UNIVERSIDADE FEDERAL DO RIO DE JANEIRO
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INSTITUTO DE BIOLOGIA
PROGRAMA DE PÓS-GRADUAÇÃO EM ECOLOGIA

INDIRECT INTERACTIONS AMONG CORALS, ALGAE AND MICROORGANISMS IN REEF ECOSYSTEMS

MARIA LUIZA ABIERI MONIZ DE SOUZA

Rio de Janeiro/RJ - Brasil

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Dissertação apresentada ao programa de Pós-Graduação em Ecologia da Universidade Federal do Rio de Janeiro, como parte dos requisitos necessários à obtenção do grau de Mestre em Ciências Biológicas (Ecologia).

Orientador: Rodrigo Leão de Moura

Co-orientador: Rodolfo Pinheiro da Rocha Paranhos

Rio de Janeiro, RJ – BRASIL
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Defendida em 30 de maio de 2016.

APROVADA POR:

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Prof. Dr. Rodrigo Leão de Moura

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"The naturalist will feel this astonishment more deeply after having examined the soft and almost gelatinous bodies of these apparently insignificant creatures, and when he knows that the solid reef increases only on the outer edge, which day and night is lashed by the breakers of an ocean never at rest".

Charles Darwin, 1842, The Structure and Distribution of Coral Reefs

"We are all mad here."

Lewis Carroll, 1865, Alice in Wonderland

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Resumo

Mudanças climáticas e estressores antropogênicos (e.g. sedimentação, nutrificação e sobre-pesca) estão levando os recifes coralíneos a um declínio global sem precedentes, com a progressiva substituição de corais construtores por organismos não-construtores (e.g. algas e esponjas). Tais mudanças são frequentemente acompanhadas do processo conhecido como "microbialização", ligado a surtos de doenças, mortalidades em massa e eutrofização do ecossistema recifal. Os microrganismos protagonizam os principais ciclos biogeoquímicos, mas diversos aspectos de seu papel na degradação dos recifes coralíneos ainda são mal esclarecidos. Neste trabalho foi investigado o acoplamento entre organismos bentônicos e assembleias microbianas na coluna d'água adjacente e em interfaces coral-alga, com foco na influência (indireta) da matéria orgânica dissolvida (MOD) exsudada pelos produtores primários. A dissertação está dividida em dois capítulos e foi baseada em experimentos de incubação de pequena escala e curta duração, empregando uma combinação única de métodos e abordagens analíticas. Mais especificamente, comparamos a diversidade e a atividade microbiana em incubações de corais, macroalgas e tufos de algas, visando avaliar se os produtores primários bentônicos dominantes estimulam as assembleias microbianas de maneira diferente (Capítulo 1), e também avaliamos a demanda energética microbiana em interfaces coral-alga (Capítulo 2). No primeiro conjunto de experimentos (Capítulo 1) foi observada expressiva saída de *Symbiodinium* sp. dos corais, e de diatomáceas/cianobactérias das incubações de algas. Mudanças significativas nas propriedades citométricas (e.g. tamanho, fluorescência) e no consumo de nutrientes também foram características das incubações de cada macróbio, com maior diversidade bacteriana nas incubações de corais. No segundo experimento (Capítulo 2), observou-se maior demanda energética nas assembleias microbianas das interfaces coral-alga do que nos macróbios em isolamento, bem como menores concentrações de oxigênio. O aumento na produção energética microbiana e o consumo de oxigênio foram significativamente correlacionados à razão entre bactérias hetero e autotróficas, mas não com a abundância microbiana total. Os dois capítulos fornecem informações relevantes quanto à influência da MOD exsudada por produtores primários e sobre o fluxo de energia através dos microbiomas bentônicos.

Palavras-chave: Bacterioplâncton, MOD, Recifes de águas turvas, Citometria, Bioenergia.

Abstract

Climate changes and anthropogenic stressors (e.g. sedimentation, nutrification, overfishing) are leading coralline reefs to an unprecedented global decline, with progressive substitution of reef-building corals by non-building organisms (e.g. algae, sponges). Such shifts are often accompanied by the so-called "microbialization" of reef ecosystems, a process connected to coral disease outbreaks, massive mortalities and eutrophication. Microorganisms catalyse Earth's key biogeochemical cycles, but several aspects of their role in coralline reef degradation are still poorly understood. Here, we investigated the coupling between benthic organisms and microbial assemblages in the water column and coral-algal interfaces, focusing on the (indirect) influence of the dissolved organic matter (DOM) exuded by different primary producers. The dissertation is divided in two chapters and is based on small-scale and short-term incubation experiments that employed a singular combination of methods and analytical approaches. Specifically, we contrasted microbial activity and diversity in coral, macroalgae and algal turf incubations, in order to verify whether such dominant benthic primary producers stimulate microbial assemblages differently (Chapter 1), and also addressed the microbial energetic demand on coral-algal interaction zones (Chapter 2). In the first set of experiments (Chapter 1), efflux from incubated macrobes included massive *Symbiodinium* sp. release by corals, and diatom/cyanobacteria release in both algal incubations. Significant changes in cytometric properties (e.g. bacterial size, fluorescence) and contrasts in nutrient utilization were also detected for each incubated macrobe, including a more diverse bacterial community in coral incubations. In the second set of experiments (Chapter 2), we recorded a high energetic demand from coral-algal interface microbial assemblages, stronger than that associated with isolated macrobes. A lower oxygen concentration at the coral-algal interface was also evident and corroborated the increase in microbial activity in contact zones. Increases in microbial power output and lower oxygen concentrations were significantly correlated with hetero:autotrophic microbes` rates, but not with total microbial abundance. The two chapters provide relevant insights on how the released DOM affects microbial assemblages and how energy flows through the coral and algal microbiomes.
Keywords: Bacterioplankton, DOM, Turbid-zone reefs, Cytometry, Bioenergy.

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Chapter 1

Introduction

Marine Ecology imprinted major, but often underrated, theoretical contributions to the broader field of Ecology (Bertness et al., 2014). Observational and manipulative experiments in marine systems founded long-standing debates about open versus closed communities (e.g. Hjort, 1914) and the relative importance of carbon fixing by terrestrial plants and aquatic microorganisms (e.g. Hensen, 1911; Partensky et al. 1999). The demonstration of the key role that biological interactions play in the regulation of natural communities is another remarkable contribution of Marine Ecology (e.g. Connell, 1961a; b; Paine, 1966). However, while top-down controls are well documented in rocky intertidal and other marine communities, the extent of bottom-up controls remains less explored as a critical dimension of benthic-pelagic coupling, despite its direct link to the escalating anthropogenic influences.

Benthic-pelagic coupling is among the major processes responsible by the remarkable secondary productivity of tropical reefs, where efficient nutrient recycling enables such high-biomass communities under oligotrophic conditions (Odum, 1956; Hatcher, 1990). The organic matter (OM) derived from macrobenthic primary producers is a major source of the biologically available organic carbon, and therefore plays a central role in biogeochemical cycles (Hegdes, 2002; Wild et al., 2004a). Besides sourcing particulate matter, benthic primary producers release a large fraction of their photosynthetically fixed carbon as dissolved OM (DOM) into the surrounding water, fuelling microbial production (e.g. Haas et al., 2010; Tanaka et al., 2011). Light and nutrient availability, coupled to temperature variations, are known to affect the amount and quality of the released OM

(Haas et al., 2010; Schoepf et al., 2013; Levas et al., 2015; Mueller et al., 2014; 2016), and recent studies are also revealing that epi and endosymbiont communities affect and are affected by the exuded OM (e.g. Morrow et al., 2011; Hester et al., 2016).

Heterotrophic Bacteria and Archaea (heretofore collectively referred to as bacteria) are the major mediators of the degradation and remineralization of OM in the Ocean (Ducklow & Carlson, 1992). This *microbial loop* enhances the bioavailability of particulate organic matter (POM) to higher trophic levels, through the recycling of the OM derived from primary producers (Azam et al., 1983). Rates of OM degradation/remineralization vary due to several factors, including the complexity of the molecular continuum and its chemical reactivity, which controls the microbial community degradability. The bacterial growth efficiency (BGE) resulting of OM consumption and biomass accretion depends on catabolism:anabolism ratios, with higher values implying in lower BGE, and vice-versa (Haas et al., 2011).

Tropical reefs are under intense anthropogenic forcing operating at local (e.g. overfishing and nutrification) and global (e.g. temperature and acidification) scales. Over the last century, these ecosystems deteriorated rapidly, resulting in the demise of more than half of the world's coral cover (Pandolfi et al., 2003; Wilkinson, 2008). Concurrently, the abundance of non-building and fast-growing organisms, such as fleshy and filamentous benthic algae, has increased dramatically (e.g. Hughes, 1994; McCook et al., 2001; Francini-Filho et al., 2013). Such acute changes in benthic cover dominance, resulting in less diverse or productive steady states, are referred to as *phase-shifts* (Done, 1992). Several studies pointed out that benthic algae influence coral health, either directly or indirectly. Direct interactions include abrasion and shading (McCook et al., 2001). Also,

algae may also function as pathogen reservoirs (Sweet et al., 2013) and may release secondary metabolites that are deleterious to corals (Morrow et al., 2011; Paul et al., 2011). An additional indirect coral-algal interaction, which constitutes the focus of the present study, is the release of reactive OM that is able to feedback microbial metabolisms and negatively affects reef-building corals (e.g. Haas et al., 2011; Barott & Rohwer, 2012; Nelson et al., 2013).

The OM released by scleractinian corals (mainly POM; Naumann et al., 2010) is less reactive and hard to degrade, and is therefore primarily degraded by specialized bacteria. Such microbial assemblages increase overall community diversity and are generally implied in higher BGE (Wild et al., 2004b; Haas et al., 2011; Nelson et al., 2013). Conversely, the OM released by fleshy and filamentous benthic algae (largely in the DOM pool; Haas et al., 2010) consists of large amounts of more labile OM, stimulating a rapid DOM uptake and favouring opportunistic microbes (Haas et al., 2010; Wild et al., 2010). This process may shift the microbial community towards a less diverse state with reduced net heterotrophy and lowered BGE (Haas et al., 2011; Nelson et al., 2013). Such latter shifts in the bacterial community may imply in a large reallocation of the available energy, resulting in the so-called microbialization of the ecosystem (McDole et al., 2012).

Phase-shifts are widely reported in degraded coral reefs from the South Atlantic, Caribbean and Indo-Pacific (Bruce et al., 2012; Jackson et al., 2014; Silveira et al., 2015). This process is associated to reductions in the resistance and resilience of reef-building corals, with consequent losses of secondary productivity and biodiversity at the ecosystem's level (Graham, 2015). Interactions between physical forcing and organism's life-history shapes ecosystem functioning and influences the response of communities to natural and

anthropogenic disturbance. Understanding how competition dynamics driven from physically and biologically-mediated interactions operate in a changing world is one of the major current challenges to marine ecology and conservation.

Study goals

The main goal of the present study was to investigate the coupling between benthic organisms and microbial assemblages, both in the water column and in coral-algal interfaces, focusing on the (indirect) influence of the dissolved organic matter (DOM) exuded by different primary producers on numerical and physiological responses in the water column microbial community subjected to these exudates.

Specifically, Chapter 1 addressed changes in composition, activity, diversity and dominance in microbial assemblages associated to the water column adjacent to the major benthic primary producers of the Abrolhos reefs, Brazil. This study was largely based on flow cytometry, which, despite providing low taxonomic resolution data, allow for the acquisition of large and low cost series of data. Chapter 2 addresses how benthic macroorganisms' interactions affect the metabolisms of reef-associated microbes, using chemo-thermokinetic methods.

Hypothesis

The dissolved OM released by fast-growing primary producers (e.g. macroalgae, turf algae) may compromise coral health and lead to overall microbialization of reef systems when top-down controls are released.

Dissertation synthesis

This dissertation consists of two chapters (formatted as individual manuscripts to be submitted for peer reviewed publication) that address relevant issues regarding the interactions between microorganisms and benthic macrobes in reef ecosystems. **Chapter one**, *Microbial activity and diversity enhanced by benthic primary producers in a turbid-zone reef*, focuses on the exudation of Dissolved Organic Carbon (DOC) by the three dominant benthic producers (coral, turf algae and macroalgae), addressing their stimuli on the activity and diversity of microorganisms. Together, these benthic macro-organisms cover more than 90% of the Abrolhos reefs (Francini-Filho et al., 2013), and we cautiously infer that the processes revealed by our incubations may reach ecosystem-level magnitudes.

This experimental approach is pioneering in Brazil, and fills important knowledge gaps about benthic-pelagic coupling in turbid-zone reefs, i.e. reefs with elevated concentrations of nutrients and high turbidity. Hence, when contrasted with the current literature, our experiments explore quite dissimilar baselines, as previous studies are based on data from oligotrophic reefs in the Caribbean and Indo-Pacific. Furthermore, the exploitation of microbial diversity from flow cytometry data represents a significant methodological contribution from this dissertation. The first chapter represents the author's main project, and was developed in collaboration with the advisor, the co-advisor and other partners (www.abrolhos.org), including major contributions from Prof. Paulo S. Salomon (IB-UFRJ).

Chapter two, *The power of microbes: Microbial bioenergetics of coral-algal interaction*s, addresses a correlate theme and it is the result of a partnership led by SDSU (San Diego

State University) colleagues. It was included in the dissertation as the author (M.L.M. Souza) gave a significant contribution to this work, which was conducted during an internship under advice of SDSU's Professor Forest Rohwer. Based on macroorganisms incubations (corals and algae) from the Indo-Pacific and artificial oligotrophic formulations of seawater, the experiments explored the thermodynamic mechanisms subjacent to the interactions between corals and algae, which are strongly mediated by microorganisms.

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Chapter 1

Microbial activity and diversity enhanced by benthic primary producers in a turbid-zone reef

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Introduction

Tropical reefs are among the ecosystems with the greatest biological diversity and productivity, providing ecosystem services and livelihoods for over 500 million people (Connell, 1978; Wilkinson, 2008). Although they occupy <0.1% of the ocean, reefs provide habitat for about 25% of marine species (Spalding et al., 2001). Half of the world's reefs disappeared in the last five decades, and most of the remaining are critically degraded by human activities and climate changes (Pandolfi et al., 2003; Wilkinson, 2008). While the effects of some stressors have been more broadly studied and emphasized (e.g. Jackson et al., 2001), the microbial-mediated effects from nutrification and eutrophication remain to be fully understood (Haas et al., 2011; Barott & Rohwer, 2012).

Despite being restricted to warm oligotrophic waters, reefs are among Earth's most productive ecosystems (Odum & Odum, 1955; Hatcher, 1990). This apparent paradox is partially explained by highly efficient nutrient recycling through a complex set of symbioses between macro and microbes (Hatcher, 1988; Wild et al., 2004; de Goeij et al., 2013). The foremost primary producers in tropical reefs are macroalgae, algal turfs and corals (Odum and Odum, 1955; Hatcher, 1988). Algal turfs are sediment-rich consortia structured by filamentous algae and cyanobacteria, encompassing diverse auto and

heterotrophic microbial assemblages (Steneck, 1988; Connell et al., 2014). Nitrogen fixation rates by algal turfs are high (Williams & Carpenter, 1998; Cardini et al., 2014), and the consortium may benefit from increased temperature and nutrient concentrations, as well as by decreased herbivory (Burkepile & Hay, 2006; Vermeij et al., 2010). Algal turfs also represent a major food source for macro-herbivores such as fishes and sea urchins (Carpenter, 1986; Lewis, 1986). Conversely, consumption of macroalgae is more limited, as they are strongly chemically defended (Rasher et al., 2013). When consumption of turf and macroalgae is reduced by overfishing or by massive mortality of herbivores (review Adam et al., 2015), these organisms may become dominant and the coralline system tends to lose diversity and resilience, a process known as *phase-shift* (Done, 1992; McManus & Polsenberg, 2004), culminating in a state with reduced secondary productivity (Graham, 2015).

Corals represent a prime example of "holobiont" (Margulis, 1991), comprising the scleractinian macrobe and a diverse assemblage of microbes that includes eukaryotes (e.g. photosynthetic dinoflagellates, fungi), prokaryotes (Bacteria and Archaea) and viruses (Rohwer et al., 2002). This mixotrophic consortium is an efficient system to obtain nutrients and energy in oligotrophic waters. Corals are important ecosystem engineers, and their architectural complexity contributes to the high diversity of coral reefs (Szmant, 1997). They also source dissolved organic carbon (DOC) and particulate organic matter (POM) to the ecosystem, this latter representing an additional functional role whose relative importance is not yet fully understood (Ducklow & Mitchell, 1979; Wild et al., 2008a). *Symbiodinium* endosymbionts are a major source of glucose to the scleractinian host (Burriesci et al., 2012), and its excess is exuded to the water column as DOC, becoming

available for heterotrophic microbial consumption (Azam et al., 1983). The acquisition of nutrients and the DOC/POM exudation comprise key processes in the homeostasis of the holobiont, and may vary according to host species and its associated microbiota, and also respond to environmental conditions (Haas et al., 2010; Tremblay et al., 2014; Levas et al., 2015).

Macroalgae, turf and corals exude at least 10, and up to 35% of the carbon that they fix, as DOC (Haas et al., 2011). The amount and chemical composition of the exudates is variable (Nelson et al., 2013; review Brocke et al., 2015) and affects the metabolic capacity of planktonic microorganisms (Haas et al., 2013a; Logue et al., 2015), ultimately determining the efficiency of the microbial loop in providing POM to higher trophic levels (Azam & Malfatti, 2007). Additionally, the exuded DOC/POM positively feeds back the growth and respiration of microorganisms, pathogens or not, increasing stress, diseases, changes in the reproductive cycle and/or localized hypoxia and tissue necrosis, comprising the DDDAM model of coral reef degradation (Dissolved organic matter, disease, direct contact, algae and microorganisms) (Barott & Rohwer, 2012), which also incorporates the effects of direct contacts of macroalgae, turf and corals, which may be physically harmful to the corals (Morrow et al., 2013; Shearer et al., 2014).

Here we evaluated changes in microbial abundance and cytometric diversity in the water column under *in vitro* exposition to exudates of corals, algal turfs and macroalgae. Incubations involved the most common macroalgae (*Dictyota* spp.) of the study area, its main reef building hermatypic coral (*Mussismilia braziliensis*), and two visually discernible types of algal turfs, which were incubated over 24 h. Besides focusing on the effects of different benthic primary producers and nutrient addition, our experiment was carried out in

a species-poor turbid-zone reef system (Perry, 2011; Browne et al., 2012), comprising a comparative baseline to be contrasted with data from the richer oligotrophic reefs in the Caribbean and the Indo-Pacific (e.g. Haas et al., 2011; Smith et al., 2013). The studied system, Abrolhos Bank, Brazil, develops under naturally high sediment/nutrient levels (Leão & Ginsburg, 1997; Bastos et al., 2015), but is rapidly declining due to increasing coral diseases (Francini-Filho et al., 2008), overfishing (Freitas et al., 2011) and sedimentation (Silva et al., 2013). It represents a phase-shifting reef (Done, 1992; Hughes, 1994) with steadily decreasing fish biomass (Francini-Filho & Moura, 2008), increasing algal-turf dominance (Francini-Filho et al., 2013), and system-wide microbialization (Bruce et al., 2012).

Methods

Study site and field sampling. Experiments were conducted in February 2014 at the Santa Barbara Island (-17.968^{o} S, -38.707^{o} W), Brazil, located within the Abrolhos National Marine Park (ANMP). The Abrolhos Bank encompasses the largest and richest reefs in the South Atlantic (Moura et al., 2013), and are remarkable for high sedimentation and nutrient levels (Leão & Ginsburg, 1997; Bastos et al., 2015; Silveira et al., 2015). The three investigated organisms are among the most abundant benthic primary producers (Francini-Filho et al., 2013): one Brazilian-endemic coral (*Mussismilia braziliensis*), the dominant fleshy macroalgae (*Dictyota* spp.) and two visually-discernible types of algal turfs (Figure 1). Specimens and control seawater were collected from 5-7 m depths in the south side of the island. In-situ light intensity (lx) and temperature (°C) were recorded with HOBO loggers, and were repeated in the incubations. A hammer and chisel were used to break off coral nubbins without damaging living tissue. The two types of algal turf were collected

with a chisel, using a 50 mL falcon tube to standardize collected volume with minimal damage to the filaments. Due to the heterogeneity and ubiquity (30-70%) of turf algae cover (Francini-Filho et al., 2013) we characterized two dominant types based on morphology and colour: Turf algae 1 (TA1) is a greyish and sediment-rich assemblage with short (<2mm) filaments, while Turf Algae 2 (TA2) is a greenish assemblage with less sediment and longer (>3 mm) filaments (Figures 1C and 1D). Fleshy macroalgae were collected still associated to the substrate, which was gently removed before incubation.

Experimental setup. Three replicate specimens of each primary producer were incubated individually in airtight sealed (low-density polyethylene film) polypropylene beakers (n=15; sulfuric acid-cleaned and seawater-leached) with 1500 mL of filtered seawater (80 μm). Incubation and seawater control triplicates were distributed in a random block design and were artificially illuminated at 25000 lx. Abiotic parameters measurements and water column sampling were carried out at four fixed times points (0, 6, 12, and 24 h) with 6 h under light, 6 h under dark and 12 h under light. Beakers were not stirred to avoid influencing organic matter release (Sharshar et al., 1996; Smith et al., 2013). Water temperature (28-30.3 ^{o}C) was similar to that of the environment in the two preceding days and did not vary among treatments and across time (Tukey's p>0.1).

Samples collection and processing. Surface area of each specimen was estimated with software Image J (National Institutes of Health, USA) from orthogonal photographs over scaled paper. For the massive and hemispheric coral colonies, area was calculated from colony geometry (Naumann et al., 2009). Dissolved oxygen (DO) and temperature (°C) were acquired with Clark-type O_2 sensor (Revsbech, 1989) and thermocouple sensors connected to an UnderWater Meter System (Unisense, Denmark) (3 readings/replicate).

Microelectrodes had 400-600 µm outside tip glass diameters, <3 sec. response time and 0.3 µM, 0.1 °C detection limit. The conversion from µvolts to µM and °C were determined as factory indication. Incubation water was sampled with HDPE syringes at the beginning and end (24 h) of the experiment into 150 mL opaque vials to nutrient analysis. Intermediary time points (6, 12 h) were sampled into 14 mL tubes. Syringes and vials were acid-washed. Ammonium and Organic Nitrogen (ON) concentrations were assessed with colorimetric methods (Grasshoff et al., 1999; Hydes et al., 2010) adapted to a SEAL AutoAnalyzer 3 (AA3, 5 channels). DOC was measured from 40 mL samples filtered through a GF/F filter (Whatman; 0.7 µm), using a CHN analyzer (Carlo Erba) as described by Rezende et al. (2010).

Protists and filamentous cyanobacteria (5–80 µm) were sampled from 5 ml water aliquots preserved in glutaraldehyde (1% final concentration) in the dark, at 4°C. Samples were analysed with a Fluid Imaging Particle Analysis system (FlowCAM) equipped with a 90 µm, field-of-view flow cell and a 10X magnification objective, at 0.1 mL min^{-1} for 30 min. Data were collected in the "autoimage" mode at 20 $frames.sec^{-1}$ (39.5% efficiency). Semi-automatic classification of particles included *Symbiodinium* sp., filamentous cyanobacteria and diatoms. Undefined particles were disregarded. Factory-provided calibration factors were used to calculate plankton abundance and dimensions.

Bacterial abundance estimates, cell fluorescence and side-scattered light were assessed from two 1.5 mL water aliquots fixed with paraformaldehyde and glutaraldehyde (1% + 0.05% final concentration). Samples were maintained in the dark for 10 min at 4 °C, frozen in liquid nitrogen and stored at -80 °C (Gasol & del Giorgio, 2000). Before processing, samples were diluted and swirled with a vortex mixer to disaggregate cell clumps. Analyses

were carried out with a Becton Dickinson flow cytometer (FACS Calibur, 488 nm excitation light), following del Giorgio et al. (1996) and Andrade et al. (2003). Flow rate (mL s^{-1}) was calibrated with fluorescent beads (FluoSpheres®, 1.0 μm: Molecular Probes) following Lebaron et al. (1994) and Li et al. (1995). Photosynthetic bacterioplankton (PB) was assessed from unstained aliquots, while total bacterioplankton (TB) was assessed from Sybr Green I stained aliquots (5 X 10^{-4} final concentration) (Andrade et al., 2003). Fluorescence and side scattered light (SSC) were measured using logarithmic amplification and recorded in relative units in a four-decade range spanned by 1024 channels onto three different photomultipliers: FL1 (530 ± 30 nm); FL2 (585 ± 42 nm) and FL3 (>650 nm). Data analysis was carried out with software WinMDI v2.8 (Joseph Trotter, Scripps Research Institute). Fluorescence centered at 585±42 nm wavelengths (FL2) was ascribed to phycoerythrin, while wavelengths >650 nm (FL3) were ascribed to chlorophylls (Gasol & del Giorgio, 2000; Marie et al., 2005). Particles with red fluorescence weaker than that emitted by *Prochlorococcus* spp. were rejected (Olson et al., 1993). Cell abundances from stained samples were determined from scatter plots of SSC (X-axis, cell size) and FL1 (Y-axis, green fluorescence related to nucleic acid content), and from unstained samples by scatter plots of SSC (X-axis, cell size) and FL3 (Y-axis, native autofluorescence related to chlorophylls) (Andrade et al., 2003). Bacterioplankton cells were ascribed to either high nucleic acid content (HDNA) or low nucleic acid content (LDNA) bacteria, the former presumed to be metabolically more active (del Giorgio et al., 1996; Gasol et al., 1999). Abundance of heterotrophic bacterioplankton (HB) was assessed by subtracting PB in TB.

Data Analyses. Means and standard errors of biotic and abiotic variables were calculated from each replicate at all time points (0, 6, 12 and 24 h), with concentrations extrapolated

to 1 L. Rates of change in DO, DOC, and nitrogen compounds (µmol $L^{-1}h^{-1}$) were calculated by dividing the difference of concentrations between adjacent time points by incubation time. Surface-area normalization was achieved by subtracting changes in control means per time point and dividing rates by the surface area of each incubated macroorganism (Dahl, 1973) (µmol h^{-1} dm^{-2}).

Calculation of diversity indices involves categorizing of each organism and rating the importance of each category (Magurran, 1988). Instead of using conventional taxonomy, we modified Li's (1997) classification scheme of cytometric properties to represent our data, using SSC (proxy for cell size) and FL1 (proxy for relative cellular DNA content) for TB, and SSC and FL3 (proxy for relative cellular chlorophyll content) for PB and photosynthetic protists. The number of criteria (variables) varies depending on the number of light sensors of the flow cytometer, while the number of categories is determined by the number of discrete channels after analog-to-digital conversion of voltage pulses by the equipment, defining the resolution at which each variable is measured (e.g. 256 - 1024 discrete values). At the lowest resolution, if only one variable is used, there would be a maximum of 256^1 categories. Conversely, if e.g. eight variables are considered simultaneously, maximum number of categories is 256^8.

Analyses were carried out at the highest instrument resolution (1024 channels), generating untreated matrices with cells represented as lines and cytometric variables as columns. Since we used two variables to characterize PB and TB, the high number of categories (1024^2) resulted in reduced relative importance of each category. Therefore, we further reduced resolution to 16 (256 categories), 32 (1024) (and 64 channels (4096) (Supplementary Figures, 1A, 1B and 1C, respectively), comparing scatterplots, number of

species (*S*) and the exponential Shannon-Wiener index ($exp^{H'}$) (Magurran, 1988) (Supplementary Figure 2), given by $\exp^{[-\sum pi * \ln(pi)]}$, where *pi* is the proportional abundance of the *ith* category. For PB samples the higher resolutions of 32 and 64 channels created a biased community differentiation, and the best fit resolution was that with 16 channels (256 categories) (Li, 1997). For TB samples, the 16 channels resolution suppressed groups with small number of cells, and we carried out further analyses with 32 channels (1024 categories). Resolution was reduced by successive nested binning of counts from adjacent channels using Microsoft Excel® function "look". Reduced variables were combined by the function "contifs", leading to a cell abundance distribution into the maximum number of categories in the light scatter-fluorescence domain of 1024 to TB and 256 to PB, where each category is equally important to the community diversity.

Relationships involving cytometric parameters corresponding to bacterial activity (SSC, FL1 and FL3) were assessed by the estimated averages without channel reduction, using linear regressions and permutational multivariate analyses of variance (PERMANOVA), performed with software PRIMER with PERMANOVA+ (Anderson et al., 2008), with time and treatments as fixed factors. Similarities among bacterioplankton communities were assessed with the Bray-Curtis similarity measure and standardized data. Variance components were used to quantify variability associated to each source (Searle et al., 1992) and were expressed in terms of their square-root ('sq. root var.'), in order to be on the same measurement scale as the original similarity measure. Pairwise tests of interactions (treatment*time*replicate) were not analysed due to few possible permutations (Anderson et al., 2008).

As our experimental design consisted of triplicates incubations nested within treatments and non-independent time-point sampling on the same incubations, linear mixed effects models (LMEs) were used to compare microbial densities, DO and nutrient concentration (Bolker et al., 2009; Zuur et al., 2009). Calculations were performed with the "lmer" function of the lmerTest package (Kuznetsova et al., 2013) and plots were built with ggplot2 package (Wickham, 2009), both in R 3.0.2 (R Development Core Team, 2014). Analyses were conducted on $\log_{10}$ transformed data to meet normality assumptions (verified by histograms and normal probability plots of residuals), but graphics and tables are based on untransformed data. LMEs account for dependencies within hierarchical groups through the introduction of *random effects* (Pinheiro & Bates, 2000) for each subject, i.e. assuming a different baseline value to the response variable and *random intercepts*. LME use the linear regression paradigm (Montgomery et al., 2007) to describe the average measurement and its temporal trajectory (i.e. contribution of time and/or time-varying variables), expressed as a linear combination of a set of independent variables. Satterthwaite approximations of degrees of freedom were used to test for significant differences between fixed effects using a stepwise model reduction (α=0.05). Analyses of variance (ANOVA) were performed on each LME, with Tukey's post hoc tests used to compare treatments and control. Means were considered statistically significant for $p < 0.05$. *M. braziliensis* responses at time point 24 h were removed from the analyses due to clear signals of degradation (highest DOC and ammonium values, lowest DO).

Results

Abiotic parameters

Surface-area normalized rates of change in DOC, DO, ammonium and ON are shown in Table 1 and Figure 2 (absolute values in Supplementary Figure 3). Rates of change in DOC concentration ranged widely, from -20.2 to 170.4 µmol $h^{-1}dm^{-2}$, and were significantly different among treatments and time points (ANOVA repeated measures, $p<0.001$) (Table 1). Highest rates were recorded in the *M. braziliensis* incubations at 6 and 12 h, followed by TA2 incubations, with significantly increased values over the first 12 h and a significant decrease in the last 12 h (Tukey's, $p=0.02$) (Figure 2A). TA1 incubations also showed positive rates over the first 6 h, followed by significant decreases. *Dictyota* sp. incubations exhibited the lowest DOC variation, with lower values under light and higher in the dark.

Rates of DO change were significantly different among treatments (ANOVA repeated measures, $p<0.001$) and time points ($p<0.05$). Rates tended to increase over the first 6 h (Figure 2B), with the exception of *M. braziliensis* incubations. Highest rates were recorded in TA2 and TA1 incubations, followed by those of *Dictyota* sp. All treatments showed negative rates during the dark cycle, with lowest values in *M. braziliensis* followed by TA2, TA1 and *Dictyota* sp. incubations. In the last 12 h, DO rates increased, remaining negative in both turf algae and becoming positive in the *Dictyota* sp. treatments. Ammonium change rates showed significant variations only among time points (ANOVA repeated measures, $p=0.03$) (Table 1). Rates increased consistently only in *M. braziliensis* incubations, while in the other treatments lower values were recorded under light (0-6 h and 12-24 h) and higher values in the dark (6-12 h) (Figure 2C). Highest rates were recorded in the TA2

incubations, followed by those of TA1 and *Dictyota* sp. Rates of change in ON concentrations were significantly different among treatments (ANOVA repeated measures, $p<0.001$), but not across time points ($p=0.12$) (Table 1). Interaction between treatment and time was significant ($p<0.03$) (Table 1). ON tended to decrease in the first 6 h and to increase during the dark cycle, with the exception of *M. braziliensis* incubations, which showed a significant initial increase, followed by a slight decrease (Figure 2D). After 24 h, rates decreased in TA1 and *Dictyota* sp. incubations, and increased in those of TA2. In control incubations, DOC, DO, ammonium and ON concentrations did not vary significantly (ANOVA repeated measures, $p>0.3$).

Biotic responses

Density of free-living *Symbiodinium* increased only on *M. braziliensis* incubations (ANOVA repeated measures, $p=0.032$), with significant increases between 0-6 and 12-24 h (Tukey's, $p<0.001$) (light cycles) (Table 2; Supplementary Figure 5). Pennate diatoms' density showed significant variation among treatments and time points (ANOVA repeated measures, $p<0.001$) (Table 2; Supplementary Figure 6A). TA2 incubations showed significant increases from 0-6 h (Tukey's, $p=0.001$), differing significantly from other treatments and control incubations ($p<0.05$). After 12 h, pennate diatom densities increased in all treatments, becoming significantly different from control in TA2, TA1 and *Dictyota* sp. incubations (Dunnet's, $p<0.05$) (Table 2). Filamentous cyanobacteria densities differed significantly among treatments (ANOVA repeated measures, $p<0.001$), but not across time ($p=0.64$) (Table 2; Supplementary Figure 6B). TA1, TA2 (one outlier removed) and *Dictyota* sp. incubations were significantly different from controls at 24 h (Dunnett's, $p<0.05$), while *M. braziliensis* incubations did not differ from controls.

Along the experiment, TB density significantly increased in all treatments and controls (ANOVA repeated measures, $p<0.001$) (Table 2; Supplementary Figure 6C). Interaction between treatment and time increased above baseline only in *M. braziliensis* incubations at 12 h (ANOVA repeated measures, $p<0.001$), with TB densities two times higher than those of the controls (Dunnett's, $p=0.018$). A dominance of HB (80% of TB) over PB was recorded in all incubations and controls. HB and TB densities were significantly correlated ($r=0.98$, $p<0.001$). PB density did not differ among treatments or across time ($p>0.1$) (Table 2; Supplementary Figure 6D). As a consequence of changes in HB concentrations over time, the PB:HB ratio decreased along the experiment, with the lowest values recorded in 12 h *M. braziliensis* incubations (0.02 ± 0.001) (Figure 3). HB and PB densities were significantly correlated only in TA1 and control incubations ($r=0.65$ and 0.73, $p=0.02$ and 0.006, respectively).

Total bacterioplankton (TB) assemblages

TB exhibited significant variation in SSC and FL1 means (PERMANOVAs, $p<0.001$) across treatments, also with significant temporal correlations (Supplementary Figures 7 and 8). *Mussismilia braziliensis* incubations had significantly higher SSC and FL1 values than those of all other treatments (PERMANOVA's pairwise test, $p<0.02$; Supplementary Figure 8). In TA2 incubations, SSC and FL1 means were also significantly different from other treatments (PERMANOVA's pairwise tests, $p<0.01$), with the exception of TA1 (similarity >70%; pairwise tests, $p>0.08$). TA1 and *Dictyota* sp. incubations did not differ from controls (PERMANOVA's pairwise tests, $p>0.22$).

Diversity and evenness of flow cytometric attributes were strongly correlated (r>0.6, p<0.05), but uncorrelated with TB density (p>0.1). Although there was a slight decline during the dark cycle (6-12 h) in all treatments, diversity and evenness tended to decrease in *Dictyota* sp. incubations and to increase in the other treatments, with the exception of TA2 incubations, which presented consistently lower values (Figure 4).

The TB assemblage structure varied significantly along the experiment (PERMANOVAs, p<0.001; Supplementary Figure 9), with most variability associated with treatment (sq. root var. 18.3 and 14.9% for treatment and time, respectively). In control incubations, there were no differences in pairwise comparisons of average Bray-Curtis sequential similarities (p>0.1), but they were significantly dissimilar (pairwise tests, p<0.001) from all but *Dictyota* sp. incubations (p=0.23) (Figure 5). TB assemblage structure changed faster in *M. braziliensis* incubations, with average dissimilarity >60% over the first 6 h (Figure 5). TA1 and TA2 incubations were similar (pairwise test, p=0.14) and both differed from control incubations and over time (Figure 5).

Photosynthetic bacterioplankton (PB) assemblages

PB exhibited significant variation between treatments and strong correlation in SSC and FL3 means (PERMANOVA, p=0.009), but no significant temporal variation (PERMANOVA's pairwise tests, p<0.1; Supplementary Figures 7 and 8). Incubations of *M. braziliensis* were significantly different from those of control, TA2 and *Dictyota* sp. (PERMANOVA's pairwise tests, p<0.03), presenting higher SSC and FL3 after 6 h (Supplementary Figure 8). Control, TA1, TA2 and *Dictyota* sp. incubations were highly similar (>90%; PERMANOVA's pairwise tests, p>0.3).

Akin to TB, diversity and evenness were strongly correlated (r>0.85, p<0.001), but uncorrelated with PB density (all p>0.8). Declines during the dark cycle (6-12 h) were less remarkable than those observed in TB. Diversity and evenness did not change in control incubations, but clearly increased in all treatments (Figure 4).

The PB assemblage structure varied significantly among treatments and along the experiment (pairwise PERMANOVA's tests, p<0.001), with exception of TA1 and TA2 incubations, which showed 77.7% average similarity between groups (pairwise test, p=0.18) (Figure 5). Conversely to TB assemblages, most PB variability was associated with time (sq. root var. 19.2 and 8.04% for time and treatment, respectively), with a sharp shift recorded in the last 12 h (Figures 4 and 5). Moreover, *Dictyota* sp. incubations were more similar to control incubations (72.1% similarity), while those of *M. braziliensis* were more dissimilar (64.71% similarity).

Discussion

The progressive increase of anthropogenic stressors and climate changes are driving a rapid deterioration of coralline reefs, resulting in dramatic coral cover declines and increased the dominance of turf, algae, sponges, and other fast-growing and non-building benthic organisms (Done, 1992; Hughes et al., 2003; McManus & Polsenberg, 2004). Overfishing, sedimentation and nutrification comprise the primary anthropogenic drivers of such acute dominance shifts (Lirman, 2001; Bellwood et al., 2004; Jessen et al., 2013), and there is mounting evidence that benthic primary producers feedback this coral reef degradation loop by stimulating community-wide microbialization and diseases (Barott & Rohwer, 2012; McDole et al., 2012;). While the relevance of such coupling between benthic and pelagic

processes has been demonstrated in oligotrophic tropical waters (Wild et al., 2008a; Kelly et al., 2012; Nelson et al., 2013), they remain poorly understood in nearshore reefs under higher sedimentation and nutrient levels, which are often incorrectly described as degraded. Besides presenting relatively lower diversity, turbid-zone reefs encompass naturally low coral and high algal cover, with long-term stable communities and high calcium carbonate mineralization rates (Perry, 2011; Browne et al., 2012). Here, by means of a controlled incubation set up in the microcosm scale and considering the ecological context of the Abrolhos Bank, a major turbid-zone reef system off Eastern Brazil, we explored how the DOM released by three of the most abundant benthic primary producers in this system affects the abundance, diversity and activity of planktonic microbial communities in the water column.

Although being less speciose than Caribbean reefs, the Abrolhos reefs are the largest and richest reefs within the South Atlantic, with elevate levels of Brazilian-endemism (e.g. >50% in reef corals and >30% in reef fishes, Moura & Sazima, 2000). Abrolhos is also remarkable for being subjected to elevate sedimentation (Leão & Ginsburg, 1997; Bastos et al., 2015). Turbidity reaches the highest recorded levels among living reefs (Leão & Ginsburg, 1997), but is not related to pulsed river sourcing, being primarily driven by sediment resuspension during winter cold fronts (Segal & Castro, 2011). Conversely, DOC, ammonium and ON concentrations are slightly higher, but within the ranges reported for oligotrophic reefs worldwide, averaging around 123 µmol $L^{-1}C$, 0.1 and 6 µmol $L^{-1}N$ (Silveira et al., 2015; Supplementary Figure 3). For instance, Sorokin (1995) reports 43-143 µmol $L^{-1}C$ for Indo-Pacific and Caribbean reefs, and Miyajima et al. (2005) report 0.2 and

4-8 µmol $L^{-1}N$ for southern Japan reefs, among other similar ranges reported from elsewhere (e.g. Larned, 1998; Suzuki & Casareto, 2011).

Our experiment was short-term conducted in controlled, closed incubations, providing conservative estimates of the effects of benthic primary producers on surrounding microorganisms, also allowing for comparisons with similar studies from more oligotrophic Caribbean and Indo-Pacific reefs (e.g. Haas et al., 2011; Smith et al., 2013). During the incubations, the most conspicuous exchange between primary producers and the water column was the massive *Symbiodinium* release from corals (Table 2; Supplementary Figure 5). Free-living *Symbiodinium* cells were found only in coral incubations, indicating their absence or very low abundance in the seawater, as well as in turf and algae, where they can be present as epibionts (Littman et al., 2008; Granados-Cifuentes et al., 2015). *Symbiodinium* release from corals is widely reported as a response to temperature and pH anomalies (Glynn & D'Croz, 1990; Pelejero et al., 2005), and we documented that it occurred in diel batches, with significant higher efflux during daytime, compared to the dark period of the incubations. The rapid change rate recorded in water-column *Symbiodinium* densities cannot be associated to reproductive activity (Fitt & Trench, 1983; Wang et al., 2008), and the recurrent daylight increases indicated that it is not exclusively associated to incubation and temperature stress, which were constant. The role of irradiance in the holobiont interactions has been less studied than that of thermal and pH anomalies (but see Santos et al., 2009). If light conditions indeed magnify symbiosis disruption, further fine-scale chronobiology studies of bleaching episodes, and the potential mitigation of bleaching with artificial shading deserve increased attention (Muller & van Woesik, 2009; Ateweberhan et al., 2013). The coral-dinoflagellate symbiosis stability is primarily

controlled by the host, including regulation of endosymbiont density by nutrient availability within tissue (Falkowski et al., 1993), digestion and symbiont shedding (Tilyanov et al., 1996; Baghdasarian & Muscatine, 2000; Garren & Azam, 2012). In our experiment, after 12 h, the high concentrations of DOC and ammonium, coupled with the resume of light irradiance, seem to have offered a favourable environment to nutrient uptake and increased *Symbiodinium* activity within the host (Pernice et al., 2012; Rädecker et al., 2015), unbalancing the symbiosis (Wiedenmann et al., 2012).

Turf algae are a globally dominant benthic cover element, and play a major role in the increasing coral-to-algae dominance shifts (Barott et al., 2009; Vermeij et al., 2010; Barott et al., 2012). For instance, the nitrogen fixing cyanobacteria that are abundant in turf mats (Den Haan et al., 2014) are favoured from increasing temperatures and nutrient availability (Bellwood et al., 2004; Vermeij et al., 2010; Mueller et al., 2016), and the entire turf consortium benefits from decreased top-down control due to declining macroherbivore densities (Francini-Filho et al., 2013; MacNeil et al., 2015; Marshell & Mumby, 2015). Despite their relevance, characterization of algal turfs is still broad and mainly based on morphology, with the common filamentous class being structured by diatoms, cyanobacteria, other microalgae groups and juvenile macroalgae stages (Steneck, 1988; Connell et al., 2014). Similar associations are observed in macroalgae, which may harbour an epibiont assemblage of diatoms and cyanobacteria (Ballantine, 1979; Gauna et al., 2015).

The increase in cyanobacteria filaments and pennate diatom cells in TA1, TA2 and *Dictyota* sp. incubations indicates that these macrobenthos may source microbes to the water column (Table 2), as the short incubation time (24 h) and light cycles are

incompatible with the growth cycle of these microplanktonic groups (Graham & Wilcox, 2000; Lee, 2008). Conversely, the low density of filamentous cyanobacteria and pennate diatoms on control and coral incubations may be related to their low presence in water column and as coral epibionts. Although filamentous cyanobacteria are dominant in Abrolhos' turf assemblages (Walter et al., in prep.), our study shows that the mean output of pennate diatoms was more intense (3.37×10^3 diatom cells and 1.01×10^3 cyanobacteria filaments L^{-1} h^{-1}). A high proportion of tychoplanktonic pennate diatoms have been observed in the coastal region of the Abrolhos reefs (Susini-Ribeiro et al., 2013). In our experiment, turbulence was minimal, and the ascent rate observed in turf and macroalgae incubations can be mainly related to mechanisms that allow the cells to float. Vertical movements of cyanobacteria and non-flagellated eukaryotic microalgae are related to cell physiology and environmental conditions (Bienfang & Harrison, 1984). Increased sinking can be either related to unhealthy cells and nutrient limitation or to the secretion of extracellular mucilage and cell aggregation (Alldredge & Silver, 1988; Lee, 2008).While physiological factors, such as buoyancy regulation through carbohydrate ballasting and exchange of intracellular ions (eg. NH_4^+ over SO_4^{2-}), also plays a role in slowing sinking rates and increasing diatom's buoyancy at the cost of steady energy expenditure (Gross & Zeuthen, 1948; Anderson & Sweeney, 1977). Likewise the presence of intracellular gas vacuoles in cyanobacterias, reduce its density aiding it flotation (Carr & Whitton, 1982; Villareal & Carpenter, 2003). The buoyancy behaviour of diatoms and cyanobacterias has important biogeochemical implications by vertical transport of matter (organic and inorganic) (Villareal et al., 1999) and potentially intensifying benthic pelagic coupling.

Despite the evident microbial planktonic flows recorded in our experiment, a part of the time-treatment divergences can be related to either sampling errors due to the microbial vertical movement, to the lack of turbulence in the incubations or to the variable species' composition in association to the benthic hosts (Connell et al., 2014; Gauna et al., 2015; Granados-Cifuentes et al., 2015).

The significant and strongly correlated increases in SSC and SYBR Green fluorescence (FL1) measured in the flow cytometer refer to larger cells with higher DNA content and higher metabolism/cellular division rates (Gasol et al., 1999; Tracy et al., 2010) (Supplementary Figure 7). Both parameters were also positively correlated with TB density and incubation time, strengthening the idea of heterotrophic metabolism stimulation from macrobenthic organic exudates. Remarkably, the overall TB increase is largely associated to HB, as there were no significant changes in PB densities (Table 2; Figure 3). Smaller chl *a* variation associated to light cycles were recorded in all treatments (Supplementary Figures 7 and 8), but PB increased significantly only in coral incubations (cell size and chl *a* content). Dissolved inorganic nitrogen (DIN) is highly limiting to autotrophy (D'Elia & Wiebe, 1990). The highly increased ammonium concentration in the coral incubations may be associated to the higher heterotrophic activity in this treatment and might have stimulated bacterial uptake and photosynthesis (Kirchman & Wheeler, 1998). While the other incubated macroorganisms (turf and macroalgae) seem to have assimilated a substantial amount of ammonium and other DIN compound (Nasr et al., 1968; Den Haan, 2015), leading to nutrient limitation to PB activity. Dissolved organic matter and DIN are sourced by a broad taxonomic spectrum of macro and micro autotrophs, and therefore present highly variable composition (Morán et al., 2002; Nelson et al., 2013). Once these

exudates fuel different HB assemblages (Hatcher, 1988; Tanaka et al., 2011; Silveira et al., 2015), they may partially account for the observed variation in PB:HB ratios and community similarity (Figures 3 and 5). Besides being less voluminous than turf and macroalgae exudations (Adey & Goertemiller, 1987; Hatcher, 1988; Wild et al., 2008a), the DOM released by corals is largely composed by lower quality ("junk food") photosynthates with high C:N ratio (Falkowski et al., 1984). Moreover, corals secrete large amounts of POM as mucus, a potentially important but yet neglected ecosystem nutrification mechanism (Ducklow & Mitchell, 1979; Wild et al., 2004; 2008b). The rates of DOC release in coral incubations were higher than those recorded in a previous study in Abrolhos (Silveira et al., 2015) and data from the Red Sea (Wild et al., 2010) and Pacific Ocean (<2.5 µmol $h^{-1}dm^{-2}$; Haas et al., 2011). Although injuries and stress to corals nubbins were minimum, the relatively short acclimatization may have increased mucus outflow (Brown & Bythell, 2005). We observed a high frequency of unspecific particulate matter aggregates in the FlowCAM imagery from *M. braziliensis* incubations (Supplementary Figure 11). POM released by scleractinian corals is less labile than DOM, and in such instances is degraded by a more specialized microbial community. In our experiment, such specific stimuli seems to have been partially responsible for DO decrease and DOC, ammonium and ON increase in coral incubations (Kristensen et al., 1995; Wild et al., 2008b). The cytometric dissimilarity (Figure 5) provides further support to the idea of bacterial selection driven by exuded DOM and POM, as it evidenced significant temporal and treatment-related changes in all incubations.

Microbial diversity reflects the complexity of community structure within dynamic ecosystems (Atlas & Bartha, 1998; Worm et al., 2002). In biologically-controlled

ecosystems, where interpopulation interactions outweigh abiotic stress, microbial diversity tends to be higher, allowing redundant responses to environmental fluctuations (Atlas & Bartha, 1998). Diversity tends to be lowered under intensified disturbance regimes that selects against generalists (Connell, 1978), but higher diversity is often associated to unhealthy environments. For instance, despite the controversy around the triggers of bacterial succession during coral disease progression (Bourne et al., 2008), it is widely reported that diseased coral holobionts exhibit higher microbial diversity than healthy ones (Reis et al., 2009; Sunagawa et al., 2009).

Investigations of bacterial composition, function and diversity are often based on ribosomal markers and metagenomics (Giovannoni & Rappé 2000; Moran, 2008). Molecular approaches are highly accurate, but are still time and resource-consuming, a fact that limits their usage when rapid microbial responses need to be assessed in a large number of samples. Also, it is remarkable that closely related taxa may differ in DOM degradation capabilities (Martiny et al., 2013; Logue et al., 2015). As an alternative, optical properties can be used to identify functional (e.g. PB:HB ratios) and structural (e.g. diversity) patterns in microbial assemblages (Li, 1997; 2002; Schiaffino et al., 2013). Diversity and evenness indexes based on optical features corroborate the occupation of different trophic niches in the size-fluorescence domain, and also afford direct comparisons of molecular properties by means of cytometric properties (García et al., 2015).

The DOM released by some benthic primary producers (e.g. corals) stimulates complex communities with high species richness, restricted energy requirements (Haas et al., 2010; 2011), and low primary productivity (Margalef, 1979). These microbial communities also exhibit selective consumption of released organic compounds and high bacterial growth

efficiency (BGE), i.e. low catabolism:anabolism ratio (Libes, 2009; Nelson et al., 2013). The strong inverse relationship between diversity and increase in DO (likely the result of oxygenic photosynthesis) was especially pronounced in our turf and macroalgae incubations. Previous studies have suggested that the DOM released by these primary producers has relatively large amounts of low-quality and highly labile DOC, engendering an inefficient bacterial growth, with increasing dominance and lowered diversity (Haas et al., 2010; 2011; Wild et al., 2010; Nelson et al., 2013; Silveira et al., 2015). Bacterial assemblages in macroalgae incubations corroborate the idea of fast DOC and DO consumption by a few populations, resulting in decreased diversity (Figure 4). When compared to macroalgae, algal turfs present higher metabolic rates (Adey & Goertemiller, 1987; Hatcher, 1988). Compositional changes of the DOM released by benthic cyanobacterial mats depend of light cycles (Brocke et al., 2015), with daytime release of photosynthates and nighttime release of products from incomplete organic matter degradation and fermentation (Kristensen et al., 1995). Accordingly, both turf algae incubations presented gradual changes in bacterial community structure (Figure 5), transitioning from slower DOC and DO consumption towards enhanced rates with no significant changes in growth, indicating lower BGE (Haas et al., 2010; 2011; 2013a).

The Abrolhos Bank exhibits the high turf/algal benthic cover typical of turbid-zone reefs. However, fleshy macroalgal cover is steadily increasing, and the system is facing a rapid coral cover decline due to diseases progression (Francini-Filho et al., 2008; 2013). The fast decrease in DOC concentration via enhanced heterotrophy and co-metabolism of refractory carbon are widely observed in turf/algal dominated reefs (Dinsdale et al., 2008; Haas et al., 2013a; Silveira et al., 2015). Higher availability of more labile DOC may indirectly affect

nearby corals by enhancing microbial growth and respiration, potentially causing localized hypoxia (Haas et al., 2013b) and benefitting opportunistic pathogens (Barott & Rohwer, 2012; Kelly et al., 2012). Bruce et al. (2012) observed shifts from photosynthetic microbial communities to heterotrophic assemblages with abundant bacterial pathogens and increased archaeal and viral DNA sequences in reefs under anthropogenic influences in the Abrolhos Bank.

Conclusion

Our study showed that benthic primary producers from turbid-zone reefs, including corals, are important POC sources to the water column in the form of living symbionts (from corals) and epibionts (from turfs and macroalgae). In addition, dissolved organic matter released by primary producers also induces significant shifts on water column microbial community structure, similarly to observations from oligotrophic reef systems, with potential consequences to nutrient recycling and dynamics of turbid-zone reefs at larger spatial scales. Shifts in benthic dominance of coral reefs imply in biodiversity loss and secondary productivity reduction (Graham, 2015), and such consequences may be aggravated in a region with high endemism levels and low diversity/functional redundancy, such as the Abrolhos Bank reefs, which also dwells under sediment and nutrient levels that are possibly near the thresholds for reef development.

Figures and Tables

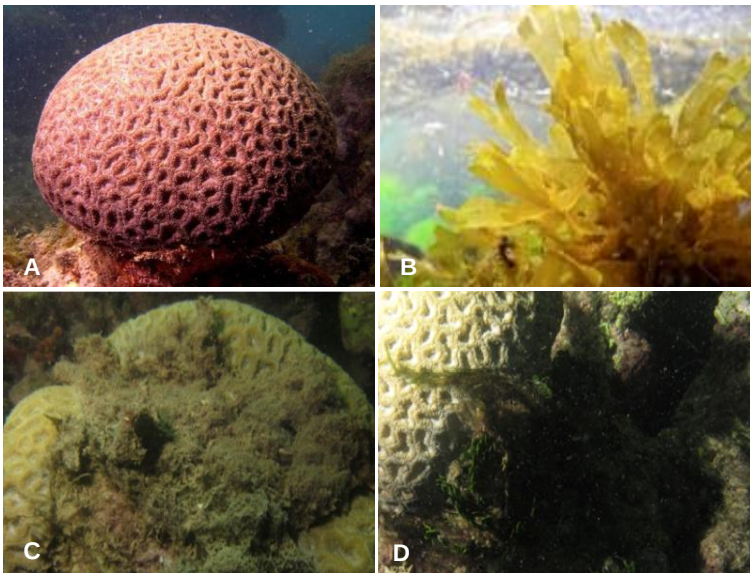


Figure 1. Primary producers investigated in this study: (A) Brazilian-endemic coral holobiont *Mussismilia braziliensis*; **(B)** dominant fleshy macroalgae *Dictyota* spp.; **(C)** Turf algae 1 (TA1); **(D)** Turf algae 2 (TA2).

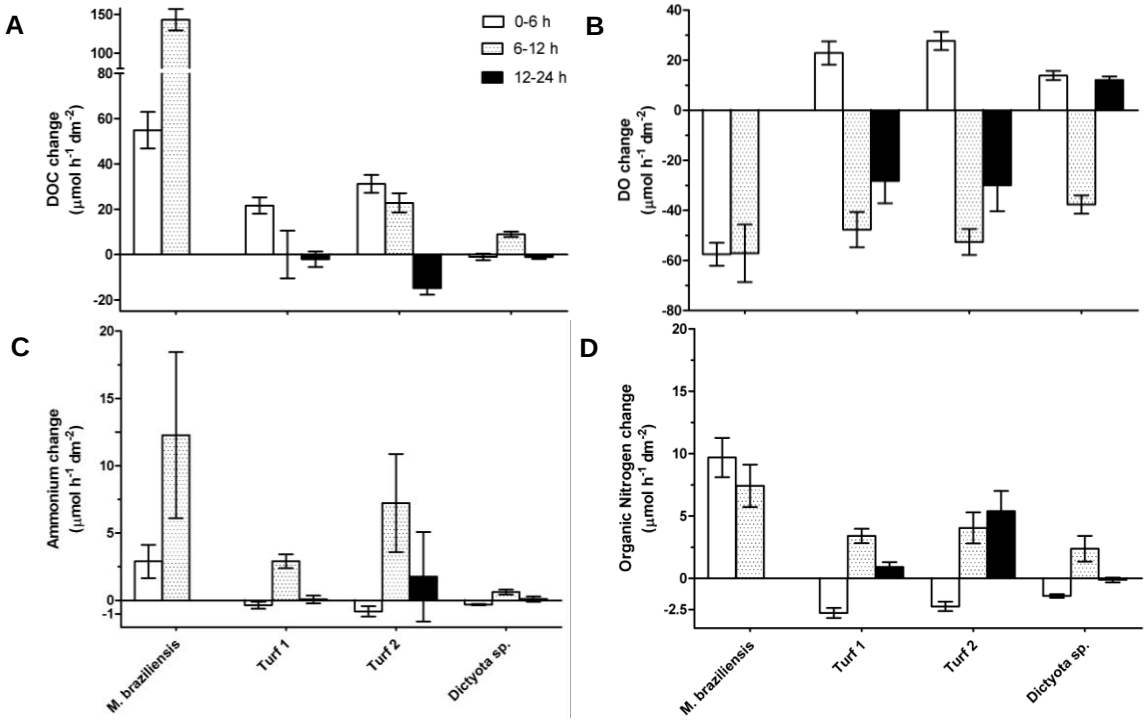


Figure 2. **Rates of change in DOC (A), DO (B), Ammonium (C) and Organic Nitrogen (D), normalized to the surface area of primary producers over a 24 h light/dark cycle.** Bars are means with standard error whiskers. Significant differences are shown in Table 1.

Table 1. Means±SE of change in DOC, DO, Ammonium and Organic Nitrogen in each treatment/time point and results of the mixed-model ANOVA. (Tukey's post hoc test $p<0.05$)

	M. braziliensis	Turf 1	Turf 2	*Dictyota* sp.	*p*
DOC (µmol h^{-1} dm^{-2})					Treatment<0.001
0-6	54.9±8.1 [a] *+	21.6±3.6 [a] ¤	31.2±3.9 [a]	-0.01±0.00 ¤*#	Time<0.001
6-12	143.2±13.8 [b] *#+	0.02±10.5 [b] ¤	22.8±4.2 [a] ¤+	0.06±0.00 ¤#	Treatment x Time
12-24	-	-2.0±3.4 [b]	-14.8±2.8 [b] +	-0.01±0.00 #	<0.001
DO (µmol h^{-1} dm^{-2})					Treatment<0.001
0-6	-57.5±4.6 [a] *#+	22.8±4.6 [a] ¤	27.7±3.7 [a] ¤+	13.9±1.8 [a] ¤#	Time=0.05
6-12	-57.1±11.5 [a]	-47.68±7.0 [b]	-52.6±5.1 [b]	-37.7±3.7 [b]	Treatment x Time
12-24	-	-28.3±8.9 [b] +	-29.9±10.3 [b] +	12.1±1.4 [a] *#	=0.002
Ammonium (µmol h^{-1} dm^{-2})					Treatment=0.85
0-6	2.9±1.2 [a] *#+	-0.4±0.3¤	-0.9±0.4¤	-0.3±0.04¤	Time=0.03
6-12	12.3±6.2 [b]	2.9±0.5	7.3±3.6	0.6±0.2	Treatment x Time
12-24	-	0.1±0.3	1.8±3.3	0.1±0.2	=0.16
Org. Nitrogen (µmol h^{-1} dm^{-2})					Treatment<0.001
0-6	9.7±1.6 *#+	-2.8±0.4¤	-2.2±0.4 [a] ¤	-1.4±0.1¤	Time=0.12
6-12	7.4±1.7	3.4±0.6	4.0±1.2 [b]	2.4±1.0	Treatment x Time
12-24	-	0.9±0.4 #	5.4±1.6 [b] *+	-0.1±0.1#	=0.03

Time points with different superscript letters within treatment columns were significantly different from each other. Significantly different means from those of: *M. braziliensis* (¤); Turf 1 (*); Turf 2 (#); *Dictyota* sp. (+). Colors represent the light/dark cycle. Values normalized to the surface area of the macroorganisms.

Table 2. Planktonic densities (means±SE) in each treatment/time point and results of the mixed-model ANOVA. (Tukey's post hoc test $p<0.05$)

	Control	*M. braziliensis*	Turf 1	Turf 2	*Dictyota* sp.	*p*
Symbiodinium (cell .10^3 L^{-1})						
0	-	0 [a]	-	-	-	
6	-	73±17 [b]	-	-	-	
12	-	76±33 [b]	-	-	-	
24	-	432±196 [c]	-	-	-	Time=0.032
Pennate Diatom (cell .10^3 L^{-1})						
0	2.6±1.3	2.6±1.3	2.6±1.3 [a]	2.6±1.3 [a]	2.6±1.3 [a]	Treatment<0.001
6	2.2±0.2 *#	1.2±0.6 *#	8.0±1.5 [b] ¤#+	**47.7±11.7** [b] ¤*+	3.87±1.7 [a] *#	Time<0.001
12	1.4±0.7 *#+	2.9±2.0 *#+	**20.3±4.2** [c] ¤#+	**82.0±16.6** [b] ¤*+	**12.2±1.9** [b] ¤*#	Treatment x Time
24	1.1±1.1 *#+	-	**26.3±5.7** [c] ¤#+	**93.6±44.5** [b] ¤*+	**14.3±11.3** [b] ¤#	<0.001
Cyanobacteria (fill .10^3 L^{-1})						
0	6.9±3.1	6.9±3.1	6.9±3.1	6.9±3.1 [a]	6.9±3.1	
6	2.4±1.6 *+	6.7±0.7 *+	**8.5±0.8** ¤	5.9±2.1 [a] +	**12.9±3.2** ¤#	
12	2.9±0.3 *+	1.7±1.7 *+	**14.6±7.7** ¤	2.9±2.0 [a] *+	**12.7±4.9** ¤#	Treatment<0.001
24	1.8±1.8 *+	-	**13.0±4.7**¤	25.7±23.6 [b]	**31.6±8.6**¤	Time=0.64
Total Bacteria (cell .10^8 L^{-1})						
0	9.3±0.3 [a]	9.3±0.3 [a]	9.3±0.3 [a]	9.3±0.3 [a]	9.3±0.3 [a]	Treatment>0.1
6	19.6±1.1 [b]	21.2±9.5 [b]	22.2±4.4 [b]	28.3±3.8 [b]	19.1±1.4 [b]	Time<0.001
12	28.6±3.5 [c] ¤	**60.0±11.2** [b]	34.1±7.4 [b]	42.2±7.9 [b]	28.0±2.6 [c]	Treatment x Time
24	38.9±4.7 [d]	-	37.6±5.6 [b]	43.6±15.3 [b]	35.4±0.9 [d]	<0.001
Photosynthetic Bacteria (cell .10^8 L^{-1})						
0	1.7±0.01	1.7±0.01	1.7±0.01	1.7±0.01	1.7±0.01	
6	2.2±0.05	0.7±0.4	2.1±0.04	2.1±0.06	1.8±0.1	
12	2.1±0.05	1.4±0.4	2.1±0.01	1.9±0.2	1.6±0.1	Treatment>0.1
24	2.1±0.08	-	2.1±0.07	1.8±0.1	1.6±0.2	Time>0.1

Time points with different superscript letters within treatment columns were significantly different from each other. Treatments that differ significantly from the control at each time set are bold. Significantly different from: *M. braziliensis* (¤); Turf 1 (*); Turf 2 (#); *Dictyota* sp. (+). Colors represent the light/dark cycle.

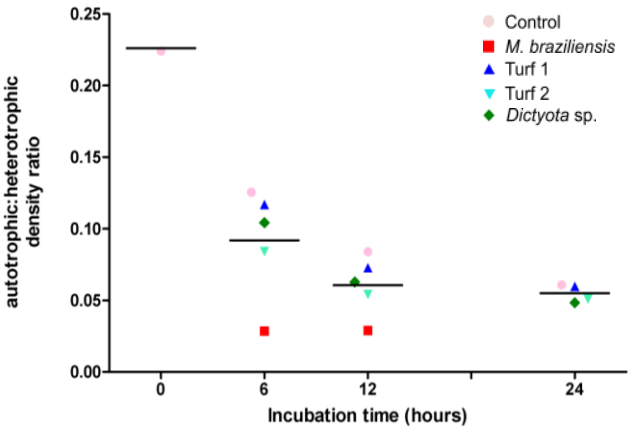


Figure 3. Autotrophic:heterotrophic ratios in the bacterioplankton. Symbols represent the means of each treatment (n=3) and horizontal bars represent the means of all samples at each time point (n=15).

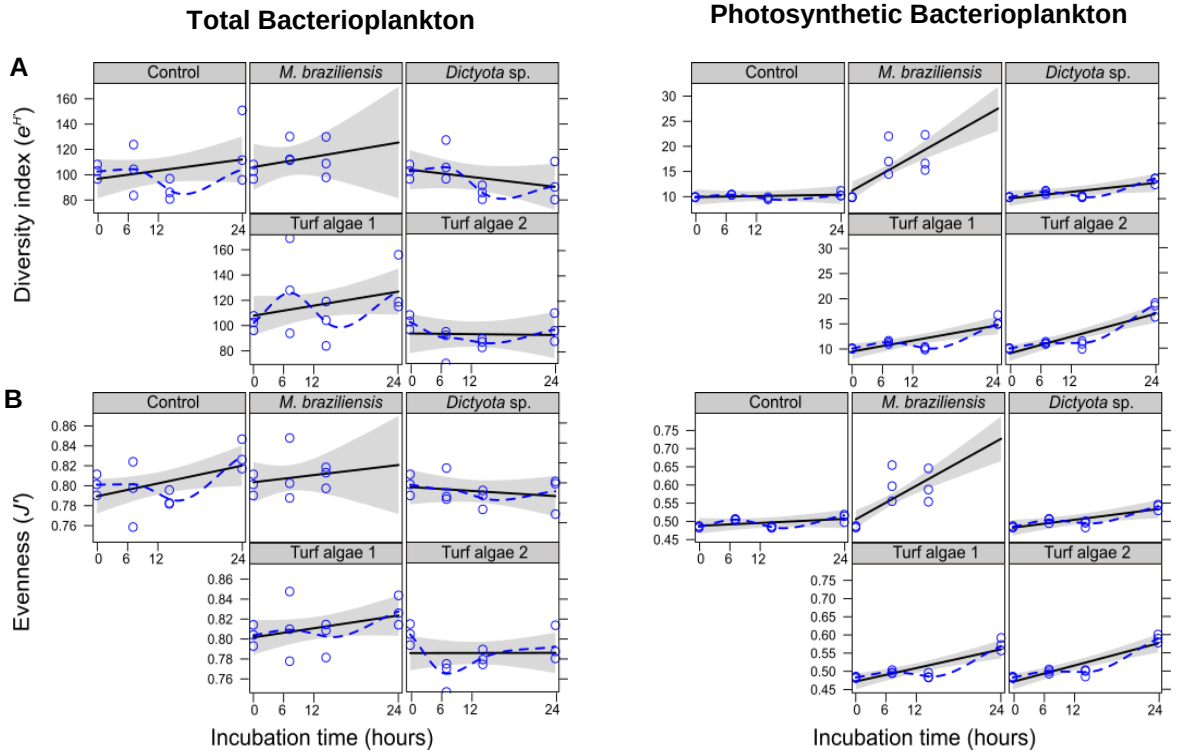


Figure 4. **Partial residual plots showing temporal trends in diversity and evenness of the bacterioplankton.** Data points from each incubation replicate. LME partial regression analysis (component+residual), where solid line, linear regression line (slope of time); dashed line, fitted spline curve and 95% confidence intervals. **(A)** Exponential Shannon Wiener diversity index (eH'), **(B)** Pielou's evenness (J'). Note that axes are in different scales.

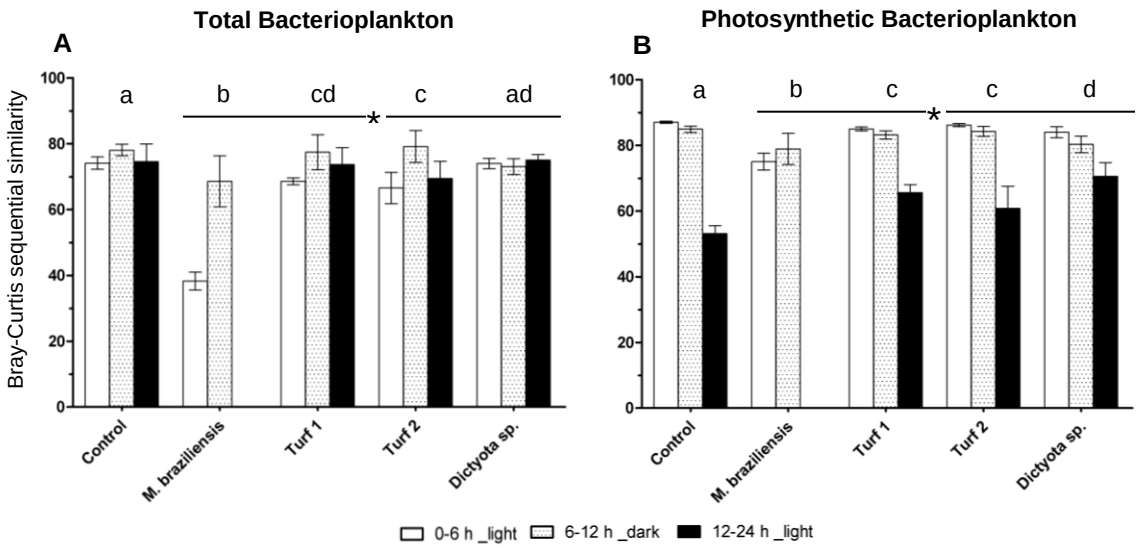
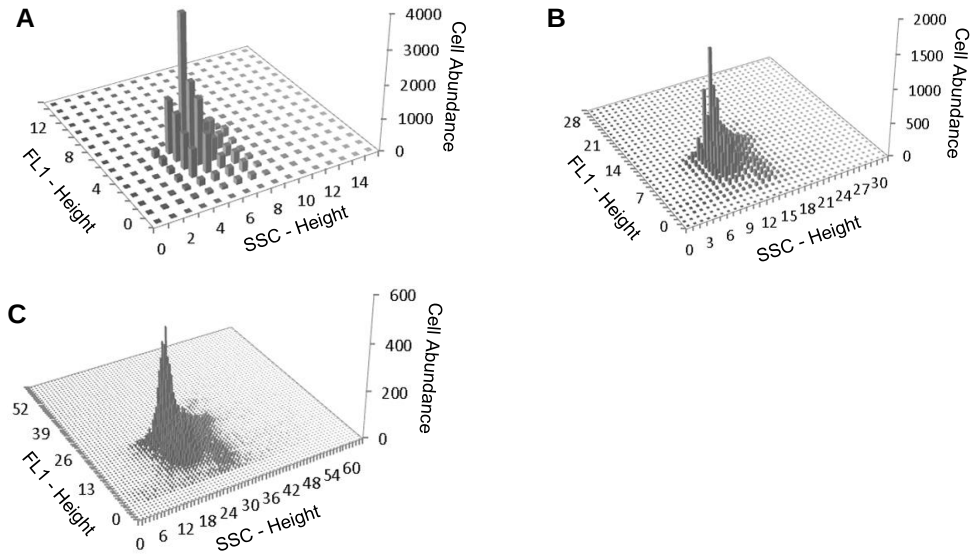
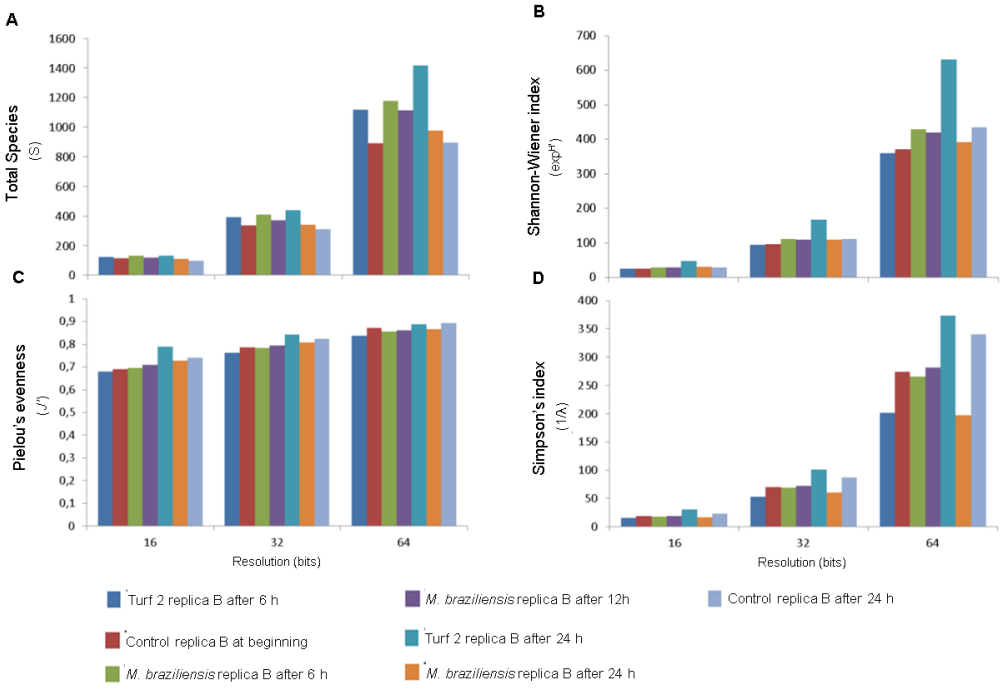


Figure 5. Temporal trends in total and photosynthetic bacterioplankton assemblages. Means of Bray-Curtis similarity (±SE) between adjacent time points (0-6, 6-12, 12-24 h). Treatments with the same letter are not significantly different (pairwise PERMANOVA $p<0.05$). *Significantly different across time.

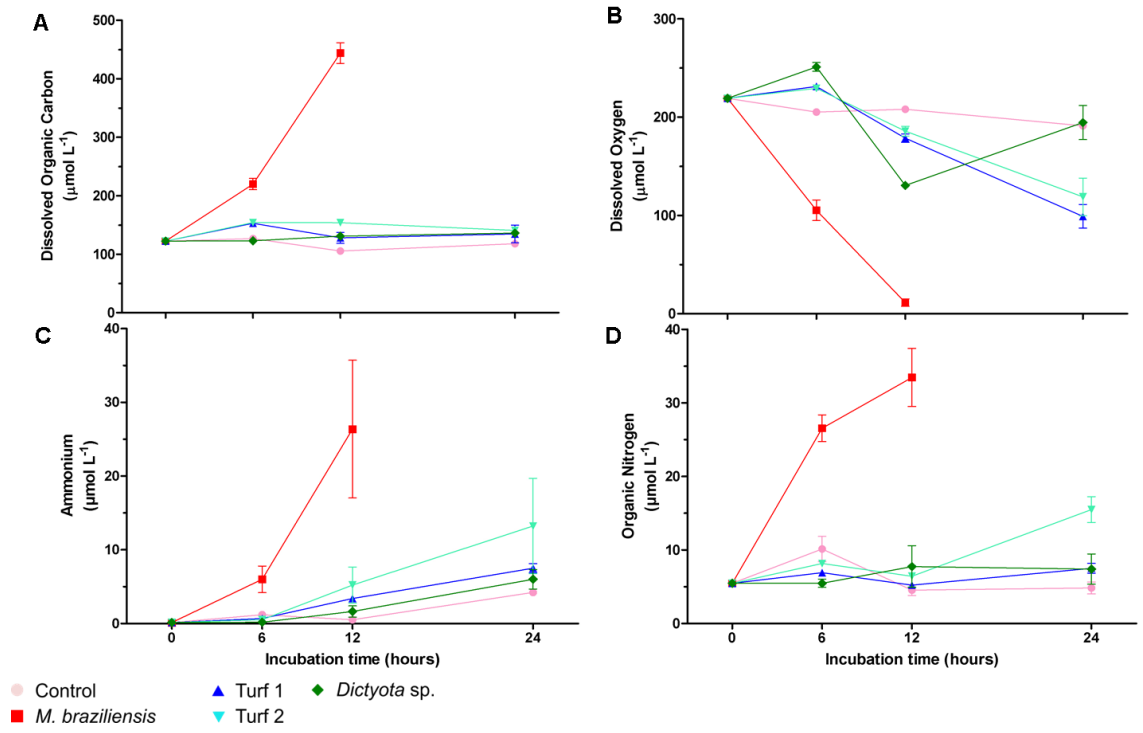
Supplementary figures



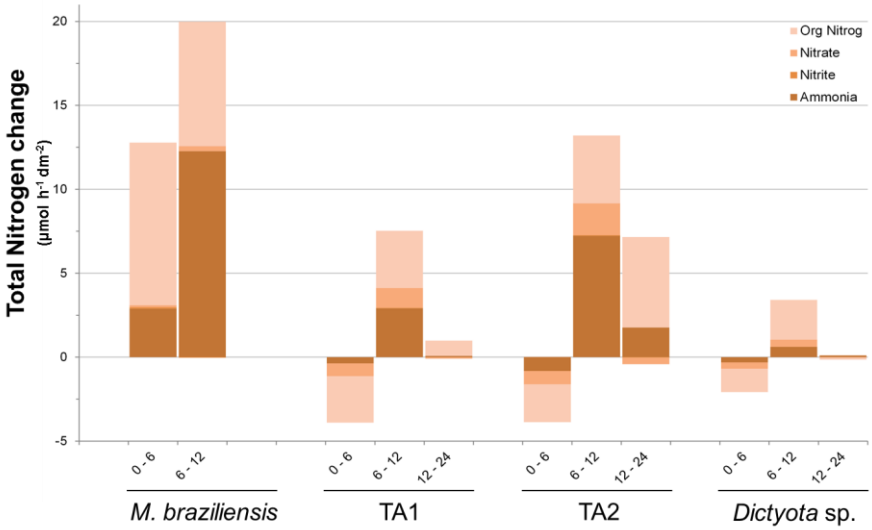
Supplementary Figure 1. Effects of resolution reduction in the number of cytometric categories. **(A)** Reduced resolution to 16 channels, **(B)** reduced resolution to 32 channels and **(C)** reduced resolution to 64 channels. From Stained sample of turf 2 after 6 h.



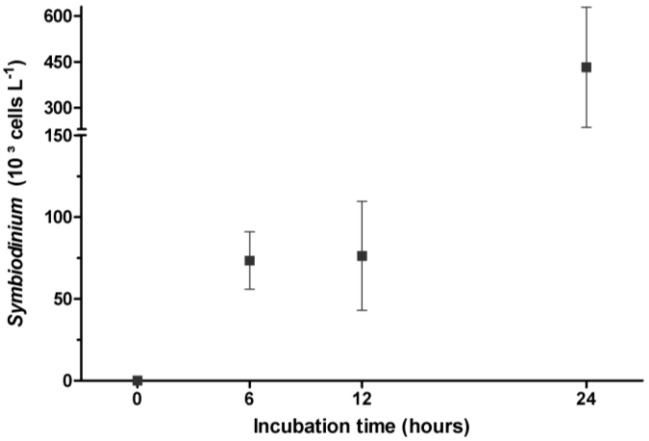
Supplementary Figure 2. Effects of resolution reduction on diversity estimates.
(A) Number of species (S), **(B)** exponential Shannon-Wiener index (expH'), **(C)** Pielou`s evenness (J') and **(D)** reciprocal of Simpson's index (1/λ). From seven stained samples. Data from each treatment/time point color coded according to the legend.



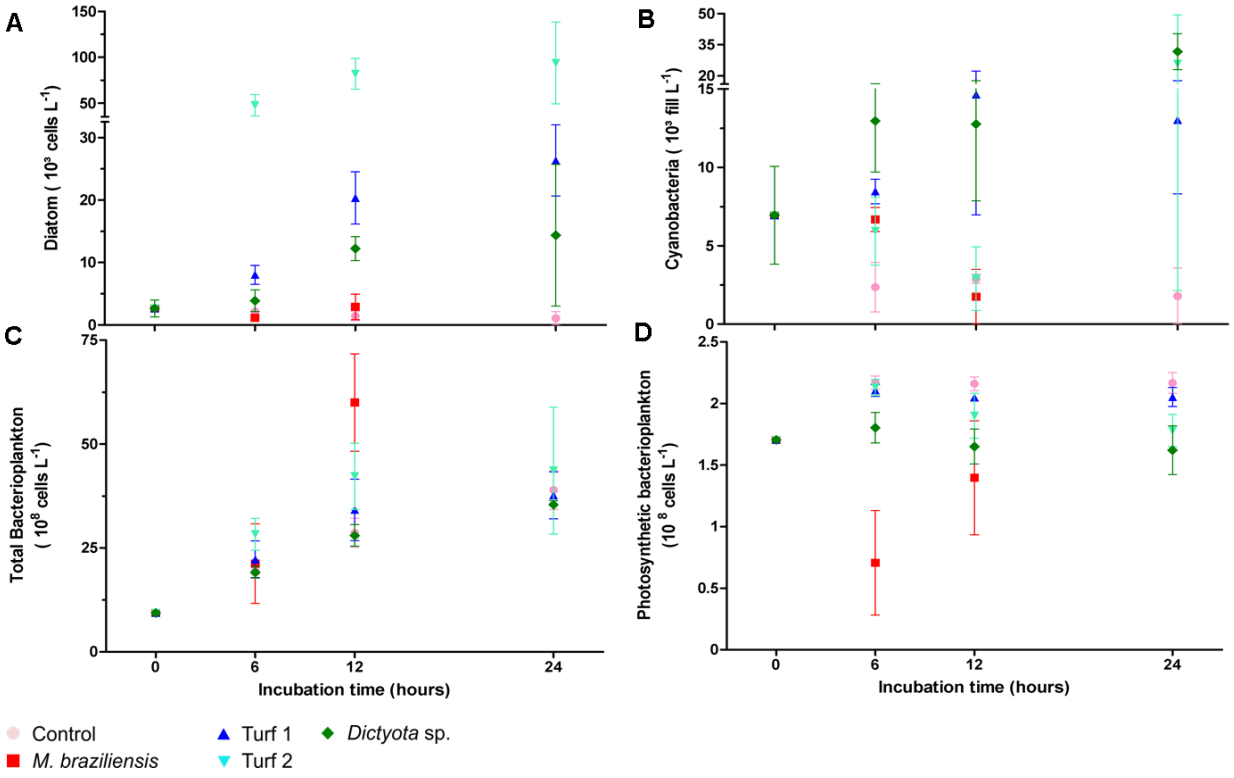
Supplementary Figure 3. DOC, DO, Ammonium and Organic Nitrogen concentrations in incubations over a 24 h light/dark cycle.



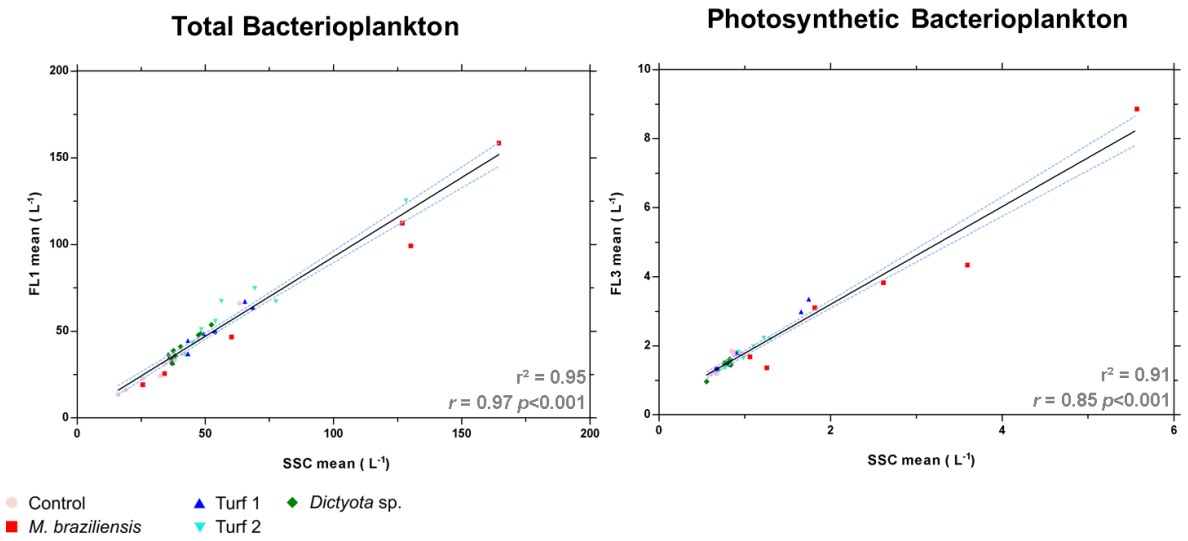
Supplementary Figure 4. Rates of change in Total Nitrogen composition, normalized to the surface area of primary producers over a 24 h light/dark cycle (mean).



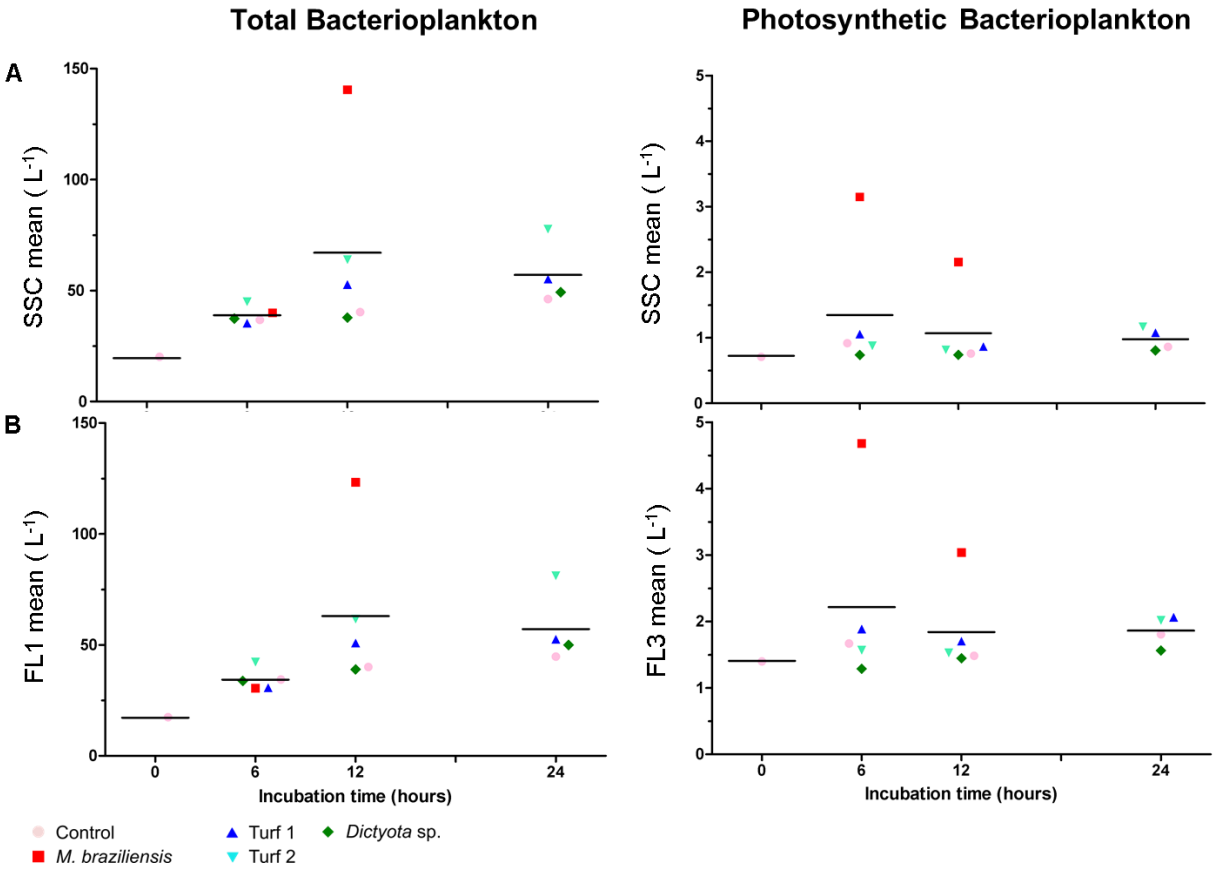
Supplementary Figure 5. *Symbiodinium* densities in *M. braziliensis* incubations (mean±SE).



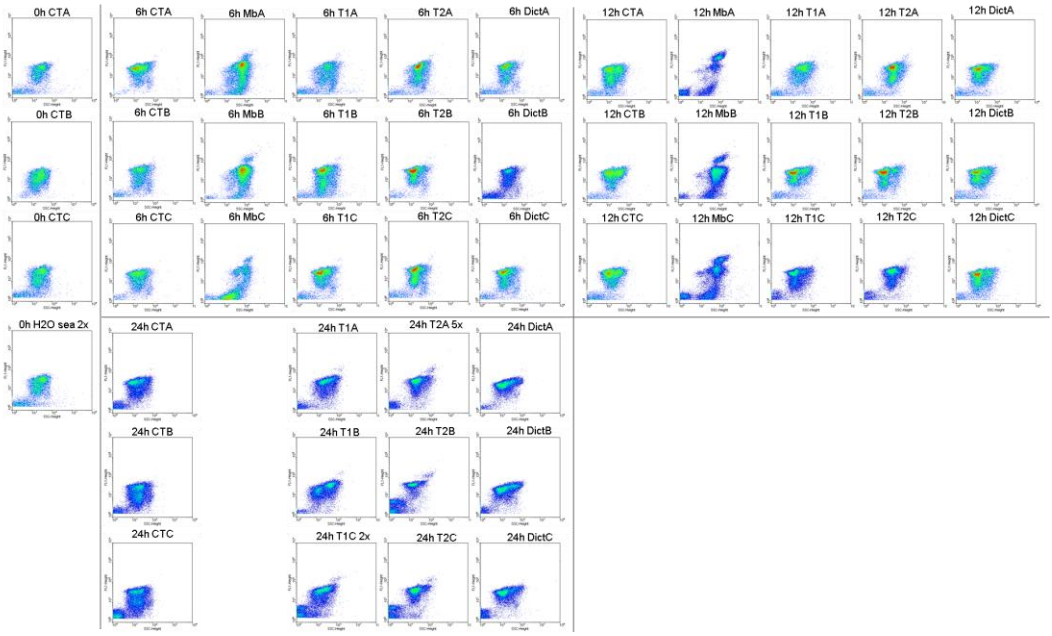
Supplementary Figure 6. Microbial densities for each treatment and time-point (mean±SE).



Supplementary Figure 7. Bacterioplankton size (SSC) and activity (FL1 and FL3).

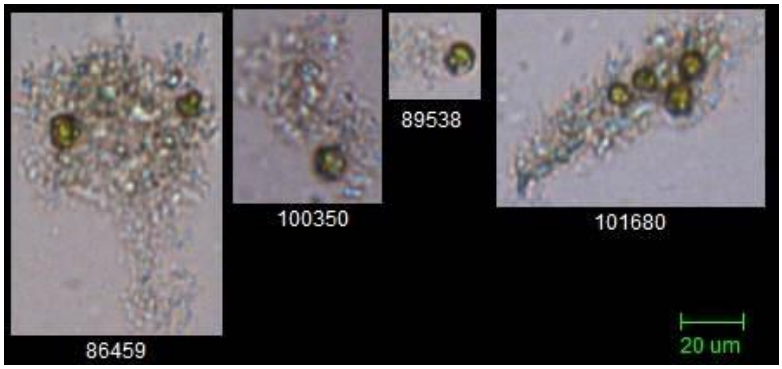


Supplementary Figure 8. Light scatter-fluorescence domain of the bacterioplankton assemblages.



Supplementary Figure 9. Density plots of the Total Bacterioplankton community.

Supplementary Figure 10. Density plots of the Photosynthetic Bacterioplankton community.



Supplementary Figure 11. Unspecific particulate matter aggregates in the FlowCAM imagery from *M. braziliensis* incubations with attached *Symbiodinium* cells.

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Chapter 2

The power of microbes: Microbial bioenergetics of coral-algal interactions

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Abstract

The incidence of coral-algal interactions is increasing with anthropogenic stressors. Utilizing a novel combination of methods, we provide a bioenergetics analysis of lab-simulated interaction zones. The energetic demands of microbial communities at the coral-algal interaction interface were higher than in the communities associated with either of the macroorganisms alone. There was also a lower oxygen concentration at interaction zones relative to areas distal from the interface. Increases in microbial power output and lower oxygen concentrations were significantly correlated with the ratio of heterotrophic to autotrophic microbes but not the total microbial abundance. Together these data shows that coral-algal interfaces harbour higher proportions of heterotrophic microbes that are optimizing maximal power output, as opposed to yield. This yield to power shift provides a thermodynamic mechanism underlying the transition from coral- to algal- dominated reef ecosystems.

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Introduction

Scleractinian corals and algae harbour diverse and abundant viral and microbial communities that are relatively species specific and distinct from the surrounding water column (Herndl & Velimirov, 1986; Rohwer et al., 2002; Wegley et al., 2004; Bourne & Munn, 2005; Ritchie, 2006; Marhaver et al., 2008; Barott et al., 2011; Morrow et al., 2012a; Nelson et al., 2013; Hester et al., 2016; Knowlton & Rohwer, 2003). These microbiomes play a major role in reef trophic dynamics (Hoogenboom et al., 2015; Tremblay et al., 2015), and the biogeochemical cycling of the surrounding environment (Selmer et al., 1993; Lesser et al., 2004; Wegley et al., 2007; Siboni et al., 2008; Raina et al., 2009; Fiore et al., 2010; Haas et al., 2010; 2011). Benthic macro- and microorganisms have also been shown to change the availability of various organic and inorganic nutrients (Wild et al., 2004), alter the oxygen availability (Wild et al., 2010; Haas et al., 2013) and concomitantly pH (Smith et al., 2013) in their immediate surrounding, and may produce hydrophilic and hydrophobic compounds which affect neighbouring organisms (Ritchie, 2006; Smith et al., 2006; Nissimov et al., 2009; Morrow et al., 2011; 2012b; Rasher et al., 2011).

Understanding the extent of microbial influences and how they differentially affect ecosystem functioning has become increasingly important during times of global shifts in benthic reef community structure. Anthropogenic influences, such as over-fishing and nutrient addition, have led to a decline of coral abundance in reef systems at a global scale (Hughes et al., 2007; Meltvedt & Jadot, 2014). As a result, turf and fleshy macroalgae are becoming the dominant benthic macroorganisms covering the substratum of formerly coral-dominated reef ecosystems (McCook, 1999; McCook et al., 2001; Hughes et al., 2003;

2007). The increased algal abundance results in a higher likelihood of coral-algal interaction events (Barott et al., 2012a). Although the course and outcome of these interactions will consequently determine the community structure of the reef environment, the mechanisms involved in ongoing interaction events are still not fully identified. Whether directly through diseases (Bourne et al., 2009), or indirectly through harmful secondary metabolites (Hay, 1996; Morrow et al., 2011) or microbe induced hypoxia (Smith et al., 2006; Barott et al., 2009; 2012b; Gregg et al., 2013; Haas et al., 2013), microbial involvement in these interaction events is unquestioned.

Smith et al. (2006) demonstrated that algal derived exudates can cause high levels of coral mortality. Moreover, they found that this algal-induced coral mortality was microbially mediated, and that the algal exudates lead to significantly lower oxygen concentrations on coral surfaces (Smith et al., 2006; Barott et al., 2009). These hypoxic conditions compromise coral to much greater extents than the algae in their competition for the limited substrate in reef environments (Haas et al., 2014). Furthermore, algal dominated patches of reefs have been found to yield more heterotrophic microbial communities, which contain a higher number of virulence factors (Dinsdale et al., 2008; Kelly et al., 2012)

The positive feedback loop resulting from these interactions was described as the Dissolved Organic Carbon (DOC), Disease, Algae, and Microbes (DDAM) model (Kline et al., 2006; Barott & Rohwer, 2012). DDAM posits that increases in the percent cover of benthic macroalgae causes enhanced release of bioavailable DOC, which cultivates a copiotrophic microbial community (Nelson et al., 2013). In turn, this change in the microbial community structure leads to coral mortality through various pathogenicity mechanisms. The increase in coral mortality frees up benthic space for more algae to inhabit, which yields a positive

feedback loop, ultimately resulting in coral death and algal dominated reef systems. Moreover, microbial communities growing on algal exudates also have higher and less effective carbon turnover rates, thereby increasing local oxygen consumption rates (Haas et al., 2011) and altering the allocation of energy toward microbial activity and away from higher trophic levels (McDole et al., 2012).

Although there is some understanding of the mechanisms by which these microbial assemblages alter the trophic dynamics (Odum & Odum, 1955; McDole et al., 2012) and energy flux (Salomonsen, 1992; Fabiano et al., 2004; Carlson et al., 2007) of the reef, little is known about the underlying thermodynamic mechanisms involved in the ongoing coral-algal interaction events. Here, we used a novel combination of methods to characterize how benthic macroorganism interactions affect the metabolisms of reef-associated microbes. A combination of microcalorimetry with planar oxygen optode imaging (Gregg et al., 2013; Haas et al., 2013), flow cytometry, and epifluorescence microscopy was used to generate a detailed bioenergetics analysis of microbial assemblages associated with coral-algal interactions. Microcalorimetry provides a thermodynamic assessment of metabolic heat dissipation by respective communities. Planar oxygen optodes allow for a fine scale 2-dimensional visualization of the metabolic effects on O_2 concentrations in the surrounding water-column (Gregg et al., 2013), and flow cytometry permits us to estimate the partitioning of autotrophic and heterotrophic microbes in these communities (Zubkov et al., 2000). Epifluorescence microscopy allows us to tie the values obtained through the previous methods to the total microbial abundance. The results of this study provide insight as to how energy flows through the varied metabolisms of coral and algal microbiomes,

thus unravelling the mechanisms responsible for determining the outcomes of coral-algal interactions.

Methods

Experimental design and sampling. Independent 10 L aquaria were filled with artificial seawater. One piece of coral (*Porites* sp.) and one mass of algae (*Chaetomorpha* sp.) (Aquatic Warehouse, San Diego, California, USA) were placed in direct contact with one another in each tank (Figure 1A). Planar oxygen optodes were fitted around the coral, algae, and interface (Figure 1A) to measure relative oxygen concentrations, which was used in a two dimensional colorimetric analysis of the Biological Oxygen Demand (BOD). Polyethelene tubing (5 mm) connected to 21 gauge luer lock needles mounted to the back of the optode sheet served as sampling ports through which water samples were collected via a 10 mL syringe. The aquaria were equilibrated for 36 hours with a constant water flow and 12-hour dark/light cycles in order for microbial communities to establish. After preliminary equilibration, water flow was stopped in all tanks to allow oxygen gradients to form. Pictures were taken of the BOD optodes, after which 5 mL of water was sampled from each of the three benthic areas (coral, algae, and interface) via the aforementioned sampling ports. Ambient water-column samples were also taken. After sampling, the water flow was returned to the aquaria. This sampling scheme was conducted on eight separate biological replicates.

Sample processing. Each sample was divided into aliquots for flow cytometry (1 mL), epifluorescence microscopy (1 mL), and calorimetry (3 mL). Flow cytometry samples were fixed with microscopy grade glutaraldehyde and the flash frozen in liquid N_2 before being

stored at -80 °C. Microscopy samples were fixed with microscopy grade paraformaldehyde, vacuum filtered onto a 0.02 µm Anodisc filter (Whatman inc., Florham Park, NJ, USA), stained with SYBR Green I (5x final concentration; Invitrogen, Carlsbad, CA, USA), and mounted on microscope slides. Calorimetry samples were weighed and placed in a TAM III isothermal heat conduction microcalorimeter (TA Instuments, New Castle, Delaware, USA) for 8 hrs.

Sample analyses. Flow cytometry samples were thawed at 37 °C and plated in a flat bottomed 96-well-plate on ice. Analysis was performed with a BD FACS-Canto via the high-throughput sampler unit for enumeration utilizing the methods of Zubkov et al. (2000). For enumeration of autotrophic microbe populations, 100 µL of sample was collected for analysis on standard mode using the 488 nm laser for excitation. Bivariate plots were used to analyse the sample for chlorophyll fluorescence (red), which were done in the PerCP-Cy5-5 channel (670 long pass filter preceded by a 655 nm long pass mirror) and the phycoerytherin (PE) channel (585/42 band pass filter preceded by a 556 nm long pass mirror). For autotrophic populations, threshold gating was determined by using 0.02 µm filtered seawater. Yellow-green fluorescent microsphere beads (0.75 µm) were used to control for sample volume analysed. To control for consistency between plates and daily runs, a standard seawater sample, collected from San Diego, California, was used. This control was used for both autotrophic and total microbe enumerations. One hundred µL sample volume was collected and analysed for SYBR fluorescence, which was excited by the 488 nm laser and detected in the FITC channel (530/30 nm band pass filter preceded by a 502 nm long pass mirror). Threshold gating for the heterotrophic populations was determined by using unstained representative coral/reef water, and to verify the amount of

background associated with the instrument. Data was collected on FACSDiva 6.1.1 and analysed using FlowJo 7.6.5. In order to enumerate heterotrophic microbe populations, the comparative autotrophic populations were subtracted from the total microbial counts (SYBR fluorescence).

Isothermal calorimetry was conducted using a TAM III multi-channel microcalorimeter. Samples were placed in the machine within an hour of the sampling process. Samples were lowered into the measurement position after a 30-minute equilibration period. Calorimetric measurements were taken continuously for each channel for 8 hours at 299 K.

Results

Macroorganism-associated microbial thermodynamics

Isothermal heat-conduction microcalorimetry was used to measure microbial power output across the coral-algal-interaction interface. The interface region demonstrated a significantly higher power output than either the coral- or algae-associated communities by themselves (Figure 1B, Supplementary Figures 1 and 2) (student T-test, d.f.=7, interface-coral p=0.04, interface-algae p=0.01). Furthermore, the coral- and algae-associated microbial communities had higher power output than the surrounding ambient tank water microbial communities (Figure 1B, Supplementary Figures 1 and 2) (student T-test, d.f.=7 coral-water p=0.001, algae-water p=0.01) and the 0.02 µm filtered seawater control (Supplementary Figure 1). Integration of the heat production over time provided further evidence that the interface-associated microbial community produced a significantly higher power output than that of the coral-, algal-, or water-column-associated microbial assemblages (Supplementary Figure 2).

This data demonstrates that there are small-scale spatial differences in the way that energy flows though macroorganism-associated microbial communities. More specifically, the microbial assemblages associated with the coral-algal interface have a higher thermodynamic power output than the microbial communities associated with either macroorganism alone.

Microbial community trophic structure

Flow cytometry analysis revealed that the interface microbial community is significantly more heterotrophic than the communities associated with coral or algae (Figure 1C) (student T-test, d.f.=7, interface-coral p=0.03, interface-algae p=0.01). This shift in community metabolism to a more heterotrophic microbial consortium at the interface correlates significantly with the total heat output of the microbial communities (Figure 2A) (R^2: 0.42; ANOVA, $F_{1,26}$=18.6 p<0.001). Whereas, total microbial abundance was not significantly different between sampling sites (Supplementary Figure 4), and was not a strong predictor of heat production (Figure 2B) (R^2: 0.04; ANOVA, $F_{1,27}$=1.76 p=0.29), indicating that the shift towards a more heterotrophic community metabolism is responsible for the increased power at the coral-algal-interaction interface.

Biological oxygen demand

Planar biological oxygen demand (BOD) optodes (Gregg et al., 2013) provided a fine scale 2-dimensional assessment of the O_2 concentrations associated with the coral-algal interactions. Supplementary Figure 3 presents representative pictures of the oxygen concentrations visualized in a typical coral-algal interaction experiment. The optodes demonstrate a clear O_2 decline at the coral-algal interface relative to the oxygen levels

associated with coral, algae, and the water column (Figure 1D and Supplementary Figure 3), supporting previous observations by Haas et al. (2013).

Discussion

Overall, our results demonstrate a shift in community metabolism towards increased net heterotrophy at the coral algal interaction interface (Figure 1C and 2A). This leads to an increase in catabolic reactions such as oxidative respiration, which use biologically available oxygen; in turn, lowering O_2 concentration at the interface between the macroorganisms (Figure 1D and Supplementary Figure 3). The unique microbial community metabolism at the interaction zone (Figure 1C) yields an increase in thermodynamic power at the interface (Figure 1B and Supplementary Figure 2).

Nonequilibrium thermodynamics and ecology

Work in the field of thermal physics has demonstrated that as open systems approach a steady state they begin to minimize energy dissipation (Onsager, 1931a; b; Prigogine & Nicolis, 1971). Dissipation is one way to measure the energy that is converted to heat and "wasted" by any process. In biological systems, thermodynamic theory (Wicken, 1980; Weber et al., 1989; Salthe, 1998) predicts that as ecosystems approach a steady state, they should foster a community which serves to minimize the dissipation and thus maximize the *yield* (amount of work done per unit energy input (J/J)) of the system (Onsager, 1931a; b; Prigogine & Nicolis, 1971). In contrast, perturbed ecosystems going toward an alternative steady state will harbour a community that maximizes the *power output* (the amount of energy used per unit time (J/s)) of the system (Odum & Odum, 1955). Systems running at maximum power do so by operating at less than maximal efficiency. Maximizing power

output is typically accomplished by proceeding at efficiencies no greater than 50% of the maximum reversible efficiency (Supplementary Figure 5) (Odum & Pinkerton, 1955). Thus, changes in the power output of an ecosystem may be a significant indicator of the current state of the system (Fath et al., 2001; Nielsen & Jørgensen, 2013).

This study demonstrated that power output at the coral-algal interaction interface is higher than in the communities associated with either of the single organisms. This was evident through higher microbial heat production (Figure 1B, Supplementary Figures 1 and 2) and lower oxygen concentration (Figure 1D and Supplementary Figure 3) at interaction zones compared to areas distal from the interface. These measurements support prior observations that coral-algae interfaces foster a unique microbial community metabolism (Barott et al., 2012b; Haas et al., 2013). This study further identifies small-scale, spatial alterations in the thermodynamics of microbial communities associated with specific benthic boundary layers. Specifically, the data demonstrates a *yield-to power switch* occurring at the interaction interface between coral and algae. A better understanding of the thermodynamic fluxes and power of an ecosystem will provide measurements that can be used for modelling these ecosystems to predict the systems' trajectories and eventually devise plans for ecological remediation.

Bioenergetic mechanisms of the DDAM loop

At the coral-algal interface, algae exude dissolved organic carbon (DOC) in close proximity to coral, which causes a shift in the microbial community toward more heterotrophic metabolisms (Figure 1C, Dinsdale et al., 2008; Kelly et al., 2012). As heterotrophic microbial metabolisms are favoured in these interaction events, the microbial community

metabolism is shifted toward more copiotrophic consumers relative to the amount of autotrophic producers (Figure 1C), which affects coral reef systems in two ways. First, these copiotrophic consumers have a greater net oxygen consumption, which causes localized hypoxia at the coral-algal interface (Figure 1D, Barott et al., 2009; Haas et al., 2013). Second, this microbial community shift toward relatively more heterotrophic consumers means that a greater portion of the available energy will be utilized by the microbial fraction of the ecosystem leaving less energy for higher trophic levels, a process referred to as microbialization (McDole et al., 2012)

Microbialization is a metric of the proportion of energy allocated to the microbial fraction of an ecosystem (i.e. a measure of the trophic cascading from microbes to macro-organisms) (McDole et al., 2012). One cause of microbialization on coral reefs is overfishing, which reduces grazing pressure on algae. Releasing macroalgae from grazing begins the DDAM positive feedback loop where DOC released by the un-grazed algae cultivates a copiotrophic, bacterial community, ultimately leading to coral mortality and more benthic space for algae. Our data suggests that a possible underlying mechanism in the DDAM loop (Kline et al., 2006; Barott & Rohwer, 2012) and the subsequent microbialization (McDole et al., 2012) of coral reef ecosystems may be the higher power output of the microbial communities at the coral-algal interaction interface. The increase in power –that is, energy used per unit time - at the interaction interface (Figure 1B, Supplementary Figures 1 and 2) stems from an augmentation in net heterotrophy at the interface (Figure 1C). This switch to a microbial community with higher thermodynamic power coupled to the increase in heterotrophic metabolisms, such as oxidative respiration,

creates localized areas of hypoxia (Figure 1D and Supplementary Figure 3), which damages the coral tissue ultimately leading to coral disease and mortality (Figure 3).

Micro-scale and whole-reef-scale dynamics

Studies have shown that increased algal cover is significantly correlated with a net increase in heterotrophic microbial communities (Dinsdale et al., 2008; Kelly et al., 2012) and a shift toward faster, lower yield metabolisms (Haas et al., 2016). Our data present evidence that this increase in power and net heterotrophy observed on a reef-wide-scale may be due to the small-scale (on the order of 10 μm – 1 mm) spatial dynamics occurring at the coral-algal interface. That is, the altered community metabolisms and bioenergetics may stem from the increased number of coral-algal interactions that occur as algae begin to increase in benthic abundance. This raises an interesting question: to what extent are the activities observed at the coral-algal interface driving the large-scale bioenergetic dynamics observed on the reef as a whole? Future work should focus on understanding the ways in which the small-scale activities at the interface scale-up to whole reef dynamics. *In silico* modelling may provide a better understanding of how small-scale interaction events affect the overall bioenergetics of reef ecosystems.

Conclusion

The findings presented here suggest that as the once stable state of coral reef ecosystems are perturbed by algal derived DOC, there is a shift in the microbial system from a community optimized for efficiency to one that is optimized to perform at maximum power. This *yield to power switch* of the microbial community allows the microbes to outcompete corals for bio-available oxygen, thus providing a competitive advantage to

surrounding macroalgae. In this way, the yield to power switch is posited to be an underlying energetic mechanism involved in the microbialization of coral reefs and the DDAM positive feedback loop (Figure 3)**.** As this change in microbial mediated reef energetics may be the mechanism leading to the ongoing algal dominations of benthic areas that were once inhabited by coral, an understanding of coral reef microbial bioenergetics may serve as an indicator of coral reef health and provide important insight to predict the future trajectory of these valuable ecosystems.

Figures

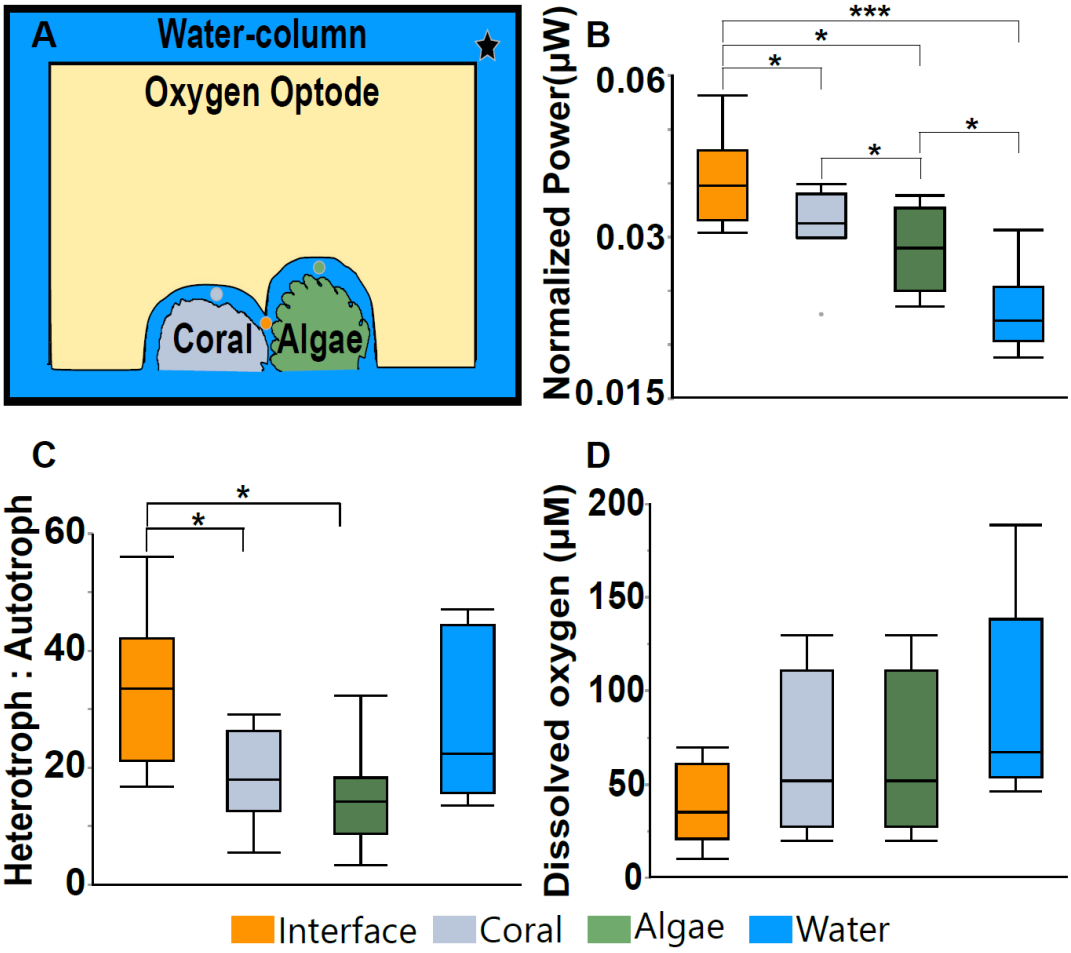


Figure 1. Microbial dynamics at the coral-algal interface. (A) Schematic of the experimental set-up. Aquaria containing coral and algae were outfitted with a planar oxygen optode mounted vertically above the benthic macroorganisms. Colored dots represent the location of the sampling ports at the interface (orange), coral (grey), and algae (green), and the star represents where water-column (blue) samples were taken. **(B)** Total power output (µW, microwatts) of microbial communities. **(C)** Heterotroph to autotroph ratio. **(D)** Dissolved oxygen concentration in µM.

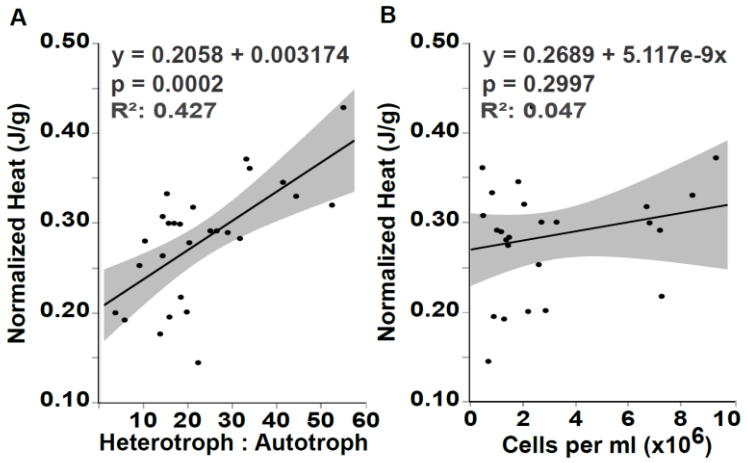


Figure 2. Total heat output of the microbial community plotted against the heterotroph autotroph ratio (A) and the cellular abundance (B).

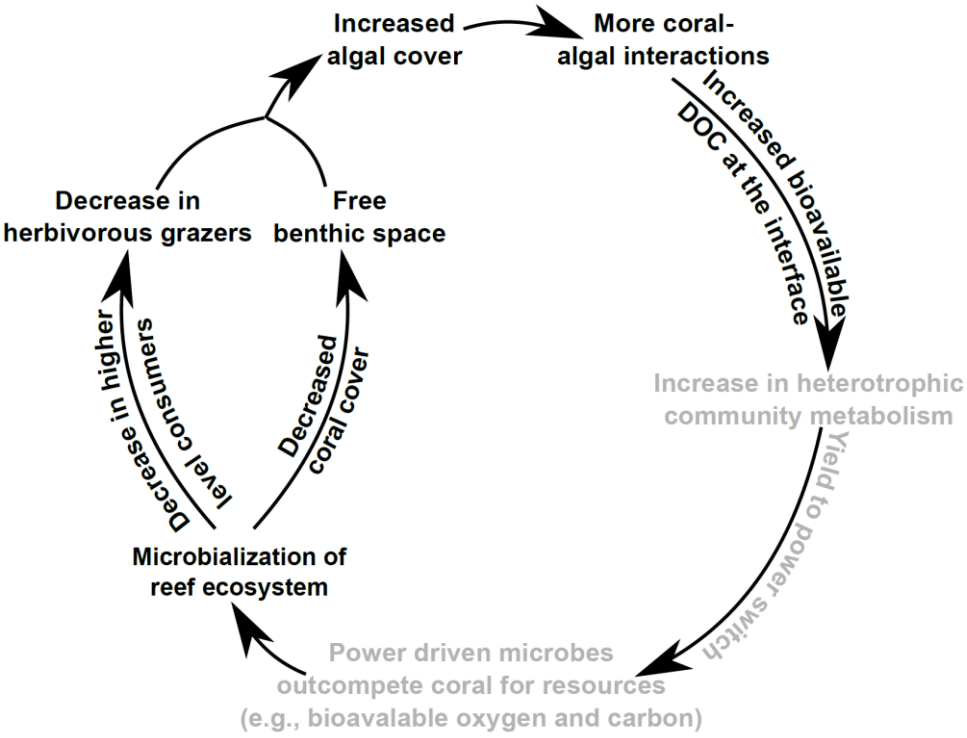
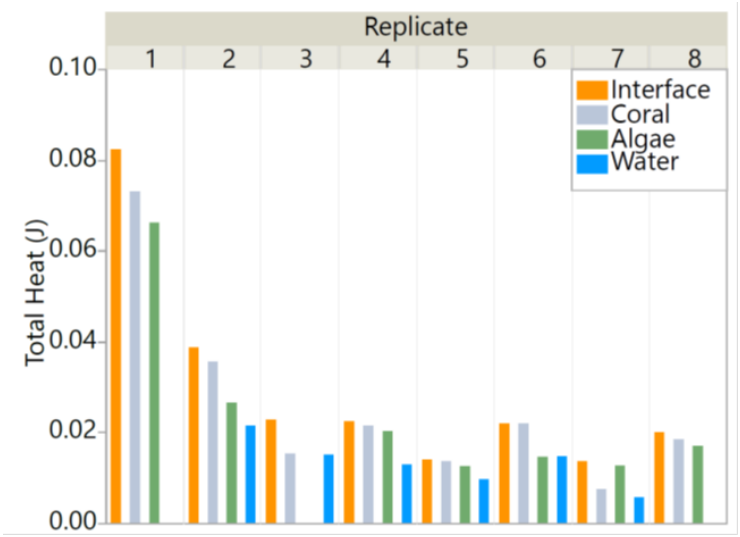
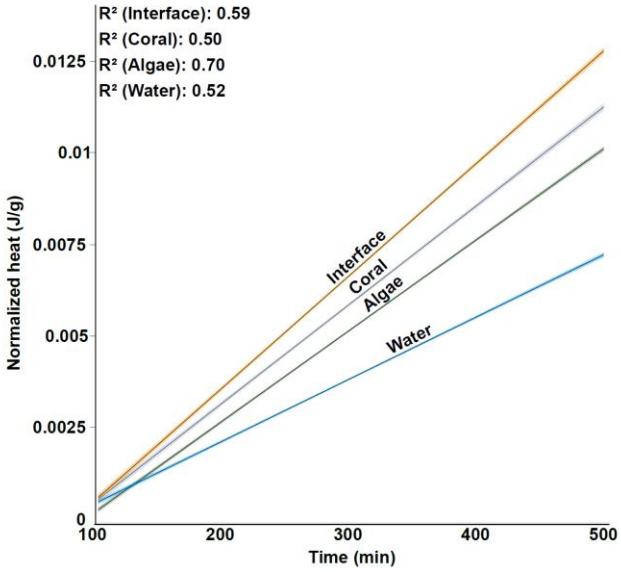


Figure 3. Conceptual depiction of the DDAM feedback model. Grey text indicates the bioenergetic and thermodynamic mechanisms associated with the DDAM loop established by this study.

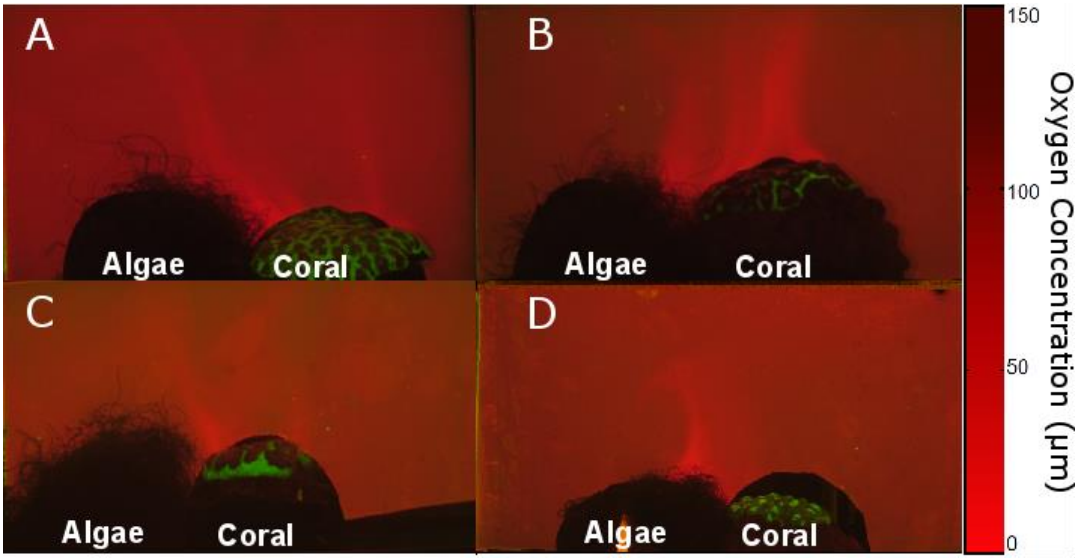
Supplementary Figures



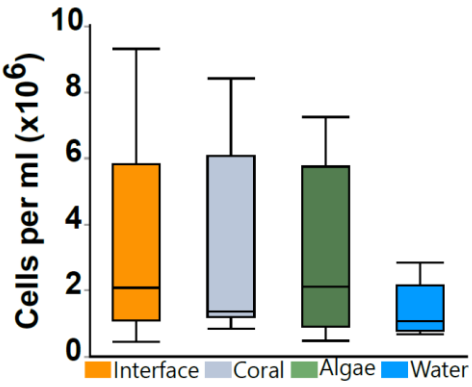
Supplementary Figure 1. Total heat output of each macroorganism-associated microbial community measured by calorimetry for all experimental replicates.



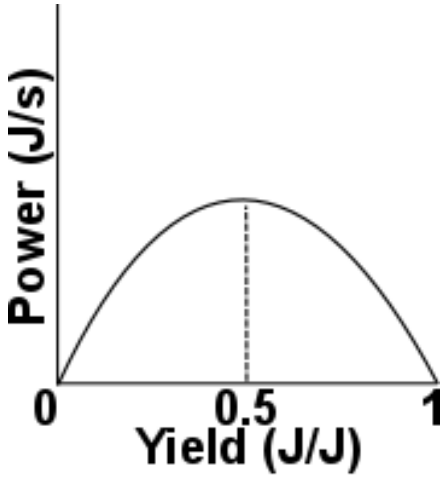
Supplementary Figure 2. Microbial heat output over time. The y-axis is the total heat (J, joules). The x-axis is time (min, minutes). The lines represent the lines of best fit for the average of the eight replicates and the shaded area represents the 95% confidence of fit.



Supplementary Figure 3. Oxygen concentrations at the coral-algal interaction interface. Representative photographs of planar oxygen optodes in coral-algal interactions. Higher red intensity indicates relatively lower oxygen concentrations.



Supplementary Figure 4. Total microbial abundances across the three benthic macroorganisms. The vertical axis represents the number of microbes per milliliter (x 10^6).



Supplementary Figure 5. Conceptual depiction of power versus yield. Power -the measure of energy output per unit time (Joules/ second) - is maximized at 50% yield - the dimensionless measure of energetic output per unit energy input (Joules/ Joules). Figure adapted and modified from Odum and Pinkerton (1955).

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