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

Author names and affiliations:

Zachary A. Quinlan[1], Kristina Remple[1], Michael D. Fox[2], Nyssa J. Silbiger[3], Thomas A. Oliver[4], Hollie M. Putnam[5], Linda Wegley Kelly[6], Craig A. Carlson[7], Megan J. Donahue[8] and Craig E. Nelson[1]*

[1] Center for Microbial Oceanography: Research and Education, Department of Oceanography and Sea Grant College Program, University of Hawai‘i at Mānoa, Honolulu, HI 96822, USA
[2] Scripps Institution of Oceanography, University of California, San Diego, CA 92093, USA
[3] Department of Biology, California State University, Northridge, CA 91330, USA
[4] National Oceanic and Atmospheric Administration, National Marine Fisheries Service Pacific Islands Fisheries Science Center, Ecosystem Sciences Division, Honolulu, HI 96818, USA
[5] Department of Biological Sciences, University of Rhode Island, Kingston, RI 02881, USA
[6] Department of Biology, San Diego State University, San Diego, CA 92182, USA
[7] Department of Ecology, Evolution and Marine Biology, University of California, Santa Barbara, CA 93016, USA
[8] Hawai‘i Institute of Marine Biology, Kāne'ohe, HI 96744, USA
* Correspondence should be addressed to craig.nelson@hawaii.edu.

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.

Scientific significance statement (125 words):
Corals and algae support reef food webs and release a significant proportion of their primary production as dissolved compounds metabolized by planktonic and benthic microbes. Little is known about the composition of these compounds, how they may differ among producers or how nutrient availability may alter their production. This experiment contrasted the composition of fluorescent organics exuded by four coral reef benthic constitutents and measured the effects of nutrient enrichment on exudate release. While all producers released significant amounts of bulk and fluorescent organics, and nutrients stimulated exudation, coral exudates were distinctly enriched in aromatic amino acid-like compounds and accumulated faster. These findings clarify a mechanism whereby anthropogenic activities that alter benthic cover and nutrient pollution on reefs will influence microbial organic matter processing.

Data availability statement:
All data and metadata from this experiment will be made publicly available before publication by the US National Science Foundation Biological and Chemical Oceanography Data Management Office (BCO-DMO) Project 675025 (https://www.bco-dmo.org/project/675025).

Abstract (150 words):
Dissolved organic matter (DOM) composition is a key determinant of microbial community metabolism and trophic nutrient transfer. 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 DOM. We conducted a mesocosm experiment to characterize and contrast DOM exudates from these four organismal groups under three levels of continuous inorganic nutrient enrichment. We measured bulk dissolved organic carbon and nitrogen and the multivariate spectral characteristics of fluorescent DOM (fDOM). Moderate nutrient enrichment enhanced DOM exudation by all producers. Corals exuded rapidly accumulating DOM with a markedly high concentration of aromatic amino acid-like fDOM components that clearly distinguishes them from algal exudates, which were dominated by humic-like fDOM components and did not accumulate significantly. Our results emphasize the differences between coral and algae in their potential to influence microbial communities and metabolism in reefs.

Keywords (3-6):
dissolved organic matter, fluorescence spectroscopy, nutrient enrichment, coral, macroalgae, reef

1. Introduction

Coral reefs are highly productive ecosystems that thrive in relatively 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), that cover dead coral pavement and rubble. A proportion of the primary production generated by these photosynthetic organisms is exuded into the water column 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 utilized directly by other sessile metazoans on the reef or 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. The composition of exudates released by different primary producers on reefs is a key determinant of the structure of the microbial communities that drive the microbial loop, including both bacterioplankton (Nelson et al. 2013; Haas et al. 2016) and and likely the diverse and abundant microbial consortia that associate with reef organisms and surfaces (Ritchie and Smith 1995; Lee et al. 2016). The metabolic activity of coral and algal-associated microbial communities directly influences competition between these benthic holobionts (Barott and Rohwer 2012). Therefore, determining how exudate DOM production and composition varies among different benthic taxa is fundamental to understanding coral reef ecology.

In addition to the need for characterizing DOM across different reef constituents, the impact of anthropogenic disturbances on DOM production and composition is also poorly understood and may be globally relevant (Hughes et al. 2017). One of the primary determinants of coral reef benthic composition and food web structure is the presence and density of human populations whose activities impact reef ecosystems (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.

Dissolved organic matter 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 (Petras et al.; Kido Soule et al. 2015). One widely used method of DOM characterization is spectral analysis of

a subset of light-absorbing DOM that is fluorescent (fDOM), allowing quantitative measurement of a suite 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 exudates in >200 water samples from aquaria containing coral, algae, rubble and sand over one month.

The present study compared the magnitude and composition of exudates from four dominant coral reef benthic primary producer constituents (coral, macroalgae, sand and rubble) factorially under three different nutrient treatments (ambient, low, and high) over four weeks. We hypothesized that bulk DOM and fDOM composition would differ consistently among benthic producer types and that nutrient enrichment would both stimulate the production of DOM exudates and alter their 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 in all constituents without modifying fDOM composition. Together these results indicate that the benthic composition of reef ecosystems will have a fundamental impact on the composition of DOM and the subsequent metabolism of organic matter by bacterioplankton.

2. Methods

2.1 Collection of major reef constituents

Three visibly healthy colonies each of *Porites compressa* and *Montipora capitata*, two

69 locally abundant hermatypic corals, were collected between 4 and 7 meters depth from fringing
70 reef immediately adjacent to the Hawai‘i Institute of Marine Biology in Kāne‘ohe Bay, Hawai‘i
71 (HIMB; 21.4326 ˚, -157.7866˚). Each colony was fragmented into 12 nubbins, mounted onto one
72 of thirty-six separate polystyrene frames (roughly 10 cm^2) using epoxy putty (one nubbin from
73 each colony per frame; 24.8 ± 5.23 g dry weight *P. compressa*, 21.9 ± 5.05 g dry weight of *M.*
74 *capitata*), and allowed to acclimate 10 days before the start of the experiment. Rubble of dead
75 skeleton from *P. compressa* skeleton was haphazardly collected in conjunction with the coral
76 collections, separated into 36 equal portions (78.9 ± 3.42 g dry weight) and contained within
77 polyethylene mesh netting containers. The macroalga *Gracilaria sp.* (Rhodophyta) was collected
78 from the north point of HIMB (21.4360˚, -157.7881˚); any visible invertebrates and epiphytes
79 within the macroalgae were removed, fronds were separated into 36 equal portions (11.0 ± 0.55 g
80 wet weight) and contained within polyethylene mesh netting mesh containers. Sand was
81 collected from the top 3 cm of aerobic reef sand on the eastern edge of HIMB (21.4350˚, -
82 157.7871˚) using a 7.5 cm diameter core and was left undisturbed in each of the 36 petri dishes
83 in which it was collected.
84
85 2.2 *Aquaria and nutrient enrichment systems*
86 Thirty-six square polycarbonate aquaria were affixed with an upper spigot drain to hold
87 water level constant at 6 L, acid washed and soaked for 72 hours in filtered sea water to leach
88 plasticizers prior to the experiment, scrubbed clean, rinsed with freshwater and dried. Each
89 aquarium was filled and placed into one of three 1300L flow-through seawater tanks (n = 12
90 aquaria per tank) as water baths to maintain stable temperature. Each tank contained one
91 replicate of each benthic group maintained at each nutrient level (Figure 1). Source water from

92 Kāne‘ohe Bay was filtered through a sand filter followed by a 20 µm polyethylene cartridge pre-
93 filter to exclude large plankton. A concentrated nutrient mix (2 mmol L^{-1} sodium nitrate and 0.67
94 mmol L^{-1} monosodium phosphate, 20L) was prepared every other day by amending seawater
95 with a frozen concentrated stock in a pre-cleaned polycarbonate carboy stored at ambient
96 temperatures in the dark. Both the source water and nutrient mixture were pumped by continuous
97 peristalsis through platinum cured silicone tubes into nutrient mixing aquaria with 90 minute
98 residence times maintained at three concentrations (ambient, low and high; mean and time series
99 concentrations in Figure 1 and Figure S2, respectively) then distributed by peristalsis to the
100 experimental aquaria maintained at a 5-hour residence time. Each week all aquaria were replaced
101 with cleaned and dried aquaria and randomly rearranged spatially within incubation tanks, but
102 maintained in three replicate experimental blocks cycled among 1300 L tanks to account for light
103 and temperature variation (means of 288 ± 354 µmol photon m^{-2} s^{-1} and 25.9 ±1.9 ˚C did not
104 differ significantly among water baths and are detailed in parallel manuscripts).
105
106 2.3 *Dissolved Organic Matter (DOM) sample collection and analysis*
107 DOM samples were collected biweekly from each aquaria using acid washed and
108 seawater leached treatment-specific, rubber free polyethylene syringes and filtered through a 0.2
109 µm polyethersulfone filter (25 mm; Sterlitech) in a polypropylene filter holder (Swin-lok;
110 Whatman). Filtrate was collected in acid washed, combusted, triple sample-rinsed amber
111 borosilicate vials with teflon septa lids and stored dark at 4˚C until analysis within 1 month of
112 collection. Dissolved organic carbon (DOC) was measured as non-purgeable organic carbon via
113 acidification, sparging and high temperature platinum catalytic oxidation on a Shimadzu TOC-V
114 (Carlson et al. 2010). Nutrient samples were collected identically, but frozen (-20 °C) in

115 polyethylene centrifuge tubes, thawed to room temperature, mixed thoroughly and analyzed on a
116 Seal Analytical Segmented Flow Injection AutoAnalyzer AA3HR for simultaneous
117 determination of soluble reactive phosphate (PO_4^{3-}), ammonium (NH^{4+}), nitrate + nitrite (N + N;
118 NO^{3-} + NO^{2-}), silicate (SiO_4) and total dissolved nitrogen and phosphorus (TDN, TDP; via in-
119 line persulfate/ultraviolet oxidation). Dissolved organic nitrogen (DON) was calculated as the
120 difference between TDN and the sum of ammonium, nitrate and nitrite.
121 Samples for fluorescence spectroscopy were measured using an Horiba Aqualog scanning
122 fluorometer following the methods of Nelson et al. (2015) and processed using a Matlab
123 (v2007b) script (available at https://github.com/zquinlan/fDOMmatlab/script.md). Six
124 PARAFAC components were validated using split half validation and outlier analysis (Figure
125 S1). All PARAFAC components had similar excitation-emission maxima and strong covariation
126 among samples with previously identified fluorophores; thus for subsequent analyses we
127 examined established fluorescence maxima from the literature (Table S1; Coble 1996; Stedmon
128 et al. 2003; Lakowicz 2010).
129
130 *2.4 Statistical Analysis*
131 For statistical analysis, concentrations or raw peak Raman units were log_{10}-transformed
132 to approximate a Gaussian distribution. For clarity, all statistical comparison of DOM
133 measurements were done at a single timepoint midway through the experiment at 2 weeks
134 continuous enrichment except time series analyses. Analysis of covariance (ANCOVA)
135 compared the means of DOM components among four benthic primary producer treatments
136 across a continuous gradient of measured nutrient inputs and their interactions, using incubation
137 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 $\alpha = 0.05$. 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 $((x-\bar{x})*\text{stdev}(x)^{-1})$.

3. Results

3.1 Combined effects of nutrients and reef constituents on DOM components

At two weeks, there was a significant effect of both reef constituent and nutrient addition on bulk dissolved organic carbon (DOC) and all fDOM components (FDR $p < 0.05$), except tyrosine-like components which were not affected by nutrients ($p_{nutrient} = 0.4668$, Table S2). No interaction effects were found to be significant ($p_{nutrient} \times p_{constituent}$ FDR $p > 0.05$), suggesting that nutrients alter the quantity but not the composition of DOM released. Concentrations of bulk DOC and DON in the influent seawater averaged ~ 70 and ~ 6 µmol L^{-1}, respectively and 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 (Figure 2a; Dunnet'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

161 significantly different from ambient in sand (both Low and High) and algae treatments (High
162 only; Dunnett's $p < 0.05$, Figure 2a). The ratio DOC:DON did not differ among benthic
163 constituents at 2 weeks but showed a significant decreasing trend with nutrient enrichment ($p =$
164 0.0006). Similar patterns in all parameters were found as early as 2 days of incubation, but
165 treatment differences among aquaria and observable concentrations declined in further sample
166 weeks and were not analyzed further except to examine temporal responses within treatments
167 (see below).
168
169 *3.2 Effects of benthic producers on fDOM concentrations*
170 Coral exuded fDOM components associated with aromatic amino acids (tyrosine-like,
171 tryptophan-like and phenylalanine-like, henceforth proteinaceous fDOM) at concentrations
172 above the influent seawater both at ambient nutrient levels and with nutrient enrichment (Figure
173 2b, Dunnett's FDR $p < 0.05$). However, none of the other major benthic constituents exuded
174 proteinaceous fDOM above the influent seawater at any nutrient level (Figure 2b, Dunnett's FDR
175 $p > 0.05$), indicating that these exudates are unique to corals. Robust gradients in humic-like
176 fDOM production with nutrient enrichment were observed across different reef constituents
177 (Figure 2c). Rubble, sand and coral each increased production of humic-like fDOM in response
178 to nutrients ($p < 0.01$; Figure S3) but there was no nutrient effect on algal exudation of fDOM
179 (regression $p > 0.05$). Both coral and sand had significantly increased production of humic-like
180 and a subset of proteinaceous fDOM components as nutrients were increased ($p < 0.05$, Figure
181 S3). Over the four weeks of sampling there was a significant decrease in the proteinaceous
182 fDOM measured from the coral aquaria at all nutrient levels (Figure 3), but no other temporal
183 effects were detected.

184

185 *3.3 Multivariate analysis of fDOM composition*

186 Samples collected at 2 weeks of incubation were hierarchically clustered according to
187 standardized concentrations of 6 fDOM components and grouped consistently with the three
188 main categories of exudation (Figure 4). Input seawater along with ambient levels of nutrients in
189 rubble macroalgae and sand aquaria all clustered similarly to each other and were interpreted as
190 background fDOM levels. At elevated levels of nutrients, both the rubble and macroalgae
191 segregated into a new cluster defined by elevated levels of humic-like fDOM components,
192 consistent with univariate statistical analyses. Conversely, coral samples were segregated from
193 input and other organisms at all levels of nutrient addition, exhibiting elevated concentrations of
194 proteinaceous fDOM signatures. Ordination by PCA showed that within clusters of elevated
195 humic exudation and elevated proteinaceous exudation there was a multivariate gradient of
196 fDOM compositional shift corresponding to increased levels of nutrient addition (Figure 5).

197

198 **4. Discussion**

199 Our results demonstrate that dominant benthic producer constituents on coral reefs
200 release DOM (Figure 2), that exudation generally increases with modest stable nutrient
201 enrichment (Figures 2, 5, S3), and that corals release fDOM that is distinct from other benthic
202 reef constituents (Figure 2c, 4, 5). Coral-derived fluorescent exudates were largely composed of
203 aromatic amino acid-like material and coral was the only reef constituent to significantly enrich
204 water column proteinaceous fDOM. This suggests that concentrations of proteinaceous fDOM
205 measured on reef environments which are enriched above water column levels may be
206 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 needed. Preliminary field sampling of DOM near corals indicates elevated proteinaceous fDOM immediately adjacent to corals (Figure 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 (Figure 2a), is consistent with previous observations of reduced lability relative to exudates 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.

Corals increase proteinaceous fDOM exudation with moderate, ecologically relevant levels of nutrient enrichment. Both rubble and sand increase humic-like fDOM release with nutrient enrichment, though much less consistently, demonstrating that nutrients enhance DOM exudation. Nutrients have been observed to stimulate both coral and algal primary production (Ferrier-Pagès et al. 2000; D'Angelo and Wiedenmann 2014), which is likely to increase the rate (or magnitude) of exudation of excess photosynthates into the water column. However, there was 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 (Figure 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. 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 rapidly metabolized 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 health and inform our understanding of how corals modulate the metabolism of microbial associates. 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

253 cycling on coral reefs worldwide.

254

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Figure Legends:

Figure 1. Experimental design. Replicate experimental units each comprising 12 aquaria (6 L flow through with 5-h residence time), were assembled in three 1300 L flowing seawater water bath to maintain thermal stability (mean 25.9 +/-1.9 ˚C). Aquaria contained one of four benthic constituents (coral, rubble, macroalgae, and sand) factorially manipulated with one of three nutrient levels (ambient, low high). Water was distributed into experimental aquaria from 10 L nutrient mixing aquaria (mixing aquaria residence time 90 minutes).

Figure 2. DOM concentrations in aquaria at 2 weeks. Concentrations of (a) DOC, (b) humic-like fDOM and (c) proteinaceous fDOM are shown as means of three replicate aquaria (whiskers are standard error of the mean) by benthic constituent and nutrient level (x-axis). Asterisks denote treatments significantly higher than influent seawater by Dunnet's *post hoc* test ($P<0.05$). Shaded horizontal bars bracket mean and standard error of ambient input seawater for each parameter for background reference. R.U. is raman units of water. Note that DOC and humic-like fDOM generally increased with nutrient additions in three of the four benthic constituent aquaria (See Figure S3) but not in mixing aquaria, and that proteinaceous fDOM was highly enriched and increased with nutrients only in coral aquaria.

Figure 3. Change in fDOM parameters over the four-week 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.

Figure 4. Clustering of fDOM samples at 2 weeks. Each sample is a row and tip on the dendrogram annotated by nutrient level and benthic constituent. Clusters are interpreted on the left for clarity according to trends among samples. Heat map illustrates standardized magnitude of fDOM component fluorescence in units of standard deviation of the mean Raman Units of Water for each component (columns). Dendrogram was generated using Ward's minimum variance method.

Figure 5. Principle Components Analysis (PCA) of fDOM exudate composition among all samples at 2 weeks. Samples are symbol coded according to nutrient level. Shaded circles annotate the clusters defined in Figure 4. Arrows indicate the magnitude of covariation of each of the six fDOM components with PCA axes. Note that while ambient nutrient treatments of macroalgae, rubble and sand cluster with input seawater, after nutrient addition rubble, algae and sand form a new cluster as they exude humic-like fDOM. Coral produces proteinaceous exudates at all levels of nutrients but exhibits a clear trend with increasing nutrients. Component axes are annotated according to the proportion of covariation explained by each. Exudate concentrations

were calculated by subtracting influent seawater concentrations and then $\log_{10}$ transformed before principle component eigenvalues were calculated.

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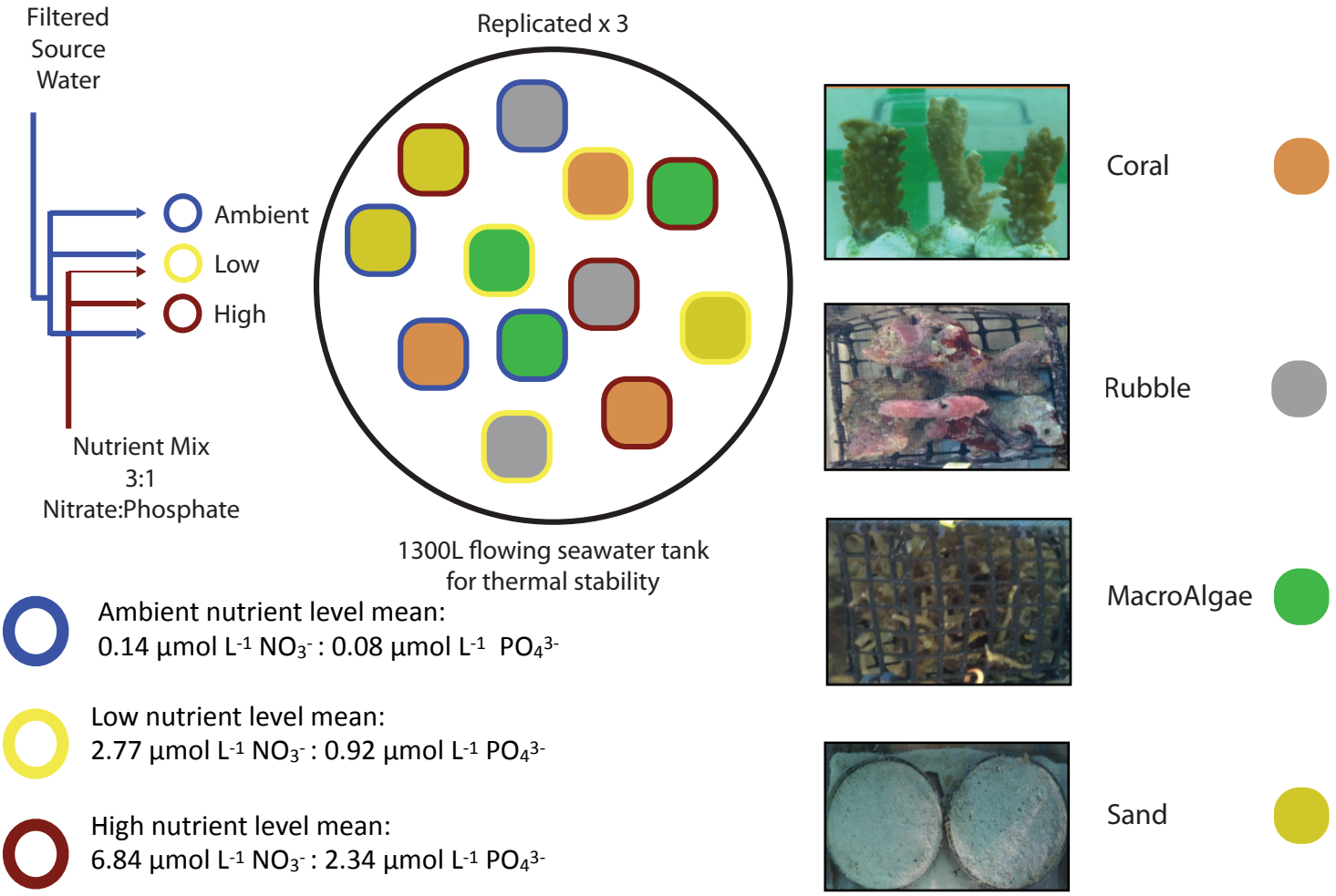

Figure 1. Experimental design. Replicate experimental units each comprising 12 aquaria (6 L flow through with 5-h residence time), were assembled in three 1300 L flowing seawater water bath to maintain thermal stability (mean 25.9 +/-1.9 ˚C). Aquaria contained one of four benthic constituents (coral, rubble, macroalgae, and sand) factorially manipulated with one of three nutrient levels (ambient, low high). Water was distributed into experimental aquaria from 10 L nutrient mixing aquaria (mixing aquaria residence time 90 minutes).

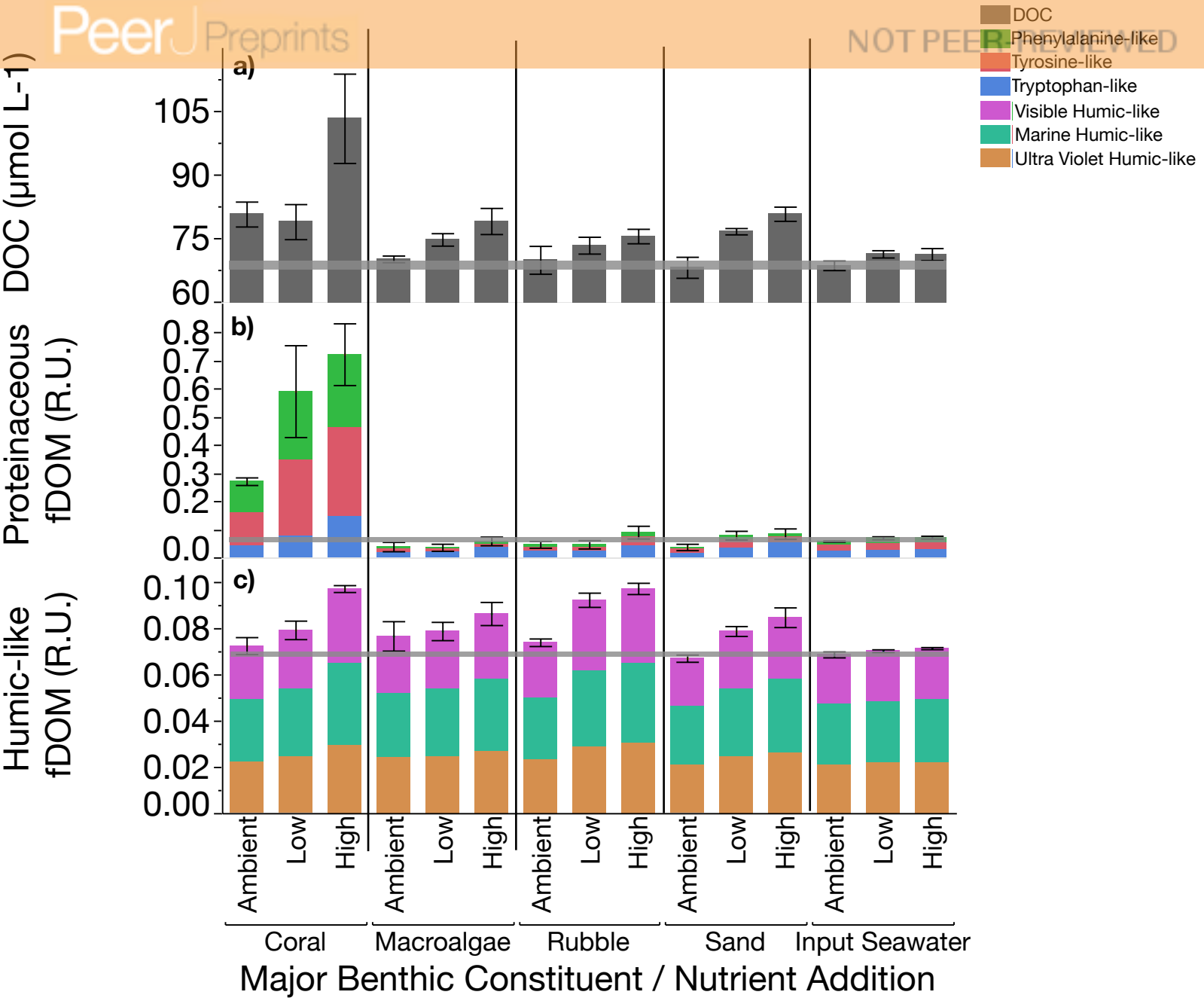

Figure 2. DOM concentrations in aquaria at 2 weeks. Concentrations of (a) DOC, (b) humic-like fDOM and (c) proteinaceous fDOM are shown as means of three replicate aquaria (whiskers are standard error of the mean) by benthic constituent and nutrient level (x-axis). Asterisks denote treatments significantly higher than influent seawater by Dunnet's *post hoc* test ($P<0.05$). Shaded horizontal bars bracket mean and standard error of ambient input seawater for each parameter for background reference. R.U. is raman units of water. Note that DOC and humic-like fDOM generally increased with nutrient additions in three of the four benthic constituent aquaria (See Figure S3) but not in mixing aquaria, and that proteinaceous fDOM was highly enriched and increased with nutrients only in coral aquaria.

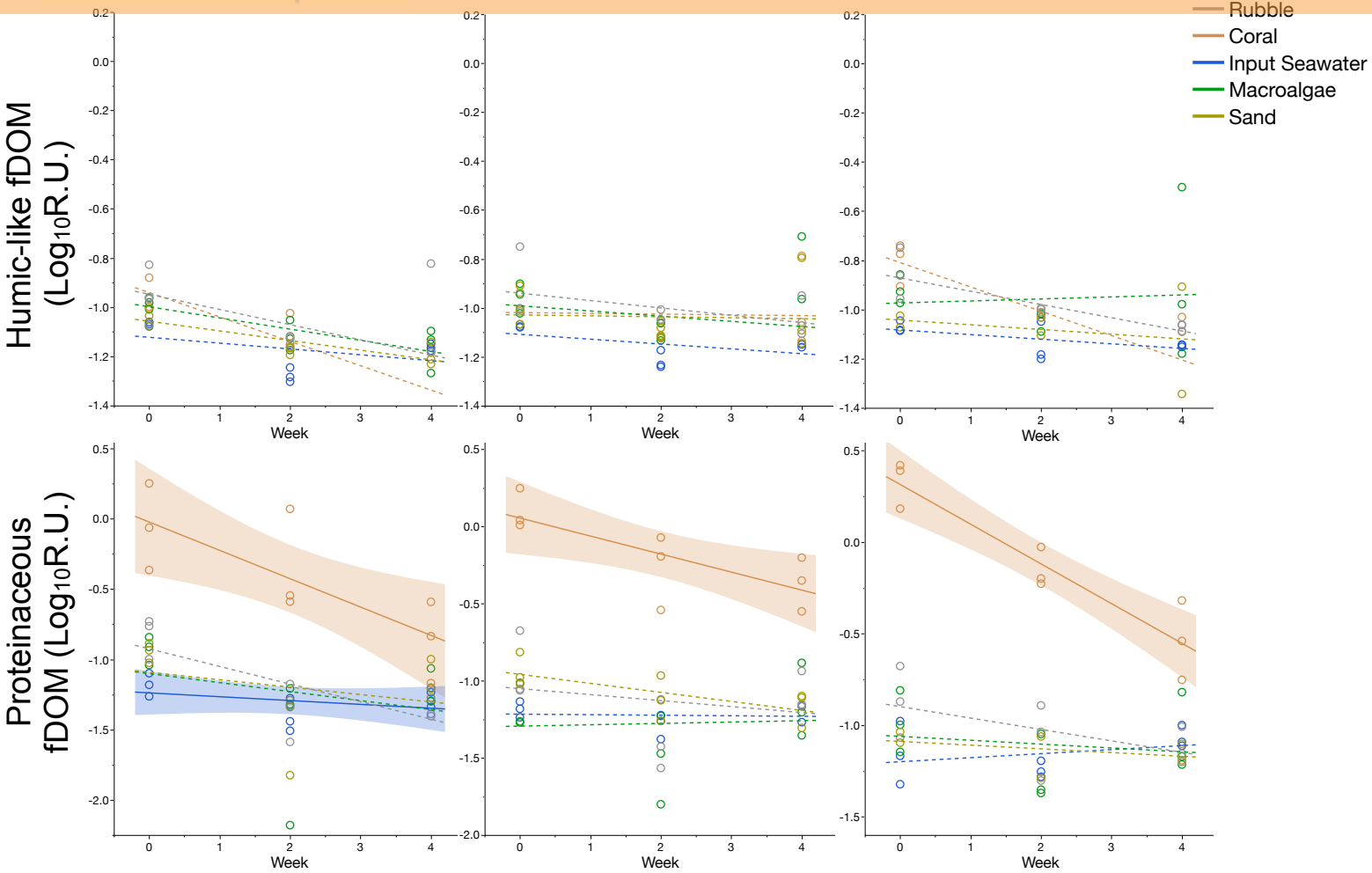

Figure 3. Change in fDOM parameters over the four-week duration of the experiment. Regression models with solid lines and shaded ranges are significant at FDR-adjusted $p < 0.05$; non-significant regressions are shown as dashed lines. Note that in general there was no temporal change in fDOM except in coral aquaria where proteinaceous fDOM declined 0.5-1.0 orders of magnitude over time in all three nutrient treatments.

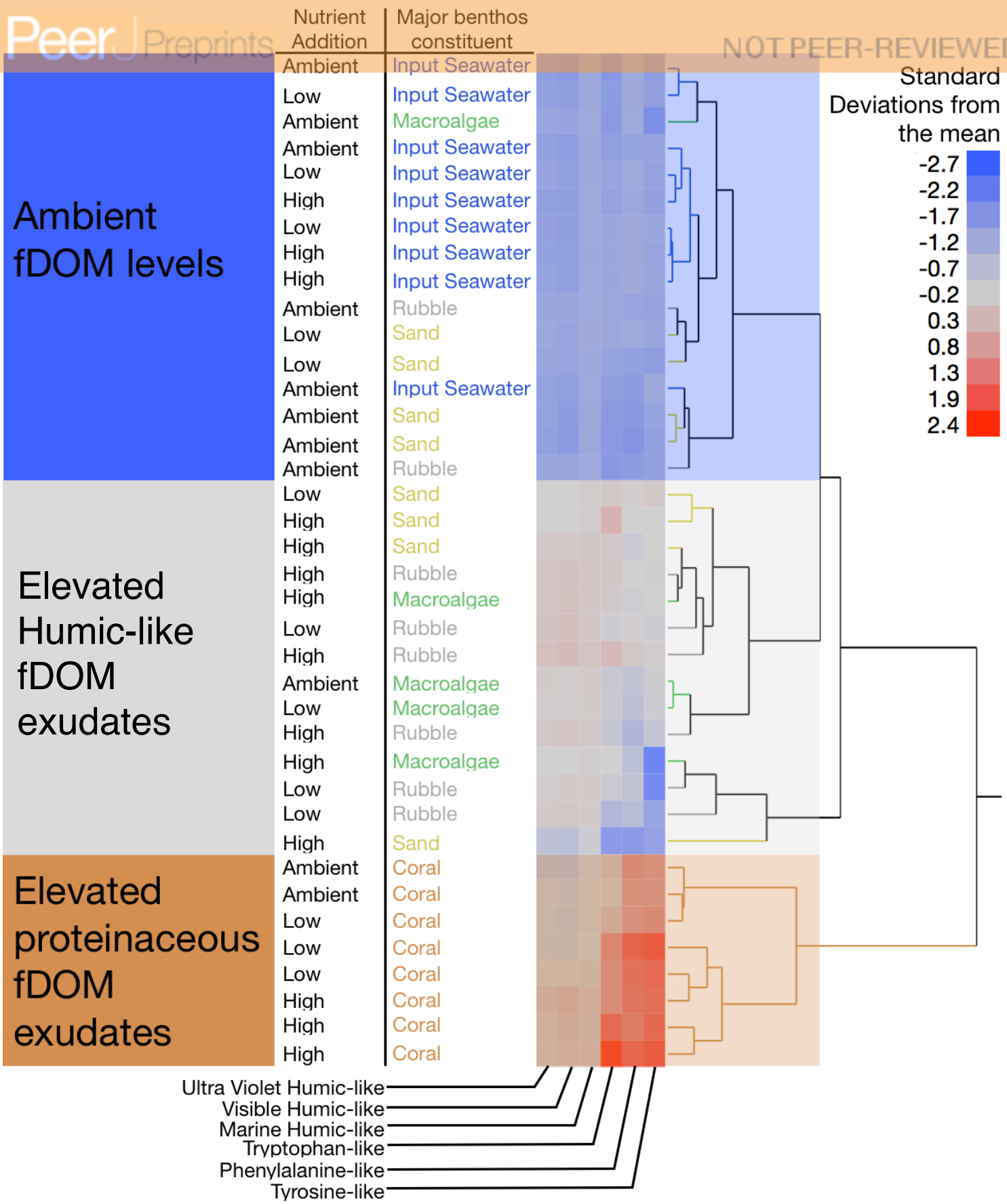

Figure 4. Clustering of fDOM samples at 2 weeks. Each sample is a row and tip on the dendrogram annotated by nutrient level and benthic constituent. Clusters are interpreted on the left for clarity according to trends among samples. Heat map illustrates standardized magnitude of fDOM component fluorescence in units of standard deviation of the mean Raman Units of Water for each component (columns). Dendrogram was generated using Ward's minimum variance method.

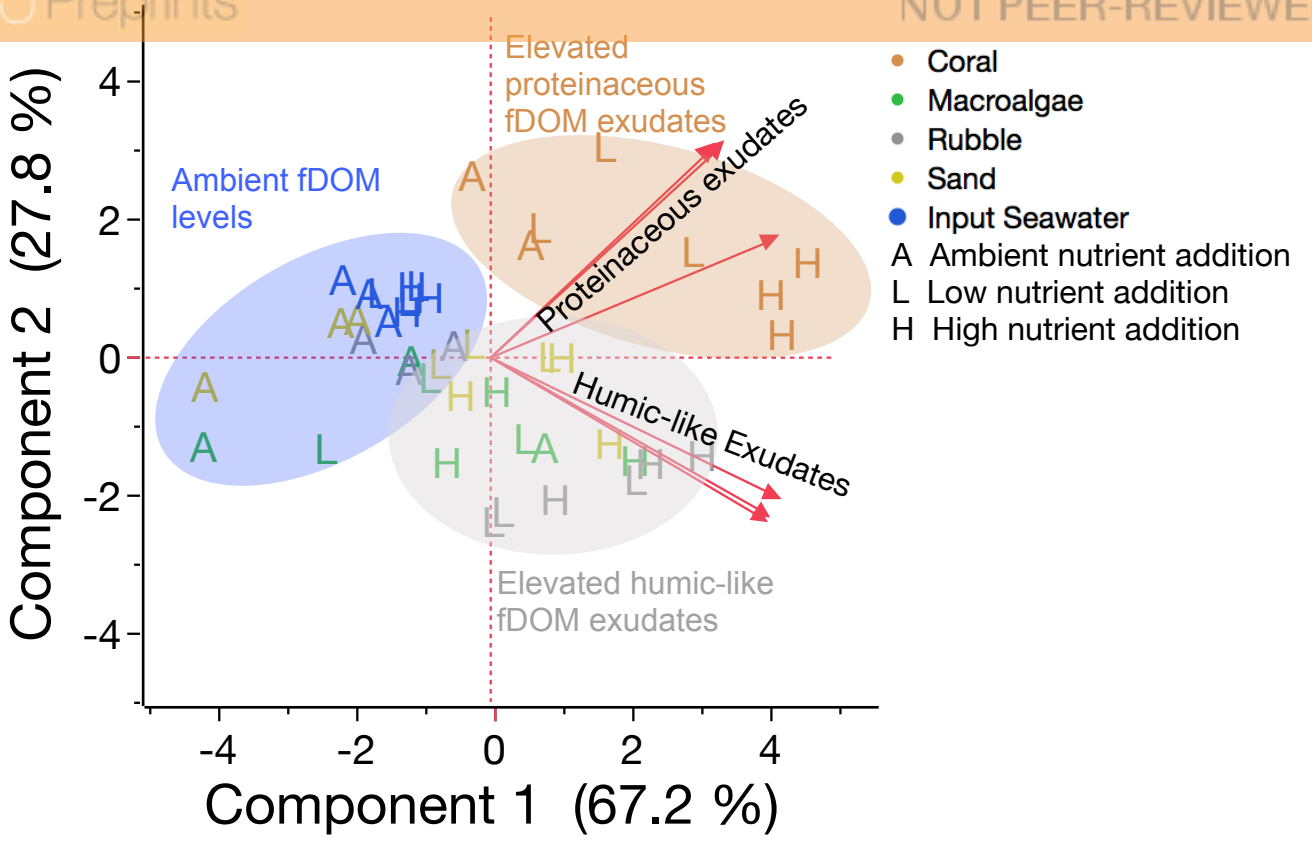

Figure 5. Principle Components Analysis (PCA) of fDOM exudate composition among all samples at 2 weeks. Samples are symbol coded according to nutrient level. Shaded circles annotate the clusters defined in Figure 4. Arrows indicate the magnitude of covariation of each of the six fDOM components with PCA axes. Note that while ambient nutrient treatments of macroalgae, rubble and sand cluster with input seawater, after nutrient addition rubble, algae and sand form a new cluster as they exude humic-like fDOM. Coral produces proteinaceous exudates at all levels of nutrients but exhibits a clear trend with increasing nutrients. Component axes are annotated according to the proportion of covariation explained by each. Exudate concentrations were calculated by subtracting influent seawater concentrations and then $\log_{10}$ transformed before principle component eigenvalues were calculated.