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Microbiome dynamics of two differentially resilient corals

Zoe A. Pratte[1,2,*], Laurie L. Richardson[1]

[1]Department of Biological Sciences, Florida International University, Miami, FL 33199, USA

[2]*Present address:* Department of Biological Sciences, Georgia Institute of Technology, 311 Ferst Drive, Atlanta, GA 30332, USA

ABSTRACT: Coral bleaching and disease are 2 common occurrences that are contributing to global coral cover decline. Understanding the interactions between the coral animal and its microbial associates, and how they may change in the presence of stressors such as warming and acidification, is a crucial component to understanding both susceptibility and resistance to disease and bleaching. The coral *Diploria labyrinthiformis* has been shown to be more disease resistant than its relative *Pseudodiploria strigosa*, providing an ideal study system for disease resistance. In this study, we examined the bacterial communities of these 2 coral species on the Florida Reef tract every 6 mo for 18 mo (*in situ* sampling), and under experimental (laboratory) thermal and pH manipulation. The *in situ* sampling encompassed wide fluctuations in temperature, including an anomalously warm summer period. The laboratory experiments involved exposure to both increased temperature (31°C) and lowered pH (7.7). The *in situ* bacterial communities of both coral species were highly similar in the winter, but diverged during summer, with the *D. labyrinthiformis* bacterial community being more stable than that of *P. strigosa.* Differences in the bacterial community between the 2 coral species included 29 operational taxonomic units (OTUs) that were specific to *D. labyrinthiformis* in all seasons, while only 2 OTUs were specific to *P. strigosa.* The comparative stability of the *D. labyrinthiformis* microbiome, in addition to harboring a more specific microbiome, may be a key component of the relative disease resistance of this coral.

KEY WORDS: *Diploria* · *Pseudodiploria* · Microbiota · Climate change · Acidification

INTRODUCTION

Coral reef degradation is occurring worldwide, and is believed to be largely the result of anthropogenic impacts (Pandolfi et al. 2011). The health of the coral is dependent upon all members of the coral holobiont, which is composed of the coral animal, algal endosymbionts (*Symbiodinium*), viruses, bacteria, archaea, and fungi (Rohwer et al. 2002, Reshef et al. 2006, Rosenberg et al. 2007). The bacterial members of the holobiont are particularly crucial, fulfilling roles such as disease resistance through production of antimicrobials (Ritchie 2006), niche occupation believed to prevent invasion by pathogens (Reshef et al. 2006), and nutrient biogeochemical cycling such as nitrogen fixation, nitrification, and sulfate reduction (Beman et al. 2007, Raina et al. 2009, Kimes et al. 2010, Lema et al. 2012, Pratte 2013, Bourne et al. 2013). Given the importance of the coral-associated bacterial community, it is essential to understand how this community might contribute to coral health, disease, and resilience in the presence of anthropogenic stressors.

Coral susceptibility to disease and bleaching (the loss of the symbiotic *Symbiodinium* from the coral tissue) is thought to be exacerbated by increased seawater temperature and acidification (Pandolfi et al. 2011). These susceptibilities are variable between

*Corresponding author: zoe.pratte@biology.gatech.edu

different coral species, as well as among colonies of the same coral species (Sutherland & Ritchie 2004, Grottoli et al. 2006, Hughes et al. 2010, Pandolfi et al. 2011). On reefs of the wider Caribbean, 2 major reef-building corals, *Pseudodiploria strigosa* (recently moved from the genus *Diploria*; Budd et al. 2012) and *Diploria labyrinthiformis*, display different bleaching and disease susceptibilities, despite being closely related. For example, *P. strigosa* was more susceptible to a yellow band disease outbreak in the US Virgin Islands (0.7% prevalence), while *D. labyrinthiformis* showed no sign of disease (Calnan et al. 2008). Similarly, *P. strigosa* has proven more susceptible to black band disease (BBD) compared to *D. labyrinthiformis* throughout the Caribbean, Bermuda, and Belize (Rützler et al. 1983, Edmunds 1991, Jones et al. 2012). Conversely, *D. labyrinthiformis* has been documented to bleach more readily than *P. strigosa* (Cook et al. 1990, Villamizar et al. 2008). Molecular comparison of the microbial communities associated with these 2 coral species may reveal differences that, when assessed in response to stressors such as temperature increase and acidification, lead to insights into possible mechanisms of disease resistance. For example, specific bacterial strains associated with rhizobial roots enables some plants to better resist drought conditions than their counterparts without these beneficial strains (Rolli et al. 2015). Identification of the presence of specific bacteria associated with *D. labyrinthiformis* may lead to understanding of its relative disease resistance. In this study, the bacterial communities of the 2 coral species *D. labyrinthiformis* and *P. strigosa* were compared seasonally for 18 mo (*in situ*), and experimentally in the laboratory in the presence of controlled elevation of temperature and acidification. The goal of the study was to identify, using 16S rRNA gene sequencing, differences in the bacterial communities that may potentially contribute to disease susceptibility and/or resistance.

MATERIALS AND METHODS

In situ sample collection

Three pairs of corals (1 *Diploria labyrinthiformis* colony and 1 *Pseudodiploria strigosa* colony per pair) were located at Horseshoe Reef (25° 8.36′ N, 80° 17.64′ W) and 3 pairs at Algae Reef (25° 8.80′ N, 80° 17.58′ W) in the Florida Keys National Marine Sanctuary. The 2 colonies in each pair occurred within 3 m of each other, thereby ensuring that both species experienced relatively consistent environmental conditions. Small samples (1 cm^2) that included coral tissue, the surface mucopolysaccharide layer (SML), and underlying skeleton were taken from each coral colony using a hammer and chisel, and immediately placed in individual Whirlpak bags, followed by patching of the sample site with non-toxic modelling clay. Samples were then promptly brought to the surface, put into 1 ml of RNA later® (Life Technologies) in a 2 ml microcentrifuge tube, and placed directly on dry ice. Samples were then transported to the laboratory where they were kept at −80°C until further processing. All samples were collected at 3 time points: August 2013 and 2014, to investigate the effects of warmer temperatures, and February 2014 (winter on these reefs). Seawater temperature, measured at the time of collection at each site, was 30°C during each August sampling, and 25°C during the winter collection. However, in August of 2014, average sea surface temperatures were recorded that were approximately 1°C higher, indicating that overall temperatures in the month of August were higher in 2014 compared to 2013, despite the single measurement being the same. At the time of collection in 2013, a coral bleaching warning was issued for the Florida Keys indicating mild thermal stress, although before and after sampling the level was lower. In August 2014, the Florida Keys were at a level 1 bleaching alert, indicating higher thermal stress, that later built to a level 2 alert following sampling (see www.coralreefwatch.noaa.gov).

Laboratory experiments

Laboratory experiments were carried out at Florida International University to compare coral microbiomes of both species in the presence of both acidification (pH 7.7) and warming (31°C) under controlled conditions. According to the prediction by the IPCC Fourth Assessment using the A1FI model, the ocean will be pH 7.7 by 2100. Three colonies of each species were collected from the Florida Keys National Marine Sanctuary Coral Nursery between November 2012 and October 2013 and fragmented into approximately 3 cm^2 pieces. Fragments were set onto small cement pedestals and allowed to recover in a large holding tank for a minimum of 3 wk (but up to 18 mo) before experimentation. Physical conditions of the holding tank were identical to control experimental tanks (described below), with the exception of volume (340 l; see Pratte & Richardson 2014 for details). All coral ap-

peared visibly healthy at the time of experimental setup. Experiments were conducted between November 2013 and June 2014. Three 19 l experimental aquaria were set up identically, each containing a recirculating filter (mechanical filtration), live rock (microbial filtration/nitrate removal), and artificial sea water (34 ppt: Instant Ocean Reef Crystals®). Aquaria were maintained on a 12 h light:12 h dark cycle under metal halide and cool white fluorescent lights. All aquaria were allowed to establish for 2 wk before coral introduction, and coral fragments were allowed to acclimate for 2 wk before experimentation. After coral introduction, 10% water changes were conducted on a weekly basis. The temperature treatment group was subject to increasing temperature from 25 to 31°C over a period of 6 wk at a rate of 1°C wk^{-1}. The second treatment group included both increasing temperature as described above and acidification from pH 8.2 to 7.7 at a rate of 0.1 units wk^{-1}. The change in pH was implemented using a CO_2 injection system (AZOO). The control group was maintained at 25°C and a pH of 8.2. In a previous publication (Pratte & Richardson 2016), 2 fragments of *D. labyrinthiformis* had been maintained in these control conditions for 9 mo. The 2 fragments remained visibly healthy and showed signs of growth, demonstrating that control conditions were sufficient to maintain healthy corals. All aquaria were set up in duplicate (pseudo-replication), with 2 to 5 fragments of each species in each aquarium. True replication (i.e. different experimental aquaria, filtration, and acidification system) was not possible due to limited space and resources. However, the treatment assigned to each tank was rotated upon replication. A total of 5 fragments of *D. labyrinthiformis* and 8 fragments of *P. strigosa* were used for each treatment. At the termination of the experiment, skeleton, tissue, and SML samples were collected as described above for the *in situ* study, and stored at −80°C in RNA later® until further processing.

DNA extraction, processing, and analysis

Samples were allowed to thaw on ice, then the entire sample, which included SML, tissue, and skeleton, was placed in a bead beating column from the FastDNA™ Spin Kit for Soil (Qbiogene) and genomic DNA was extracted according to the manufacturer's protocol. DNA was then quantified using the Qubit® 2.0 Fluorometer (Life Technologies) and pooled according to species for each treatment or time point. All pooled genomic DNA samples were diluted to a concentration of 20 ng μl^{-1}. To identify the bacterial population associated with samples, the V4 and V5 region of the 16S rRNA gene was amplified via PCR using primers F563/BSR926 (Claesson et al. 2010). PCR reaction conditions were 1× PCR Buffer, 2.5 mM $MgCl_2$, 0.5 µM of each forward and reverse primer, 0.25 U GoTaq® Hot Start Polymerase (Promega), and 10 ng genomic DNA. The total volume was brought up to 20 µl with DNA-grade sterile water. The PCR amplifications were conducted in a Peltier Thermal Cycler (PTC-200; MJ Research) under the following conditions: 94°C for 8 min; followed by 35 cycles of 94°C for 1 min, 45°C for 1 min, and 72°C for 1 min; with a final extension at 72°C for 10 min. All reactions were run in duplicate, and products verified on a 1.8% Tris-borate-EDTA agarose gel with GelRed™ (Biotium). Total DNA was quantified using the Qubit® 2.0 Fluorometer. Duplicate reactions were pooled and each species and/or treatment was given a separate barcode using the Ion Xpress RNA-Seq Barcode 01-16 Kit (Life Technologies). Each of the 3 time points from *in situ* sampling, and all laboratory samples, were run on a separate Ion 314 Chip v2 (Life Technologies). The Ion Torrent PGM (Life Technologies) performed 200 base-read sequencing using the Ion PGM Sequencing 200 Kit v2 (Life Technologies). Ion Torrent PGM sequences were filtered using Torrent Suite v4.2 software to remove polyclonal and low quality sequences with a Phred-like score less than 15 (Torrent Suite v4.2 software does not directly utilize the Phred scoring system), and .fastq files were generated. All sequence files are available under project ID 12497 on the MG-RAST server.

Data analysis

In situ and laboratory datasets were processed separately to produce 2 separate operational taxonomic unit (OTU) tables. The .fastq files generated by the Ion Torrent server were processed through Quantitative Insights Into Microbial Ecology (QIIME; Caporaso et al. 2010). Sequences were filtered for a minimum length of 100 bp, and checked for chimeras using USEARCH v6.1 (SILVA; Edgar 2010). Sequences that were less than 100 bp or identified as chimeras were removed. Reads sharing 97% sequence similarity were clustered using the open-reference algorithm (Edgar 2010) using the SILVA database as taxonomic assignment (Glöckner et al. 2017). OTUs identifying as 'chloroplast', 'mitochondria', or 'other' at the phylum level were removed.

Considering the wide spread of sequencing depth (see Weiss et al. 2017), each OTU table was rarified independently to the lowest number of reads (23 959 reads for the *in situ* table, and 1053 for the laboratory table) using QIIME. Each OTU table was then exported for further statistical analysis using PRIMER 6 software (Primer-E). Sequence abundances were square-root transformed and clustered using Bray-Curtis similarity matrices. Using PRIMER software, similarity profile (SIMPROF) analysis was performed to identify significantly different communities, non-metric multidimensional scaling (nMDS) plots were created, and the average dispersion (distance from sample type centroid) was calculated for each species using a test for homogeneity of multivariate dispersions (PERMDISP). Each OTU table was then imported into R using the 'Phyloseq' package (McMurdie & Holmes 2013), and alpha diversity statistics were calculated. Then the R package 'DESeq2' (Love et al. 2014) was used for each OTU table in a pairwise fashion to determine OTUs that differed significantly in relative abundance between either coral species (all seasons pooled or all laboratory conditions pooled), or conditions (both species pooled for each OTU table). Additionally, individual OTUs that were present in only 1 species or condition (season or laboratory condition) were identified for further analysis, as well as OTUs present in all samples regardless of species or condition.

RESULTS

Next generation sequencing and alpha diversity

Altogether, 1 466 829 raw sequence reads were obtained, 1 440 516 of which passed the Torrent Suit™ quality control (QC) check, and 339 693 of which passed the minimum read length requirement of 100 base pairs. After QC, the *in situ* samples contained between 23 959 and 70 426 reads, and the laboratory samples contained between 1053 and 22 373 reads (Table 1). OTU tables were rarefied to a uniform sequence depth; 23 959 for the seasonal table and 1053 for the experimental table. Observed OTUs ranged from 633 (summer 2014, *Pseudodiploria strigosa*) to 4954 (summer 2013, *P. strigosa*) for the *in situ* data and 317 (control, *Diploria labyrinthiformis*) to 3730 (temperature treatment, *D. labyrinthiformis*) for the laboratory data. Chao1 diversity estimates were lower on average *in situ* (2171.7) compared to experimental estimates (2301.0), although this was

Table 1. Read abundance, quality statistics, and Chao1 diversity estimates. Each Ion 314 Chip had 2 barcodes for *in situ* data, and 6 barcodes for laboratory data. QC: quality control; QUIME: Quantitative Insights Into Microbial Ecology (Caporaso et al. 2010); OTUs: operational taxonomic units; *H*: Shannon diversity index

Sample	Raw reads (Ion 314)	(QC > 20) (Torrent Suite v4.2)	QIIME after filtering (≥100 bp)	Observed OTUs	Diversity (SE)	*H*
***In situ* field surveys**						
Summer 2013	397187					
Diploria labyrinthiformis		162094	58652	1251	1939 (77)	2.66
Pseudodiploria strigosa		221716	67163	4954	4969 (4)	6.93
Winter	458039					
D. labyrinthiformis		210801	23959	1016	1722 (83)	3.00
P. strigosa		247238	70436	1546	1921 (42)	2.93
Summer 2014	323978					
D. labyrinthiformis		138108	43947	1286	1734 (52)	3.01
P. strigosa		179723	24098	633	745 (22)	3.16
Laboratory study						
D. labyrinthiformis	287625					
Control (25°C, pH 8.2)		6416	1053	317	700 (77)	4.26
Temperature (31°C, pH 8.2)		123660	22373	3730	3949 (23)	6.95
Temp + pH (31°C, pH 7.7)		15772	3562	1342	2393 (105)	6.51
P. strigosa						
Control (25°C, pH 8.2)		58939	12120	2028	2482 (44)	6.21
Temperature (31°C, pH 8.2)		45818	12739	1189	1592 (47)	4.16
Temp + pH (31°C, pH 7.7)		30231	8102	1919	2690 (70)	4.26
Total	1466829	1440516	348204			

not significant (*t*-test, p = 0.43). However, Shannon diversity indices were significantly higher (*t*-test, p = 0.03) for experimental corals, indicating an increased evenness across OTU abundancies (Table 1). *In situ*, average Shannon diversity was also substantially lower for *D. labyrinthiformis* (2.89) compared to *P. strigosa* (4.34). The opposite was true for experimental corals, where Shannon diversity indices were highest in *D. labyrinthiformis* for the 2 treatments with increased temperatures, although the control treatment was not markedly increased (Table 1).

Community similarity, composition, and beta diversity

Two Bray-Curtis similarity matrixes were calculated, one from *in situ* data and the other from laboratory data, and used to create NMDS plots and cluster dendrograms to compare similarity between bacterial communities. All *in situ D. labyrinthiformis* microbial communities clustered closely together with a calculated dispersion of 19.826, compared to the widely dispersed *P. strigosa* (41.409; Fig. 1). No significant differences were detected between winter samples of both species and the *D. labyrinthiformis* community from the 2013 summer sampling (Fig. 1). All other *in situ* communities were significantly different from all other samples (SIMPROF test; $p < 0.05$). As with the *in situ* microbial communities, *D. labyrinthiformis* had a lower calculated dispersion in the manipulative experiments compared to *P. strigosa* (24.614 and 27.722 respectively; Fig. 2). Microbial communities of *P. strigosa* in the control and temperature/acidification treatment were statistically indistinguishable from one another, as were microbial communities of *D. labyrinthiformis* in temperature and temperature + pH conditions (SIMPROF test; $p < 0.05$). Other communities were significantly different from all other samples (Fig. 2).

A total of 110 bacterial classes were detected, 98 in the *in situ* communities, and 90 in laboratory communities. The relative proportions of the most dominant classes are shown in Figs. 3 (*in situ*) & 4 (laboratory). The microbial community of *P. strigosa* during summer 2013 was represented by 90 classes, while all other *in situ* bacterial communities comprised 18 to 37 classes. In the laboratory experiment, the control *D. labyrinthiformis* was only represented by 37 bacterial classes. All remaining laboratory samples contained between 58 and 77 classes.

'DESeq2' results

In total, 'DESeq2' identified 20 OTUs as significantly different, 14 between *in situ* conditions and

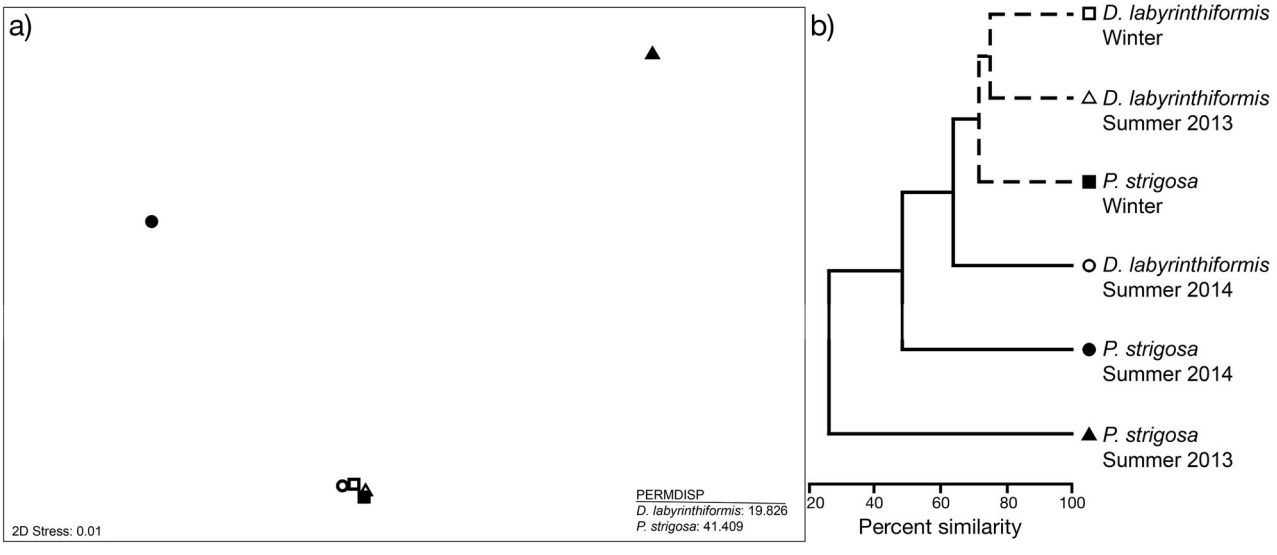

Fig. 1. (a) Non-metric multidimensional scaling (nMDS) plot and (b) cluster dendrogram of *in situ* (summer 2013, winter 2014, and summer 2014) bacterial communities associated with 2 coral species, *Diploria labyrinthiformis* and *Pseudodiploria strigosa*. Clustering based upon a Bray-Curtis dissimilarity matrix, and significantly similar groups calculated using similarity profile (SIMPROF; dashed lines in cluster dendrogram), and dispersion calculated for all seasons for each species using a test for homogeneity of multivariate dispersions (PERMDISP)

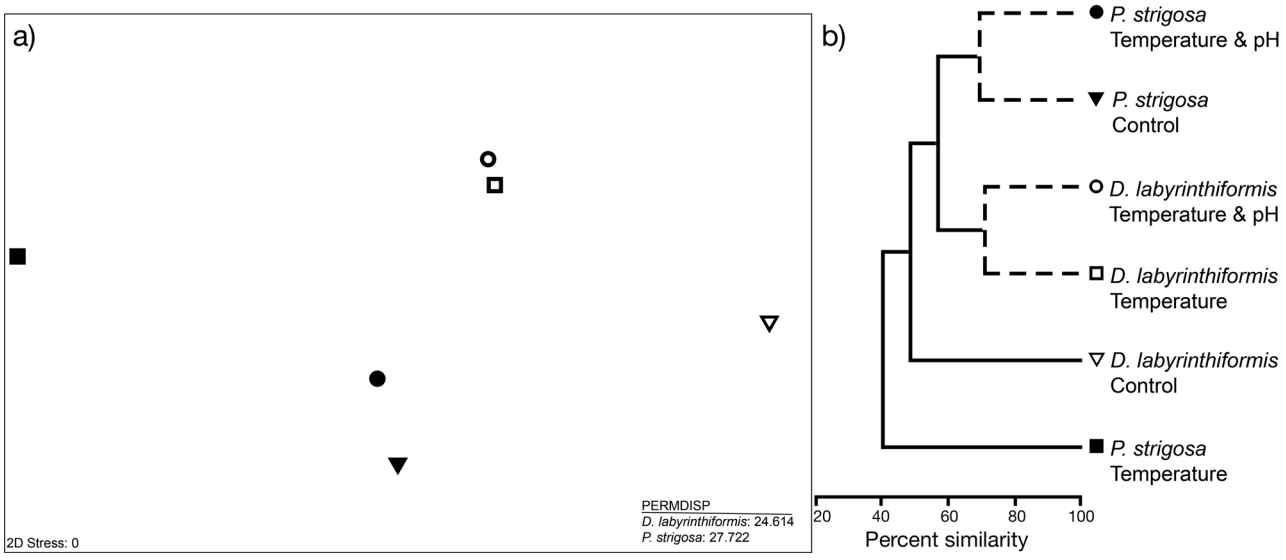

Fig. 2. (a) Non-metric multidimensional scaling (nMDS) plot and (b) cluster dendrogram of laboratory bacterial communities associated with 2 coral species, *Diploria labyrinthiformis* and *Pseudodiploria strigosa*, subjected to 3 treatments: control (25°C, pH 8.2), temperature (31°C, pH 8.2), and temperature + pH (31°C, pH 7.7). Clustering was based upon a Bray-Curtis dissimilarity matrix, significantly similar groups calculated using a similarity profile SIMPROF (dashed lines in cluster dendrogram), and dispersion calculated for all treatments for each species using a test for homogeneity of multivariate dispersions (PERMDISP)

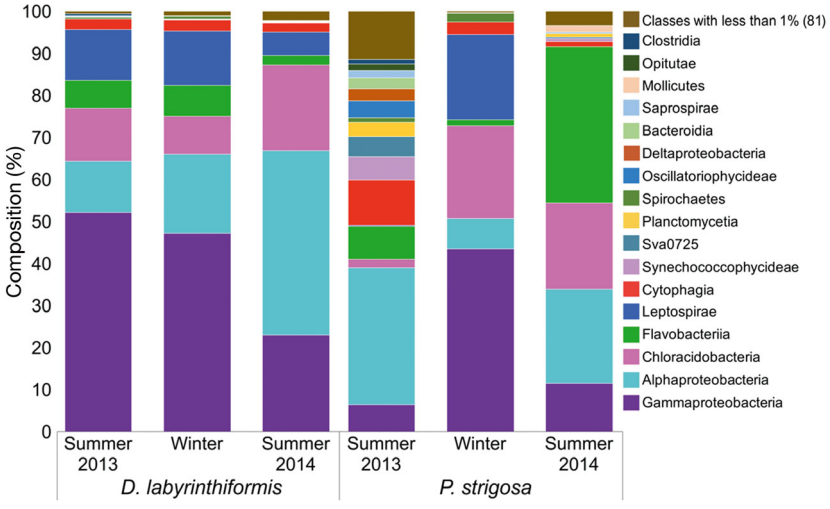

Fig. 3. Percent composition (by class) of the *in situ* bacterial communities associated with 2 coral species, *Diploria labyrinthiformis* and *Pseudodiploria strigosa*, for 3 different seasons (summer 2013, winter 2014, and summer 2014)

6 between laboratory conditions (Table 2). A total of 11 classes were represented in the 20 significantly different OTUs: *Sva0725*, *Bacteroidia*, *Cytophagia*, *Chlorobia*, *Synechococcophycideae*, *Fibrobacteria*, *Clostridia*, *Alphaproteobacteria*, *Gammaproteobacteria*, *Leptospirae*, and *Mollicutes*. *In situ*, the majority (13 out of 14) of significantly different OTUs were more abundant in the summer of 2013 compared to either winter or summer 2014 (Table 2), including OTUs from the genera *Bacteroides* (class *Bacteroidia*), *Inquilinus* (class *Alphaproteobacteria*), and *Hahella* (class *Gammaproteobacteria*). From the laboratory, 4 OTUs were significantly more abundant in increased temperature conditions when compared to the control, including 3 OTUs from *Prosthecochloris* (class *Chlorobia*) and one from *WH1-8* (class *Clostridia*). Two OTUs belonging to the genera *Leptospira* (class *Leptospirae*) and the order *Ucp1540* (class *Fibrobacteria*) were significantly more abundant in *D. labyrinthiformis* compared to *P. strigosa* (all laboratory treatments combined).

Sample type specific OTUs

In the *in situ* data, 29 OTUs were specific to *D. labyrinthiformis*, and only 2 OTUs were specific to *P. strigosa* (Table 3). A total of 161 OTUs were only detected in winter samples, the majority of which were in the 86 OTUs belonging to the order *Chromatiales* (class *Gammaproteobacteria*) and 31 OTUS

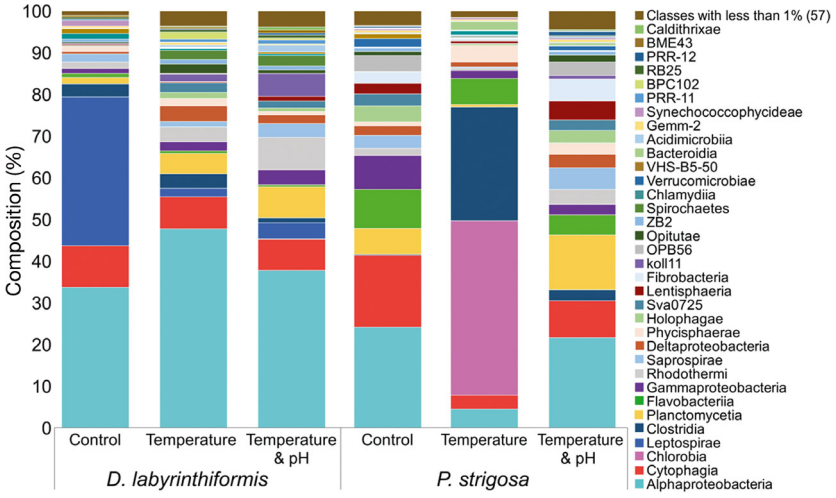

Fig. 4. Percent composition (by class) of the bacterial communities associated with 2 coral species, *Diploria labyrinthiformis* and *Pseudodiploria strigosa*, for 3 different treatments: control (25°C, pH 8.2), temperature (31°C, pH 8.2), and temperature + pH (31°C, pH 7.7)

from the genus *Leptospira* (class *Leptospirae*). Fifty-one OTUs were specific to the summer of 2013, and 29 to the summer of 2014. Thirty OTUs were detected in all species and all seasons, including 12 from the *Amoebophilaceae* family (class *Cytophagia*), 4 from the *Kiloniellales* order (class *Alphaproteobacteria*), and 6 from the order *Chromatiales* (class *Gammaproteobacteria*; Table 3).

From laboratory experiments, 9 OTUs were specific to *D. labyrinthiformis*, including 3 from the genus *Leptospira* (class *Leptospirae*), while 7 OTUs across 5 classes were specific to *P. strigosa* (Table 4). Nine OTUs were only found in control conditions, 4 in increased temperature (3 belonging to the *Planctomycetia* class), and 26 across 13 classes in increased temperature and lowered pH. Five OTUs were detected in all laboratory conditions and species, from the *Rhodothermaceae* (class *Rhodothermi*) and *Amoebophilaceae* families (class *Cytophagia*), *Alphaproteobacteria* class, and *Kiloniellales* order (class *Alphaproteobacteria*).

DISCUSSION

The present study is the first to use high-throughput sequencing to examine seasonal changes in the tropical coral microbial associates of 2 closely related, but differentially resilient, corals. The *in situ* bacterial community of *Pseudodiploria strigosa* was highly dispersed and did not cluster according to summer season (2013 or 2014), or to coral species (Fig. 1). *In situ* microbial communities from *Diploria labyrinthiformis* proved to be less variable and less diverse both in terms of alpha diversity and dispersion. Additionally, microbial communities associated with *D. labyrinthiformis* in summer 2013 were statistically indistinguishable from the winter communities of both species (SIMPROF; $p \geq 0.05$), suggesting that *D. labyrinthiformis* may be more stable in stressed *in situ* conditions relative to *P. strigosa*. Although microbial communities associated with *D. labyrinthiformis* in summer 2014 were significantly different from all others, they did cluster relatively close to the winter samples, supporting this hypothesis (Fig. 1). Bourne et al. (2016) also hypothesized that the stability of a microbiome may vary according to host species, and here we provide the first evidence. The relative stability of the *in situ D. labyrinthiformis* microbiome may also play a role in the lower disease incidence reported for this species relative to *P. strigosa*. In the summer of 2014, a level 1 bleaching alert was issued, indicating these corals were thermally stressed at the time of collection. Shortly after collection the bleaching alert was raised to level 2, and a mass bleaching event occurred. We suggest that bleaching may be predicted prior to the event by specific shifts in the coral microbiota of those corals that have a relatively stable microbiome, as evidenced by the shift seen in *D. labyrinthiformis* in August 2014 prior to a mass bleaching event, although this remains to be proven. Microbial shifts as predictive of coral bleaching have previously been proposed by Bourne et al. (2008).

Differences between the microbial communities of *D. labyrinthiformis* and *P. strigosa* were only detected in the summer samples. The 2 coral species possessed similar bacterial communities during the winter, and were not statistically distinguishable as shown by SIMPROF analysis (Fig. 1). Although corals maintain distinct bacterial communities that vary among coral species (Rohwer et al. 2002), corals within the same genera are highly similar (Littman et al. 2009). Thus, the similarity between the microbial communities of *Diploria* and *Pseudodiploria* may occur for various reasons, including phylogenetic similarity (Budd et al. 2012). Alternatively, similarities could be driven by location (as in Littman et al. 2009) and/or seasonal water column parameters with

Table 2. Pairwise 'DESeq2' results. Rows with identical taxonomy indicate multiple operational taxonomic units (OTUs) within that taxonomic assignment. No OTUs could be identified to species level

Phylum	Class	Order	Family	Genus	Relative abundance		Log_2 fold change	Adj. p-value
					Increased	Decreased		
Acidobacteria	*Sva0725*	*Sva0725*			Summer 2013	Winter	23.08	<0.001
					Summer 2013	Summer 2014	23.46	<0.001
Acidobacteria	*Sva0725*	*Sva0725*			Summer 2013	Winter	24.24	<0.001
					Summer 2013	Summer 2014	24.58	<0.001
Bacteroidetes	*Bacteroidia*	*Bacteroidales*	*Bacteroidaceae*	*Bacteroides*	Summer 2013	Winter	23.42	<0.001
					Summer 2013	Summer 2014	23.61	<0.001
Bacteroidetes	*Cytophagia*	*Cytophagales*	*Flammeovirgaceae*		Summer 2013	Winter	23.32	<0.001
					Summer 2013	Summer 2014	23.34	<0.001
Chlorobi	*Chlorobia*	*Chlorobiales*	*Chlorobiaceae*	*Prosthecochloris*	Temperature	Temp + pH	27.84	<0.001
					Temperature	Control	24.01	<0.001
Chlorobi	*Chlorobia*	*Chlorobiales*	*Chlorobiaceae*	*Prosthecochloris*	Temperature	Control	23.96	<0.001
Chlorobi	*Chlorobia*	*Chlorobiales*	*Chlorobiaceae*	*Prosthecochloris*	Temperature	Control	24.79	<0.001
Cyanobacteria	*Synechococco-phycideae*	*Pseudanabaenales*	*Pseudanabaenaceae*		Summer 2013	Winter	24.66	<0.001
					Summer 2013	Summer 2014	25.13	<0.001
Fibrobacteres	*Fibrobacteria*	*Ucp1540*			*P. strigosa* (laboratory)	*D. labyrinthiformis* (laboratory)	10.00	0.01
Firmicutes	*Clostridia*	*Clostridiales*	*Acidaminobacteraceae*	*WH1-8*	Temperature	Control	22.99	<0.001
Firmicutes	*Clostridia*	*Clostridiales*	*Clostridiaceae*		Summer 2013	Summer 2014	21.69	<0.001
Proteobacteria	*Alphaproteobacteria*	*Rhodospirillales*	*Rhodospirillaceae*	*Inquilinus*	Summer 2013	Winter	22.96	<0.001
Proteobacteria	*Alphaproteobacteria*	*Rhodospirillales*			Summer 2013	Winter	23.19	<0.001
					Summer 2013	Summer 2014	23.45	<0.001
Proteobacteria	*Alphaproteobacteria*	*Rhodospirillales*			Summer 2013	Winter	23.10	<0.001
					Summer 2013	Summer 2014	23.26	<0.001
Proteobacteria	*Alphaproteobacteria*				Summer 2013	Winter	23.93	<0.001
					Summer 2013	Summer 2014	24.31	<0.001
Proteobacteria	*Alphaproteobacteria*				Summer 2013	Winter	22.91	<0.001
					Summer 2013	Summer 2014	23.25	<0.001
Proteobacteria	*Alphaproteobacteria*				Summer 2013	Winter	23.93	<0.001
					Summer 2013	Summer 2014	24.28	<0.001
Proteobacteria	*Gammaproteobacteria*	*Oceanospirillales*	*Hahellaceae*	*Hahella*	Summer 2013	Winter	24.42	<0.001
					Summer 2013	Summer 2014	24.86	<0.001
Spirochaetes	*Leptospirae*	*Leptospirales*	*Leptospiraceae*	*Leptospira*	*D. labyrinthiformis* (laboratory)	*P. strigosa* (laboratory)	10.62	0.01
Tenericutes	*Mollicutes*	*Acholeplasmatales*	*Acholeplasmataceae*	*Acholeplasma*	Summer 2014	Winter	26.52	<0.001
					Summer 2014	Summer 2013	22.70	<0.001

Table 3. Operational taxonomic units (OTUs) only detected in one *in situ* sample type (one species across all seasons, or both species in one season), all summer samples (both coral species), or present in all coral species in all seasons (considered the 'core' microbiome)

Phylum	Class	Order	Family	Genus (species if known)	*D. labyrinthiformis*	*P. strigosa*	Winter	Summer 2013	Summer 2014	Summer 2013 and 2014	All seasons and species
Acidobacteria	*Chloracidobacteria*	*RB41*	*Ellin6075*		1 OTU	1 OTU	7 OTUs		4 OTUs		2 OTUs
Acidobacteria	*DA052*	*Ellin6513*							6 OTUs		
Acidobacteria	*RB25*							1 OTU			
Bacteroidetes	*Rhodothermi*	*Rhodothermales*	*Balneolaceae*	*Balneola*	1 OTU						
Bacteroidetes	*Saprospirae*	*Saprospirales*	*Saprospiraceae*					4 OTUs			
Bacteroidetes	*BME43*							1 OTU			
Bacteroidetes	*Cytophagia*	*Cytophagales*	*Amoebophilaceae*						1 OTU		12 OTUs
Bacteroidetes	*Cytophagia*	*Cytophagales*	*Amoebophilaceae*	*SGUS912*			2 OTU				3 OTUs
Bacteroidetes	*Cytophagia*	*Cytophagales*	*Flammeovirgaceae*					5 OTUs			
Bacteroidetes	*Cytophagia*	*Cytophagales*	*Flammeovirgaceae*	*Fulvivirga*			1 OTU	1 OTU			
Bacteroidetes	*Flavobacteriia*	*Flavobacteriales*	*Cryomorphaceae*		2 OTUs				1 OTU	1 OTU	
Bacteroidetes	*Flavobacteriia*	*Flavobacteriales*	*Flavobacteriaceae*					1 OTU			
Bacteroidetes	*Flavobacteriia*	*Flavobacteriales*	*Flavobacteriaceae*	*Flavobacterium*				1 OTU	2 OTUs		
Cyanobacteria	*Oscillatoriophycideae*	*Oscillatoriales*	*Phormidiaceae*	*Oscillatoria*				2 OTUs			
Cyanobacteria	*Oscillatoriophycideae*	*Chroococcales*	*Xenococcaceae*					1 OTU			
Firmicutes	*Clostridia*	*Clostridiales*	*Clostridiaceae*	*Clostridium*				1 OTU			
Firmicutes	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*Epulopiscium*				2 OTUs			
OD1	*ZB2*							1 OTU			
Proteobacteria	*Alphaproteobacteria*				13 OTUs		16 OTUs	4 OTUs	3 OTUs		
Proteobacteria	*Alphaproteobacteria*	*Kiloniellales*			3 OTUs		1 OTU	1 OTU	1 OTU		4 OTUs
Proteobacteria	*Alphaproteobacteria*	*Rhizobiales*					1 OTU				
Proteobacteria	*Alphaproteobacteria*	*Rhizobiales*	*Phyllobacteriaceae*				1 OTU				
Proteobacteria	*Alphaproteobacteria*	*Rhizobiales*	*Bradyrhizobiaceae*						1 OTU		
Proteobacteria	*Alphaproteobacteria*	*Rhodobacterales*	*Rhodobacteraceae*		2 OTUs		3 OTUs	3 OTUs			1 OTU
Proteobacteria	*Alphaproteobacteria*	*Rhodobacterales*	*Rhodobacteraceae*	*Rhodobacter* (*sphaeroides*)			1 OTU				
Proteobacteria	*Alphaproteobacteria*	*Rhodospirillales*						5 OTU			
Proteobacteria	*Alphaproteobacteria*	*Rhodospirillales*	*Rhodospirillaceae*					1 OTU			
Proteobacteria	*Alphaproteobacteria*	*Rickettsiales*	*Pelagibacteraceae*		1 OTU				2 OTUs		
Proteobacteria	*Betaproteobacteria*	*Burkholderiales*	*Burkholderiaceae*	*Burkholderia* (*tuberum*)					2 OTUs		
Proteobacteria	*Betaproteobacteria*	*Burkholderiales*	*Comamonadaceae*	*Curvibacter*					1 OTU		
Proteobacteria	*Betaproteobacteria*	*Burkholderiales*	*Oxalobacteraceae*	*Ralstonia*					1 OTU		1 OTU
Proteobacteria	*Betaproteobacteria*	*Burkholderiales*	*Comamonadaceae*	*Delftia*							1 OTU
Proteobacteria	*Betaproteobacteria*	*Rhodocyclales*	*Rhodocyclaceae*	*Dechloromonas*			2 OTUs				
Proteobacteria	*Deltaproteobacteria*	*Myxococcales*						2 OTUs			
Proteobacteria	*Deltaproteobacteria*	*NB1-j*	*JTB38*					1 OTU			
Proteobacteria	*Gammaproteobacteria*	*Alteromonadales*	*HTCC2188*					1 OTU			
Proteobacteria	*Gammaproteobacteria*	*Chromatiales*			4 OTUs		86 OTUs		3 OTUs		6 OTUs
Proteobacteria	*Gammaproteobacteria*	*Chromatiales*	*Ectothiorhodospiraceae*					1 OTU			
Proteobacteria	*Gammaproteobacteria*	*Legionellales*	*Francisellaceae*							1 OTU	
Proteobacteria	*Gammaproteobacteria*	*Oceanospirillales*	*Halomonadaceae*	Candidatus *Portiera*			1 OTU				
Proteobacteria	*Gammaproteobacteria*	*Pseudomonadales*	*Pseudomonadaceae*	*Pseudomonas*					1 OTU		
Proteobacteria	*Gammaproteobacteria*	*Thiotrichales*	*Piscirickettsiaceae*			1 OTU					
Proteobacteria	*Gammaproteobacteria*	*Vibrionales*	*Vibrionaceae*	*Vibrio* (*shilonii*)				1 OTU			
Spirochaetes	*Leptospirae*	*Leptospirales*	*Leptospiraceae*	*Leptospira*	2 OTUs		31 OTUs				
Spirochaetes	*Spirochaetes*	*Spirochaetales*	*Spirochaetaceae*				8 OTUs	1 OTU			
Verrucomicrobia	*Pedosphaerae*	*Pedosphaerales*						5 OTUs			
Verrucomicrobia	*Opitutae*	*Puniceicoccales*	*Puniceicoccaceae*	*Coraliomargarita*				3 OTUs			
ZB3	*BS119*							1 OTU			

Table 4. Operational taxonomic units (OTUs) only detected in one sample type (one species across all laboratory conditions, or both species in one laboratory condition), both elevated temperature conditions (both coral species), or present in all coral species in all conditions (considered the 'core' microbiome)

Phylum	Class	Order	Family	Genus	*D. labyrinthiformis*	*P. strigosa*	Control (25°C, pH 8.2)	Temp (31°C, pH 8.2)	Temp +pH (31°C, pH 7.7)	All elevated temps (31°C, pH 7.7 + 8.2)	All conditions and species
Bacteroidetes	*Rhodothermi*	*Rhodothermales*	*Balneolaceae*	*Balneola*					1 OTU		
Bacteroidetes	*Rhodothermi*	*Rhodothermales*	*Rhodothermaceae*		1 OTU				3 OTUs	1 OTU	1 OTU
Bacteroidetes	*Saprospirae*	*Saprospirales*	*Saprospiraceae*						1 OTU		
Bacteroidetes	*BME43*				1 OTU						
Bacteroidetes	*Cytophagia*	*Cytophagales*	*Flammeovirgaceae*		3 OTUs						
Bacteroidetes	*Cytophagia*	*Cytophagales*	*Amoebophilaceae*				1 OTU				1 OTU
Bacteroidetes	*Cytophagia*	*Cytophagales*	*Amoebophilaceae*	*Ucs1325*			1 OTU				
Bacteroidetes	*Flavobacteriia*	*Flavobacteriales*	*Flavobacteriaceae*			1 OTU	1 OTU				
Cyanobacteria						1 OTU					
Fibrobacteres	*Fibrobacteria*	*Ucp1540*				1 OTU					
Gemmatimonadetes	*Gemm-2*						1 OTU				
Lentisphaerae	*Lentisphaeria*	*Lentisphaerales*	*Arctic95B-10*			1 OTU			4 OTUs		
OD1	*ZB2*						1 OTU				
OP3	*PBS-25*								1 OTU		
Planctomycetes	*C6*	*d113*							1 OTU		
Planctomycetes	*Phycisphaerae*	*Phycisphaerales*							1 OTU		
Planctomycetes	*Phycisphaerae*	*Phycisphaerales*	*Phycisphaeraceae*				1 OTU				
Planctomycetes	*Pla3*								1 OTU		
Planctomycetes	*Planctomycetia*	*Pirellulales*	*Pirellulaceae*					3 OTUs	2 OTUs		
Proteobacteria	*Alphaproteobacteria*				1 OTU		1 OTU	1 OTU	2 OTUs	2 OTUs	1 OTU
Proteobacteria	*Alphaproteobacteria*	*Kiloniellales*				2 OTUs			1 OTU		2 OTUs
Proteobacteria	*Alphaproteobacteria*	*Rhodospirillales*							1 OTU		
Proteobacteria	*Alphaproteobacteria*	*Rhodospirillales*	*Rhodospirillaceae*				2 OTUs		1 OTU		
Proteobacteria	*Deltaproteobacteria*					1 OTU			1 OTU		
Spirochaetes	*Leptospirae*	*Leptospirales*	*Leptospiraceae*	*Leptonema*					1 OTU		
Spirochaetes	*Leptospirae*	*Leptospirales*	*Leptospiraceae*	*Leptospira*	3 OTUs						
Spirochaetes	*Spirochaetes*	*Spirochaetales*	*Spirochaetaceae*						1 OTU		
Verrucomicrobia	*Verruco-5*	*SS1-B-03-39*							3 OTUs		

stress in the summer playing a greater role in differentiating the microbiome composition than in the winter.

SIMPROF analysis of all laboratory treatments of both coral species revealed no clear pattern, including no significant differences detected between the control (25°C) and elevated temperature/acidification treatment for *P. strigosa* as well as between the control and temperature treatments for *D. labyrinthiformis* (Fig. 2), indicating possible clustering by coral species. The coral-associated bacterial community shifts dramatically when placed in aquaria, and requires a 2 wk acclimation period to stabilize (Kooperman et al. 2007, Pratte et al. 2015). Although coral microbial communities were allowed to stabilize for 2 wk (see Pratte et al. 2015), we cannot definitively rule out tank effect as a possible cause for the lack of clear clustering patterns.

With the exception of summer samples from *P. strigosa* in 2013, *in situ* Shannon diversity indices were similar to those previously reported for *Montastraea annularis* (Barott et al. 2009), Pacific *Porites* sp. (Pratte et al. 2018), *Montastraea faveolata*, and were slightly higher than *Porites asteroides* (Morrow et al. 2012; Table 1). In addition to an elevated Chao1 estimate, *P. strigosa* communities from the 2013 summer also had a relative increase in *Alphaproteobacteria* compared to the winter and summer of 2014 (Fig. 3). Increases in alpha diversity, dispersion, and *Alphaproteobacteria* can be an indication of stress and disease (Sekar et al. 2008, Sunagawa et al. 2009, Zaneveld et al. 2016, Pratte et al. 2018). The elevated diversity metrics and *Alphaproteobacteria* detected in *P. strigosa*

communities from summer 2013 may be representative of a subclinical infection or decline in overall health, despite the colonies being visually healthy (Reed et al. 2010). As such, data from *P. strigosa* colonies in the summer of 2013 were interpreted with caution. These results also indicate the need to acquire multiple time points when assessing the coral microbial community, to be able to identify anomalies such as those described here.

Seasonal shifts could be seen at the class taxonomic level (Fig. 3), which may be a result of changes in environmental conditions, a change in the bacterial source (i.e. water column), or changes in coral physiology or mucus composition leading to changes in the bacterial community. Typically, shifts in the coral microbiota are associated with bleaching or disease, and are known to correlate with relatively warmer water temperatures (Pantos & Bythell 2006, Bourne et al. 2008), and previous seasonal shifts in coral microbial communities have been described (Chen et al. 2011, Chiu et al. 2012, Ceh et al. 2012, Li et al. 2014, Sharp et al. 2017). These community shifts may be a normal component of a healthy coral microbiome, such as the seasonal shifts shown in the maple sap microbiota (Filteau et al. 2010), rhizosphere microbiota (Gomes et al. 2001), and high mountain lake microbiota (Pernthaler et al. 1998). Seasonal microbial evaluation of 3 sponges revealed that although the core microbiota remained relatively stable, minor shifts were detected in microbes present in lower abundance (Erwin et al. 2012). Seasonal patterns in the microbiota associated with several different coral species have also been shown (Chiu et al. 2012, Ceh et al. 2012). As with this study, Ceh et al. (2012) demonstrated strong seasonal clustering rather than clustering by coral species, although Chiu et al. (2012) saw an increase in *Alphaproteobacteria* in the colder seasons, which did not occur in the present study.

Next, patterns in the presence or absence of OTUs across seasons, species, and treatments were investigated. The summer microbiomes of *P. strigosa* and *D. labyrinthiformis* were not enriched in any particular taxa and contained more OTUs at lower abundance that were not detected in winter samples (Table 3). Examining OTUs only detected in one species (but detected in ALL seasons and years), 29 OTUs were detected in all *D. labyrinthiformis* (and not *P. strigosa*) while only 2 OTUs were detected in only *P. strigosa* samples. OTUs only detected in *D. labyrinthiformis* include 4 in the *Chromatiales* order, and 2 in the genus *Leptospira*. Both *P. strigosa* and *D. labyrinthiformis* were comparatively enriched in OTUs from the order *Chromatiales* (class *Gammaproteobacteria*) in the winter (Table 3), and OTUs from the genus *Leptospira* (class *Leptospirae*) tended to be absent in the summers. A relative increase of *Leptospira* in the winter microbiota of gorgonians has also been reported (van de Water et al. 2018). Although *Leptospira* are commonly associated with disease, non-pathogenic saprophytic species exists, and have been detected previously in healthy organisms such as jellyfish (Cleary et al. 2016), the coral *Balanophyllia europaea* (Meron et al. 2011), and gorgonians (*Eunicella* spp., *Corallium rubrum*; van de Water et al. 2018). The functional role *Leptospira* play in the anthozoid microbiome has yet to be determined.

The majority of OTUs (13 of 14) identified as significantly different by 'DESeq2' were between the summers of 2013 and 2014, likely because of the highly diverse *P. strigosa* 2013 summer sampling. However, the mollicute *Acholeplasma* was significantly increased in both coral species in the summer of 2014 compared to the summer of 2013. *Acholeplasma* have been detected previously in deep water corals (Kellogg et al. 2009), and are thought to be symbionts of some marine bryozoans (Boyle et al. 1987).

Overall, communities in laboratory aquaria had higher Chao1 estimates (Table1) and a higher relative abundance of *Alphaproteobacteria* compared to those communities seen in the *in situ* samples (Figs. 3 & 4), and were more similar to those previously published by Sunagawa et al. (2009). pH had no apparent effect on the bacterial communities of either coral species, with the exception of one OTU, *Prosthecochloris* in the *Chlorobia* class (Table 2). The increase in *Prosthecochloris* in acidified conditions is unsurprising, considering optimal pH growth conditions for other members of the genus is 6.8 (Keppen et al. 2008). In similar studies with decreased pH, an increase in *Alphaproteobacteria*, *Vibrionaceae*, and *Alteromonadaceae* was documented when *Acropora eurystoma* was subjected to a pH of 7.3 (Meron et al. 2011), and an increase in *Bacteroidetes*, *Chlorobi*, *Cyanobacteria*, and *Spirochaetes* was seen in *Porites compressa* (Vega Thurber et al. 2009). However, only a shift in one *Chlorobia* OTU was detected in the present study, possibly because of differences in the levels of acidification (7.7 in the current study vs. 7.3 and 6.8 in the above-mentioned studies). It is also possible that subtle shifts in the microbiota occurred that were too small to be detected or below the cut-off value of number of reads (1053) utilized in the laboratory component of this study.

While the overall bacterial communities associated with each coral species in summer 2013 and summer

2014 were largely dissimilar (Fig. 1), there were some similarities. A 'core' of 30 OTUs present in all seasons and species was detected, including members from the classes *Gammaproteobacteria*, *Betaproteobacteria*, *Alphaproteobacteria*, *Cytophagia*, and *chloracidobacteria* (Table 3). One of these OTUs belongs to the genus *Ralstonia* (*Betaproteobacteria*), which has been previously described as a ubiquitous core microbial member of several coral species and is thought to be an endosymbiotic bacterium associated with zooxanthellae (Ainsworth et al. 2015). Both laboratory and *in situ* corals contained core OTUs from the *Chromatiales* order (*Gammaproteobacteria*), *Amoebophilaceae* family (*Cytophagia*), and *Kiloniellales* family (*Alphaproteobacteria*), indicating that these taxa may be a critical component to *Diploria* and *Pseudodiploria* health.

In total, 13 OTUs associated with the family *Endozoicimonaceae* (class *Gammaproteobacteria*) were detected in the *in situ* data, and in all but one case, each OTU was only detected in one season and species (data not shown). No *Endozoicimonaceae* were detected in the either species in the winter, nor in the summer 2013 samples for *P. strigosa*, and was most abundant in *P. strigosa* 2014 samples (0.7%). Interestingly, *Endozoicimonaceae*, a family that is typically in high relative abundance (often greater than 50%) and associated with healthy corals (Bayer et al. 2013, Neave et al. 2017, Pratte et al. 2018), was not detected in any winter samples, a time period in which disease prevalence is lowest (Zvuloni et al. 2009, Heron et al. 2010). Similarly, no reads from the *Endozoicimonaceae* family were detected in any of the laboratory conditions. As such, it can be concluded that differences in disease susceptibility is not a direct result of differences in relative abundance of beneficial *Endocoicimonaceae*.

The genus *Vibrio* is commonly associated with coral disease, and tends to increase in abundance either from primary infection (Kushmaro et al. 2001, Ben-Haim et al. 2003) or with bleaching (Bourne et al. 2008) and in the summer (Koren & Rosenberg 2006). Members of the *Vibrio* genus have also been associated with apparently healthy coral microbial communities (Ritchie 2006, Littman et al. 2011). It is undisputed that vibrios play crucial roles in coral health, in particular in bleaching, and were therefore examined in the present study. *Vibrio* spp. were not present in high abundance (<3%) in any of the laboratory treatments, while all other *in situ* samples contained <0.05% *Vibrio.* An OTU similar to *V. shilonii*, a known coral pathogen, was present in both *P. strigosa* and *D. labyrinthiformis* in summer 2013. However, only a single read was detected in each of these samples, which is not indicative of an infection. Similarly, only 2 reads in the genus *Vibrio* were detected in the entirety of the laboratory data, both associated with *P. strigosa* in the elevated temperature treatment. Vega Thurber et al. (2009) did not detect a significant shift in *Vibrio* abundance in bleached corals, although it should be considered that expression of virulence genes may increase without an increase in the overall population (Vega Thurber et al. 2009). The relative lack of *Vibrio* reads detected in this study indicate that the comparative susceptibility of *D. labyrinthiformis* to bleaching in comparison to *P. strigosa* is not likely the result of higher *Vibrio* populations associated with the species.

The present study demonstrates that corals harbor dynamic microbiomes. Our results suggest that winter coral-associated bacterial communities may be more similar between species, while the summer bacterial communities are more variable. This variability may potentially be linked to observed differential resistance to the stress of warm summertime temperatures (Pratte & Richardson 2014). The *P. strigosa* microbiome was more variable and less specific (in terms of OTUs) than the *D. labyrinthiformis* microbiome, whether by mucus composition, coral exudates, other residential microbes, or another yet unpredicted mechanism. This variability and relative lack of specific OTUs may contribute to *P. strigosa*'s comparative susceptibility to disease on the Florida Keys reef tract.

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