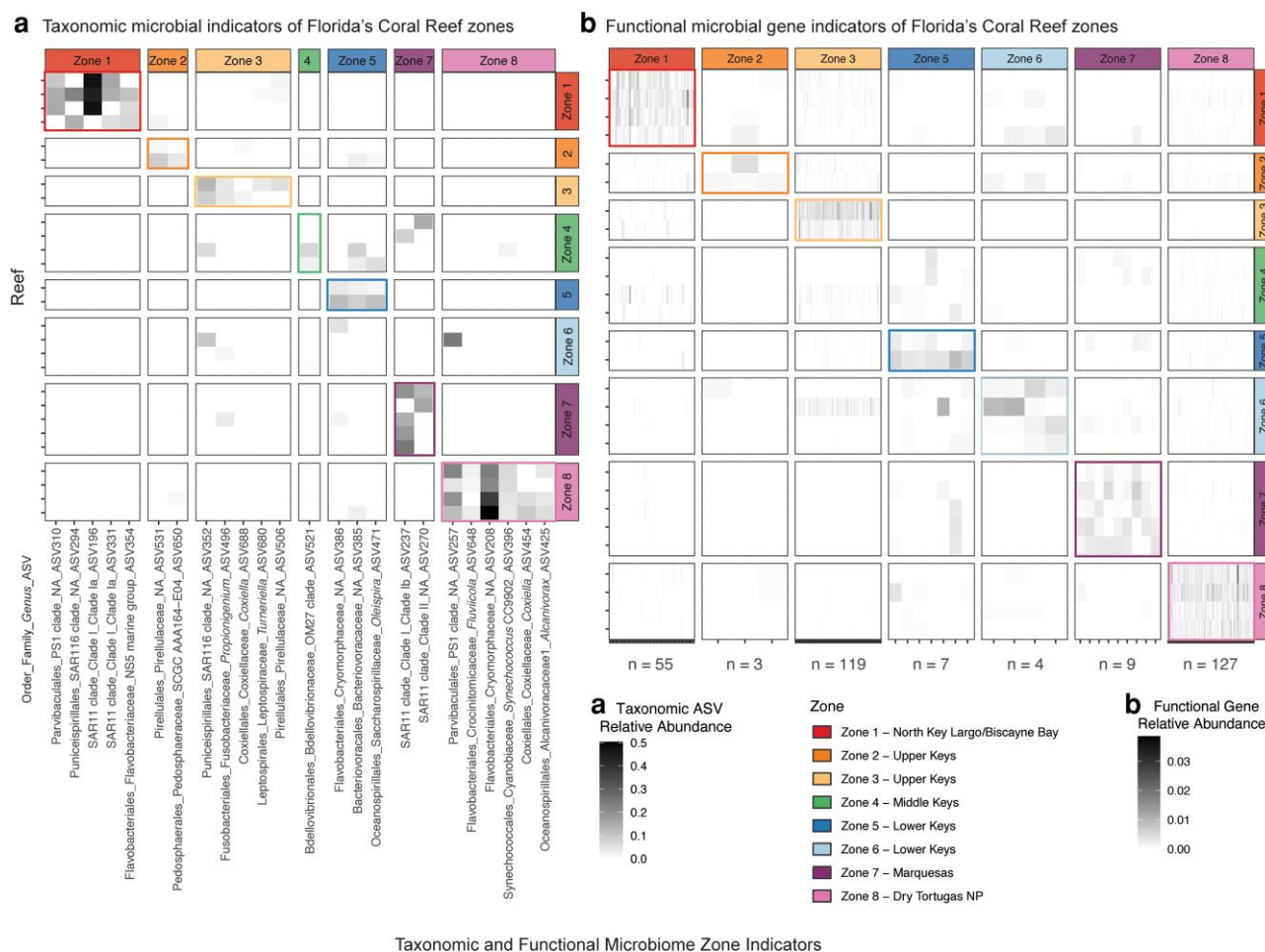


Fig. 4. Indicator microbial taxa and functional genes across FCR zones. (a) Relative abundance across all reefs of ASVs diagnostic of individual reef zones and ASV identifier on the x-axis. (b) Relative abundance across all reefs of bacterial functional genes diagnostic of individual reef zones. Number of functional genes indicative in each grouping labeled on x-axis, with full summary of genes in [File S04](#). All indicator taxa and functional genes determined by indicator value analysis ($A \geq 0.6$, $B \geq 0.6$, and $P < 0.05$). Taxa and functional genes are grouped by the associated zone they are indicative of.

The biogeographic zone emerged as strongly associated to microbiome and metabolome composition from individual reefs in Florida. These differential patterns across zones, as well as emergent habitat and environmental features, are summarized in [Fig. S10](#). For example, omic signatures in zones 1 and 8 were often opposing and distinct. Indeed, Dry Tortugas National Park (zone 8) emerged as a unique habitat, containing low levels of MTA, a distinct microbial community with low abundances of heterotrophic microbes, and high numbers of functional and taxonomic microbial indicators, including *Synechococcus* and *Acanthorax*, compared to zone 1, North Key Largo and Biscayne Bay ([Fig. S10](#)). Within the Upper to Lower Keys and Marquesas, seawater signatures were likewise distinct, and these zonal signatures were even captured within apparently healthy coral host-associated microbial taxa that shifted across reef zones.

These zonal changes agree with the established importance of biogeography for microorganisms in reef seawater (26, 27, 30). While microbiome studies abound, understanding of biogeographic signatures of reef metabolites is still in its infancy. Similar to our findings, Yamashita et al. (56) found significant shifts in optical properties of seawater dissolved organic matter (DOM) across regions in FCR, largely attributed to differences in anthropogenic and terrestrial influences. In contrast, a study of the high-quality reefs in the Cuban Jardines de la Reina

archipelago using the same mass spectrometry-based methods as the present paper did not find a strong biogeographic signature (22). These differences could be related to variation in anthropogenic influence, hydrogeography, and overall quality between the FCR and Jardines de la Reina reef systems (31). As reef metabolomics (both targeted and untargeted approaches) begin to gain traction in reef ecology (18), we anticipate future analyses of dissolved reef metabolites will provide insight into the mechanisms and consequences of such metabolomic and microbial biogeography.

Reef water environmental features of FCR habitats, which changed zonally, explained a larger portion of the variation in microbiomes and metabolomes than other parameters like benthic organisms. The abundance of heterotrophic microbes and the degree to which they outnumbered their photosynthetic counterparts (*Prochlorococcus* and *Synechococcus*) significantly increased northeast along FCR, suggesting the northern region may be more heterotrophic, or the types of autotrophs could be shifting to different phytoplankton groups. Additionally, high TOC concentrations and heterotrophic microbial abundances have been previously documented on Floridian reefs (31), and the pattern of higher TOC in zones 1 and 2 in the Upper Keys and North Key Largo/Biscayne Bay area compared to Dry Tortugas reflects patterns found from years of water quality monitoring in the region

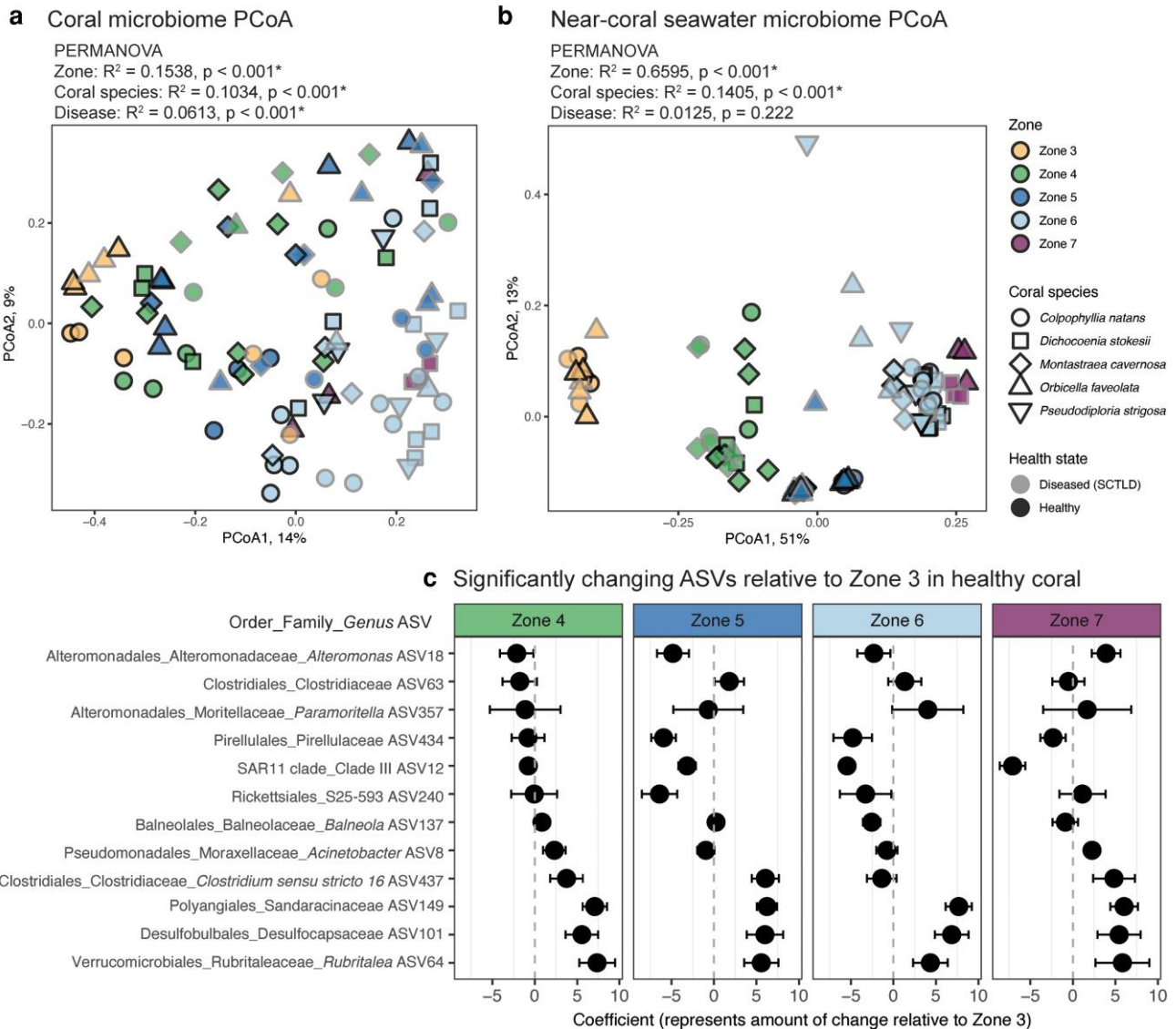


Fig. 5. Coral and near-coral seawater taxonomic microbiomes are influenced by biogeography. Principal coordinates analysis (PCoA) of (a) coral and (b) near-coral seawater taxonomic microbiome beta diversity (Bray–Curtis dissimilarity). Results from a permutational ANOVA (PERMANOVA) displayed above each graph indicate coral microbiomes are significantly different across zones, coral species, and between apparently healthy and diseased corals, while near-coral seawater microbiomes are significantly different across zones and coral species ($P < 0.05$). Symbol outlines denote health state. (c) 12 ASVs significantly differed relative to zone 3, within apparently healthy coral microbiomes as identified by differential abundance tests (Benjamini–Hochberg adjusted $P < 0.05$; see [Methods S02](#)).

(Fig. S11) (38). The increased heterotrophic microbes and TOC in that region explained metabolomic and microbiome compositions of zone 1 (Fig. 1) and was further validated by the five indicator microbes for North Key Largo and Biscayne Bay, which are all known heterotrophs (SAR116, SAR11, NS5 marine group, and Parvibaculales) (Fig. 5). Interestingly, SAR11 clade 1a ASVs were indicators for zone 1, while clades Ib and II were indicative of zone 7, likely reflecting physiological adaptations to the different oceanographic influences of the two regions, or potentially the differing anthropogenic influences between the regions (57). At the western end of FCR, at Dry Tortugas National Park (zone 8), concurrence in the microbial patterns emerged, where *Synechococcus* cells were most abundant and related to microbiome composition. Strain CC9902 was also a microbial indicator for that zone. Fundamental differences in coral growth (40) and nutrient (inorganic and organic) regimes exist between Dry Tortugas, a more

offshore environment, and the nearshore North Key Largo/Biscayne Bay area (38), which supports the stark microbial community differences between these habitats.

TOC significantly changed across FCR, but TOC is a single value that does not capture the complex dynamics of thousands of low-molecular-weight dissolved and particulate compounds. After using both targeted and untargeted approaches, we found that dissolved targeted metabolites related well to the benthic and biogeochemical habitats, while the dissolved untargeted metabolome (in both positive and negative ion modes) significantly related to a few measured biogeochemical parameters. Within the untargeted metabolome (positive and negative ion modes), 56 total metabolites shifted across reef zones, in patterns that varied broadly from the concentration of TOC. Two metabolites from the targeted metabolomic analysis, taurocholic acid and MTA, changed significantly across the zones in FCR. Taurocholic acid

is a bile salt, which is released and used as an olfactory cue by fishes, and the varied concentrations may reflect differences in fish communities (58), though we did not measure fish biomass or diversity in this study. MTA, a sulfur-containing metabolite, is more concentrated in water overlying reefs compared to deeper, off-reef waters (22) and is cycled by reef sponges (59). Concentrations of MTA in the Marquesas and the Dry Tortugas National Park were similar to the off-reef water measured by Weber et al. (22), perhaps due to the similar open-ocean hydrodynamic regimes. MTA is cycled by several pathways intracellularly, and microbial proteins involved in these pathways were encoded for the seawater metagenomes, including proteins that produce MTA (polyamine aminopropyltransferase, COG0421, EC 2.5.1.16 and EC 2.5.1.104; isovaleryl-homoserine lactone synthase, COG3916, EC 2.3.1.228) and proteins that use MTA (MTA phosphorylase, COG0005, EC 2.4.2.28; MTA/SAM nucleosidase, COG0775, EC 3.2.2.9; MTA/SAM deaminase, COG0402, EC 3.5.4.28). In the present study, the negative relationship between decreasing MTA concentrations and increasing abundances of genes for proteins that use MTA followed an expected pattern, but the opposite correlation was true for proteins that produce MTA (Fig. S9). While we measured extracellular MTA concentrations, our genetic data did not identify any removal pathways from cells (i.e. transporters) and instead identified intracellular proteins found in many bacteria and archaea for recycling MTA so that it does not accumulate in cells and inhibit other cellular pathways (60, 61). Ultimately, given the presence of MTA and MTA cycling genes in reef seawater metagenomes and the known ecological relevance of taurocholic acid, further experimental research on these and other metabolites is warranted to demonstrate the role these metabolites play in reef habitats. With this information, we will be better equipped to disentangle the implications of these metabolite patterns on reef quality.

FCR has been impacted by a years-long highly contagious SCTLD outbreak (41, 62). After sampling apparently healthy and diseased tissue across five zones and five coral species, surprisingly, reef zone was a stronger driver of coral microbiome structure than species and disease, which are known to impact coral microbiomes (46, 63). This zonal influence mirrored changes we also identified in reef water microbiomes and metabolomes (Fig. 1). Geography is increasingly important to coral microbiome signatures, showing temporal stability (64) that extends for years in aquaria-raised corals (65). Within the overall changes, we identified 12 microorganisms from multiple species of apparently healthy corals that shifted across reef zones regardless of species. Many of these microbes frequently associate with corals, including *Paramoritella* (66), *Acinetobacter* (67, 68), *Alteromonas* (69), *Balneola* (70), and *Rubritalea* (71). Given the strong influence of zones on host microbiomes, further studies on microbiomes associated with coral diseases should consider and control for regional changes in microbial communities.

In addition to zone and microbial parameters, both the taxonomic microbiome and targeted metabolome were related to the benthic environment, with both related to hard and soft coral cover. While omic data were driven by zone, benthic community cover was not (Figs. S1 and S4), which may indicate the benthic habitat influences omic parameters in ways distinct from biogeographic signatures. These patterns are likely due to the known impact of coral-derived metabolites on reef water microbial communities (20, 23, 25). An additional interesting relationship emerged between hard coral species richness and the taxonomic microbiome, which likely reflected the heightened

hard coral species richness and distinct coral community composition at Dry Tortugas, a region known to promote coral growth that historically had higher hard coral cover (40, 53). Another component of the benthic habitat that was related to omic data was coral disease (SCTLD) prevalence, which significantly changed across zones and peaked in Zone 5, potentially contributing to the biogeographic signatures we documented in reef water microbiomes and metabolomes. For example, one ASV indicator in zone 5 was Cryomorpaceae, a microbe that has been associated with SCTLD disease lesions in previous studies (47, 72). As coral reefs continue to degrade in part due to infectious diseases, implementing omics in reef water in addition to coral evaluations may help capture reef ecosystem changes. While some programs already monitor specific groups of microorganisms of public health interest (73), expanding to multiple omic strategies that could capture microorganisms implicated in marine diseases may shed light into potential mechanisms leading to the reef's condition.

While the benthic community changed minimally over the eight zones we studied, the microorganisms, metabolites, and organic nutrients we measured within the overlying water column differed across the zones. For example, at one end of FCR, Dry Tortugas National Park, the waters harbored increased *Synechococcus* and lowered heterotrophic microbes and more diverse microbial taxonomic and biosynthetic pathways indicative of the region (Fig. 6). At the northeastern end of our study area, North Key Largo and Biscayne Bay harbored low abundances of *Prochlorococcus* and increased heterotrophic microorganisms, lipid biosynthesis, and less diverse microbial indicator taxa (Fig. 6). Metabolite and nutrient signatures were also distinct between the two regions (Fig. 6). These seawater-bound features are connected through benthic–pelagic coupling with the underlying reef habitat which varies across reef regions and are likely influenced by anthropogenic impacts like development and population density (26, 70), though that was not measured here. One of the major differences in the benthic habitat was disease history, which may have contributed to these signatures in the reef water. North Key Largo/Biscayne Bay is near the initial outbreak area of SCTLD in 2014 (62) and has therefore experienced multiple years of disease pressure, while Dry Tortugas had no SCTLD. Dry Tortugas corals contracted SCTLD in 2021 (74), and thus our data set captured the multi omic profile of this region ahead of the disease front.

Metabolome and microbiome analyses have the potential to revolutionize our understanding of reef processes and energetics, but our understanding of the current data and their implications is limited, and their inclusion into monitoring programs and research studies comes with significant challenges. Metabolomics alone requires specialized equipment and expertise from sampling to mass spectrometric analysis, and access to and use of such mass spectrometry facilities can be costly and analytically and computationally challenging. To begin, conducting experiments with targeted compounds, like MTA and taurocholic acid, could offer an initial glimpse into how they are implicated in reef processes. Targeted metabolomics utilizes authentic standards to quantify each metabolite and is adaptable and comparable between mass spectrometry facilities. Microbiome analyses are even more developed and accessible, with more facilities and at a lower cost than metabolomics. Characterizing taxonomic microbiomes (via 16S rRNA gene sequencing) is more cost-effective compared to shotgun metagenomic sequencing and is even adaptable to in-the-field use, though at the loss of potentially

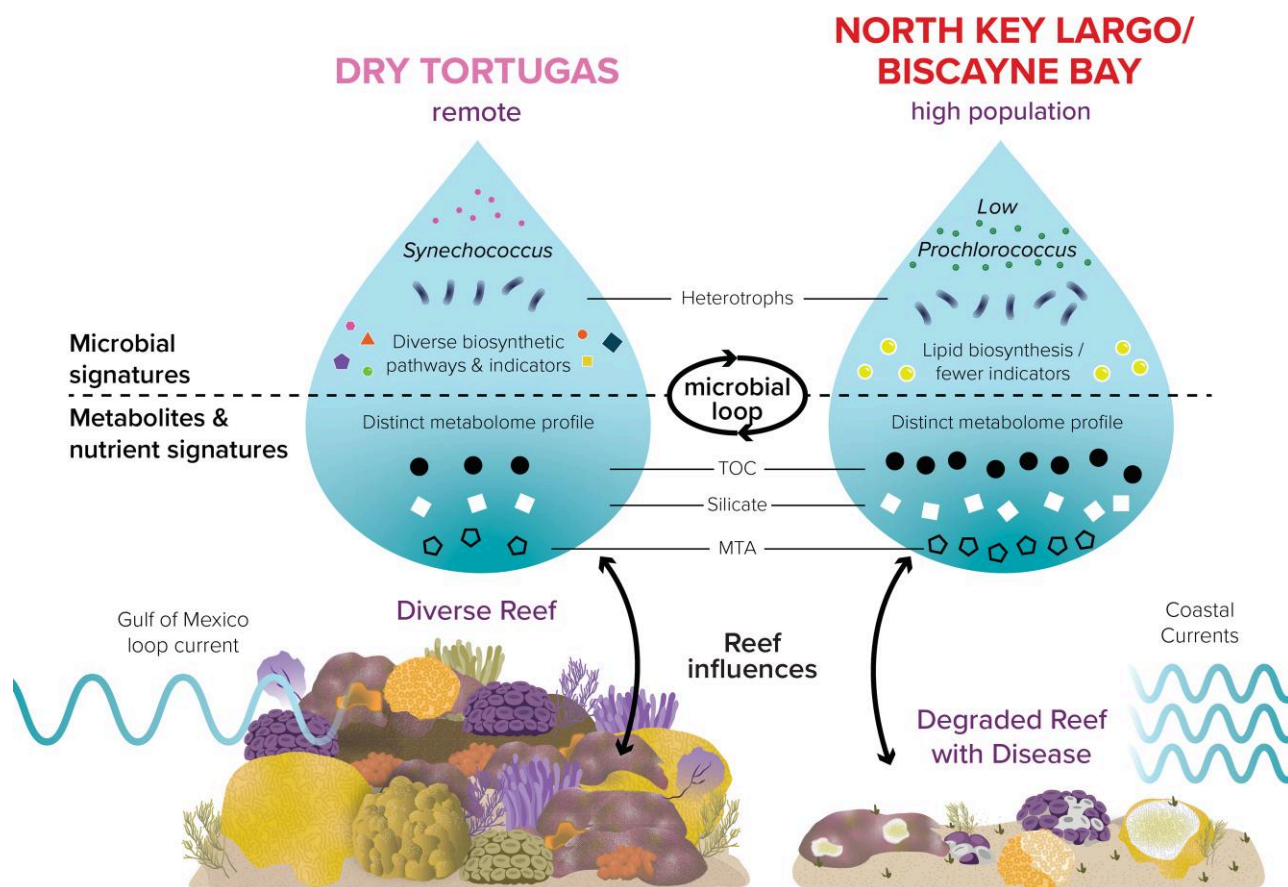


Fig. 6. Conceptual diagram of how differential seawater omic signatures depict ocean environment and ecosystem conditions in two contrasting Florida's Coral Reef habitats, Dry Tortugas (zone 8) and North Key Largo/Biscayne Bay (zone 1). Metabolites and microorganisms that are measured by omics and other approaches are connected via the microbial loop. Within two different habitats, the omic signatures become unique as they are influenced by reef habitat and likely by parameters not examined here, such as prevailing water currents and human population density. While habitat conditions influenced seawater omics, next steps include experimental investigations into the mechanisms related to these signatures and determining if these can serve as diagnostic tools for reefs.

useful functional information (46, 75). Regardless, given the significant associations and indicators of the taxonomic microbiome with reef zones, benthic organisms, environmental features in this data set, and the relative ease of use compared to other omic techniques, characterizing them in a time-series context with existing monitoring programs could be highly informative for understanding reef dynamics.

In conclusion, this novel omic data set of reef microorganisms and metabolites enhances our understanding of the chemical and microbial habitat within FCR. Further, it provides insights into specific compounds (e.g. MTA and taurocholic acid) and microbial indicators for focus in follow-up experimental studies to better understand the dynamics of these indicators within the reef habitat. Outside of experimental work, continued investigations of reef microorganisms and metabolites are needed. In particular, those in a time-series context that link microbial and metabolite patterns to habitat characteristics or external stressors will help elucidate the mechanisms behind patterns we observed and how these features may contribute to reef decline or growth. For example, currently (summer 2023) FCR is experiencing unprecedented levels of seawater warming and coral bleaching. Follow-up omic studies could identify the nonvisible, ecological impacts of the predicted coral reef ecosystem mortality. While metabolomics and microbial analyses probe the smallest components of a reef, they also reflect larger habitat patterns in reef

environments, warranting further inclusion of these techniques in studying Earth's most biodiverse ocean ecosystem.

Materials and methods

Study area and sampling

We sampled coral reef benthic environments during a research cruise aboard the M/V Alucia between 2019 June 5 and 2019 June 19 (Fig. 1; File S03). We targeted 85 reefs that were separated into eight zones (between 8 and 14 reefs per zone), based on historical management areas that line up with subregions of Biscayne National Park and North Key Largo area; Upper, Middle, and Lower Keys; the Marquesas; and Dry Tortugas National Park, similar to those reported in publications and used in the Disturbance Response Monitoring program (52, 53, 76) (see Methods S02). At 85 reefs, 30-min roving diver surveys were used to measure the prevalence of SCTLD. As of June 2019, none of the colonies surveyed had received any treatment for SCTLD. Within a 100 m² plot, high-resolution imagery for benthic composition was captured on 45 reefs. We collected 30–40 mL of seawater via SCUBA at all 85 reefs at reef depth for inorganic nutrients and organic carbon and total nitrogen (TN). Inorganic nutrient samples were frozen after 1.4 mL subsamples were removed and fixed with paraformaldehyde (1% final concentration) to quantify cell

abundances. Organic carbon and TN samples were acidified and kept at room temperature or 4°C until analysis.

At 13 reefs, we collected triplicate 1.7 L seawater samples for metabolomic analysis (targeted and untargeted) via SCUBA. The seawater was prefiltered (0.1 µm) to remove microbial biomass and acidified before low-molecular-weight organic metabolites were concentrated using solid-phase extraction (SPE) cartridges. At 27 reefs (including the 13 sampled for metabolomics), we collected two 4 L benthic seawater samples. We filtered 2 L at a time using a 0.2 µm filter (microbiome analysis) or GF/F filter (chlorophyll analysis). Filters and SPE cartridges were frozen at -80°C for analysis at the Woods Hole Oceanographic Institution (WHOI).

On 11 reefs with active SCTLD, we collected coral tissue with 10-mL Luer Slip syringes and near-coral seawater with 60-mL Luer Lok syringes from separate apparently healthy and SCTLD-afflicted coral colonies. We targeted the following coral species: *Colpophyllia natans*, *Dichocoenia stokesii*, *Montastraea cavernosa*, *Orbicella faveolata*, and *Pseudodiploria strigosa*. The seawater was hand-filtered (0.2 µm), and all samples (filters and coral tissue) were frozen at -80°C until analysis at WHOI. Further details are included in the [Methods S02](#).

Benthic and seawater environmental sample processing

Photomosaics at 45 reefs were analyzed for benthic composition as described in Fox et al. (77). The number of diseased and apparently healthy coral colonies were counted and used to generate a proportion of colonies at each reef with SCTLD. Samples collected for quantifying microbial cell abundances were processed at the University of Hawaii, as described previously (78). Nonpigmented prokaryotes were used as a proxy for heterotrophic bacterial and archaeal cells (79, 80), referred to as “heterotrophic microbes”. Nonpurgeable TOC, TN, and inorganic nutrients (phosphate, ammonium, silicate, nitrite, and nitrate) were analyzed as described previously (31). TON was calculated by subtracting ammonium and nitrite plus nitrate from TN. Chlorophyll analysis involved a 24-h 90% acetone extraction, followed by fluorometric analysis. See [Methods S02](#) for further details.

Metabolomic and microbiome laboratory processing

SPE cartridges were processed following the methods in Weber et al. (22). For untargeted metabolite analysis, eluted and resuspended extracts were measured using an ultrahigh-performance liquid chromatography system (Vanquish UHPLC) coupled with an Orbitrap Fusion Lumos Tribrid mass spectrometer that was run in both positive and negative ion modes (see [Methods S02](#)). Processing the untargeted data using XCMS ([Methods S02](#)) resulted in a final chemical feature table with unique mass-to-charge ratios and retention times for each feature as well as the corresponding peak intensity for that feature across samples. To prepare the untargeted metabolomic data for statistical analyses, data were filtered of blank-associated features and features with low coefficient of variation across samples and then normalized to seawater volume, keeping 1,428 (negative ion mode) and 2,759 (positive ion mode) features. Extracts for targeted metabolomics were analyzed using UHPLC (Accela Open Autosampler and Accela 1250 Pump) coupled with a H-ESI and a triple stage quadrupole mass spectrometer (TSQ Vantage) that was operated in selective reaction monitoring mode. Targeted compounds included a range of environmentally relevant vitamins, amino acids, and

other metabolites that have been detected in marine microorganisms, their culture media, and reef seawater (59, 81, 82). After conversion of instrument files, final concentrations were corrected for limit of detection and extraction efficiency (83). See [Methods S02](#) for further details.

We extracted DNA from 0.2 µm filters used for 1.7 L seawater collections and four control blank filters using PowerBiofilm kits (Qiagen, Germantown, MD, USA) following manufacturer protocols. The resulting DNA was used as the template for both 16S rRNA gene sequencing of bacteria and archaea as well as shotgun sequencing. For 16S rRNA gene sequencing for the taxonomic microbiome, we followed methods previously reported (78) and sequenced 2 × 250 bp on an Illumina MiSeq. A library for shotgun metagenomics (functional microbiome) was prepared using the Illumina DNA Prep following manufacturer protocols. Samples were sequenced 2 × 150 bp on a P3 flow cell on an Illumina NextSeq 2000. See [Methods S02](#) for library preparation details.

To expedite the turnaround time between sample collection and taxonomic microbiome results for disease diagnostics, we performed on-ship processing and 2 × 150 bp sequencing on the Illumina iSeq100 system for near-coral seawater samples from five reefs from 2019 June 9 to 2019 June 10. All remaining coral and near-coral seawater samples were processed at WHOI. On-ship and at-WHOI methods followed in-the-field microbiome preparation protocols described previously (46) and are detailed further in the [Methods S02](#).

Bioinformatics

Sequence reads from the Illumina MiSeq and iSeq runs were separately run through the DADA2 pipeline to generate ASVs (v1.18.0) (84). We removed mitochondrial and chloroplast ASVs and filtered out low-abundant ASVs (average count <0.5). DNA extraction and PCR controls were removed for data analysis. ASVs were given a unique number, and sequences associated with the IDs were saved.

Shotgun sequencing on the Illumina NextSeq yielded 13,592,782 ± 3,357,010 paired-end sequence reads per reef seawater microbial community sample. Due to sequencing errors, six reefs were singulars, while all others had duplicate sequence samples. We quality filtered the sequence reads and coassembled them with MegaHit (v1.2.9) (85), as this assembler has been shown to generate more genes that could be successfully annotated in complex environments such as ocean and soil samples compared to metaSPAdes (86). We annotated the assembly and calculated gene abundances. We kept only genes with known Clusters of Orthologous Genes (COG) identifiers and further filtered to remove low-abundance genes, leaving 103,665 genes. All resultant gene and taxa data tables were transformed to relative abundance and log transformed after adding a pseudocount of 1. See [Methods S02](#) for detailed bioinformatic packages, parameters, and links to scripts.

Statistical analysis

Benthic cover, hard coral richness, organic and inorganic nutrients, cell abundances, and disease prevalence data were measured from 45 reefs (benthic cover) or 85 reefs (all other parameters) in FCR across eight zones. We used a Kruskal-Wallis test in R (v4.0.3) to compare changes across zones with a Bonferroni-corrected P-value to account for multiple comparisons (Fig. 2). We followed with a Wilcoxon rank sum test for pairwise comparisons (significant at $P < 0.05$ after a Benjamini-Hochberg FDR adjustment) ([File S05](#)). To examine the changes in benthic

composition and hard coral species composition across FCR, we conducted a principal component analysis, followed by a PERMANOVA test by zone. Wilcoxon rank sum tests were used to compare environmental variables at Dry Tortugas National Park to all other zones. Additional linear regressions were conducted between environmental variables (see [Methods S02](#)).

A dbRDA was calculated to evaluate how geography (zone) and environmental conditions (TOC, heterotrophic microbes, *Prochlorococcus*, *Synechococcus*, picoeukaryotes, hard coral cover, algae cover, soft coral cover, sponge cover, and hard coral species richness) explained changes in metabolomes and microbiomes. dbRDAs were calculated for each data set with either gower (targeted metabolomes) or Bray–Curtis (other omic data sets) dissimilarities. Significance of individual environmental variables within the analysis was evaluated with an ANOVA using the parameter by = “terms”.

To test for metabolites that changed across zones, sites in zones 2 and 3 as well as zones 5 and 6 were grouped to ensure each zone had more than one reef. Kruskal–Wallis or ANOVA tests were used to evaluate significantly changing metabolites across zones (Bonferroni-corrected $P < 7.246 \times 10^{-5}$ and 3.378×10^{-5} for negative and positive metabolites, respectively), followed by pairwise Wilcoxon rank sum tests between zones (FDR-adjusted $P < 0.05$). The 56 untargeted features were z-score standardized for visualization. Model II ordinary least squares linear regressions were used to relate average relative abundance of MTA source and sink genes in seawater metagenomes to dissolved concentration of MTA.

To identify seawater taxa and functional microbiome indicators of FCR, we used the multilevel pattern analysis, `multipatt()` function in the R package `indicspecies` (v1.7.12). Indicators were reported if they had a positive predictor value (*A*) over 0.6 and sensitivity (*B*) over 0.6 and had a $P < 0.05$. Indicator ASVs and functional genes were visualized with heat maps and summarized in [File S04](#).

Coral and near-coral seawater taxonomic microbiome beta diversity was calculated with Bray–Curtis dissimilarity and visualized with principal coordinates analysis. A PERMANOVA test was used to identify the influence of zone, disease, and coral species on microbiome composition. Apparently healthy coral microbiomes were subset from the larger data set for differential abundance analysis to test which ASVs significantly changed by zone and controlling for the effect of species using `comcob` (87). All statistical tests are detailed further in the [Methods S02](#).

Acknowledgments

We thank the crew of the M/V *Alucia* and OceanX for logistical, diving, and sampling support. We thank Katey Lesneski, Sam Clements, Clinton Edwards, and Hanna Koch for sampling assistance, Carolyn Miller for support with preparing iSeq 100 protocols for on-ship sequencing, Gretchen Swarr and the WHOI FT-MS facility for assistance with metabolomic sample processing and analysis, Natalie Renier and Jennifer Richards from the WHOI Graphics Studio for illustration support, Erin McParland for assistance with identifying MTA cycling genes, and Ceiba Becker, Laura Gibson, and Esmeralda Alcantar for their work on benthic composition analysis. We thank the National Park Service, the Florida Fish and Wildlife Conservation Commission, the Florida Department of Environmental Conservation, the NOAA Coral Reef Conservation Program, Les Kauffman, Arthur Gleason, and Mote Marine Laboratory for assistance with reef site selection. Corals and seawater were collected under Dry Tortugas National Park permit, DRTO-2019-SCI-0010, Florida Keys

National Marine Sanctuary permit, FKNMS-2019-046, John Pennekamp Coral Reef State Park permit, 05131925, and Biscayne National Park permit, BISC-2018-SCI-0029.

Supplementary material

[Supplementary material](#) is available at PNAS Nexus online: S01 - Supplementary Tables and Figures, S02 - Methods, S03 - Metadata, S04 - Microbial indicators, and S05 - Pairwise Wilcoxon tests.

Funding

This work was supported by the Dalio Foundation (now “OceanX”), The Tiffany & Co. Foundation, the National Science Foundation (OCE-1736288) (to A.A.) and NOAA OAR Cooperative Institutes award to A.A. and E.B.K. (#NA19OAR4320074).

Author contributions

C.C.B., L.W., B.Z., S.S., E.M., A.S.C., E.B.K., and A.A. conceived and designed the sampling scheme. C.C.B., L.W., B.Z., C.S., A.S.C., and E.M. collected the data. A.S.C. and E.M. processed and contributed disease data. B.Z., C.S., and S.S. processed and contributed benthic photomosaic data. L.W., M.C.K.S., and K.L. processed and contributed metabolomic data. C.C.B. and L.W. processed and contributed microbiome data. C.C.B. performed the analysis and wrote the manuscript. L.W., B.Z., C.S., S.S., A.S.C., E.M., M.C.K.S., K.L., E.B.K., and A.A. provided helpful comments and edits on analyses and the manuscript.

Data availability

Metabolomic data files are uploaded to MetaboLights database and are under curation under accession MTBLS6574. All sequence data (metagenomic and 16S rRNA) are uploaded to the NCBI Sequence Read Archive under BioProject numbers PRJNA910824 and PRJNA742384. Environmental data and metabolite and sequence data accessions can be accessed within BCO-DMO project 746196 under data set 890979. Code for reproducing statistical analyses and main figures in the manuscript are uploaded to <https://github.com/CynthiaBecker/FL-reef-omics>.

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