Spatial and vertical biogeography of coral reef sediment bacterial and diazotroph communities

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ABSTRACT: Coral reefs are globally important marine ecosystems as sites of high biotic diversity. Reef flat sediments are reasonably homogeneous in composition. This substratum is subject to gradients in water motion, grazing pressure and benthic productivity. This study used DNA fingerprinting techniques, automated rRNA intergenic spacer analysis (ARISA), and terminal restriction fragment length polymorphism (TRFLP) to examine patterns of bacterial assemblage composition within Heron Reef flat sediments, targeting the entire bacterial community (not including Archaea) and nitrogen-fixing bacteria. ARISA fingerprints contained between 51 and 148 operational taxonomic units (OTU) per surface sediment sample. The mean whole-community similarity between adjacent sites along a transect from Heron Island to the reef crest was 0.50 ± 0.03 (mean ± SE) Whittaker Index (comparison of relative abundances) and 0.46 ± 0.03 Jaccard Index (presence/absence only). Comparison of sediment community fingerprints to a water column community fingerprint collected above the reef flat at high tide indicated that sediments contained different assemblages to the water column (mean similarity between sediments and water column assemblage = 0.15 ± 0.01 Jaccard Index); however, assemblage composition did not differ significantly from that expected by random association. Vertical sediment cores showed dissimilarity between surface (0 to 3 cm) and deep (3 to 5 cm) community fingerprints, which was probably related to redox state. Comparison of fingerprints of fecally-derived sediments from the sea cucumber *Holothuria atra* and surrounding sediments indicated that the impact of metazoan grazing could not be distinguished from ambient small spatial scale variability in sediment assemblage composition. The *nifH*-TRFLP fingerprints of sediment prokaryotic assemblages (amplified from all samples, but only in sufficient quantities for analysis from 7 locations) indicated the presence of ubiquitous, potentially diverse diazotroph communities, with little assemblage similarity between locations. Our results suggested that while bacterial assemblages (including communities of nitrogen-fixing bacteria) in sediments of a coral reef are diverse, their assemblage composition is not related to grazing pressure but may be influenced by other biotic and abiotic environmental conditions such wave energy and sediment depth.

KEY WORDS: Coral reefs · Bacteria · Disturbance · Diversity · *Holothuria atra*

INTRODUCTION

Coral reefs are marine habitats of high biotic diversity and are among the most productive ecosystems on earth. Most studies of diversity in coral reefs have focused on the biogeography of macroorganisms, e.g. scleractinian corals and macroalgae (Glynn 1976), and the impact of human activities on coral reef ecosystems (Hughes et al.

2003). Prokaryotic (Bacterial and Archaeal) assemblages associated with coral tissue surfaces are abundant, extremely diverse (e.g. Rohwer et al. 2002), and within sediments are productive (Rasheed et al. 2004, Wild et al. 2004). Sediments in coral reefs contain large biomasses of microalgal chlorophyll *a* (Heil et al. 2004), and nitrogen fixation has been measured within coral sediments in at least 1 study (Capone 1996), suggesting that coral

reef atolls and reef flats are active sites of biogeochemistry relative to chlorophyll-poor surrounding waters. Despite the biogeochemical importance of reef flat sediments in the oligotrophic ocean, there have been no previous studies to examine spatial patterns of sediment bacterial assemblages.

Microbial diversity in the ocean has been studied from several perspectives that have targeted ecological and biogeochemical sources of variation in community structure (Giovannoni et al. 1990, Fuhrman & Davis 1997, Venter et al. 2004). Early studies examining the diversity of bacteria in soils demonstrated that the same bacteria could be identified in enrichment cultures of geographically distant samples, which led to the maxim that 'everything is everywhere' and 'the environment selects' (Beijerinck 1913). The former of these ideas has not been tested in the marine environment, but observation of phylogenetically-close relatives of SAR-11 group bacteria at diverse global locations (Morris et al. 2002), novel candidate divisions of deep sediment bacteria at widespread sites (Webster et al. 2004), and closely related Group I Archaea in mesopelagic waters of the North Atlantic and Pacific (Fuhrman & Davis 1997) suggests some overlap of at least similar species in extremely widespread locations. Selection leading to particular bacterial assemblages has not been adequately addressed in marine sediments, but environmental specificity of bacterioplankton assemblage composition has been previously investigated in estuaries (Hewson & Fuhrman 2004), and metazoan specificity of coral surface-associated bacteria in geographically distant locations has been observed (Rohwer et al. 2002). The hypothesis that similar environmental conditions select for similar assemblage structures can be easily examined in marine sediments. Marine sediments are thought to be heterogenous and patchy habitats, characterised by micrometer- to millimeter-scale geochemical gradients. However, sediments on coral reef flats can have remarkably deep but variable (i.e. 1 to 3 cm) oxic layers (Rasheed et al. 2004), experience similar levels of radiative energy flux, are of remarkably consistent geological composition, and are relatively immobile in space and time. Little is known of sediment microbial assemblage patterns and dynamics, nor the factors controlling them.

Two types of scientific investigation provide insight into factors shaping biological diversity. The first of these is observation of communities over natural gradients, while the second is direct manipulative experimentation. Since bacteria are subject to significant bottle effects during containment (Hewson et al. 2003), experimental manipulation is not ideal for examining ecological interactions within sediments if the time required to observe experimental effects exceeds the time within which bacteria will be influenced by con-

tainment. In contrast, observation of environmental gradients in bacterial diversity allows inference of community dynamics in response to environmental stimuli. In the absence of significant trophic gradients (and hence differences in resource availability), factors shaping coral reef sediment microbial communities that co-vary with trophic conditions in other environments (e.g. estuaries) can be examined independently. Due to relatively consistent illumination and chemical inputs across reef flats, gradients result from physical energy flux (and concomitant disturbance) and biological reworking by macrofauna, which in turn causes increased oxygen flux (via bioturbation and bioirrigation) and input of fecal materials. Irradiative flux to the sea surface is constant across the reef flat, but varies locally in the benthos as punctuated by coral and algal cover.

The aim of this study was to identify geographical patterns in bacterial and diazotroph assemblages in unconsolidated sediments, and to discuss their potential relation to environmental gradients on the Heron Reef flat. This study examined sediments on the Heron Reef flat, a coral cay of ~28 ha on the southern Great Barrier Reef, Australia, which exhibits the characteristic coral zonation of coral cays worldwide (Glynn 1976). Diversity of scleractinian corals has been investigated on this reef over the past 3 decades, which has led to classical ecological hypotheses such as those by Connell (1978). This reef is also one of the loci of research on the effects of global warming upon coral reef bleaching (Hughes et al. 2003). To examine sediment bacterial communities in this ecosystem, we used 2 culture-independent fingerprinting approaches that offer high resolution at both total and functional group levels: (1) automated rRNA intergenic spacer analysis (ARISA) (Fisher & Triplett 1999), which amplifies 16S–23S rRNA internally transcribed spacer (ITS) DNA fragments, was used to examine total bacterial community structure, and (2) terminal restriction fragment length polymorphism (TRFLP) of a portion of the *nifHDK* operon was used to a analyze nitrogen-fixing functional group composition (Widmer et al. 1999). These techniques allow observation of clear differences in assemblage composition among samples; although they can also be used to provide identification of organisms, this application requires clone libraries of 16S rRNA and *nifH* genes (Brown et al. 2005), which have not yet been compiled for our study environment.

MATERIALS AND METHODS

Sample collection. All samples were collected in early January 2003 on the Heron Island Reef Flat, Great Barrier Reef, Australia (23° 27' S, 151° 55' E). Samples were collected at low tide along a 140 m tran-

sect from Heron Island to the reef crest every 20 m (Fig. 1). When referring to sample location along the transect line, 0 m hereby denotes the reef crest and −140 m denotes the sample location adjacent to Heron Island. Small-scale spatial variability was also determined by collecting duplicate samples within a 10 cm^2 sediment patch in Shark Bay. Sediment samples were collected at low tide using a 10 ml syringe with the needle end removed. The plunger on the sediment corer was placed flat on the sediment surface and barrel pushed into sediments to a depth of 1.2 cm, thus enclosing 1 cm^3 of sediment. The syringe corer was then withdrawn and sediments extruded into 15 ml sterile centrifuge tubes. Vertical profile samples were collected in the same way, but to a depth of 5 cm, and the entire sediment core was sectioned at 1 cm intervals using a sterile razor blade. To investigate the effects of holothurian grazing upon sediment bacterial communities, we collected (1) sediments immediately

in front of *Holothuria atra* (i.e. within 2 cm of an animal's mouth), and (2) semi-consolidated fecal pellets immediately behind the animal. To investigate water column bacterial communities, 20 l of surface water was collected from the reef flat at high tide using an acid- and seawater-rinsed low density polyethylene cubitainer. Surface water was subsequently filtered over a 47 mm A/E Glass Fiber Filter (1.2 µm nominal pore size) to remove metazoa and algae, and then over a 0.2 µm Durapore (Millipore Corp Type GVWP) filter to collect bacterial DNA. All samples were maintained at −80°C prior to laboratory analysis at the University of Southern California.

DNA extraction. Sediment subsamples, holothurian fecal pellets, and the water column Durapore filter were extracted using BIO101 Soil DNA extraction kits (QBIOGENE), where DNA was eluted in a final extracted volume of 50 µl. DNA was quantified using PICO Green Fluorescence (Molecular Probes) on a Stratagene MX3000 quantitative fluorometer. For the water column filter, the initial agitation step with lysing matrix was replaced by placing the folded filter filtration-side out into an eppendorf tube and boiling for 60 s in the presence of buffer PBR and MT (manufacturer-supplied reagents).

Microbial community fingerprinting. ARISA was conducted on 10 ng extracted DNA as measured by Pico Green (Molecular Probes) fluorescence (Fisher & Triplett 1999) with modifications as described previously (Hewson et al. 2003). The ITS region (plus ~285 bases of 16S and 23S rRNA) of DNA extracts was amplified using the polymerase chain reaction (PCR). PCR was carried out in 50 µl reactions using 1 × PCR buffer (Promega), 2.5 mM $MgCl_2$ (Promega), 250 µM of each deoxynucleotide (Promega), 200 nM each of universal primer 16s−1392F (5'-G(C/T)-ACACACCGCCCGT-3') and bacterial primer 23s−125R labeled with a 5' TET (5'- GGGTT(C/G/T)CCCCATTC(A/G)-G- 3'), 2.5 U *Taq* polymerase (Promega) and BSA (40 ng µl^{-1} final concentration, Sigma #A-7030). These primers specifically targeted bacteria, hence archaea are not included in our analysis. Thermocycling was preceded by a 3 min heating step at 94°C, followed by 30 denaturing cycles at 94°C for 40 s, annealing at 55°C for 40 s, extension at 72°C for 90s, with a final extension step
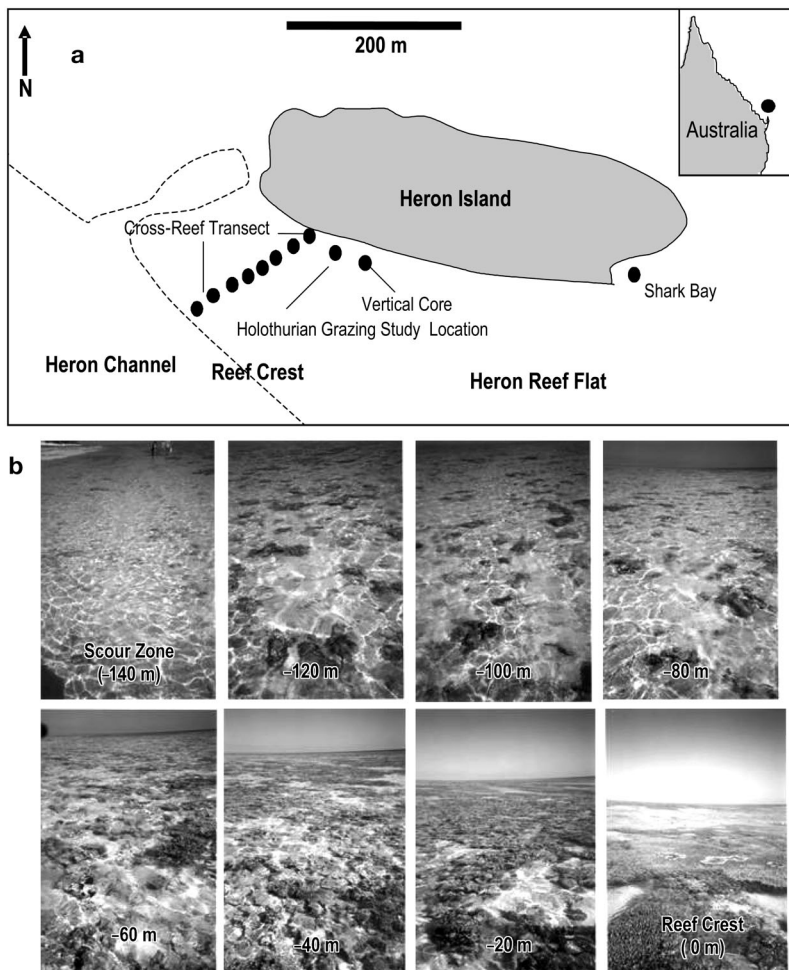

Fig. 1. (a) Sampling location on Heron Island (Great Barrier Reef, Australia) showing cross-reef transect sampling sites, holothurian grazing study sites, vertical core site and Shark Bay site. (b) Eight transect locations demonstrating change in coral cover

of 5 min at 72°C. The calculated melting temperatures of both primers were approximately 52°C. PCR amplification products were purified in Zymo Clean & Concentrator kits, then diluted to 5 ng μl^{-1} as measured by Pico Green fluorescence.

TRFLP was conducted on *nifH* gene fragments on 20 ng extracted DNA as described previously (Widmer et al. 1999). Nested amplification of *nifH* gene fragments using degenerate primers comprised 2 steps (Zehr et al. 1998). Amplification consisted of the same thermocycling protocol and contained identical PCR reagent mixtures as ARISA amplification. For the first amplification step, PCR primers *nifH*3 (5′-ATRTTRT-TNGCNGCRTA-3′) and *nifH*4 (5′-TTYTAYGGNAAR-GGNGG-3′) were used. PCR products (4 µl) from the first round of amplification were then transferred to a new reaction and re-amplified using the nested degenerate primers *nifH*1 (5′-TET-TGYGAYCCNAARGC-NGA-3′) and *nifH*2 (5′-ANDGCCATCATYTCNCC-3′), where TET indicated the fluorochrome labeled primer. PCR products were run on 1% SeaKem GTC Agarose Gels (1 × TBE) at 100V for 1 h, and gels were post-stained with SYBR Gold (1 µl ml^{-1} of manufacturer's stock, final concentration) and visualized under UV excitation on a transilluminator. Bands at ca. 370 bp were excised using a sterile razor blade, and DNA was extracted from agarose plugs using Zymo gel recovery kits. Samples containing 25 ng of purified PCR product were then restriction digested into terminal restriction fragments using *HaeIII* at 36°C. Fragments were digested overnight (12 h) and reactions stopped by heating to 60°C for 5 min. Pellets of DNA were then resuspended in 5 µl deionised H$_2$O. Products from both ARISA and *nifH*-TRFLP were run for 5 h on an ABI 377XL automated sequencer with ABI 2500 bp FAM-labeled 2500 bp standards. The sequencer electropherograms were then analyzed using ABI Genescan software.

Statistical analysis of ARISA and TRFLP data. Outputs from the ABI Genescan software were transferred to Microsoft Excel for subsequent analysis. Peaks less than 5 times the baseline fluorescence intensity were considered to be 'noise' and discarded. With this criterion, the practical detection limit for 1 operational taxonomic unit (OTU) was approximately 0.09% of the total amplified DNA. The area under each peak was expressed as a percentage of the total integrated area under the electropherogram. Simpson's indices (*D*) were calculated manually using descriptions given in Legendre & Legendre (1998) according to the following equation:

$$D = \sum_{i=1}^{n}(p_i)^2 \qquad (1)$$

Whole communities (i.e. all OTU each comprising >0.09% of total amplified DNA) were compared by calculating the Jaccard Index of similarity and the Whittaker Index of similarity (S_w) using the following equations (Legendre & Legendre 1998):

$$\text{Jaccard Index} = W(a_1 + a_2 - W)^{-1} \qquad (2)$$

$$S_w = 1 - \sum_{i=1}^{n} \frac{|(b_{i1} - b_{i2})|}{2} \qquad (3)$$

where p_i is the fraction of each peak of total integrated area, *W* is the number of shared ITS peaks between assemblages 1 and 2, a_1 and a_2 are the total number of ITS or TRF lengths in assemblages 1 and 2, respectively, and where b_1 and b_2 are the percentage contributions to amplified DNA of the *i*th OTU or TRF in samples 1 and 2, respectively. These indices were scaled from 1 (completely identical) to 0 (completely different). Peaks were placed in bins at intervals ±1 bp for ITS lengths <700 bp, ±2.5 for ITS lengths 700 to 1000 bp, and ±5 bp for ITS lengths >1000 bp. To account for possible lumping and splitting of peaks, binning was repeated 10 times (maximum bin size), each time starting bins +1 bp from the preceding repetition (Hewson & Fuhrman 2005). All Jaccard and Whittaker Indices indicated here represent the maximum similarity of those 10 bin frames. Agglomerative hierarchical cluster analysis was conducted using the XLStat (AddinSoft SARL) program via unweighted pair-group mean average method (UPGMA).

RESULTS AND DISCUSSION

Bacterial assemblage fingerprints, representing all taxa that comprise >0.09% of total assemblage DNA (Hewson & Fuhrman 2004) each contained 51 to 148 distinct OTU. The richness of fingerprints was higher than those reported in other fingerprinting studies using ARISA from pelagic (Hewson & Fuhrman 2004) and sediment environments (Hewson et al. 2003); however, they were lower than estimates of richness using other methods (Torsvik et al. 2002). Previous research on coral-associated bacteria (using cloning and sequencing) has reported a high diversity of assemblages (Rohwer et al. 2002), and our results were consistent with previous studies that found higher richness and diversity in sediment assemblages than in overlying water (Hewson et al. 2003). Similarly, sediment diazotroph (i.e. nitrogen fixing) assemblage fingerprints contained 53 distinct OTU, which was more than detected using ARISA in other environments (e.g. rice paddies; Tan et al. 2003), but significantly lower than fingerprints from marine sediments and plankton that were determined by cloning and sequencing (which offer higher phylogenetic resolution than fingerprints) (Zehr et al. 1998, Burns et al. 2002).

Bacterial assemblage fingerprints along the sediment transect shared 0.50 ± 0.03 Whittaker Index (mean ± SE) and 0.46 ± 0.03 Jaccard Index (mean ± SE, n = 32 pairwise comparisons); however, they displayed less similarity to the single water column fingerprint (0.14 ± 0.01 Whittaker Index and 0.15 ± 0.01 Jaccard Index) (Fig. 2a). Samples collected 10 cm apart in Shark Bay had a Whittaker Index of 0.70 and a Jaccard Index of 0.60. In a previous study of coastal bacterioplankton in Moreton Bay, which contains several different habitats (riverine, estuarine and open ocean), assemblages had an average similarity of 0.63 ± 0.01 Whittaker Index and 0.48 ± 0.10 Jaccard Index (Hew-

son & Fuhrman 2004). Diazotrophic assemblage fingerprints were also dissimilar to each other and shared a Jaccard Index of 0.38 ± 0.05 (mean ± SE) (Fig. 2c). Four fingerprints from within the gutter zone (separated by ca. 30 m) were similarly heterogeneous and shared a Whittaker Index of 0.43 ± 0.03 and a Jaccard Index of 0.45 ± 0.04 (mean ± SE). This may indicate that assemblages in coral reef flat sediments are patchy on scales of cm, consistent with previous high-resolution studies of *Pseudomonas* spp. in soils (Cho & Tiedje 2000). We believe this patchiness demonstrates that the habitat of coral reef flat sediments, along with homogeneous benthic primary productivity (Heil et al. 2004) and
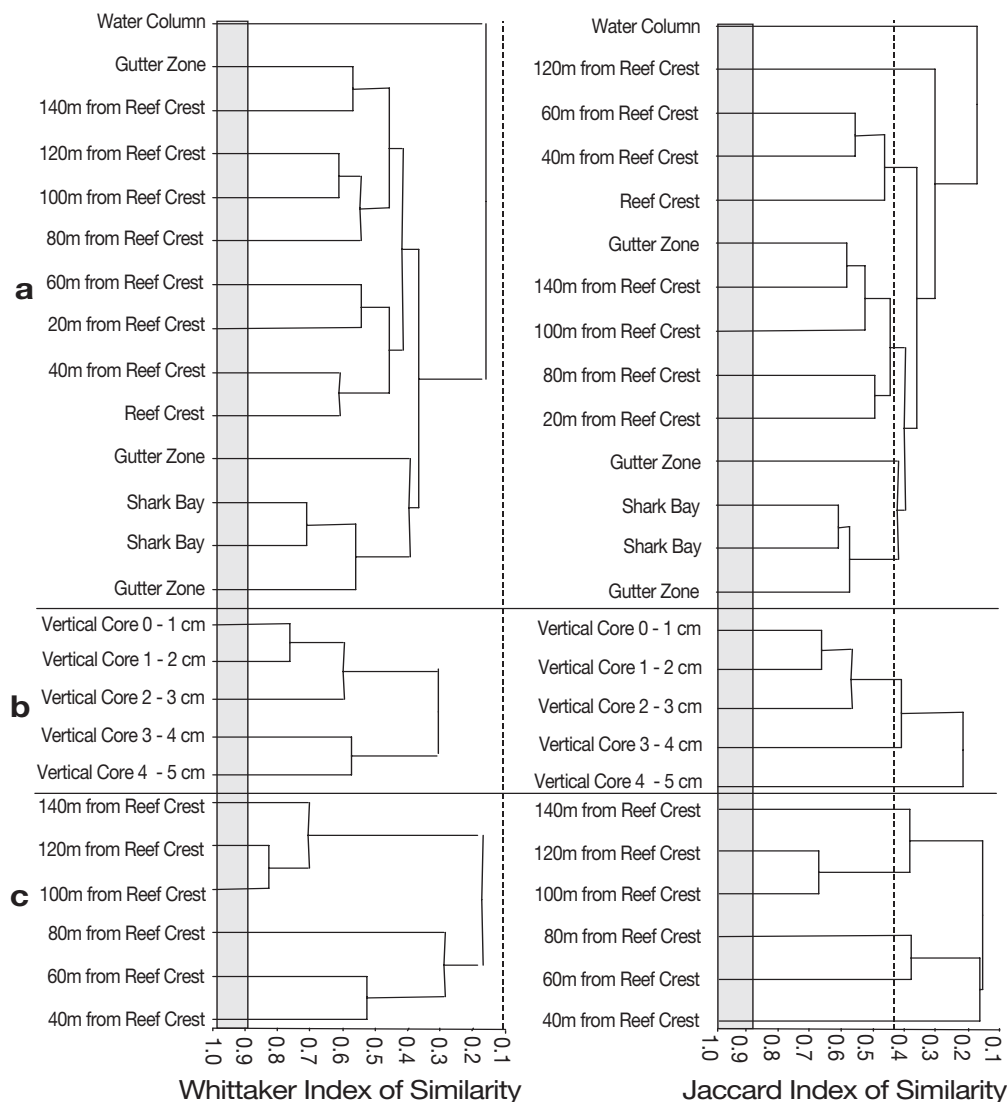

Fig. 2. Cluster analyses of reef flat microbial communities: (a) spatial and (b) vertical automated rRNA intergenic spacer analysis (ARISA) and (c) spatial *nifH* terminal restriction fragment length polymorphism (TRFLP) analyses. Clustering derived from the Whittaker Index of Similarity, the Jaccard Index, and unweighted pair-group-mean analysis. Light grey bar indicates average replicate fingerprint similarity. Dashed line on left indicates 95% of 1250 Monte Carlo generated random communities pairwise comparisons based upon random number of fragments (from 5 to 75), random fragment sizes from 400 to 1200 bp (i.e. similarity expected by chance alone), and random proportion of amplified DNA (exponentially distributed) (Hewson & Fuhrman 2005)
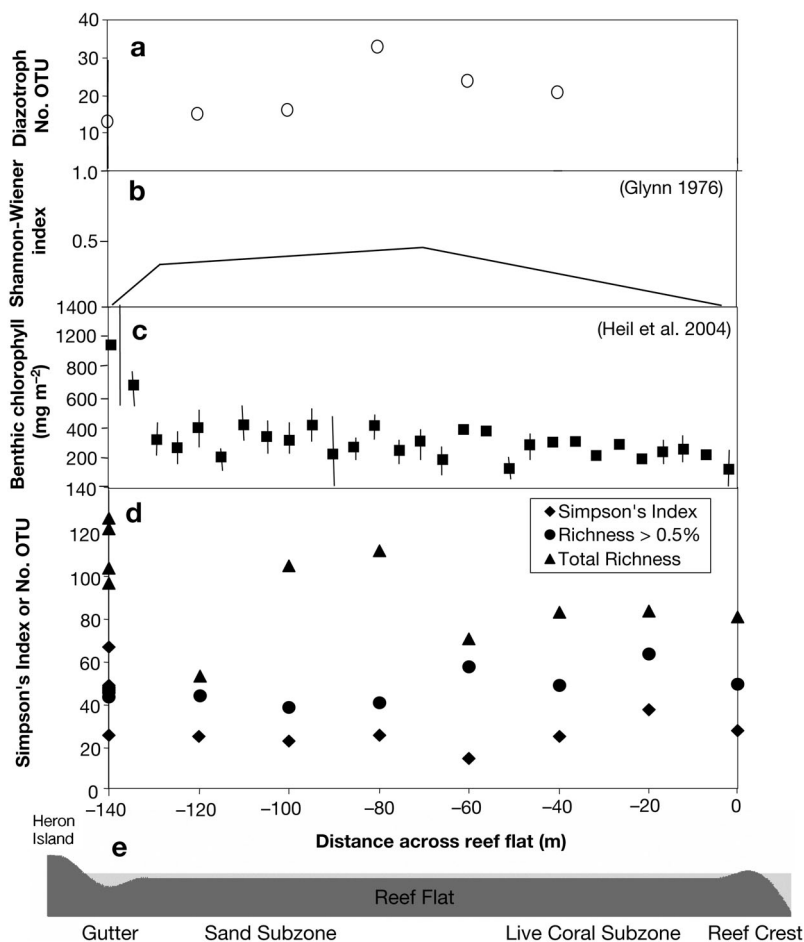
Fig. 3. Cross-reef flat richness of (a) *nifH* TRFLP fingerprints, (b) coral diversity redrawn from Glynn (1976), (c) benthic microalgal productivity (redrawn from Heil et al. 2004), and (d) diversity and richness of ARISA community fingerprints across (e) reef flat. Error bars indicate SE. OTU: operational taxonomic unit

On the Heron Reef, a scour zone or 'gutter', the result of breaching the reef crest to provide a boating channel in 1920, is adjacent to the island (–140 m) and experiences strong currents between tides. Sediment microalgal productivity is highest in the gutter zone, which is believed to be due to greater sediment-surface boundary layer breakdown and concomitant enhanced nutrient availability compared to other reef flat locations (Heil et al. 2004). The richness of the whole bacterial assemblage fingerprints decreased from Heron Island to the reef crest, with the exception of the sample collected –120 m from the reef crest (Pearson's correlation coefficient (r) = –0.84, p < 0.01 excluding –120 m) (Fig. 3). In contrast, the richness of each OTU that comprised >0.5% of total amplified DNA (i.e. 'major' taxa) increased significantly from Heron Island to the reef crest (p < 0.05, n = 4; Student's *t*-test comparing variance in –140 to –80 m and –60 to 0 m). All fingerprints from the gutter region and Shark Bay were of greater richness than fingerprints generated from –60 to 0 m. This suggests that physical disturbance may select for a few OTU which are adapted to elevated water motion environments. Coral diversity is greatest when intermediately disturbed (Connell 1978), and is least diverse when high disturbance causes dominance by *r*-selected (i.e. fast growing, opportunistic) species (e.g. *Pocillopora* spp.). Connell's (1978) study confirmed earlier work (Glynn 1976) that demonstrated high coral diversity away from the high water motion reef crests, but dominance by *Pocillopora* spp. on the crest itself. Our results are consistent with work on scleractinian corals, as bacterial fingerprints contained a higher relative abundance of some bacteria (i.e. 'dominant' bacteria) that caused an overall lower diversity index at the location of the highest water motion regime. The fingerprint Simpson's Index was moderately higher –20 m from the reef crest, which may reflect that this zone experiences intermediate disturbance (Fig. 3).

Physical energy flux potentially shapes bacterial assemblages. However, biotic interactions may play a more important role in shaping sediment bacterial assemblages. Since benthic microalgal photosynthesis corresponds with elevated bacterial production on the reef flat (Moriarty & Hansen 1990), benthic microalgal productivity probably plays an important role in determining

substratum type, may be subject to heterogeneous unmeasured factors (e.g. resource quality; Torsvik et al. 2002) which influence assemblage diversity.

Waves cause resuspension of sediments; however, resuspension varies with tides and location on the reef flat. This flux has various impacts upon microbial assemblages within sediments, and results e.g. in greater oxygen penetration, greater delivery of detritus, and removal of DOC and nutrients from sediments. Wave energy represents physical energy flux (e.g. water motion) that in turn creates a gradient of disturbance from reef crest to island. Areas closer to the reef crest experience physical energy primarily in the form of wave motion, while those closer to the island experience energy mainly as laminar flow. Since laminar flow of water (currents) probably does not disturb sediments as greatly as wave action, or at least does so uniformly over small scales, sediment microbial assemblages near the reef crest experience greater physical disturbance than those closer to Heron Island.
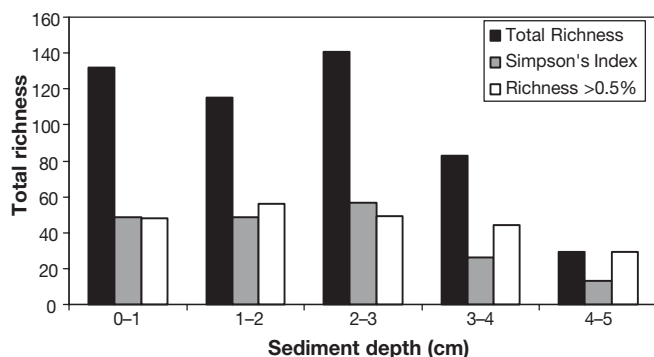
Fig. 4. ARISA fingerprint richness and diversity in vertical profile at 1 station in the gutter zone

sediment bacterial assemblages. Unfortunately, since productivity was elevated only in the gutter (Heil et al. 2004), we were unable to collect sufficient samples to examine this relationship.

Two biotic factors have been demonstrated to influence bacterial communities at Heron Island and elsewhere: these are (1) the chemical environment (redox state and electon acceptor/donor speciation) of sediments (a consequence of biological activities), and (2) grazing by macrofauna (Moriarty et al. 1985). Since there is no appreciable cross-reef flat gradient in nutrient availability, as reflected by nutrient limitation to all benthic microalgae (Heil et al. 2004), geochemical gradients are primarily vertical. We investigated the relationships among assemblage fingerprints in vertical profile at a single location in the gutter zone. Richness of bacterial OTU demonstrated a subsurface maximum, which perhaps corresponded to the oxic/anoxic boundary (Fig. 4). Assemblages closer to the surface (0 to 3 cm) were more similar to each other (Whittaker Index: 0.78 ± 0.12; Jaccard Index 0.74 ± 0.07, mean ± SE) than to deeper communities (Whittaker Index: 0.35 ± 0.03; Jaccard Index of 0.29 ± 0.06) (Fig. 2b).

The tropical sea cucumber *Holothuria atra* has been demonstrated to cause significant stimulation of both bacterial (Moriarty et al. 1985) and benthic microalgal (Uthicke 2001) production via grazing activities. These deposit-feeding organisms are most abundant close to Heron Island and are believed to graze a large percentage of total reef flat area daily (Moriarty et al. 1985). We collected sediments within 2 cm of the mouth of 2 individuals and also collected unconsolidated fecal pellets. Samples collected within 10 cm of each other (i.e. a similar distance to that between food and feces of *H. atra*) elsewhere on the reef flat (Shark Bay) shared a Whittaker Index of 0.70 and a Jaccard Index of 0.60, therefore we expected differences between food sediment and holothurian feces to reflect grazing impact. Assemblage fingerprints of bacteria in fecal pellets and surrounding sediments shared a Whittaker Index of

0.51 ± 0.10 and a Jaccard Index of 0.40 ± 0.14 (mean ± SE), indicating that grazing impact could not be distinguished from the substantial ambient small-scale variability.

Our results relied on assemblage fingerprints, for which there may be questions regarding the applicability of this approach to quantitative ecological studies. Assemblage fingerprinting techniques are not perfect due to biases inherent in the PCR (Suzuki & Giovannoni 1996), variation in operon copy number (Klappenbach et al. 2000), and differences in cell extractability among taxa (Polz & Cavanaugh 1998). However, fingerprints are reproducible and allow clear differences among communities to be observed (Hewson & Fuhrman 2004). Furthermore, a recent ARISA study of OTU within bacterioplankton assemblage fingerprints corresponding to *Prochlorococcus* spp. compared remarkably favorably to flow cytometric enumeration ($r^2 = 0.86$) from 4 yr of monthly time series data from the San Pedro Ocean Time Series (Brown et al. 2005). In this study, we used fingerprinting techniques to compare bacterial communities in different samples, and our conclusions did not fully rely on relative abundance data and were also supported by presence/absence data (Jaccard Index changes).

Coral reef flat sediment bacterial assemblages showed variability among sites both in terms of common (i.e. >1% of total amplified DNA in fingerprints) and rare (<1% of total amplified DNA) bacterial OTU, and were therefore patchy when entire assemblage fingerprints were examined. While we cannot exclude the effects of unmeasured variables upon the structure of sediment bacterial assemblages, this study demonstrated that patterns of assemblage variability are consistent with gradients in physical energy flux. Finally, this study demonstrated the utility of fingerprinting techniques for comparing sediment bacterial assemblages.

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