



3 Supplementary Figure 1. Experimental setup.

In order to simulate the effects of overfishing and/or nutrient pollution, $3 \text{ m x } 3 \text{ m } (9 \text{ m}^2)$ 4 experimental plots were created to exclude herbivores nested within control or nutrient-5 enriched areas. **a**, Arrangement of each plot. Each 9 m^2 plot was delineated into 1 m^2 6 plots with metal nails driven into the reef at the corners and center of each plot. Within 7 8 each 9 m² plot, we nested two 1 m² herbivore exclosures and two 1 m² exclosure controls 9 (three-sided, open-topped partial exclosures, to control for experimental artifacts). All 10 exclosures were made of plastic-coated wire mesh with 2.5 cm diameter holes. This mesh 11 diameter excludes all herbivorous fishes >10 cm total length, but allows in smaller 12 juvenile herbivorous fishes. Exclosures and exclosure controls were constructed within each 9 m^2 plot so as to maximize similarity in initial algal abundance, community 13 structure, and rugosity. For the enrichment treatment, Osmocote[®] (19-6-12, N-P-K) slow-14 release garden fertilizer was placed in 15 cm diameter PVC tubes with 10, 1.5 cm holes. 15 These enrichment tubes were attached to each metal nail within the 9 m^2 enrichment plots 16 for a total of 25 enrichment tubes per enrichment plot. Nutrient diffusers were replaced 17 18 every 30-40 days to ensure continued delivery of N and P. b. Arrangement of plots in experiment. Eight of these 9 m² plots were constructed, four enriched with nitrogen and 19 20 phosphorous (N) and four left at ambient levels of nutrient loading to act as control plots

Reef

4N

- 21 (C). These plots were developed and maintained for ~3 years near Pickles Reef (N
- 22 24.99430, W 080.40650). The plot enlarged in panel **a** is shaded in blue in panel **b** in
- 23 order to show its relationship to the rest of the experiment. Plots are not drawn to scale. **c**,
- 24 Map of the experimental site. Pickles Reef is a 5-6 m deep spur and groove reef system
- 25 located just east of Key Largo, Florida, USA, and is representative of coral depauperate
- habitats common in South Florida. Map data are from Google Maps 2012-2014 and
- 27 partner INEGI.
- 28



30 Supplementary Figure 2. Treatments alter algal community composition over time.

a, Algal abundance over time. Relative cover of each algal taxon or functional group

32 surveyed over time (see also Supplementary Data 1a,b). Cover is normalized to 100% for

- 33 visual clarity (see Figure 1a for overall changes in absolute cover). CCA, crustose
- 34 coralline algae. Most algal types responded significantly to exclosure of herbivores or
- nutrient loading (see Supplementary Data 1c for mixed-effects model results). **b**, Algal
- 36 community change. Change in algal communities over time is summarized as a PCoA
- 37 plot of Bray-Curtis divergences between all algal communities exposed to each treatment
- 38 (the first two PCoA axes are plotted against time). Treatment significantly altered overall 39 community composition (PERMANOVA, 1000 iterations, pseudo-F = 97.11, p = 0.001).
- 40 Colors represent treatments; the wireframe box surrounds pretreatment communities.



Supplementary Figure 3. Microbial phyla in the coral surface microbiome are altered by algal competition, temperature extremes, microbial community evenness,

46 and microbial community richness.

43

47 **a-d**, Abundance of microbial phyla. Plots display microbial relative abundance in the 48 coral surface mucus layer as a function of deciles of upright algal cover (macroalgae, turf 49 algae, and cyanobacteria), temperature, quartiles of microbial community richness, or 50 quartiles of microbial community evenness. Minor phyla (<1% average relative 51 abundance) are not shown, and only samples with associated temperature or algal 52 metadata are included (n = 435 samples). Significance was assessed by Spearman 53 correlation and a permutational significance test, and multiple comparisons addressed 54 with an FDR q-value threshold of 0.05 (Supplementary Data 3b). a, Increasing levels of 55 upright algal cover significantly reduced the abundance of Actinobacteria, 56 Cyanobacteria, Bacteroidetes. In contrast, Planctomycetes and Unclassified bacteria 57 increased in abundance as algal cover increased. Elevated temperatures significantly 58 reduced the abundance of Actinobacteria, Unassigned microbes, Bacteroidetes, and 59 *Firmicutes* (Supplementary Table 3c). **b**, In some cases, taxa appeared to respond to 60 temperature extremes rather than temperature *per se*. This was quantified by regressing 61 microbial abundance against the squared deviation of temperature from 28 °C 62 (Supplementary Table 3d). Proteobacteria significantly increased at temperature 63 extremes by this measure, while *Cyanobacteria* decreased. For panels c and d, all phyla 64 with >1% average abundance were tested for differences in abundance in microbial 65 communities with varying evenness and richness. All phyla tested significantly differed 66 in abundance across quartiles of evenness and richness (Kruskal-Wallis test, p < 0.05,

- 67 FDR q < 0.05). *Proteobacteria* accounted for 91.9% of the least even communities (1^{st})
- 68 quartile of evenness). In contrast *Proteobacteria* made up only 44-52% of communities
- 69 in the 2^{nd} , 3^{rd} , and 4^{th} quartiles of evenness, where *Cyanobacteria* were more abundant.
- 70 More taxonomically rich communities had significantly fewer *Cyanobacteria* (the richest
- 71 quartile was 10.7% Cyanobacteria; the least rich 24.0%) and Actinobacteria (2.7% in the
- most rich quartile vs. 4.0% in the least rich quartile) but more *Proteobacteria* (52.7% vs.
- 73 46.4%), *Bacteroidetes* (14.7% vs. 13.1%) and *Planctomyces* (7.6% vs. 1.8%).
- 74



Supplementary Figure 4. *Actinobacteria* and blooms of opportunists in the coral
 mucus microbiome.

78 Each point represents a single 16S rRNA sample from coral mucus. Coordinates plot the

79 abundance of Acidimicrobiales (Actinobacteria) on the x-axis vs. the relative abundance

80 of Alteromonadales, Vibrionales, or Rhodobacterales on the y-axis. Outbreaks of these

81 opportunists to abundances above 25% of the community (horizontal blue gradient) were

82 not observed when *Acidimicrobiales* were present at >2.5% abundance. Reductions of

83 Acidimicrobiales below ~2.5% appear to allow blooms of Vibrionales, Rhodobacterales,

84 and *Alteromonadales* opportunists that rose as high as 80% of total community

85 composition (vertical red gradient). See Supplementary Data 3e for data on

86 environmental conditions favoring dominance by specific groups.





88 Supplementary Figure 5. PICRUSt predicted changes in coral microbiome gene 89 function correlated with increasing upright algal cover or temperature extremes.

90 **a-b**, Predicted microbial functions. Functional profiles (KEGG orthology groups) for

- 91 each sample were imputed based on comparison of microbial community composition
- 92 with sequenced bacteria and archaea in the PICRUSt software package. These profiles
- 93 were summarized into KEGG categories. Selected KEGG categories that showed

- 94 significant positive or negative correlations (Pearson correlation, FDR q < 0.05) with
- 95 increasing upright algal cover (panel **a**) or temperature extremes (panel **b**) are shown.
- 96 Temperature extremes were calculated as the mean squared deviation from 28 $^{\circ}$ C , a non-
- 97 stressful temperature that reflected the mean temperature across samples (this also
- 98 approximated the overall annual average at the site). For the full set of categories
- 99 significantly correlated with upright algal cover or temperature extremes, see
- 100 Supplementary Data 4a,b.
- 101





Supplementary Figure 6. β-diversity of coral microbiomes relative to treatment,
 evenness, algal competition, or thermal stress.

- 105 **a-d,** PCoA plots of coral mucus microbiome β -diversity using Weighted UniFrac distances between 16S rRNA gene amplicon libraries, showing PC axes 2.3 and 4 (PC 1, 106 107 which captures differences between Synechococcus and Proteobacteria dominated 108 communities is shown in Fig. 2). The plots are colored by treatment (panel **a**), microbial 109 community evenness (panel b) algal contact (panel c), or temperature (panel d). Points 110 representing samples where a metadata classification was unavailable were excluded 111 from plots, for example in cases where coral-algal contact could not be assessed. 112 Subpanels reproduce main plots, but separated by selected metadata categories. Evenness 113 in panel **b** represents quartiles of microbial community equitability. While no clustering 114 by treatment was observed in panel **a**, microbial β -diversity was significantly higher in 115 corals contacting algae than in those that did not (non-parametric t-test on Weighted 116 Unifrac distances, p = 0.001) in panel c, and in panel d corals at high temperatures (>30) 117 °C; p = 0.001) or low temperatures (<24 °C; p = 0.002, not shown) had higher microbial 118 β -diversity relative to samples collected when sea surface temperatures were between 24-119 30 °C. Thus, interactions with algae and temperature extremes increase β -diversity in
- 120 coral microbiomes.





Corals, by macroalgae contacted







123 with macroalgae.

124 **a-b.** Effects of algal competition on coral microbiome β -diversity in this experiment are 125 shown for all macroalgal contacts (panel a) and contact with prevalent macroalgal genera 126 (panel **b**). All microbial distances are Weighted UniFrac distances. Overall, contact with 127 macroalgae increased microbial beta-diversity (non-parametric t-test, 1000 replicates, p = 128 0.001). When split into categories by macroalgal genera, large significant increases in 129 microbial variability were observed in corals contacting Dictyota algae (non-parametric t-130 test, 1000 replicates, p = 0.001) or multiple types of macroalgae (p = 0.001). In contrast, 131 competition with *Amphiroa* algae marginally reduced β -diversity (p = 0.057) while corals 132 in competition with *Halimeda* showed significantly reduced β -diversity (p = 0.001). c, 133 Data on microbial β -diversity caused by algal contact based on a re-analysis of Vega Thurber *et al.*, 2012^{23} . In that experiment, samples were collected from macroalgae, 134 135 Porites astreoides corals alone, or P. astreoides placed in direct competition with 136 macroalgae. Box plots show β -diversity of corals, algae, or corals in competition with 137 algae. Algal contact significantly increases coral microbiome β-diversity above that of 138 either coral alone or algae alone. P-values reflect Bonferroni-corrected permutational t-139 tests of Bray-Curtis distances between samples. Bray-Curtis distances were used here as measures of β-diversity because Weighted UniFrac could not be calculated for T-RFLP 140 141 data.

142







Supplementary Figure 8. Coral mortality as a function of contact with algae or sediment.

147 Panels show the percentage of corals that died over the course of the experiment as a

148 function of contact with sediment or different types of algae. **a**, Coral morality by coral

149 genus over the course of the experiment as a function of algal contact. *Porites* corals

150 showed significantly elevated mortality with algal contact, while Agaricia corals showed

a similar pattern but no significant difference. *Siderastrea* corals suffered no mortality

regardless of algal contact. **b**, coral morality by coral genus over the course of the

153 experiment as a function of algal and sediment contact. *Agaricia* corals showed greater

154 susceptibility to the combination of sediment and algae than to algae alone. In most cases,

155 competition with algae or contact with sediment increased coral mortality. **c**, coral

mortality subdivided by algae contacted. In some cases, corals contacted more than one

157 type of algae or algae and sediment making these categories not mutually exclusive.

158 Many algal taxa did not contact corals frequently enough for meaningful statistics (e.g.

159 cyanobacteria, *Stypopodium zonale*). P-values reflect Fisher's Exact Test.



Supplementary Figure 9. Dark Spot Syndrome in *Siderastrea* corals increases with algal contact and is associated with increased prevalence and extent of tissue loss.

164 **a**, Prevalence of Dark Spot Syndrome (DSS) across *Siderastrea siderea* corals contacting

165 or not contacting algae. Algal contact increases the prevalence of DSS. **b**, Prevalence of

166 DSS in Control vs. Nutrient Enriched plots. Nutrient enrichment did not significantly

167 increase DSS as we had shown in Vega Thurber *et al.* (2014), although the pattern is the

same as in our previous study. These data reflect only corals within the plots themselves.Our previous work showed similar and highly significant increases in the prevalence of

170 DSS in radial surveys around the same enriched plots studied here that included 3-4 times

more corals than within these plots. Thus, the lack of significance within plots may

172 reflect the lower number of *Siderastrea* corals within plots than in radial surveys around

173 the plots as in 7 . **c**, Tissue loss prevalence in *Siderastrea* corals with or without DSS.

174 Corals with visible DSS were more likely to lose tissue. **d**, Average extent of growth

175 (positive numbers) or tissue loss (negative numbers) in corals with or without DSS.

176 While *Siderastrea* without DSS gained tissue on average, those with DSS lost tissue.

177 Statistics are from Fisher's exact test **a-c** or ANOVA **d**.



180 Supplementary Figure 10. Temperature, thermal stress, and effects on the coral microbiome. a, Temperature time-series based on the Pathfinder V5.2 dataset (Methods). 181 182 The left vertical axis and thin solid black lines shows temperatures in °C. The horizontal 183 orange dotted line indicate the maximum monthly mean (MMM) temperature of 29.26 184 °C. This is calculated as the average temperature of the warmest month in available 185 climatological data (1982-2008; i.e. excluding the study period). The red horizontal dotted line indicates the MMM +1 °C (30.26 °C), which is often used as a temperature 186 187 threshold at which coral thermal stress begins to accumulate in predictions of coral 188 bleaching. For purposes of predicting coral bleaching, coral thermal stress is typically 189 measured in units of Degree Heating Weeks (DHWs, °C-weeks). DHWs are usually calculated relative to the MMM +1 °C, and are the accumulation of temperatures above 190 191 this threshold. As a hypothetical example, if temperatures exceeded the MMM + 1 by 0.2192 C for 3 weeks, 0.6 DHWs would accumulate. The accumulation of degree heating weeks 193 due to temperatures above the MMM + 1 °C is shown by the solid red line and the 194 secondary vertical axis on the right hand side of the plot (DHWs (MMM +1)). Because 195 vulnerability to pathogenic bacteria is thought to occur at lower levels of thermal stress 196 than coral bleaching, we also plotted DHWs calculated relative to the MMM. This is 197 shown by the orange line (DHWs (MMM)). We emphasize that all DHW values 198 presented in the main text are calculated based on the MMM + 1 °C (red lines). **b.** Coral 199 microbiomes vs. temperature. Microbial community evenness (left axis, blue circles) and the relative abundance of Proteobacteria and Synechococcus cyanobacteria (right axis, 200 201 orange triangles and cyan squares, respectively). The x-axis shows sea-surface 202 temperatures. Regression lines show the loess regression (span = 0.25) for each data 203 series (evenness, dotted lines; Proteobacteria, dashed line; Synechococcus dot-dashed 204 line). Gray shading around each line indicates twice the standard error of the regression. 205 Vertical lines indicate metrics from the thermal stress calculation. The orange vertical 206 line is the MMM, the red one is the MMM + 1 $^{\circ}$ C. Notably, the abundance of Proteobacteria increase, and overall community evenness decrease around the MMM of 207 208 29.26 °C.

	Percent Change in Relative Abundance Compared to Control		
Taxonomy (Microbial Order)	Nutrient	Exclosure	Exclosure & Nutrient
Archaea Crenarchaeota Thaumarchaeota Cenarchaeales	-20.79%	61.83%	0.78%
Acidobacteria Sva0725 Sva0725	4.75%	111.13%	45.47%
Bacteroidetes Bacteroidia Bacteroidales	-19.33%	41.36%	9.40%
Bacteroidetes Flavobacteriia Flavobacteriales	-3.31%	29.07%	22.62%
Chlamydiae Chlamydiia Chlamydiales	49.44%	149.04%	60.60%
Cyanobacteria Synechococcophycideae Synechococcales	5.42%	-22.24%	-21.25%
Fusobacteria Fusobacteriia Fusobacteriales	-19.08%	39.01%	-16.80%
Gemmatimonadetes Gemm-2	31.21%	142.33%	102.66%
Lentisphaeraec Lentisphaeria Lentisphaerales	-16.45%	32.89%	30.42%
Planctomycetes BD7-11	-32.83%	125.51%	-17.17%
Planctomycetes C6od113	-17.83%	107.33%	41.01%
Planctomycetes OM190 agg27	-4.80%	58.00%	21.55%
Planctomycetes Planctomycetia Pirellulales	-10.75%	70.81%	11.03%
Proteobacteria Alphaproteobacteria Rhodospirillales	-17.96%	54.61%	86.92%
Proteobacteria Alphaproteobacteria Rickettsiales	83.04%	14.69%	-5.89%
Proteobacteria Deltaproteobacteria Myxococcales	-6.81%	28.66%	13.02%
Proteobacteria Deltaproteobacteria NB1-j	-3.92%	109.96%	67.54%
Proteobacteria Deltaproteobacteria Spirobacillales	-6.86%	43.69%	22.35%
Proteobacteria Gammaproteobacteria Chromatiales	-32.93%	40.63%	-5.00%
Proteobacteria Gammaproteobacteria HOC36	-5.57%	5.91%	65.07%
Proteobacteria Gammaproteobacteria Marinicellales	-15.52%	23.66%	18.56%
Proteobacteria Gammaproteobacteria Thiohalorhabdales	-45.08%	17.75%	-7.73%
Proteobacteria Gammaproteobacteria Thiotrichales	-15.76%	37.10%	11.54%
Proteobacteria Gammaproteobacteria; Alteromonadales	12.96%	-6.25%	-6.25%
Spirochaetes Spirochaetes Spirochaetales	0.60%	111.06%	18.23%

Positive	Negative		
>100%	0 to -15%		
50 to 99%	-16 to 30%		
0 to 49%	-31 to -50%		

209 Supplementary Table 1. Response of microbial orders to treatment.

210 Table reports microbial orders that were significantly different (Kruskal-Wallis, FDR q \leq

211 0.05) across treatments. Values report percent changes compared to corals in control

212 plots. Cold colors are reductions compared to controls and hot colors represent increases

213 compared to controls. In general, corals in herbivore exclosures had large elevations in

214 many taxa as compared to controls while nutrient pollution caused small to moderate

215 reductions in various taxa. The Taxonomy column gives phylum, class, and order based

- on Greengenes taxonomy.
- 217

218 Supplementary Notes

219220 Natural history of the study site

221 This experiment was conducted in the area of Pickles Reef (N 24.99430, W 222 80.40650), located east of Key Largo, Florida in the United States. The Florida Keys reef 223 tract consists of a large bank reef system located approximately 8 km offshore of the 224 Florida Keys, USA, and paralleling the island chain. Our study reef is a 5-6 m deep spur 225 and groove reef system within this reef tract. The reefs of the Florida Keys have robust herbivorous fish populations¹ and are relatively oligotrophic². Coral cover on most reefs 226 227 in the Florida Keys, including our site, is 5-10%, while macroalgal cover averages ~15%, but ranges from 0-70% depending on location and season³⁻⁵. Parrotfishes (Scaridae) and 228 229 surgeonfishes (Acanthuridae) are the dominant herbivores on these reefs as fishing for 230 them was banned in 1981. The other important herbivore on Caribbean reefs, the urchin 231 Diadema antillarum, remains at low densities across the Florida Keys following the mass 232 mortality event in 1982-3⁶.

233

234 Tests of the experimental design and implementation

235 *Nutrient enrichment in seawater and algal tissue samples*

236 Sampling of water column nutrients showed that enrichment increased both 237 dissolved inorganic nitrogen (3.91 µM vs. 1.15 µM in enriched vs. control) and soluble 238 reactive phosphorus (0.27 μ M vs. 0.035 μ M in enriched vs. control) in the water column'. 239 Levels of both DIN and SRP in the control plots were within the range of concentrations 240 for offshore reefs, as measured in a 15-year water-monitoring program in the Florida 241 Keys⁸. Levels of DIN and SRP in the enriched treatment were similar to those reported 242 from other anthropogenically-impacted reefs located around the world⁹. Additionally, 243 nitrogen concentrations in the tissues of the common alga Dictyota menstrualis were 20% 244 higher in the enriched plots compared to the control plots, suggesting that the nutrients 245 from the enrichment were consistently available to benthic organisms⁷.

246

247 Herbivorous fish community assessments

Herbivorous fish biomass averaged 50.9 ± 5.7 g/m² over the course of our sampling, which is among the higher values seen across the Caribbean^{10,11}. There were no differences in biomass across different sampling periods (ANOVA: F = 0.23, p = 0.880). On average, parrotfishes and surgeonfishes represented 55% and 45% of total herbivorous fish biomass respectively. We did not quantify *D. antillarum* as they were rarely encountered.

254

255 Tests for unintended experimental exclosure effects

256 One potential concern with using exclosures to manipulate herbivore access is that 257 they can potentially alter water flow regimes or sedimentation inside of the full 258 exclosures, creating experimental artifacts in patterns of algal community structure. We 259 saw no differences between full exclosures, exclosure controls, or open areas for either sedimentation rates measured via sediment traps (e.g. 12 ; one-way ANOVA, F = 0.37, p = 260 0.70) or for bulk flow rates measured via clod cards (e.g. 13 ; one-way ANOVA, F = 3.01, 261 p = 0.10). Exclosures were scrubbed every 4-6 weeks to minimize growth of fouling 262 organisms and minimize changes in flow. However, these exclosures do decrease light 263

availability to the benthos by 15% (e.g. ¹⁴). Given that the light availability common at
these shallow depths saturates the photosystems of primary producers¹⁵, the slight
decrease in light availability likely had minimal impact on primary production or
interactions among benthic organisms.

Further, we saw no differences in damselfish densities (primarily the bicolor damselfish *Stegastes partitus*) between exclosures vs. exclosure controls (one-way ANOVA, F = 0.43, p = 0.54) or between enriched vs. ambient nutrient plots (one-way ANOVA, F = 0.16, p = 0.71). Thus, these territorial and aggressive fishes that can impact how larger herbivorous fishes feed (e.g. ¹⁶) likely did not differently affect the treatments. Further, we are confident that our treatments imparted minimal caging artifacts as others have shown minimal artifacts from using similar designs (e.g. ¹⁷⁻²⁰).

275

276 Benthic community surveys

277 Shifts in algal community composition

278 Increases in total algal cover corresponded to alterations in algal community 279 composition (Supplementary Figure 2a,b). Control plots were marked by high 280 abundances of closely cropped filamentous turf algae and crustose coralline algae. On 281 healthy reefs a high abundance of these two algal functional groups, along with low 282 abundance of macroalgae, makes for productive reefs and prime habitat for coral recruitment, growth, and reproduction²¹. Nutrient pollution and/or herbivore exclusion 283 284 decreased cover of both of these groups while also increasing the abundance of algae that 285 can be harmful to corals. Herbivore exclusion in general led to increases in several groups of algae, such as Sargassum spp., Amphiroa spp., tall filamentous turf, Dictytoa 286 spp., and Halimeda spp., known to both harm corals in direct competition^{22,23} and 287 strongly impact the coral microbiome²³⁻²⁵. Nutrient enrichment also increased the 288 abundance of important algal competitors such as *Dictyota* spp., tall filamentous turf, and 289 290 Lyngbia spp. cyanobacteria, which are noted for producing chemicals harmful to corals 291 and their microbes 26 .

292

293 Microbial community analysis

294 *Results of coral microbiome function prediction*

295 When we assessed predicted microbiome function in response to algal abundance, 76 functional categories were significantly correlated with upright algal cover (Pearson 296 297 correlation, FDR q < 0.05), with correlation coefficients ranging from -0.23 to 0.18 298 (Supplementary Figure 5a, Supplementary Data 4a). High levels of macroalgae increased 299 functional categories associated with opportunism (Bacterial Invasion of Epithelial Cells, 300 Bacterial Motility Proteins, Bacterial Chemotaxis, Cell Motility and Secretion), and 301 decreased the abundance of pathways involved in antibiotic production (here the single 302 category tetracycline biosynthesis). The strongest single correlate of increased algal cover was a predicted decrease in microbial genes for linoleic acid (C18:2, cis-cis-9,12) 303

- 304 metabolism.
- 305 Extremes of temperature significantly altered the predicted abundance of 124 KEGG 306 Pathways (Pearson correlation, p < 0.05, FDR q < 0.05). Extremes of temperature

307 increased the predicted genomic abundance of pathways for bacterial chemotaxis,

- 308 motility, environmental sensing and secretion often observed to increase with
- 309 opportunism (Supplementary Figure 5b, Supplementary Table 4b). Previous work has

310 shown that elevated temperature increases DMSP release from the coral holobiont, which

in turn guides chemotaxis by several marine microorganisms²⁷, including the coral 311 pathogen Vibrio corallyticus, to the coral surface²⁸. 312

313 Conversely, five categories of antibiotic/antimicrobial production decreased in 314 extreme temperature (Novobiocin biosynthesis, Streptomycin biosynthesis, isoquinoline 315 alkaloid biosynthesis, Biosynthesis of vancomycin group antibiotics, Tetracycline 316 biosynthesis), as did several pathways associated with photosynthesis (Photosynthesis

317 proteins, photosynthesis- antennae proteins, carbon fixation in photosynthetic organisms,

318 porphyrin and chlorophyll metabolism). These changes may reflect shifts from a

319 community rich in phototrophs (*Synechococcus*) and defensive symbionts (e.g.

320 Actinobacteria) to one more strongly dominated by a variety of mostly proteobacterial 321 opportunists.

- 322
- 323

324

Comparison of predicted functional profiles for corals under a range of temperatures with metagenomic data from laboratory temperature manipulations

325 KEGG categories associated with increasing temperature in the present study tended 326 to change in the same direction as observed in Vega Thurber *et al.*, 2009^{29} . These 327 commonalities were striking given that one data set represents genome-based predictions 328 from field 16S rRNA data, while the other represents pooled metagenomes from a 329 laboratory experiment. To quantify these trends we used sign tests to compare the direction of change with temperature for the 24 level 3 KEGG functional categories that 330 331 changed by >1% with elevated temperature in Vega Thurber et al., 2009. We compared 332 these categories with either: a) the set of 13 KEGG categories significantly associated 333 with temperature in this study (dropping pathways that did not significantly change here) 334 or b) all 24 KEGG categories enriched by temperature in the previous study.

335 Regardless of the method used, KEGG categories showed a significant tendency to 336 change in the same direction with temperature. Among the set of all 24 KEGG pathways 337 increased by temperature in Vega Thurber 2009, 17 of 24 changed in the same direction 338 in this study. Thus, the direction of change with temperature was significant overall 339 between these two very different experiments (one-sided sign test, 17 successes, 24 trials, 340 p = 0.032). The 13 KEGG Pathways that changed by >1% in Vega Thurber 2009 and also 341 changed significantly in this study (Pearson correlation, FDR q < 0.05) also had a 342 significant tendency to change in the same direction (one sided sign test, 10 trials, 13 343 successes, p = 0.046).

344

345 *Confirmation of macroalgal contact as a driver of microbial beta diversity*

346 To confirm that direct macroalgal contact was the driver of changes in the microbial 347 β -diversity seen in this work, we reanalyzed data from a previous experiment²³ at the 348 same study site that placed replicate *Porites* corals in contact with macroalgae. Three 349 categories from that experiment were compared: corals alone, algae alone, or corals in 350 competition with algae (Supplementary Figure 7c). Corals in contact with macroalgae 351 showed higher β -diversity than corals without macroalgae (Bonferroni-corrected 352 permutational t-test, p = 0.009). β -diversity in corals in contact with macroalgae was also 353 greater than for the algae themselves (Bonferroni-corrected permutational t-test, p = 354 0.003).

356 Effects of algal competition on microbial beta-diversity within coral genera

357 To test whether increasing β -diversity with algal competition is a consistent feature 358 of the coral genera in our study, the effects of algal competition on microbial β -diversity 359 was assessed within each coral genus. In each case, mean microbial community β diversity was greater when corals were in competition with macroalgae, although when 360 361 *Porites* was considered alone this difference was not significant (permutational t-tests; 362 Agaricia, p = 0.006; Siderastrea, 1 p = 0.005, Porites p = 0.74). However, the variability 363 imparted to Porites microbiomes from parrotfish bites and nutrient enrichment could 364 have confounded the impact of macroalgae. Similarly, competition with *Dictyota* algae 365 significantly increased microbial β -diversity in all coral genera (*Agaricia* p = 0.001; Siderastrea p = 0.001; Porites p = 0.002). These findings do not exclude the likely 366 367 possibility that the microbes associated with many coral genera have partially species-368 specific interactions with particular algae.

369

370 Comparison of temperature vs. seasonal effects on microbial beta diversity

Temperature was an important correlate of many aspects of microbial community
 structure. Because temperature was not experimentally manipulated, and many other
 oceanographic parameters fluctuate seasonally, we sought to test whether apparent
 changes in temperature might be due to unrelated seasonal changes.

375 We first tested whether temperature increased microbial β -diversity within seasons 376 as well as between seasons. We reasoned that if overall seasonal changes drove microbial 377 β-diversity, and correlations with temperature were an incidental byproduct, then withinseason effects of temperature on microbial β-diversity should be weak or non-existent. 378 379 Instead, we found that even within summer and fall samples (considered separately), high 380 temperatures (>30 °C) resulted in greater β -diversity than non-stressful temperatures (24-381 29 °C), indicating that thermal stress influences microbial communities within as well as 382 between seasons (Summer: p = 0.001, permutational t-test; Fall p= 0.001, permutational 383 t-test). Consistent with either warm or cold temperatures disrupting coral microbiomes, 384 low (< 24 °C) temperatures in winter were associated with significantly greater β -385 diversity than 24-29 °C winter samples (p = 0.003, permutational t-test).

To address the possibility that some other seasonal environmental factor besides 386 387 short-term temperature changes might be the main driver of microbial β-diversity, we 388 examined 40 environmental parameters measured seasonally by the SERC Water Quality 389 Monitoring Network in South Florida, including dissolved inorganic nitrogen, total 390 organic carbon, chlorophyll a, turbidity, SiO_2 etc. (Supplemental Data 3k). To test 391 whether differences in these parameters might explain microbial β-diversity, we 392 constructed Euclidean distance matrices for each parameter across samples, and used 393 Mantel tests, a permutational procedure for comparing two distance matrices, to test 394 whether any of these environmental parameters significantly correlated with microbial β-395 diversity. No correction for multiple comparisons was performed in this instance, since 396 we did not wish to miss a relevant parameter that might falsify our interpretation of the 397 role of temperature.

398 Among the measured parameters and temperature, only three correlated significantly 399 with microbial β -diversity. Daily temperature had the greatest influence (r = 0.127, p = 400 0.01), with surface measurements of total organic carbon (TOC; r = 0.045, p = 0.02), and 401 turbidity (r = 0.09, p = 0.04) playing secondary roles. Notably, although daily 402 temperature measurements (HCOM_temp_0m_degrees) were well correlated with
403 microbial community structure, seasonal temperature measurements (collected by SERC
404 quarterly at a single time point per season) were not (SERC_TEMP_B). This may
405 indicate that short-term changes in temperature are important, above and beyond typical
406 seasonal temperature trends.

407 A final possibility that we considered was that the apparent effects of temperature on 408 β -diversity might be explained by the relatively modest seasonal variation in upright algal 409 cover (which includes both tall turf algae and macroalgae). To disentangle the effects of 410 temperature from upright algal cover on microbial β -diversity, we conducted a partial 411 Mantel test examining the relationship between temperature and microbial β -diversity 412 while normalizing for the effect of upright algal cover. We found that temperature was 413 still significantly correlated with β -diversity (Partial Mantel test; r = 0.12 p = 0.01) after 414 accounting for the effects of upright algal cover (Supplemental Data 3k).

415 We do not interpret these results to mean that temperature is the only influence on 416 microbial communities. For example, oceanographic parameters not significant on a 417 quarterly basis may have important short-term or spatially localized effects that could be 418 uncovered with high-resolution sampling. However, these results taken together argue 419 against the possibility that the observed correlations between temperature and microbial 420 community structure are artifacts of seasonal fluctuations in water chemistry or algal 421 cover. Instead, short-term temperature variation appears to be an important factor 422 influencing coral microbiome stability. This analysis also identified seasonal variation in 423 dissolved organic carbon and turbidity as additional influences on coral microbial community structure, consistent with the effects of these parameters on corals and coral 424 microbiomes in laboratory experiments^{30,31}. 425

427 SUPPLEMENTARY REFERENCES 428 1 Burkepile, D. E. et al. Nutrient supply from fishes facilitates macroalgae and 429 suppresses corals in a Caribbean coral reef ecosystem. Sci Rep 3, 1493, 430 doi:10.1038/srep01493 (2013). 431 2 Briceno, H. O. B., J. N. . Annual report of the water quality monitoring project for 432 the water quality protection program of the Florida Keys National Marine 433 Sanctuary (Southeast Environmental Research Center, Florida International 434 University, http://serc.fiu.edu/wqmnetwork/ 2012). 435 3 Paddack, M., Cowen, R. & Sponaugle, S. Grazing pressure of herbivorous coral 436 reef fishes on low coral-cover reefs. Coral Reefs 25, 461-472, 437 doi:10.1007/s00338-006-0112-y (2006). 438 4 Schutte, V. G., Selig, E. R. & Bruno, J. F. Regional spatio-temporal trends in 439 Caribbean coral reef benthic communities. Mar Ecol Prog Ser 402, 115-122 440 (2010). 441 5 Maliao, R., Turingan, R. & Lin, J. Phase-shift in coral reef communities in the 442 Florida Keys National Marine Sanctuary (FKNMS), USA. Mar Biol 154, 841-443 853, doi:10.1007/s00227-008-0977-0 (2008). 444 6 Chiappone, M., Swanson, D. W. & Miller, S. L. Density, spatial distribution and 445 size structure of sea urchins in Florida Keys coral reef and hard-bottom habitats. 446 Mar Ecol Prog Ser 235, 117-126 (2002). 447 7 Vega Thurber, R. et al. Chronic nutrient enrichment increases prevalence and 448 severity of coral disease and bleaching *Global Change Biology* **20**, 544-554, 449 doi:10.1111/gcb.12450 (2014). 450 8 Briceno, H. O. & Boyer, J. N. Annual report of the water quality monitoring 451 project for the water quality protection program of the Florida Keys National 452 Marine Sanctuary Southeast Environmental Research Center, Florida 453 International University, 82 http://serc.fiu.edu/wgmnetwork/ (2012). 454 9 Dinsdale, E. A. et al. Microbial Ecology of Four Coral Atolls in the Northern Line 455 Islands. PloS one 3, -, doi:ARTN e1584 456 DOI 10.1371/journal.pone.0001584 (2008). 457 10 Burkepile, D. E. et al. Nutrient supply from fishes facilitates macroalgae and 458 suppresses corals in a Caribbean coral reef ecosystem. *Scientific Reports* **3**, 459 doi:10.1038/srep01493 (2013). 460 11 Newman, M. J. H., Paredes, G. A., Sala, E. & Jackson, J. B. C. Structure of Caribbean coral reef communities across a large gradient of fish biomass. *Ecology* 461 462 Letters 9, 1216-1227 (2006). 463 12 Gleason, D. Sedimentation and distributions of green and brown morphs of the Caribbean coral Porites astreoides Lamarck. Journal of Experimental Marine 464 465 Biology and Ecology 230, 73-89 (1998). Jokiel, P. L. & Morrissey, J. I. Water motion on coral reefs - evaluation of the 466 13 clod card technique. Marine Ecology-Progress Series 93, 175-181 (1993). 467 468 14 Ferrari, R., Gonzalez-Rivero, M., Ortiz, J. C. & Mumby, P. J. Interaction of 469 herbivory and seasonality on the dynamics of Caribbean macroalgae. Coral Reefs 470 **31**, 683-692, doi:10.1007/s00338-012-0889-9 (2012).

471	15	Carpenter, R. C. Relationships between primary production and irradiance in
472		coral reef algal communities. <i>Limnology and Oceanography</i> 30 , 784-793 (1985).
473	16	Hixon, M. A. & Bronstoff, W. N. Damselfish as keystone species in reverse:
474		intermediate distrubance and diversity of reef algae. Science 220, 511-513 (1983).
475	17	Smith, J., Smith, C. & Hunter, C. An experimental analysis of the effects of
476		herbivory and nutrient enrichment on benthic community dynamics on a
477		Hawaiian reef. Coral Reefs 19, 332-342 (2001).
478	18	Burkepile, D. & Hay, M. Predator release of the gastropod Cyphoma gibbosum
479		increases predation on gorgonian corals. <i>Oecologia</i> 154 , 167-173 (2007).
480	19	Miller, M. & Hay, M. Effects of fish predation and seaweed competition on the
481		survival and growth of corals. <i>Oecologia</i> 113 , 231-238 (1998).
482	20	Miller, M. et al. Effects of nutrients versus herbivores on reef algae: A new
483		method for manipulating nutrients on coral reefs. Limnology and Oceanography
484		44 , 1847-1861 (1999).
485	21	Birrell, C. L., McCook, L. J., Willis, B. L. & Diaz-Pulido, G. A. Effects of
486		benthic algae on the replenishment of corals and the implications for the resilience
487		of coral reefs. Oceanography and Marine Biology: An Annual Review 46, 25-63
488		(2008).
489	22	Rasher, D. B. & Hay, M. E. Chemically rich seaweeds poison corals when not
490		controled by herbivores. Proceedings of the National Academy of Science 107,
491		9683-9688 (2010).
492	23	Vega Thurber, R. et al. Macroalgae Decrease Growth and Alter Microbial
493		Community Structure of the Reef-Building Coral, Porites astreoides. <i>PloS one</i> 7,
494		e44246 (2012).
495	24	Smith, J. E. et al. Indirect effects of algae on coral: algae-mediated, microbe-
496		induced coral mortality. Ecology Letters 9, 835-845 (2006).
497	25	Morrow, K. M., Liles, M. R., Paul, V. J., Moss, A. & Chadwick, N. E. Bacterial
498		shifts associated with coral-macroalgal competition in the Caribbean Sea. Mar
499		<i>Ecol Prog Ser</i> 488 , 103-117 (2013).
500	26	Morrow, K. M., Paul, V. J., Liles, M. R. & Chadwick, N. E. Allelochemicals
501		produced by Caribbean macroalgae and cyanobacteria have species-specific
502		effects on reef coral microorganisms. Coral Reefs 30, 309-320,
503		doi:10.1007/s00338-011-0747-1 (2011).
504	27	Seymour, J. R., Simo, R., Ahmed, T. & Stocker, R. Chemoattraction to
505		dimethylsulfoniopropionate throughout the marine microbial food web. Science
506		329 , 342-345, doi:10.1126/science.1188418 (2010).
507	28	Garren, M. et al. A bacterial pathogen uses dimethylsulfoniopropionate as a cue
508		to target heat-stressed corals. The ISME Journal 8, 999-1007,
509		doi:10.1038/ismej.2013.210 (2013).
510	29	Thurber, R. V. et al. Metagenomic analysis of stressed coral holobionts.
511		Environmental Microbiology 11, 2148-2163 (2009).
512	30	Kuntz, N. M., Kline, D. I., Sandin, S. A. & Rohwer, F. Pathologies and mortality
513		rates caused by organic carbon and nutrient stressors in three Caribbean coral
514		species. Mar Ecol Prog Ser 294, 173-180, doi:10.3354/meps294173 (2005).

- 515 31 Kline, D. I., Kuntz, N. M., Breitbart, M., Knowlton, N. & Rohwer, F. Role of
 516 elevated organic carbon levels and microbial activity in coral mortality. *Mar Ecol*517 *Prog Ser* **314**, 119-125 (2006).