

1 Microbial community shifts associated with the ongoing stony coral 2 tissue loss disease outbreak on the Florida Reef Tract

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14 15 ABSTRACT:

16 As many as 22 of the 45 coral species on the Florida Reef Tract are currently affected by stony
17 coral tissue loss disease (SCTLD). The ongoing disease outbreak was first observed in 2014 in
18 Southeast Florida near Miami and as of early 2019 has been documented from the northernmost
19 reaches of the reef tract in Martin County down to Key West. We examined the microbiota
20 associated with disease lesions and apparently healthy tissue on diseased colonies of
21 *Montastraea cavernosa*, *Orbicella faveolata*, *Diploria labyrinthiformis*, and *Dichocoenia*
22 *stokesii*. Analysis of differentially abundant taxa between disease lesions and apparently healthy
23 tissue identified five unique amplicon sequence variants enriched in the diseased tissue in three
24 of the coral species, namely an unclassified genus of Flavobacteriales and sequences identified as
25 *Fusibacter* (Clostridiales), *Planktotalea* (Rhodobacterales), *Algicola* (Alteromonadales), and
26 *Vibrio* (Vibrionales). In addition, several groups of likely opportunistic or saprophytic colonizers
27 such as Epsilonbacteraeota, Patescibacteria, Clostridiales, Bacteroidetes, and Rhodobacterales
28 were also enriched in SCTLD disease lesions. This work represents the first microbiological
29 characterization of SCTLD, as an initial step toward identifying the potential pathogen(s)
30 responsible for SCTLD.

31 32 KEYWORDS:

33 scleractinian coral, white syndrome, dysbiosis, Caribbean, coral microbiome

34

35 INTRODUCTION

36

37 An ongoing outbreak of coral disease termed stony coral tissue loss disease (SCTLD) is currently
38 impacting at least twenty coral species on the Florida Reef Tract (Florida Keys National Marine
39 Sanctuary, 2018; Lunz et al., 2017). Outbreak levels of disease were first observed in late 2014
40 off Virginia Key, FL, and over the next year the disease was detected both north and south along
41 the Florida Reef Tract (Precht et al., 2016). Active monitoring by county, state, and federal
42 agencies in Southeast Florida and the Florida Keys National Marine Sanctuary has since
43 recorded disease occurrence from northern Martin County south and west through the Florida
44 Reef Tract to Key West. The geographic extent, the number of coral species impacted, and the
45 modeling of disease incidence all support the conclusion that this is a highly contagious disease
46 (Muller et al., 2018).

47

48 Monitoring efforts and early studies indicate that not all coral species are equally susceptible to
49 SCTLD, with corals like *Dendrogyra cylindrus*, *Dichocoenia stokesii*, and *Meandrina*
50 *meandrites* succumbing very rapidly to the disease (Florida Keys National Marine Sanctuary,
51 2018). Important reef-building species like *Montastraea cavernosa* and *Siderastrea siderea* have
52 also been heavily impacted (Walton et al., 2018). In addition to variability in response among
53 different coral species, environmental factors may influence disease severity. For example, the
54 highest prevalence of SCTLD in Southeast Florida during surveys from 2012 to 2016 also
55 coincided with the highest prevalence of coral bleaching (Walton et al., 2018) in response to
56 elevated sea surface temperatures in the summer and winter of 2014 (Manzello, 2015). In
57 addition, dredging operations between 2013 and 2015 in the channel at the Port of Miami were
58 correlated with increased sedimentation and partial coral mortality near the site where the disease
59 outbreak was detected (Miller et al., 2016). Responses within species may also be constrained by
60 local reef conditions. In the Upper Keys, monitored colonies of *S. siderea* and *Pseudodiploria*
61 *strigosa* experienced higher mortality from white plague-like disease (prior to the onset of
62 SCTLD incidence) in offshore reefs dominated by octocorals and macroalgae in comparison to
63 inshore patch reefs with higher coral cover (Rippe et al., 2019).

64

65 Currently, it is unclear whether all of the impacted species are suffering from the same disease,
66 and there are no existing diagnostic tools to positively identify SCTLD, as is the case for most
67 coral diseases. Several enigmatic tissue loss diseases, also known as white syndromes as they are
68 characterized by the white skeleton left exposed by the disease, have been identified in corals
69 around the world, most without the identification of a definitive causative agent (Mera and
70 Bourne, 2018). In the Caribbean, potential pathogens have been identified for three coral tissue
71 loss diseases: white band disease type II, white plague disease type II, and white pox disease.
72 White band disease type II has been attributed to *Vibrio harveyi* (Gil-Agudelo et al., 2006;
73 Ritchie and Smith, 1998) and is part of the suite of white band diseases that contributed to the
74 drastic decline in *Acropora palmata* and *A. cervicornis* in the 1980s (Aronson and Precht, 2001).
75 White plague disease type II has also reportedly impacted dozens of other Caribbean stony coral
76 species (Weil et al., 2006), and an outbreak of this disease in the 1990s was attributed to an
77 alphaproteobacterium (Richardson et al., 1998), and the potential pathogen, named
78 *Aurantimonas coralicida*, was subsequently isolated from *Dichocoenia stokesii* (Denner et al.,
79 2003). A later study using both traditional clone libraries of small subunit ribosomal genes and
80 microarray assays of Caribbean *Orbicella faveolata* affected by white plague disease type II

81 failed to detect the putative pathogen *Aurantimonas coralicida* (Sunagawa et al., 2009). White
82 pox disease (acroporid serratiosis), which exclusively impacts *Acropora palmata* corals in the
83 Caribbean, has been attributed to the opportunistic bacterial pathogen *Serratia marcescens*
84 (Patterson et al., 2002), which may have originated from human sewage (Sutherland et al., 2010).
85 In contrast, putative pathogens have not been identified for most common coral diseases (Weil et
86 al., 2006).

87
88 To aid in the discovery of the pathogen or pathogens responsible for the current SCTL
89 outbreak, we characterized the microbiomes associated with four coral species with SCTL
90 from sites in Southeast Florida and the Middle Keys. For each diseased colony, we examined
91 microbiome composition at the disease lesion, in apparently healthy polyps immediately adjacent
92 to the lesion, and in apparently healthy polyps far from the disease lesion. Neighboring corals
93 that had no apparent disease lesions were also sampled when available. This strategy was used to
94 determine the microbiome shifts associated with SCTL, which may contribute to a better
95 understanding of the disease ecology of the current devastating outbreak on the Florida Reef
96 Tract.

97 98 MATERIALS AND METHODS

99
100 Coral colonies with SCTL were identified in Southeast Florida offshore from Ft. Lauderdale
101 and in the Middle Florida Keys near Long Key Bridge. *Montastraea cavernosa* and *Orbicella*
102 *faveolata* were sampled from SE Florida in July and December 2017. *Diploria labyrinthiformis*
103 and *Dichocoenia stokesii* were sampled in the Middle Keys in December 2017. For *D.*
104 *labyrinthiformis*, and *D. stokesii*, the disease lesions were peripheral or apical, with multiple
105 lesions often proceeding to coalescence (Figure 1). In all sampled *M. cavernosa* and *O.*
106 *faveolata*, lesions were clearly preceded by a row of bleached and/or partially bleached polyps
107 (Figure 1A).

108
109 Coral mucus/tissue samples were collected while SCUBA diving using agitation of the coral
110 surface and aspiration with a needleless syringe. Care was taken to collect all of the released
111 particulates via the syringe to prevent the spread of diseased material. Samples were collected at
112 the lesion, at the first row of healthy-looking polyps adjacent to the lesion, and as far as possible
113 away from the lesion in apparently healthy tissue from nine colonies of *M. cavernosa* and four
114 colonies each of *D. labyrinthiformis*, and *D. stokesii* (Table S1). Six healthy neighboring corals
115 of *M. cavernosa* and one healthy neighboring coral of *D. labyrinthiformis* were also sampled
116 (Table S1). Samples were kept on ice until reaching shore where mucus and tissue were
117 separated by settling out of the seawater and frozen until extraction of DNA.

118
119 Extractions of genomic DNA were performed with a DNeasy Powersoil kit (Qiagen,
120 Germantown, MD) according to the manufacturer's instructions, with bead beating for 10 min.
121 The V4 region of the 16S rRNA gene was amplified in triplicate for each sample using the 515F
122 (Parada et al., 2016) and 806RB (Apprill et al., 2015) Earth Microbiome primers and
123 thermocycler protocol (Caporaso et al., 2012) in 25- μ l reactions containing Phusion High-fidelity
124 Master Mix (New England Biolabs, Ipswich, MA), 0.25 μ M of each primer, 3% dimethyl
125 sulfoxide (as recommended by the manufacturer of the polymerase), and 2 μ l of DNA template.
126 Triplicate samples were consolidated and cleaned with a MinElute PCR purification kit (Qiagen)

127 and quantified with a Nanodrop 1000 (Thermo-Fisher Scientific, Waltham, MA). DNA
128 extraction kit blanks were produced alongside regular DNA extractions without the addition of
129 any starting coral biomass. Two extraction kit blanks and one PCR blank were processed
130 alongside coral samples through sequencing with unique Earth Microbiome barcodes. One final
131 amplicon pool containing 240 ng of each sample library was submitted to the University of
132 Florida Interdisciplinary Center for Biotechnology Research for sequencing. The amplicon pool
133 was quantified using quantitative PCR and QUBIT for quality control and size selected with ELF
134 to produce fragment sizes in the desired range for amplicon sequencing. Sequencing was
135 performed on an Illumina MiSeq with the 2x150bp v. 2 cycle format.

136
137 Adapters and primers were removed from raw sequencing reads with cutadapt v. 1.8.1 (Martin,
138 2011). Sequencing reads are available in NCBI's Sequence Read Archive under Bioproject
139 PRJNA521988. Further processing of amplicon libraries was completed in R v. 3.5.1. All R
140 scripts and data needed to recreate the figures in this manuscript are available on GitHub
141 (<https://github.com/meyermicrobiolab/Stony-Coral-Tissue-Loss-Disease-SCTLD-Project>).
142 Quality filtering, error estimation, merging of reads, dereplication, removal of chimeras, and
143 selection of amplicon sequence variants (ASVs) (Callahan et al., 2017) were performed with
144 DADA2 v. 1.6.0 (Callahan et al., 2016), using the filtering parameters truncLen=c(150,150),
145 maxN=0, maxEE=c(2,2), truncQ=2, rm.phix=TRUE to remove all sequences with ambiguous
146 basecalls and phiX contamination. Taxonomy was assigned in DADA2 to ASVs using the
147 SILVA small subunit ribosomal RNA database v. 132 (Yilmaz et al., 2014). The ASV and
148 taxonomy tables, along with associated sample metadata were imported into phyloseq v. 1.22.3
149 (McMurdie and Holmes, 2013) for community analysis. Sequences that could not be assigned as
150 bacteria or archaea and sequences identified as chloroplasts or mitochondria were removed from
151 further analysis.

152
153 ASVs with a mean read count across all samples of less than 5 were removed from the analysis.
154 Zero counts were transformed using the count zero multiplicative method with the
155 zCompositions package in R (Palarea-Albaladejo and Martín-Fernández, 2015). The zero-
156 replaced read counts were transformed with the centered log-ratio transformation, and the
157 Aitchison distance metric was calculated with CoDaSeq (Gloor et al., 2017). Principal
158 component analysis of the Aitchison distance was performed with prcomp in R and plotted with
159 ggplot2 (Wickham, 2016). Analysis of similarity (ANOSIM) and permutational multivariate
160 analysis (PERMANOVA) were performed on the Aitchison distance with vegan (Dixon, 2003)
161 with 999 permutations. Multivariate dispersions of the Aitchison distance were calculated with
162 the betadisper function in vegan and fitted to a linear model to test the significance of coral
163 species and sample condition.

164
165 Differential abundance of ASVs between diseased tissue and apparently healthy tissue on
166 diseased colonies was determined with DESeq2 v. 1.20.0 (Love et al., 2014). Differential
167 abundance was assessed within each of the coral species *M. cavernosa*, *D. labyrinthiformis*, and
168 *D. stokesii*, using the lesion samples and the samples farthest from the lesions. Original count
169 data was used after filtering rows with fewer than 5 counts over the entire row and using the
170 parametric Wald test in DESeq2.

171
172

173 RESULTS

174

175 In the colonies sampled for this study, the disease appeared to progress more slowly in *M.*
176 *cavernosa* as the exposed skeleton was already colonized by turf algae at the time of collection.
177 In contrast, *D. labyrinthiformis* and *D. stokesii* displayed bare skeleton over most of the colony at
178 the time of sampling, suggesting a more rapid progression of the tissue loss on these highly
179 susceptible species (Figure 1B and 1C). These observations are consistent with reports from the
180 Coral Reef Evaluation and Monitoring Project in Southeast Florida and the Florida Keys
181 (Brinkhuis and Huebner, 2016; Walton et al., 2018).

182

183 Microbiomes were characterized from a total of 62 coral samples (Table S1). After quality-
184 filtering and joining, an average of 42,560 reads (2,358 - 233,271) per coral sample were used in
185 the analysis. A total of 128 archaeal ASVs and 11,189 bacterial ASVs were detected in coral
186 samples. The three control samples, two from the extraction kit through sequencing and one from
187 PCR through sequencing, were also sequenced and after quality-filtering and joining had an
188 average of 10,144 reads (258-18,348) per control sample, which were classified as 94 bacterial
189 ASVs (Table S2). Overall, microbial community structure differed by coral species (ANOSIM R
190 = 0.407, p = 0.001) (Figure 2). Alpha- and Gammaproteobacteria, Bacteroidetes (Bacteroidia),
191 and Cyanobacteria (Oxyphotobacteria) were commonly detected in all samples (regardless of
192 disease state) from the four coral species (Figure 3), consistent with previous studies of coral
193 microbiomes (Huggett and Apprill, 2018). Amplicon sequence variants (ASVs) classified as
194 Proteobacteria that appeared to be coral mitochondrial sequences based on BLASTn searches
195 were only in *D. stokesii* (Figure 3D). ASVs classified simply as Bacteria in both *D.*
196 *labyrinthiformis* (Figure 3C), and *D. stokesii* (Figure 3D) were unique sequences, as described in
197 further detail below. Campylobacteria were detected in higher relative abundances in and near
198 disease lesions in *M. cavernosa*, *D. labyrinthiformis*, and *D. stokesii*, while Deltaproteobacteria
199 were also detected in higher relative abundances in and near lesions of *M. cavernosa* (Figure 3).

200

201 Disease often has a stochastic effect on microbiome composition (Zaneveld et al., 2017),
202 therefore the dispersion of beta diversity was examined according to health condition (Figure 4).
203 Neighboring healthy colonies were sampled as available and only *M. cavernosa* had a sufficient
204 number of sampled healthy colonies for comparison. In *M. cavernosa*, there is a pattern of health
205 state and stochastic effects on microbiome composition, as healthy colonies had lower variation
206 in their microbiomes, while diseased tissue and apparently healthy tissue on diseased colonies
207 had higher variability in their microbiome composition. In contrast, beta diversity between
208 disease lesions and apparently healthy tissue on diseased colonies of all four coral species were
209 similarly variable.

210

211 Common taxa detected in all samples of *D. labyrinthiformis* from the middle Keys included
212 Rhodobacteraceae of the genus *Shimia* and Cyanobiaceae, namely *Prochlorococcus* strain
213 MIT9313 and *Synechococcus* strain CC9902 (Figure S2). The healthy neighboring *D.*
214 *labyrinthiformis* colony and the healthy tissue farthest from the lesion on one of the diseased *D.*
215 *labyrinthiformis* colonies had high relative abundances of sequences classified as family
216 Simkaniaceae, genus *Candidatus Fritschea* in the Phylum Chlamydiae; members of this group
217 are known as insect endosymbionts (Everett et al., 2005). The healthy colony and healthy tissues
218 also tended to have higher relative abundances of an ASV classified only as Bacteria, that was

219 99% identical to both a clone library sequence (GenBank Accession KC668983) detected in
220 *Stylophora pistillata* coral from the Red Sea (Bayer et al., 2013) and to a clone (GenBank
221 Accession GQ413901) detected in *Porites cylindrica* in the Philippines (Garren et al., 2009).

222
223 Common taxa detected in apparently healthy samples of *D. stokesii* from the middle Keys
224 included an ASV classified only as Bacteria, that was 97% similar to a clone library sequence
225 (GenBank Accession HQ189553) from an estuarine anemone, *Nematostella vectensis*, sampled
226 from Cape Cod (Har et al., 2015). Other common taxa in *D. stokesii* included the Cyanobiaceae
227 strains *Prochlorococcus* MIT9313 and *Synechococcus* CC9902 and an unclassified
228 betaproteobacterial genus of Rhodocyclaceae that was detected in every *D. stokesii* sample
229 (Figure S3). The closest BLASTn matches to this Rhodocyclaceae ASV were 98% similar to
230 sequences recovered from soils. High relative abundances of an ASV that was an exact match to
231 the type species of *Vibrio ishigakensis* from Japanese coral reef seawater (Gao et al., 2016) were
232 detected in the disease lesions of *D. stokesii* colonies as well as in apparently healthy tissues at
233 lower relative abundances.

234
235 While microbial community structure changed with sample condition (diseased, apparently
236 healthy tissue on diseased coral, or healthy neighboring coral), the effect size was small
237 (PERMANOVA $R^2 = 0.074$, $p = 0.001$) when compared to the effect of site (PERMANOVA R^2
238 $= 0.154$, $p = 0.001$) or coral species (PERMANOVA $R^2 = 0.223$, $p = 0.001$). Therefore, we
239 looked for taxa that were statistically more abundant (enriched) as determined by DESeq2
240 analysis in disease lesions within each coral species (Figure S4). In *M. cavernosa*, 119 ASVs
241 were enriched in disease lesions versus healthy tissue on diseased colonies out of 121 ASVs that
242 were detected as differentially abundant. In *D. labyrinthiformis*, 124 of 132 differentially
243 abundant ASVs were enriched in disease lesions. In *D. stokesii*, 69 of 161 differentially abundant
244 ASVs were enriched in disease lesions. Of these, 30 ASVs were enriched in disease lesions of at
245 least two coral species. One of the 30 ASVs, classified as Rhodobacteraceae, was also detected
246 in the PCR blank sample (Table S2). Only five ASVs were enriched in the disease lesions of all
247 three coral species (Table 1, Figure 5). These five ASVs were not detected in any of the control
248 samples (Table S2). The disease-enriched ASVs from all three coral species included an
249 unclassified genus of Flavobacteriales and sequences identified as *Fusibacter* (Clostridiales),
250 *Planktotalea* (Rhodobacterales), *Algicola* (Alteromonadales), and *Vibrio* (Vibrionales).

251
252 The Flavobacteriales ASV was identified to family Cryomorphaceae, but not assigned to a
253 genus. This ASV had only one exact BLASTn match to a clone sequence associated with marine
254 dinoflagellates (GenBank Accession KY281634). This Cryomorphaceae ASV was up 6.3%
255 relative abundance in disease lesions of *D. labyrinthiformis* and *D. stokesii* (Figure 5, panel A).
256 The Cryomorphaceae ASV was detected in 10 out of 19 lesion samples, six out of 18 samples of
257 apparently healthy tissue near the disease lesion, and in one out of seven undiseased coral
258 samples. It was not detected in the 18 samples of apparently healthy tissue far from the disease
259 lesion.

260
261 The *Fusibacter* ASV was an exact match to a clone library sequence (GenBank Accession
262 KC527495) from a white plague disease infected *Porites lutea* coral from Thailand (Roder et al.,
263 2014) in addition to closely matching (99.6% identical) sequences from Black Band Disease
264 infected corals in the Red Sea (GenBank Accession GU472060, EF089469) (Arotsker et al.,

265 2015). The *Fusibacter* ASV was up to 3.5% relative abundance in the disease lesions of *M.*
266 *cavernosa* and was detectable at lower levels in disease lesions of *D. labyrinthiformis* and *D.*
267 *stokesii* (Figure 5, panel B). It was detected in four of 19 lesion samples and two of 18 near
268 lesion samples, but not detected in samples far from the lesion or undiseased corals.

269
270 BLASTn searches of the sequences revealed that the *Planktotalea* ASV is a commonly detected
271 strain in marine environments, including in sediments and associated with invertebrates and is
272 also an exact match to an unpublished sequence (GenBank Accession EF033448) associated with
273 an outbreak of white-plague-like disease in the Flower Garden Banks National Marine
274 Sanctuary. The *Planktotalea* ASV was up to 1.5% relative abundance in the disease lesions of *D.*
275 *labyrinthiformis* and was more abundant in lesions versus non-lesions in *M. cavernosa* and *D.*
276 *stokesii* (Figure 5, panel C). It was detected in six of 19 lesions and three of 18 near lesion
277 samples, but not detected in samples far from the lesion or undiseased corals.

278
279 The *Algicola* ASV was an exact match to a clone library sequence (GenBank Accession
280 GU471978) from a Black Band Disease mat on Red Sea *Favia* sp. (Arotsker et al., 2015) as well
281 as to a clone library sequence (GenBank Accession FJ403097) from an unpublished study of
282 diseased and healthy Caribbean *Orbicella faveolata*. The *Algicola* ASV was up to 1.2% relative
283 abundance in both apparently healthy and diseased tissues of *M. cavernosa* and was more
284 abundant in lesions versus non-lesions in *D. labyrinthiformis* and *D. stokesii* (Figure 5, panel D).
285 It was detected in four of 19 lesions and one sample near the lesion and one sample far from the
286 lesion in *M. cavernosa*.

287
288 The *Vibrio* ASV was an exact match to clone library sequences from a Black Band Disease mat
289 on Caribbean *Pseudodiploria strigosa* (GenBank Accession HM768606) (Klaus et al., 2011),
290 from a Black Band Disease mat on Red Sea *Favia* sp. (GenBank Accession GU472063)
291 (Arotsker et al., 2015), and from white plague disease type II in Caribbean *Orbicella faveolata*
292 (GenBank Accession FJ202106) (Sunagawa et al., 2009). The highest relative abundances of the
293 *Vibrio* ASV were 0.5% in the disease lesion of one *M. cavernosa* colony and was more abundant
294 in lesions versus non-lesions in *D. labyrinthiformis* and *D. stokesii* (Figure 5, panel E). It was
295 detected in seven of 19 lesions and four of 18 near lesion samples, but not detected in samples
296 far from lesions or undiseased corals.

297
298 While only five unique ASV sequences were detected at statistically higher abundance in the
299 disease lesions of all three coral species, some groups were consistently detected in higher
300 abundances in disease lesions, albeit with varying ASV sequences. Epsilonbacteraeota,
301 especially Campylobacterales of the genus *Arcobacter*, were more abundant in lesions from all
302 three coral species (Figure S4), including seven ASVs in *M. cavernosa*, six ASVs in *D.*
303 *labyrinthiformis*, and two ASVs in *D. stokesii*. Only one of these *Arcobacter* ASVs was enriched
304 in more than one coral species (*M. cavernosa* and *D. labyrinthiformis*). In addition, other
305 Campylobacterales were enriched in disease lesions, including *Sulfurospirillum* and *Sulfurovum*
306 in *M. cavernosa*, *Thiovulum* in *D. labyrinthiformis*, and an Rs-M59_termite_group ASV in *D.*
307 *stokesii*. Likewise, Patescibacteria were also enriched in disease lesions of all three coral species.
308 Of the 39 ASVs classified as JGI_0000069-P22 in the phylum Patescibacteria, seven were
309 enriched in lesions. Only one ASV classified as JGI_0000069-P22 was enriched in more than
310 one coral species (*D. labyrinthiformis* and *D. stokesii*), which was 96% similar to a sequence

311 (GenBank Accession EU183997) recovered from heat-stressed *Rhopaloeides odorabile* sponge
312 (Webster et al., 2008). In addition, one ASV classified as Gracilibacteria in the phylum
313 Patescibacteria was enriched in lesions on *M. cavernosa* that is an exact match to a clone library
314 sequence (GenBank Accession KC527297) from a white plague disease infected *Pavona*
315 *duerdeni* coral from Thailand (Roder et al., 2014). One ASV classified as *Fusibacter*
316 (Clostridiales) enriched in lesions from both *M. cavernosa* and *D. labyrinthiformis* was 99.6%
317 identical to the ASV sequence that was differentially abundant in all three coral species. In
318 addition, the *Fusibacter* ASV enriched in lesions from both *M. cavernosa* and *D.*
319 *labyrinthiformis* was an exact match to three different clone library sequences (GenBank
320 Accessions MH341654, GU472060, EF089469) from Black Band Disease infected corals in the
321 Red Sea (Arotsker et al., 2015; Hadaidi et al., 2018).

322
323 In addition to the Flavobacteriales (Cryomorphaceae) ASV enriched in the disease lesions of all
324 three coral species, other Bacteroidetes ASVs were also consistently enriched in disease lesions
325 across all three coral species, including 16 ASVs classified as Bacteroidales, 12 from
326 Chitinophagales, 25 from Cytophagales, and 22 from Flavobacteriales. Of the 16 Bacteroidales
327 ASVs enriched in disease samples, six were exact matches to sequences previously detected in
328 coral diseases, including Black Band Disease in Caribbean *Pseudodiploria strigosa* (GenBank
329 Accession AY497296) (Frias-Lopez et al., 2004), white plague disease in *Porites lutea* from
330 Thailand (GenBank Accession KC527457, KC527469) (Roder et al., 2014), white plague disease
331 type II in Caribbean *Orbicella faveolata* (GenBank Accession FJ202346, FJ202437, FJ202774)
332 (Sunagawa et al., 2009), and Black Band Disease infected corals in the Red Sea (GenBank
333 Accession MH341668, MH341680) (Hadaidi et al., 2018). This included two ASVs with exact
334 matches to sequences from two different disease studies. In addition, one Bacteroidales ASV was
335 a close match (99.6% identical) to sequences (GenBank Accession MH341668, KC527457) from
336 these studies. Three Flavobacteriales ASVs were also matches to sequences previously detected
337 in coral diseases, including an exact match to an unpublished sequence (GenBank Accession
338 EU780277) associated with the disease margin of *Tubinaria mesenterina* and close matches
339 (99.6%) to sequences from Black Band Disease infected *Favia* sp. in the Red Sea (GenBank
340 Accession GU472416, GU471979) (Arotsker et al., 2015).

341
342 In addition to the *Planktotalea* (Rhodobacteraceae) enriched in the disease lesions of all three
343 coral species, five additional ASVs classified as Rhodobacteraceae were enriched in the disease
344 lesions of both *M. cavernosa* and *D. labyrinthiformis*, three of which match sequences previously
345 associated with coral disease. Two Rhodobacteraceae ASVs were exact matches to clone library
346 sequences from Black Band Disease (GenBank Accession DQ446109, MH341655) (Hadaidi et
347 al., 2018; Sekar et al., 2008) and one Rhodobacteraceae ASV was an exact match to a clone
348 library sequence from white plague disease type II (GenBank Accession FJ203176) (Sunagawa
349 et al., 2009).

350
351 In addition to the *Vibrio* ASV enriched in the disease lesions of all three coral species, one
352 additional *Vibrio* ASV was enriched in the disease lesions of *M. cavernosa* and *D.*
353 *labyrinthiformis* that was an exact match to a sequence (GenBank Accession MK168657)
354 identified as *V. coralliilyticus* strain 2214. While this particular strain is not known to be
355 pathogenic, other strains within this species are known pathogens of coral and shellfish
356 (Ushijima et al., 2014, 2018). It should be noted that this *Vibrio* ASV is 253 bp long and is an

357 equally good match to dozens of other *Vibrio* species and strains, making an exact identification
358 of *Vibrio* strain impossible with the V4 region of the 16S small subunit ribosomal gene.

359

360 DISCUSSION

361

362 Consistent with other microbial investigations of coral diseases, we found a shift in the
363 microorganisms present in stony coral tissue loss disease (SCTLD) disease lesions versus
364 apparently healthy coral tissue. The disruption, or dysbiosis, of the normal, healthy microbiota,
365 which in corals is often dominated by a small number of bacterial groups, is often paralleled by
366 an increase in microbial diversity. This increased diversity is often stochastic, so that each altered
367 microbiome is unique (Zaneveld et al., 2017), while the original healthy microbial community
368 has a stable composition of lower diversity. In the current study, this pattern held true for *M.*
369 *cavernosa*, from which we sampled several undiseased colonies. The undiseased colonies as well
370 as apparently healthy tissue on diseased colonies sampled in summer from *M. cavernosa* were
371 dominated by the gammaproteobacterial genera *Halomonas* and *Shewanella*. In contrast, the
372 apparently healthy tissue on diseased colonies sampled in winter were much more variable, like
373 the microbiota associated with lesions. In *D. labyrinthiformis* and *D. stokesii*, both of which were
374 sampled in winter, apparently healthy tissue on diseased colonies as well as disease lesions
375 hosted diverse microbial communities, and no difference was seen in the dispersion of beta
376 diversity between healthy and diseased tissue on diseased colonies. This suggests that when
377 sampling when the disease appears to be more active and when sampling from more susceptible
378 coral species (Florida Keys National Marine Sanctuary, 2018), the microbiome of the entire
379 colony is already disrupted by SCTLD. The December sampling also followed extreme
380 environmental conditions and changes in salinity, turbidity, and sedimentation caused by
381 Hurricane Irma in September 2017, which could have also affected microbiome composition of
382 all the corals sampled in winter.

383

384 A single potential pathogen was not identified; however, five unique ASV sequences were
385 enriched in the SCTLD lesions of three different coral species, and all but one of these sequences
386 were exact matches to sequences previously associated with coral disease. These sequences were
387 classified as Flavobacteriales, Clostridiales, Rhodobacterales, Alteromonadales, and Vibrionales,
388 groups which have all been consistently detected in coral diseases, as well as in apparently
389 healthy coral tissues. Previous investigations of white plague disease type II in Caribbean *O.*
390 *faveolata* also found an enrichment of the same five bacterial orders (Sunagawa et al., 2009). The
391 *Fusibacter* ASV sequence enriched in the lesions of all three coral species is an exact match to a
392 sequence from coral infected with white plague disease in the Pacific (Roder et al., 2014). The
393 unique *Planktotalea* sequence (Rhodobacterales) was an exact match to an unpublished sequence
394 previously detected in a white-plague-type outbreak in the Gulf of Mexico, and the *Vibrio* ASV
395 sequence enriched in the lesions of all three coral species is an exact match to a sequence
396 enriched in the white plague disease type II lesions from the Sunagawa *et al.* study. However, the
397 enrichment of these groups is not confined to “white syndrome” coral diseases. Previous work
398 has also identified the enrichment of Flavobacteriales, Clostridiales, Rhodobacterales,
399 Alteromonadales, and Vibrionales associated with Black Band Disease mats (Miller and
400 Richardson, 2011). Indeed, ASV sequences enriched in disease lesions of SCTLD were also
401 exact matches to sequences enriched in Black Band Disease from geographically disparate
402 tropical regions.

403
404 Collectively, the presence of similar microbes in several coral diseases from around the world
405 can be interpreted in multiple ways. These microbes may be the pathogens that initiate coral
406 disease, however, their presence in multiple, visually distinct coral diseases suggests that this
407 may not be the case. In addition to primary pathogens, these microbes may be opportunistic or
408 secondary pathogens that infect immunocompromised or otherwise vulnerable corals (Lesser et
409 al., 2007), such as those stressed by elevated water temperatures or sedimentation. The current
410 outbreak was preceded by both higher sea surface temperatures in the summer and winter of
411 2014 (Manzello, 2015) and higher sedimentation on corals due to dredging operations between
412 2013 and 2015 in the channel at the Port of Miami (Miller et al., 2016). Coral microbiome
413 diversity increases with environmental stresses such as climate change, water pollution, and
414 overfishing and is accompanied by an increase of bacteria classified as *Vibrionales*,
415 *Flavobacteriales*, *Rhodobacterales*, *Alteromonadales*, *Rhizobiales*, *Rhodospirillales*, and
416 *Desulfovibrionales* (McDevitt-Irwin et al., 2017). Here, we detected an enrichment of the first
417 four of these bacterial orders in the disease lesions of SCTLD. Organisms that are enriched in the
418 disease state may be resident bacteria that are already present in healthy corals and grow in
419 response to changes in the host during disease progression. Recent experimental infections of
420 *Acropora cervicornis* with white plague disease revealed both colonizers present in the diseased
421 coral tissue that transferred to and increased in newly infected corals as well as responders that
422 were present in healthy tissue and increased during disease progression in newly infected corals
423 (Gignoux-Wolfsohn et al., 2017).

424
425 Alternatively, the disease-enriched microbes may be saprophytic colonizers that increase in
426 numbers as decaying coral tissue fuels their growth (Egan and Gardiner, 2016). In particular,
427 many of the enriched groups detected here are associated with the anoxic conditions that
428 accompany tissue decay, such as the Epsilonbacteraeota (Campbell et al., 2006), Clostridiales
429 such as *Fusibacter* (Ravot et al., 1999), and Patescibacteria. The superphylum Patescibacteria is
430 comprised of three environmentally widespread phyla that to-date have no cultured
431 representatives, but metagenomic analyses have revealed that Patescibacteria lack TCA cycle
432 genes and therefore must be strict anaerobic fermenters (Wrighton et al., 2012) and that they tend
433 to have reduced genomes, characteristic of parasitic or symbiotic microbes (Sánchez-Osuna et
434 al., 2017). Our study may be the first to specifically detect this relatively newly named
435 superphylum (Rinke et al., 2013) as present in coral disease lesions; however, one
436 Patescibacteria ASV sequence detected here matched a clone library sequence from white plague
437 disease infected *Pavona duerdeni* coral from Thailand (Roder et al., 2014), suggesting that these
438 cryptic yet environmentally widespread taxa may also be common in coral disease.

439
440 The enrichment of disease-associated bacteria in the lesions of corals with SCTLD is not
441 definitive proof that the pathogen is bacterial. However, disease progression in laboratory and
442 field trials appears to slow or stop with the application of antibiotics (Florida Keys National
443 Marine Sanctuary, 2018), strongly suggesting that bacteria are involved with disease progression.
444 The isolation and culturing of a bacterial pathogen(s) would be invaluable to the development of
445 diagnostic methods and targeted mitigation efforts. Therefore, attempts at isolation of the
446 potential pathogen and establishing new infections with isolates is ongoing by our group. In
447 addition, the pathogen or pathogens responsible for SCTLD may be viral or eukaryotic, which
448 may only be uncovered through techniques such as shotgun metagenomics. Metagenomic

449 analysis of samples from corals impacted by SCTL D may provide additional information
450 regarding the nature of the pathogen, whether bacterial, viral, or eukaryotic, as well as the
451 mechanisms used by the pathogen(s) to infect and kill corals.

452
453

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464

465

466 AUTHOR CONTRIBUTIONS STATEMENT

467

468 Experimental design was determined collectively by JM, GA, CH, BU, and VP. Field collections
469 were conducted by JM and VP. Molecular lab work and analysis was performed by JM and JC.
470 JM drafted the manuscript and all authors contributed to interpretations and revisions.

471

472

473 CONFLICT OF INTEREST STATEMENT

474

475 The authors declare no conflicts of interest associated with this project.

476

477

478 CONTRIBUTION TO THE FIELD STATEMENT

479

480 The Caribbean is known as a hotspot for coral diseases that have dramatically reduced
481 populations of reef-building corals. Currently, half of the coral species in the Florida Reef Tract
482 are impacted by an ongoing outbreak of stony coral tissue loss disease that was first detected in
483 late 2014. This work presents the first characterization of the changes in microbiological
484 communities associated with the disease in four stony coral species. Disease lesions consistently
485 supported microbial communities enriched with bacterial taxa that have previously been detected
486 in coral diseases, including white-plague-type diseases and Black Band Disease. Enriched taxa in
487 disease lesions may be causing the primary infection or may be secondary infections. Additional
488 studies are needed to determine the roles of bacteria enriched in diseased tissue and to evaluate
489 the potential for non-bacterial pathogens such as viruses.

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495 REFERENCES

496

- 497 Apprill, A., McNally, S., Parsons, R., and Weber, L. (2015). Minor revision to V4 region SSU
498 rRNA 806R gene primer greatly increases detection of SAR11 bacterioplankton. *Aquat.*
499 *Microb. Ecol.* 75, 129–137.
- 500 Aronson, R. B., and Precht, W. F. (2001). “White-band disease and the changing face of
501 Caribbean coral reefs,” in *The Ecology and Etiology of Newly Emerging Marine Diseases*,
502 ed. J. W. Porter (Dordrecht: Springer Netherlands), 25–38.
- 503 Arotsker, L., Kramarsky-Winter, E., Ben-Dov, E., Siboni, N., and Kushmaro, A. (2015).
504 Changes in the bacterial community associated with black band disease in a Red Sea coral,
505 *Favia* sp., in relation to disease phases. *Dis. Aquat. Organ.* 116, 47–58.
- 506 Bayer, T., Neave, M. J., Alsheikh-Hussain, A., Aranda, M., Yum, L. K., Mincer, T., et al. (2013).
507 The microbiome of the Red Sea coral *Stylophora pistillata* is dominated by tissue-
508 associated *Endozoicomonas* bacteria. *Appl. Environ. Microbiol.* 79, 4759–4762.
- 509 Brinkhuis, V., and Huebner, L. (2016). Grecian Rocks Disease Outbreak 7/16/2016. Florida Fish
510 and Wildlife Conservation Commission.
- 511 Callahan, B. J., McMurdie, P. J., and Holmes, S. P. (2017). Exact sequence variants should
512 replace operational taxonomic units in marker-gene data analysis. *ISME J.* 11, 2639–2643.
- 513 Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., and Holmes, S. P.
514 (2016). DADA2: High-resolution sample inference from Illumina amplicon data. *Nat.*
515 *Methods* 13, 581–583.
- 516 Campbell, B. J., Engel, A. S., Porter, M. L., and Takai, K. (2006). The versatile epsilon-
517 proteobacteria: key players in sulphidic habitats. *Nat. Rev. Microbiol.* 4, 458–468.
- 518 Caporaso, J. G., Lauber, C. L., Walters, W. A., Berg-Lyons, D., Huntley, J., Fierer, N., et al.
519 (2012). Ultra-high-throughput microbial community analysis on the Illumina HiSeq and
520 MiSeq platforms. *ISME J.* 6, 1621–1624.
- 521 Denner, E. B. M., Smith, G. W., Busse, H.-J., Schumann, P., Narzt, T., Polson, S. W., et al.
522 (2003). *Aurantimonas coralicida* gen. nov., sp. nov., the causative agent of white plague
523 type II on Caribbean scleractinian corals. *Int. J. Syst. Evol. Microbiol.* 53, 1115–1122.
- 524 Dixon, P. (2003). VEGAN, a package of R functions for community ecology. *J. Veg. Sci.* 14,
525 927–930.
- 526 Egan, S., and Gardiner, M. (2016). Microbial Dysbiosis: Rethinking Disease in Marine
527 Ecosystems. *Front. Microbiol.* 7, 991.
- 528 Everett, K. D. E., Thao, M., Horn, M., Dyszynski, G. E., and Baumann, P. (2005). Novel
529 chlamydiae in whiteflies and scale insects: endosymbionts “*Candidatus Fritschea bemisiae*”

- 530 strain Falk and “Candidatus Fritschea eriococci” strain Elm. *Int. J. Syst. Evol. Microbiol.*
531 55, 1581–1587.
- 532 Florida Keys National Marine Sanctuary (2018). Case Definition: Stony Coral Tissue Loss
533 Disease (SCTLD). National Oceanic and Atmospheric Administration Available at:
534 [https://nmsfloridakeys.blob.core.windows.net/floridakeys-prod/media/docs/20181002-](https://nmsfloridakeys.blob.core.windows.net/floridakeys-prod/media/docs/20181002-stony-coral-tissue-loss-disease-case-definition.pdf)
535 [stony-coral-tissue-loss-disease-case-definition.pdf](https://nmsfloridakeys.blob.core.windows.net/floridakeys-prod/media/docs/20181002-stony-coral-tissue-loss-disease-case-definition.pdf).
- 536 Frias-Lopez, J., Klaus, J. S., Bonheyo, G. T., and Fouke, B. W. (2004). Bacterial community
537 associated with black band disease in corals. *Appl. Environ. Microbiol.* 70, 5955–5962.
- 538 Gao, F., Al-Saari, N., Rohul Amin, A. K. M., Sato, K., Mino, S., Suda, W., et al. (2016). *Vibrio*
539 *ishigakensis* sp. nov., in *Halioticoli* clade isolated from seawater in Okinawa coral reef area,
540 Japan. *Syst. Appl. Microbiol.* 39, 330–335.
- 541 Garren, M., Raymundo, L., Guest, J., Harvell, C. D., and Azam, F. (2009). Resilience of coral-
542 associated bacterial communities exposed to fish farm effluent. *PLoS One* 4, e7319.
- 543 Gignoux-Wolfsohn, S. A., Aronson, F. M., and Vollmer, S. V. (2017). Complex interactions
544 between potentially pathogenic, opportunistic, and resident bacteria emerge during infection
545 on a reef-building coral. *FEMS Microbiol. Ecol.* 93. doi:10.1093/femsec/fix080.
- 546 Gil-Agudelo, D. L., Smith, G. W., and Weil, E. (2006). The white band disease type II pathogen
547 in Puerto Rico. *Revista de Biología Tropical* 54, 59–67.
- 548 Gloor, G. B., Macklaim, J. M., Pawlowsky-Glahn, V., and Egozcue, J. J. (2017). Microbiome
549 datasets are compositional: and this is not optional. *Front. Microbiol.* 8, 2224.
- 550 Hadaidi, G., Ziegler, M., Shore-Maggio, A., Jensen, T., Aeby, G., and Voolstra, C. R. (2018).
551 Ecological and molecular characterization of a coral black band disease outbreak in the Red
552 Sea during a bleaching event. *PeerJ* 6, e5169.
- 553 Har, J. Y., Helbig, T., Lim, J. H., Fernando, S. C., Reitzel, A. M., Penn, K., et al. (2015).
554 Microbial diversity and activity in the *Nematostella vectensis* holobiont: insights from 16S
555 rRNA gene sequencing, isolate genomes, and a pilot-scale survey of gene expression. *Front.*
556 *Microbiol.* 6, 818.
- 557 Huggett, M. J., and Apprill, A. (2018). Coral microbiome database: Integration of sequences
558 reveals high diversity and relatedness of coral-associated microbes. *Environ. Microbiol.*
559 *Rep.* doi:10.1111/1758-2229.12686.
- 560 Klaus, J. S., Janse, I., and Fouke, B. W. (2011). Coral black band disease microbial communities
561 and genotypic variability of the dominant Cyanobacteria (CD1C11). *Bull. Mar. Sci.* 87,
562 795–821.
- 563 Lesser, M. P., Bythell, J. C., Gates, R. D., Johnstone, R. W., and Hoegh-Guldberg, O. (2007).
564 Are infectious diseases really killing corals? Alternative interpretations of the experimental
565 and ecological data. *J. Exp. Mar. Bio. Ecol.* 346, 36–44.

- 566 Love, M., Anders, S., and Huber, W. (2014). Differential analysis of count data--the DESeq2
567 package. *Genome Biol.* 15, 10–1186.
- 568 Lunz, K., Landsberg, J., Kiryu, Y., and Brinkhuis, V. (2017). Investigation of the Coral Disease
569 Outbreak Affecting Scleractinian Coral Species along the Florida Reef Tract. Florida
570 Department of Environmental Protection Available at:
571 <https://floridadep.gov/sites/default/files/FWRI-Diseases-Report.pdf>.
- 572 Manzello, D. P. (2015). Rapid recent warming of coral reefs in the Florida Keys. *Sci. Rep.* 5,
573 16762.
- 574 Martin, M. (2011). Cutadapt removes adapter sequences from high-throughput sequencing reads.
575 *EMBnet.journal* 17, 10–12.
- 576 McDevitt-Irwin, J. M., Baum, J. K., Garren, M., and Vega Thurber, R. L. (2017). Responses of
577 Coral-Associated Bacterial Communities to Local and Global Stressors. *Frontiers in*
578 *Marine Science* 4, 262.
- 579 McMurdie, P. J., and Holmes, S. (2013). phyloseq: an R package for reproducible interactive
580 analysis and graphics of microbiome census data. *PLoS One* 8, e61217.
- 581 Mera, H., and Bourne, D. G. (2018). Disentangling causation: complex roles of coral-associated
582 microorganisms in disease. *Environ. Microbiol.* 20, 431–449.
- 583 Miller, A. W., and Richardson, L. L. (2011). A meta-analysis of 16S rRNA gene clone libraries
584 from the polymicrobial black band disease of corals. *FEMS Microbiol. Ecol.* 75, 231–241.
- 585 Miller, M. W., Karazsia, J., Groves, C. E., Griffin, S., Moore, T., Wilber, P., et al. (2016).
586 Detecting sedimentation impacts to coral reefs resulting from dredging the Port of Miami,
587 Florida USA. *PeerJ* 4, e2711.
- 588 Muller, E., van Woesik, R., and Sartor, C. (2018). Spatial Epidemiology Modeling of the Florida
589 Coral Disease Outbreak. Florida Department of Environmental Protection Available at:
590 [https://floridadep.gov/sites/default/files/Spatial-Epidemiology-Modeling-of-the-FRT-Coral-](https://floridadep.gov/sites/default/files/Spatial-Epidemiology-Modeling-of-the-FRT-Coral-Disease-Outbreak.pdf)
591 [Disease-Outbreak.pdf](https://floridadep.gov/sites/default/files/Spatial-Epidemiology-Modeling-of-the-FRT-Coral-Disease-Outbreak.pdf).
- 592 Palarea-Albaladejo, J., and Martín-Fernández, J. A. (2015). zCompositions — R package for
593 multivariate imputation of left-censored data under a compositional approach.
594 *Chemometrics Intellig. Lab. Syst.* 143, 85–96.
- 595 Parada, A. E., Needham, D. M., and Fuhrman, J. A. (2016). Every base matters: assessing small
596 subunit rRNA primers for marine microbiomes with mock communities, time series and
597 global field samples. *Environ. Microbiol.* 18, 1403–1414.
- 598 Patterson, K. L., Porter, J. W., Ritchie, K. B., Polson, S. W., Mueller, E., Peters, E. C., et al.
599 (2002). The etiology of white pox, a lethal disease of the Caribbean elkhorn coral, *Acropora*
600 *palmata*. *Proc. Natl. Acad. Sci. U. S. A.* 99, 8725–8730.

- 601 Precht, W. F., Gintert, B. E., Robbart, M. L., Fura, R., and van Woesik, R. (2016).
602 Unprecedented disease-related coral mortality in Southeastern Florida. *Sci. Rep.* 6, 31374.
- 603 Ravot, G., Magot, M., Fardeau, M. L., Patel, B. K., Thomas, P., Garcia, J. L., et al. (1999).
604 *Fusibacter paucivorans* gen. nov., sp. nov., an anaerobic, thiosulfate-reducing bacterium
605 from an oil-producing well. *Int. J. Syst. Bacteriol.* 49 Pt 3, 1141–1147.
- 606 Richardson, L. L., Goldberg, W. M., Kuta, K. G., Aronson, R. B., Smith, G. W., Ritchie, K. B.,
607 et al. (1998). Florida's mystery coral-killer identified. *Nature* 392, 557.
- 608 Rinke, C., Schwientek, P., Sczyrba, A., Ivanova, N. N., Anderson, I. J., Cheng, J.-F., et al.
609 (2013). Insights into the phylogeny and coding potential of microbial dark matter. *Nature*
610 499, 431–437.
- 611 Rippe, J. P., Kriefall, N. G., Davies, S. W., and Castillo, K. D. (2019). Differential disease
612 incidence and mortality of inner and outer reef corals of the upper Florida Keys in
613 association with a white syndrome outbreak. *Bulletin of Marine Science* 95, 305–316.
- 614 Ritchie, K. B., and Smith, G. W. (1998). Type II White-Band Disease. *Revista de Biología*
615 *Tropical* 46, 199–203.
- 616 Roder, C., Arif, C., Bayer, T., Aranda, M., Daniels, C., Shibl, A., et al. (2014). Bacterial
617 profiling of White Plague Disease in a comparative coral species framework. *ISME J.* 8,
618 31–39.
- 619 Sánchez-Osuna, M., Barbé, J., and Erill, I. (2017). Comparative genomics of the DNA damage-
620 inducible network in the Patescibacteria. *Environ. Microbiol.* 19, 3465–3474.
- 621 Sekar, R., Kaczmarek, L. T., and Richardson, L. L. (2008). Microbial community composition
622 of black band disease on the coral host *Siderastrea siderea* from three regions of the wider
623 Caribbean. *Mar. Ecol. Prog. Ser.* 362, 85–98.
- 624 Sunagawa, S., DeSantis, T. Z., Piceno, Y. M., Brodie, E. L., DeSalvo, M. K., Voolstra, C. R., et
625 al. (2009). Bacterial diversity and White Plague Disease-associated community changes in
626 the Caribbean coral *Montastraea faveolata*. *ISME J.* 3, 512–521.
- 627 Sutherland, K. P., Porter, J. W., Turner, J. W., Thomas, B. J., Looney, E. E., Luna, T. P., et al.
628 (2010). Human sewage identified as likely source of white pox disease of the threatened
629 Caribbean elkhorn coral, *Acropora palmata*. *Environ. Microbiol.* 12, 1122–1131.
- 630 Ushijima, B., Richards, G. P., Watson, M. A., Schubiger, C. B., and Häse, C. C. (2018). Factors
631 affecting infection of corals and larval oysters by *Vibrio coralliilyticus*. *PLoS One* 13,
632 e0199475.
- 633 Ushijima, B., Videau, P., Burger, A. H., Shore-Maggio, A., Runyon, C. M., Sudek, M., et al.
634 (2014). *Vibrio coralliilyticus* strain OCN008 is an etiological agent of acute *Montipora*
635 white syndrome. *Appl. Environ. Microbiol.* 80, 2102–2109.

- 636 Walton, C. J., Hayes, N. K., and Gilliam, D. S. (2018). Impacts of a Regional, Multi-Year, Multi-
637 Species Coral Disease Outbreak in Southeast Florida. *Frontiers in Marine Science* 5, 323.
- 638 Webster, N. S., Cobb, R. E., and Negri, A. P. (2008). Temperature thresholds for bacterial
639 symbiosis with a sponge. *ISME J.* 2, 830–842.
- 640 Weil, E., Smith, G., and Gil-Agudelo, D. L. (2006). Status and progress in coral reef disease
641 research. *Dis. Aquat. Organ.* 69, 1–7.
- 642 Wickham, H. (2016). *ggplot2: Elegant Graphics for Data Analysis*. Springer.
- 643 Wrighton, K. C., Thomas, B. C., Sharon, I., Miller, C. S., Castelle, C. J., VerBerkmoes, N. C., et
644 al. (2012). Fermentation, hydrogen, and sulfur metabolism in multiple uncultivated bacterial
645 phyla. *Science* 337, 1661–1665.
- 646 Yilmaz, P., Parfrey, L. W., Yarza, P., Gerken, J., Pruesse, E., Quast, C., et al. (2014). The
647 SILVA and “All-species Living Tree Project (LTP)” taxonomic frameworks. *Nucleic Acids*
648 *Res.* 42, D643–8.
- 649 Zaneveld, J. R., McMinds, R., and Vega Thurber, R. (2017). Stress and stability: applying the
650 Anna Karenina principle to animal microbiomes. *Nat Microbiol* 2, 17121.
- 651

652 TABLES

653

654 Table 1. Amplicon sequence variants detected as differentially abundant in stony coral tissue loss
 655 disease lesions compared to apparently healthy tissue on diseased corals in *Montastraea*
 656 *cavernosa*, *Diploria labyrinthiformis*, and *Dichocoenia stokesii*.

657

Amplicon sequence variant	Family	Genus	Source of exact sequence match in Genbank	Genbank Accession of exact sequence match
TACGGAGGGTCCAAGCGTTATCCGG ATTTATTGGGTTTAAAGGGTCCGTA GGCGGGGTTTTAAGTCAGTGGTGAA AGCCTACAGCTCAACTGTAGAAGCTG CCATTGAAACTGGAAGCTCTGAATG TGATTGAGGTAGGCGGAATATGTCA TGTAGCGGTGAAATGCTTAGATATG ACATAGAACACCGATAGCGAAGGC AGCTTACCAGTCATTATTGACGCT GATGGACGAAAGCGTGGGGAGCGA ACAGG	Cryomorphaeae	unclassified	dinoflagellate	KY281634
TACGTAGGGGGCAGCGTTATCCG GAATCACTGGGCGTAAAGGGTGCG TAGGCGGTTTTTCAAGTCAGAAGTG AAAGGCTATGGCTCAACCATAGTA AGCTTTTGAAACTGTTAAACTTGAG TGCAGGAGAGGAAAGTGGAATTCC TAGTGTAGAGGTGAAATTCGTAGAT ATTAGGAGGAACACCAGTGGCGAA GGCGACTTCTGGACTGTAAGTAC GCTGAGGCACGAAAGCGTGGGGAG CGAACAGG	Clostridiales Family_XII	<i>Fusibacter</i>	Pacific white plague disease, <i>Porites lutea</i>	KC527495
TACGGAGGGGGTTAGCGTTGTTCGG AATTACTGGGCGTAAAGCGCACGT AGGCGGATTAGTCAGTCAGAGGTG AAATCCCAGGGCTCAACCCTGGAA CTGCCTTTGATACTGCTAGTCTTGA GTTTCGAGAGAGGTGAGTGGGAATTC CGAGTGTAGAGGTGAAATTCGTAG ATATTCGGAGGAACACCAGTGGCG AAGGCGGCTCACTGGCTCGATACTG ACGCTGAGGTGCGAAAGTGTGGGG AGCAAACAGG	Rhodobacteraceae	<i>Planktotalea</i>	white-plague-like outbreak, Flower Garden Banks National Marine Sanctuary	EF033448
TACGGAGGGTGCGAGCGTTAATCG GAATTACTGGGCGTAAAGCGTACG CAGGCGGTTAAGTCAAGTCAGATGTG AAAGCCCCGGGCTCAACCTGGGAA CTGCATTTGAAACTGGCTAACTAGA GTGCGACAGAGGGTGGTAGAATTT CAGGTGTAGCGGTGAAATGCGTAG AGATCTGAAGGAATACCGATGGCG AAGGCAGCCACCTGGGTGACACT GACGCTCATGTACGAAAGCGTGGG TAGCAAACAGG	Pseudoalteromonadaceae	<i>Algicola</i>	Black Band Disease, Red Sea coral; Caribbean disease-associated <i>Orbicella faveolata</i>	GU471978; FJ403097
TACGGAGGGTGCGAGCGTTAATCG GAATTACTGGGCGTAAAGCGCATG CAGGTGGTTTGTAAAGTCAGATGTG AAAGCCCTGGGCTCAACCCGGGAA GGTCATTTGAAACTGGCAAGCTAGA GTACTGTAGAGGGGGTGAATTTTC AGGTGTAGCGGTGAAATGCGTAGA GATCTGAAGGAATACCGTGGCGA AGGCGGCCCCCTGGACAGATACTG CACTCAGATGCGAAAGCGTGGGG AGCAAACAGG	Vibrionaceae	<i>Vibrio</i>	Black Band Disease, <i>Pseudodiploria strigosa</i>	HM768606

658 FIGURE LEGENDS

659

660 Figure 1. Stony coral tissue loss disease appearance in three coral species from the Florida Reef
661 Tract: *Montastraea cavernosa* (A), *Diploria labyrinthiformis* (B), and *Dichocoenia stokesii* (C).

662

663 Figure 2. Principal component analysis of microbial community structure in disease lesions
664 (DD), apparently healthy tissue on diseased corals (DH), and apparently healthy neighboring
665 corals with no signs of disease (H).

666

667 Figure 3. Relative abundance of amplicon sequence variants, colored by Class, in undiseased
668 neighboring corals, apparently healthy tissue far from the disease lesion, apparently healthy
669 tissue near the disease lesion, and disease lesions in *Montastraea cavernosa* (A), *Orbicella*
670 *faveolata* (B), *Diploria labyrinthiformis* (C), and *Dichocoenia stokesii* (D) with stony coral tissue
671 loss disease.

672

673 Figure 4. The dispersion of beta diversity shown as the distance to the centroid. Panel (A)
674 compares dispersion of beta diversity in microbial communities from disease lesions (DD),
675 apparently healthy tissue on diseased corals (DH), and apparently healthy neighboring corals
676 with no signs of disease (H), with points colored to indicate coral species. Panel (B) compares
677 dispersion of beta diversity in microbial communities from *Montastraea cavernosa*, *Orbicella*
678 *faveolata*, *Diploria labyrinthiformis*, and *Dichocoenia stokesii*, with points colored to indicate
679 disease condition.

680

681 Figure 5. Relative abundance of the three amplicon sequence variants that were differentially
682 abundant in disease lesions versus apparently healthy (non-lesion) tissues of *Montastraea*
683 *cavernosa* (M_{cav}), *Orbicella faveolata* (O_{fav}), *Diploria labyrinthiformis* (D_{lab}), and
684 *Dichocoenia stokesii* (D_{sto}).

685

686

687 Supplemental Information:

688

689 Figure S1. Relative abundance of amplicon sequence variants, colored by Family, in undiseased
690 neighboring corals, apparently healthy tissue far from the disease lesion, apparently healthy
691 tissue near the disease lesion, and disease lesions in *Montastraea cavernosa* with stony coral
692 tissue loss disease. Samples with names beginning with PB were collected in July 2017 and
693 samples with names beginning with a number were collected December 2017.

694

695 Figure S2. Relative abundance of amplicon sequence variants, colored by Family, in undiseased
696 neighboring corals, apparently healthy tissue far from the disease lesion, apparently healthy
697 tissue near the disease lesion, and disease lesions in *Diploria labyrinthiformis* with stony coral
698 tissue loss disease.

699

700 Figure S3. Relative abundance of amplicon sequence variants, colored by Family, in apparently
701 healthy tissue far from the disease lesion, apparently healthy tissue near the disease lesion, and
702 disease lesions in *Dichocoenia stokesii* with stony coral tissue loss disease.

703

704 Figure S4. Differentially abundant amplicon sequence variants (ASVs) in stony coral tissue loss
705 disease. Positive log₂FoldChange values correspond to ASVs that are more abundant in disease
706 lesions compared to apparently healthy tissue on diseased corals in *Montastraea cavernosa*.

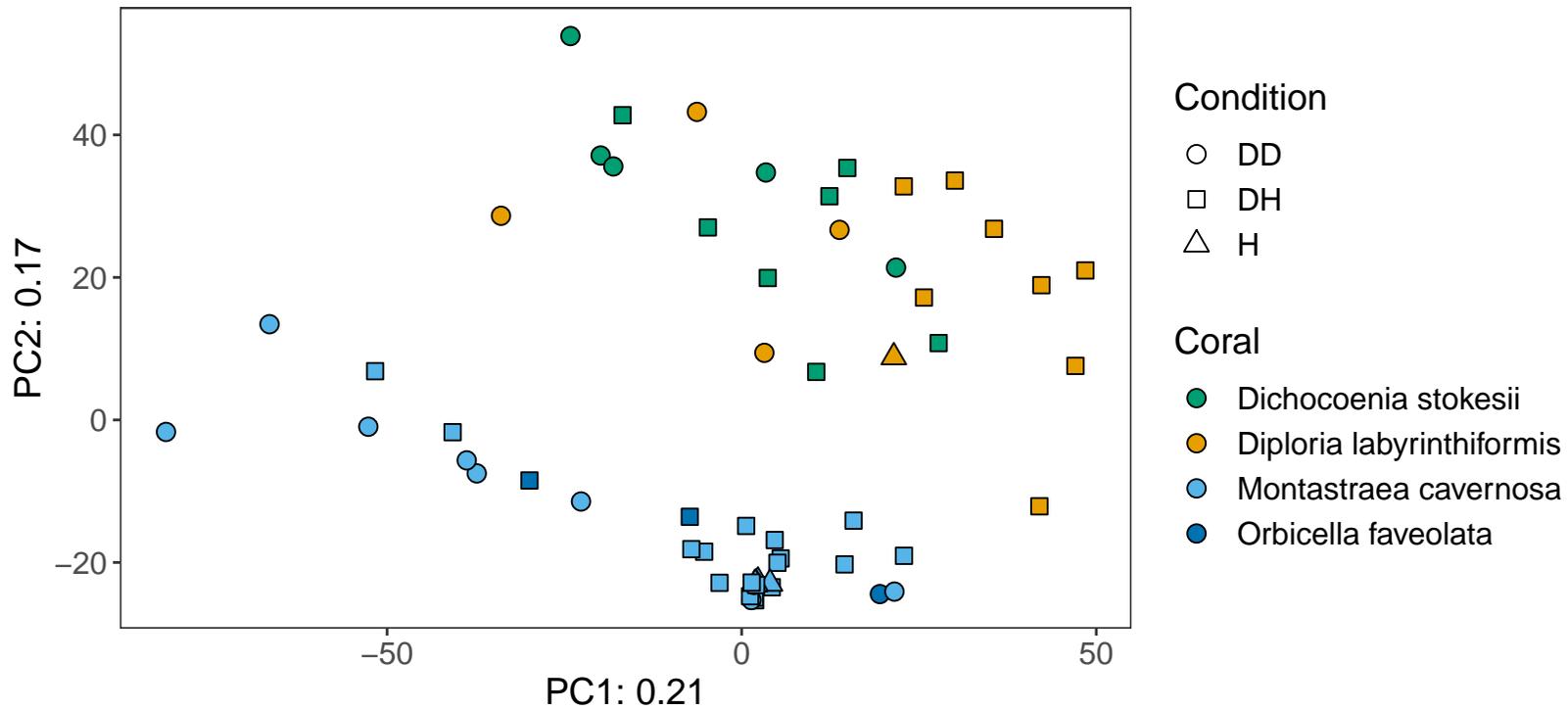
707
708 Figure S5. Differentially abundant amplicon sequence variants (ASVs) in stony coral tissue loss
709 disease. Positive log₂FoldChange values correspond to ASVs that are more abundant in disease
710 lesions compared to apparently healthy tissue on diseased corals in *Diploria labyrinthiformis*.

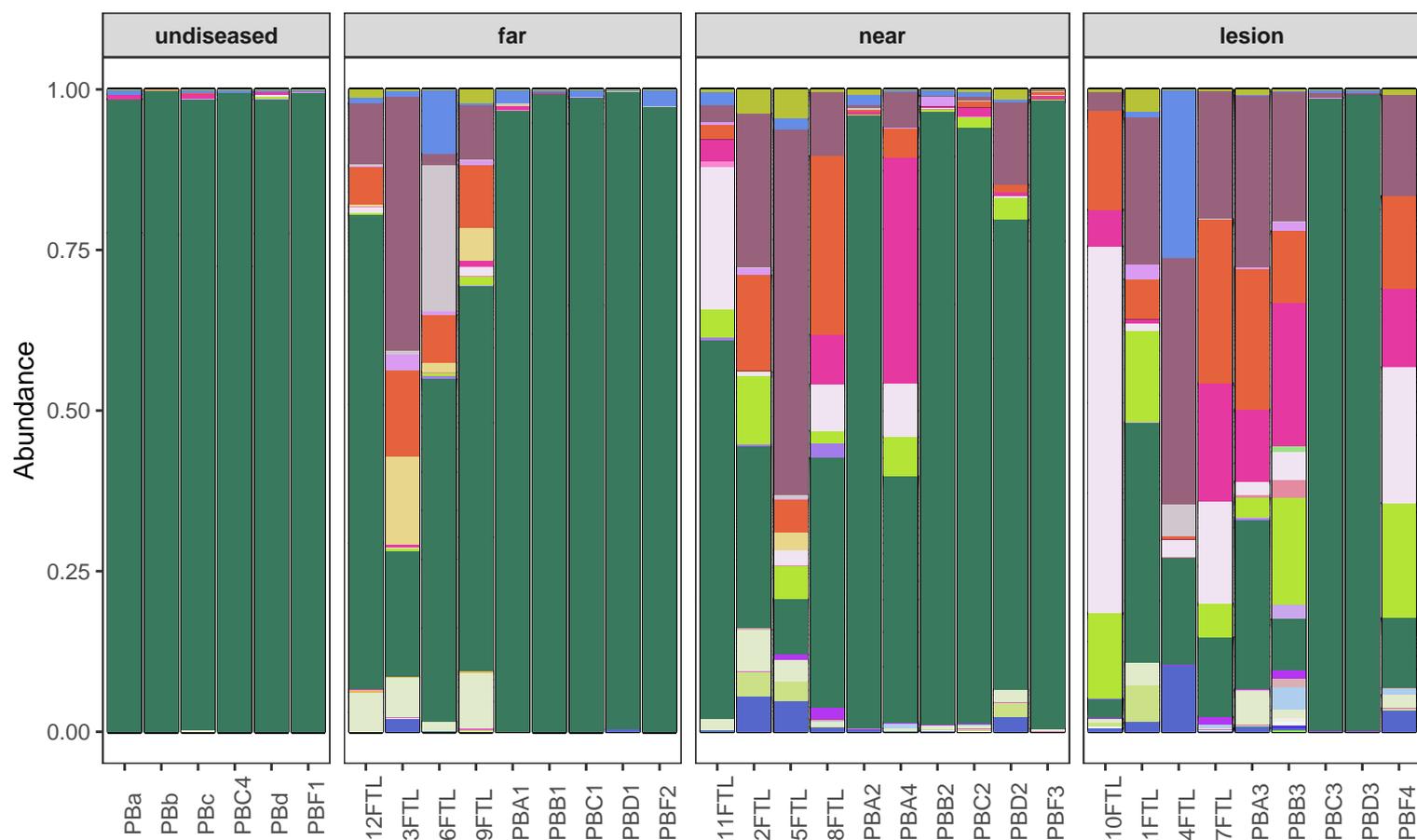
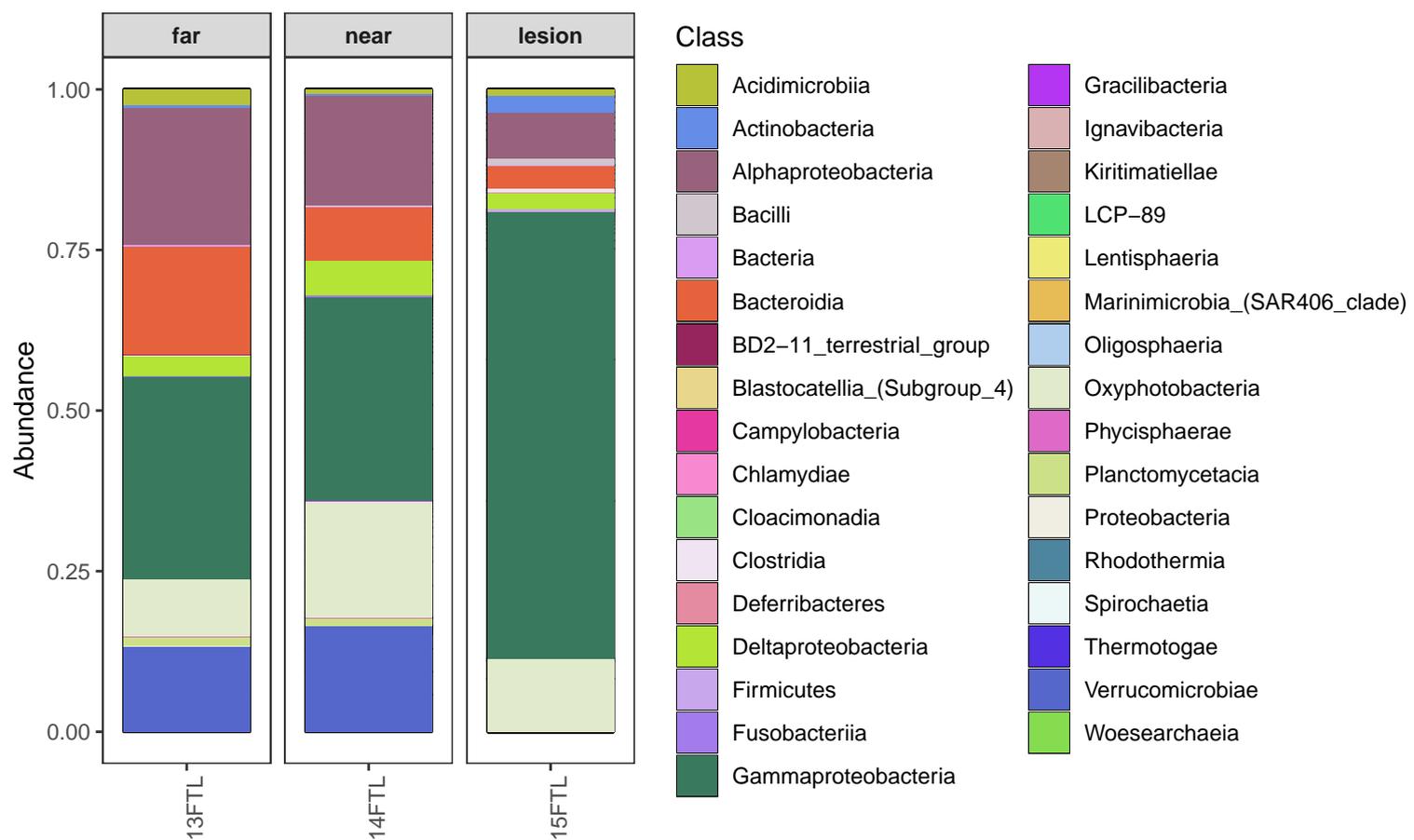
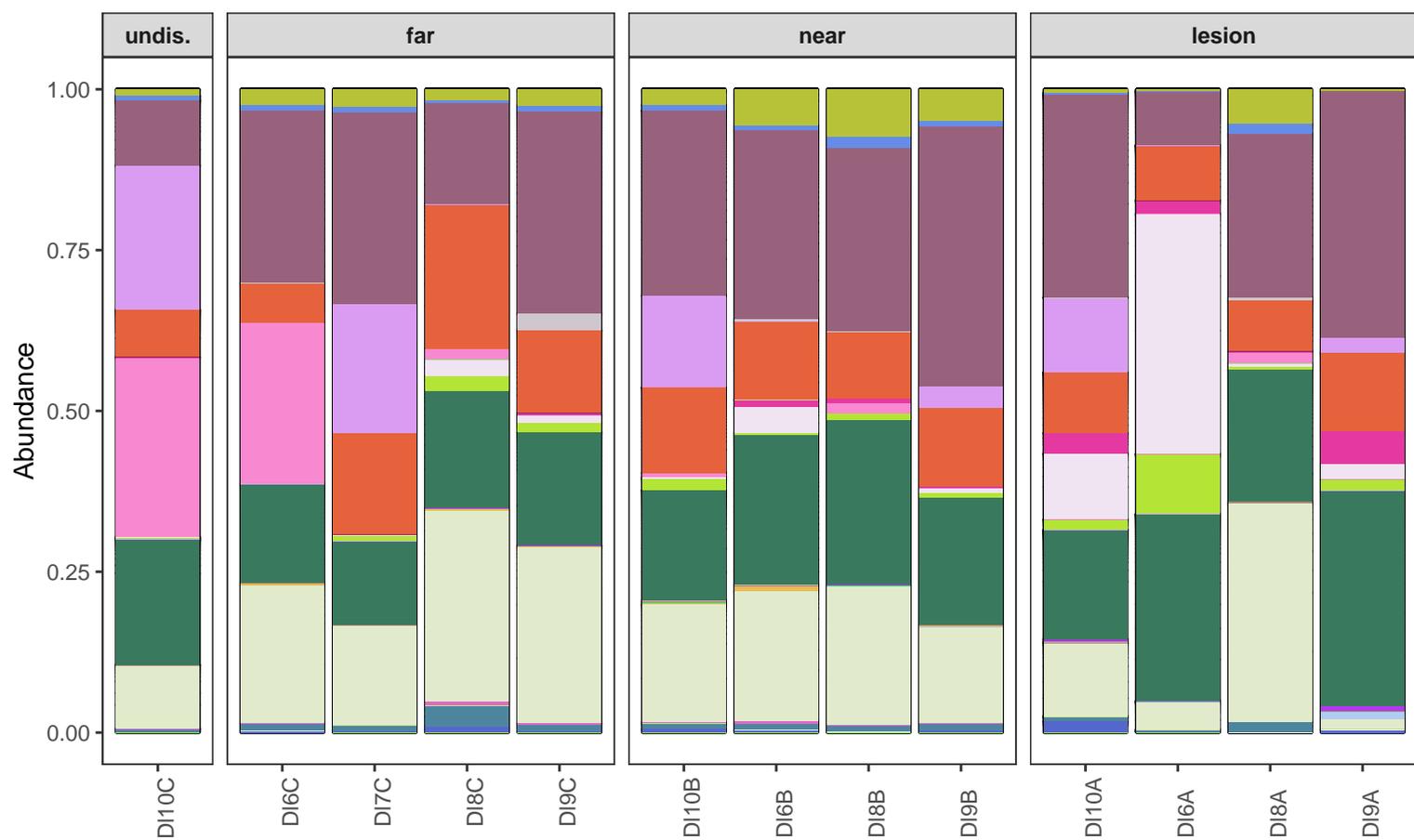
711
712 Figure S6. Differentially abundant amplicon sequence variants (ASVs) in stony coral tissue loss
713 disease. Positive log₂FoldChange values correspond to ASVs that are more abundant in disease
714 lesions compared to apparently healthy tissue on diseased corals in *Dichocoenia stokesii*.

715
716 Table S1. Sample metadata for microbial communities collected from diseased and apparently
717 healthy tissue associated with stony coral tissue loss disease. Condition DD indicates disease
718 lesion, DH indicates apparently healthy tissue on diseased colonies (and is further parsed as near
719 or far from the lesion), H indicates apparently healthy neighboring corals. Up to 3 samples were
720 collected per coral colony, as indicated by the colony name. Raw reads (with adapters removed)
721 are deposited in NCBI under Bioproject Accession # PRJNA521988.

722
723 Table S2. Amplicon sequence variants detected in three control samples. Blank 1 (B1) and Blank
724 2 (B2) were no template controls from DNA extraction kit through sequencing. Blank 3 (B3) was
725 a no template PCR control that was cleaned and sequenced.





A *Montastraea cavernosa***B** *Orbicella faveolata***C** *Diploria labyrinthiformis***D** *Dichocoenia stokesii*