nature microbiology

ARTICLES

PUBLISHED: 25 APRIL 2016 | ARTICLE NUMBER: 16042 | DOI: 10.1038/NMICROBIOL.2016.42

Global microbialization of coral reefs

Andreas F. Haas[1*†], Mohamed F. M. Fairoz[2†], Linda W. Kelly[1], Craig E. Nelson[3], Elizabeth A. Dinsdale[1], Robert A. Edwards[1], Steve Giles[4], Mark Hatay[1], Nao Hisakawa[1], Ben Knowles[1], Yan Wei Lim[1], Heather Maughan[5], Olga Pantos[6], Ty N. F. Roach[1], Savannah E. Sanchez[1], Cynthia B. Silveira[1], Stuart Sandin[7], Jennifer E. Smith[7] and Forest Rohwer[1]

Microbialization refers to the observed shift in ecosystem trophic structure towards higher microbial biomass and energy use. On coral reefs, the proximal causes of microbialization are overfishing and eutrophication, both of which facilitate enhanced growth of fleshy algae, conferring a competitive advantage over calcifying corals and coralline algae. The proposed mechanism for this competitive advantage is the DDAM positive feedback loop (dissolved organic carbon (DOC), disease, algae, microorganism), where DOC released by ungrazed fleshy algae supports copiotrophic, potentially pathogenic bacterial communities, ultimately harming corals and maintaining algal competitive dominance. Using an unprecedented data set of >400 samples from 60 coral reef sites, we show that the central DDAM predictions are consistent across three ocean basins. Reef algal cover is positively correlated with lower concentrations of DOC and higher microbial abundances. On turf and fleshy macroalgal-rich reefs, higher relative abundances of copiotrophic microbial taxa were identified. These microbial communities shift their metabolic potential for carbohydrate degradation from the more energy efficient Embden–Meyerhof–Parnas pathway on coral-dominated reefs to the less efficient Entner–Doudoroff and pentose phosphate pathways on algal-dominated reefs. This 'yield-to-power' switch by microorganism directly threatens reefs via increased hypoxia and greater CO_2 release from the microbial respiration of DOC.

As the world's most productive and diverse marine ecosystems, coral reefs have a wide variety of mechanisms for cycling energy and nutrients. Benthic primary producers, namely zooxanthellae-harbouring corals and crustose coralline algae (CCA), form the base of the pristine coral reef food web[1]. In addition to direct trophic transfer of primary production through herbivory, a number of processes recycle organic matter in reef ecosystems.

Background

Organic carbon fluxes on coral reefs. Coral-derived organic matter forms viscous mucus that traps nutrients and is subsequently recycled in the calcareous reef sands or by cryptic benthic consumers[1,2]. A significant proportion (up to 40%; ref. 3) of coral and algal primary production is also released as dissolved organic carbon (DOC), a heterogeneous mixture of polysaccharides, proteins and lipids. Scleractinian corals produce DOC that is more similar in neutral sugar composition to reef water and offshore water carbon pools, whereas turf and fleshy macroalgal-derived DOC is enriched in labile sugars and supports higher microbial growth rates[3,4]. DOC forms the basis of the microbial loop and is recycled through microbial biomass into higher trophic levels. Marine DOC is broadly divided into three pools based on its usability by microorganism. Labile DOC is rapidly degraded and has a very short half-life (for example, free glucose is metabolized within minutes of being released[5]). In contrast, refractory DOC can exist for many to thousands of years[5]. Semi-labile DOC is degradable in certain circumstances, including conditions where the microbial communities are primed with excesses of labile DOC (ref. 6).

Changes in reef benthic community composition. As coral reefs degrade, the dominant benthic macroorganisms shift from calcifying organisms (for example, corals, CCA and *Halimeda* spp.) to non-calcifying turfing algae (moderately disturbed reef) and fleshy macroalgae (highly disturbed reefs)[7,8]. The primary local cause of this shift is overfishing, which releases turf and fleshy macroalgae from grazing controls[9]. Eutrophication also contributes to turf and fleshy macroalgal growth, especially on overfished reefs[10]. Global stressors, such as temperature changes and ocean acidification, weaken coral holobionts via numerous mechanisms including bleaching[11] and increased herpes viral production[12]. All of these stressors promote higher abundances of potential pathogens and disease prevalence[13,14].

Changes in reef microbial communities. Turf and fleshy macroalgae release significantly higher amounts of bioavailable DOC per unit surface area or biomass than corals[3,15,16], and this algal-derived DOC supports copiotrophic bacterial growth[3,4,17]. These copiotrophs have the potential to remineralize available organic nutrients at a higher rate[18] and encode greater numbers of potential virulence factor genes[4]. They can harm and/or kill corals by creating zones of prolonged hypoxia and presumably through other, uncharacterized pathogenic mechanisms[4,19–21]. As the corals die, more space is created for algae, thereby creating a positive feedback loop called DDAM (DOC, disease, algae, microorganism)[13,22]. Turf algae are the early successors on moderately stressed reefs and primarily compete with corals through DDAM mechanisms[22]. On highly degraded reefs, fleshy macroalgae become more common and compete with corals by

[1]Department of Biology, San Diego State University, San Diego, California 92182, USA. [2]Faculty of Fisheries and Marine Sciences, Ocean University of Sri Lanka, Tangalle, Sri Lanka. [3]Center for Microbial Oceanography: Research and Education, Department of Oceanography and Sea Grant College Program, School of Ocean and Earth Science and Technology, University of Hawai'i at Mānoa, Hawaii 96822, USA. [4]Big Rose Web Design, 8550 Greenway Blvd, LLC, Madison, Wisconsin 53562, USA. [5]Ronin Institute, Montclair, New Jersey 07043, USA. [6]Global Change Institute, University of Queensland, St Lucia, Queensland 4072, Australia. [7]Scripps Institution of Oceanography, University of California San Diego, La Jolla, California 92093, USA. †These authors contributed equally to this work. *e-mail: andreas.florian.haas@gmail.com

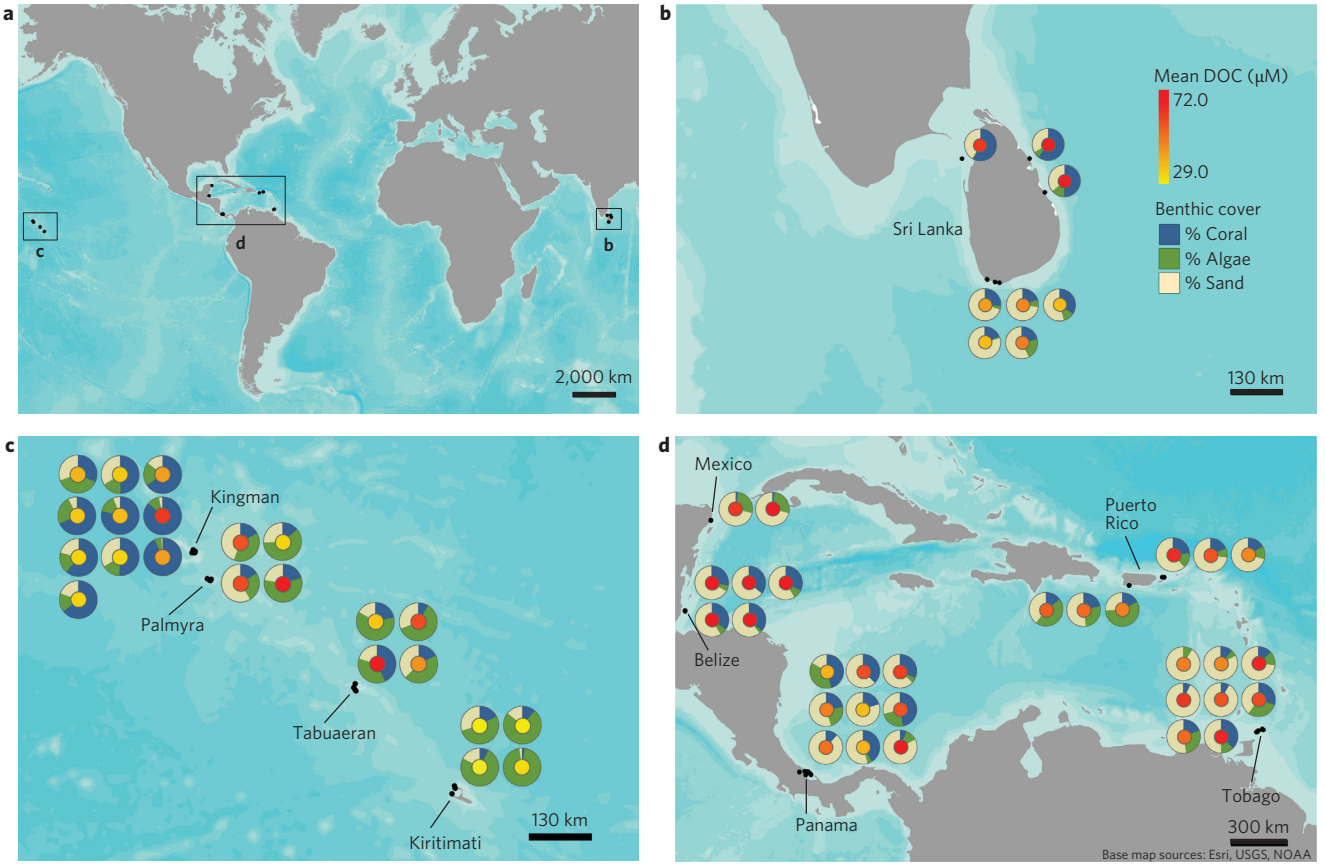

Figure 1 | Sampling sites. a, World map showing the three ocean systems sampled for the study. **b–d**, Enlarged maps, showing mean DOC concentrations (ring centre) and percentage of benthic cover (outer ring: blue = coral plus CCA, green = algae, white = sand/rock) at each sampling location ($n = 60$) in the Indian Ocean (**b**), the Central Pacific (**c**)[13] and the Caribbean (**d**).

shading and physical abrasion[23], as well as hydrophobic and hydrophilic algal exudates[22].

Testing the central predictions of the DDAM model. The DDAM model predicts that reefs with elevated algal cover will have higher microbial biomass, depleted stocks of DOC and microbial communities dominated by copiotrophic and potentially pathogenic taxa. Measurements of DOC concentrations and microbial abundances from more than 400 samples across 60 coral reef sites in three different ocean basins demonstrate that the occurrence of DDAM-predicted increases in microbial abundances and depletion of DOC on algal-dominated reefs is a global phenomenon. Metagenomes generated from a subset of sampling sites identified metabolic shifts in microbial communities associated with different functional groups of primary producers: on turf and fleshy macroalgal-rich reefs, microbial communities were characterized by higher proportions of less energy-efficient carbohydrate metabolisms (Entner–Doudoroff (ED) and pentose phosphate (PP) pathways), revealing a mechanism by which greater quantities of DOC could be remineralized more quickly and less efficiently to support the observed microbialization[24].

Results and discussion

DOC concentrations decrease with increasing algal cover. A counterintuitive component of the DDAM model is that high production of DOC by turf and/or fleshy macroalgae leads to an overall depletion of DOC standing stock (compared with either coral-dominated sites or offshore waters)[22,25]. To test whether this 'more DOC gives less DOC'[26] observation is a global phenomenon, DOC concentrations were measured on 60 reefs in the Indian, Pacific and Atlantic oceans (Fig. 1). DOC concentrations on reefs ranged from very low (32.3 ± 1.5 μmol l^{-1} in Kiritimati , Republic of Kiribati, Central Pacific Ocean) to 68.5 ± 1.7 μmol l^{-1} (in Belize, Caribbean). Correlation of DOC concentration with the percentage cover of turf and fleshy macroalgae benthic cover produced a significant negative relationship in all three oceanic systems (Caribbean: $r = -0.17$, $P = 0.0192$; Central Pacific: $r = -0.30$, $P < 0.0001$; Indian Ocean: $r = -0.34$, $P = 0.0317$; Fig. 2a and Supplementary Table 1). For every 10% increase in percentage cover of turf and fleshy macroalgae, DOC standing stocks decreased by ~2 μmol l^{-1}. These measurements support the prediction that more labile DOC production results in lower overall DOC concentrations on coral reefs globally.

Microbial abundances increase with reef algal cover. The observation of 'more DOC gives less DOC' can be attributed to priming[27,28], where labile DOC from the turf and fleshy macroalgae stimulate microorganism to remineralize semi-labile DOC pools. Further degradation of the DOC standing stock is predicted to increase community carrying capacity, thus supporting more microorganism[29]. Consistent with this prediction, microbial cell abundances are positively correlated with the percentage of turf and fleshy macroalgae cover in all three ocean systems (Caribbean: $r = 0.22$, $P = 0.0275$; Central Pacific: $r = 0.35$, $P < 0.0001$; Indian Ocean: $r = 0.36$, $P = 0.0424$; Fig. 2b and Supplementary Table 1). In short, there was an order of magnitude increase in microbial abundances from a near-pristine coral atoll like Palmyra (Central Pacific, median density of microorganism $1.7 \times 10^5 \pm 0.9 \times 10^5$ cells ml^{-1}) to the relatively degraded Panama reefs ($1.5 \times 10^6 \pm 0.1 \times 10^6$ cells ml^{-1}).

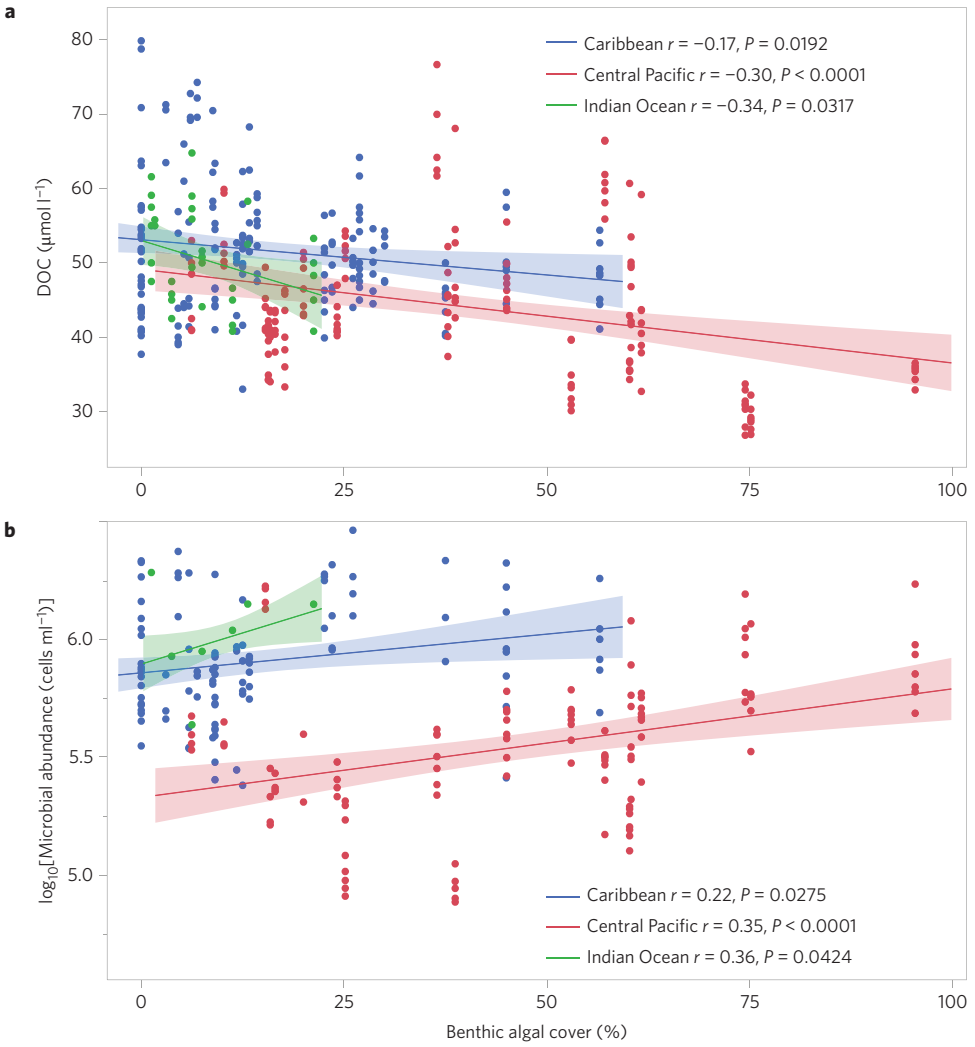

Figure 2 | Algal cover compared to DOC and microbial abundance. a,**b**, Correlation of percentage of algal cover and DOC water column concentrations (**a**) and percentage of algal cover and $\log_{10}$-transformed microbial cell abundances (**b**) with 95% confidence interval. *r* and *P* values are given for each ocean system.

The decrease in DOC standing stocks and increase in microbial abundances found across these reefs match results from incubation experiments performed with coral and algal exudates and reef microbial communities[3,18]. These experiments showed that a surplus of 2 μmol l^{-1} of degradable DOC increased the carrying capacity of a microbial community by $\sim 1.8 \times 10^5$ cells ml^{-1}. Furthermore, exudates collected from fleshy algae elicited a rapid draw down of surplus DOC (ref. 3). Calculations based on the survey measurements from this study combined with empirically derived rates of exudate release by tropical benthic primary producers[3,18] and marine microbial carbon demand[4,30] suggest that some of the algae-dominated sites have negative water column carbon fluxes of up to 20 μmol l^{-1} d^{-1} (Supplementary Fig. 1) with as much as 4 μmol carbon l^{-1} stored in microbial biomass. Experiments have demonstrated that this pelagic heterotrophy is fueled by allochthonous DOC sources and that the overall reef system remains net autotrophic[18].

These estimates of carbon flow through the microbial food web do not account for all of the carbon remineralization that occurs on reefs because viruses[31], heterotrophic protists[5], suspension feeders[2,32], as well as physical and chemical factors, all contribute to DOC standing stocks. To evaluate the role of other abiotic forcings on DOC concentrations and microbial abundances on these reefs, multiple regression analysis was conducted in four separate models (one for each ocean basin and one combined model), each including latitude, longitude, particulate organic carbon (POC), dissolved inorganic phosphorus (DIP) and dissolved inorganic nitrogen (DIN) as covariates. The analysis showed that algal cover remained a robust predictor of both DOC and microbial abundance after accounting for abiotic factors ($P < 0.01$ for the algal cover term within the multivariate and residual models, except DOC in the Pacific where $P = 0.44$, Supplementary Table 1), emphasizing the independent role of algal cover as a factor in reef microbialization.

Copiotrophic microorganism and virulence factors are more abundant with increasing algal cover. A second prediction of the DDAM model is that increased algal cover will lead to increased copiotrophic microorganism with greater potential to cause disease. To investigate differences in microbial community structure and metabolic potential on reefs with varying degrees of coral and algal cover, we sequenced and annotated microbial metagenomes from nine reefs spanning a wide range in algal cover. Microbial taxonomy and metabolism were analysed using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database[33]. The taxonomic composition of the microbial community was significantly correlated to the percent cover of turf and fleshy macroalgal (permutational multivariate analysis of variance, PERMANOVA; $P < 0.03$, $R^2 = 0.23$–0.26 at genus, family and order levels). Algal-dominated sites were enriched in

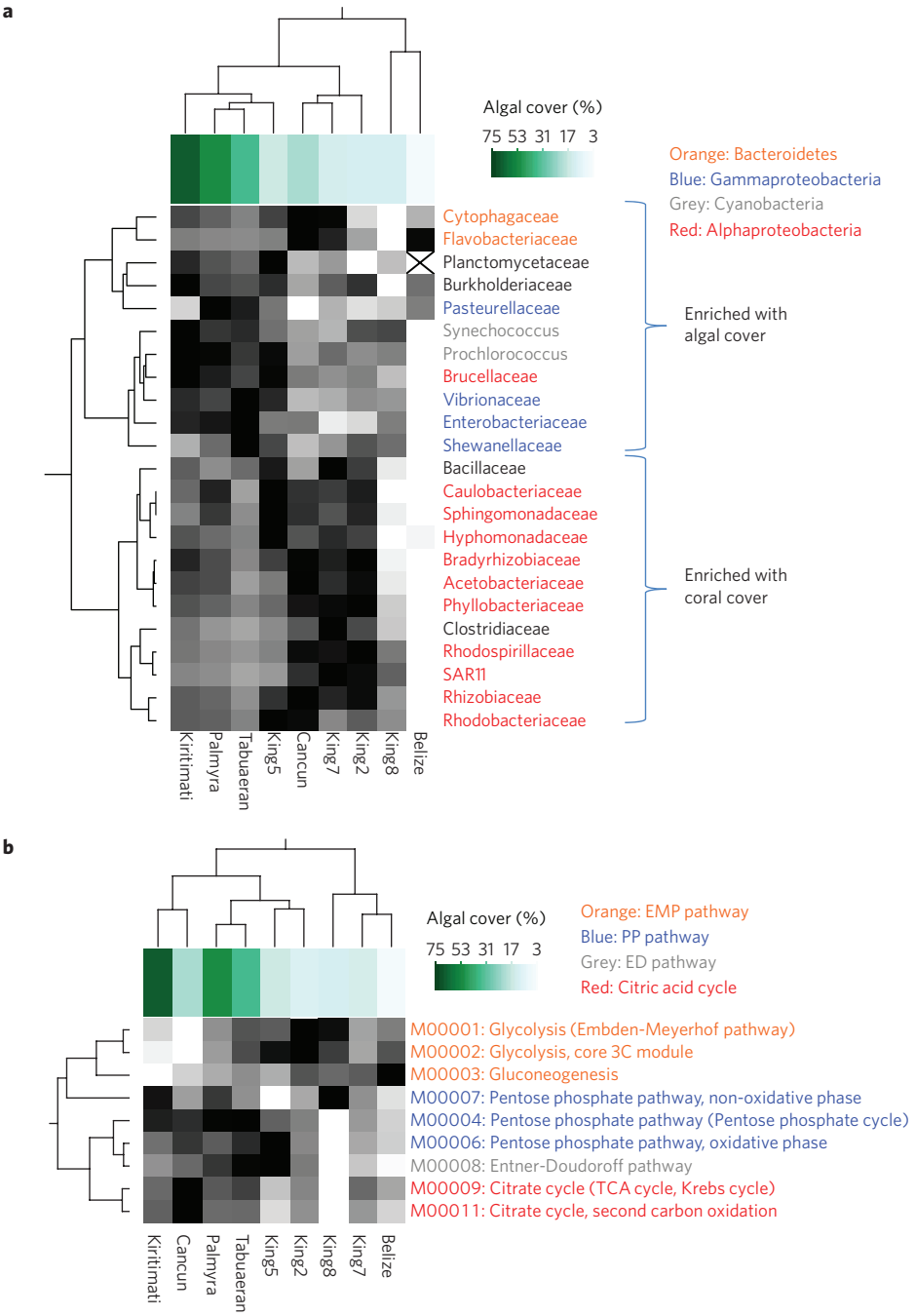

Figure 3 | Metagenomic taxonomic and functional analysis of reef-associated microbial communities. a,**b**, Samples are clustered by community composition via KEGG taxonomy families (**a**) and KEGG carbohydrate metabolism pathway modules according to algal cover (in green at top) among nine metagenomes (**b**). Note that samples cluster similarly for both community composition and for carbohydrate metabolism and that algal-dominated samples are relatively enriched in ED/PP pathway modules and a suite of dominant taxa. Samples (columns) are hierarchically clustered according to gene relative abundance for each parameter (rows) using relative enrichment hierarchical clustering (Ward's minimum variance method). Only families that reached 1% of community in at least one sample and were found in every sample are shown. All data were arcsin (square root) transformed before standardization for clustering. Heatmaps are relative enrichment standardized by each parameter by subtracting the column mean and dividing by the column standard deviation.

copiotrophic taxa[34] (Fig. 3a), including Gammaproteobacterial families (Enterobacteriaceae, Vibrionaceae, Shewanellacaeae and Pasteurellaceae), Bacteriodetes (Cytophagaceae and Flavobacteriaceae) and families from several other phyla (for example, Planctomycetaceae, Brucellaceae and Burkholderiaceae). Coral-dominated reefs were enriched in oligotrophic Alphaproteobacteria families, which are widespread in oceanic waters and coral exudates[4] (Fig. 3a). The relative abundances of four of the most abundant orders increased linearly and significantly ($P < 0.05$) with algal cover across all nine metagenomes (Supplementary Fig. 2).

The DDAM model also predicts that microorganism with a greater capacity to cause disease will be enriched on degraded reefs. To determine whether more potential pathogens were present on algal-dominated reefs, the metagenomic libraries were compared against a curated database of bacterial virulence factor genes. Microbial communities carried higher relative abundances

of virulence genes as reefs increased in algal cover (Pearson correlation $P < 0.0001$; Supplementary Table 2). Furthermore, the relative abundances of 53 specific virulence factors (out of 496 tested) exhibited significant positive relationships with percentage cover of turf and fleshy macroalgae (Pearson correlation $P < 0.05$; Supplementary Table 2). Finally, the relative abundance of total virulence factor genes was also positively correlated with the prevalence of coral disease (percentage of corals showing signs of disease at each site; $P < 0.0001$; Supplementary Table 2). This finding provides further support for a mechanistic relationship whereby algae facilitate the growth of microbial taxa with higher abundances of virulence factors (as shown experimentally in ref. 4), which may contribute to the observed disease prevalence via opportunistic pathogens[35] or through indirect mechanisms (for example, hypoxia[20]). The relationships between algal cover, bacterial virulence and coral disease shown here are merely correlative. Accordingly, these patterns warrant further investigations to characterize disease aetiologies on algae-dominated reefs.

ED and PP genes are more abundant with increasing algal cover. When the functional potentials of the metagenomes were compared, only the central carbohydrate metabolism showed significant differences across the reef-associated microbial communities (out of 26 KEGG pathway modules tested). Algal cover was a strong and significant linear predictor of the multivariate composition of the central carbohydrate metabolism among the nine metagenomes (PERMANOVA, $P = 0.0322$, $R^2 = 0.32$). Specifically, the proportional abundances of the Embden–Meyerhof–Parnas (EMP), ED and PP pathways differed significantly between algal-dominated and coral-dominated reefs ($P = 0.0079$, $R^2 = 0.42$), with relative enrichment in the EMP pathway modules (M1, M2 and M3) on coral-dominated reefs and a contrasting enrichment of the alternative ED and PP pathways on algal-dominated reefs (Fig. 3b and Supplementary Fig. 3). Microorganism primarily use these catabolic pathways to produce pyruvate[36], which is then terminally remineralized to CO_2 in the tricarboxylic acid (TCA) cycle[36]. The EMP pathway enriched on coral-dominated reefs is the most efficient based on adenosine triphosphate (ATP) production[37], whereas the direct breakdown of sugars in the ED pathway produces pyruvate faster, requiring up to 3.5 times lower enzyme levels, but at an energetic cost in terms of ATP generation[38–40]. This more thermodynamically favourable catabolic route allows the microorganism to utilize more oxidized carbon sources with lower free enthalpies[39].

Yield to power switch. Sacrificing energetic efficiency[39] to outgrow and deprive competitors of food is a common ecological strategy referred to as the 'yield to power' switch[41,42]. Furthermore, microorganism using the PP pathway for catabolism are predicted to metabolize a greater variety of carbohydrates, thus diversifying the menu available to the microorganism[43,44]. Particularly important are the pentose sugars present in the turf and fleshy macroalgae-derived DOC (refs 45,46), which can be shunted directly in the PP pathway[44]. Finally, the significant enrichment of genes encoding for the TCA cycle on algal-dominated reefs corroborates the increased potential of this microbial community to rapidly remineralize the available organic carbon (Fig. 3b and Supplementary Fig. 4). Taken together, the metagenomic analyses show that benthic communities dominated by turf and fleshy macroalgae support a copiotrophic microbial community with the metabolic capacity to metabolize a greater diversity of carbon substrates faster, thereby promoting enhanced degradation of DOC standing stocks.

Reef microbialization occurs globally. These findings further support previous calculations that demonstrated that microbialization occurs more often with cumulative human impacts[47,48]. Here, we provide geochemical and microbiological evidence that coral reef microbialization occurs on a global scale, and suggest a mechanism for the observed changes in the allocation of metabolic energy between microorganism and macrobes on degraded reefs, with microorganism inefficiently remineralizing organic nutrients and reducing the availability of this energy to higher trophic levels.

Conclusion

This global data set supports the main predictions of the DDAM feedback model: labile DOC exuded by fleshy algae feeds copiotrophic, pathogen-like microorganism, which threaten corals[20,21]. These microbial communities are genomically adapted for rapid but inefficient metabolism to further degrade labile and semi-labile marine DOC pools. Increased microbial respiration of organic carbon to CO_2 will contribute to localized acidification[49,50], making it even harder for reef-building corals to stand their ground.

Methods

Sampling sites. Data were collected between September 2000 and August 2007 at multiple sampling sites in ten different regions across three ocean systems (Central Pacific: Kiritimati, Tabuaeran, Palmyra and Kingman; Caribbean: Belize, Tobago, Panama, Puerto Rico and Mexico; Indian Ocean: Sri Lanka; Fig. 1). Geographic coordinates for each of the 60 sampling sites are provided in Supplementary Table 3.

Benthic cover. Benthic covers were assessed using photo-quadrats[51]. For each site, ten photo-quadrats were randomly selected along each of two 25 m transects. Pictures were taken with a digital camera connected to a tripod (1 m high) and a frame (0.9×0.6 m or 0.54 m^2). Images were later analysed using Photogrid 1.0. Each photograph was analysed with 100 stratified random points per image. All organisms marked by a point (2,000 per transect) were identified to the finest level of taxonomic resolution possible. The percentages of reef building taxa (including scleractinian corals and CCA) and fleshy algal (including macro- and turf algae) cover were calculated by dividing the number of points that were assigned to the respective functional group by the total number of points counted for each photo-quadrat. Average percentage cover per site was calculated by taking the mean of group abundances across the 20 photo-quadrats.

Water sample collection. Sample collection for DOC concentration, microbial counts and metagenomic analysis was conducted as described in ref. 52. Briefly, pre-washed diver-adapted 2 l polycarbonate Niskin bottles were used to collect seawater samples at each site from ~25 cm above the reef surface at a water depth of 10 m. Bottles remained capped until the sampling procedure. Before sampling, all collection and sample processing devices that could potentially contact the sample (except the GF/F filter) were submerged for 24 h in a 5% hydrochloric acid (HCl) bath to leach out any contaminating DOC. After each sampling procedure, devices were rinsed with the 5% HCl solution to prevent carbon contamination. Niskin bottles were opened just before submerging and their insides were flushed twice with sample water from that site. Special care was taken that sampling sites were upstream from boats or other divers to avoid contamination.

Sample processing. The water samples were processed within 1 h of collection to generate subsamples for later DOC analysis and direct counts of Bacteria and Archaea (microorganism). The Niskin bottles were connected directly to a positive-pressure filtration system that minimizes external contamination. All fixtures were made from polycarbonate or silicone. Acid-washed tubing and filter cassettes were flushed with 200 ml of the sampled water before sample processing began. Samples were processed under positive pressure by applying 0.2 bar of pressure to the Niskin bottles using a SCUBA tank.

Measurement of DOC concentrations. For DOC analysis, samples were filtered through a pre-combusted Whatman GF/F glass fibre filter and collected in pre-combusted amber glass vials (Wheaton) with acid-washed Teflon-lined lids. The bottles and lids were rinsed three times with the filtrate before collection of the sample for analysis. Samples were acidified (~pH 2) with analytical-grade 30% HCl (Fluka) and stored at 4 °C. Subsequently, DOC concentrations were measured by Expert Chemical Analysis using the high-temperature combustion method and an O.I. Analytical Model 1010 TOC analyser. To ensure quality control, DOC consensus reference materials (CRM: DSW Lot 05-05, 45–46 μM carbon; LCW Lot 12-01, 2 μM carbon, supplied by W. Chen, University of Miami; ref. 53) were used and the high-carbon standard was run every six samples. Low carbon water references (<4.17 μM) were measured as 2.89 ± 0.72 μM (mean ± standard error), and deep sea water references (45.83 μM) were measured as 45.54 ± 0.38 μM (mean ± standard error) over the course of the sample analysis. Both standards were consistently in the target range: low carbon standard (<4.17 μM) and deep sea standard (<1% deviation) (Supplementary Table 3). Compared to oceanic DOC concentrations established for surface seawater around the globe[54], the values measured here are relatively low for surface waters of tropical oceans. However, this is unlikely to affect the relative differences among samples and the statistical inferences, because all samples were

collected using an identical protocol and were analysed by the same laboratory using the identical TOC analyser with stable reference standards.

Enumeration of microbial abundances. The number of microorganism in each water column sample was determined via direct counts by epifluorescent microscopy using 500 µl of a 5× SYBR Gold stained reef water sample filtered through a 0.02 µm Anodisc (Whatman), as described in ref. 52.

Data processing and statistical analysis

DOC concentration. Reduced Major Axis (Model II) linear regression was used to build linear models and determine how much variation in DOC concentration and microbial cell numbers could be explained by benthic cover. The significance of each regression and a 95% confidence interval for the slope were calculated. Regressions were considered significant if $P < 0.05$. Regressions were evaluated separately for each ocean system because microbial community dynamics depend on a multitude of local variables such as nutrient availability, seawater temperature, salinity, light availability and micronutrient concentration[55].

Our data set included synoptic measurements of POC and dissolved inorganic phosphate (DIP) and nitrate (DIN, including nitrite). To evaluate whether algal cover is a significant predictor of DOC and microbial abundance after accounting for these key covariates, we used multiple regression to evaluate both the significance of algal cover in a least-squares multivariate model as well as the significant correlation of algal cover with the residuals of a model including all other factors in each ocean basin. Our results are detailed in Supplementary Table 1. Briefly, algal cover was a significant predictor of both DOC and microbial abundance ($P < 0.01$) in four separate models that each included latitude, longitude, POC, DIP and DIN as covariates, one model for all data and three models for each ocean basin separately (there was one exception: $P = 0.44$ for DOC in the Pacific Ocean). These models explained a significant proportion of variation in DOC and microbial abundance, with R^2 ranging from 0.24 to 0.88. In addition, in each ocean basin and across all data, algal cover was strongly and significantly correlated ($P < 0.05$, $|r| = 0.33$–0.41; one exception, $P = 0.64$, $r = 0.04$ for DOC in the Pacific Ocean), with the residuals of multiple regression models predicting DOC and microbial abundance from the covariates listed above.

Metagenomes. The metagenomes analysed from nine reef sites, seven in the Central Pacific and two in the Caribbean, used in this study are publicly available through the MG-RAST server under project name 'Global Microbialization On Reefs' with the IDs listed in Supplementary Table 4. Sequence data were processed using PRINSEQ (ref. 56) to remove low-quality and short reads. Pre-processed high-quality sequence data were then compared against the KEGG protein database[33] using BLASTx (ref. 57). The blast output for each metagenome was analysed using the HUMAnN software[58] to generate relative pathway and module abundances (dividing module read abundance by total read abundance for each metagenome). The normalized abundance values enable comparisons between samples and were therefore used for downstream statistical analyses. In addition, pre-processed sequence data were taxonomically annotated using the KEGG database within the MG-RAST software environment (http://www.biomedcentral.com/1471-2105/9/386). Module and taxon relative abundance data were arcsine-square root transformed before all linear statistical analyses to better approximate normal distributions (a standard transformation for relative abundance data). Analyses were restricted to Bacteria and Archaea, excluding hits to Eukarya, Viruses or unclassified reads.

An estimation of putative virulence factors (VFs) in reef microbial communities was conducted through sequence comparisons to a curated database for bacterial virulence factor genes (VFDB, http://www.mgc.ac.cn/VFs/)[59] using BLASTP, with an E value of 0.0001. The number of putative VFs per metagenome was enumerated based on the best blast hit (lowest E value) for each significant protein sequence similar to the VF database. The VF database comprises all experimentally validated bacterial VFs (traits encoded by 'virulence genes', which pathogenic microorganism use to cause infection, such as genes involved in adhesion and invasion, secretion systems and toxin production).

Metabolic pathways. Based on our previous experimental and observational results[4,18] we hypothesized that benthic cover would influence the relative abundances of microbial carbohydrate metabolism pathways in reef ecosystems, that is, those genes associated with catabolic oxidation of three to six carbon sugars, specifically the PP pathway, the TCA cycle, the ED pathway and the EMP pathway. We tested our prediction that modules associated with carbohydrate metabolism were more likely to be related to benthic cover than those associated with other pathways by comparing mean probabilities of regression significance among pathway groups. First, we regressed relative abundances of each of 216 KEGG modules (sets of gene orthologues associated into a defined pathway) found in our data set against percentage of algae cover at each sampling site. The P values were collated and an analysis of variance (ANOVA) was run to evaluate differences in mean P value among 42 KEGG module hierarchical groups (P values were roughly normally distributed and were not transformed). The ANOVA was significant ($P = 0.0312$), and analysis of means[60] *post hoc* multiple comparisons analysis was used to test whether any of the module groups were significantly different from the overall mean ($\alpha = 0.05$). Out of 26 pathway module groups tested, only the central carbohydrate metabolism was significantly lower in mean regression P value than the overall mean (group mean of 0.17 was below the lower decision limit of 0.18 when $n = 9$ and grand mean = 0.45), indicating modules in this pathway module grouping were significantly more likely to be related to percentage algae cover than modules in other pathway groupings. Other KEGG module grouping (structural complexes, functional sets) found in our metagenomes ($n = 16$) showed no significant differences in mean algal cover regression P values (ANOVA $P = 0.41$). Based on this result we narrowed our subsequent statistical investigations to only the KEGG modules associated with the central carbohydrate metabolism. Statistical evaluation of these relations was conducted using JMP 10 software (SAS Institute 2012).

In the sampled metagenomes, the central carbohydrate metabolism was represented by nine modules in four pathways: the EMP pathway (M1, M2 and M3), the PP pathway (M4, M6, and M7), the ED pathway (M8) and the TCA cycle (M9 and M11). To test whether algal cover was a significant predictor of multivariate carbohydrate metabolism composition or multivariate taxonomic composition, relative abundances of all taxonomic annotations (summed by genus $n = 476$, family $n = 232$ or order $n = 116$, separately) or all carbohydrate module annotations ($n = 9$) were converted to among-sample Bray–Curtis distance matrices and regressed against proportional algal cover using permutational multivariate analysis of variance via the adonis command in the vegan package for R.

To evaluate the quantitative linkage between dominant benthic cover and carbohydrate metabolism more specifically, we used a series of tests comparing the relative abundances of (1) each of the nine individual modules or (2) groups of modules representing each of the four pathways listed above, for significant differences between coral-dominated and algae-dominated reefs. These tests were performed in R version 2.9.2 (http://www.r-project.org). For both (1) and (2), we used a bootstrapping approach to test for differences among groups. This approach was chosen because of the relatively small number of data points and the lack of clear expectations regarding probabilistic distributions of the relative abundance data. As a summary metric of difference values we used a response ratio, calculated as the difference in natural logarithms of the group-specific means of the abundance data from sites with dominant benthic cover being algae and coral, respectively. These means were defined differently for each of the two analyses. For (1), the means were averaged values of relative abundance estimates for each module. For (2), the means were summed values of the estimated means for each of the constituent modules in the focal pathway. Bootstrapping the data generated a null distribution of response ratio values expected for each module (for (1)) or each pathway (for (2)), when the dominant benthic cover had no effect on module or pathway mean relative abundance. To determine whether each module showed differences (1), we assembled 100,000 virtual collections of data for each module based on sampling with replacement from the nine actual values from the metagenomes. Bootstrapped sample sizes were constrained to equal those of the real data set (that is, $n = 5$ for 'coral' and $n = 4$ for 'algae'). To answer whether each pathway showed differences (2), we calculated the null similarly, but we summed the bootstrapped means across the constituent modules of the pathway for each simulated data set. In both cases, the bootstrapped values were used to generate a distribution to which we could compare the estimated response ratio from the true data. Statistical significance was met if the estimated value lay in the most extreme 0.025 of the null distribution (consistent with two-tailed test with $\alpha = 0.05$).

Accession codes. The metagenomes used in this study are publicly available through the MG-RAST server under the project name 'Global Microbialization On Reefs' (http://metagenomics.anl.gov/linkin.cgi?project=16895). MG-RAST IDs: 4449259.3, 4449268.3, 4442643.3, 4442652.3, 4442653.3, 4466740.3, 4466742.3, 4466813.3 and 4466841.3.

Received 7 October 2015; accepted 2 March 2016; published 25 April 2016

References

1. Wild, C. *et al.* Coral mucus functions as an energy carrier and particle trap in the reef ecosystem. *Nature* **428,** 66–70 (2004).
2. De Goeij, J. M. *et al.* Surviving in a marine desert: the sponge loop retains resources within coral reefs. *Science* **342,** 108–110 (2013).
3. Haas, A. F. *et al.* Effects of coral reef benthic primary producers on dissolved organic carbon and microbial activity. *PLoS ONE* **6,** e27973 (2011).
4. Nelson, C. E. *et al.* Coral and macroalgal exudates vary in neutral sugar composition and differentially enrich reef bacterioplankton lineages. *ISME J.* **7,** 962–979 (2013).
5. Carlson, C. in *Biogeochemistry of Marine Dissolved Organic Matter* (eds Hansell, D. A. & Carlson, C. A.) 123–124 (Academic, 2014).
6. Hansell, D. A. & Carlson, C. A. Localized refractory dissolved organic carbon sinks in the deep ocean. *Glob. Biogeochem. Cycles* **27,** 20067 (2013).
7. Vroom, P. S. 'Coral dominance': a dangerous ecosystem misnomer? *J. Mar. Biol.* **2011,** 164127 (2011).
8. Sandin, S. A. *et al.* Baselines and degradation of coral reefs in the northern Line Islands. *PLoS ONE* **3,** e1548 (2008).
9. Hughes, T. P. *et al.* Phase shifts, herbivory, and the resilience of coral reefs to climate change. *Curr. Biol.* **17,** 360–365 (2007).
10. Nyström, M., Folke, C. & Moberg, F. Coral reef disturbance and resilience in a human-dominated environment. *Trends Ecol. Evol.* **15,** 413–417 (2000).

11. Anthony, K. R., Kline, D. I., Diaz-Pulido, G., Dove, S. & Hoegh-Guldberg, O. Ocean acidification causes bleaching and productivity loss in coral reef builders. *Proc. Natl Acad. Sci. USA* **105,** 17442–17446 (2008).
12. Thurber, R. L. V. *et al.* Metagenomic analysis indicates that stressors induce production of herpes-like viruses in the coral *Porites compressa*. *Proc. Natl Acad. Sci. USA* **105,** 18413–18418 (2008).
13. Dinsdale, E. A. *et al.* Microbial ecology of four coral atolls in the northern Line Islands. *PLoS ONE* **3,** 1584 (2008).
14. Kelly, L. W. *et al.* Black reefs: iron induced phase-shift on coral reefs. *ISME J.* **6,** 638–649 (2012).
15. Wild, C., Niggl, W., Naumann, M. S. & Haas, A. F. Organic matter release by Red Sea coral reef organisms—potential effects on microbial activity and *in-situ* O_2 availability. *Mar. Ecol. Prog. Ser.* **411,** 61–71 (2010).
16. Haas, A. F., Jantzen, C., Naumann, M. S., Iglesias-Prieto, R. & Wild, C. Organic matter release by the dominant primary producers in a Caribbean reef lagoon: implication for *in-situ* O_2 availability. *Mar. Ecol. Prog. Ser.* **409,** 27–39 (2010).
17. Morrow, K. M. *et al.* Allelochemicals produced by Caribbean macroalgae and cyanobacteria have species-specific effects on reef coral microorganisms. *Coral Reefs* **30,** 309–320 (2011).
18. Haas, A. F. *et al.* Influence of coral and algal exudates on microbially mediated reef metabolism. *PeerJ* **1,** e108 (2013).
19. Kline, D. I., Kuntz, N. M., Breitbart, M., Knowlton, N. & Rohwer, F. Role of elevated organic carbon levels and microbial activity in coral mortality. *Mar. Ecol. Prog. Ser.* **314,** 119–125 (2006).
20. Smith, J. E. *et al.* Indirect effects of algae on coral: algae-mediated, microbe-induced coral mortality. *Ecol. Lett.* **9,** 835–845 (2006).
21. Kuntz, N. M. *et al.* Pathologies and mortality rates caused by organic carbon and nutrient stressors in three Caribbean coral species. *Mar. Ecol. Prog. Ser.* **294,** 173–180 (2005).
22. Barott, K. L. & Rohwer, F. L. Unseen players shape benthic competition on coral reefs. *Trends Microbiol.* **20,** 621–628 (2012).
23. McCook, L., Jompa, J. & Diaz-Pulido, G. Competition between corals and algae on coral reefs: a review of evidence and mechanisms. *Coral Reefs* **19,** 400–417 (2001).
24. Jackson, J. B. C. *et al.* Historical overfishing and the recent collapse of coastal ecosystems. *Science* **293,** 629–637 (2001).
25. Nelson, C. E., Alldredge, A. L., McCliment, E. A., Amaral-Zettler, L. A. & Carlson, C. A. Depleted dissolved organic carbon and distinct bacterial communities in the water column of a rapid-flushing coral reef ecosystem. *ISME J.* **5,** 1374–1387 (2011).
26. Thingstad, T. F. *et al.* Counterintuitive carbon-to-nutrient coupling in an Arctic pelagic ecosystem. *Nature* **455,** 387–390 (2008).
27. Geller, A. Comparison of mechanisms enhancing biodegradability of refractory lake water constituents. *Limnol. Oceanogr.* **31,** 755–764 (1986).
28. van Nugteren, P. *et al.* Seafloor ecosystem functioning: the importance of organic matter priming. *Mar. Biol.* **156,** 2277–2287 (2009).
29. Zweifel, U. L., Norrman, B. & Hagstrom, A. Consumption of dissolved organic carbon by marine bacteria and demand for inorganic nutrients. *Mar. Ecol. Prog. Ser.* **101,** 23–32 (1993).
30. Del Giorgio, P. A., Cole, J. J. & Cimbleris, A. Respiration rates in bacteria exceed phytoplankton production in unproductive aquatic systems. *Nature* **385,** 148–151 (1997).
31. Weinbauer, M. G., Chen, F. & Wilhelm, S. W. in *Microbial Carbon Pump in the Ocean* (eds Jiao, N., Azam, F. & Sanders, S.) 54–56 (AAAS, 2011).
32. Gili, J. M. & Coma, R. Benthic suspension feeders: their paramount role in littoral marine food webs. *Trends Ecol. Evol.* **13,** 316–321 (1998).
33. Kanehisa, M. & Goto, S. KEGG: Kyoto encyclopedia of genes and genomes. *Nucleic Acids Res.* **28,** 27–30 (2000).
34. Lauro, F. M. *et al.* The genomic basis of trophic strategy in marine bacteria. *Proc. Natl Acad. Sci. USA* **106,** 15527–15533 (2009).
35. Harvell, C. D. *et al.* Emerging marine diseases—climate links and anthropogenic factors. *Science* **285,** 1505–1510 (1999).
36. Kim, B. H. & Gadd, G. M. *Bacterial Physiology and Metabolism* (Cambridge Univ. Press, 2008).
37. Romano, A. H. & Conway, T. Evolution of carbohydrate metabolic pathways. *Res. Microbiol.* **147,** 448–455 (1996).
38. Flamholz, A., Noor, E., Bar-Even, A., Liebermeister, W. & Milo, R. Glycolytic strategy as a tradeoff between energy yield and protein cost. *Proc. Natl Acad. Sci. USA* **110,** 10039–10044 (2013).
39. Westerhoff, H. V., Hellingwerf, K. J. & Van Dam, K. Thermodynamic efficiency of microbial growth is low but optimal for maximal growth rate. *Proc. Natl Acad. Sci. USA* **80,** 305–309 (1983).
40. Stettner, A. I. & Segrè, D. The cost of efficiency in energy metabolism. *Proc. Natl Acad. Sci. USA* **110,** 9629–9630 (2013).
41. Pfeiffer, T. & Schuster, S. Game-theoretical approaches to studying the evolution of biochemical systems. *Trends Biochem. Sci.* **30,** 20–25 (2005).
42. Odum, H. & Pinkerton, R. C. Time speed regulator: the optimum efficiency for the maximum power output in physical and biological systems. *Am. Sci.* **43,** 331–343 (1955).
43. Hankinson, O. Mutants of the pentose phosphate pathway in *Aspergillus nidulans*. *J. Bacteriol.* **117,** 1121–1130 (1974).
44. Sprenger, G. A. Genetics of pentose-phosphate pathway enzymes of *Escherichia coli* K-12. *Arch. Microbiol.* **164,** 324–330 (1995).
45. Haas, A. F. & Wild, C. Composition analysis of organic matter released by cosmopolitan coral reef-associated green algae. *Aquat. Biol.* **10,** 131–138 (2010).
46. Milner, H. W. in *Algal Culture from Laboratory to Pilot Plant* Vol. 600 (ed., Burlew, J. F.) 285–302 (Carnegie Institution of Washington, 1953).
47. McDole, T. *et al.* Assessing coral reefs on a Pacific-wide scale using the microbialization score. *PLoS ONE* **7,** e43233 (2012).
48. Halpern, B. S. *et al.* A global map of human impact on marine ecosystems. *Science* **319,** 948–952 (2008).
49. Joint, I., Doney, S. C. & Karl, D. M. Will ocean acidification affect marine microbes? *ISME J.* **5,** 1–7 (2011).
50. Price, N. N., Martz, T. R., Brainard, R. E. & Smith, J. E. Diel variability in seawater pH relates to calcification and benthic community structure on coral reefs. *PLoS ONE* **7,** e43843 (2012).
51. Preskitt, L. B., Vroom, P. S. & Smith, C. M. A rapid ecological assessment (REA) quantitative survey method for benthic algae using photo quadrats with SCUBA. *Pacif. Sci.* **58,** 201–209 (2004).
52. Haas, A. F. *et al.* Unraveling the unseen players in the ocean—a field guide to water chemistry and marine microbiology. *J. Vis. Exp.* **93,** e52131 (2014).
53. Sharp, J. H. *et al.* Final dissolved organic carbon broad community intercalibration and preliminary use of DOC reference materials. *Mar. Chem.* **77,** 239–253 (2002).
54. Hansell, D. A., Carlson, C. A., Repeta, D. J. & Schlitzer, R. Dissolved organic matter in the ocean: a controversy stimulates new insights. *Oceanography* **22,** 202–211 (2009).
55. Kelly, L. W. *et al.* Local genomic adaptation of coral reef-associated microbiomes to gradients of natural variability and anthropogenic stressors. *Proc. Natl Acad. Sci. USA* **111,** 10227–10232 (2014).
56. Schmieder, R. & Edwards, R. Quality control and preprocessing of metagenomic datasets. *Bioinformatics* **27,** 863–864 (2011).
57. Camacho, C. *et al.* BLAST+: architecture and applications. *BMC Bioinformatics* **10,** 421 (2009).
58. Abubucker, S. *et al.* Metabolic reconstruction for metagenomic data and its application to the human microbiome. *PLoS Comput. Biol.* **8,** e1002358 (2012).
59. Chen, L. *et al.* VFDB: a reference database for bacterial virulence factors. *Nucleic Acids Res.* **33,** 325–328 (2005).
60. Nelson, P. R., Wludyka, P. S. & Copeland, K. A. *The Analysis of Means: A Graphical Method for Comparing Means, Rates, and Proportions* (SIAM, 2005).

Acknowledgements

Reef water samples were collected during two research expeditions to the Line Islands funded by the National Geographic Society, the Moore Family Foundation, the Fairweather Foundation, the Marine Managed Areas Science Project of Conservation International, Scripps Institution of Oceanography, E. Scripps, I. Gayler and several private donors. The authors thank the captain and crew of the RV White Holly and MV 'Searcher' for logistical support and hospitality. Field support was also provided by S. Ahamed and the Co-operative Society of Tourist Boat Operators, Pigion Island, in Sri Lanka. This work was carried out under research permits from the US Fish and Wildlife Service to operate in the Kingman Atoll National Wildlife Refuge and the Environment and Conservation Division of the Republic of Kiribati. The authors thank The Nature Conservancy and the Palmyra Atoll Research Consortium for additional field support. This research was sponsored by the Marine Microbial Initiative of the Gordon and Betty Moore Foundation and by GBMF Investigator Award 3781 as well as a Canadian Institute for Advanced Research Integrated Microbial Biodiversity Program Fellowship 141679 and the Pew Charitable Trusts award MASTER 666/PROJ 28972 (to F.R.). It was funded by the US National Science Foundation awards OCE–1538567 (to L.W.K.), OCE–1538393 (to C.E.N.) and DUE–1323809 (to E.A.D.). C.E.N. was funded in part by a grant/cooperative agreement from the National Oceanic and Atmospheric Administration, Project A/AS-1, which is sponsored by the University of Hawaii Sea Grant College Program, SOEST, under Institutional Grant No. NA14OAR4170071 from NOAA Office of Sea Grant, Department of Commerce. The views expressed herein are those of the author(s) and do not necessarily reflect the views of NOAA or any of its subagencies. This manuscript is UH SOEST publication no. 9575 and UH Sea Grant publication number UNIHI-SEAGRANT-JC-15-12. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Author contributions

A.F.H., M.F.M.F., L.W.K. and C.E.N. conceptualized the study, analysed data and wrote the paper. E.A.D., R.A.E., S.G., M.H., N.H., B.K., Y.W.L., H.M., O.P., T.N.F.R., S.E.S., C.B.S., S.S. and J.E.S. performed experiments and analysed data. F.R. contributed to the concept and design of the study, data analysis and manuscript writing.

Additional information

Supplementary information is available online. Reprints and permissions information is available online at www.nature.com/reprints. Correspondence and requests for materials should be addressed to A.F.H.

Competing interests

The authors declare no competing financial interests.