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ORIGINAL ARTICLE

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;* [2]*Department of Ecology, Evolution and Marine Biology, University of California, Santa Barbara, CA, USA;* [3]*The Josephine Bay Paul Center for Comparative Molecular Biology and Evolution, Marine Biological Laboratory, Woods Hole, MA, USA and* [4]*Department of Geological Sciences, Brown University, Providence, RI, USA*

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 (DOM) in the interaction between reefs and the surrounding ocean remains limited. In this study, we present the results of a 4-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 5.5×10^8 cells l^{-1} offshore and 68 μmol l^{-1} DOC and 3.1×10^8 cells l^{-1} over the reef, respectively) across a 4-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 Betaproteobacteria, 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.

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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 have 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

Correspondence: CE Nelson, Marine Science Institute, University of California, Santa Barbara, CA 93106, USA.
E-mail: cr_nelson@lifesci.ucsb.edu
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the microbial loop (Pomeroy, 1974; Azam *et al.*, 1983). Both theory (Ducklow, 1990; Sorokin, 1990; Crossland *et al.*, 1991), and field-based models (Grigg *et al.*, 1984; Arias-Gonzalez *et al.*, 1997) 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 (Wiebe *et al.*, 1975; Lesser *et al.*, 2004) 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 site in Moorea, French Polynesia. The Moorea Coral Reef Long Term Ecological Research 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 4 years along depth profiles in three nearshore habitats (forereef, backreef and bay) and $\sim$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 physicochemical gradients.

Materials and 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, Figure 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 $\sim$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 3-day period two to three times each year from 2005 through 2009. DOC and bacterioplankton were collected in 10 depth-profile time-series sampling events over this

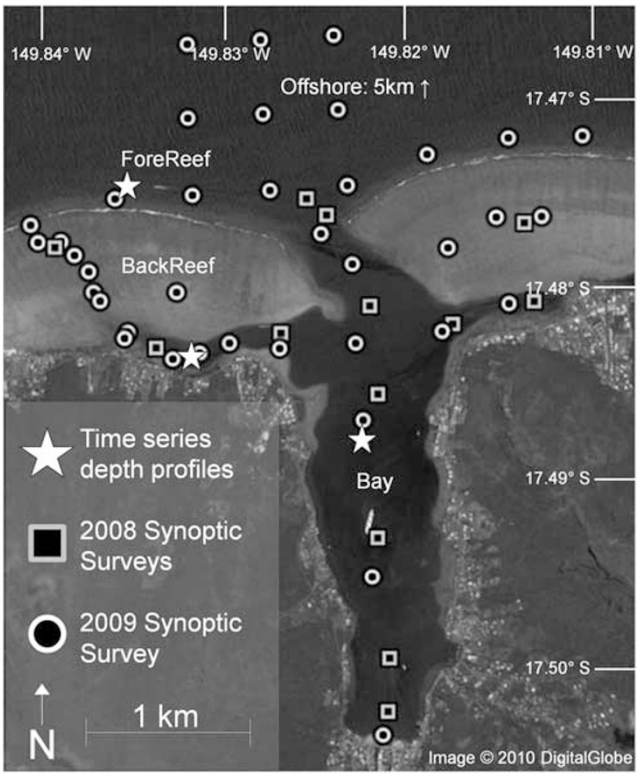

Figure 1 A satellite photograph of Paopao Bay, Moorea with sampling locations identified according to time and type of sampling. Offshore sampling locations (four sample stations in 2009 and one time-series depth profiling station) are within ~6 km north of the reef crest and are excluded from this figure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

period and two additional high-resolution grid surveys (August–September 2008 and 2009; Figure 1). All samples were stored at *in situ* temperatures in the dark for up to 2 h before processing. Seasonal time-series samples were collected at discrete depths (1 m, 5 m and 10 m) via 8 l 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, Whatman Inc., Piscataway, NJ, USA), whereas in seasonal time-series sampling total organic carbon (TOC) was sampled directly from Niskin bottles (General Oceanics Inc., Miami, FL, USA) without filtration. Particulate organic carbon is a small component of the TOC pool of Moorean waters (averaging 3–5% both offshore and in the reef environments) and does not differ significantly between offshore and back reef habitats ($n=21$, $P=0.12$), thus the temporal and spatial dynamics of the TOC pool are primarily because of the 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 high-density polyethylene 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 min of fixation. Nucleic acid samples from synoptic Austral winter surveys (August–September 2008 and 2009) were collected by gravity filtering 0.8–1.5 l water through a 0.2 μm polyethersulfone filter cartridge (Millipore Sterivex model SVGP; Millipore, Billerica, MA, USA), 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 (January 2008) collected duplicate 1 l 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 Inc., Valencia, CA, USA) 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 (Shimadzu Scientific Instruments, Columbia, MD, USA) modified according to Carlson *et al.* (2010). Ultraviolet-oxidized deionized water with organics removed (Barnstead, Nanopure Diamond, Thermo Scientific, Asheville, NC, USA) was used for blank correction for all samples. Each system run was calibrated with both potassium hydrogen pthalate standards (four point curve 25–100 μM) referenced against low carbon deep Sargasso Sea reference waters (2600 m) and surface Sargasso Sea water every six to eight analyses (Hansell and Carlson, 1998; Carlson *et al.*, 2004) calibrated with DOC consensus reference waters (Hansell, 2005).

Bacterioplankton abundance measurement

Fixed samples were thawed, mixed, stained with 1× SYBR Green I (Invitrogen Corporation, Carlsbad, CA, USA) 30 min (dark room temperature) and analyzed within 3 h. We empirically determined that the integrity of the stain yielded consistent abundance measurements throughout a minimum of 3 h measured at 20 min intervals. Samples were counted using a flow cytometer (LSR II; BD Biosciences, San Jose, CA, USA) equipped with a high throughput sampler, coherent sapphire 488 nm laser and a default suite of six detectors (side-scatter and forward-scatter photodiodes and green, orange, red and far-red photomultipliers). Using the high throughput sampler 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 s. 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 versus 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 epifluorescence microscopy (Porter and Feig, 1980) yielded a strong relationship between the two measurements, with cytometry counts ~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 the 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 August–September 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 (5′-AGRGTTYGATYMTGGCTCAG-3′) and 519r (5′-GWATTACCGCGGCKGCTG-3′) was used to amplify the 16S rRNA gene (30 cycles of 94 °C 30 s, 57 °C 60 s, 72 °C 120 s) according to Nelson (2009). Products were gel-extracted via QiaEx (Qiagen) and digested 4 h at 37 °C with enzyme HaeIII (New England Biolabs, Ipswich, MA, USA) followed by enzyme inactivation (20 min 80 °C). Fragment analysis of formamide-saturated and heat-denatured samples via capillary sequencer (Applied Biosystems, Carlsbad, CA, USA; 3730XL) was conducted at the UC Berkeley DNA Sequencing Facility using a custom-sizing standard (20 sizes over the range 30–650 base pairs; Bioventures, BioVentures Inc., Murfreesboro, TN, USA). Electropherogram peak areas in the 30 550 bp range were relativized by sample totals, aligned and analyzed according to Nelson, (2009), with peaks <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 January 2008 (Supplementary 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 Sequence Read Archive under the accession number SRA030397. All statistical analyses and heatmaps were conducted using JMP (v. 8; SAS Institute Inc.); unless otherwise noted, *P*-values for differences between habitats are derived from analysis of variance with Tukey *post hoc* tests to control for multiple comparisons. All community structure analyses were performed with Primer-E (v. 6; Clarke and Gorley, 2006). All contour plots were generated with Ocean Data View v4.3 (Schlitzer 2010) using DIVA gridding with 30×30 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 (Figure 2 and Supplementary Figure S1). In these surface surveys, DOC concentrations in the forereef and bay were intermediate between backreef and offshore end points, whereas 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 (Supplementary Figures 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 to 2009 (Figure 3). The backreef environment was significantly lower in DOC concentration relative to offshore waters over the 2005–2009 sampling period regardless of season (analysis of variance with Tukey *post hoc* tests comparing concentrations in each habitat $P < 0.05$; Figures 3a and b) and was consistently depleted in bacterioplankton relative to all other habitats (Figures 3c and d). 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; Figure 3b). Winter bacterioplankton densities in the bay were elevated relative to all other habitats and exceeded summer bay bacterioplankton densities (Figure 3d). DOC and bacterioplankton vertical variability on any sampling date was much smaller than lateral variability among habitats from backreef through offshore (for example, Supplementary Figure S1a) with no statistical effect of sampling depth on later habitat differentiation across dates (analysis of

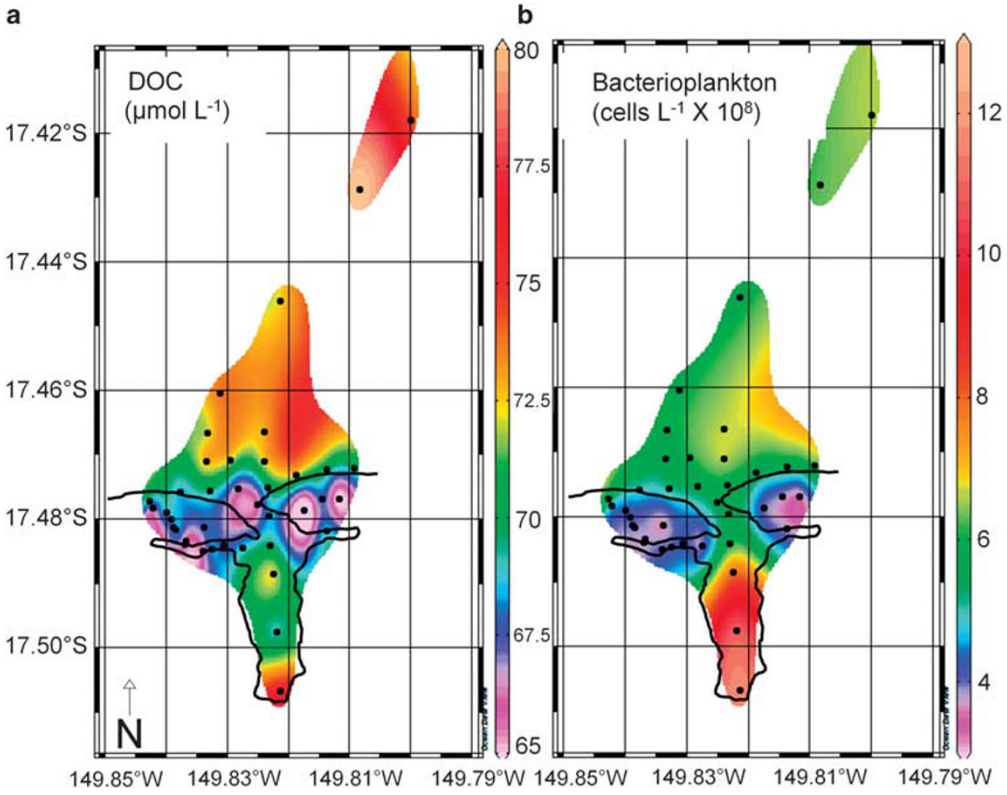

Figure 2 DOC (**a**) and bacterioplankton (**b**) concentrations measured during a synoptic survey of surface waters in the vicinity of Paopao Bay, Moorea, 1 September 2009. The black line gives a rough outline of the bay and reef crest. Note that both DOC and bacterioplankton are depleted behind the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

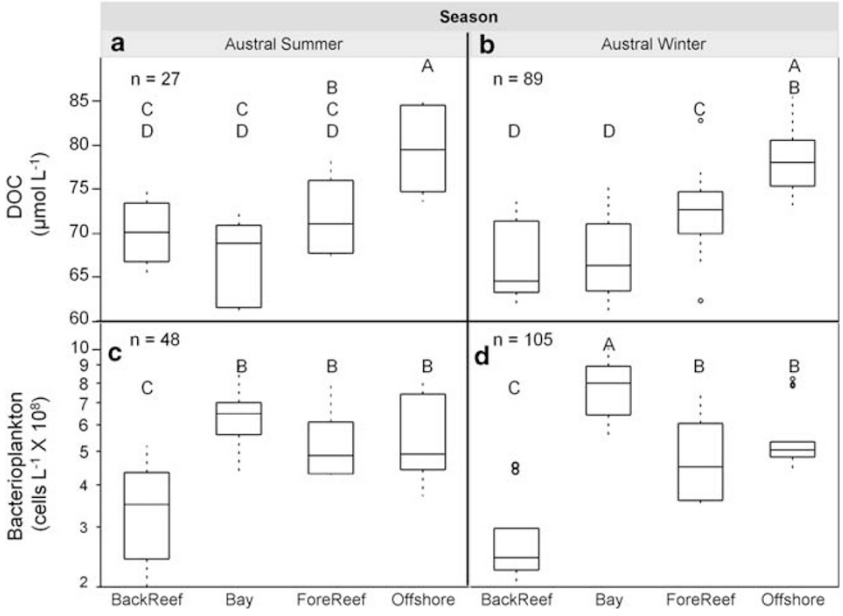

Figure 3 DOC (**a**, **b**) and bacterioplankton (**c**, **d**) concentrations averaged across 1, 5 and 10 m discrete depth samples two to three times annually at four sampling locations 2005–2009 in the vicinity of Paopao Bay, Moorea (see Figure 1 for profile locations). Data are separated by season (Summer **a**, **c**; Winter **b**, **d**) 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.

covariance 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; $P = 0.02$).

Concentrations of phosphate, nitrite and silica did not differ significantly among the four habitats over the 2005–2009 time series averaged over the upper 10 m (Supplementary Figure S2, $P > 0.10$ in either season or grouped across seasons). In winter only, nitrate concentrations were greater on average in the backreef (mean 0.46 μmol l^{-1}) than offshore (mean 0.13 μmol l^{-1}; $P = 0.012$, $n = 23$). Particulate organic stocks (carbon, nitrogen and chlorophyll *a*) were significantly higher within the bay relative to other locations ($P < 0.05$) across the seasonal dataset but not significantly different between forereef, backreef and offshore sampling points in either season or grouped across seasons ($P > 0.05$).

Synoptic spatial differentiation of bacterioplankton community structure

Bacterioplankton community structure was found to be significantly different among the offshore, backreef, forereef and bay habitats on multiple dates and using different methods of community characterization, including TRFLP, cloning and amplicon pyrosequencing (Figures 4–6, Supplementary Figures S3–5).

TRFLP fingerprinting

Synoptic winter surveys in August–September of 2008 and 2009 revealed significant differences between habitats each year in TRFLP fingerprints of bacterioplankton community structure (Figures 4 and Supplementary Figure S3; two-way nested analysis of similarities tested the significance of clustering by habitat within years $R = 0.76$, $P < 0.001$). Hierarchical clustering of surface samples collected 1 September 2009 according to relative abundance of TRFLP phylotypes (Figure 4) matched habitat clustering patterns observed during smaller surveys in 2008 (Supplementary Figure S3a) and showed minimal depth variation (Supplementary Figure S3b). The dominant non-metric multidimensional scaling axis of community variation (53.8% variation) paralleled the onshore to offshore habitat gradient in both years when ordinated together. Although the relationships between habitats were consistent between 2008 and 2009, the 2 years differed significantly overall (analysis of similarities tested the significance of clustering by year $R = 0.60$, $P < 0.001$). As with patterns of bacterioplankton and DOC depletion, these spatial patterns in community

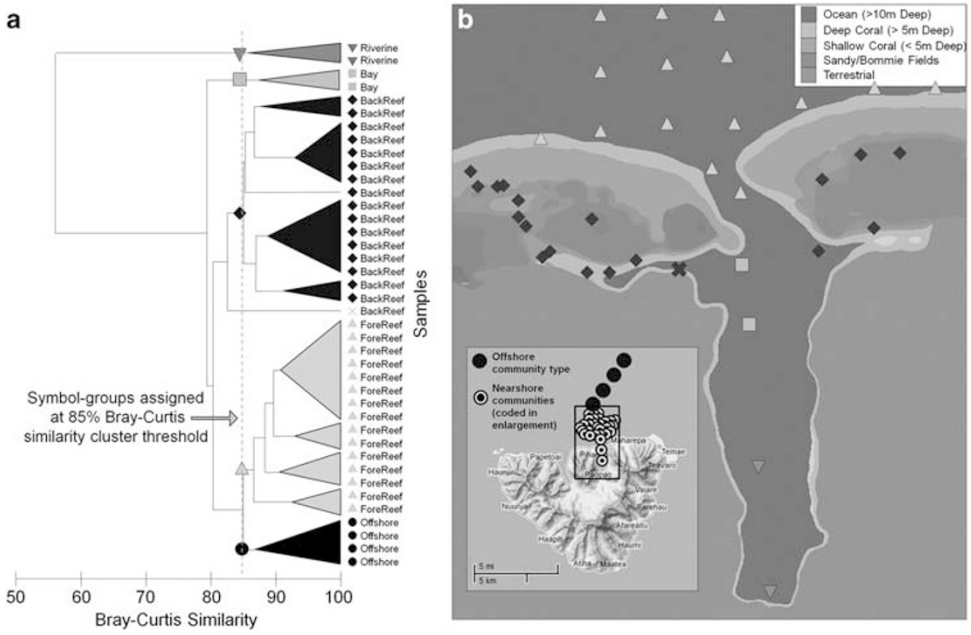

Figure 4 Spatial distribution of bacterioplankton community types in the 2009 synoptic survey. Surface DNA samples are symbol/color coded according to five community types defined as 85% Bray–Curtis similarity group average (unweighted pair group method with arithmetic mean) clusters of 16S bacterial rRNA gene amplicon TRFLP fingerprints (**a**; vertical line demarks 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 ~6 km of Moorea in >200 m deep water. A color reproduction of this figure is available on the html full text version of the manuscript.

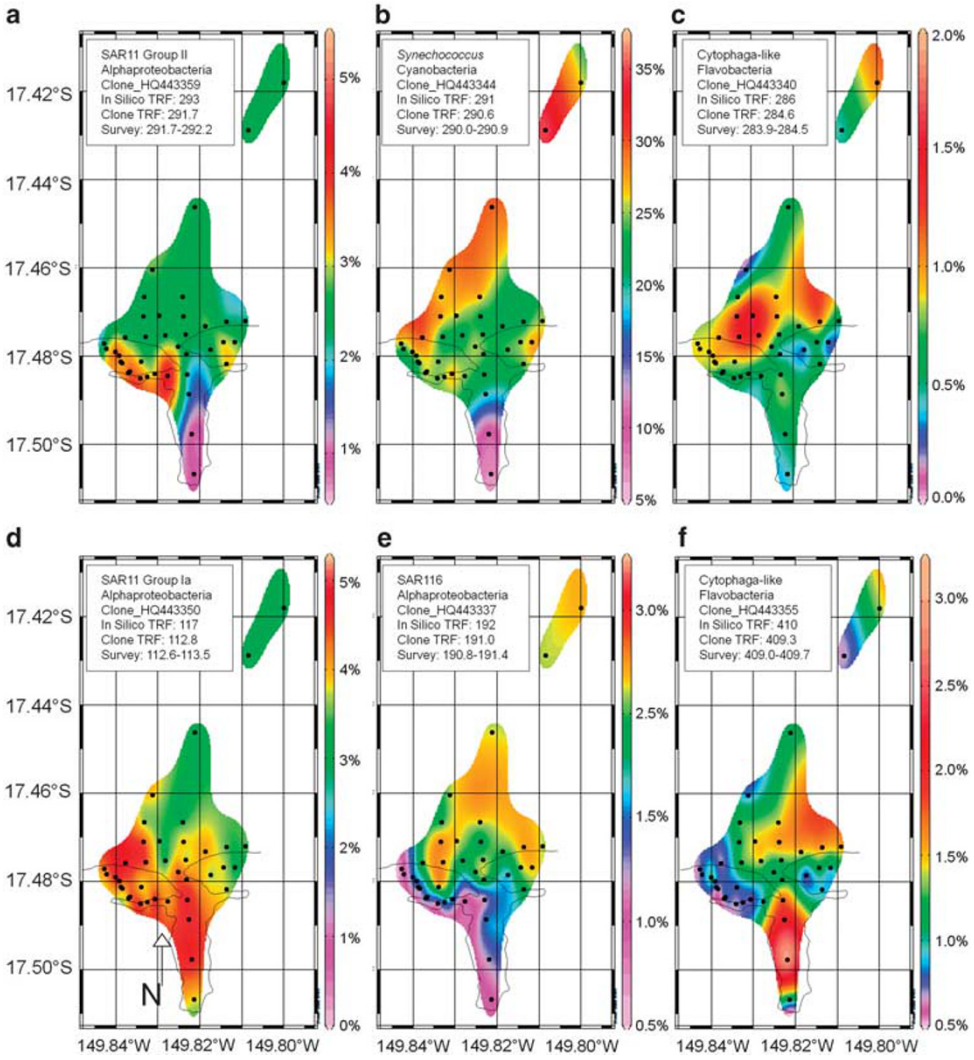

Figure 5 Spatial distribution of bacterial phylotypes in the vicinity of Paopao Bay 1 September 2009. Each plot shows shaded contours of the relative abundance of terminal restriction fragments (TRFs), which were putatively identified with a cloned sequence from Moorea (Supplementary Figure S5). Each phylotype distribution displayed here is unambiguously represented by a cloned sequence with a measured TRF falling within the 1 bp range of environmental TRFs and for which the closest matching full-length clone in the greengenes database (DeSantis *et al*. 2006a, b) 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 (117 bp) and clone TRF lengths (113 bp) as shown by Morris *et al*. (2005). Note that SAR11 clades are relatively enriched in the backreef (**a**, **d**), whereas 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**), whereas the two Flavobacteria are enriched in the forereef (**c**) and bay (**f**), respectively.

differentiation held constant over two adjacent sampling dates in 2008 separated by a significant storm event (Supplementary Figures S3c, d).

Clone libraries

Using a random clone library, phylogenetic classifications were putatively assigned to 33 of 120 terminal restriction fragments found in the 2008–2009 synoptic surveys by measuring terminal restriction fragment lengths of cloned 16S amplicons phylogenetically resolved into clades using maximum likelihood (Guindon and Gascuel, 2003; Dereeper *et al.*, 2008) (Supplementary Figure 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 (Figures 5a and d, respectively). *Synechococcus* were relatively dominant throughout the surface waters but increased in relative abundance offshore (Figure 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 (Figure 5e). Two distinct members of the Flavobacteriaceae showed contrasting distributions, with one enriched only in the forereef (Figure 5c) and another depleted only in the backreef

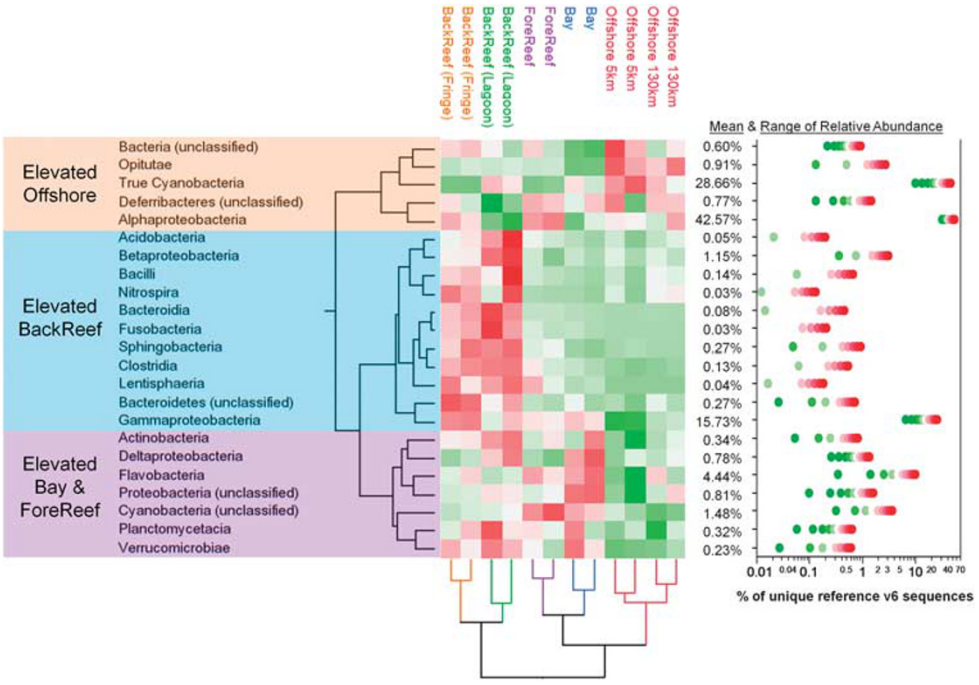

Figure 6 Spatial variability in relative abundance of bacterial classes derived from pyrosequencing of environmental 16S rRNA V6 amplicons sampled in the vicinity of Moorea 11–13 January 2008. Replicate samples are labeled (top) according to collection location (see Supplementary Table S1) and clustered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

(Figure 5f). A resemblance matrix comprised solely of these six taxa was correlated with overall community resemblance among sampling locations and years ($r_{\text{Mantel}}=0.82$, $P<0.01$), demonstrating that the variation in these six taxa matched the overall community differentiation patterns among habitats.

Pyrosequencing

The 16S rRNA gene amplicon sequence data also revealed similar habitat partitioning to that demonstrated in TRFLP analyses (Figure 6) based on >237 000 V6 tags analyzed among six habitats along the reef-offshore gradient (Supplementary Table S1). Methodological replicate samples (~20 000 sequences each) were not significantly different ($P>0.05$ by similarity profiling using the SIMPROF algorithm in Primer-E v6) but the community structure of each nearshore habitat was significantly different (SIMPROF $P<0.05$, Figure 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 operational taxonomic units (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 OTU relative abundance or presence/absence data (Supplementary Figure 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 (Figure 6). Backreef habitats were relatively enriched in Beta- and Gammaproteobacteria, 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 >1% of sequences on average 16%, 1.2% and 4.4% respectively; Figure 6). The majority of bacterial classes found via pyrosequencing were present at low abundances (<0.5% of sequences; Figure 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 and Bacteroidia), in the nearshore habitats relative to the open ocean.

Discussion

Our seasonal and synoptic surveys comprised >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 (Figures 2, 3, Supplementary Figure 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 (Figures 4–6, Supplementary Figure 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 (Scheffers *et al.*, 2004; Houlbreque *et al.*, 2006; Genin *et al.*, 2009).

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 (Suzuki *et al.*, 2001; Dinsdale *et al.*, 2008). Instead most studies in rapidly flushed reefs show either diel increases in DOC above offshore concentrations (Van Duyl and Gast, 2001; Hata *et al.*, 2002) 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 and Ducklow, 1996; Cherrier *et al.*, 1996; Carlson, 2002; Carlson *et al.*, 2004). 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 versus 27.81 °C) and less saline (salinities of 35.99 versus 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 September 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 20 m; 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 (Passow and Alldredge, 1994; Verdugo *et al.*, 2004; Mari *et al.*, 2007) 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; Supplementary Figure 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 (Suess, 1970; Hillgärtner *et al.*, 2001). However, this process is difficult to distinguish from heterotrophic reef sediment biofilms that can remove DOM (Wild *et al.*, 2004, 2006). Although 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 (Supplementary Figure S2); dilution would be expected to non-selectively 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 (Figures 4–6, Supplementary Figures S3–S4), implying selective pressures within the water column operating on bacterioplankton at reef residence timescales. Third, DOC and bacterioplankton depletion patterns seem 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 (Figure 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 h before 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 (Lenhardt, 1991; Delesalle and Sournia, 1992; 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; Van Duyl and Gast, 2001; Hata *et al.*, 2002; Nakajima *et al.*, 2009). Recent work has demonstrated the potential for sponges to consume both DOC and bacterioplankton at biogeochemically significant rates (Yahel *et al.*, 2003; Van Duyl *et al.*, 2006; de Goeij and Van Duyl, 2007; De Goeij *et al.*, 2008). 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 $<1\%$ 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 (Richter *et al.*, 2001; Scheffers *et al.*, 2004; de Goeij and Van Duyl, 2007).

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 semilabile 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 (Ducklow, 1990; Smith *et al.*, 2006; Dinsdale *et al.*, 2008; Barott *et al.*, 2009). Bacterial production rates are typically elevated in reef environments (Moriarty *et al.*, 1985; Torréton and Dufour, 1996; Gast *et al.*, 1999; 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 (Figures 4–6, Supplementary Figure S3) were clear and consistent among years (2008 and 2009; Figures 4 and Supplementary Figure S3), seasons (austral summer and winter 2008; (Figures 5 and Supplementary Figure S3), and methods (16S rRNA V6 amplicon pyrosequencing and TRFLP fingerprinting; Figures 4, 6, Supplementary Figure 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 (Supplementary Figure 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 (Figures 5a, d). 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 (Figure 5e). Consistent with the pyrosequencing results, both Flavobacterial phylotypes (Figures 5c and f) 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, Supplementary 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 (Figure 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 and 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 (Supplementary Figure S2). The three reef water column environments sampled by pyrosequencing (forereef, backreef: lagoon and backreef: fringe) showed markedly higher numbers of bacterial OTUs for equal sampling intensity (sequence reads) compared with offshore and bay habitats (Supplementary 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 (Van Duyl and Gast, 2001; Seymour *et al.*, 2005a, 2005b) 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; Yoshinaga *et al.*, 1991; Torréton and Dufour, 1996; Torréton *et al.*, 1997; Sakka *et al.*, 2002). 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; Pagès and Andréfouët, 2001; Pagès *et al.*, 2001; Torréton *et al.*, 2002; Mari *et al.*, 2007). 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 (Torréton, 1999).

The removal of semilabile 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 (Ware *et al.*, 1992; Gattuso *et al.*, 1996, 1999; Suzuki and Kawahata, 2003). 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 micronutrients 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 ($\sim$14% of coral net daily production) indicate that planktonic bacterial communities have 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 having 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.

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