Fluorescent organic exudates of corals and algae in tropical reefs are compositionally distinct and increase with nutrient enrichment

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Abstract

Dissolved organic matter (DOM) composition influences microbial community metabolism and benthic primary producers are a source of DOM in coral reefs. As reef benthic communities change, in part due to nutrient pollution, understanding impacts on reef microbial processes requires knowledge of DOM sources and composition. We conducted a multi-week mesocosm experiment dosing four coral reef benthic constituents with three levels of nitrate and phosphate to contrast exudate composition and quantify the effects of nutrient enrichment on exudate release. Moderate nutrient enrichment enhanced bulk dissolved organic carbon exudation by all producers. Corals exuded rapidly accumulating DOM with characteristics that clearly distinguish them from algal exudates; coral exudates also accumulated throughout the experiment whereas algal exudates did not. Our results clarify a mechanism whereby anthropogenic activities that alter benthic cover and nutrient pollution on reefs may alter microbial communities and metabolism in reefs.

Scientific Significance Statement

On coral reefs, four primary groups of benthic organisms dominate photosynthetic production: corals, macroalgae, microphytobenthos, and encrusting algae on rubble—all of which exude significant quantities of dissolved organic matter (DOM). However, little is known about whether and how DOM exudates differ among these four groups and whether nutrient enrichment alters exudate quantity or composition. Our mesocosm experiment showed that nutrients stimulated exudation in all groups, but there were key differences in composition among the groups. Corals exuded DOM with characteristics that clearly distinguish them from algal exudates; coral exudates also accumulated throughout the experiment whereas algal exudates did not. Our results clarify a mechanism whereby anthropogenic activities that alter benthic cover and nutrient pollution on reefs may alter microbial communities and metabolism in reefs.

Author Contribution Statement

ZAQ and CAC conducted the laboratory measurements. ZAQ analyzed the data and conducted all spectral modeling. KR, NJS, MJD, and CEN designed the experiments. KR, MDF, TAO, HMP, and CEN ran the experiment. All authors contributed to data interpretation and edited the manuscript. ZAQ and CEN wrote the paper and are accountable for the integrity of the data, analysis and presentation of findings as a whole.

Data Availability Statement: All data and metadata from this experiment has been made publicly available via the US National Science Foundation Biological and Chemical Oceanography Data Management Office Dataset 723868 (https://www.bco-dmo.org/dataset/723868).

Additional Supporting Information may be found in the online version of this article.

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which were dominated by humic-like fluorescent components and did not accumulate significantly. Our results indicate that corals and algae release DOM of different quality and the quantity of DOM release increases with inorganic nutrient availability.

Coral reefs are highly productive ecosystems that thrive in oligotrophic tropical waters, in part by intense recycling of limited nutrients through a highly diverse and active microbial community (Raina et al. 2009; Cardini et al. 2015). Benthic primary producer communities in tropical reef ecosystems are dominated by hermatypic corals, macroalgae, sand-associated microphytobenthos, and a variety of encrusting organisms, primarily crustose coralline algae (CCA) and mixed turf algal assemblages (Hatcher 1988). A proportion of their photosynthate is exuded as dissolved organic matter (DOM; Crossland 1987; Ferrier-Pages et al. 1998; Wild et al. 2010; Haas et al. 2011), exometabolites which are differentially utilized by microorganisms (Haas et al. 2011, 2013; Nelson et al. 2013) or by other reef metazoans and their microbial symbionts (Stephens 1960; de Goeij et al. 2008). The quantity and composition of these DOM exudates may be a key indicator of benthic organismal metabolism and/or health. Exudate composition influences the structure of the microbial communities that drive the microbial loop, including both bacterioplankton (Nelson et al. 2013; Haas et al. 2016) and biofilms associated with reef organisms and surfaces (Ritchie and Smith 1995; Lee et al. 2016). Coral- and algal-associated microbial communities directly influence competition between these benthic holobionts (Barott and Rohwer 2012), with evidence that labile carbon such as algal exudates enriches virulence factors in microbial communities (Nelson et al. 2013; Sweet et al. 2013; Cárdenas et al. 2017). Determining how exudate DOM production and composition vary among benthic taxa is fundamental to understanding coral reef ecology.

The impact of anthropogenic disturbances on DOM production and composition is also poorly understood and may be globally relevant (Hughes et al. 2017). The presence and density of human populations whose activities impact reef ecosystems influences coral reef benthic composition and food web structure (Jackson et al. 2001; Sandin et al. 2008; Smith et al. 2016). One well-documented impact to reef benthic community structure and function is anthropogenic nutrient inputs, including groundwater contamination, wastewater discharge, terrigenous fertilizer and sediment runoff (Smith et al. 1981; Shahidul Islam and Tanaka 2004; Wear and Vega Thurber 2015). Nutrients have been shown to differentially stimulate algal production (Lapointe 1997; Littler et al. 2006), but how nutrients impact DOM exudation by benthic primary producers in reef ecosystems is poorly understood. Because both the quantity and composition of exudates may be altered by nutrient enrichment, examining how different benthic producers alter exudate composition in response to nutrients may provide an indicator of anthropogenic nutrient enrichment of reefs, which can be difficult to measure directly.

DOM in natural aquatic ecosystems is an exceedingly complex mixture of compounds dictated by diverse sources and subsequent abiotic and microbial alterations (Benner 2002; Moran et al. 2016). Methods of querying the composition of DOM vary widely (Repeta 2015), from relatively coarse bulk characterization of elemental content (C, N, P, etc.) to more fine-scale multivariate methods that analyze a subset of extracted compounds, ranging from chromatographic analysis of sugars (Goldberg et al. 2009; Nelson et al. 2013) or amino acids (Yamashita and Tanoue 2003), to high resolution mass spectrometry (Kido Soule et al. 2015; Petras et al. 2017). A subset of light-absorbing DOM is fluorescent (fDOM), allowing multivariate spectral characterization of putative humic-like, fulvic-like and aromatic proteinaceous compounds (Coble 2014).

In this study, we coupled scanning fluorescence and absorbance spectroscopy with bulk DOM measurements to characterize the magnitude and composition of exudates in >200 water samples from aquaria containing one of four dominant coral reef benthic primary producer constituents (coral, macroalgae, sand, and rubble) factorially treated with three different inorganic nutrient treatments (ambient, low, and high) over 4 weeks. We hypothesized that DOM composition would differ consistently among benthic producer types. Because reef organisms release a relatively stable and organism-specific proportion of their primary production as DOM (Haas et al. 2011), we further hypothesized that nutrient enrichment would stimulate exudate production and speculated on the potential to alter exudate composition. Our results demonstrate that corals continuously release DOM with strong fluorescence from aromatic amino acid-like compounds that accumulates faster than algal DOM, suggesting it may be more refractory. These compounds are clearly distinguishable from humic-like fDOM exudates released by algal benthic reef constituents which do not accumulate as rapidly. Finally, nutrient enrichment significantly enhanced exudation of DOM by all benthic constituents without modifying fDOM composition. Together these results indicate that changing benthic composition of reef ecosystems will have a fundamental impact on the composition of DOM and the subsequent metabolism of organic matter by reef microbial communities.

**Methods**

**Collection of major reef constituents**

Three visibly healthy colonies each of *Porites compressa* and *Montipora capitata*, two locally abundant hermatypic
corals, were collected between 4 m and 7 m depth from the fringing reef immediately adjacent to the Hawai‘i Institute of Marine Biology in Kāne‘ohe Bay, Hawai‘i (HIMB; 21.435°, −157.787°) between 12 October 2015 and 16 October 2015. Each colony was fragmented into 36 nubbins and one nubbin from each colony was mounted onto each of 36 polystyrene frames (roughly 10 cm²) using epoxy putty. Each frame had six nubbins (three Porites, three Montipora); one nubbin from each colony per frame; 24.8 ± 5.23 g dry weight P. compressa, 21.9 ± 5.05 g dry weight of M. capitata. Corals were allowed to acclimate 10 d before the start of the experiment. Skeletal rubble from P. compressa was collected haphazardly in conjunction with the coral collections, separated into 36 equal portions (78.9 ± 3.42 g dry weight) and contained within polyethylene mesh netting containers. Sand was collected from the top 3 cm of aerobic reef sand using a 7.5 cm diameter core and was left undisturbed in each of the 36 petri dishes in which it was collected. The macroalga Grateloupia sp. (Rhodophyta) was collected from the north point of HIMB (21.436°, −157.788°); any visible invertebrates and epiphytes within the macroalgae were removed, fronds were separated into 36 equal portions (11.0 ± 0.55 g wet weight) and contained within polyethylene mesh netting mesh containers.

Aquaria and nutrient enrichment systems

Square polycarbonate aquaria (n = 36), affixed with an upper drain to maintain 6 L, were acid washed and soaked for 72 h in flowing sea water to leach plasticizers prior to the experiment, scrubbed clean, rinsed with freshwater and dried. Each aquarium was filled with four benthic constituent units (either four coral frames, four algal or rubble mesh portion containers or four sand dishes) and placed into one of three 1300 L flow-through seawater tanks (12 aquaria per tank) as water baths to maintain stable temperature. Each tank thus contained one replicate aquarium of each benthic group maintained at each nutrient level (Fig. 1). Source water from Kāne‘ohe Bay was filtered through a sand filter followed by a 20 μm polyethylene cartridge pre-filter to exclude large plankton. A concentrated nutrient mix (2 mmol L⁻³ sodium nitrate and 0.67 mmol L⁻³ monosodium phosphate, 20 L) was prepared every other day by amending seawater with a frozen concentrated stock in a pre-cleaned polycarbonate carboy stored at ambient temperatures in the dark. Both the source water and nutrient mixture were pumped by continuous peristalsis through platinum cured silicone tubes into nutrient mixing aquaria with 90 min residence time maintained at three concentrations (ambient, low and high; mean and time series concentrations in Fig. 1, Supporting Information Fig. S2, respectively) then distributed by peristalsis to the experimental aquaria maintained at a 5-h residence time. Each week all aquaria were replaced with cleaned and dried aquaria and randomly rearranged spatially within incubation tanks, but maintained in three replicate experimental blocks cycled among 1300 L tanks to account for light and temperature variation; means of 288 ± 354 μmol photon m⁻² s⁻¹ and 25.9 ± 1.9°C did not differ significantly among water baths and are detailed in a companion manuscript (Silbiger et al., unpubl.).

DOM sample collection and analysis

DOM samples were collected biweekly over a period of 4 weeks from each aquaria using acid washed and seawater leached treatment-specific, rubber free polyethylene syringes and filtered through a 0.2 μm polyethersulfone filter (25 mm; Sterlitech) in a polypropylene filter holder (Swin-lok; Whatman). Filtrate was collected in acid washed, combusted, triple sample-rinsed amber borosilicate vials with teflon septa lids and stored dark at 4°C until analysis within 1 month of collection. Dissolved organic carbon (DOC) was measured by high temperature combustion using a Shimadzu TOC-V (Carlson et al. 2010). Nutrient samples were collected identically, but frozen (−20°C) in polyethylene centrifuge tubes, thawed to room temperature, mixed thoroughly and analyzed on a Seal Analytical Segmented Flow Injection AutoAnalyzer AA3HR for simultaneous determination of soluble reactive PO₄³⁻, NH₄⁺, NO₃⁻, NO₂⁻, H₂SiO₄ and total dissolved nitrogen and phosphorus (TDN, TDP; via in-line persulfate/ultraviolet oxidation). Dissolved organic nitrogen (DON) was calculated as the difference between TDN and the sum of NH₄⁺ + NO₃⁻ + NO₂⁻. Samples for fluorescence spectroscopy were measured using an Horiba Aqualog scanning fluorometer following the methods of Nelson et al. (2015) and processed using a MATLAB (v2007b) script (https://github.com/zquinlan/IDOMmatlab/ script.md). Six PARAFAC components were validated using split half validation and outlier analysis (Supporting Information Fig. S1). All PARAFAC components had similar excitation-emission maxima and strong covariation among samples with previously identified fluorophores; thus for subsequent analyses we examined established fluorescence maxima from the literature (Supporting Information Table S1; Coble 1996; Stedmon et al. 2003; Lakowicz 2010).

Statistical analysis

For statistical analysis, concentrations of fDOM peak Raman Units were log₁₀-transformed to approximate a Gaussian distribution. For clarity, all statistical comparison of DOM measurements was done at a single timepoint midway through the experiment at 2 weeks continuous enrichment except time series analyses. Analysis of covariance (ANCOVA) compared the means of DOM components among four benthic primary producer treatments across a continuous gradient of measured nutrient inputs and their interactions, using incubation tank blocks as a random effect to control for experimental blocking. One-way ANOVA was used to assess differences among benthic constituents within each nutrient level or vice versa; Dunnett’s post hoc tests were used to assess significance differences from the influent seawater at α = 0.05. Effect of nutrient enrichment on each DOM component was assessed with least squares linear regression analysis using measured
input TDP as a continuous metric of practical nutrient enrichment. For multiple univariate testing of different DOM components we controlled the false discovery rate (FDR) by adjusting p-values (Benjamini and Hochberg 1995). For multivariate analysis and visualization, we used Wards’ minimum variance hierarchical clustering and principal components analysis (PCA) with input data standardized to units of standard deviation using z-scores ((x-x)*/SD*(x)^-1).

**Results**

**Combined effects of nutrients and reef constituents on DOM components**

At 2 weeks both reef constituent and nutrient addition significantly affected bulk DOC and all fDOM components (FDR p<0.05; except tyrosine-like components which were not affected by nutrients p_nutrient = 0.4668, Supporting Information Table S2). DOC and DON in the influent seawater averaged ~ 70 μmol L^-1 and ~ 6 μmol L^-1, respectively; DOC, DON and each of the six fDOM components did not differ among nutrient level mixing tanks in the influent seawater (ANOVA p > 0.05). In the coral aquaria, DOC was significantly elevated above influent seawater (Fig. 2a; Dunnett’s p = 0.0002). Among algae, sand and rubble aquaria DOC and DON did not differ among benthic constituents within each nutrient level (ANOVA p > 0.05); nutrients generally increased both DOC and DON, though this effect was only significantly different from ambient in sand (both Low and High) and algae treatments (High only; Dunnett’s p < 0.05, Fig. 2a).
ratio DOC : DON did not differ among benthic constituents at 2 weeks but showed a significant decreasing trend with nutrient enrichment ($p = 0.0006$). Finally, no interaction effects between nutrient enrichment and benthic constituent treatments were found ($p_{\text{nutrient} \times p_{\text{constituent}}}$ FDR $p > 0.05$). Taken together, these results show that while constituents were compositionally distinct in exudation of various DOM components, and nutrients increased DOM exudation, there was no evidence that nutrients would change differential exudation of any given component of the DOM pool, implying that nutrients alter the quantity but not the composition of exudates relative to other benthic constituents. Similar patterns in all parameters were found as early as 2 d of incubation, but treatment differences among aquaria and observable concentrations declined in further sample weeks and were not analyzed further except to examine temporal responses within treatments (see below). Coral and macroalgae both maintained a positive growth rate, measured via change in buoyant weight, throughout the 6-week incubation period in all treatments (Silbiger et al., unpubl.).

**Effects of benthic producers on fDOM concentrations**

Coral exuded fDOM components associated with aromatic amino acids (tyrosine-like, tryptophan-like and phenylalanine-like, henceforth proteinaceous fDOM) at concentrations above the influent seawater both at ambient nutrient levels and with nutrient enrichment (Fig. 2b, Dunnett’s FDR $p < 0.05$). However, none of the other major benthic constituents exuded proteinaceous fDOM above the influent seawater at any nutrient level (Fig. 2b, Dunnett’s FDR $p > 0.05$), suggesting that proteinaceous fDOM exudates may be primarily associated with corals or other reef animals. Robust increases in humic-like fDOM production with nutrient enrichment were observed across different reef constituents (Fig. 2c). Rubble, sand, and coral each increased production of humic-like fDOM in response to nutrients (regression $p < 0.01$; Supporting Information Fig. S3) but there was no nutrient effect on algal exudation of fDOM (regression $p > 0.05$; Supporting Information Fig. S3). Both coral and sand also increased production of some proteinaceous fDOM components as nutrients were increased ($p < 0.05$, Supporting Information Fig. S3). Over the 4 weeks of sampling there was a significant decrease in the proteinaceous fDOM measured from the coral aquaria at all nutrient levels (Fig. 3), but no other temporal effects were detected.

**Multivariate analysis of fDOM composition**

Samples collected at 2 weeks of incubation were hierarchically clustered according to standardized concentrations of six fDOM components and grouped consistently into three main categories of exudation (Fig. 4). Input seawater along with ambient levels of nutrients in rubble, macroalgae and sand aquaria all clustered similarly to each other and were interpreted as background fDOM levels. At elevated levels of nutrients, both the rubble and macroalgae segregated into a new cluster defined by elevated levels of humic-like fDOM components. Conversely, coral samples were segregated from input and other organisms at all levels of nutrient addition, exhibiting elevated concentrations of proteinaceous fDOM signatures. Ordination by PCA further clarified that within clusters of elevated humic exudation and elevated proteinaceous exudation there was a compositional shift in fDOM with nutrient enrichment (Fig. 5).

**Discussion**

Our results demonstrate that dominant benthic producer constituents on coral reefs release DOM (Fig. 2), that exudation generally increases with modest stable nutrient enrichment (Figs. 2, 5, Supporting Information Fig. S3), and that corals release fDOM that is distinct from other benthic reef constituents (Figs. 2, 4, 5). Coral-derived fDOM was dominated by aromatic amino acid-like material and coral was the only reef constituent to significantly enrich water column proteinaceous fDOM, suggesting proteinaceous fDOM in reefs may be predominantly coral-derived. Monitoring fDOM characteristics on reefs may be useful in assessing reef community composition and/or nutrient pollution, though further physiological analysis of this phenomenon would be
Preliminary field sampling of DOM near corals indicates elevated proteinaceous fDOM immediately adjacent to corals (Supporting Information Fig. S4), suggesting future work on DOM plumes around corals is warranted (Walsh et al. 2017).

Our observation that coral exudate accumulation outpaces consumption, as evidenced by consistently elevated bulk DOC across nutrient treatments (Fig. 2a), is consistent with previous observations of reduced lability of coral exudates relative to those of algae (Nelson et al. 2013). The fact that these coral exudates were markedly enriched in fDOM components associated with aromatic amino acids indicates that at least a portion of the accumulated carbon contains nitrogenous compounds. This result agrees with prior reports of elevated tryptophan-like exudates on coral reef environments (Matthews et al. 1996); however, our findings link detection of these compounds in the marine environment with direct production of tryptophan-like, tyrosine-like, and phenylalanine-like specifically by corals. Exudation of relatively recalcitrant proteinaceous fDOM may fuel coral-associated biofilm communities with reduced nitrogen compounds; if biofilms are able to utilize these compounds more efficiently than planktonic communities this may be advantageous for corals. Freshwater biofilms have been found to utilize humic DON in carbon limited conditions while preferentially taking up inorganic nitrogen when carbon limitation is alleviated (Ghosh and Leff 2013); were such a process occurring here both the presence of labile algal exudates and/or nitrogen pollution would destabilize this interaction, with implications for coral-algal competition on degraded reefs.

Corals, rubble, and sand variously increased fDOM exudation with ecologically relevant levels of nutrient enrichment, demonstrating that nutrients broadly enhance DOM exudation. Nutrients can stimulate both coral and algal primary production (Ferrier-Pagès et al. 2000; D’Angelo and Wiedenmann 2014); given our previous observations that many reef organisms, including the CCA and turfing algae dominant on rubble, exude a consistent proportion of their photosynthesis as DOM (Haas et al 2011) stimulation of primary productivity by nutrients is likely to increase exudation.

Fig. 3. Change in fDOM parameters over the 4 weeks duration of the experiment. Regression models with solid lines and shaded ranges are significant at FDR-adjusted $p < 0.05$; non-significant regressions are shown as dashed lines. Note that in general there was no temporal change in fDOM except in coral aquaria where proteinaceous fDOM declined 0.5–1.0 orders of magnitude over time in all three nutrient treatments.
However, we found no evidence that increased production stimulated by nutrient enrichment would alter the composition of exudates, suggesting that compositional differences in exudates among corals and algae have a complex physiological basis uncoupled from raw photosynthate release. Further investigation of the effects of nutrient enrichment on the physiology of corals and algae in the context of exudation, as well as differences in microbial utilization of this exuded DOM under nutrient enrichment may further illuminate this dynamic.

The steady and significant decrease in observed proteinaceous fDOM over the month-long experiment in the coral aquaria (Fig. 3) indicates either a decline in fDOM production by coral or a change in rates of microbial consumption or transformation of aromatic amino acid-like fDOM components. Bacterioplankton or coral microbial symbiont community acclimatization to enhanced metabolism of proteinaceous exudates over the course of the experiment is one mechanism to explain this pattern, and analysis of compositional shifts in bacterioplankton communities may shed
light on the role of microbial remineralization processes in driving this steady decline in stocks of proteinaceous fDOM in the coral aquaria. Further studies to directly measure microbial consumption of proteinaceous fDOM and subsequent growth patterns will shed light on the implications of this pool of coral-derived DOM on reef community metabolism. Macroalgal fDOM release was highly variable and not significantly related to nutrients or consistently different from the influent seawater, potentially because the DOM produced by macroalgae did not have major fluorescent components and/or because exudates produced were more rapidly metabolized than coral exudates as observed in other studies (Haas et al. 2011; Nelson et al. 2013).

In conclusion, the stable and compositionally distinct exudation of proteinaceous fDOM by corals is an intriguing observation with potential to illuminate coral physiology and inform our understanding of how corals modulate the metabolism of microbial associates and the microbial metabolism of the larger reef ecosystem. The observation that other primary producers exude measurable quantities of humic-like fDOM and that both corals and algae increase exudation in response to nutrient enrichment suggests that DOM quantity and composition are sensitive to changing benthic community composition as well as nutrient enrichment, two of the central management issues in coral reefs today. Further investigation of the physiological basis for compositional differences in exudates and the microbial responses to exudates are warranted and are likely to better understand biogeochemical cycling on coral reefs worldwide.

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