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Energetic differences between bacterioplankton trophic groups and coral reef resistance

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Coral reefs are among the most productive and diverse marine ecosystems on the Earth. They are also particularly sensitive to changing energetic requirements by different trophic levels. Microbialization specifically refers to the increase in the energetic metabolic demands of microbes relative to macrobes and is significantly correlated with increasing human influence on coral reefs. In this study, metabolic theory of ecology is used to quantify the relative contributions of two broad bacterioplankton groups, autotrophs and heterotrophs, to energy flux on 27 Pacific coral reef ecosystems experiencing human impact to varying degrees. The effective activation energy required for photosynthesis is lower than the average energy of activation for the biochemical reactions of the Krebs cycle, and changes in the proportional abundance of these two groups can greatly affect rates of energy and materials cycling. We show that reef-water communities with a higher proportional abundance of microbial autotrophs expend more metabolic energy per gram of microbial biomass. Increased energy and materials flux through fast energy channels (i.e. water-column associated microbial autotrophs) may dampen the detrimental effects of increased heterotrophic loads (e.g. coral disease) on coral reef systems experiencing anthropogenic disturbance.

1. Introduction

The major pathways for energy and materials flux in the ocean are primarily through the viruses, microbes and protists [1–3]. Even though oceanic phytoplankton represent less than 1% of the global primary producer biomass, as a guild they are responsible for nearly half of the total net primary production (NPP) on the planet [4–6]. In coral reef ecosystems, cyanobacteria of the genera *Prochlorococcus* and *Synecchococcus* are typically responsible for 50–80% of total phytoplankton primary production [7]. Similarly, heterotrophic microbes are responsible for most of the carbon and nutrient reminerialization in the marine environment [8,9]. Combined, these two trophic guilds are responsible for most of the energy and materials flux in the world's oceans. Only in certain cases do macrobes significantly contribute to energy flows. These *macrobial ecosystems* occur in near shore environments, where large concentrations of pelagic and benthic macro-organisms congregate. Among macrobial marine ecosystems, coral reefs are the most productive and appear to be particularly sensitive to changing energetic requirements by different trophic levels. Owing to high mass-specific metabolic requirements and rapid biomass turnover, small changes in microbial biomass have large effects on total energy flux. A proportionately greater increase in the metabolic demands of microbes relative to macrobes is

called *microbialization* [10,11]. Microbialization and human impact are strongly correlated on coral reefs [10].

The disease, dissolved organic carbon (DOC), algae and microbe (DDAM) model is a mechanistic hypothesis explaining how coral reefs decline in response to shifting energy fluxes. This positive feedback model has significant support ranging from ecological surveys to laboratory studies (reviewed in Barott & Rohwer [12]) [12–17]. DDAM predicts that stressors such as overfishing and eutrophication release turf and fleshy macroalgae from grazing control, leading to increased release rates of labile DOC. DOC stimulates heterotrophic microbial growth [18–21], some of which are opportunistic coral pathogens [22]. These pathogens kill corals and create more space for the algae.

The relationship between benthic and water-column microbial processes on coral reef systems is not well understood. In oligotrophic waters surrounding coral reef ecosystems and lagoons, *Synecchococcus* and *Prochlorococcus* spp. are the predominant microbial autotrophs [7]. Cyanobacteria within these genera are generally not considered pathogenic and are very fast energy channels. In aquatic ecosystems, phenomena which dampen vertical energy flux (either by impeding transfer between trophic levels or by reducing the efficiency with which energy is passed through a trophic level) tend to reduce the destabilizing effect of increased energy flux up a food chain [23,24]. It has also been shown that food webs are more stable when the majority of energy flows through the fastest basal channel in both aquatic and terrestrial ecosystems [25].

Marine microbial growth rates are traditionally measured by incorporation assays using radiolabelled nucleotides or amino acids [26,27]. Because of the regulatory issues associated with radiative materials, these methods are not easily amendable to large-scale surveys of coral reefs. Non-radioactive methods have been developed. However, their success in remote sites has been variable [28,29]. An alternative approach is to use the metabolic theory of ecology (MTE) to predict community-level energy flux from body mass and abundance. MTE is a conceptual framework for ecological energetics that can be used to explore ecosystem structure and dynamics in terms of flux, transformation, and storage of energy and materials. This empirically supported mathematical equation (equation (1.1)) predicts individual metabolic rate (I) from the combined effects of body mass (M) and temperature (T), where M is the wet weight of the organism in grams, and i_0 and α (scaling exponent) are fitted intercept and slope coefficients that vary depending on organismal group (i.e. fishes microbes) and physiological state (i.e. basal versus active metabolic rate) [30,31].

$$I = i_0 M^{\alpha} e^{-E/kT}. \tag{1.1}$$

For microbes, individual metabolic rate scales super-linearly (as opposed to sub-linearly) with body mass [31].

The effects of temperature and trophic strategy on individual metabolic rate are accounted for by $e^{-E/kT}$ [32,33], where E is the activation energy, k is Boltzmann's constant (8.62×10^{-5} eV K^{-1}) and T is the water temperature at the site at the time of collection (in Kelvin). Our metabolic rate predictions are dependent on the assumption that the effective activation energy for oxygenic photosynthesis ($E = 0.32$ eV, range = 0–30°C) is lower than the average energy of activation for the biochemical reactions of the Krebs cycle ($E = 0.61$ eV). This assumption is empirically supported [34–36] and has been used to predict metabolic rates for planktonic organisms in both freshwater and marine ecosystems [33,36,37]. Increasing T and/or reducing E increases chemical reaction rates (equation (1.1)). Thus, at a fixed temperature and mass, an autotrophic bacterium requires more metabolic energy than a heterotrophic bacterium. As E occurs in the exponent (equation (1.1)), a small increase in the proportional abundance of microbial autotrophs may profoundly affect rates of energy and materials cycling within a reef system.

In this study, we use a combination of flow cytometry and MTE to quantify the relative contributions of two broad groups of bacterioplankton: autotrophs versus heterotrophs, to metabolic energy flux on 27 Pacific coral reefs experiencing varying degrees of human impact. We provide support for the hypothesis that autotrophic-driven microbialization may slow the rise of heterotrophic microbes, including heterotrophic coral pathogens, by serving as an energy 'sink' on impacted coral reefs. We also identify new bioenergetics-based indices that can be used to differentiate high and low human-impacted coral reef systems.

2. Material and methods

(a) Sample collections and preparation

All 27 islands were surveyed following the National Oceanic and Atmospheric Association (NOAA)'s Rapid Ecological Assessment (REA) protocol as part of the Pacific Reef Assessment and Monitoring Program (Pacific RAMP) [38,39]. Multiple sites (depth: 10–15 m) were sampled at each island in four broad regional groups: the Main Hawaiian Islands (2008), Guam and the Mariana Islands (2009), the American Samoa region (2010) and the Pacific Remote Island Areas (PRIAs) (2010). At each site diver-deployable, 2 l Niskin bottles were filled with water from approximately 1 m above the reef benthos. For nutrient analysis, plastic scintillation vials were rinsed 3× with the water sample, filled and then frozen at −20°C. Concentrations of nitrite, nitrate and phosphate were analysed at NOAA's Pacific Marine Environmental Laboratory (PMEL) (detection limits: NO_x, 0.01 µM; PO_4^{3-}, 0.01 µM). Water for local chlorophyll *a* analysis was collected concurrently and processed using standard fluorometric methods [40].

(b) Flow cytometry analysis

Water samples for flow cytometry were passed through a 20 µm pore size filter (Whatman Nucleopore Track-Etched membrane; GE Healthcare Life Sciences, USA). The filtrate, containing micro- and picoautotrophs, was fixed in electron microscopy grade glutaraldehyde (0.125% final concentration; Electron Microscopy Sciences, Hatfield, PA, USA) for 15 min at room temperature in the dark. Aliquots (1 ml) were frozen in liquid nitrogen, and stored at −80°C. On the day of analysis, samples were thawed at 37°C and split into 2 × 500 µl aliquots. One aliquot was stained with SYBR Green I (10× final concentration; Invitrogen Molecular Probes, Carlsbad, CA, USA) and used to analyse the total number of microbes in each sample [41]. The other aliquot was left unstained and used to determine the proportion of autotrophs (micro- and pico-phytoplankton). Using a BD FACSCanto with a high throughput sampler (Becton Dickinson Biosciences, San Jose, CA, USA), samples were excited using a blue laser line (488 nm), and thresholds were set to chlorophyll fluorescence (back to back long pass mirrors resulting in a range of 675–735 nm), and FITC fluorescence (530/30), for unstained and stained aliquots, respectively (electronic supplementary material, figure S1). A channel for chlorophyll and a channel for phycoerytherin (585/42) were used for the detection of autotrophs. Autotrophic counts were subtracted from SYBR-stained total counts to give

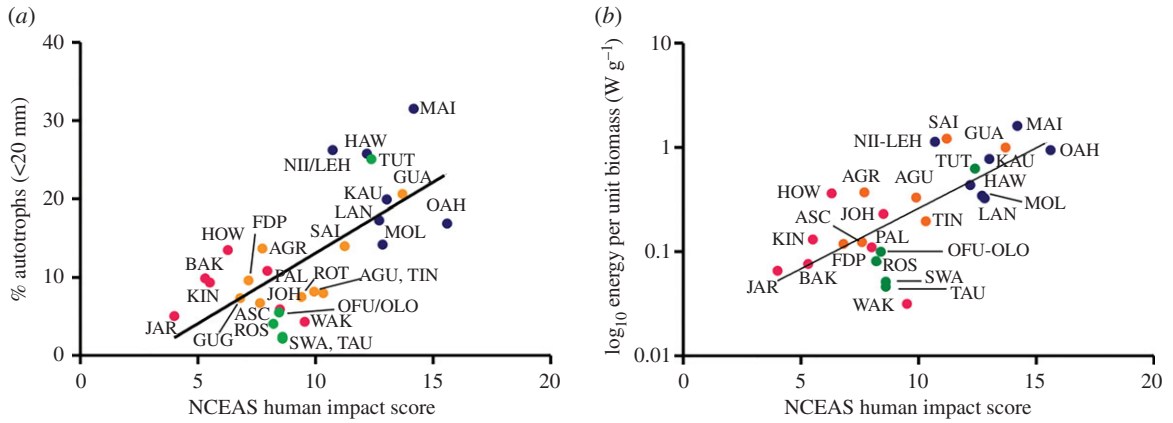

Figure 1. (*a*) The relationship between the island-level NCEAS cumulative human impact scores and the relative abundance of microbial autotrophs (micro- and picoautotrophs; less than 20 µm) in reef-water ($n = 27$, $y = 1.89x - 6.0$, $r^2 = 0.46$). Colour denotes oceanographic region: Guam and the Mariana Islands (orange circles), the Main Hawaiian Islands (blue circles), Pacific Remote Islands Areas (pink circles) and American Samoa (green circles). For three-letter island codes, see the electronic supplementary material, table S2. (*b*) The amount of energy consumed per gram of microbial biomass supported (mass-specific metabolic rate) versus the NCEAS cumulative human impact score ($n = 26$, $y = 0.12x - 1.74$; $r^2 = 0.50$).

heterotrophic counts. Not all *Prochlorococcus* cells could be quantified because dimly fluorescing cells were below the noise threshold. Data (.fcs) files were analysed using FlowJo v. 7.6.5 software (Treestar Inc., Ashland Oregon).

(c) Calculating autotrophic and heterotrophic energy use

The average energy of activation (E) for respiration (0.61 eV) was used to predict metabolic rates for heterotrophic microbes [31]. The effective activation energy for the light reactions of photosynthesis is 0.32 eV [33]. In *Prochlorococcus* spp., these two activities have opposite expression patterns relative to the light–dark cycle [42]. Therefore, an average value of $E = 0.46$ eV was used to predict metabolic rates for the autotrophic fraction. Metabolic rates for microbial autotrophs were also calculated separately for night ($E = 0.61$ eV) and day ($E = 0.32$ eV) and then averaged (caveats, electronic supplementary material, table S1). A scaling exponent (α) of 1.72 (basal metabolic rate for microbes) was used for both trophic components [31]. Individual mass values from McDole *et al*. [10] were used to calculate site-level metabolic rates using only the mass-dependent side of the MTE equation ($i_0 M^{\alpha}$) [10]. Site-level values were then averaged to the island level. These values were then multiplied by island-level mean per cent autotrophs or heterotrophs (equation (2.2)) and the temperature-dependent side of equation (1.1) ($e^{-E/kT}$) using the respective values for the energy of activation ($E = 0.46$ or 0.61 eV), and the island-level mean value for temperature (T).

$$I_{\text{autotrophs}} = i_0 M^{\alpha} \left(\frac{\text{cells}_{\text{unstained}}}{\text{cells}_{\text{stained}}} \right). \tag{2.1}$$

(d) Human impact and oceanographic data

The level of human impact was quantified using the cumulative global human impact map generated by the National Center for Ecological Analysis and Synthesis (NCEAS; http://www.nceas.ucsb.edu/globalmarine/impacts). Mean impact scores were calculated in ArcGIS v. 9.3 using previously described methods [10].

Primary productivity estimates were derived from Moderate Resolution Imaging Spectroradiometer (MODIS) satellite data using the Vertically Generalized Production Model (VGPM; www.science.oregonstate.edu/ocean.productivity/standard.product.php) [43]. As these satellite datasets are less accurate for near shore measurements, satellite-based NPP values were estimated for a 50 km radius ring surrounding each island, with the first 10 km around each island removed [44]. Soluble iron deposition rates were extracted with ArcMap from Mahowald *et al*. [44] using the global map file for Scenario III (atmospheric processing + direct emissions) [44]. Site-level values were then averaged to the island level.

3. Results

(a) Water-column associated microbial autotrophs are not likely to be fish or coral pathogens

Although resident heterotrophic microbes probably play a key role in limiting the abundance of pathogenic microbes on corals under normal conditions [45], the majority of the reef-associated pathogens that have been described to date belong to this trophic group. Electronic supplementary material, table S2 lists the causative agents for all coral diseases listed in the Global Coral Disease Database (http://coraldisease.org/) and includes a long list of opportunistic pathogens in the Family *Vibrionacae* that infect both fishes and corals. Microbial mats dominated by benthic, filamentous species of cyanobacteria also play a large role in coral diseases leading to reef decline (e.g. Black Band Disease); however, the predominant microbial autotrophs in oligotrophic waters surrounding coral reef ecosystems and lagoons are *Synecchococcus* and *Prochlorococcus* spp., genera which are not likely to be pathogens in the marine environment [7].

(b) Human impact and autotrophic microbes

The proportional abundance of micro- and picoautotrophs in reef-water was significantly positively correlated with the NCEAS cumulative human impact score at the Pacific-wide scale (Pearson's correlation: $r = 0.66$, $n = 27$, $p < 0.0001$, two tails). At regional scales, the highest proportion of microbial autotrophs also occurred on the most impacted locations in the Mariana (Guam and Saipan) and the American Samoa regions (Tutuila) (figure 1*a*, electronic supplementary material, table S3). These results support the hypothesis that increasing human activities favour planktonic autotrophs over heterotrophs up to a certain (undefined) point.

(c) Effects of island size

Regardless of geographical region, larger islands are more densely populated and hence experience higher levels of

human influence. The NCEAS human impact scores were highly correlated with log land area (km^2) (Pearson's correlation: $r = 0.84$, $n = 27$, $p < 0.0001$, two tails). Therefore, island size may confound the positive correlation between the level of human impact and the per cent autotrophs (figure 1*a*). To address this issue, land area (square kilometres) and reef area (square kilometres) were included as possible predictor variables in multiple linear regression analysis [46]. This resulted in four models of interest: per cent autotrophs (y) = $\beta_0 + \beta_1$ (NCEAS), $y = \beta_0 + \beta_1$ (NCEAS) + β_2 (log land area), $y = \beta_0 + \beta_1$ (NCEAS) + β_2 (log reef area), $y = \beta_0 + \beta_1$ (NCEAS) + β_2 (log land area) + β_3 (log reef area). The regression coefficients (β) represent the relative contribution of each of the independent variables to the prediction of the dependent variable (y), where y = per cent autotrophs.

In the first model (per cent autotrophs = $\beta_0 + \beta_1$ (NCEAS)), the NCEAS score was significant ($p < 0.0001$). Using a *t*-test, and given that the NCEAS score is included in the model, the *p*-values associated with land area (but not reef area) were significant in both the two and three parameter models ($p < 0.01$). Using a model selection criteria, the model with the smallest Akaike information criteria value included land area as an additional variable ($y = \beta_0 + \beta_1$ (NCEAS) + β_2 (log land area)) [47]. This model explained 51% of the variability in the per cent autotrophs; an improvement over the simplest model ($y = \beta_0 + \beta_1$ (NCEAS)) which explained 45% of the variability (electronic supplementary material, table S5).

One explanation for the positive relationship between the relative abundance of phytoplankton, human impact and island size, might be land-based sources of nutrient input. To investigate this hypothesis, NCEAS layers for nutrient input (based on average annual use of fertilizer) and non-point source inorganic pollution (urban run-off) were obtained from http://globalmarine.nceas.ucsb.edu [48]. In the Marianas Islands, there was a strong positive relationship between inorganic pollution and the per cent of micro- and picoautotrophs (linear regression: $n = 5$, $y = 213.0x + 5.98$, $r^2 = 0.87$; electronic supplementary material, figure S2). In the Main Hawaiian Islands, per cent micro- and picoautotrophs were not correlated with either variable. No significant relationships were found to exist between the abundance of microbial autotrophs and the nutrient levels (PO_4^{3-}, SiO_2 or NO_x) in the surrounding reef-water at the time of sample collection in any of the regions. To look more closely at whether the per cent autotrophs might be explained by the surrounding ocean, we tested for between island-groups differences. In summary, the pattern in per cent autotrophs (figure 1*a*) does not appear to be driven by differences in the regional oceanographic parameters of any one island group (electronic supplementary material, figure S3). It should be noted that the PRIAs islands span a particularly wide latitudinal range (approx. 0°–20° N) and examination of boxplots showed large variation in NPP, NO_x and PO_4^{3-} for this group.

(d) Higher NCEAS score reefs support less microbial biomass per unit of energy flux

Mean microbial biomass at each location was used to calculate a mass-specific metabolic rate for the entire microbial community (i.e. Watts of energy consumed per gram of microbial tissue supported). Mass-specific metabolic rate ($W\,g^{-1}$) was calculated by dividing the total predicted metabolic rate (this study) by the total microbial biomass for each island

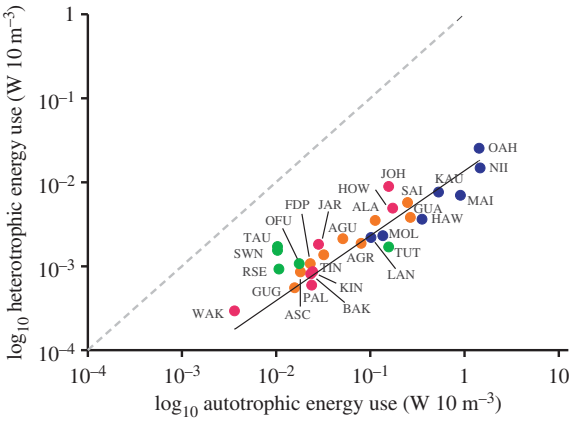

Figure 2. Least-squares regression analysis on log-transformed energy use predictions for autotrophic and heterotrophic microbes (less than 20 µm fraction) in the reef-water column ($n = 27$, $y = 0.77x - 1.86$; $r^2 = 0.80$). The dotted line has a slope of unity. Colours represent oceanographic regions as shown in figure 1.

($g\,10\,m^{-3}$; adapted from McDole *et al.* [10]). At the Pacific-wide scale, reefs with higher NCEAS scores harboured microbial communities that required more energy per gram of tissue supported (figure 1*b*). For example, on Tau Island (NCEAS score = 8.6), microbial autotrophs were approximately 2% of the total bacterioplankton community but required approximately 5× more energy per second than heterotrophs. By comparison, on Maui (NCEAS score = 14.2) autotrophs were 31.5% of the population and required 128× more energy per second than heterotrophs; a 30-fold increase in the amount of energy required per gram (Tau = $0.05\,W\,g^{-1}$; Maui = $1.6\,W\,g^{-1}$). Although heterotrophic cells comprised greater than 75% of the total bacterial community abundance in all samples (88% on average), small increases in the proportional abundance of microbial autotrophs had profound effects on the amount of energy fluxed by the microbial community.

In general, this relationship was also found at regional levels. In the Marianas region, rates of energy flux per gram of microbial biomass were highest on the most impacted islands of Guam and Saipan. Similarly, the mass-specific metabolic rate of the microbial community on Tutuila (the most impacted island in American Samoa) was an order of magnitude higher than on less impacted locations in the same region (e.g. Swain's and Rose Island). Within the Main Hawaiian Islands, water-column associated microbes were fluxing the most energy per gram on Maui, Oahu and Niihau. Maui and Oahu have the highest NCEAS scores in the 27 island dataset, while Niihau-Lehua reef area is the least populated location within the group. This suggests there may be factors affecting ecological dynamics in this location that are not captured by the NCEAS framework. As microbial metabolic rates scale as individual body mass to the 1.7 power (super-linear scaling), a higher proportion of copiotrophic bacteria, which are typically larger cells, would exacerbate (and not compensate for) any autotrophic-driven increase in energy use, further increasing mass-specific metabolic requirements at more impacted sites.

In figure 2, we show that metabolic rate predictions for autotrophs and heterotrophs follow each other. When metabolic requirements increase for microbial phytoplankton, energy use predictions for heterotrophic microbes also rise, indicative of the trophic energy transfer that is occurring

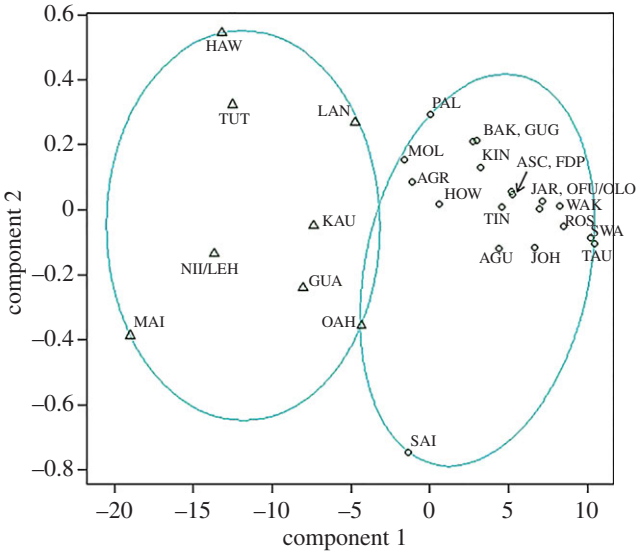

Figure 3. Visualization of results obtained with PAM clustering. The relative abundance of microbial autotrophs (%) and mass-specific metabolic rate (W g^{-1}) were used as *x*- and *y*-coordinates. The analysis includes 27 island locations from four different regional groups: the Main Hawaiian Islands ($n = 7$), the American Samoa region ($n = 5$), the Marianas region ($n = 8$) and PRIAs group ($n = 7$).

between these two bacterioplankton groups. The less than unity slope signifies that metabolic activity by microbial heterotrophs declines relative to that of autotrophic microbes as total metabolic demands increase. Similar relationships in which bacterioplankton biomass is 'dampened in amplitude but coherent in the direction of change' relative to chlorophyll *a* concentration have been described [49–51].

(e) Bioenergetics-based classification

We previously showed that the microbialization score is a useful metric for capturing the widespread, between-region degradation that is currently occurring on Pacific coral reefs [10]. To further investigate the use of bioenergetics-based indices for assessing coral reef health, the relative abundance of microbial autotrophs (%) and mass-specific metabolic rate (W g^{-1}) were selected as *x*- and *y*-coordinates and partitioning around meteoids (PAM), a more robust version of *k*-means, was used to pick the optimal number of clusters based on average silhouettes ([R], http://www.R-project.org). The correct choice of *k* was shown to be 2 or 8–10 (electronic supplementary material, figure S4). We selected $k = 2$ as the more appropriate value for our 27 island dataset; when *k* goes from 2 to 10, observations cluster into successively smaller subsets which are hard to explain. The Wilcoxon signed-ranks test [R] was used to assess whether differences in treatment means for the NCEAS cumulative human impact scores or log land area could explain the results of the cluster analysis. The *p*-value was highly significant for both 'test' variables ($p = 1.72 \times 10^{-5}$ and $p = 1.08 \times 10^{-5}$, respectively).

Cluster 1 contains 20 island locations and includes representatives from all four regional groups (figure 3). With the exception of Molokai (Main Hawaiian Islands; MHI), Saipan and Tinian (Marianas), all islands in this group have NCEAS scores less than 10 and are smaller than 45 km^{-2}. Cluster 1 also includes islands located in some of the most nutrient-poor (e.g. Wake Island and the American Samoa region) and nutrient-rich regions of the Pacific (e.g. Jarvis), providing additional support for the hypothesis that the microbial energy use patterns in the water column defined here are largely driven by human impact and/or island size. Interestingly, Saipan does not localize with the rest of this cluster, demonstrating the ability of the analysis to detect outliers. Based on its position, Saipan may be an example of an island that is currently transitioning to an increasingly disturbed state. Cluster 2 contains all locations from the Main Hawaiian Islands except for Molokai (MOL), as well as the most impacted reef systems from both the Marianas and American Samoa regions (Guam and Tutuila, respectively). Even though six of eight locations are from the same regional group (MHI), the intra-group proximity within cluster 2 is relatively low. This finding supports the hypothesis that chronic human impacts probably alter Pacific coral reefs in such a way that they are no longer reflective of the background environments in which they reside [52].

4. Discussion

The relationship between benthic and water-column microbial processes on coral reef systems is essentially a black box. There is a clear relationship between changing numbers and biomass of water-column microbes and the makeup of the benthos (e.g. microbialization); however, microbes in the water column are very different in terms of types and metabolic potential than those on the benthic surface [7,53,54]. The work presented here takes a more in depth look at microbes in the water column on coral reefs experiencing different levels of microbialization. Figure 4 shows how coral reef benthic condition can influence autotrophic activity in the water column. In a healthy reef system, calcifiers and benthic planktivores (i.e. wall of mouths) continually absorb organic and inorganic carbon, as well as nutrients from the water column. This limits energy and materials available to the bacterioplankton and microbialization remains relatively low. As calcifiers decrease, there is a corresponding increase in the turf and fleshy algae, benthic cyanobacteria, macro-borers, etc., all of which promote the dissolution of the reef, releasing organic and inorganic carbon, as well as the previously stored nutrients [55,56]. The reef has switched from a growing ecosystem that assimilates resources to a rotting corpse that releases resources into the water column. We hypothesize that this switch from calcifying to dissolving system promotes the observed increase in planktonic autotrophy.

The DDAM model predicts that overfishing and/or nutrient-loading promote the growth of benthic turf and fleshy macroalgae [57–59], leading to increased amounts of algal-derived DOC thereby fuelling heterotrophic microbial growth and coral disease. Fluxing a larger proportion of energy and materials through fast, non-pathogenic energy channels (i.e. water-column associated microbial autotrophs) may be one way human-impacted reef ecosystems resist the rise of microbial heterotrophs, including potential coral pathogens [60]. Our calculations provide support for this hypothesis as reefs with higher NCEAS scores tended to harbour microbial communities with a greater proportional abundance of microbial phytoplankton, but supported less total microbial biomass per unit of energy fluxed (figure 1*a*,*b*).

Dinsdale *et al*. [61] also documented increases in the proportion of microbial autotrophs along an increasing gradient of human activity (Kingman < Palmyra < Fanning) [61]. At the most impacted atoll, Christmas (Kiritimati), there was an increased number of heterotrophic microbes which could be

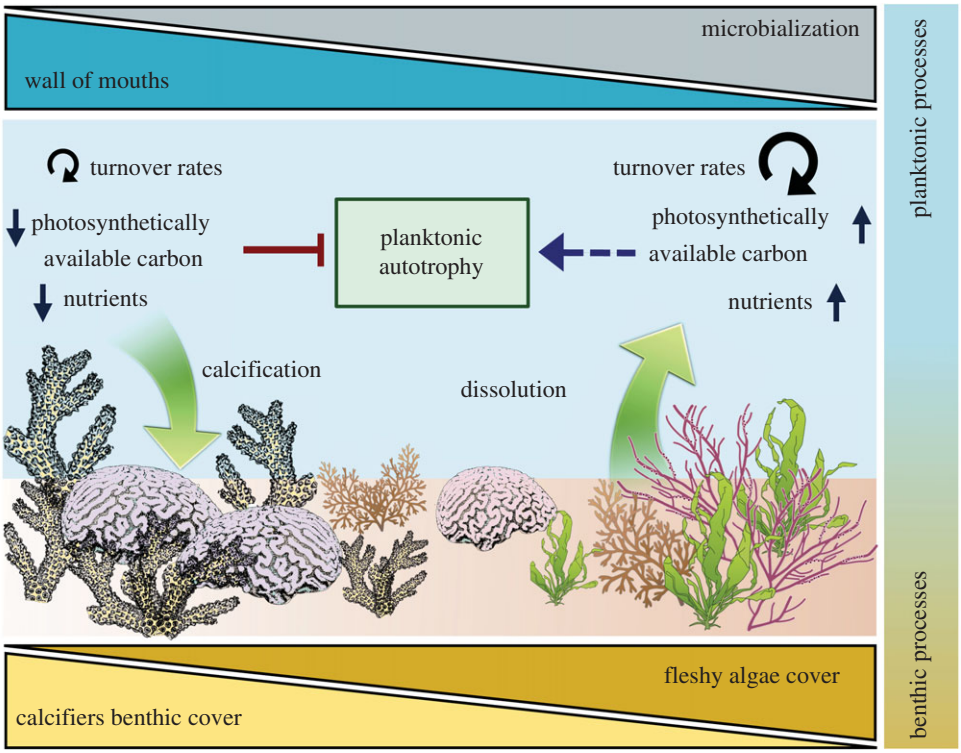

Figure 4. Planktonic autotrophy on coral reefs experiencing different levels of microbialization: from reef-building ecosystem to rotting corpse. In a healthy reef system, microbialization in the water column remains relatively low as benthic planktivores (i.e. wall of mouths) continually absorb photosynthetically available carbon and nutrients. As a reef degrades, the landscape shifts from calcifiers to fleshy algae, resulting in the dissolution of previously stored carbon and nutrients, fuelling autotrophic driven microbialization in the water column.

classified as opportunistic pathogens on the basis of 16S rDNA. This shift was correlated with the removal of fish herbivores which typically graze on benthic reef algae [17]. Kelly *et al.* [54] also estimated the composition of natural reef-associated microbial communities and found that uninhabited reefs associated with a higher per cent cover of reef-building calcifiers were characterized by higher abundances of cyanobacteria [54]. In this study, we found no evidence linking the relative abundance of cyanobacterial sequences obtained from publically available microbial metagenomes to the NCEAS human impact score (methods described in Knowles *et al.* [62]). However, relative abundances of reef-associated cyanobacteria from direct counts (27 islands) and reef-associated microbial metagenomes (from 21 of the same islands) fell within a similar range (4.1%–26.2% and 6.5%–30%, respectively; electronic supplementary material, tables S3 and S4). On average, *Synecchococcus* and *Prochlorococcus* spp. accounted for 95% of water-column associated cyanobacterial populations (electronic supplementary material, table S4).

Viruses also play a large role in mediating energy and materials flux through the base of the food web in marine ecosystems. In oligotrophic environments, where bacterial growth is limited by nitrogen, phosphorous or organic carbon, the viral shunt is thought to be 'beneficial' for remineralization purposes [63]. However, owing to feedback between host-lysis and nutrient pools, viral-shunt dynamics are highly complex and nonlinear, and efforts to incorporate viruses into whole ecosystem trophic models have been limited. Recently, a multi-trophic model of a microbial ecosystem in the surface ocean found that the inclusion of viruses in the model resulted in higher bacterial densities, increased cyanobacterial turnover and reduced trophic transfer [64]. This finding suggests that the viral shunt stimulates microbial production and also serves as an energy 'sink'. Another study found that on highly degraded coral reefs with elevated microbial abundances, the prevalence of viral lysogeny increases [62]. The authors suggest that lysogenic conversion of microbes on degraded reefs could promote the DDAM mechanism by resulting in an increased prevalence of bacteria with viral-encoded functions including pathogenicity genes.

There are many examples where human activities change ecosystem dynamics in both positive and negative ways [65,66]. Sulfates and other anthropogenic aerosols scatter incoming solar radiation back into space, reducing the effects of global warming [67,68] and global warming may increase system tolerance to eutrophication for a wide range of ecosystem types [66].

The outcome of human activity not only depends on the absolute degree of human impact, but also on the rate(s) of change. Autotrophic-driven microbialization may slow the transition from coral to algal dominance, which could make it easier for a reef system to return to a less-degraded state [69]. However, once in an 'extremely degraded' state, it is unlikely that the system will return to a less-degraded state owing to DDAM feedback.

(a) Caveats

The major caveat with this study is that the energy of activation (E) can significantly influence metabolic rate predictions because it occurs in the exponent. Rubisco activity has long been considered the main limiting factor of photosynthesis under saturating irradiance and limiting CO_2 concentrations for higher plants, algae and cyanobacteria [70–73]. For this reason, an $E = 0.32$ eV for photosynthesis was used in this study. However, some studies have raised the possibility that another reaction in the Calvin cycle may limit the rate of photosynthesis in cyanobacteria [74,75]. If Rubisco concentration is

not the main rate limiting factor for photosynthesis in cyanobacteria it remains unclear what it is. Thus, our MTE predictions of autotrophs versus heterotrophs are dependent on the assumption that the effective activation energy for oxygenic photosynthesis is lower than the average energy of activation for aerobic respiration.

In this study, the effect of the light–dark cycle on microbial autotrophs was accounted for using an average activation energy value for respiration and photosynthesis ($E = 0.46$ eV). Alternatively, metabolic rates during the day ($E = 0.32$ eV) and night ($E = 0.61$ eV) can be calculated separately and then averaged. When this was done, daily mean metabolic rates for microbial autotrophs were higher than those obtained using the averaged activation energy of 0.46 eV by approximately two orders of magnitude (electronic supplementary material, table S1). One of the most significant parameters influencing the metabolic rate of the microbial community is the energy of activation, and this large range in values represents uncertainty in our measurements. Given these two alternatives, we consider the more conservative calculations to be the 'best' values to report. A third caveat is that changes in light levels can lead to variations in the rate of photosynthesis. It is likely that light becomes rate limiting in 'extremely' degraded (and highly turbid) reef systems; our sampling primarily took place in reef locations with high water clarity (depth: 10–15 m).

5. Conclusion

Energy is an important property of any ecosystem. Developing bioenergetics-based indices for REA are of particular urgency for Pacific coral reefs, which are incredibly biodiverse yet extremely sensitive to human activity. We show that microbial energy use patterns in the water column defined here appear to be largely driven by human impact and/or island size and that these types of metabolic indices can be used to differentiate high and low human-impacted Pacific coral reef systems. We provide strong support for the hypothesis that increased autotrophic activity by *Synecchoccus* and *Proclorococcus* spp. disproportionately alters energy flux in coral reef ecosystems and may provide a resistance mechanism by serving as an energy 'sink' on impacted coral reefs. However, microbialization and the underlying DDAM dynamics still pose a very real threat to these ecosystems.

Data accessibility. The datasets supporting this article have been uploaded as part of the electronic supplementary material.

Authors' contributions. Conceived and designed experiments: F.R., T.M.S., M.H. Performed experiments: T.M.S. Collected microbial data: T.M.S., K.B., C.S. Analysed data: T.M.S., B.H., B.N., C.B.S., J.G., N.H. Contributed reagents/materials/analysis tools: R.E.B. Wrote the paper: T.M.S., F.R. Oversaw metabolic rate calculations: J.N. Oversaw statistical analyses: B.B. All authors gave final approval for publication.

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