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

Craig E. Nelson*[1], Alice L. Alldredge[1,2], Elizabeth A. McCliment[3], Linda A. Amaral-Zettler [3,4], and Craig A. Carlson[1,2]

[1] Marine Science Institute, University of California, Santa Barbara, CA, USA, 93106

[2] Department of Ecology, Evolution and Marine Biology, University of California, Santa Barbara, CA, USA, 93106

[3] The Josephine Bay Paul Center for Comparative Molecular Biology and Evolution, Marine Biological Laboratory, Woods Hole, MA, USA, 02543

[4] Department of Geological Sciences, Brown University, Providence, RI 02912, USA

* Correspondence: CE Nelson, Marine Science Institute, University of California, Santa Barbara, CA 93106, USA. E-mail: cr_nelson@lifesci.ucsb.edu

Acknowledgements: We thank K. Seydel, A. Brooks, and A. Jani for assistance with field, boating, and SCUBA logistics in Moorea; R. Morris for preliminary sampling and amplicon cloning; L. Washburn, M. Fewings, and C. Gottschalk for thoughtful discussions and analysis of wave height measurements; the staff of the UC Berkeley Gump station for logistical support, and E. Halewood and R. Henry for laboratory assistance with DOC and TRFLP respectively. We thank A. Jani and two anonymous reviewers for their improvements to the manuscript. This project was supported by the US National Science Foundation Moorea Coral Reef Long Term Ecological Research project (NSF OCE-0417412) through minigrants to CAC and NSF OCE-0927411 to CAC as well as the MIRADA-LTERs program (NSF DEB-0717390 to LAZ).

Abstract:

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

Introduction:

Coral reefs are highly productive ecosystems that develop and thrive within the oligotrophic tropical and subtropical oceans (Darwin, 1889). Understanding the sources of nutrients and organic material that support coral reefs is central to predicting and managing how these ecosystems will respond to global change (Sorokin, 1990). Microbial communities play a dominant biogeochemical role in both reef and open-ocean environments, with heterotrophic microbial communities recycling more than half of net productivity in both ecosystem types (Cho and Azam, 1990; Ducklow, 1990). The largest pool of organic matter found in the ocean is a heterogenous mixture of dissolved compounds, a small portion of which is bioavailable to bacterioplankton on time scales of hours to days (Carlson, 2002). This bioavailable component of dissolved organic carbon (DOC) is a key component of the microbial loop (Azam *et al.*, 1983; Pomeroy, 1974). Both theory (Crossland *et al.*, 1991; Ducklow, 1990; Sorokin, 1990), and field-based models (Arias-Gonzalez *et al.*, 1997; Grigg *et al.*, 1984) indicate the importance of microbes to reef food webs and that understanding microbial processes is central to understanding the links between reef and ocean ecosystems.

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

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

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

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

Methods :

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

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

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

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

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

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

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

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

Results:

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

The gradients of DOC concentrations and bacterioplankton densities observed during the synoptic spatial survey (Austral winter 2008-2009) were also maintained through time as revealed from the seasonal sampling of bay, reef and offshore habitats from 2005-2009 (Fig 3). The Backreef environment was significantly lower in DOC concentration relative to Offshore waters over the 2005-2009 sampling period regardless of season (ANOVA with Tukey post hoc tests comparing concentrations in each habitat $p < 0.05$; Figs. 3a and 3b) and was consistently depleted in bacterioplankton relative to all other habitats (Figs. 3c and 3d). During austral winter differentiation between habitats was more pronounced, with elevated DOC in the Forereef relative to the other nearshore habitats (but still less than offshore; Fig. 3b). Winter bacterioplankton densities in the Bay were elevated relative to all other habitats and exceeded summer Bay bacterioplankton densities (Fig. 3d). DOC and bacterioplankton vertical variability on any sampling date was much smaller than lateral variability among habitats from Backreef through Offshore (e.g. Fig. S1a) with no statistical effect of sampling depth on later habitat differentiation across dates (ANCOVA was used to test the significance of interaction between habitat and depth in explaining variation in DOC and bacterioplankton concentrations; habitat*depth $p = 0.19$ and 0.37 respectively). Moreover, there was no evidence for persistent stratification of concentrations in the upper 10 m of Forereef, Backreef, or Offshore habitats across seasons (although surface bacterioplankton concentrations in the Bay 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 µmol L^{-1}) than Offshore (mean 0.13 µmol
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

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

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

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

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

Discussion:

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

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

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

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

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

sands common in the Backreef habitats of Moorea is another abiotic removal process that may be important and has been demonstrated in similar environments (Hillgärtner *et al.*, 2001; Suess, 1970). However, this process is difficult to distinguish from heterotrophic reef sediment biofilms that can remove DOM (Wild *et al.*, 2006; Wild *et al.*, 2004). While the Backreef habitats in Moorea have abundant carbonate sands, preliminary results show no difference in DOC concentrations in these surficial sediments (data not shown).

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

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

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

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

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

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

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

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

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

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

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

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

References

Adjeroud M (1997). Factors influencing spatial patterns on coral reefs around Moorea, French Polynesia. *Marine Ecology Progress Series* **159:** 105-119.

Arias-Gonzalez J, Delesalle B, Salvat B, Galzin R (1997). Trophic functioning of the Tiahura reef sector, Moorea Island, French Polynesia. *Coral Reefs* **16:** 231-246.

Ayukai T (1995). Retention of phytoplankton and planktonic microbes on coral reefs within the Great Barrier Reef, Australia. *Coral Reefs* **14:** 141-147.

Azam F, Fenchel T, Field J, Gray J, Meyer-Reil L, Thingstad F (1983). The ecological role of water-column microbes in the sea. *Marine Ecology Progress Series* **10:** 257-263.

Barott K, Smith J, Dinsdale E, Hatay M, Sandin S (2009). Hyperspectral and Physiological Analyses of Coral-Algal Interactions. *PLoS One* **4:** e8043.

Beman J, Roberts K, Wegley L, Rohwer F, Francis C (2007). Distribution and diversity of archaeal ammonia monooxygenase genes associated with corals. *Applied and Environmental Microbiology* **73:** 5642.

Carlson C (2002). Production and removal processes. *Biogeochemistry of marine dissolved organic matter***:** 91–151.

Carlson C, Ducklow H (1996). Growth of bacterioplankton and consumption of dissolved organic carbon in the Sargasso Sea. *Aquatic Microbial Ecology* **10:** 69-85.

Carlson C, Giovannoni S, Hansell D, Goldberg S, Parsons R, Vergin K (2004). Interactions among dissolved organic carbon, microbial processes, and community structure in the mesopelagic zone of the northwestern Sargasso Sea. *Limnology and Oceanography* **49:** 1073-1083.

Carlson C, Hansell D, Nelson N, Siegel D, Smethie W, Khatiwala S *et al* (2010). Dissolved organic carbon export and subsequent remineralization in the mesopelagic and bathypelagic realms of the North Atlantic basin. *Deep Sea Research Part II: Topical Studies in Oceanography* **57:** 1433-1445

Cherrier J, Bauer J, Druffel E (1996). Utilization and turnover of labile dissolved organic matter by bacterial heterotrophs in eastern North Pacific surface waters. *Marine Ecology Progress Series* **139:** 267-279.

Cho BC, Azam F (1990). Biogeochemical significance of bacterial biomass in the ocean's euphotic zone. *Marine Ecology Progress Series* **63**: 253–259

Clarke, KR, Gorley, RN, 2006. PRIMER v6: User Manual/Tutorial. PRIMER-E, Plymouth.

Cottrell M, Kirchman D (2003). Contribution of major bacterial groups to bacterial biomass production (thymidine and leucine incorporation) in the Delaware estuary. *Limnology and Oceanography* **48:** 168-178.

Crossland C, Barnes D (1983). Dissolved nutrients and organic particulates in water flowing over coral reefs at Lizard Island. *Marine and Freshwater Research* **34:** 835-844.

Crossland C, Hatcher B, Smith S (1991). Role of coral reefs in global ocean production. *Coral Reefs* **10:** 55-64.

Darwin C (1889). *The structure and distribution of coral reefs*. D. Appleton and company.

de Goeij J, Van den Berg H, Van Oostveen M, Epping E, Van Duyl F (2008). Major bulk dissolved organic carbon (DOC) removal by encrusting coral reef cavity sponges. *Marine Ecology Progress Series* **357:** 139-151.

de Goeij J, Van Duyl F (2007). Coral cavities are sinks of dissolved organic carbon (DOC). *Limnology and Oceanography* **52:** 2608-2617.

Delesalle B, Sournia A (1992). Residence time of water and phytoplankton biomass in coral reef lagoons. *Continental Shelf Research* **12:** 939-949.

DeSantis TZ, Hugenholtz P, Keller K, Brodie E, Larsen N, Piceno Y, et al. (2006). NAST: a multiple sequence alignment server for comparative analysis of 16S rRNA genes. *Nucleic Acids Research* **34**: W394.

DeSantis TZ, Hugenholtz P, Larsen N, Rojas M, Brodie E, Keller K, et al. (2006). Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. *Applied and Environmental Microbiology* **72**: 5069.

Dereeper A, Guignon V, Blanc G, Audic S, Buffet S, Chevenet F et al (2008). Phylogeny. fr: robust phylogenetic analysis for the non-specialist. *Nucleic Acids Research* **36**: W465-W469.

Dinsdale E, Pantos O, Smriga S, Edwards R, Angly F, Wegley L *et al* (2008). Microbial ecology of four coral atolls in the Northern Line Islands. *PLoS One* **3:** 1584.

Ducklow H (1990). The biomass, production and fate of bacteria in coral reefs. *Ecosystems of the world.* **25:** 265-289.

Fabricius K (2005). Effects of terrestrial runoff on the ecology of corals and coral reefs: review and synthesis. *Marine Pollution Bulletin* **50:** 125-146.

Ferrier-Pages C, Gattuso J, Cauwet G, Jaubert J, Allemand D (1998). Release of dissolved organic carbon and nitrogen by the zooxanthellate coral Galaxea fascicularis. *Marine Ecology Progress Series* **172:** 265-274.

Fichez R, Harris P, Cauwet G, Dejardin P (1996). Dissolved carbon in pore waters from the carbonate barrier reef of Tahiti (French Polynesia) and its basalt basement. *Aquatic Geochemistry* **2:** 255-271.

Gast G, Jonkers P, van Duyl F, Bak R (1999). Bacteria, flagellates and nutrients in island fringing coral reef waters: influence of the ocean, the reef and eutrophication. *Bulletin of Marine Science* **65:** 523-538.

Gast G, Wiegman S, Wieringa E, van Duyl F, Bak R (1998). Bacteria in coral reef water types: removal of cells, stimulation of growth and mineralization. *Marine Ecology Progress Series* **167:** 37-45.

Gattuso J, Frankignoulle M, Smith S (1999). Measurement of community metabolism and significance in the coral reef CO2 source-sink debate. *Proceedings of the National Academy of Sciences of the United States of America* **96:** 13017.

Gattuso J, Pichon M, Delesalle B, Canon C, Frankignoulle M (1996). Carbon fluxes in coral reefs. I. Lagrangian measurement of community metabolism and resulting air-sea CO 2 disequilibrium. *Marine ecology progress series* **145:** 109-121.

Genin A, Monismith S, Reidenbach M, Yahel G, Koseff J (2009). Intense benthic grazing of phytoplankton in a coral reef. *Limnology and Oceanography* **54:** 938-951.

Giovannoni S, Stingl U (2005). Molecular diversity and ecology of microbial plankton. *Nature* **437:** 343-348.

Grigg R, Polovina J, Atkinson M (1984). Model of a coral reef ecosystem. *Coral Reefs* **3:** 23-27.

Guindon S, Gascuel O (2003). A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Systematic Biology* **52**: 696-704.

Hansell D (2005). Dissolved organic carbon reference material program. *Eos Transactions* **86:** 318.

Hansell D, Carlson C (1998). Net community production of dissolved organic carbon. *Global Biogeochemical Cycles* **12:** 443-453.

Hata H, Kudo S, Yamano H, Kurano N, Kayanne H (2002). Organic carbon flux in Shiraho coral reef (Ishigaki Island, Japan). *Marine Ecology Progress Series* **232:** 129-140.

Hatcher B. (1983). The role of detritus in the metabolism and secondary production of coral reef ecosystems. In: *Proceedings of the Inaugural Great Barrier Reef Conf.* Baker J, Carter R, Sammarco P and Stark K (eds). James Cook University Press, pp 317-325.

Hench J, Leichter J, Monismith S (2008). Episodic circulation and exchange in a wave-driven coral reef and lagoon system. *Limnology and Oceanography* **53:** 2681-2694.

Hillgärtner H, Dupraz C, Hug W (2001). Microbially induced cementation of carbonate sands: are micritic meniscus cements good indicators of vadose diagenesis? *Sedimentology* **48:** 117-131.

Houlbreque F, Delesalle B, Blanchot J, Montel Y, Ferrier-Pagès C (2006). Picoplankton removal by the coral reef community of La Prévoyante, Mayotte Island. *Aquatic Microbial Ecology* **44:** 59-70.

Huber JA, Mark Welch DB, Morrison HG, Huse SM, Neal PR, Butterfield DA et al (2007). Microbial population structures in the deep marine biosphere. *Science* **318**: 97-100.

Lenhardt X (1991). *Hydrodynamique des lagons d'atoll et d'île haute en Polynésie française*, vol. Doctorat. Editions de l'ORSTOM-Institut français de recherche scientifique pour le développement en coopération: Paris, 156pp.

Lesser M, Mazel C, Gorbunov M, Falkowski P (2004). Discovery of symbiotic nitrogen-fixing cyanobacteria in corals. *Science* **305:** 997.

Linley E, Koop K (1986). Significance of pelagic bacteria as a trophic resource in a coral reef lagoon, One Tree Island, Great Barrier Reef. *Marine Biology* **92:** 457-464.

Mari X, Rochelle-Newall E, Torréton J, Pringault O, Jouon A, Migon C (2007). Water residence time: a regulatory factor of the DOM to POM transfer efficiency. *Limnology and Oceanography* **52:** 808-819.

Moriarty D, Pollard P, Hunt W (1985). Temporal and spatial variation in bacterial production in the water column over a coral reef. *Marine Biology* **85:** 285-292.

Morris R, Vergin K, Cho J, Rappé M, Carlson C, Giovannoni S (2005). Temporal and spatial response of bacterioplankton lineages to annual convective overturn at the Bermuda Atlantic Time-series Study site. *Limnology and Oceanography* **50:** 1687–1696.

Nakajima R, Yoshida T, Azman B, Zaleha K, Othman B, Toda T (2009). In situ release of coral mucus by Acropora and its influence on the heterotrophic bacteria. *Aquatic Ecology* **43:** 815-823.

Neall V, Trewick S (2008). The age and origin of the Pacific islands: a geological overview. *Philosophical Transactions of the Royal Society B: Biological Sciences* **363:** 3293.

Nelson C (2009). Phenology of high-elevation pelagic bacteria: the roles of meteorologic variability, catchment inputs and thermal stratification in structuring communities. *The ISME journal* **3:** 13-30.

Odum H, Odum E (1955). Trophic structure and productivity of a windward coral reef community on Eniwetok Atoll. *Ecological Monographs* **25:** 291-320.

Pagès J, Andréfouët S (2001). A reconnaissance approach for hydrology of atoll lagoons. *Coral Reefs* **20:** 409-414.

Pagès J, Andrefouët S, Delesalle B, Prasil V (2001). Hydrology and trophic state in Takapoto Atoll lagoon: comparison with other Tuamotu lagoons. *Aquating Living Resources* **14:** 183-193.

Passow U, Alldredge A (1994). Distribution, size and bacterial colonization of transparent exopolymer particles(TEP) in the ocean. *Marine Ecology Progress Series* **113:** 185-198.

Pomeroy L (1974). The ocean's food web, a changing paradigm. *Bioscience* **24:** 499-504.

Porter K, Feig Y (1980). The use of DAPI for identifying and counting aquatic microflora. *Limnology and Oceanography* **25:** 943-948.

Reidenbach M, Monismith S, Koseff J (2002). Reefs Revealed Using the Control Volume Approach. *Oceanography* **15:** 90.

Richter C, Wunsch M, Rasheed M, Kötter I, Badran M (2001). Endoscopic exploration of Red Sea coral reefs reveals dense populations of cavity-dwelling sponges. *Nature* **413:** 726-730.

Rohwer F, Breitbart M, Jara J, Azam F, Knowlton N (2001). Diversity of bacteria associated with the Caribbean coral Montastraea franksi. *Coral Reefs* **20:** 85-91.

Rougerie F, Fagerstrom J, Andrie C (1992). Geothermal endo-upwelling: a solution to the reef nutrient paradox? *Continental Shelf Research* **12:** 785-798.

Sakka A, Legendre L, Gosselin M, Niquil N, Delesalle B (2002). Carbon budget of the planktonic food web in an atoll lagoon (Takapoto, French Polynesia). *Journal of Plankton Research* **24:** 301.

SAS Institute Inc., JMP, v8.0, Cary, NC, 1989-2007

Scheffers S, Nieuwland G, Bak R, Van Duyl F (2004). Removal of bacteria and nutrient dynamics within the coral reef framework of Curaçao (Netherlands Antilles). *Coral Reefs* **23:** 413-422.

Schlitzer, R., Ocean Data View, v4.3, http://odv.awi.de, 2010

Seymour J, Patten N, Bourne D, Mitchell J (2005a). Spatial dynamics of virus-like particles and heterotrophic bacteria within a shallow coral reef system. *Marine Ecology Progress Series* **288:** 1-8.

Seymour J, Seuront L, Mitchell J (2005b). Microscale and small-scale temporal dynamics of a coastal planktonic microbial community. *Marine Ecology Progress Series* **300:** 21-37.

Smith J, Shaw M, Edwards R, Obura D, Pantos O, Sala E *et al* (2006). Indirect effects of algae on coral: algae mediated, microbe induced coral mortality. *Ecology letters* **9:** 835-845.

Sorokin Y (1973). On the feeding of some scleractinian corals with bacteria and dissolved organic matter. *Limnology and Oceanography* **18:** 380-385.

Sorokin Y (1990). Aspects of trophic relations, productivity and energy balance in coral-reef ecosystems. *Ecosystems of the world*. **25:** 401-418.

Southwell M, Weisz J, Martens C, Lindquist N (2008). In situ fluxes of dissolved inorganic nitrogen from the sponge community on Conch Reef, Key Largo, Florida. *Limnology and Oceanography* **53:** 986-996.

Suess E (1970). Interaction of organic compounds with calcium carbonate--I. Association phenomena and geochemical implications.*Geochimica et Cosmochimica Acta* **34:** 157-168.

Suzuki A, Kawahata H (2003). Carbon budget of coral reef systems: An overview of observations in fringing reefs, barrier reefs and atolls in the Indo-Pacific regions. *Tellus* **55:** 428-444.

Suzuki Y, Casareto B, Kurosawa K. (2001). Import and export fluxes of HMW-DOC and LMW-DOC in coral reef at Miyako Island, Okinawa. In:*Proceedings of the 9th International Coral Reef Symposium*. Bali, Indonesia.

Torréton J (1999). Biomass, production and heterotrophic activity of bacterioplankton in the Great Astrolabe Reef lagoon (Fiji). *Coral Reefs* **18:** 43-53.

Torréton J, Dufour P (1996). Temporal and spatial stability of bacterioplankton biomass and productivity in an atoll lagoon. *Aquatic Microbial Ecology* **11:** 251-261.

Torréton J, Dufour P (1996). Bacterioplankton production determined by DNA synthesis, protein synthesis, and frequency of dividing cells in Tuamotu atoll lagoons and surrounding ocean. *Microbial Ecology* **32:** 185-202.

Torréton J, Pagès J, Dufour P, Cauwet G. (1997). Bacterioplankton carbon growth yield and DOC turnover in some coral reef lagoons. In:*Proceedings of the 8th International Coral Reef Symposium*, pp. 947–952.

Torréton J, Pagès J, Talbot V (2002). Relationships between bacterioplankton and phytoplankton biomass, production and turnover rate in Tuamotu atoll lagoons. *Aquatic Microbial Ecology* **28:** 267-277.

Torréton J, Rochelle-Newall E, Jouon A, Faure V, Jacquet S, Douillet P (2007). Correspondence between the distribution of hydrodynamic time parameters and the distribution of biological and chemical variables in a semi-enclosed coral reef lagoon. *Estuarine, Coastal and Shelf Science* **74:** 766-776.

Van Duyl F, Gast G (2001). Linkage of small-scale spatial variations in DOC, inorganic nutrients and bacterioplankton growth with different coral reef water types. *Aquatic Microbial Ecology* **24:** 17-26.

Van Duyl F, Scheffers S, Thomas F, Driscoll M (2006). The effect of water exchange on bacterioplankton depletion and inorganic nutrient dynamics in coral reef cavities. *Coral Reefs* **25:** 23-36.

Verdugo P, Alldredge A, Azam F, Kirchman D, Passow U, Santschi P (2004). The oceanic gel phase: a bridge in the DOM-POM continuum. *Marine Chemistry* **92:** 67-85.

Ware J, Smith S, Reaka-Kudla M (1992). Coral reefs: sources or sinks of atmospheric CO_2? *Coral Reefs* **11:** 127-130.

Wegley L, Edwards R, Rodriguez Brito B, Liu H, Rohwer F (2007). Metagenomic analysis of the microbial community associated with the coral Porites astreoides. *Environmental Microbiology* **9:** 2707-2719.

Weinbauer M, Kerros M, Motegi C, Wilhartitz I, Rassoulzadegan F, Torréton J *et al* (2010). Bacterial community composition and potential controlling mechanisms along a trophic gradient in a barrier reef system. *Aquatic Microbial Ecology* **60:** 15-28.

Wiebe W, Johannes R, Webb K (1975). Nitrogen fixation in a coral reef community. *Science* **188:** 257-259.

Wild C, Laforsch C, Huettel M (2006). Detection and enumeration of microbial cells within highly porous calcareous reef sands. *Marine and Freshwater Research* **57:** 415-420.

Wild C, Rasheed M, Werner U, Franke U, Johnstone R, Huettel M (2004). Degradation and mineralization of coral mucus in reef environments. *Marine Ecology Progress Series* **267:** 159-171.

Yahel G, Sharp J, Marie D, Häse C, Genin A (2003). In situ feeding and element removal in the symbiont-bearing sponge Theonella swinhoei: Bulk DOC is the major source for carbon. *Limnology and Oceanography* **48:** 141-149.

Yoshinaga I, Fukami K, Ishida Y (1991). Comparison of DNA and protein synthesis rates of bacterial assemblages between coral reef waters and pelagic waters in tropical ocean. *Marine Ecology Progress Series* **76:** 167-174.

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.

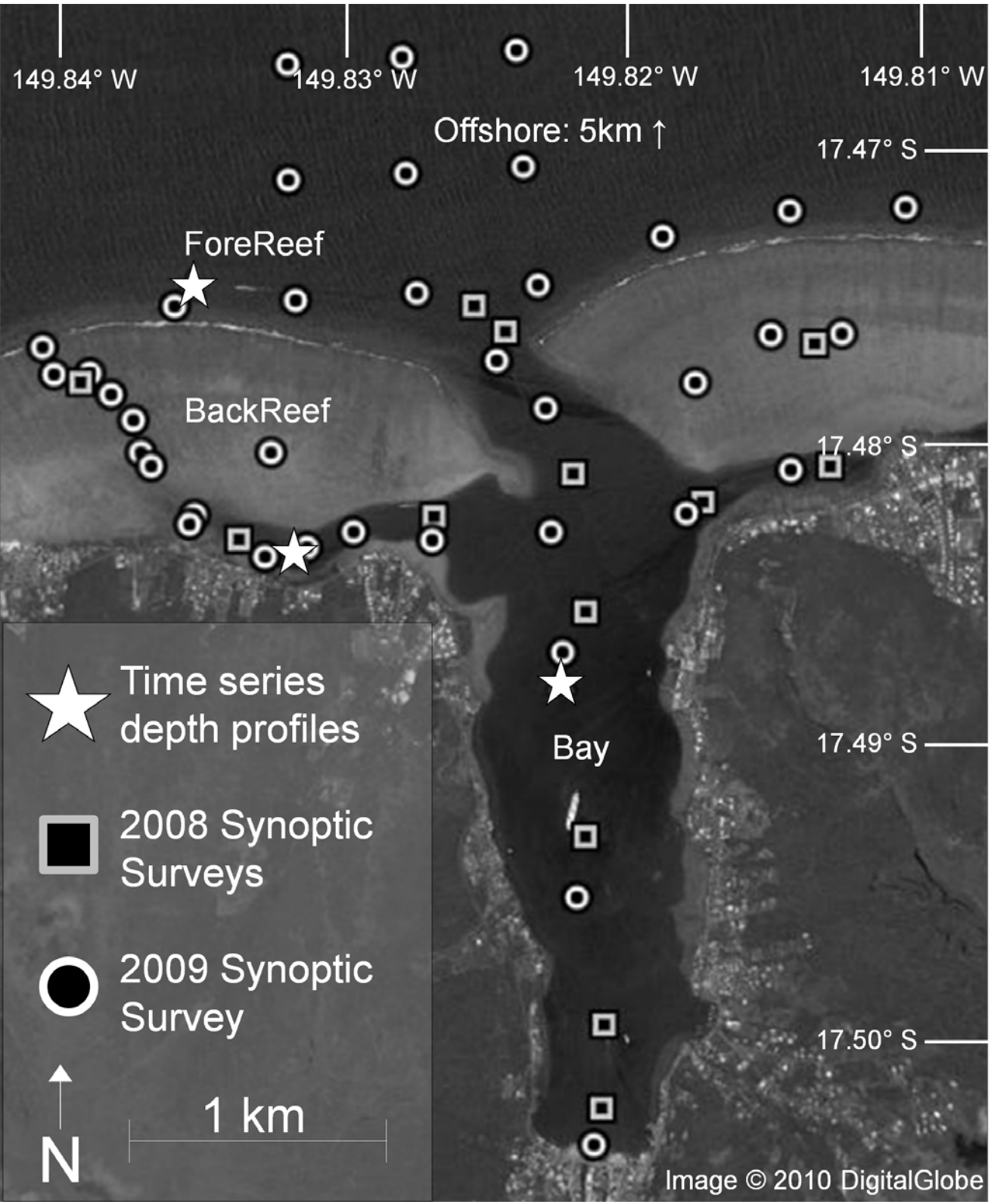
Fig 3. DOC and bacterioplankton concentrations averaged across 1, 5, and 10 m discrete depth samples 2-3 times annually at four sampling locations 2005-2009 in the vicinity of Paopao Bay, Moorea (see Fig. 1 for profile locations). Data are separated by season to test for significant differences when 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.

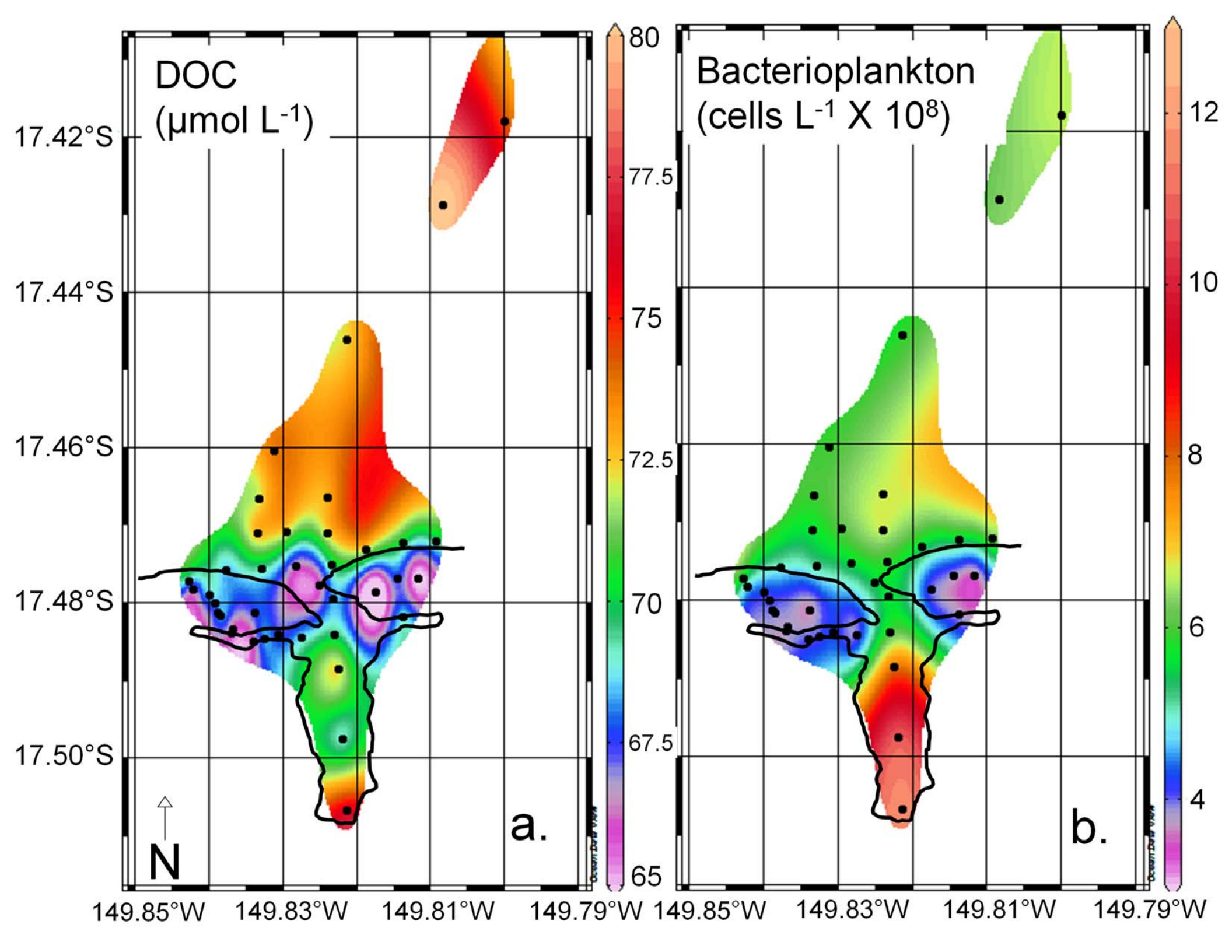
Fig 4. Spatial distribution of bacterioplankton community types in the 2009 synoptic survey. Surface DNA samples are symbol/color coded according to 5 community types defined as 85% Bray-Curtis similarity group average (UPGMA) clusters of 16S bacterial rRNA gene amplicon TRFLP fingerprints (a; vertical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

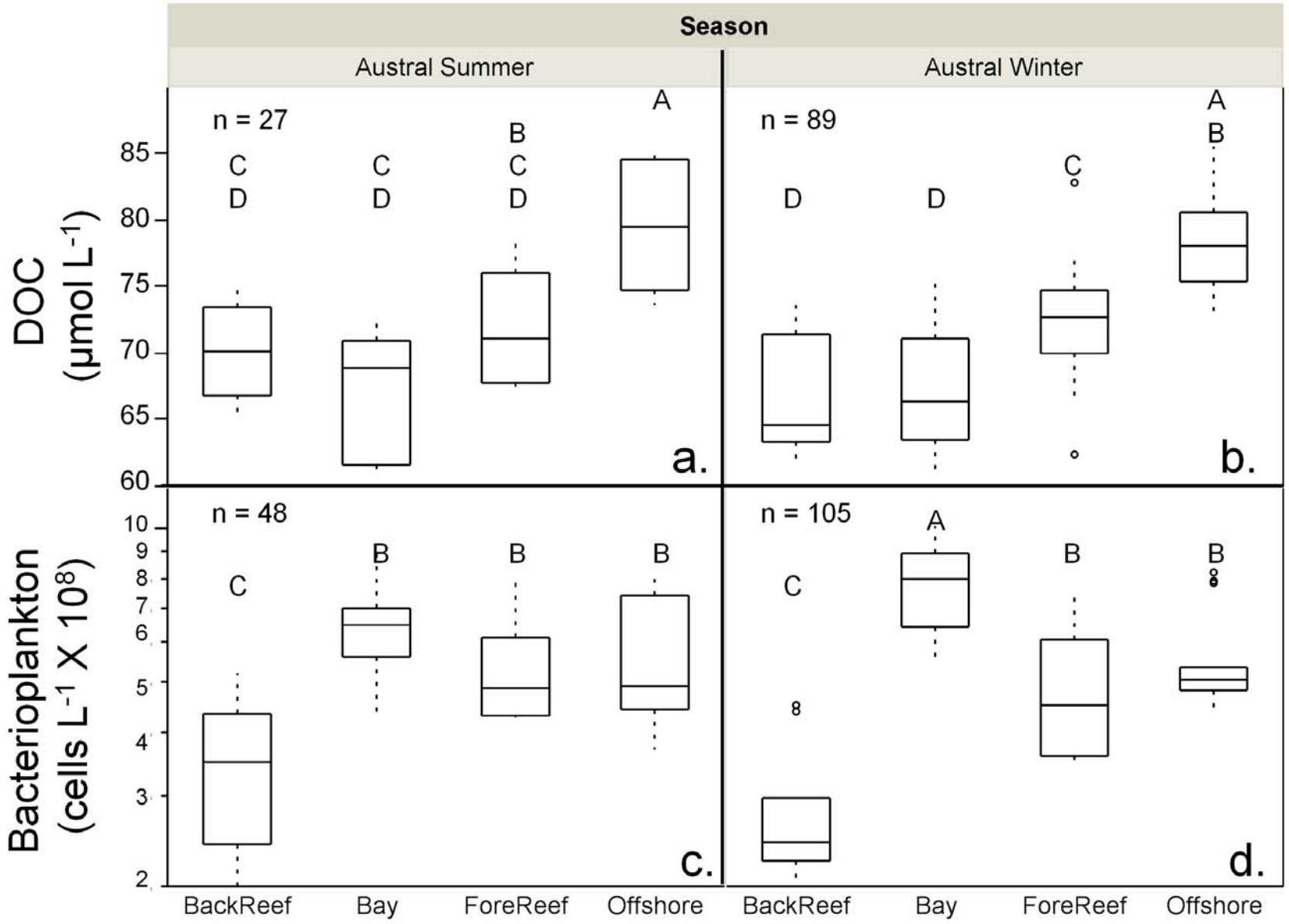
habitats for clarity. The map (b) is loosely shaded according to depth and substrate type keyed at the upper right, with samples symbol-coded according to TRFLP cluster. The inset map in (b) shows community types found at the offshore sampling locations, which were within ~6km of Moorea in >200m deep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

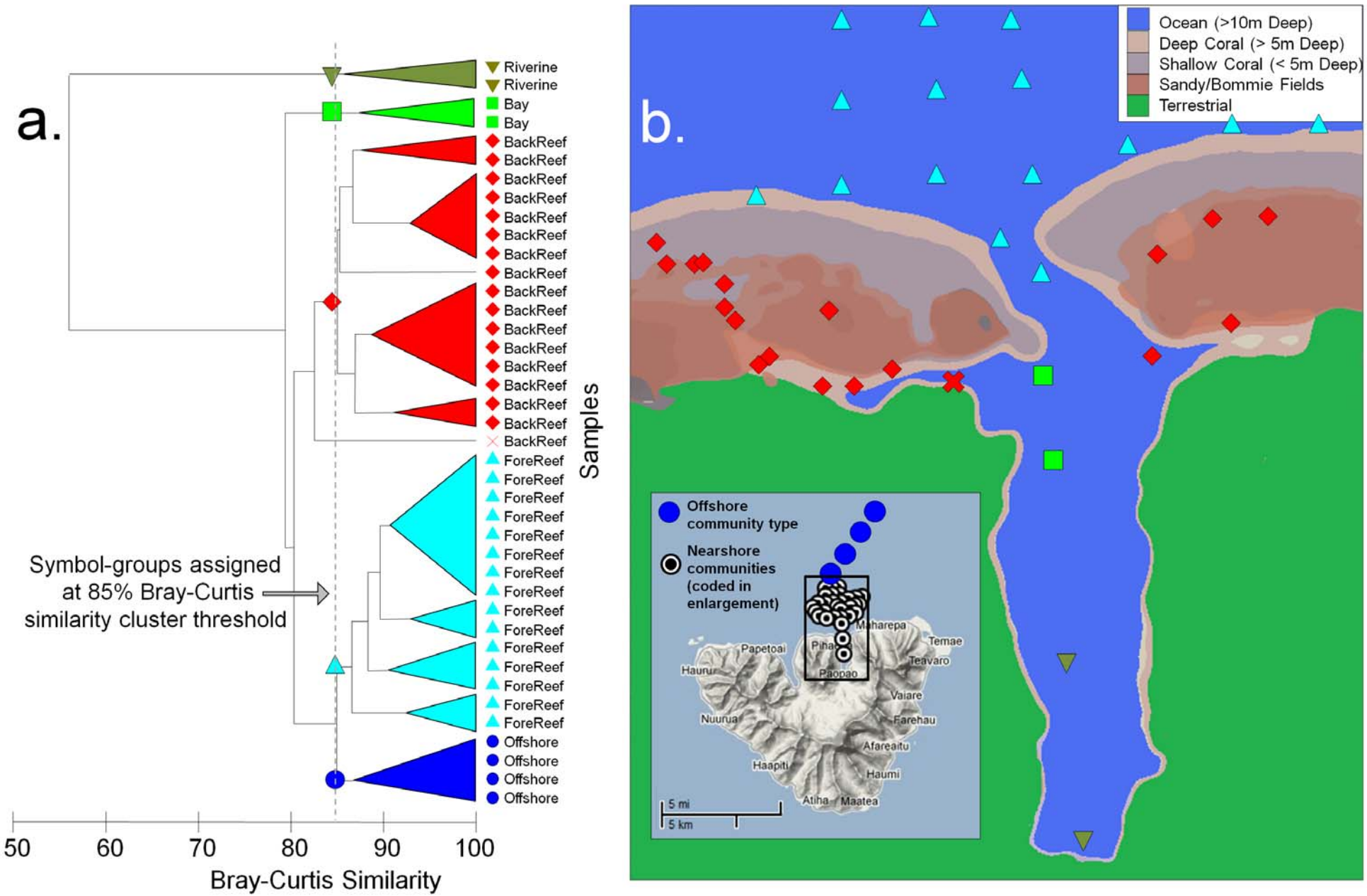
Fig 5. Spatial distribution of bacterial phylotypes in the vicinity of Paopao Bay 1 Sept 2009. Each plot shows shaded contours of the relative abundance of terminal restriction fragments (TRFs) which were putatively identified with a cloned sequence from Moorea (Fig S5). Each phylotype distribution displayed 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.

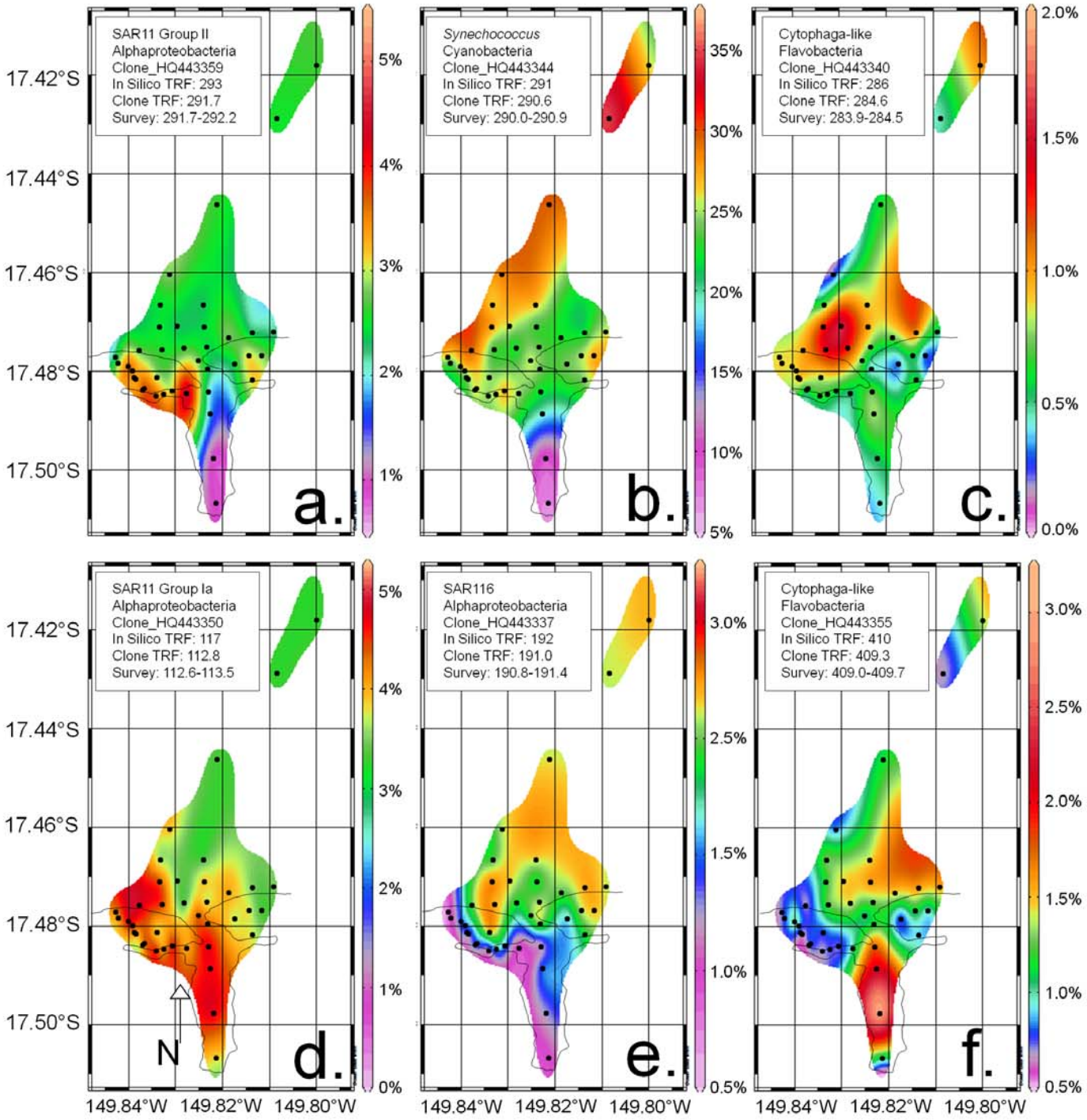
Fig 6. Spatial variability in relative abundance of bacterial classes derived from pyrosequencing of environmental 16S rRNA V6 amplicons sampled in the vicinity of Moorea 11-13 Jan 2008. Replicate samples are labeled (top) according to collection location (see Table S1) and clustered (bottom) by relative 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.

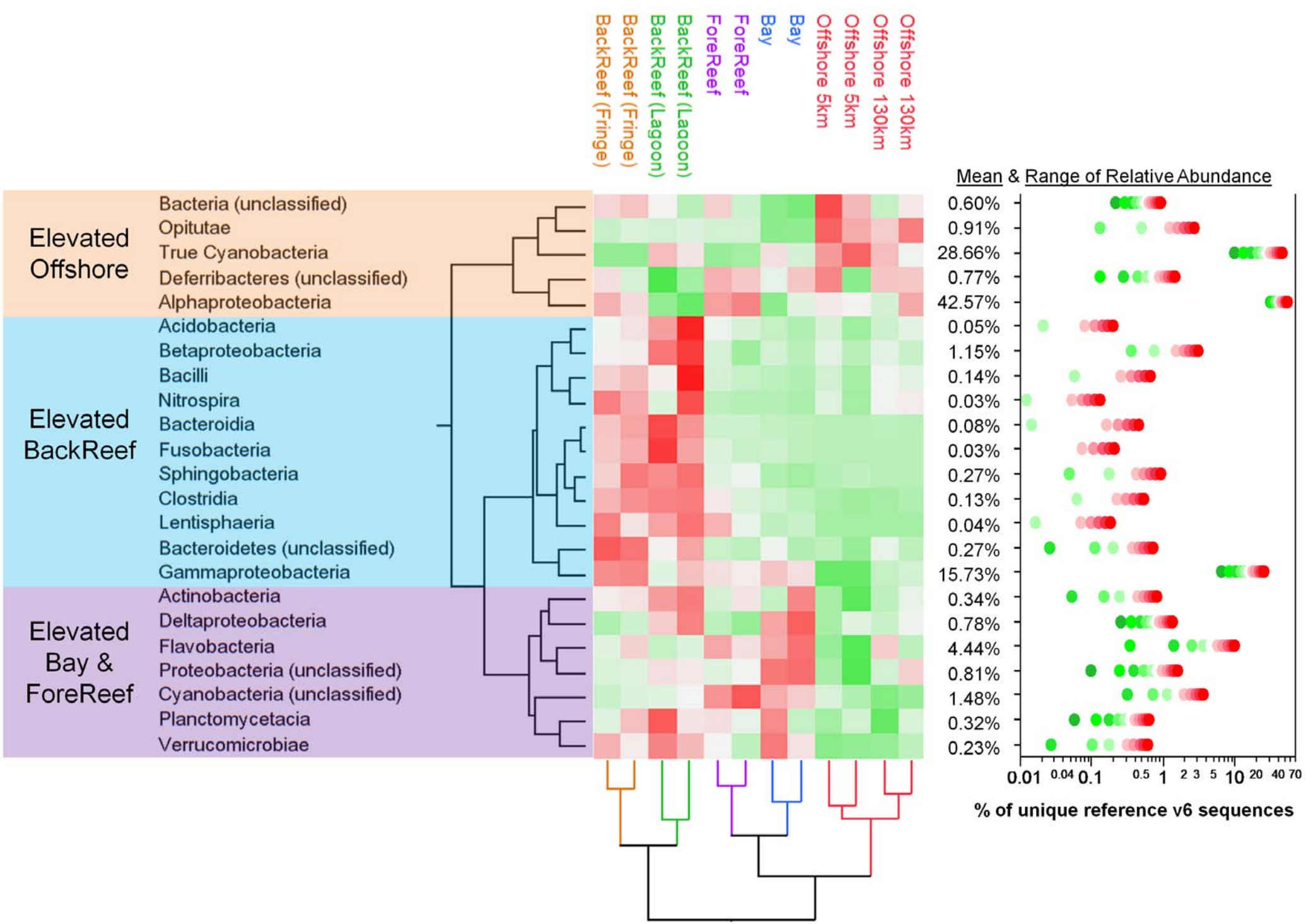

149.84° W
149.83° W
149.82° W
149.81° W
Offshore: 5km ↑
17.47° S
ForeReef
BackReef
17.48° S
Bay
17.49° S
17.50° S
Time series depth profiles
2008 Synoptic Surveys
2009 Synoptic Survey
N
1 km
Image © 2010 DigitalGlobe


DOC
(µmol L-1)
Bacterioplankton
(cells L-1 X 10^8)
17.42°S
17.44°S
17.46°S
17.48°S
17.50°S
149.85°W
149.83°W
149.81°W
149.79°W
80
77.5
75
72.5
70
67.5
65
12
10
8
6
4
N
a.
b.


Season
Austral Summer
Austral Winter
n = 27
n = 89
n = 48
n = 105
DOC (µmol L-1)
Bacterioplankton (cells L-1 X 10^8)
a.
b.
c.
d.
BackReef
Bay
ForeReef
Offshore


a.
Riverine
Riverine
Bay
Bay
BackReef
BackReef
BackReef
BackReef
BackReef
BackReef
BackReef
BackReef
BackReef
BackReef
BackReef
BackReef
BackReef
BackReef
BackReef
ForeReef
ForeReef
ForeReef
ForeReef
ForeReef
ForeReef
ForeReef
ForeReef
ForeReef
ForeReef
ForeReef
ForeReef
ForeReef
Offshore
Offshore
Offshore
Offshore
Samples
Symbol-groups assigned at 85% Bray-Curtis similarity cluster threshold
50
60
70
80
90
100
Bray-Curtis Similarity
b.
Ocean (>10m Deep)
Deep Coral (> 5m Deep)
Shallow Coral (< 5m Deep)
Sandy/Bommie Fields
Terrestrial
Offshore community type
Nearshore communities (coded in enlargement)
Maharepa
Temae
Papetoai
Pihaa
Teavaro
Hauru
Paopao
Vaiare
Nuurua
Farehau
Afareaitu
Haapiti
Haumi
Atiha
Maatea
5 mi
5 km


SAR11 Group II
Alphaproteobacteria
Clone_HQ443359
In Silico TRF: 293
Clone TRF: 291.7
Survey: 291.7-292.2
a.
Synechococcus
Cyanobacteria
Clone_HQ443344
In Silico TRF: 291
Clone TRF: 290.6
Survey: 290.0-290.9
b.
Cytophaga-like
Flavobacteria
Clone_HQ443340
In Silico TRF: 286
Clone TRF: 284.6
Survey: 283.9-284.5
c.
SAR11 Group Ia
Alphaproteobacteria
Clone_HQ443350
In Silico TRF: 117
Clone TRF: 112.8
Survey: 112.6-113.5
d.
SAR116
Alphaproteobacteria
Clone_HQ443337
In Silico TRF: 192
Clone TRF: 191.0
Survey: 190.8-191.4
e.
Cytophaga-like
Flavobacteria
Clone_HQ443355
In Silico TRF: 410
Clone TRF: 409.3
Survey: 409.0-409.7
f.
17.42°S
17.44°S
17.46°S
17.48°S
17.50°S
N
149.84°W
149.82°W
149.80°W
5%
4%
3%
2%
1%
0%
35%
30%
25%
20%
15%
10%
5%
2.0%
1.5%
1.0%
0.5%
0.0%
3.0%
2.5%
2.0%
1.5%
1.0%
0.5%


BackReef (Fringe)
BackReef (Fringe)
BackReef (Lagoon)
BackReef (Laqoon)
ForeReef
ForeReef
Bay
Bay
Offshore 5km
Offshore 5km
Offshore 130km
Offshore 130km
Mean & Range of Relative Abundance
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
0.60%
0.91%
28.66%
0.77%
42.57%
0.05%
1.15%
0.14%
0.03%
0.08%
0.03%
0.27%
0.13%
0.04%
0.27%
15.73%
0.34%
0.78%
4.44%
0.81%
1.48%
0.32%
0.23%
0.01 0.04 0.1 0.5 1 2 3 5 10 20 40 70
% of unique reference v6 sequences