

Depleted dissolved organic carbon and distinct Bacterial communities in the water column of a rapid-flushing coral reef ecosystem

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1 **Abstract:**

2 Coral reefs are highly productive ecosystems bathed in unproductive, low-nutrient
3 oceanic waters, where microbially-dominated food webs are supported largely by
4 bacterioplankton recycling of dissolved compounds. Despite evidence that benthic reef
5 organisms efficiently scavenge particulate organic matter and inorganic nutrients from
6 advected oceanic waters, our understanding of the role of bacterioplankton and dissolved
7 organic matter in the interaction between reefs and the surrounding ocean remains
8 limited. Here we present the results of a four-year study conducted in a well-
9 characterized coral reef ecosystem (Paopao Bay, Moorea, French Polynesia) where
10 changes in bacterioplankton abundance and dissolved organic carbon (DOC)
11 concentrations were quantified and bacterial community structure variation was
12 examined along spatial gradients of the reef:ocean interface. Our results illustrate that the
13 reef is consistently depleted in concentrations of both DOC and bacterioplankton relative
14 to offshore waters (averaging $79 \mu\text{mol L}^{-1}$ DOC and $5.5 \times 10^8 \text{ cells L}^{-1}$ offshore and 68
15 $\mu\text{mol L}^{-1}$ DOC and $3.1 \times 10^8 \text{ cells L}^{-1}$ over the reef, respectively) across a four year time
16 period. In addition, using a suite of culture-independent measures of bacterial community
17 structure, we found consistent differentiation of reef bacterioplankton communities from
18 those offshore or in a nearby embayment across all taxonomic levels. Reef habitats were
19 enriched in Gamma-, Delta-, and Beta-proteobacteria, Bacterioidetes, Actinobacteria and
20 Firmicutes. Specific bacterial phylotypes, including members of the SAR11, SAR116,
21 Flavobacteria, and *Synechococcus* clades, exhibited clear gradients in relative abundance
22 among nearshore habitats. Our observations indicate that this reef system removes
23 oceanic DOC and exerts selective pressures on bacterioplankton community structure on
24 timescales approximating reef water residence times, observations which are notable both
25 because fringing reefs do not exhibit long residence times (unlike those characteristic of
26 atoll lagoons) and because oceanic DOC is generally recalcitrant to degradation by
27 ambient microbial assemblages. Our findings thus have interesting implications for the
28 role of oceanic DOM and bacterioplankton in the ecology and metabolism of reef
29 ecosystems.
30

31 **Introduction:**

32 Coral reefs are highly productive ecosystems that develop and thrive within the
33 oligotrophic tropical and subtropical oceans (Darwin, 1889). Understanding the sources
34 of nutrients and organic material that support coral reefs is central to predicting and
35 managing how these ecosystems will respond to global change (Sorokin, 1990).

36 Microbial communities play a dominant biogeochemical role in both reef and open-ocean
37 environments, with heterotrophic microbial communities recycling more than half of net
38 productivity in both ecosystem types (Cho and Azam, 1990; Ducklow, 1990). The largest
39 pool of organic matter found in the ocean is a heterogenous mixture of dissolved
40 compounds, a small portion of which is bioavailable to bacterioplankton on time scales of
41 hours to days (Carlson, 2002). This bioavailable component of dissolved organic carbon
42 (DOC) is a key component of the microbial loop (Azam *et al.*, 1983; Pomeroy, 1974).
43 Both theory (Crossland *et al.*, 1991; Ducklow, 1990; Sorokin, 1990), and field-based
44 models (Arias-Gonzalez *et al.*, 1997; Grigg *et al.*, 1984) indicate the importance of
45 microbes to reef food webs and that understanding microbial processes is central to
46 understanding the links between reef and ocean ecosystems.

47

48 Odum and Odum (1955) put forward a widely cited theory for how reefs acquire the
49 necessary macronutrients to sustain high productivity, positing that high flow rates and
50 surface area allow reefs to concentrate nutrients and organic matter from dilute oceanic
51 water, and specifically emphasizing the probable importance but largely unknown role of
52 dissolved organic matter within the reef. Nutrient inputs from terrestrial sources
53 (Fabricius, 2005), nitrogen-fixation (Lesser *et al.*, 2004; Wiebe *et al.*, 1975) or even
54 geothermal endo-upwelling (Rougerie *et al.*, 1992) cannot balance the nutrient
55 requirements of coral reef systems (Crossland and Barnes, 1983). Understanding the
56 interaction of bacterioplankton and dissolved organic matter (DOM) at the ocean:reef
57 interface is important to interpreting nearshore ecosystem productivity and organic
58 recycling. This is especially true if coral reefs are supported by oceanic subsidies through
59 continual scavenging and transformation of nutrients and biomass from offshore waters.

60

61 Tropical reef ecosystems support a diverse and active microbial community both directly
62 associated with corals and in the surrounding water column (Ducklow, 1990). Recent
63 research has emphasized the specificity and metabolic integration of surficial microbial
64 communities associated with corals, sponges, and other key reef benthic macroorganisms
65 (Rohwer *et al.*, 2001; Wegley *et al.*, 2007), yet we have a poor grasp of the composition
66 of the planktonic microbial community (Dinsdale *et al.*, 2008; Weinbauer *et al.*, 2010).
67 The community structure of the heterotrophic bacterioplankton is fundamentally linked to
68 the bioavailability, composition, and metabolism of DOM and availability of inorganic
69 nutrients in aquatic habitats (Cottrell and Kirchman, 2003; Giovannoni and Stingl, 2005),
70 thus defining community connectivity and variation among nearshore habitats is
71 important in clarifying the metabolic role of bacterioplankton in the reef ecosystem.

72

73 We surveyed concentrations of bacterioplankton and DOC in a barrier/fringing reef-
74 embayment site of the Moorea Coral Reef Long Term Ecological Research (MCR-
75 LTER) site in Moorea, French Polynesia. The MCRLTER is an interdisciplinary,
76 decadal-scale research program seeking to understand the processes that modulate
77 ecosystem function, shape community structure and diversity, and determine abundance
78 and dynamics of the coral reef communities of the South Pacific. Samples were collected
79 seasonally over four years along depth profiles in three nearshore habitats (Forereef,
80 Backreef, and Bay) and ~5 km Offshore. In addition, multiple synoptic surface surveys
81 were conducted across the reef-ocean interface to characterize spatial gradients in DOC
82 and bacterioplankton community structure. Our goal was to develop a solid foundation of
83 spatiotemporal variability in DOC and bacterioplankton community structure at the reef-
84 ocean interface in the context of physical processes. We investigate the concept of the
85 reef platform as a source or sink of water column DOC and bacterioplankton as oceanic
86 inputs flow through the nearshore environment by answering three central questions: 1)
87 whether reef environments contain concentrations of DOC that differ from their oceanic
88 inputs, 2) whether bacterioplankton densities on the reef correlate with spatial patterns of
89 DOC at the reef-ocean interface, and 3) whether bacterioplankton communities on coral
90 reefs differ systematically from offshore habitats despite a seemingly high flushing rate.

91 We aimed to contextualize these questions through time and space in a system with
92 consistent reef-ocean connectivity and well-defined physico-chemical gradients.
93
94

95 **Methods :**

96 *Study location* – This study was carried out in the vicinity of Paopao Bay on the north
97 shore of the island of Moorea, French Polynesia (-17.48, -149.82, Fig. 1). Moorea is 1.5 -
98 2 million years old (Neall and Trewick, 2008) with barrier reefs cresting within 1 km of
99 the shore. Reef pass channels occur roughly every 5-10 km around the circumference of
100 the island, typically corresponding to embayments of varying size, of which Paopao (aka
101 Cook’s Bay) is one of the two largest: the Bay averages 25-30 m depth and Avaroa Pass
102 is ~35 m deep (Hench *et al.*, 2008). The Forereef slope has relatively high coral density
103 and drops steeply (average slope 1:8) to depths exceeding 500 m within 1 km offshore.
104 The Backreef platform includes a shallow (< 3 m) lagoon region comprising a mixture of
105 dense corals and barren sands interspersed with massive coral “bommies” as well as a
106 deeper (10-12 m) fringing reef region bordering the island. Waves drive water from the
107 Forereef across the reef crest (averaging 0.2 m s^{-1} with negligible tidal influence) that
108 rapidly drains laterally, mixing with the Bay and forming a steady offshore jet exiting
109 through the pass (Hench *et al.*, 2008). These three hydraulically interconnected habitats
110 (Bay, Forereef, and Backreef), as well as Offshore locations 1-6 km north of the island,
111 are referred to throughout the manuscript and both synoptic and time-series sampling
112 strategies were designed to clarify temporal and spatial variation among the habitats.

113
114 *Sample collection and storage* – Samples were collected over a three-day period 2 to 3
115 times each year from 2005 through 2009. DOC and bacterioplankton were collected in
116 ten depth-profile time-series sampling events over this period and two additional high-
117 resolution grid surveys (Aug.-Sep. 2008 and 2009; Fig. 1). All samples were stored at *in*
118 *situ* temperatures in the dark for up to 2 hours before processing. Seasonal time-series
119 samples were collected at discrete depths (1, 5 and 10 m) via 8L teflon-coated acid-rinsed
120 Niskin bottles and synoptic grid samples were hand-collected at ~0.1 m depth in acid-
121 washed polycarbonate bottles. In synoptic grid surveys DOC was sampled directly from
122 the collection bottle through combusted glass fiber filters (Whatman GF/F) while in
123 seasonal time-series sampling total organic carbon (TOC) was sampled directly from
124 Niskin bottles without filtration. Particulate organic carbon is a small component of the
125 TOC pool of Moorean waters (averaging 3% to 5% both offshore and in the reef

126 environments) and does not differ significantly between Offshore and BackReef habitats
127 ($n = 21$, $p = 0.12$), thus the temporal and spatial dynamics of the TOC pool are primarily
128 due to changes in the DOC pool (Hansell and Carlson, 1998) and the measurement of
129 TOC from the seasonal sampling is henceforth referred to as DOC throughout this
130 manuscript. All DOC samples were collected into acid-leached, Nanopure flushed,
131 sample-rinsed 60 mL HDPE bottles and stored frozen at $-20\text{ }^{\circ}\text{C}$ until analysis (Carlson *et*
132 *al.*, 2010). Unfiltered samples for bacterioplankton abundance were fixed with
133 paraformaldehyde (0.4% final concentration) and stored frozen ($-80\text{ }^{\circ}\text{C}$) within 30
134 minutes of fixation. Nucleic acid samples from synoptic Austral winter surveys (Aug.-
135 Sep. 2008 and 2009) were collected by gravity-filtering 0.8-1.5 L water through a $0.2\text{ }\mu\text{m}$
136 polyethersulfone filter cartridge (Millipore Sterivex), preserved frozen with 1.7 mL
137 sucrose lysis buffer (for fingerprinting; 40 mmol L^{-1} ethylenediaminetetraacetic acid, 50
138 mmol L^{-1} Tris-HCl, 750 mmol L^{-1} sucrose, 400 mmol L^{-1} NaCl, pH 8.0). A single Austral
139 summer sampling event for pyrosequencing (Jan. 2008) collected duplicate 1L whole
140 water samples in sterile polyethylene terephthalate bottles from the upper 5 m. Samples
141 were filtered and stored as above except that Puregene Lysis Buffer (Qiagen) was used in
142 place of sucrose lysis buffer.

143

144 *DOC concentration measurement* – Samples were thawed at room temperature, vortexed
145 to mix thoroughly, decanted into precombusted borosilicate vials with acid-washed
146 teflon-lined lids, and analyzed via high temperature oxidation on a modified Shimadzu
147 TOC-V modified according to Carlson *et al.* (2010). UV- oxidized deionized water with
148 organics removed (Barnstead Nanopur Diamond) was used for blank correction for all
149 samples. Each system run was calibrated with both potassium hydrogen phthalate
150 standards (4 point curve $25 - 100\text{ }\mu\text{M}$) referenced against low carbon deep Sargasso Sea
151 reference waters (2600 m) and surface Sargasso Sea water every 6 – 8 analyses (Carlson
152 *et al.*, 2004; Hansell and Carlson, 1998) calibrated with DOC Consensus Reference
153 Waters (Hansell, 2005).

154

155 *Bacterioplankton abundance measurement* – Fixed samples were thawed, mixed, stained
156 with 1X SYBR[®] Green I (Invitrogen) 30 minutes (dark room temperature) and analyzed

157 within 3 hours. We empirically determined that the integrity of the stain yielded
158 consistent abundance measurements throughout a minimum of three hours measured at
159 20 minute intervals. Samples were counted using a flow cytometer (LSR II; BD
160 Biosciences) equipped with a high throughput sampler (HTS), Coherent Sapphire 488nm
161 laser, and a default suite of 6 detectors (side-scatter and forward-scatter photodiodes and
162 green, orange, red, and far-red photomultipliers). Using the HTS syringe pumps, a known
163 sample volume (45 μL) was injected at a steady rate ($0.5 \mu\text{L sec}^{-1}$) such that data
164 acquisition was maintained at $<1000 \text{ events sec}^{-1}$ and $>10,000$ bacterial events were
165 recorded for each sample over a period of at least 90 sec. A minimum green fluorescence
166 threshold (channel 200) was assigned to exclude unstained particles and photomultiplier
167 voltages were adjusted upward such that $\sim 10\%$ of events were visible as noise on each
168 channel to increase signal:noise and the clarity of population differentiation. Two
169 dimensional gating was applied on graphs of scatter vs. green fluorescence to remove
170 noise (populations averaging zero side scatter). Bacterial concentration calculations were
171 corrected for minor dilution with stain and fixative. A subset of samples counted both by
172 flow cytometry and 4',6-diamidino-2-phenylindole (DAPI) epifluorescence microscopy
173 (Porter and Feig, 1980) yielded a strong relationship between the two measurements, with
174 cytometry counts approximately 20% less than microscopy counts (Model II regression
175 slope = 0.82, $n = 75$, $r^2 = 0.64$, $p < 0.001$).

176

177 *Bacterial community structure measurement* – We used two culture-independent
178 approaches to assess bacterial community structure from 16S rRNA gene sequence
179 information in DNA extracted from 0.2 μm membranes. Terminal restriction fragment
180 length polymorphism (TRFLP) was used to analyze ~ 100 samples collected synoptically
181 in Aug.-Sep. of 2008 and 2009 according to Nelson (2009). In brief, filtered cells were
182 lysed by incubating preserved filters amended to 1% sodium dodecyl sulfate and 8 μg
183 mL^{-1} Proteinase K at 60°C and a portion was extracted using the DNEasy kit (Qiagen).
184 The polymerase chain reaction with primers 8f (AGRGTTYGATYMTGGCTCAG) and
185 519r (GWATTACCGCGGCKGCTG) was used to amplify the 16S rRNA gene (30
186 cycles of 94°C 30 sec, 57°C 60 sec, 72°C 120 sec) according to Nelson (2009). Products
187 were gel-extracted via QiaEx (Qiagen) and digested 4 hours at 37°C with enzyme HaeIII

188 (New England Biolabs) followed by enzyme inactivation (20 min 80°C). Fragment
189 analysis of formamide-saturated and heat-denatured samples via capillary sequencer
190 (Applied Biosystems 3730XL) was conducted at the UC Berkeley DNA Sequencing
191 Facility using a custom sizing standard (20 sizes over the range 30 to 650 base pairs;
192 Bioventures). Electropherogram peak areas in the 30-550 bp range were relativized by
193 sample totals, aligned and analyzed according to Nelson (2009), with peaks less than
194 0.5% of total peak area excluded from analysis. Clone libraries (sequences of 100 random
195 16S rRNA amplicons using identical primers from water collected from the Backreef in
196 March of 2007: Genbank accession numbers HQ443320-HQ443409) were used to assign
197 putative sequence-based phylogenetic information to terminal restriction fragments of
198 interest as previously described (Nelson, 2009). Amplicon pyrosequencing of the V6
199 hypervariable region of the bacterial 16S rRNA gene was conducted on samples collected
200 Jan. 2008 (Table S1) using bacterial primers 967f and 1046r on DNA extracted and
201 amplified according to (Huber et al., 2007). These 16S rRNA gene V6 amplicon
202 sequences have been deposited in the National Center for Biotechnology Information
203 (NCBI) Sequence Read Archive under the accession number SRPXXXXXX. All
204 statistical analyses and heatmaps were conducted using JMP (v. 8; SAS Institute); unless
205 otherwise noted, p-values for differences between habitats are derived from ANOVA
206 with Tukey post hoc tests to control for multiple comparisons. All community structure
207 analyses were performed with Primer-E (v. 6; Clarke *et al.*, 2006). All contour plots were
208 generated with Ocean Data View v4.3 (Schlitzer 2010) using DIVA gridding with 30X30
209 scale-length to avoid overinterpolation, a method well-optimized for sampling points
210 which show spatial variation in density.

211 **Results:**

212 *Spatial gradients of DOC and bacterioplankton concentrations-* Both surface DOC and
213 bacterioplankton concentrations were depleted in the Backreef relative to Offshore waters
214 during synoptic sampling surveys in September of 2008 and 2009 (Figs. 2 and S1). In
215 these surface surveys DOC concentrations in the Forereef and Bay were intermediate
216 between Backreef and Offshore endpoints while bacterioplankton abundances were
217 elevated in the Bay relative to other habitats. These spatial patterns held constant over
218 two adjacent sampling dates in 2008 between which a common strong southerly wind
219 (known locally as a mara'amu) produced substantial surface waves and sediment
220 resuspension (Figs. S1b-e).

221
222 The gradients of DOC concentrations and bacterioplankton densities observed during the
223 synoptic spatial survey (Austral winter 2008-2009) were also maintained through time as
224 revealed from the seasonal sampling of bay, reef and offshore habitats from 2005-2009
225 (Fig 3). The Backreef environment was significantly lower in DOC concentration relative
226 to Offshore waters over the 2005-2009 sampling period regardless of season (ANOVA
227 with Tukey post hoc tests comparing concentrations in each habitat $p < 0.05$; Figs. 3a and
228 3b) and was consistently depleted in bacterioplankton relative to all other habitats (Figs.
229 3c and 3d). During austral winter differentiation between habitats was more pronounced,
230 with elevated DOC in the Forereef relative to the other nearshore habitats (but still less
231 than offshore; Fig. 3b). Winter bacterioplankton densities in the Bay were elevated
232 relative to all other habitats and exceeded summer Bay bacterioplankton densities (Fig.
233 3d). DOC and bacterioplankton vertical variability on any sampling date was much
234 smaller than lateral variability among habitats from Backreef through Offshore (e.g. Fig.
235 S1a) with no statistical effect of sampling depth on later habitat differentiation across
236 dates (ANCOVA was used to test the significance of interaction between habitat and
237 depth in explaining variation in DOC and bacterioplankton concentrations; habitat*depth
238 $p = 0.19$ and 0.37 respectively). Moreover, there was no evidence for persistent
239 stratification of concentrations in the upper 10 m of Forereef, Backreef, or Offshore
240 habitats across seasons (although surface bacterioplankton concentrations in the Bay
241 exceeded those at 10 m when grouped across the time series; $p = 0.02$).

242

243 Concentrations of phosphate, nitrite, and silica did not differ significantly among the four
244 habitats over the 2005-2009 time series averaged over the upper 10 m (Fig. S2. $p > 0.10$
245 in either season or grouped across seasons). In winter only, nitrate concentrations were
246 greater on average in the Backreef (mean $0.46 \mu\text{mol L}^{-1}$) than Offshore (mean $0.13 \mu\text{mol}$
247 L^{-1} ; $p = 0.012$, $n = 23$). Particulate organic stocks (carbon, nitrogen, and chlorophyll *a*)
248 were significantly higher within the Bay relative to other locations ($p < 0.05$) across the
249 seasonal dataset but not significantly different between Forereef, Backreef, and Offshore
250 sampling points in either season or grouped across seasons ($p > 0.05$).

251

252 *Synoptic spatial differentiation of bacterioplankton community structure -*

253 Bacterioplankton community structure was found to be significantly different among the
254 Offshore, Backreef, Forereef and Bay habitats on multiple dates and using different
255 methods of community characterization, including TRFLP, cloning, and amplicon
256 pyrosequencing (Figs. 4-6, S3-5).

257

258 *TRFLP fingerprinting-* Synoptic winter surveys in Aug.-Sep. of 2008 and 2009 revealed
259 significant differences between habitats each year in TRFLP fingerprints of
260 bacterioplankton community structure (Figs. 4 and S3; 2-way nested ANOSIM tested the
261 significance of clustering by habitat within years $R = 0.76$, $p < 0.001$). Hierarchical
262 clustering of surface samples collected 1 Sep 2009 according to relative abundance of
263 TRFLP phylotypes (Figure 4) matched habitat clustering patterns observed during
264 smaller surveys in 2008 (Fig. S3a) and showed minimal depth variation (Fig. S3b). The
265 dominant nonmetric multidimensional scaling axis of community variation (53.8%
266 variation) paralleled the onshore to offshore habitat gradient in both years when ordinated
267 together. While the relationships between habitats were consistent between 2008 and
268 2009 the two years differed significantly overall (ANOSIM tested the significance of
269 clustering by year $R = 0.60$, $p < 0.001$). As with patterns of bacterioplankton and DOC
270 depletion, these spatial patterns in community differentiation held constant over two
271 adjacent sampling dates in 2008 separated by a significant storm event (Figs. S3c-d).

272

273 *Clone libraries* - Using a random clone library, phylogenetic classifications were
274 putatively assigned to 33 of 120 terminal restriction fragments (TRFs) found in the 2008-
275 09 synoptic surveys by measuring TRF lengths of cloned 16S amplicons (Fig. S5). The
276 two ecotypes of SAR11 found in the clone library showed different spatial patterns of
277 relative abundance: Group Ia was relatively homogeneously distributed but slightly
278 enriched in the nearshore and Group II was contrastingly rare in the Bay but markedly
279 enriched within the Backreef (Figs. 5d and 5a, respectively). *Synechococcus* were
280 relatively dominant throughout the surface waters but increased in relative abundance
281 offshore (Fig. 5b). An unidentified member of the SAR116 clade also showed a marked
282 increase in relative abundance offshore, becoming relatively rare in the Backreef and Bay
283 habitats (Fig. 5e). Two distinct members of the Flavobacteriaceae showed contrasting
284 distributions, with one enriched only in the Forereef (Fig. 5c) and another depleted only
285 in the Backreef (Fig. 5f). A resemblance matrix comprised solely of these six taxa was
286 correlated with overall community resemblance among sampling locations and years
287 ($r_{\text{Mantel}} = 0.82$, $p < 0.01$), demonstrating that the variation in these six taxa matched the
288 overall community differentiation patterns among habitats.

289

290 *Pyrosequencing*- 16S rRNA gene amplicon sequence data also revealed similar habitat
291 partitioning to that demonstrated in TRFLP analyses (Fig 6) based on > 237,000 v6 tags
292 analyzed among six habitats along the reef-offshore gradient (Table S1). Methodological
293 replicate samples (~ 20,000 sequences each) were not significantly different (SIMPROF
294 $p > 0.05$) but the community structure of each nearshore habitat was significantly
295 different (SIMPROF $p < 0.05$, Fig 6). Spatial differences in community structure were
296 due to changes in the presence or absence of broad Bacteria phylotypes rather than minor
297 shifts in the relative abundance of taxonomically similar OTUs, as patterns in community
298 differentiation among habitats were consistent whether data were analyzed at very fine or
299 coarse taxonomic scale (reference OTUs or Order level) and whether analyzed using
300 sequence relative abundance or presence/absence data (Fig S4). These sensitivity
301 comparisons were only carried out using pyrosequencing data, as fingerprinting methods
302 (such as TRFLP) lack the phylogenetic resolution needed to contrast taxonomic levels

303 and lack the sequence frequency resolution necessary to declare a taxon absent in
304 presence/absence analyses.

305

306 We identified three primary community types at the 90% Bray-Curtis similarity level
307 when samples were clustered according to sequence frequency of bacterial Classes (Fig.
308 6). Backreef habitats were relatively enriched in Beta- and Gamma-proteobacteria,
309 Firmicutes, and Bacteroidetes and Forereef/Bay habitats were relatively enriched in
310 Actinobacteria, Deltaproteobacteria, and Planctomycetes compared with offshore
311 habitats. All samples were dominated by Alphaproteobacteria (ranging from 36 to 48%
312 and averaging 42.6%) and Cyanobacteria (ranging from 21 to 39% and averaging 28.7%)
313 with Gammaproteobacteria, Betaproteobacteria, and Flavobacteria also contributing more
314 than 1% of sequences on average 16%, 1.2%, and 4.4% respectively; Fig. 6). The
315 majority of bacterial classes found via pyrosequencing were present at low abundances (<
316 0.5% of sequences; Fig 6), suggesting that they were not included in TRFLP analyses. As
317 expected, we found elevated levels of bacterial classes known to contain various human
318 pathogens, environmental copiotrophs, and coral-associated microbes, including various
319 Gram-positive groups (Bacilli, Clostridia, Actinobacteria), Gammproteobacteria, and
320 Bacteroidetes (Flavobacteria, Sphingobacteria, Bacteroidia), in the nearshore habitats
321 relative to the open ocean.

322

323

324

325 **Discussion:**

326 Our seasonal and synoptic surveys comprised more than 100 independent samples and
327 unambiguously demonstrated that the Backreef platform behind the crest is consistently
328 depleted in both DOC and bacterioplankton relative to the open ocean and Forereef slope
329 habitats across seasons and years (Figs. 2, 3, S1). Using multiple culture-independent
330 methods to characterize bacterial community structure, we found distinct community
331 differentiation among nearshore habitats in synoptic surveys at different times of year,
332 with clear spatial gradients in identified clades, as well as distinct nearshore-offshore
333 trends in relative abundance of broad bacterial Classes (Figs. 4-6, S3-3). Together these
334 observations are notable because they indicate that reef physical and biological processes
335 work rapidly in maintaining a planktonic microbial ecosystem fundamentally altered
336 from the surrounding oceans (residence times of Moorea's reefs have been estimated on
337 the order of hours to days; Delesalle and Sournia, 1992; Hench *et al.*, 2008; Lenhardt,
338 1991). The potential for reefs to rapidly alter the density of bacterioplankton is well
339 supported by studies reporting both depletion of bacterioplankton in reef water columns
340 relative to oceanic waters (Ayukai, 1995; Gast *et al.*, 1998) and enhanced removal of
341 bacterioplankton biomass with proximity to reef benthic organisms (Genin *et al.*, 2009;
342 Houlbreque *et al.*, 2006; Scheffers *et al.*, 2004).

343
344 Our observations of altered bacterioplankton community structure over the reef further
345 suggest that such removal processes may be selective or complemented by increased
346 abundance of reef-specific taxa. However, we are not aware of another study
347 demonstrating consistently depleted DOC in reef environments relative to the open
348 ocean, although recent observations indicate the potential for the phenomenon to be
349 widespread (Dinsdale *et al.*, 2008; Suzuki *et al.*, 2001). Instead most studies in rapidly
350 flushed reefs show either diel increases in DOC above offshore concentrations (Hata *et al.*,
351 2002; Van Duyl and Gast, 2001) or consistently elevated concentrations of DOC
352 (Torréton *et al.*, 1997). Reef DOC depletion on residence timescales of hours to days is
353 surprising and has significant biogeochemical implications because the bulk DOC pool in
354 the surface waters of subtropical gyres (such as those surrounding Moorea) has been
355 reported to be recalcitrant material resistant to rapid microbial degradation by surface

356 water microbial assemblages (Carlson, 2002; Carlson and Ducklow, 1996; Carlson *et al.*,
357 2004; Cherrier *et al.*, 1996). Our results suggest that benthic and/or planktonic
358 communities within the reef ecosystem have the potential to rapidly and efficiently
359 consume both dissolved material and bacterioplankton cells, but both biogeochemical and
360 physical processes must also be considered as mechanisms to explain the patterns
361 observed.

362

363 *Evidence for physical mechanisms of DOC and bacterioplankton community alteration*
364 *on the reef* - Dilution of nearshore waters by groundwater, terrestrial runoff, or
365 geothermal endo-upwelling (Rougerie *et al.*, 1992) could potentially cause reduced DOC
366 concentrations and altered bacterioplankton community structure within the nearshore
367 environment, but three lines of evidence rule this mechanism out. First, any dilution
368 would be evident in salinity or temperature, but neither show differences in mean values
369 between Backreef and Offshore waters through time, although riverine inputs do exert a
370 small but significant influence on the Bay, making it slightly warmer (28.17 vs 27.81°C)
371 and less saline (salinities of 35.99 vs 36.05) than the other three habitats on average ($p <$
372 0.01). Second, the concentration of DOC in Paopao stream (the primary freshwater
373 source for the system) in Sept 2008 was 34.2 $\mu\text{mol L}^{-1}$, markedly lower than the surface
374 ocean but concentrated enough to require an unreasonably large freshwater input to yield
375 the ~13% (~8 $\mu\text{mol L}^{-1}$) average DOC depletion observed in the nearshore regions. Third,
376 DOC concentrations in island porewaters in neighboring Tahiti increase dramatically
377 with depth (exceeding 2 mmol L^{-1} within 20m; Fichez *et al.*, 1996), suggesting that
378 groundwater inputs would increase DOC concentrations rather than contribute to
379 depletion.

380

381 DOC and bacterioplankton depletion in the Backreef could be caused by aggregation of
382 organic particles (Mari *et al.*, 2007; Passow and Alldredge, 1994; Verdugo *et al.*, 2004)
383 and subsequent flux to the sediment or adsorption onto reef structures. However,
384 increased aggregation should be reflected in elevated concentrations of particulate
385 organic carbon on the reef (which is not observed; Fig S2) unless aggregates are rapidly
386 consumed by metazoans within the reef. DOM adsorption to the high-porosity carbonate

387 sands common in the Backreef habitats of Moorea is another abiotic removal process that
388 may be important and has been demonstrated in similar environments (Hillgärtner *et al.*,
389 2001; Suess, 1970). However, this process is difficult to distinguish from heterotrophic
390 reef sediment biofilms that can remove DOM (Wild *et al.*, 2006; Wild *et al.*, 2004).

391 While the Backreef habitats in Moorea have abundant carbonate sands, preliminary
392 results show no difference in DOC concentrations in these surficial sediments (data not
393 shown).

394

395 *Evidence for biological mechanisms of DOC and bacterioplankton community alteration*
396 *on the reef*—Three lines of evidence indicate that DOC and bacterioplankton depletion
397 are the result of selective biological removal processes rather than physical dilution or
398 aggregation mechanisms. First, we found no evidence of similar reef depletion in
399 inorganic nutrients or particulate organic matter relative to offshore waters (Fig S2);
400 dilution would be expected to nonselectively alter concentrations of many solutes and
401 aggregation would be expected to decrease nearshore particle abundance through sinking
402 export. Second, the Forereef, Backreef, Bay, and Offshore habitats support distinct
403 bacterioplankton communities (Figs. 4-6, S3-S4), implying selective pressures within the
404 water column operating on bacterioplankton at reef residence timescales. Third, DOC and
405 bacterioplankton depletion patterns appear to be regulated in part by reef water residence
406 time, implying a mechanism of active removal. The difference between offshore and
407 backreef DOC and bacterioplankton concentrations is significantly less when wave
408 energy was greatest in the Austral summer (Fig. 3, (Hench *et al.*, 2008) and wave energy
409 flux (the product of the square of significant wave height and the wave period averaged
410 over the 24 hours prior to sampling) was a strong and significant predictor of Backreef
411 DOC and bacterioplankton proportional depletion (Backreef:Offshore) among sampling
412 dates 2005-2009 (DOC: $n = 7$, $r^2 = 0.63$, $p = 0.032$; Bacterioplankton: $n = 9$, $r^2 = 0.66$, p
413 $= 0.008$). In addition, the potential for water exiting the reef passes to be retained and
414 recycled back across the reef crest (Hench *et al.*, 2008) has the potential to increase the
415 practical reef residence time of water beyond estimates based solely on flushing rates or
416 control volumes (Delesalle and Sournia, 1992; Lenhardt, 1991; Reidenbach *et al.*, 2002;
417 Torrétón *et al.*, 2007), thus increasing contact time with reef heterotrophic organisms.

418

419 *Benthic and planktonic processes removing DOC and altering reef bacterioplankton*
420 *communities* – Biological processes contributing to DOC and bacterioplankton depletion
421 and alteration of bacterioplankton community structure in the backreef may be associated
422 with the planktonic environment, reef sediments, or diverse benthic filter-feeding
423 metazoans. Corals may rapidly consume DOC and bacterioplankton (Sorokin, 1973)
424 although many recent studies show corals to be sources, rather than sinks, for DOC
425 (Ferrier-Pages *et al.*, 1998; Hata *et al.*, 2002; Nakajima *et al.*, 2009; Van Duyl and Gast,
426 2001). Recent work has demonstrated the potential for sponges to consume both DOC
427 and bacterioplankton at biogeochemically significant rates (De Goeij *et al.*, 2008; de
428 Goeij and Van Duyl, 2007; Van Duyl *et al.*, 2006; Yahel *et al.*, 2003). However,
429 conspicuous sponge taxa, which exhibit the highest filtration rates (Southwell *et al.*,
430 2008), are virtually absent from our study area, and even inconspicuous benthic sponges
431 cover less than 1% of the reef benthos in Moorea on average (Adjeroud, 1997,
432 <http://mcr.lternet.edu/data/>), although cryptic coelobite communities can increase reef
433 surface area sevenfold and rapidly remove both DOC and bacterioplankton (de Goeij and
434 Van Duyl, 2007; Richter *et al.*, 2001; Scheffers *et al.*, 2004).

435

436 Accumulated DOM in the surface waters of the tropical and subtropical oceanic gyres has
437 been shown to be resistant to rapid utilization by extant microbial assemblages (Carlson
438 2002, Carlson *et al.*, 2004). Our study suggests that the water overlying reefs exhibits a
439 different bacterioplankton community from that maintained in the open ocean, and given
440 the depletion of DOC relative to the offshore waters that bathe and exchange with the
441 reef system our study indicates that these communities may be able to consume semi-
442 labile dissolved compounds from oceanic waters more rapidly and efficiently than
443 communities outside of the reef. Labile DOM derived from coral or algae may facilitate
444 the co-metabolism of recalcitrant DOM by reef bacterioplankton communities (Barott *et*
445 *al.*, 2009; Dinsdale *et al.*, 2008; Ducklow, 1990; Smith *et al.*, 2006). Bacterial production
446 rates are typically elevated in reef environments (Gast *et al.*, 1999; Moriarty *et al.*, 1985;
447 Torr eton and Dufour, 1996; Van Duyl and Gast, 2001), and understanding the sources of

448 DOM supporting this production and the fate of this heterotrophic productivity is crucial
449 to developing a coral reef ecosystem model.

450

451

452 *Nearshore bacterioplankton community differentiation by habitat* - The observed
453 gradients in the relative abundance of specific bacterioplankton phylotypes among
454 Offshore, Forereef, Backreef, and Bay habitats (Figs. 4-6, S3) were clear and consistent
455 among years (2008 and 2009; Figs 4 and S3), seasons (austral summer and winter 2008;
456 (Figs 5 and S3), and methods (16S rRNA V6 amplicon pyrosequencing and TRFLP
457 fingerprinting; Figs. 4, 6, S3). The community differences were not solely a result of
458 variations in relative abundance of taxa as showed similar habitat differentiation patterns
459 when analyzed using presence/absence data across a wide range of taxonomic
460 aggregations (Fig. S4). These results are consistent with the patterns observed by
461 (Weinbauer *et al.*, 2010) in a lagoonal system with much longer residence time. Two
462 phylotypes belonging to different alphaproteobacterial SAR11 sub-clades (Group Ia and
463 Group II) increased in relative abundance within the reef relative to the open ocean (Figs.
464 5a, 5d). Notably, only the Group Ia phylotype was also elevated in the freshwater-
465 influenced bay samples. A member of a second alphaproteobacterial clade, SAR116, did
466 not show this pattern of nearshore persistence, instead it exhibited higher relative
467 abundance offshore, suggesting that this phylotype may be selectively grazed or a poor
468 competitor for substrates in the nearshore habitats (Fig. 5e). Consistent with the
469 pyrosequencing results, both Flavobacterial phylotypes (Figs. 5c and 5f) were relatively
470 enriched in the Bay and Forereef environments, indicating that this group may thrive in
471 the deeper, more particle-rich waters found in these regions relative to the shallower
472 Backreef lagoons.

473

474 The deep-pyrosequencing approach (averaging 40,000 sequences per habitat, Table S1)
475 elucidated clear gradients in rare taxa, many of which were < 0.5% of total sequences
476 (and thus undetectable by TRFLP, which excluded fragments < 0.5% relative
477 abundance), even when aggregated at the Class level (Fig. 5). The rare bacterial classes
478 showing clear evidence of enrichment in the Backreef relative to offshore waters included

479 a number of groups containing potential pathogens of Metazoa (Bacilli, Clostridia,
480 Actinobacteria, Bacteroidia, Sphingobacteria), as well as several groups associated more
481 with environmental samples or specific redox transformations (Acidobacteria, Nitrospira,
482 Fusobacteria, Verrucomicrobia, Planctomycetes, Lentisphaeria). Elevated levels of
483 nitrifying bacteria have been reported in other reef habitats (Beman *et al.*, 2007; Wegley
484 *et al.*, 2007) and may provide a mechanism explaining the elevated winter concentrations
485 of nitrate in the Backreef (Fig. S2). The three reef water column environments sampled
486 by pyrosequencing (Forereef, Backreef: Lagoon, and Backreef: Fringe) showed markedly
487 higher numbers of bacterial taxa (OTUs) for equal sampling intensity (sequence reads)
488 compared with Offshore and Bay habitats (Table S1). This elevated richness in reef
489 microorganismal communities would be consistent with the macroorganismal dogma of
490 reefs harboring a greater diversity of organisms and microhabitats than the surrounding
491 oceans.

492

493 *Implications for coral reef microbial and ecosystem ecology* - Reefs are frequently
494 declared to have elevated concentrations of dissolved organic matter relative to offshore
495 waters (Hatcher, 1983; Torr ton *et al.*, 1997), but our data suggest that rapidly flushed
496 reefs may exhibit depleted DOC. A similar discrepancy exists in the literature for
497 bacterioplankton, with evidence for corals enhancing reef bacterial density (Seymour *et*
498 *al.*, 2005a; Seymour *et al.*, 2005b; Van Duyl and Gast, 2001) or reducing reef bacterial
499 density (Ayukai, 1995; Gast *et al.*, 1998). Many previous studies of DOC and
500 bacterioplankton have focused on atoll lagoon systems with relatively long residence
501 times and potential accumulation of organic material, explaining the widespread
502 perception that reefs exhibit elevated levels of organic matter and bacteria (Linley and
503 Koop, 1986; Sakka *et al.*, 2002; Torreton and Dufour, 1996; Torr ton *et al.*, 1997;
504 Yoshinaga *et al.*, 1991). Our results fit well with observations that indicators of
505 eutrophication (concentrations of DOM and particulate organics, bacterial and
506 phytoplankton biomass and production, and rates of organic aggregate formation)
507 increase along a continuum of increasing reef residence time and declining oceanic
508 connectivity (from rapid-flushing fringing reefs to isolated atoll lagoons; Mari *et al.*,
509 2007; Pages and Andr fou t, 2001; Pag s *et al.*, 2001; Torr ton *et al.*, 2002). Further

510 development of comparative models integrating reef habitats of varying residence time
511 would help clarify the degree to which different reefs are supported by oceanic DOM
512 inputs and planktonic microbial recycling (Torreton, 1999).

513

514 The removal of semi-labile oceanic DOC by reefs suggests an unrecognized potential for
515 net heterotrophy of the nearshore ecosystem. Although reef ecosystems exhibit some of
516 the highest rates of gross primary production on Earth (Sorokin, 1990), their net
517 ecosystem metabolism is frequently estimated as only weakly positive because of the
518 intense heterotrophic processes associated with reef organic matter recycling (Ducklow,
519 1990). In fact, a number of studies have suggested reefs to be net heterotrophic, acting as
520 sources of carbon dioxide to the atmosphere (Gattuso *et al.*, 1999; Gattuso *et al.*, 1996;
521 Suzuki and Kawahata, 2003; Ware *et al.*, 1992). Recent modeling studies have indicated
522 that more than half of reef primary production enters the food web through microbial
523 consumption processes, potentially reducing overall energetic efficiency but retaining
524 valuable macro- and micro-nutrients within the system (Sorokin, 1990; Arias-Gonzalez *et*
525 *al.*, 1997). The results of Ferrier-Pages *et al.* (1998) demonstrating rapid uptake of coral-
526 released DOM by bacterioplankton (~14% of coral net daily production) indicate that
527 planktonic bacterial communities play a key role in coral reef food webs. Our results lend
528 support to this conceptualization of reefs as efficient scavengers and recyclers of organic
529 material with an active planktonic bacterial community unique from the open ocean
530 playing a key role in nearshore ecosystem function.

531

532 *Conclusions* – Our study combines long-term, spatially explicit data with high-resolution
533 synoptic surveys to present clear evidence that the fringing and barrier reef habitats of
534 Moorea are depleted in DOC and bacterioplankton relative to the surrounding ocean. In
535 addition, we show clear patterns in bacterioplankton community structure, with
536 differentiation of Offshore, Forereef, Backreef and Bay communities maintained in
537 different seasons and assessed by different culture-independent methods. Our results
538 indicate that the fringing reefs of Moorea are a sink for DOC and bacterial inputs from
539 the open ocean and that reefs alter the composition of the overlying bacterioplankton
540 communities. The reef communities are enriched in several classes of bacteria uncommon

541 in open ocean waters, including clades containing various copiotrophs and potential
542 pathogens. Furthermore, the consistent differentiation of communities among Backreef,
543 Forereef, Bay, and Offshore habitats emphasizes the utility of bacterioplankton
544 communities in illustrating unseen biogeochemical or ecological gradients among
545 nearshore environments. Our results support the concept of even rapidly-flushed reefs as
546 sites of intense microbial activity, resulting in enhanced rates of DOM metabolism and
547 shifts in bacterioplankton community structure relative to the surrounding ocean.

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Figure Legends:

Fig 1. A satellite photograph of Paopao Bay, Moorea with sampling locations identified according to time and type of sampling. Offshore sampling locations (4 sample stations in 2009 and 1 time-series depth profiling station) are within ~6 km North of the reef crest and are excluded from this figure (see Fig. 4 inset map).

Fig 2. DOC (a) and bacterioplankton (b) concentrations measured during a synoptic survey of surface waters in the vicinity of Paopao Bay, Moorea, 1 Sept 2009. The black line gives a rough outline of the bay and reef crest. Note that both DOC and bacterioplankton are depleted behind the reef crest.

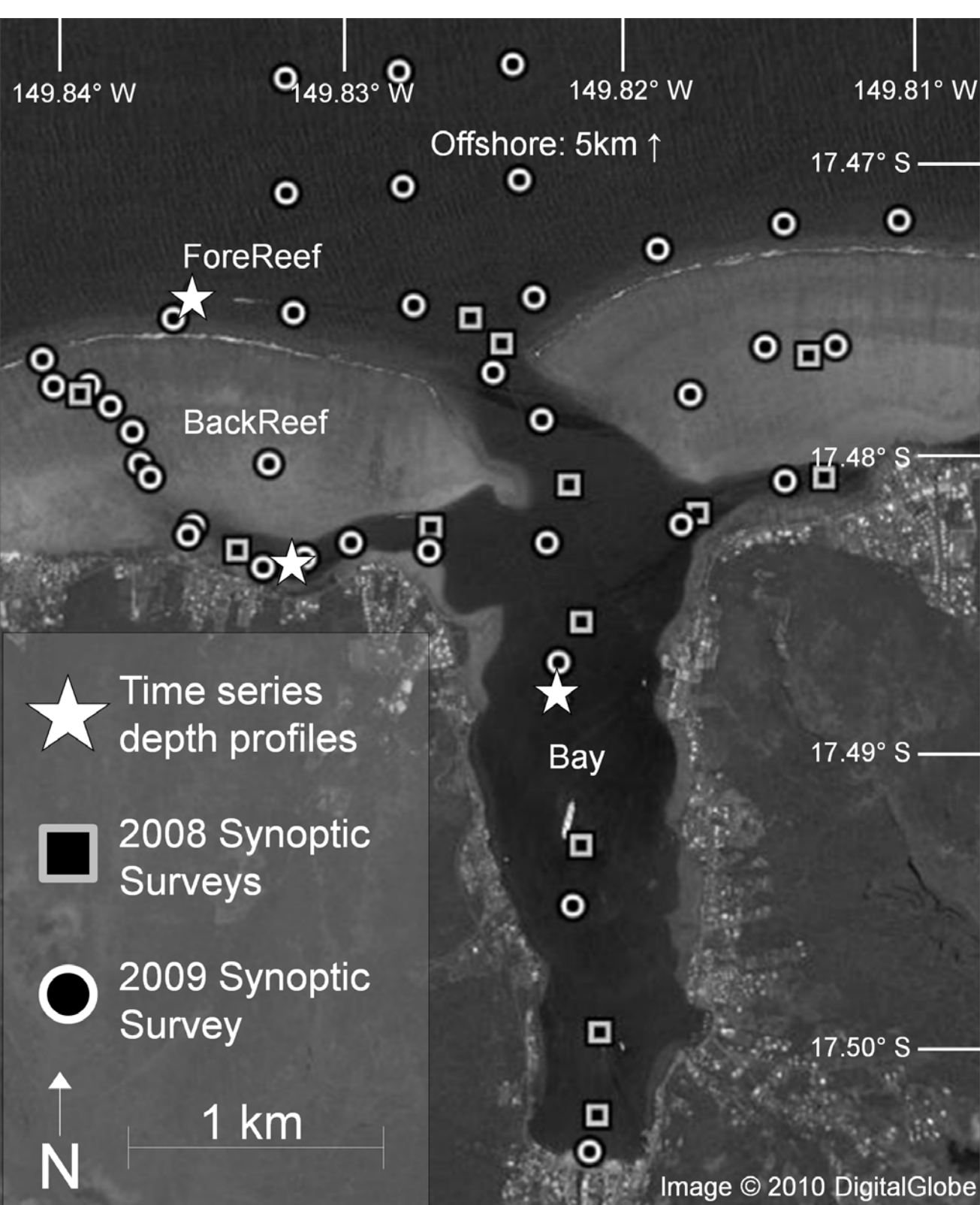
Fig 3. DOC and bacterioplankton concentrations averaged across 1, 5, and 10 m discrete depth samples 2-3 times annually at four sampling locations 2005-2009 in the vicinity of Paopao Bay, Moorea (see Fig. 1 for profile locations). Data are separated by season to test for significant differences when waves are highest during austral summer. Box plots represent mean, quartiles, and 90% ranges of data averaged at each location over time and depth. Letters denote significant differences among all averages across seasons for each parameter (means with no letters in common are significantly different at the 95% confidence level via Tukey post hoc tests). Note that Backreef is always depleted relative to Offshore and differentiation among habitats is more pronounced in Winter than Summer. Offshore DOC is always higher than all other nearshore habitats, and the only seasonal difference within a given habitat is higher bacterioplankton concentration in the Bay in Winter.

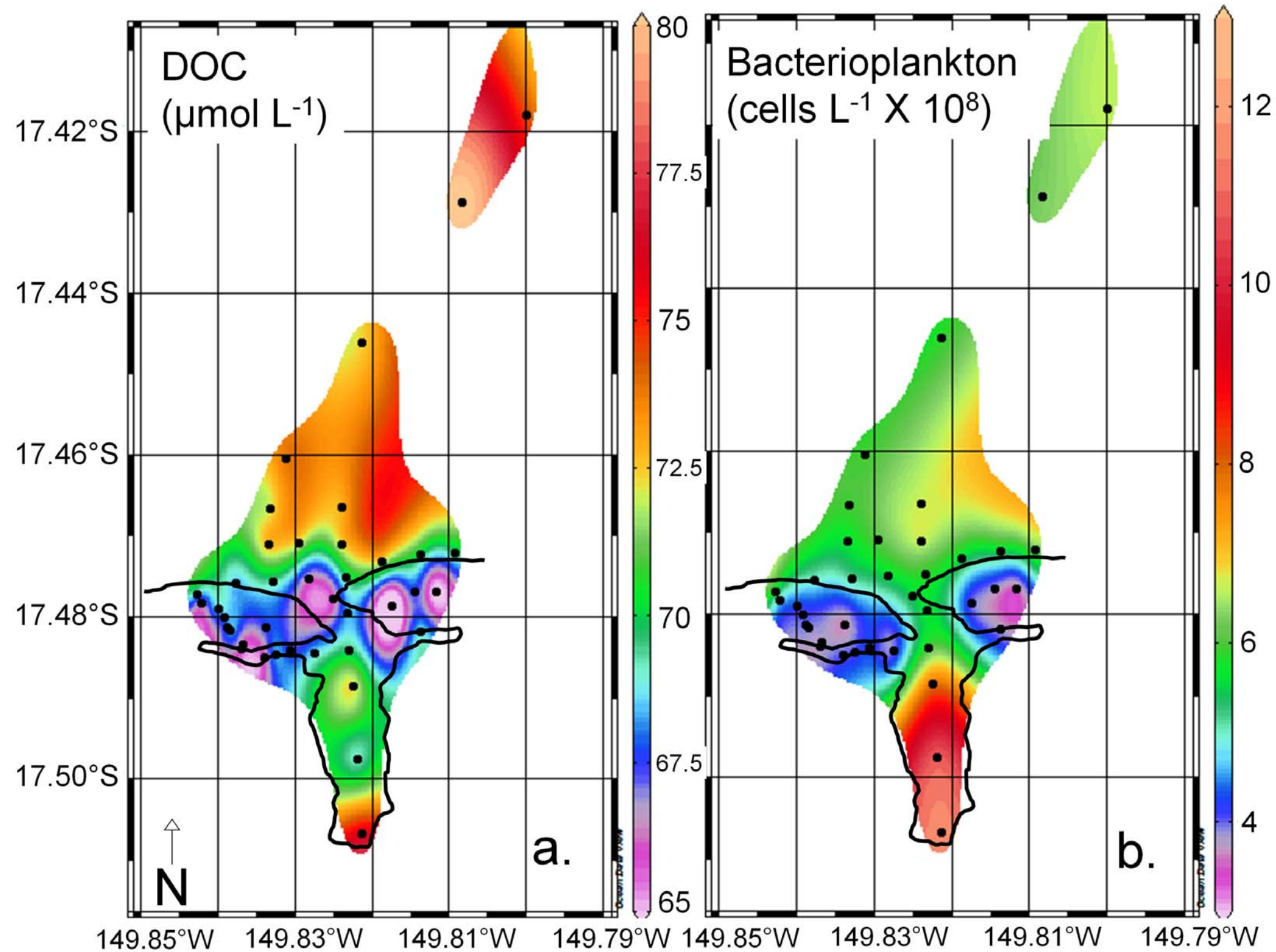
Fig 4. Spatial distribution of bacterioplankton community types in the 2009 synoptic survey. Surface DNA samples are symbol/color coded according to 5 community types defined as 85% Bray-Curtis similarity group average (UPGMA) clusters of 16S bacterial rRNA gene amplicon TRFLP fingerprints (a; vertical line demarcates the 85% cluster threshold, triangles indicate samples without significant differences by SIMPROF bootstrapping). Samples are annotated in the dendrogram according to nominal sample

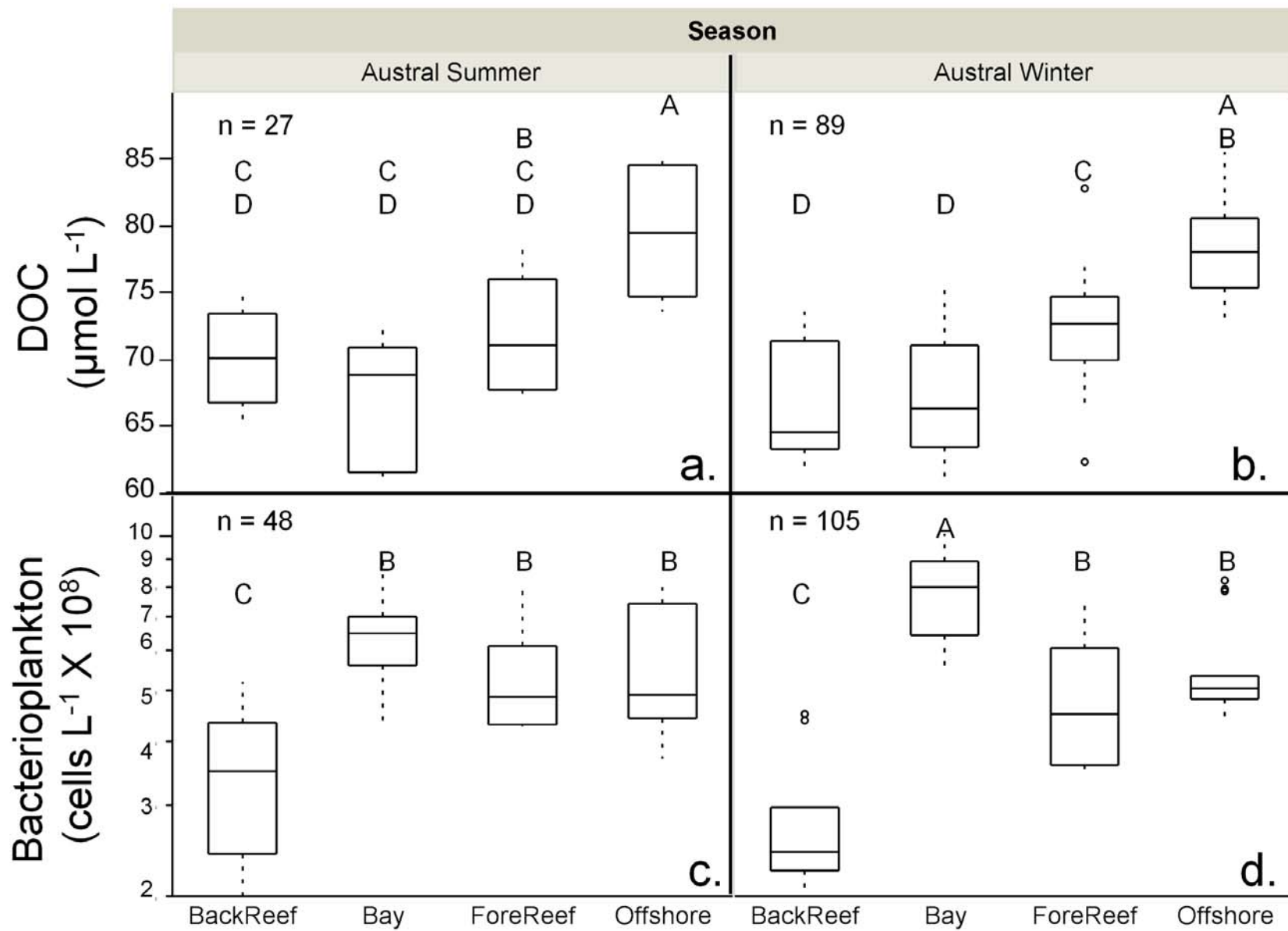
habitats for clarity. The map (b) is loosely shaded according to depth and substrate type keyed at the upper right, with samples symbol-coded according to TRFLP cluster. The inset map in (b) shows community types found at the offshore sampling locations, which were within ~6km of Moorea in >200m deep water.

Fig 5. Spatial distribution of bacterial phylotypes in the vicinity of Paopao Bay 1 Sept 2009. Each plot shows shaded contours of the relative abundance of terminal restriction fragments (TRFs) which were putatively identified with a cloned sequence from Moorea (Fig S5). Each phylotype distribution displayed here is unambiguously represented by a cloned sequence with a measured TRF falling within the 1bp range of environmental TRFs and for which the closest matching full-length clone in the greengenes database (DeSantis, et al. 2006b) has an identical *in silico* TRF and taxonomic classification, with the exception of SAR11 Group Ia which has an established consistent disparity between *in silico* TRF lengths (117bp) and clone TRF lengths (113bp) as shown by Morris, *et al.* (2005). Note that SAR11 clades are relatively enriched in the Backreef (a and d) while Cytophaga and SAR116 are relatively depleted (c, e, f). *Synechococcus*, SAR116, and SAR11 Group II are relatively depleted within the Bay and increase offshore (b, e, a), while the two Flavobacteria are enriched in the Forereef (c) and Bay (f), respectively.

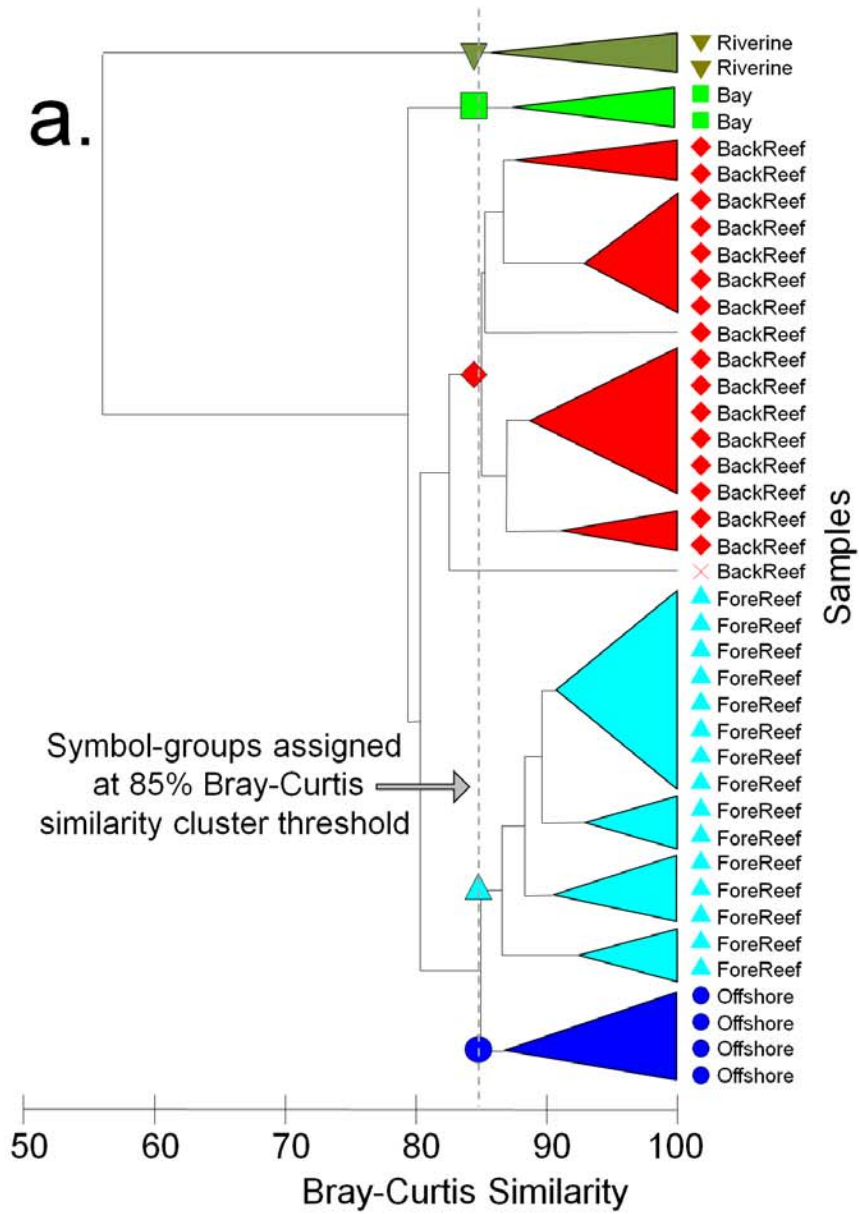
Fig 6. Spatial variability in relative abundance of bacterial classes derived from pyrosequencing of environmental 16S rRNA V6 amplicons sampled in the vicinity of Moorea 11-13 Jan 2008. Replicate samples are labeled (top) according to collection location (see Table S1) and clustered (bottom) by relative abundance of sequences matching reference OTUs aggregated by Class (cluster lines are colored the same when there is no significant difference in communities; SIMPROF $p > 0.05$). Classes are clustered (left) according to relative variance across the spatial gradient, with green below average and red above average. Mean and ranges of relative abundance of each class across the dataset are given at right with color codes matching the heat map. Clustering and heatmaps were generated in the JMP v8 statistical package using group average clustering of samples according to class relative abundances between samples.



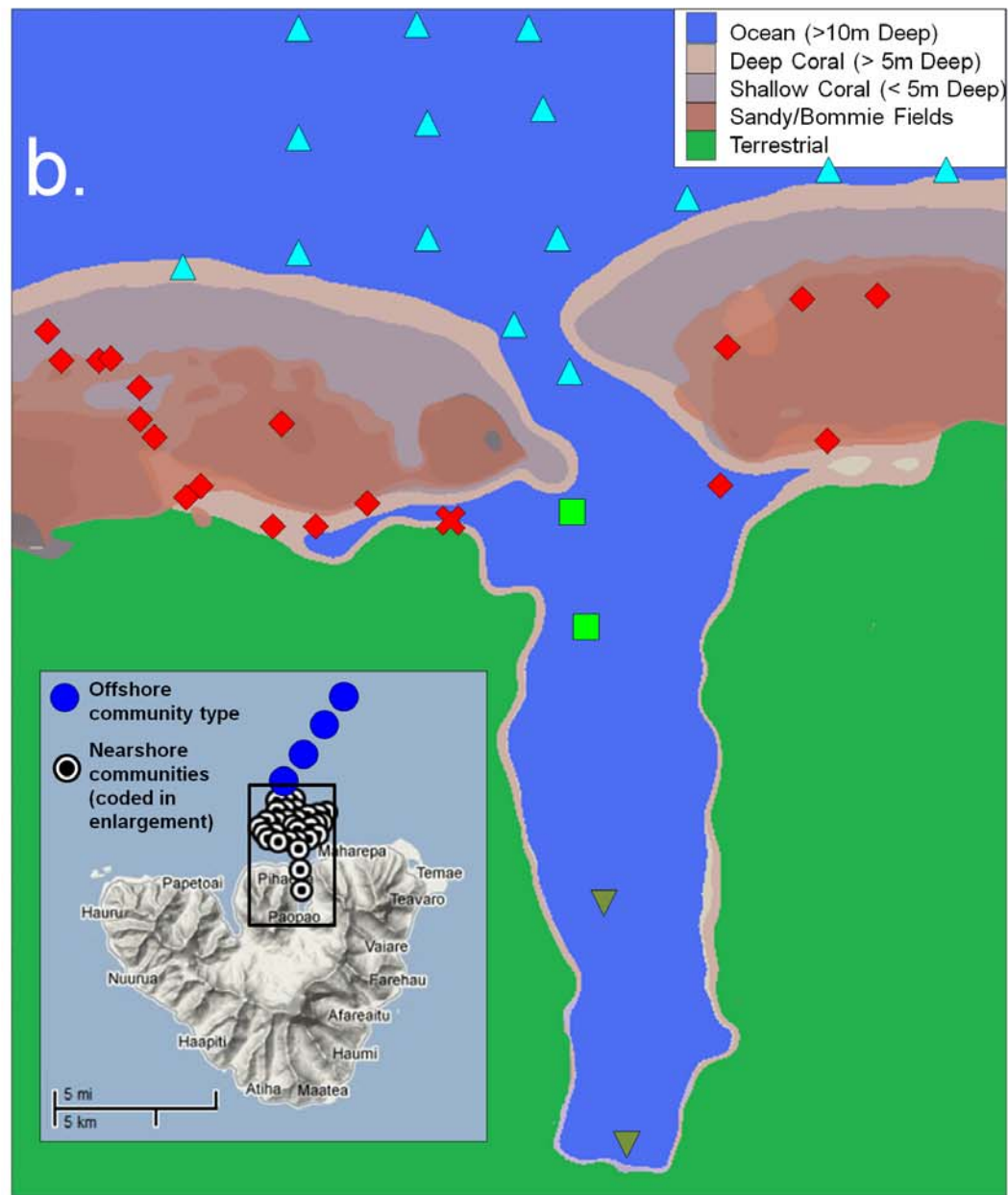


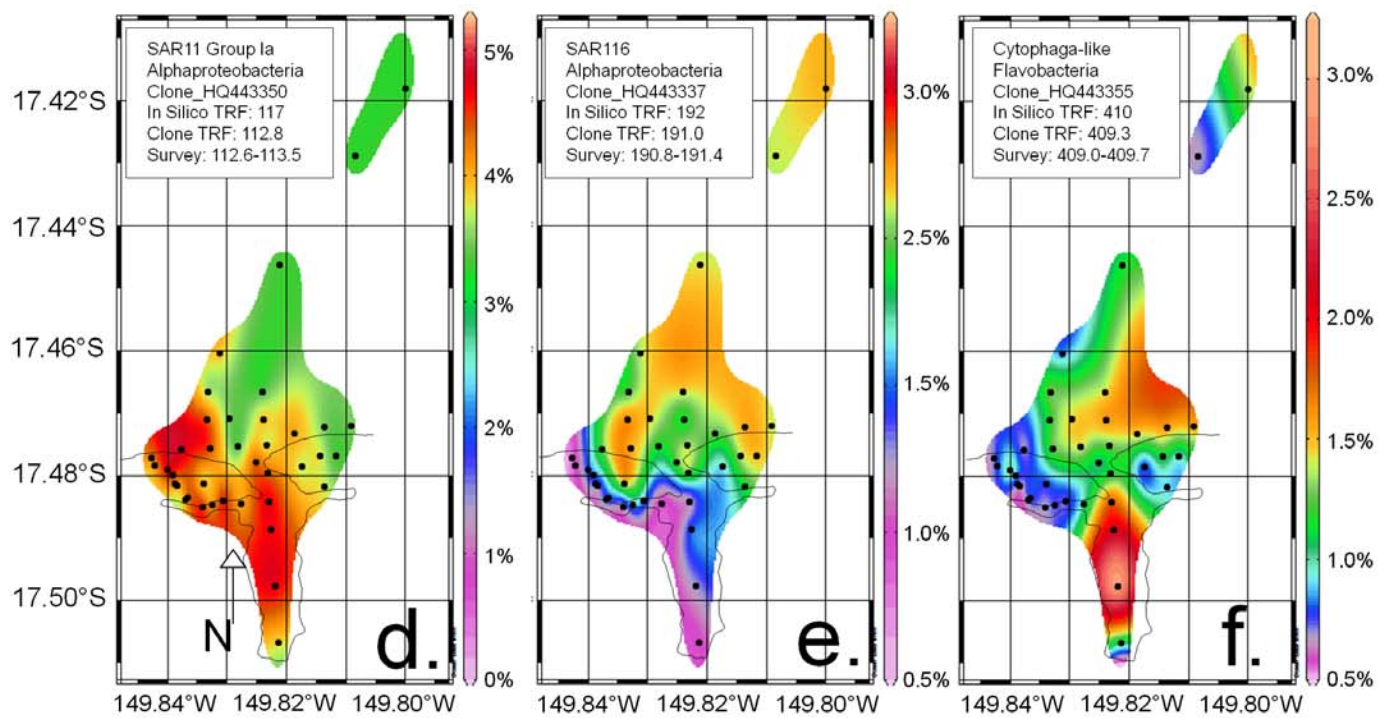
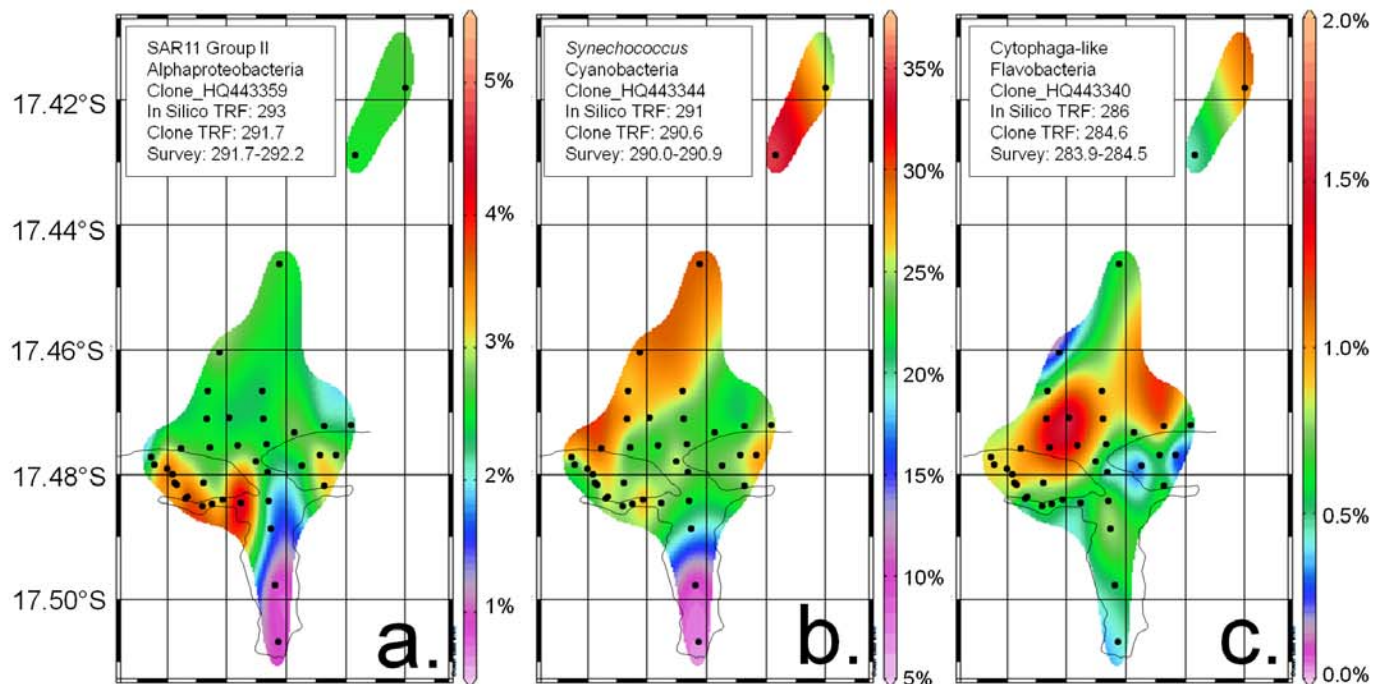


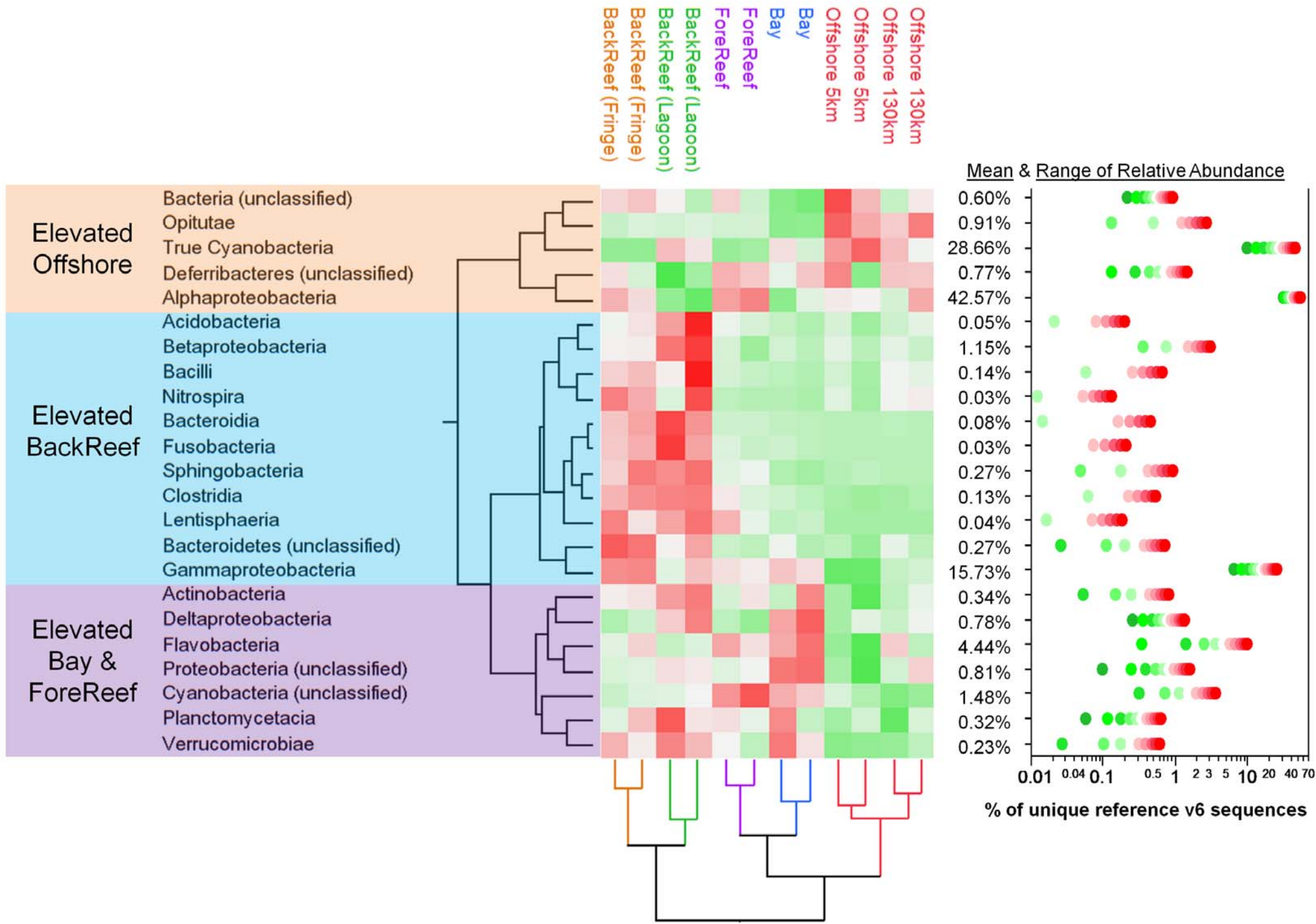
a.



b.







Elevated Offshore

- Bacteria (unclassified)
- Opitutae
- True Cyanobacteria
- Deferribacteres (unclassified)
- Alphaproteobacteria

Elevated BackReef

- Acidobacteria
- Betaproteobacteria
- Bacilli
- Nitrospira
- Bacteroidia
- Fusobacteria
- Sphingobacteria
- Clostridia
- Lentisphaeria
- Bacteroidetes (unclassified)
- Gammaproteobacteria

Elevated Bay & ForeReef

- Actinobacteria
- Deltaproteobacteria
- Flavobacteria
- Proteobacteria (unclassified)
- Cyanobacteria (unclassified)
- Planctomycetacia
- Verrucomicrobiae

Offshore 130km
Offshore 130km
Offshore 5km
Offshore 5km
Bay
Bay
ForeReef
ForeReef
BackReef (Lagoon)
BackReef (Lagoon)
BackReef (Fringe)
BackReef (Fringe)