Microbiome dynamics in the tissue and mucus of acroporid corals differ in relation to host and environmental parameters

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Abstract

Corals are associated with diverse microbial assemblages; however, the spatial-temporal dynamics of intra-species microbial interactions are poorly understood. The coral-associated microbial community varies substantially between tissue and mucus microhabitats, however, the factors controlling the occurrence, abundance, and distribution of microbial taxa over time have rarely been explored for different compartments simultaneously. Here, we test 1) differentiation in microbiome diversity and composition between coral compartments (surface mucus and tissue) of two *Acropora* hosts (*A. tenuis* and *A. millepora*) common along inshore reefs of the Great Barrier Reef, as well as 2) the potential linkage between shifts in individual coral microbiome families and underlying host and environmental parameters. Amplicon based 16S ribosomal RNA gene sequencing of 136 samples collected over 14 months, revealed significant differences in bacterial richness, diversity and community structure among mucus, tissue and the surrounding seawater. Seawater samples were dominated by members of the Synechococcaceae and Pelagibacteraceae bacterial families. The mucus microbiome of *Acropora* spp. was dominated by members of Flavobacteriaceae, Synechococcaceae and Rhodobacteraceae and the tissue was dominated by Endozoicimonaceae. Mucus microbiome in both *Acropora* species was primarily correlated with seawater parameters including levels of chlorophyll *a,* ammonium, particulate organic carbon and the sum of nitrate and nitrite. In contrast, the correlation of the tissue microbiome to the measured environmental (i.e. seawater parameters) and host health physiological factors differed between host species, suggesting host-specific modulation of the tissue-associated microbiome to intrinsic and extrinsic factors. Furthermore, the correlation between individual coral microbiome members and environmental factors provides novel insights into coral microbiome-by-environment dynamics and hence has potential implications for current reef restoration and management efforts (e.g. microbial monitoring / observatory programs).

Introduction

Coral microbiomes include the well-characterized endosymbiotic dinoflagellates of the family Symbiodiniaceae, and a vast diversity of bacteria and archaea (Bourne, Morrow & Webster, 2016; Frade et al., 2016a; Rowher et al., 2002). The microbiome has a fundamental role in the health and stability of the coral holobiont; it recycles nutrients, removes waste products and

53   defends against pathogens (Lema, Willis & Bourne, 2012; Morris et al., 2011; Rädecker et al.,

54   2015; Rosado et al., 2019). The coral microbiome is influenced by a variety of intrinsic and

55   extrinsic factors. Coral microbiomes are host species-specific and were thought to remain

56   relatively stable over space and time (Frias-Lopez et al., 2002; Rowher et al., 2002). However,

57   recent studies have proposed that spatial-temporal factors such as environmental parameters

58   (Chen et al., 2011), depth (Glasl et al., 2017), geography (Hong et al., 2009; Littman et al.,

59   2009a), seasonality (Ceh, van Keulen & Bourne, 2011; Chen et al., 2011; Hong et al., 2009;

60   Koren & Rosenberg, 2006), coastal pollution (Klaus et al., 2007), and the physiological status of

61   the host (Grottoli et al., 2018; Littman, Willis & Bourne, 2009b) can also influence the

62   occurrence and relative abundance of microbial taxa. For instance, Li et al. (2015) reported a

63   dynamic relationship between the community structure of coral-associated bacteria and the

64   seasonal variation in environmental parameters such as dissolved oxygen and rainfall. Glasl et al.

65   (2019a) showed that although host-associated microbiomes were five-times less responsive to

66   the environment compared to the seawater microbiome, they were still affected by environmental

67   factors (e.g. temperature, turbidity, and nutrient concentration).

68   The coral provides different microhabitats for its microbial associates, including the

69   surface mucus layer, coral tissue, skeleton and gastrovascular cavity, each differing in microbial

70   richness, diversity and community structure, often assessed through alpha- and beta-diversity

71   metrics (Agostini et al., 2012; Engelen et al., 2018; Pollock et al., 2018; Sweet, Croquer &

72   Bythell, 2011). Each microhabitat has a unique set of biochemical features and harbors a specific

73   microbial community (Engelen et al., 2018; Pollock et al., 2018; Sweet, Croquer & Bythell,

74   2011). Hence, revealing microhabitat-specific host-microbiome associations and their specific

75   sensitivities to environmental fluctuations is crucial to our understanding of coral holobionts. For

76   example, the coral surface mucus layer is a polysaccharide-protein-lipid complex that provides

77   an interface between the coral epithelium and the surrounding seawater (Brown & Bythell,

78   2005). Here microbes take advantage of a nutrient-rich medium and particular microbiome

79   members found in the coral mucus overlap with both the tissue and the seawater microbial

80   communities (Bourne & Munn, 2005; Brown & Bythell, 2005; Glasl, Herndl & Frade, 2016;

81   Sweet, Croquer & Bythell, 2011). In contrast to the extracellular polymeric nature of the surface

82   mucus layer, the coral tissue consists of two distinct layers (epidermis and gastrodermis) and a

83   connective-tissue layer, the mesoglea (Muller-Parker, D'Elia & Cook, 2015). The coral tissue

84 harbors photosymbiotic dinoflagellates (family Symbiodiniaceae), that can provide up to 100%

85 of energy required by their coral host (Muller-Parker, D'Elia & Cook, 2015). The

86 Symbiodiniaceae community has been shown to vary in tandem with the bacterial community in

87 early life stages of corals (Quigley et al., 2019) and this may be caused by the release of complex

88 organic molecules such as the organosulfur compound dimethylsulfoniopropionate (DMSP;

89 Bourne et al., 2013; Frade et al., 2016b). The coral tissue microbiome is mostly represented by

90 bacteria belonging to the phyla Proteobacteria and Actinobacteria. For example, the

91 gammaproteobacterial *Endozoicomonas* are abundant in the coral's endodermal tissue and are

92 often considered 'true' coral symbionts (Bayer et al., 2013; Glasl et al., 2019b; Neave et al.,

93 2016, 2017). When compared to the surface mucus layer, the microbial community in the tissue

94 is significantly less dense and diverse (Bourne & Munn, 2005; Koren & Rosenberg, 2006), likely

95 attributed to the more spatially stable and host controlled environment (Bourne & Munn, 2005),

96 although divergent evidence suggests the mucus is less diverse than the tissue (Pollock et al.,

97 2018). Furthermore, tissue-associated bacterial communities form aggregations within the coral

98 cell layers, also referred to as coral-associated microbial aggregates (CAMAs), and are often co-

99 localized near algal symbiont cells, highlighting potential metabolic interactions between

100 symbionts (Wada et al., 2019).

101   In this study, we test the hypotheses that different coral compartments (surface mucus

102 layer and tissue) of *Acropora* spp. harbor distinct microbial communities and that different

103 intrinsic and extrinsic factors explain microbiome dynamics within these compartments.

104 Furthermore, we aim to identify significant correlations of individual bacterial families

105 associated with coral tissue and mucus with host-physiological and seawater parameters.

106

Materials & Methods

108 **Sample collection**

109 Samples of *Acropora millepora* (Ehrenberg, 1834)*, Acropora tenuis* (Dana, 1846) and seawater

110 were collected monthly, at Geoffrey Bay (Magnetic Island) in the Great Barrier Reef

111 (Supplementary Figure S1), between February 2016 and March 2017, for amplicon based 16S

112 ribosomal RNA (rRNA) gene sequencing along with environmental metadata. All samples were

113 collected under the permit G16/38348.1 issued by the Great Barrier Reef Marine Park Authority.

114   Samples (n = 3 per sample type and per sampling event) for molecular analysis were

115   collected as part of the Australian Microbiome Initiative and the sample procedure has

116   previously been outlined by Glasl et al. (2019a). In brief, coral nubbins (approximately 5 cm tall)

117   of both *Acropora* species were collected, rinsed with 0.2 μm filter-sterilized seawater and placed

118   into cryogenic vials. Coral mucus from the same specimens was collected with sterile cotton

119   swabs as previously described by Glasl, Herndl & Frade (2016). Seawater samples for molecular

120   analysis were collected in sterile collapsible bags, pre-filtered through a 50 μm filter mesh to

121   remove large particles, and subsequently filtered onto a 0.2 μm Sterivex filter (Millipore). Coral

122   nubbins, mucus swabs and Sterivex filters were immediately snap frozen in liquid nitrogen after

123   collection and stored at -80°C until further processing. To acquire environmental information,

124   water and sediment samples were collected in duplicate for each sampling event as described in

125   Glasl et al. (2019a) and further analyzed according to the standard procedures of Australian

126   Institute of Marine Science (Devlin & Lourey, 2000). The environmental information processed

127   includes common reef water quality measures such as salinity, particulate organic carbon, total

128   suspended solids, concentrations of chlorophyll *a,* ammonium, the sum of nitrite and nitrate,

129   particulate nitrogen, nitrite, total nitrogen, non-purgeable organic carbon, non-purgeable

130   inorganic carbon, phosphate and silica as well as total organic carbon in the sediment, total

131   organic nitrogen in the sediment and grainsize percentage of sediments < 0.63 μm, between 0.63

132   μm and 2 mm, and > 2 mm. Seawater temperatures and daylight hours were obtained from

133   AIMS long-term monitoring temperature records (http://eatlas.org.au).

134

135   **Sample preparation and genetic assays**

136   Frozen coral tissue was airbrushed into a *ziploc* bag with phosphate-buffered saline (PBS)

137   solution added until all tissue was removed from the skeletal fragment (PBS volume noted). The

138   resulting tissue slurry was homogenized for 1 min at 12,500 rpm using a hand-held tissue

139   homogenizer (Heidolph Silent Crusher M), pelleted (10 min at 16,000 rcf) and snap frozen in

140   liquid nitrogen. DNA from the tissue and mucus samples was extracted using the DNeasy

141   PowerBiofilm kit (QIAGEN). DNA extracts were sent on dry ice to the Ramaciotti Centre for

142   Genomics (Sydney, Australia) for sequencing. The bacterial 16S rRNA gene was sequenced

143   using the 27F (Lane, 1991) and 519R (Turner et al., 1999) primers on the Illumina MiSeq

144   platform using a dual indexed 2 x 300 bp paired-end approach. Primers pairs were selected to

145 warrant comparability across datasets of the Australian Microbiome Initiative

146 (https://www.australianmicrobiome.com).

147

148 **Sequence analysis**

149 Sequencing data were analyzed as single nucleotide variants following the standardized platform

150 of the Australian Microbiome Initiative (Brown et al., 2018). In brief, paired-end reads were

151 merged using FLASH software (Magoc & Salzberg, 2011) and FASTA formatted sequences

152 were extracted from FASTQ files. Sequences < 400 bp in length, and / or containing one or more

153 N's, or homopolymer runs of > 8 bp were removed with MOTHUR (v1.34.1; Schloss et al.,

154 2009). Sequences were de-replicated and ordered by abundance using USEARCH (64 bit

155 v10.0.240; Edgar, 2010). Sequences with less than 4 representatives and Chimeras were

156 removed, and the quality-filtered sequences were mapped to chimera-free zero-radius operational

157 taxonomic units (zOTUs). A table containing the samples and their read abundances was created

158 and the zOTUs were taxonomically classified with SILVA v132 database (Yilmaz et al., 2014)

159 using MOTHUR's implementation of the Wang classifier (Wang et al., 2007) and a 60%

160 Bayesian probability cut-off. This sequencing dataset has already been used in a previous

161 contribution by the research group (Glasl et al., 2019a), but in the current study it is analyzed

162 from a different perspective aiming at comparing temporal microbiome dynamics between two

163 distinct coral compartments.

164   Chloroplasts and mitochondria derived reads were removed from the dataset and

165 remaining data was rarefied to a sequencing depth of 3,500 reads per sample in R (R Core Team,

166 2015) using subset_taxa () function in the phyloseq package (McMurdie & Holmes, 2013). Read

167 counts per sample were transformed into relative abundances.

168

169 **Coral holobiont photopigment quantification**

170 Photopigment (chlorophyll *a*) concentrations in the tissue of corals were quantified using a

171 spectrophotometric approach (Glasl et al., 2019b). Tissue pellets were thawed on ice to avoid

172 sample degradation and resuspended in 1 ml of 90% ethanol. Samples were sonicated for 1 min

173 and centrifuged for 5 min at 10,000 rcf. Subsequently, 700 µl of the supernatant was removed

174 and transferred to a new tube. The resuspension, sonication and centrifugation were repeated on

175 the remainder of the pellet. The supernatant was recovered again, combined with the previous

176   extraction and mixed by inversion. Sample extract and 90% ethanol (blank read) were loaded in

177   triplicate (200 µl each) to a 96-well plate and the absorbance was recorded at 470, 632, 649, 665,

178   696 and 750 nm in a Cytation 3 multi-mode microplate reader (BioTek, Winooski, USA) and

179   analyzed using the software Gen5 (BioTek, Winooski, USA). Blank corrected absorbance

180   measures were used to calculate chlorophyll *a* concentrations (Supplementary Equation S1).

181

182   **Coral protein quantification**

183   Soluble protein concentrations of coral tissue samples were quantified using a colorimetric

184   protein assay kit (Pierce BCA Protein Assay Kit; Glasl et al., 2019b). Tissue pellets were thawed

185   on ice and resuspended in 1 ml PBS. The resuspension (25 µl) was added to 200 µl of working

186   reagent from the kit in a 96-well plate. The plate was mixed thoroughly on a plate shaker for 30

187   seconds and then incubated at 37°C for 30 min. The plate was cooled down at room temperature.

188   The absorbance was measured at 563 nm in a Cytation 3 multi-mode microplate reader (BioTek,

189   Winooski, USA) and analyzed using the software Gen5 (BioTek, Winooski, USA).

190   Measurements of the standards and samples were blank corrected to remove background

191   absorbance. For each plate, a protein standard curve was obtained using bovine serum albumin

192   (BSA) solution at concentrations between 25 and 2,000 µg ml^{-1}.

193

194   **Symbiodiniaceae cell counting**

195   To determine cell numbers of Symbiodiniaceae in the coral tissue, the tissue pellet was thawed

196   on ice, resuspended in 1 ml of 0.2 µl filtered seawater and fixed in 2% formaldehyde (final

197   concentration) to preserve the symbiont cells. The solution was passed through a syringe needle

198   to reduce cell agglomeration and diminish the bias from cell clumps. Samples were then mixed

199   for 1 min and 10 µl of the homogenate was loaded onto a Neubauer haemocytometer (0.100 mm

200   depth). Symbiodiniaceae cells were counted under 40 x magnification with an Olympus CX31

201   light microscope. In total, six independent haemocytometer loadings (24 squares each with 0.1 µl

202   volume) were used per sample to ensure robustness of density determinations.

203

204   **Statistical analyses**

205   Statistical analyses were performed using RStudio (v1.1.463). Analyses of microbial

206   communities were performed on rarefied relative abundance data at zOTU level. zOTU richness

207   and Shannon-Weaver diversity were compared across host compartments, host species and

208   reference seawater samples using non-parametric Analysis of Variance (Kruskal-Wallis test

209   using function kruskal.test), followed by Dunn's test for multiple comparisons (function

210   dunn.test). All p-values were adjusted using the Benjamini-Hochberg multiple comparison

211   correction method to decrease the false discovery rate (Benjamini & Hochberg, 1995). A Venn

212   diagram was constructed to describe the shared and unique zOTUs among mucus, tissue and

213   seawater microbiomes using VennDiagram package (Chen & Boutros, 2011) and visualized

214   using eulerr package (Larsson, 2020). Non-Metric Multidimensional Scaling (NMDS) was used

215   to illustrate the microbial community structure among host species and host compartments based

216   on Bray-Curtis dissimilarities (phyloseq package, McMurdie & Holmes, 2013). Permutational

217   Multivariate Analysis of Variance (PERMANOVA, 999 permutations) was used to test for

218   differences in microbial structure between host species and host compartments using the adonis2

219   function of the vegan package (Oksanen et al., 2013).

220   Physiological variables were normalized (i.e., chlorophyll *a* normalized to protein content,

221   chlorophyll *a* normalized to Symbiodiniaceae numbers, Symbiodiniaceae density normalized to

222   protein content) following common procedures in coral physiology studies (Frade et al., 2008,

223   Iglesias-Prieto & Trench, 1997). Due to fragmentation of the collected coral branches, coral

224   surface area could not be measured. Environmental and physiological variables were

225   standardized and checked for collinearity using the Pearson correlation coefficient. Redundant

226   variables based on Pearson's correlation (> 0.7 or < -0.7; Dormann et al., 2013) were removed

227   from the analysis. Non-correlated variables were then used in a Bray-Curtis distance-based

228   Redundancy Analysis (db-RDA), which quantifies the impact of the explanatory variables on the

229   microbiome (dis)similarities (Legendre & Anderson, 1999). zOTU relative abundance,

230   environmental and physiological metadata were used for db-RDA using the phyloseq package

231   (McMurdie & Holmes, 2013). The analysis tests the statistical relationship between microbial

232   community composition and the environmental/physiological variables for each coral

233   compartment and host species combination. A model selection tool (ordiR2step function in the

234   vegan package, *sensu* Blanchet, Legendre & Borcard, 2008) was performed to select the best db-

235   RDA model (i.e., the best explanatory variables) for variation in microbiome composition of

236   each coral compartment (mucus and tissue) in each host species (Johnson & Omland, 2004). The

237   significance of each explanatory variable was confirmed with an ANOVA-like permutational test

238   (function permutest") for dbRDA. The explanatory value (in %) of significant explanatory

239   variables (e.g. environmental and physiological parameters) on each microbiome was assessed

240   with Variation Partitioning Analysis of the vegan package (Oksanen et al., 2013). A correlation

241   matrix (based on the default Pearson correlation) between the relative abundance of the 20 most

242   abundant microbial families and significant environmental variables was generated using the R

243   package MicrobiomSeq (Ssekagiri, Sloan & Ijaz, 2017), for which p-values were adjusted using

244   the Benjamini-Hochberg multiple comparison correction (Benjamini & Hochberg, 1995).

245

246   **Results**

247   **Composition of coral tissue and mucus microbiomes**

248   The bacterial 16S rRNA genes of 136 samples, including coral tissue (n = 24 for *A. millepora*; n

249   = 30 for *A. tenuis*), coral mucus layer (n = 24 for *A. millepora*; n = 28 for *A. tenuis*) and seawater

250   (n = 30; used as reference samples) were sequenced and 12,051 zOTUs identified at single

251   nucleotide variants.

252   zOTU richness differed significantly among mucus, tissue and seawater microbiomes

253   (Kruskal-Wallis $Chi^2_{(2,133)}$ = 57.74, p = 2.89x10^{-13}), but not between seasons (see

254   Supplementary Table S1). Coral zOTU richness differed between species (*A. millepora vs A.*

255   *tenuis*; Kruskal-Wallis $Chi^2_{(1,134)}$ = 12.23, p = 0.00047). Seawater harbored the richest microbial

256   community (558 zOTU ± 54.6), followed by the mucus (*A. millepora,* 220 zOTU ± 188; *A.*

257   *tenuis* 511 zOTU ± 234) and tissue (*A. millepora,* 125 zOTU ± 31.6; *A. tenuis,* 173 zOTU ± 146;

258   Supplementary Table S1). Alpha diversity based on Shannon Index also differed significantly

259   among microbiomes from mucus, tissue and seawater (Kruskal-Wallis $Chi^2_{(2,133)}$ = 53.37, p =

260   2.57x10^{-12}), but not between seasons (see Supplementary Table S1). Coral zOTU Shannon

261   differed between species (*A. millepora vs A. tenuis*; Kruskal-Wallis $Chi^2_{(1,134)}$ = 6.002, p =

262   0.01429). Alpha diversity measures of mucus samples were not significantly different (Shannon

263   Index: *A. millepora*, 4.18 ± 0.83; *A. tenuis*, 5.15 ± 0.69) from seawater samples (Shannon Index:

264   4.40 ± 0.209; Supplementary Table S1). In contrast, the tissue microbiome was dramatically

265   different from the mucus and seawater microbiomes and harbored the lowest microbial diversity

266   (Shannon Index: *A. millepora*, 3.35 ± 0.63; *A. tenuis*, 3.54 ± 0.84).

267   Sequences affiliated to the phyla Proteobacteria dominated the microbial community of

268   all samples (average relative abundance ± SD; mucus: 44.1 ± 11.5%; tissue: 62.8 ± 2%;

269   seawater: 39.6 ± 3.1), followed in dominance by Bacteroidetes (mucus: 27.5 ± 13.0%; tissue: 9.6

270   ± 10.9%; seawater: 12.0 ± 11.4%) and Cyanobacteria (mucus: 14.4 ± 9.0%; tissue: 9.8 ± 11.0%;

271   seawater: 38.5 ± 4.0%). Mucus microbiomes for both *Acropora* species (Figure 1) were

272   characterized mostly by members of the family Flavobacteriaceae (average relative abundance ±

273   SD; for *A. tenuis*: 17.3 ± 9.1%; *A. millepora*: 17.3 ± 12.7%), Synechococcaceae (*A. tenuis*: 12.3

274   ± 7.8%; *A. millepora*: 13.1 ± 10.2%) and Rhodobacteraceae (*A. tenuis*: 5.7 ± 3.0%; *A. millepora*:

275   6.4 ± 6.4%; Figure 1). In contrast, the Endozoicimonaceae family dominated the tissue

276   microbiome (*A. tenuis*: 43.2 ± 31.7%; *A. millepora*: 20.5 ± 19.7%), with additional

277   representation of Flavobacteriaceae (*A. tenuis*: 7.9 ± 9.6%; *A. millepora*: 7.2 ± 9.6%),

278   Synechococcaceae (*A. tenuis*: 5.5 ± 6.8%; *A. millepora*: 12.3 ± 14.5%) and Rhodobacteraceae (*A.*

279   *tenuis*: 6.5 ± 10.4%; *A. millepora*: 5.3 ± 8.5%; Figure 1) families. Seawater samples were mostly

280   characterized by members of Synechococcaceae (36.6 ± 3.9%) and Pelagibacteraceae (18.6 ±

281   4.9%), but also by Rhodobacteraceae (8.6 ± 4.8%) and Flavobacteriaceae (8.0 ± 2.6%; Figure 1).

282   Tissue and mucus microbiomes exclusively shared 1,193 zOTU (9.9%), mucus and seawater

283   microbiomes exclusively shared 1,458 zOTU (12.1%), whereas the tissue and seawater

284   microbiome shared only 66 zOTU (0.6%; Figure 2).

285   Microbial community composition (beta-diversity) significantly differed among mucus,

286   tissue and seawater (Figure 3; PERMANOVA, *pseudo-F*$_{(2,126)}$ = 14.53, p = 0.001), between

287   *Acropora* species (PERMANOVA, *pseudo-F*$_{(1,126)}$ = 4.42, p = 0.001), and between seasons

288   (PERMANOVA, *pseudo-F*$_{(1,126)}$ = 1.90, p = 0.011). Interaction between species and

289   compartment was also significant (PERMANOVA, *pseudo-F*$_{(1,126)}$ = 3.07, p = 0.002; other

290   interactions were not significant; Supplementary Table S2).

291

292   **Explanatory variables of coral tissue and mucus microbiomes**

293   Physiological parameters of the tissue (i.e., chlorophyll *a* normalized to protein content,

294   chlorophyll *a* normalized to Symbiodiniaceae numbers, Symbiodiniaceae density normalized to

295   protein content) remained stable between host species.

296   Out of a total of 20 environmental variables measured for seawater and sediment, 6

297   variables were non-mutually collinear and were thus included in the db-RDA analysis. Selected

298   variables were salinity, concentration of particulate organic carbon (POC), total suspended solids

299   (TSS), chlorophyll *a* (Chl*a*), ammonium (NH$_4^+$) and the sum of nitrite and nitrate concentrations

300   (i.e., NO$_2^-$/NO$_3^-$; Supplementary Table S3).

301   Environmental/physiological parameters investigated in this study explained a limited

302   amount of variation in the microbial community of mucus and tissue of the two *Acropora* species

303   studied (Figure 4). For example, seawater parameters explained 14% (Chl*a*, NH$_4^+$ and NO$_2^-$/NO$_3^-$

304   ) and 10% (POC and NO$_2^-$/NO$_3^-$) of the compositional variability for the mucus microbiome in

305   *A. tenuis* and *A. millepora,* respectively (ANOVA-like permutational test for dbRDA;

306   Supplementary Table S4); NO$_2^-$/NO$_3^-$ was the only explanatory environmental variable common

307   to the mucus microbiome of both *Acropora* species (5% of compositional variability explained in

308   each species). In comparison, for the seawater microbiome, environmental parameters (NO$_2^-$

309   /NO$_3^-$, TSS, POC, Salinity and Chl*a*) explained 32% of the compositional variability of the

310   microbiome (Supplementary Figure S2), suggesting greater environmental sensitivity by the

311   microbial community in the seawater compared to the coral-associated communities.

312   In contrast, tissue microbiomes of *A. millepora* and *A. tenuis* differed substantially in

313   their response to environmental and/or to physiological parameters. While host physiology (i.e.,

314   Symbiodiniaceae density normalized to protein contents) and environment (TSS and Chl*a*)

315   explained 6% and 10%, respectively, of the variation of the tissue microbiome in *A. tenuis*, in *A.*

316   *millepora*, the compositional variation was solely explained (10%) by environmental parameters

317   (NO$_2^-$/NO$_3^-$ and TSS; Variation Partitioning Analysis and ANOVA-like permutational test for

318   dbRDA; Supplementary Table S4). TSS was the only explanatory environmental variable

319   common to the tissue microbiomes of both *Acropora* species (total of 5% and 4% in *A. tenuis*

320   and in *A. millepora*, respectively).

321

322   **Correlation between bacterial families and environmental/physiological parameters**

323   The relative abundance of Synechococcaceae in tissues of both *Acropora* species and the mucus

324   of *A. tenuis* was negatively correlated with TSS (p = 0.025-0.039; Figure 5 and Supplementary

325   Tables S5 and S6). In contrast, Synechococcaceae was positively correlated to total NO$_2^-$/NO$_3^-$ in

326   both species (mucus of *A. tenuis*, p = 0.002, Supplementary Table S6; and tissue of *A. millepora*,

327   p = 0.024, Supplementary Table S5). For *A. tenuis*, Synechococcaceae abundance in the tissue

328   correlated negatively with the only significant physiological parameter (Symbiodiniaceae density

329   normalized to protein contents; p = 0.025). In the mucus of *A. millepora,* the abundance of

330   Pirellulaceae was positively correlated with NO_2^-/NO_3^- (p = 0.035) and negatively correlated

331   with TSS (p = 0.019), while OCS155 was positively correlated to NO_2^-/NO_3^- (p = 0.015).

332   Proteobacteria from the mucus of *A. tenuis*, Pelagibacteraceae and Halomonadaceae, were both

333   strongly negative correlated with chlorophyll *a* in the seawater (Pelagibacteraceae, p = 0.013;

334   Halomonadaceae, p = 0.008). Additionally, Halomonadaceae correlated negatively with NH_4^+ (p

335   = 0.005; Figure 5 and Supplementary Tables S5 and S6).

336   Tissue-associated Endozoicimonaceae showed a strong significant positive correlation

337   with Symbiodiniaceae density normalized to protein content in *A. tenuis* (p = 0.0003). In

338   contrast*,* in the tissue of *A. millepora*, Endozoicimonaceae were negatively correlated with NO_2^-

339    $/NO_3^-$ (p = 0.020), whereas the abundance of Cryomorphaceae family was negatively correlated

340   with TSS (p = 0.020; Figure 5, Supplementary Table S5).

341

342   **Discussion**

343   Microbial communities associated with corals are continually exposed to fluctuations in the

344   surrounding environment and the physiology of their host. Previous studies have demonstrated

345   changes in the coral microbiome in response to thermal stress (Ainsworth & Hoegh-Guldberg,

346   2009; Grottoli et al., 2018; Lee et al., 2015; Thurber et al., 2009), ocean acidification (Grottoli et

347   al., 2018; Thurber et al., 2009), organic matter enrichment (Garren & Azam, 2012), bleaching

348   events (Bourne et al., 2008) and other environmental and physiological factors (Glasl et al.,

349   2019a; Guppy & Bythell, 2006; Kelly et al., 2014; Li et al., 2015; Pollock et al., 2018). However,

350   the coral microbiome is not homogenous across the animal and an improved understanding of the

351   sensitivity of the microorganisms inhabiting each coral compartment is needed. This study

352   highlights compositional differences in the bacterial communities associated with coral mucus

353   and coral tissue, as well as with the surrounding seawater, findings that are largely consistent

354   with previous studies (Apprill, Weber & Santoro, 2016; Bourne & Munn, 2005; Engelen et al.,

355   2018; Pollock et al., 2018; Sweet, Croquer & Bythell, 2011). Furthermore, the high similarity

356   between mucus and seawater microbiomes (see Supplementary Tables S1 and S2, Figures 2 and

357   3) and the high dissimilarity between tissue and seawater microbiomes suggests that the mucus

358   microbial community is more strongly influenced by the external environment than the tissue

359   community. Similar results have been reported for other coral species in the Caribbean

360   (*Orbicella faveolata*, *Diploria strigosa*, *Montastraea cavernosa*, *Porites porites* and *Porites*

361   *astreoides*), where mucus and seawater shared significantly more microbial taxa than those

362   shared by tissue and seawater microbiomes (Apprill, Weber & Santoro, 2016). Our results also

363   support that mucus microbiomes are richer and more diverse than tissue microbiomes, which is a

364   pattern corroborated by many studies (Bourne & Munn, 2005; Koren & Rosenberg, 2006).

365   Despite the host species-specificity of the coral microbiomes, some bacterial taxa were

366   ubiquitously associated with a particular coral compartment. For example, Flavobacteriaceae and

367   Synechococcaceae dominated the mucus of both species, while Endozoicimonaceae dominated

368   the tissue microbiome of both *Acropora* species. However, overall microbiome composition also

369   showed some overlap between host compartments, consistent with previous reports of overlap

370   between the mucus and tissue microbiomes of other coral species (Engelen et al., 2018; Sweet,

371   Croquer & Bythell, 2011). This intersection is a natural feature of the coral holobiont as both

372   compartments are within the same host and because the constituents of the surface mucus layer

373   are originally produced inside the tissue (Bythell & Wild, 2011). The sharing of some microbial

374   taxa between compartments may also arise due to methodological challenges associated with

375   retrieving samples that are exclusively mucus or coral tissue (Sweet, Croquer & Bythell, 2011).

376   Similar methodological limitations could also obscure differences between the mucus and

377   seawater microbiomes (Brown & Bythell, 2005).

378

379   **Explanatory factors of mucus microbiome variation**

380   We hypothesized that the coral mucus microbiome, which is in direct contact with seawater,

381   would be primarily correlated with seawater parameters, whereas the tissue microbiome would

382   be most affected by the physiological state of the coral host. Mucus is highly hydrated: mucocyte

383   cells release their secretions in a condensed form which then undergo a massive swelling upon

384   hydration, forming a visco-elastic gel (Brown & Bythell, 2005). Surface mucus can therefore be

385   influenced by the presence of nutrients dissolved in the surrounding seawater (Tanaka, Ogawa &

386   Miyajima, 2010). As expected, environmental factors (i.e. seawater parameters) were influential

387   in shaping the mucus microbiome of both species (*A. millepora* and *A. tenuis*), consistent with

388   recent studies relating changes in the mucus microbiome with environmental perturbations (Li et

389   al., 2015; Pollock et al., 2018). However, the extent of influence from environmental parameters

390   (10% of variation) on the mucus microbiome was much lower than the influence of environment

391   on the seawater microbiome (32% of variation), suggesting that other factors also play a role in

392   modulating the mucus microbiome. For instance, the surrounding environment may interact with

393   host physiology and together they alter the bacterial community structure of the mucus. Mucus is

394   a nutrient-rich medium fueled by the photosynthetic activity of the Symbiodiniaceae (Brown &

395   Bythell, 2005) and therefore it is expected that some degree of variation in its chemical

396   composition is explained by host-Symbiodiniaceae factors. For example, *A. millepora* and *A.*

397   *tenuis* at the sampling site (Geoffrey Bay at Magnetic Island) associate with distinct

398   Symbiodiniaceae (LaJeunesse et al., 2018; Ulstrup & van Oppen, 2003; van Oppen et al., 2001).

399   *A. millepora* colonies were associated with *Durusdinium* (van Oppen et al., 2001) whereas *A.*

400   *tenuis* harbored *Cladocopium* spp. (Ulstrup & van Oppen, 2003). Links between mucus chemical

401   composition and microbiome community structure have been proposed (Tremblay et al., 2011).

402   Physiological factors regulating the dynamics of production and release of the surface mucus

403   layer could also contribute to regulating mucus microbial composition (Glasl, Herndl & Frade,

404   2016).

405   Fluctuations of NH_4^+, NO_2^-/NO_3^-, Chl*a* and POC in the surrounding seawater

406   significantly correlated with the mucus microbiome variation in *Acropora* species. Li et al.

407   (2015) and Chen et al. (2011) suggested that rainfall had a crucial effect on bacterial community

408   variation in the coral microbiome, being mostly associated with an increase in the relative

409   abundance of the *Bacilli* group (Chen et al., 2011; Li et al., 2015). In the present study, NO_2^-

410   /NO_3^- (and its collinear variables daylight, particulate nitrogen and grainsize of sediments;

411   Supplementary Table S3) had the greatest influence on microbiome structure, being a significant

412   factor for both studied species. The link between rainfall and increasing nutrients (such as NO_2^-

413   /NO_3^-) is well established for inshore reefs (Fabricius, 2005). In the current study, higher

414   amounts of particulate and dissolved nutrients (but a decrease in TSS), corresponded to an

415   increase in mucus-associated Synechococcaceae, Pirellulaceae, OCS155 and Rhodobacteraceae

416   and a decrease in Halomonadaceae. For instance, Synechococcaceae in the mucus was highly

417   positively correlated with NO_2^-/NO_3^- and negatively correlated with TSS. These findings

418   corroborate previous work in which the abundance of free-living *Synechococcus* in shallow

419   coastal waters decreased significantly under lower nutrient (especially nitrate) and higher TSS

420   concentrations (Uysal & Köksalan, 2006).

421   Dissolved nutrients, such as nitrogen and phosphorus, can affect coral physiology and

422   drive changes in the associated microbial community (Shaver et al., 2017; Thompson et al.,

423   2015). For example, organic-rich nutrients from terrestrial run-off negatively affect the health of

424   corals and promote rapid growth of opportunistic heterotrophic bacteria (e.g. Vibrionales,

425   Flavobacteriales and Rhodobacterales), thus affecting the overall composition of the coral

426   microbiome (McDevitt-Irwin et al., 2017; Weber et al., 2012). In our study, the abundance of

427   Flavobacteriaceae and Rhodobacteraceae in the mucus of *A. tenuis* correlated with TSS and

428   NH_4^+, respectively. The coral holobiont, including cyanobacteria related to *Synechococcus* spp.

429   (Lesser et al., 2004), can also efficiently take up inorganic nitrogen, for example, as nitrogen is

430   required by the photosynthesis production of its Symbiodiniaceae symbionts (Yellowlees, Rees

431   & Leggat, 2008). In fact, NH_4^+ can be assimilated by both coral and its Symbiodiniaceae

432   (Pernice et al., 2012), and recent work has implicated bacteria such as *Vibrio* and *Alteromonas* in

433   the incorporation and translocation of NH_4^+ into coral tissues and associated Symbiodiniaceae

434   (Ceh et al., 2013). Nitrifying members of the mucus microbiome, such as ammonium oxidizing

435   bacteria (e.g., Pirelullaceae) and archaea, are fueled by NH_4^+ (Beman et al., 2007; Siboni et al.,

436   2008; Yang et al., 2013), and NO_2^-/NO_3^- can be respired by nitrate reducers putatively active in

437   coral microbiomes (Siboni et al., 2008; Yang et al., 2013). Interestingly, Pirellulaceae

438   abundances in the mucus of *A. millepora* positively correlated with concentrations of

439   environmental NO_2^-/NO_3^-, the products of ammonium oxidation. These nitrogen-cycling

440   processes mediated by microbes are highly dependent on oxygen availability, but because

441   oxygen concentration in the mucus shows strong diel fluctuations (Shashar, Cohen & Loya,

442   1993), it is possible that both aerobic (e.g., nitrification) and anaerobic (e.g., denitrification)

443   processes happen within the mucus layer at different times of the day. Temporal dynamics in the

444   coral mucus microbiome are thus likely influenced by the individual and collective metabolic

445   capabilities of the diverse assemblage of microbes and by nutrient availability in the surrounding

446   waters.

447

448   **Explanatory factors of tissue microbiome variation**

449   The statistical relation between the coral tissue microbiome and the environmental and

450   physiological parameters differed between coral species. Whereas the tissue microbiome of *A.*

451   *tenuis* corresponded to both environment and host physiology, *A. millepora* correlated only with

452   environmental parameters. This difference may be associated to specific features of each species,

453   through which *A. millepora* could modulate the internal environment and create more stable

454   intra-tissue conditions than *A. tenuis* (e.g., via skeletal light modulation, host morphology and

455   tissue thickness, *sensu* Enriquez, Mendez & Iglesias-Prieto, 2005). A non-mutually exclusive

456   alternative explanation is the influence of the algal symbiont (Symbiodiniaceae) genotype

457   associated to the host. Little et al. (2004) investigated Symbiodiniaceae communities associated

458   with *A. millepora* and *A. tenuis* on Magnetic Island demonstrating that the coral-algal

459   endosymbiotic relationship in *Acropora* spp. is distinct between species, dynamic and flexible

460   (corals associate with different Symbiodiniaceae types at different life stages, for example), and

461   contributes significantly to physiological attributes of the coral holobiont. For example, different

462   algal genotypes can affect the nutrient availability (e.g. carbon and nitrogen) in the coral

463   holobiont (Pernice et al., 2015; Bayliss et al., 2019). Environmental factors such as seawater

464   temperature can also lead to temporal changes in the symbiont community (Cooper et al., 2011;

465   Howells et al., 2012; Rocker, Willis & Bay, 2012). As the microbiome is strongly associated to

466   the coral holobiont, any disturbance in the host-Symbiodiniaceae relationship may have indirect

467   effects on the microbial composition and its response to environmental and physiological factors.

468   Other studies demonstrate the influence of Symbiodiniaceae on the host microbial community

469   and also support the idea that these two components of the coral holobiont are finely tuned (Glasl

470   et al., 2017; Grottoli et al., 2018; Littman, Bourne & Willis, 2010; Littman, Willis & Bourne,

471   2009b; Quigley et al., 2019). In the present study, Endozoicimonaceae were strongly positively

472   correlated with the Symbiodiniaceae density in the tissue of *A. tenuis* and negatively correlated

473   with NO_2^-/NO_3^- in *A. millepora* (see Figure 5). These results are to some extent at odds with

474   experimental results showing a stable dominance of Endozoicimonaceae in tissues of

475   *Pocillopora verrucosa* irrespective of excess dissolved organic nitrogen and despite a bleaching

476   phenomenon concomitant with structural changes in its Symbiodiniaceae community (Pogoreutz

477   et al., 2018).

478   Besides the diversity of Symbiodiniaceae associated to each coral species, other factors

479   can affect the coral and its response to environmental parameters, such as photochemical

480   efficiency (Fv/Fm) and symbiont density (Cunning & Baker, 2014; Da-Anoy, Cabaitan &

481   Conaco, 2019). For instance, Da-Anoy, Cabaitan & Conaco (2019) demonstrated a greater

482   reduction of Fv/Fm in *A. tenuis* in response to elevated temperatures compared to *A. millepora*

483   and the temperature responses of the corals did not directly correlate with their associated

484   seemingly associated Symbiodiniaceae. This suggests that other species-specific physiological

485 factors could modulate the responses of the coral to the environment and, indirectly, influence

486 the tissue-associated microbiome. One such factor is the way coral-associated microbial

487 aggregates (CAMAs) are distributed throughout the tissue, which varies within populations and

488 can vary among coral species (Work & Aeby, 2014; Wada et al., 2019).

489   Total suspended solids (TSS) was the only environmental parameter measured in the

490 present study that significantly related to the tissue microbiome of both coral species. TSS can

491 impact corals by limiting light availability for photosynthesis and decreasing Symbiodiniaceae

492 densities, which can indirectly affect microbial communities (Fabricius, 2005; Pollock et al.,

493 2014). High levels of suspended solids characterize the environment of inshore reefs such as

494 those found around Magnetic Island. The decrease in TSS is strongly associated with an increase

495 in the abundance of tissue-associated Synechococcaceae and Cryomorphaceae. Cryomorphaceae

496 are typical copiotrophs in the phylum Bacteroidetes and their increase in the tissue of *A.*

497 *millepora* could relate to declines in coral holobiont health.

498

499 **Conclusions**

500 This study highlights that microbiomes inhabiting different physical microniches within the coral

501 holobiont differ in their linkage between host and environmental factors. Microbiomes of

502 *Acropora* spp. differed significantly among host compartments (surface mucus layer and tissue)

503 and species (*A. tenuis* and *A. millepora*). Seawater parameters had the greatest influence on the

504 mucus microbiome in both species whereas the tissue microbiomes showed differential patterns

505 to environmental/host-physiological parameters, suggesting host-specific modulation of the

506 tissue microbiome. While further research is needed to unequivocally define the drivers of coral

507 microbiome variation, by investigating temporal variation in water quality and coral health

508 measures and correlating these with microbial community dynamics across distinct host

509 compartments in closely related species, this study has identified several intrinsic and extrinsic

510 factors that contribute to microbiome composition in corals.

511

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520   **References**

521   Agostini, S., Suzuki, Y., Higuchi, T., Casareto, B. E., Yoshinaga, K., Nakano, Y., & Fujimura,

522   H. (2012). Biological and chemical characteristics of the coral gastric cavity. *Coral Reefs*,

523   *31*(1), 147–156. https://doi.org/10.1007/s00338-011-0831-6

524

525   Ainsworth, T. D., & Hoegh-Guldberg, O. (2009). Bacterial communities closely associated with

526   coral tissues vary under experimental and natural reef conditions and thermal stress.

527   *Aquatic Biology*, *4*, 289–296. https://doi.org/10.3354/ab00102

528

529   Ainsworth, T. D., Krause, L., Bridge, T., Torda, G., Raina, J.-B., Zakrzewski, M., Gates, R. D.,

530   Padilla-Gamiño, J. L., Spalding, H. L., Smith, C., Woolsey, E. S., Bourne, D. G.,

531   Bongaerts, P., Hoegh-Guldberg, O., & Leggat, W. (2015). The coral core microbiome

532   identifies rare bacterial taxa as ubiquitous endosymbionts. The ISME Journal, 9(10),

533   2261–2274. https://doi.org/10.1038/ismej.2015.39

534

535   Apprill, A., Weber, L. G., & Santoro, A. E. (2016). Distinguishing between Microbial Habitats

536   Unravels Ecological Complexity in Coral Microbiomes. *MSystems*, *1*(5), e00143-16,

537   /msys/1/5/e00143-16.atom. https://doi.org/10.1128/mSystems.00143-16

538

539   Bayer, T., Neave, M. J., Alsheikh-Hussain, A., Aranda, M., Yum, L. K., Mincer, T., Hughen, K.,

540   Apprill, A., & Voolstra, C. R. (2013). The Microbiome of the Red Sea Coral Stylophora

541   pistillata Is Dominated by Tissue-Associated Endozoicomonas Bacteria. *Applied and*

542   *Environmental Microbiology*, *79*(15), 4759–4762. https://doi.org/10.1128/AEM.00695-

543   13

544

545 Bayliss, S.L.J., Scott, Z.R., Coffroth, M.A., & terHorst, C.P. (2019) Genetic variation in
546   *Breviolum antillogorgium*, a coral reef symbiont, in response to temperature and
547   nutrients. *Ecology and Evolution, 9*, 2803-2813. https://doi.org/10.1002/ece3.4959

549 Beman, J. M., Roberts, K. J., Wegley, L., Rohwer, F., & Francis, C. A. (2007). Distribution and
550   Diversity of Archaeal Ammonia Monooxygenase Genes Associated with Corals. *Applied*
551   *and   Environmental   Microbiology,   73*(17),   5642–5647.
552   https://doi.org/10.1128/AEM.00461-07

554 Benjamini, Y., & Hochberg, Y. (1995). Controlling the false discovery rate: a practical and
555   powerful approach to multiple testing. *Journal of the Royal statistical society: series B*
556   *(Methodological), 57*(1), 289-300.

558 Blanchet, F. G., Legendre, P., & Borcard, D. (2008). Forward selection of explanatory variables.
559   *Ecology*, *89*(9), 2623–2632. https://doi.org/10.1890/07-0986.1

561 Bourne, D. G., Dennis, P. G., Uthicke, S., Soo, R. M., Tyson, G. W., & Webster, N. (2013).
562   Coral reef invertebrate microbiomes correlate with the presence of photosymbionts. *The*
563   *ISME Journal*, *7*(7), 1452–1458. https://doi.org/10.1038/ismej.2012.172

565 Bourne, D. G., Morrow, K. M., & Webster, N. S. (2016). Insights into the Coral Microbiome:
566   Underpinning the Health and Resilience of Reef Ecosystems. *Annual Review of*
567   *Microbiology*, *70*(1), 317–340. https://doi.org/10.1146/annurev-micro-102215-095440

569 Bourne, D. G., & Munn, C. B. (2005). Diversity of bacteria associated with the coral Pocillopora
570   damicornis from the Great Barrier Reef. *Environmental Microbiology*, *7*(8), 1162–1174.
571   https://doi.org/10.1111/j.1462-2920.2005.00793.x

573 Bourne, D., Iida, Y., Uthicke, S., & Smith-Keune, C. (2008). Changes in coral-associated
574   microbial communities during a bleaching event. *The ISME Journal*, *2*(4), 350–363.
575   https://doi.org/10.1038/ismej.2007.112

576

577 Brown, B., & Bythell, J. (2005). Perspectives on mucus secretion in reef corals. *Marine Ecology*
578 *Progress Series*, *296*, 291–309. https://doi.org/10.3354/meps296291

579

580 Brown, M. V., van de Kamp, J., Ostrowski, M., Seymour, J. R., Ingleton, T., Messer, L. F.,
581 Jeffries, T., Siboni, N., Laverock, B., Bibiloni-Isaksson, J., Nelson, T. M., Coman, F.,
582 Davies, C. H., Frampton, D., Rayner, M., Goossen, K., Robert, S., Holmes, B., Abell, G.
583 C. J., … Bodrossy, L. (2018). Systematic, continental scale temporal monitoring of
584 marine pelagic microbiota by the Australian Marine Microbial Biodiversity Initiative.
585 *Scientific Data*, *5*(1), 180130. https://doi.org/10.1038/sdata.2018.130

586

587 Bythell, J. C., & Wild, C. (2011). Biology and ecology of coral mucus release. *Journal of*
588 *Experimental Marine Biology and Ecology*, *408*(1–2), 88–93.
589 https://doi.org/10.1016/j.jembe.2011.07.028

590

591 Ceh, J., Kilburn, M. R., Cliff, J. B., Raina, J.-B., van Keulen, M., & Bourne, D. G. (2013).
592 Nutrient cycling in early coral life stages: *Pocillopora damicornis* larvae provide their
593 algal symbiont (*Symbiodinium*) with nitrogen acquired from bacterial associates.
594 *Ecology and Evolution*, *3*(8), 2393–2400. https://doi.org/10.1002/ece3.642

595

596 Ceh, J., van Keulen, M., & Bourne, D. G. (2011). Coral-associated bacterial communities on
597 Ningaloo Reef, Western Australia: Coral bacterial communities, Ningaloo Reef. *FEMS*
598 *Microbiology Ecology*, *75*(1), 134–144. https://doi.org/10.1111/j.1574-
599 6941.2010.00986.x

600

601 Chen, C.-P., Tseng, C.-H., Chen, C. A., & Tang, S.-L. (2011). The dynamics of microbial
602 partnerships in the coral Isopora palifera. *The ISME Journal*, *5*, 728–740.

603

604 Chen, H., & Boutros, P. C. (2011). *VennDiagram: A package for the generation of highly-*
605 *customizable Venn and Euler diagrams in R. 12*(1), 35.

606

607 Commonwealth of Australia. (2019). http://www.bom.gov.au/?ref=logo

609 Cooper, T. F., Berkelmans, R., Ulstrup, K. E., Weeks, S., Radford, B., Jones, A. M., Doyle, J.,
610   Canto, M., O'Leary, R. A., & van Oppen, M. J. H. (2011). Environmental Factors
611   Controlling the Distribution of Symbiodinium Harboured by the Coral Acropora
612   millepora on the Great Barrier Reef. *PLoS ONE*, *6*(10), e25536.
613   https://doi.org/10.1371/journal.pone.0025536

615 Cunning, R., & Baker, A. C. (2014). Not just who, but how many: the importance of partner
616   abundance in reef coral symbioses. *Frontiers in microbiology, 5*, 400.

618 Da-Anoy, J. P., Cabaitan, P. C., & Conaco, C. (2019). Species variability in the response to
619   elevated temperature of select corals in north-western Philippines. Journal of the Marine
620   Biological Association of the United Kingdom, 99(6), 1273-1279.

622 Dana, J. D. (1846). *United States Exploring Expedition during the years 1838-1842. 7*, 1–740.

624 Devlin M.J. & Lourey M.J. (2000) Water quality - field and analytical procedures. In: L–
625   tMotGB R, editor. Standard Operational Procedure, vol. 6. Townsville: Australian
626   Institute of Marine Science.

628 Dormann, C. F., Elith, J., Bacher, S., Buchmann, C., Carl, G., Carré, G., Marquéz, J. R. G.,
629   Gruber, B., Lafourcade, B., Leitão, P. J., Münkemüller, T., McClean, C., Osborne, P. E.,
630   Reineking, B., Schröder, B., Skidmore, A. K., Zurell, D., & Lautenbach, S. (2013).
631   Collinearity: A review of methods to deal with it and a simulation study evaluating their
632   performance. *Ecography*, *36*(1), 27–46. https://doi.org/10.1111/j.1600-
633   0587.2012.07348.x

635 Edgar, R. C. (2010). Search and clustering orders of magnitude faster than BLAST.
636   *Bioinformatics*, *26*(19), 2460–2461. https://doi.org/10.1093/bioinformatics/btq461

638   Ehrenberg, C. (1834). *Contributions to the physiological knowledge of the coral animals in*
639   *general, and especially of the Red Sea, together with an experiment on the physiological*
640   *systematics of the same. 1*, 225–380.

641

642   Engelen, A. H., Aires, T., Vermeij, M. J. A., Herndl, G. J., Serrão, E. A., & Frade, P. R. (2018).
643   Host Differentiation and Compartmentalization of Microbial Communities in the
644   Azooxanthellate Cupcorals Tubastrea coccinea and Rhizopsammia goesi in the
645   Caribbean. *Frontiers in Marine Science*, *5*, 391.
646   https://doi.org/10.3389/fmars.2018.00391

647

648   Enriquez, S., Mendez, E. R., & Iglesias-Prieto, R. I. (2005). Multiple scattering on coral
649   skeletons enhances light absorption by symbiotic algae. *Limnology and Oceanography*,
650   *50*(4), 1025–1032. https://doi.org/10.4319/lo.2005.50.4.1025

651

652   Fabricius, K. E. (2005). Effects of terrestrial runoff on the ecology of corals and coral reefs:
653   Review and synthesis. *Marine Pollution Bulletin*, *50*(2), 125–146.
654   https://doi.org/10.1016/j.marpolbul.2004.11.028

655

656   Frade, P. R., Bongaerts, P., Winkelhagen, A. J. S., Tonk, L., Bak, R. P. M. (2008). In
657   situ photobiology of corals over large depth ranges: A multivariate analysis on the
658   roles of environment, host, and algal symbiont. *Limnology and Oceanography,*
659   *53*(6), 2711-2723. https://doi.org/10.4319/LO.2008.53.6.2711

660

661   Frade, P. R., Roll, K., Bergauer, K., & Herndl, G. J. (2016a). Archaeal and bacterial
662   communities associated with the surface mucus of Caribbean corals differ in their degree
663   of host specificity and community turnover over reefs. *PLOS ONE*, *11*(1), e0144702.
664   https://doi.org/10.1371/journal.pone.0144702

665

666   Frade, P. R., Schwaninger, V., Glasl, B., Sintes, E., Hill, R. W., Simó, R., & Herndl, G. J.
667   (2016b). Dimethylsulfoniopropionate in corals and its interrelations with bacterial

668   assemblages in coral surface mucus. *Environmental Chemistry*, *13*(2), 252.

669   https://doi.org/10.1071/EN15023

670

671   Frias-Lopez, J., L. Zerkle, A., T. Bonheyo, G., & W. Fouke, B. (2002). Partitioning of bacterial

672   communities between seawater and healthy, black band diseased, and dead coral surfaces.

673   *Applied and Environmental Microbiology*, *68*(5), 2214–2228.

674   https://doi.org/10.1128/AEM.68.5.2214–2228.2002

675

676   Garren, M., & Azam, F. (2012). Corals shed bacteria as a potential mechanism of resilience to

677   organic matter enrichment. *The ISME Journal*, *6*(6), 1159–1165.

678   https://doi.org/10.1038/ismej.2011.180

679

680   Glasl, B., Bongaerts, P., Elisabeth, N. H., Hoegh-Guldberg, O., Herndl, G. J., & Frade, P. R.

681   (2017). Microbiome variation in corals with distinct depth distribution ranges across a

682   shallow–mesophotic gradient (15–85 m). *Coral Reefs*, *36*(2), 447–452.

683   https://doi.org/10.1007/s00338-016-1517-x

684

685   Glasl, B., Bourne, D. G., Frade, P. R., Thomas, T., Schaffelke, B., & Webster, N. S. (2019a).

686   Microbial indicators of environmental perturbations in coral reef ecosystems.

687   *Microbiome*, *7*(1), 94. https://doi.org/10.1186/s40168-019-0705-7

688

689   Glasl, B., Herndl, G. J., & Frade, P. R. (2016). The microbiome of coral surface mucus has a key

690   role in mediating holobiont health and survival upon disturbance. *The ISME Journal*,

691   *10*(9), 2280–2292. https://doi.org/10.1038/ismej.2016.9

692

693   Glasl, B., Smith, C. E., Bourne, D. G., & Webster, N. S. (2019b). Disentangling the effect of

694   host-genotype and environment on the microbiome of the coral *Acropora tenuis*. *PeerJ*,

695   *7*, e6377. https://doi.org/10.7717/peerj.6377

696

697   Grottoli, A. G., Dalcin Martins, P., Wilkins, M. J., Johnston, M. D., Warner, M. E., Cai, W.-J.,

698   Melman, T. F., Hoadley, K. D., Pettay, D. T., Levas, S., & Schoepf, V. (2018). Coral

699   physiology and microbiome dynamics under combined warming and ocean acidification.

700   *PLOS ONE*, *13*(1), e0191156. https://doi.org/10.1371/journal.pone.0191156

701

702 Guppy, R., & Bythell, J. (2006). Environmental effects on bacterial diversity in the surface

703   mucus layer of the reef coral *Montastraea faveolata*. *Marine Ecology Progress Series*,

704   *328*, 133–142. https://doi.org/10.3354/meps328133

705

706 Hong, M.-J., Yu, Y.-T., Chen, C. A., Chiang, P.-W., & Tang, S.-L. (2009). Influence of species

707   specificity and other factors on bacteria associated with the coral *Stylophora pistillata* in

708   Taiwan. *Applied and Environmental Microbiology*, *75*(24), 7797–7806.

709   https://doi.org/10.1128/AEM.01418-09

710

711 Howells, E. J., Beltran, V. H., Larsen, N. W., Bay, L. K., Willis, B. L., & van Oppen, M. J. H.

712   (2012). Coral thermal tolerance shaped by local adaptation of photosymbionts. *Nature*

713   *Climate Change*, *2*(2), 116–120. https://doi.org/10.1038/nclimate1330

714

715 Iglesias-Prieto, R. & Trench R. K. (1997) Acclimation and adaptation to irradiance in symbiotic

716   dinoflagellates. II. Response of chlorophyll-protein complexes to different photon-flux

717   densities. *Marine Biology,* 130(1), 23-33.

718

719 Johnson, J. B., & Omland, K. S. (2004). Model selection in ecology and evolution. *Trends in*

720   *Ecology & Evolution*, *19*(2), 101–108. https://doi.org/10.1016/j.tree.2003.10.013

721

722 Kelly, L. W., Williams, G. J., Barott, K. L., Carlson, C. A., Dinsdale, E. A., Edwards, R. A.,

723   Haas, A. F., Haynes, M., Lim, Y. W., McDole, T., Nelson, C. E., Sala, E., Sandin, S. A.,

724   Smith, J. E., Vermeij, M. J. A., Youle, M., & Rohwer, F. (2014). Local genomic

725   adaptation of coral reef-associated microbiomes to gradients of natural variability and

726   anthropogenic stressors. *Proceedings of the National Academy of Sciences*, *111*(28),

727   10227–10232. https://doi.org/10.1073/pnas.1403319111

728

729 Klaus, J. S., Janse, I., Heikoop, J. M., Sanford, R. A., & Fouke, B. W. (2007). Coral microbial

730   communities, zooxanthellae and mucus along gradients of seawater depth and coastal

731   pollution. *Environmental Microbiology*, *9*(5), 1291–1305. https://doi.org/10.1111/j.1462-

732   2920.2007.01249.x

733

734 Koren, O., & Rosenberg, E. (2006). Bacteria Associated with Mucus and Tissues of the Coral

735   *Oculina patagonica* in Summer and Winter. *Applied and Environmental Microbiology*,

736   *72*(8), 5254–5259. https://doi.org/10.1128/AEM.00554-06

737

738 LaJeunesse, T. C., Parkinson, J. E., Gabrielson, P. W., Jeong, H. J., Reimer, J. D., Voolstra, C.

739   R., & Santos, S. R. (2018). Systematic revision of Symbiodiniaceae highlights the

740   antiquity and diversity of coral endosymbionts. *Current Biology, 28*(16), 2570-2580.

741

742 Lane, D. J. (1991). *16S/23S rRNA sequencing*. 115–175.

743

744 Larsson, J. (2020). eulerr: Area-proportional euler and venn diagrams with ellipses. R package

745   version 6.1.0, https://cran.r-project.org/package=eulerr.

746

747 Lee, S. T. M., Davy, S. K., Tang, S.-L., Fan, T.-Y., & Kench, P. S. (2015). Successive shifts in

748   the microbial community of the surface mucus layer and tissues of the coral *Acropora*

749   *muricata* under thermal stress. *FEMS Microbiology Ecology*, *91*(12), fiv142.

750   https://doi.org/10.1093/femsec/fiv142

751

752 Legendre, P., & Anderson, M. J. (1999). Distance-based redundancy analysis: testing

753   multispecies responses in multifactorial ecological experiments. *Ecological Monographs*,

754   *69*(1), 1–24. https://doi.org/10.1890/0012-9615(1999)069[0001:DBRATM]2.0.CO;2

755

756 Lema, K. A., Willis, B. L., & Bourne, D. G. (2012). Corals form characteristic associations with

757   symbiotic nitrogen-fixing bacteria. *Applied and Environmental Microbiology*, *78*(9),

758   3136–3144. https://doi.org/10.1128/AEM.07800-11

759

760 Lesser M.P., Mazel C.H., Gorbunov M.Y., Falkowski P.G. (2004). Discovery of symbiotic
761 nitrogen-fixing cyanobacteria in corals. *Science*, *305*, 997–1000
762
763 Li, J., Chen, Q., Long, L.-J., Dong, J.-D., Yang, J., & Zhang, S. (2015). Bacterial dynamics
764 within the mucus, tissue and skeleton of the coral *Porites lutea* during different seasons.
765 *Scientific Reports*, *4*(1), 7320. https://doi.org/10.1038/srep07320
766
767 Little, A. F. (2004). Flexibility in algal endosymbioses shapes growth in reef corals. *Science*,
768 *304*(5676), 1492–1494. https://doi.org/10.1126/science.1095733
769
770 Littman, R. A., Bourne, D. G., & Willis, B. L. (2010). Responses of coral-associated bacterial
771 communities to heat stress differ with *Symbiodinium* type on the same coral host.
772 *Molecular Ecology*, *19*(9), 1978–1990. https://doi.org/10.1111/j.1365-
773 294X.2010.04620.x
774
775 Littman, R. A., Willis, B. L., Pfeffer, C., & Bourne, D. G. (2009a). Diversities of coral-
776 associated bacteria differ with location, but not species, for three acroporid corals on the
777 Great Barrier Reef: Diversity of coral-associated bacteria. *FEMS Microbiology Ecology*,
778 *68*(2), 152–163. https://doi.org/10.1111/j.1574-6941.2009.00666.x
779
780 Littman, R., Willis, B., & Bourne, D. G. (2009b). Bacterial communities of juvenile corals
781 infected with different Symbiodinium (dinoflagellate) clades. *Marine Ecology Progress
782 Series*, *389*, 45–59. https://doi.org/10.3354/meps08180
783
784 Magoc, T., & Salzberg, S. L. (2011). FLASH: Fast length adjustment of short reads to improve
785 genome assemblies. *Bioinformatics*, *27*(21), 2957–2963.
786 https://doi.org/10.1093/bioinformatics/btr507
787
788 McDevitt-Irwin, J. M., Baum, J. K., Garren, M., & Vega Thurber, R. L. (2017). Responses of
789 coral-associated bacterial communities to local and global stressors. *Frontiers in Marine
790 Science*, *4*, 262. https://doi.org/10.3389/fmars.2017.00262

791

792 McMurdie, P. J., & Holmes, S. (2013). phyloseq: An R package for reproducible interactive

793   analysis and graphics of microbiome census data. *PLoS ONE*, *8*(4), e61217.

794   https://doi.org/10.1371/journal.pone.0061217

795

796 Morris, J. J., Johnson, Z. I., Szul, M. J., Keller, M., & Zinser, E. R. (2011). Dependence of the

797   cyanobacterium *Prochlorococcus* on hydrogen peroxide scavenging microbes for growth

798   at the ocean's surface. *PLoS ONE*, *6*(2), e16805.

799   https://doi.org/10.1371/journal.pone.0016805

800

801 Muller-Parker, G., D'Elia, C. F., & Cook, C. B. (2015). Interactions between corals and their

802   symbiotic algae. In C. Birkeland (Ed.), *Coral Reefs in the Anthropocene* (pp. 99–116).

803   Springer Netherlands. https://doi.org/10.1007/978-94-017-7249-5_5

804

805 Neave, M. J., Apprill, A., Ferrier-Pagès, C., & Voolstra, C. R. (2016). Diversity and function of

806   prevalent symbiotic marine bacteria in the genus Endozoicomonas. *Applied Microbiology*

807   *and Biotechnology*, *100*(19), 8315–8324. https://doi.org/10.1007/s00253-016-7777-0

808

809 Neave, M. J., Rachmawati, R., Xun, L., Michell, C. T., Bourne, D. G., Apprill, A., & Voolstra,

810   C. R. (2017). Differential specificity between closely related corals and abundant

811   Endozoicomonas endosymbionts across global scales. *The ISME Journal*, *11*(1), 186–

812   200. https://doi.org/10.1038/ismej.2016.95

813

814 Oksanen J., Blanchet F.G., Kindt R., Legendre P., Minchin P.R., O'Hara R.B., Simpson G.L.,

815   Solymos P., Stevens M.H.H., Wagner H. (2013). *vegan: community ecology package.* R

816   packageversion 2.0-9. *Available at http://CRAN.R-project.org/package=vegan.*

817

818 Pernice, M., Meibom, A., van den Heuvel, A., Kopp, C., Domart-Coulon, I., Hoegh-Guldberg,

819   O., & Dove, S. (2012). A single-cell view of ammonium assimilation in coral–

820   dinoflagellate symbiosis. *The ISME Journal*, *6*(7), 1314–1324.

821   https://doi.org/10.1038/ismej.2011.196

822

823   Pernice, M., Dunn, S.R., Tonk, L., Dove, S., Domart-Coulon, I., Hoppe, P., Schintlmeister, A.,

824   Wagner, M., & Meibom, A. (2015) A nanoscale secondary ion mass spectrometry study

825   of dinoflagellate functional diversity in reef-building corals. *Environmental Microbiology*

826   *17*(10), 3570–3580. doi:10.1111/1462-2920.12518

827

828   Pogoreutz C, Radecker N, Cardenas A, Gardes A, Wild C, and Voolstra CR. 2018. Dominance

829   of Endozoicomonas bacteria throughout coral bleaching and mortality suggests structural

830   inflexibility of the Pocillopora verrucosa microbiome. *Ecology and Evolution* 8:2240-

831   2252. 10.1002/ece3.3830

832

833   Pollock, F. J., Lamb, J. B., Field, S. N., Heron, S. F., Schaffelke, B., Shedrawi, G., Bourne, D.

834   G., & Willis, B. L. (2014). Sediment and turbidity associated with offshore dredging

835   increase coral disease prevalence on nearby reefs. *PLoS ONE*, *9*(7), e102498.

836   https://doi.org/10.1371/journal.pone.0102498

837

838   Pollock, F. J., McMinds, R., Smith, S., Bourne, D. G., Willis, B. L., Medina, M., Thurber, R. V.,

839   & Zaneveld, J. R. (2018). Coral-associated bacteria demonstrate phylosymbiosis and

840   cophylogeny. *Nature Communications*, *9*(1), 4921. https://doi.org/10.1038/s41467-018-

841   07275-x

842

843   Quigley, K. M., Alvarez-Roa, C., Torda, G., Bourne, D. G., & Willis, B. L. (2019). Co-dynamics

844   of Symbiodiniaceae and bacterial populations during the first year of symbiosis with

845   *Acropora tenuis* juveniles. *MicrobiologyOpen*. https://doi.org/10.1002/mbo3.959

846

847   R Core Team. (2015). *R: A language and environment for statistical computing*. R foundation

848   for statistical computing, Vienna, Austria. *Available at http://www.R-project.org/*.

849

850   Rädecker, N., Pogoreutz, C., Voolstra, C. R., Wiedenmann, J., & Wild, C. (2015). Nitrogen

851   cycling in corals: The key to understanding holobiont functioning? *Trends in*

852   *Microbiology*, *23*(8), 490–497. https://doi.org/10.1016/j.tim.2015.03.008

853

854 Rocker, M. M., Willis, B. L., & Bay, L. K. (2012). Thermal stress-related gene expression in
855   corals with different Symbiodinium types. *In Proc 12th Int Coral Reef Symp 9*, 1-5).

856

857 Rosado, P. M., Leite, D. C. A., Duarte, G. A. S., Chaloub, R. M., Jospin, G., Nunes da Rocha,
858   U., P. Saraiva, J., Dini-Andreote, F., Eisen, J. A., Bourne, D. G., & Peixoto, R. S. (2019).
859   Marine probiotics: Increasing coral resistance to bleaching through microbiome
860   manipulation. *The ISME Journal*, *13*(4), 921–936. https://doi.org/10.1038/s41396-018-
861   0323-6

862

863 Rohwer, F., Seguritan, V., Azam, F., & Knowlton, N. (2002). Diversity and distribution of coral-
864   associated bacteria. *Marine Ecology Progress Series, 243*, 1-10.

865

866 Schloss, P. D., Westcott, S. L., Ryabin, T., Hall, J. R., Hartmann, M., Hollister, E. B.,
867   Lesniewski, R. A., Oakley, B. B., Parks, D. H., Robinson, C. J., Sahl, J. W., Stres, B.,
868   Thallinger, G. G., van Horn, D. J., & Weber, C. F. (2009). Introducing mothur: Open-
869   Source, Platform-Independent, Community-Supported Software for Describing and
870   Comparing Microbial Communities. *Applied and Environmental Microbiology*, *75*(23),
871   7537–7541. https://doi.org/10.1128/AEM.01541-09

872

873 Shashar, N., Cohen, Y., & Loya, Y. (1993). Extreme diel fluctuations of oxygen in diffusive
874   boundary layers surrounding stony corals. *The Biological Bulletin*, *185*(3), 455–461.
875   https://doi.org/10.2307/1542485

876

877 Shaver, E. C., Shantz, A. A., McMinds, R., Burkepile, D. E., Vega Thurber, R. L., & Silliman, B.
878   R. (2017). Effects of predation and nutrient enrichment on the success and microbiome of
879   a foundational coral. *Ecology*, *98*(3), 830–839. https://doi.org/10.1002/ecy.1709

880

881 Siboni, N., Ben-Dov, E., Sivan, A., & Kushmaro, A. (2008). Global distribution and diversity of
882   coral-associated *Archaea* and their possible role in the coral holobiont nitrogen cycle.

883   *Environmental Microbiology*, *10*(11), 2979–2990. https://doi.org/10.1111/j.1462-
884   2920.2008.01718.x

885

886   Ssekagiri, A., Sloan, W. T., & Ijaz, U. Z. (2017). microbiomeSeq: An R package for analysis of
887   microbial communities in an environmental context. https://doi.org/10.13140/rg.2.2.17108.71047

888

889

890   Sweet, M. J., Croquer, A., & Bythell, J. C. (2011). Bacterial assemblages differ between
891   compartments within the coral holobiont. *Coral Reefs*, *30*(1), 39–52.
892   https://doi.org/10.1007/s00338-010-0695-1

893

894   Tanaka, Y., Ogawa, H., & Miyajima, T. (2010). Effects of nutrient enrichment on the release of
895   dissolved organic carbon and nitrogen by the scleractinian coral *Montipora digitata*.
896   *Coral Reefs*, *29*(3), 675–682. https://doi.org/10.1007/s00338-010-0639-9

897

898   Thompson, J. R., Rivera, H. E., Closek, C. J., & Medina, M. (2015). Microbes in the coral
899   holobiont: Partners through evolution, development, and ecological interactions.
900   *Frontiers in Cellular and Infection Microbiology*, *4*.
901   https://doi.org/10.3389/fcimb.2014.00176

902

903   Thurber, R. V., Willner-Hall, D., Rodriguez-Mueller, B., Desnues, C., Edwards, R. A., Angly,
904   F., Dinsdale, E., Kelly, L., & Rohwer, F. (2009). Metagenomic analysis of stressed coral
905   holobionts. *Environmental Microbiology*, *11*(8), 2148–2163.
906   https://doi.org/10.1111/j.1462-2920.2009.01935.x

907

908   Tremblay, P., Weinbauer, M. G., Rottier, C., Guérardel, Y., Nozais, C., & Ferrier-Pagès, C.
909   (2011). Mucus composition and bacterial communities associated with the tissue and
910   skeleton of three scleractinian corals maintained under culture conditions. *Journal of the
911   Marine Biological Association of the United Kingdom*, *91*(3), 649–657.
912   https://doi.org/10.1017/S002531541000130X

913

914   Turner, S., Pryer, K. M., Miao, V. P., & Palmer, J. D. (1999). Investigating deep phylogenetic
915   relationships among cyanobacteria and plastids by small subunit rRNA sequence analysis
916   1. *Journal of Eukaryotic Microbiology, 46*(4), 327-338.

918   Ulstrup, K. E., & Van Oppen, M. J. H. (2003). Geographic and habitat partitioning of genetically
919   distinct zooxanthellae (Symbiodinium) in Acropora corals on the Great Barrier Reef.
920   *Molecular Ecology, 12*(12), 3477-3484.

922   Uysal, Z., & Köksalan, İ. (2006). The annual cycle of Synechococcus (cyanobacteria) in the
923   northern Levantine Basin shelf waters (Eastern Mediterranean). *Marine Ecology*, *27*(3),
924   187-197.

926   van Oppen, M. J., Palstra, F. P., Piquet, A. M. T., & Miller, D. J. (2001). Patterns of coral–
927   dinoflagellate associations in Acropora: significance of local availability and physiology
928   of Symbiodinium strains and host–symbiont selectivity. *Proceedings of the Royal Society*
929   *of London. Series B: Biological Sciences, 268*(1478), 1759-1767.

931   Wada, N., Ishimochi, M., Matsui, T., Pollock, F. J., Tang, S. L., Ainsworth, T. D., Willis,
932   B. L., Mano, N., & Bourne, D. G. (2019). Characterization of coral-associated
933   microbial aggregates (CAMAs) within tissues of the coral *Acropora hyacinthus*.
934   *Scientific Reports*, *9*(1), 14662. doi:10.1038/s41598-019-49651-7

936   Wang, Q., Garrity, G. M., Tiedje, J. M., & Cole, J. R. (2007). Naive Bayesian classifier for
937   rapid assignment of rRNA sequences into the new bacterial taxonomy. *Applied and*
938   *Environmental Microbiology*, *73*(16), 5261–5267. https://doi.org/10.1128/AEM.00062-
939   07

941   Weber, M., de Beer, D., Lott, C., Polerecky, L., Kohls, K., Abed, R. M. M., Ferdelman, T. G., &
942   Fabricius, K. E. (2012). Mechanisms of damage to corals exposed to sedimentation.
943   *Proceedings of the National Academy of Sciences*, *109*(24), E1558–E1567.
944   https://doi.org/10.1073/pnas.1100715109

945

946 Work, T., & Aeby, G. (2014). Microbial aggregates within tissues infect a diversity of corals
947   throughout the Indo-Pacific. *Marine Ecology Progress Series 500*, 1-9.

948

949 Yang, S., Sun, W., Zhang, F., & Li, Z. (2013). Phylogenetically diverse denitrifying and
950   ammonia-oxidizing bacteria in corals *Alcyonium gracillimum* and *Tubastraea coccinea*.
951   *Marine Biotechnology*, *15*(5), 540–551. https://doi.org/10.1007/s10126-013-9503-6

952

953 Yellowlees, D., Rees, T. A. V., & Leggat, W. (2008). Metabolic interactions between algal
954   symbionts and invertebrate hosts. *Plant, Cell & Environment*, *31*(5), 679–694.
955   https://doi.org/10.1111/j.1365-3040.2008.01802.x

956

957 Yilmaz, P., Parfrey, L. W., Yarza, P., Gerken, J., Pruesse, E., Quast, C., Schweer, T., Peplies, J.,
958   Ludwig, W., & Glöckner, F. O. (2014). The SILVA and "All-species Living Tree Project
959   (LTP)" taxonomic frameworks. *Nucleic Acids Research*, *42*(D1), D643–D648.
960   https://doi.org/10.1093/nar/gkt1209

961

Figure 1

Compartment-specific microbiome composition of *Acropora tenuis* and *Acropora millepora*.

Microbial community composition (size indicates mean relative abundance and color represents standard error) resolved for the surface mucus layer and tissue of two *Acropora* coral species (*A. tenuis* and *A. millepora*), and surrounding seawater, based on partial 16S rRNA gene amplicon sequencing. Only the 25 most abundant families across all samples are represented.
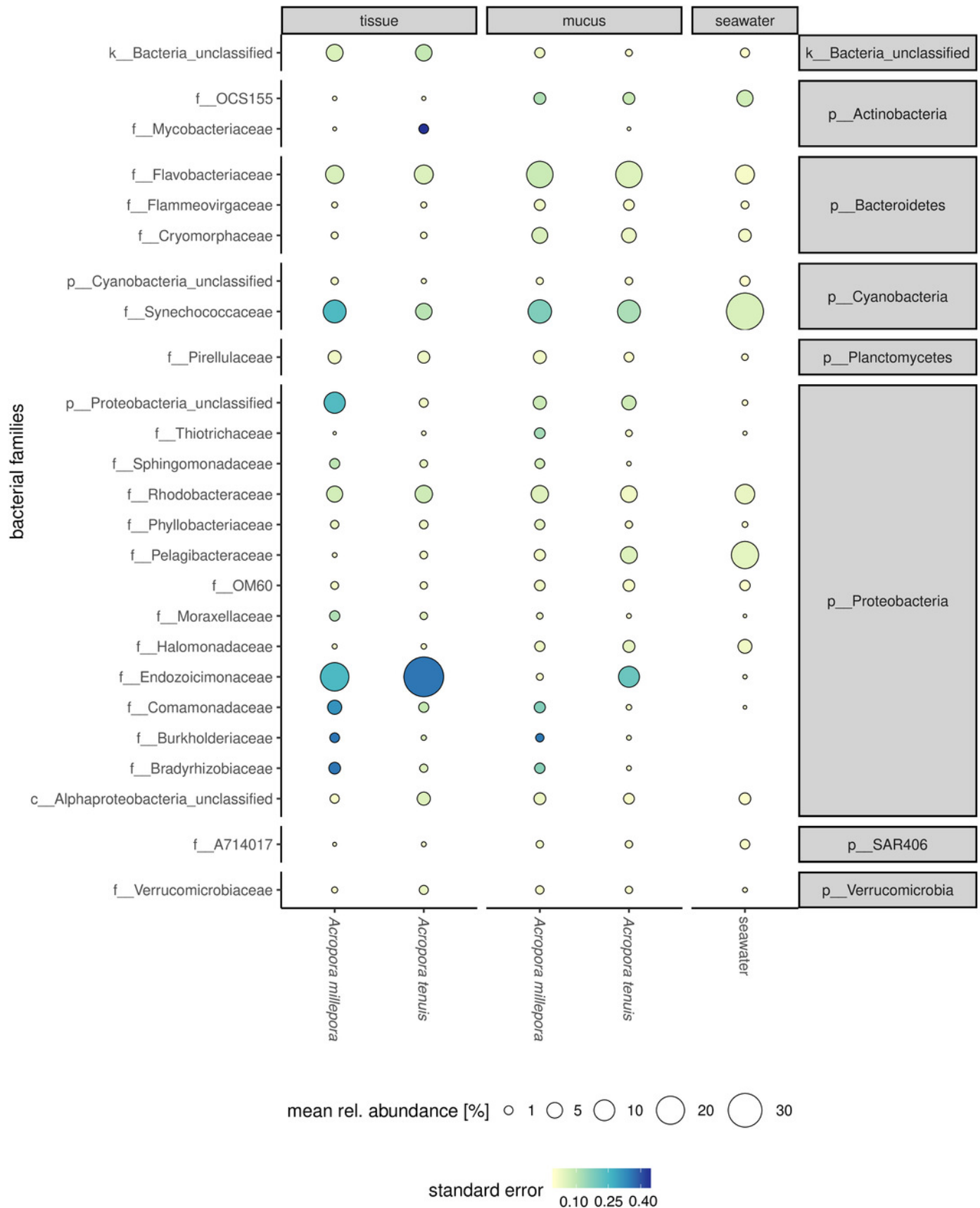
Figure 2

Alpha diversity measures of coral mucus, coral tissue, and seawater microbiomes.

(A) Venn diagram displaying the number of shared, unique, and ubiquitous zOTUs among mucus, tissue and seawater microbiomes. Two *Acropora* species (*A. tenuis* and *A. millepora*) are pooled for the tissue and mucus microbiomes. (B) zOTU richness and (C) Shannon diversity index of microbiomes associated with tissue and mucus of *A. millepora* and *A. tenuis*, as well as with seawater.
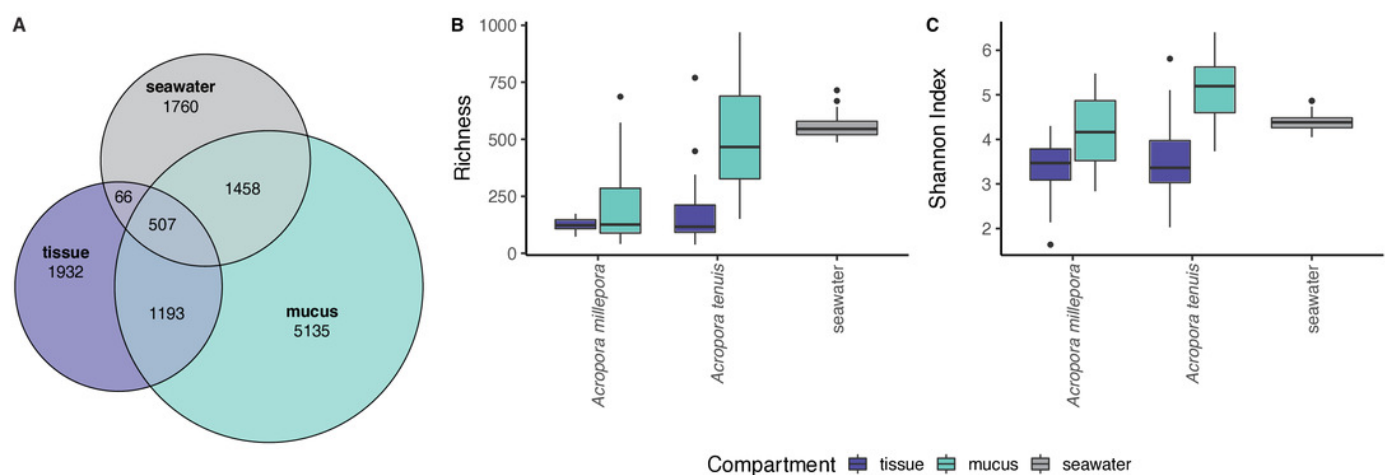
Figure 3

Compositional variability of microbiomes associated with distinct coral-compartments and the ambient seawater.

Two-dimensional non-metric multidimensional scaling (nMDS) ordination depicting variation in microbial community structure between coral compartments (mucus and tissue) of *Acropora tenuis and Acropora millepora*, and seawater samples. "k" is the number of dimensions.
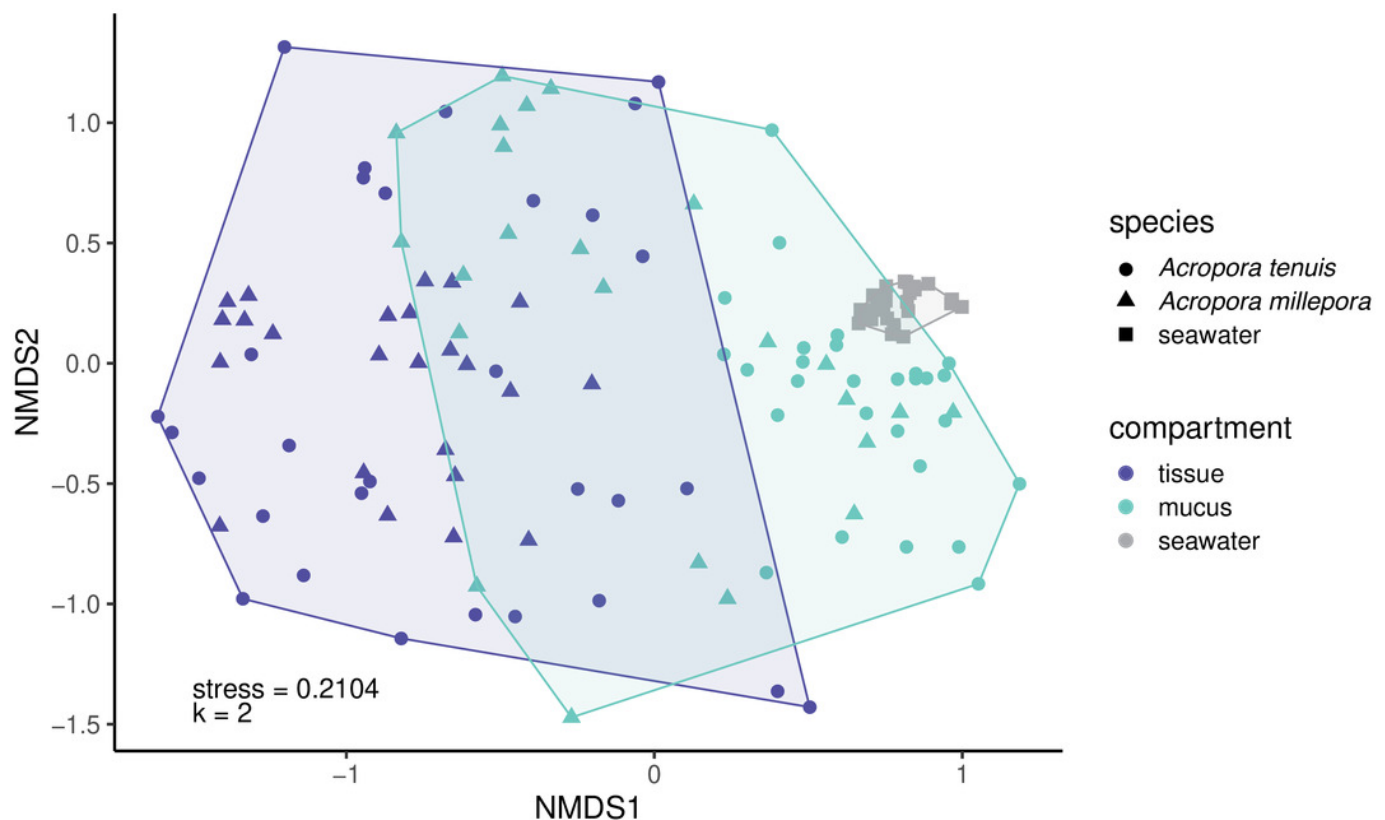
Figure 4

Environmental and physiological drivers of the *Acropora tenuis* and *Acropora millepora* microbiomes.

Distance-based redundancy analysis (db-RDA) of the relationship between environmental/physiological variables and the relative abundance of bacteria in (A) mucus and (B) tissue microbiome of *A. millepora*, and (C) mucus and (D) tissue microbiome of *A. tenuis*. Arrow length indicates the strength of the correlation between the variables and the samples (note only significant variables are shown). The selected variables explain a total of (A) 14.98%, (B) 16.44%, (C) 10.63% and (D) 10.97% of the observed variance, respectively. Environmental/physiological variables represented are the sum of nitrite and nitrate concentrations (NO2/NO3), particulate organic carbon (POC), total suspended solids (TSS), ammonium concentration (NH_4), chlorophyll *a* concentration (Chla) in seawater and Symbiodiniaceae density normalized to protein contents (Zoox/Pro) of coral tissue.
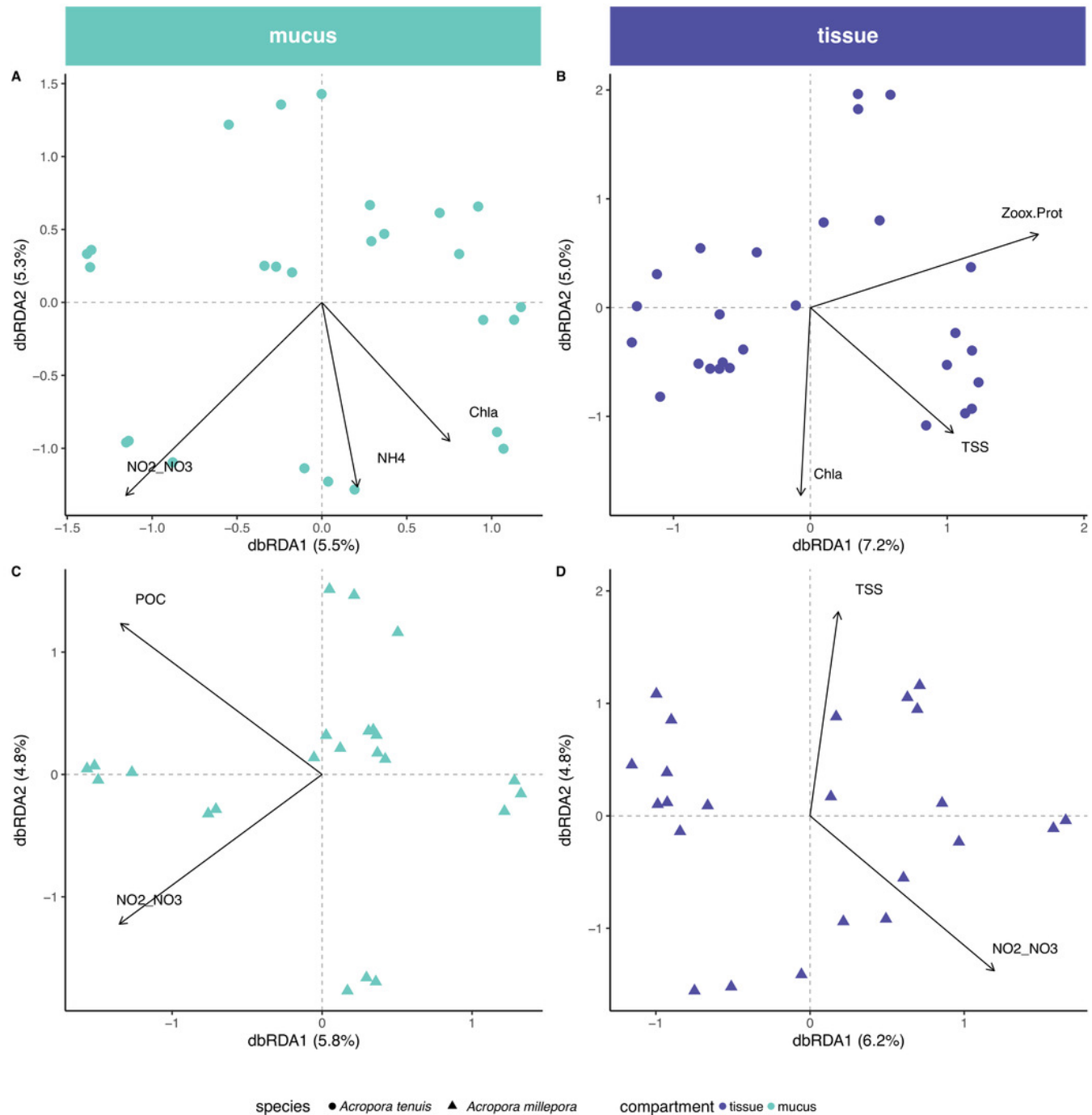
Figure 5

Bacterial taxa significantly correlated with environmental and physiological variables.

Pearson's coefficient based correlation matrix between the 20 most abundant bacterial families and environmental/physiological variables having a significant effect on the microbiome associated to tissue and surface mucus of (A) *Acropora millepora* (n_{tissue}= 24, n_{mucus}= 24)and (B) *Acropora tenuis* (n_{tissue}= 30, n_{mucus}= 28). Significant correlations indicated by asterisks at different levels of significance (* for $p < 0.05$, ** for $p < 0.01$, *** for $p < 0.001$) after correction for multiple comparisons (using Benjamini-Hochberg correction). Environmental/physiological variables represented are the sum of nitrite and nitrate concentrations (NO2/NO3), particulate organic carbon (POC), total suspended solids (TSS), ammonium concentration (NH_4), chlorophyll *a* concentration (Chla) in seawater and Symbiodiniaceae density normalized to protein contents(Zoox/Pro) of coral tissue.