Received: 30 October 2018 | Revised: 11 May 2020 | Accepted: 26 May 2020

DOI: 10.1111/mec.15497

ORIGINAL ARTICLE

MOLECULAR ECOLOGY WILEY

Patterns of environmental variability influence coral-associated bacterial and algal communities on the Mesoamerican Barrier Reef

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Funding information
UNC Dissertation Completion Fellowship; U.S. Department of Defense; Simmons Family Foundation; Carl Tryggers Foundation; Division of Ocean Sciences, Grant/Award Number: 1459522; Rufford Foundation, Grant/Award Number: 15802-1

Abstract

A coral's capacity to alter its microbial symbionts may enhance its fitness in the face of climate change. Recent work predicts exposure to high environmental variability may increase coral resilience and adaptability to future climate conditions. However, how this heightened environmental variability impacts coral-associated microbial communities remains largely unexplored. Here, we examined the bacterial and algal symbionts associated with two coral species of the genus *Siderastrea* with distinct life history strategies from three reef sites on the Belize Mesoamerican Barrier Reef System with low or high environmental variability. Our results reveal bacterial community structure, as well as alpha- and beta-diversity patterns, vary by host species. Differences in bacterial communities between host species were partially explained by high abundance of *Deltaproteobacteria* and *Rhodospirillales* and high bacterial diversity in *Siderastrea radians*. Our findings also suggest *Siderastrea* spp. have dynamic core bacterial communities that probably drive differences observed in the entire bacterial community, which may play a critical role in rapid acclimatization to environmental change. Unlike the bacterial community, *Symbiodiniaceae* composition was only distinct between host species at high thermal variability sites, suggesting that different factors shape bacterial versus algal communities within the coral holobiont. Our findings shed light on how domain-specific shifts in dynamic microbiomes may allow for unique methods of enhanced host fitness.

KEYWORDS

community assembly, DNA barcoding, ecological genomics, *Siderastrea*, symbioses

1 | INTRODUCTION

The coral holobiont is comprised of the coral animal and its phylogenetically diverse symbiotic partners including algae, bacteria, archaea, fungi, viruses, and protists (Rohwer, Seguritan, Azam, & Knowlton, 2002). Coral-associated bacterial communities are incredibly diverse (Huggett & Apprill, 2018; Morrow, Moss, Chadwick, & Liles, 2012; Rohwer, Breitbart, Jara, Azam, & Knowlton, 2001; Sharp, Pratte, Kerwin, Rotjan, & Stewart, 2017; Sunagawa, Woodley, & Medina, 2010) and may play essential roles throughout the coral's life cycle, as different coral life stages correspond with variations in bacterial community structure stability (Apprill, Marlow, Martindale, & Rappé, 2009; Littman, Willis, Pfeffer, & Bourne, 2009; Sharp, Distel, & Paul, 2012; Thompson, Rivera, Closek, & Medina, 2014; Williams, Brown, Putchim, & Sweet, 2015). Bacterial symbionts are involved in sulphur and nitrogen cycling (Rädecker, Pogoreutz,

Voolstra, Wiedenmann, & Wild, 2015; Raina, Tapiolas, Willis, & Bourne, 2009), and coral heterotrophy (Bourne, Morrow, & Webster, 2016). They also prevent disease by occupying niches that could otherwise be colonized by opportunistic pathogens, as well as by producing antimicrobials (Ritchie, 2006; Rypien, Ward, & Azam, 2010), and acting as a pseudoadaptive immune system for the entire holobiont (Palmer, 2018). Disease and reduced fitness often result when coral-bacterial associations break down due to biotic or abiotic stressors (Hughes et al., 2003; Sogin, Putnam, Nelson, Anderson, & Gates, 2017). Corals also maintain important relationships with photosynthetic algae (family *Symbiodiniaceae*; LaJeunesse et al., 2018), which provide their hosts with photosynthates—a source of nutrients and energy in otherwise oligotrophic tropical waters (Sogin et al., 2017; Thornhill, Howells, Wham, Steury, & Santos, 2017). Different genera within *Symbiodiniaceae* display a range of stress tolerances, growth rates, and either heat or cold tolerances (Berkelmans & Van Oppen, 2006; Hume et al., 2015; Little, Van Oppen, & Willis, 2004; Thornhill et al., 2008), and studies suggest the composition of coral-algal communities may shift in response to environmental stress (Thornhill et al., 2017).

Host-associated communities are not always stable and can exhibit compositional shifts in response to environmental changes (Leggat et al., 2011; Tanner, Hughes, & Connell, 1996). Dynamic microbiomes may increase the ability of the eukaryotic host to acclimatize to environmental stressors in weeks as opposed to relying on adaptation through genetic changes, which occurs over many generations (Reshef, Koren, Loya, Zilber-Rosenberg, & Rosenberg, 2006). Reef-building coral bacterial community composition (BCC) can quickly change in response to many factors including disease (Cooney et al., 2002; Sunagawa et al., 2009), the identity of major benthic functional groups such as other corals and sponges (Kelly et al., 2014), pH change (Meron et al., 2011; Thurber et al., 2009), and temperature change—linked to seasons or otherwise (Gajigan, Diaz, & Conaco, 2017; Reshef et al., 2006; Sharp et al., 2017; Thurber et al., 2009). Other research has demonstrated that for some corals, bacterial community structure is resistant to short-term temperature increases, yet sensitive to prolonged exposure to elevated temperatures (Gajigan et al., 2017). Because bacterial communities can quickly change, their functions may be equally dynamic (Ainsworth, Thurber, & Gates, 2010). Thus, bacterial community compositional changes may serve as a pseudoadaptive immune system in response to stressors and environmental changes (Palmer, 2018; Reshef et al., 2006) and may be the key to corals' continued survival (Bourne et al., 2009).

Anthropogenic warming caused by increased greenhouse gas emissions poses a significant threat to coral reef health and function (Donner, Skirving, Little, Oppenheimer, & Hoegh-Guldberg, 2005; Hughes et al., 2003). However, recent work suggests exposure to moderate degrees of thermal variability, which is predicted to increase under future climate change scenarios, may help corals adapt to future climate conditions (Barshis et al., 2013; Oliver & Palumbi, 2011; Soto, Muller Karger, Hallock, & Hu, 2011). Reef building corals are able to survive across environments with unique local thermal regimes that have been shown to influence their thermal tolerance (Barshis et al., 2013; Oliver & Palumbi, 2011; Soto et al., 2011). Several studies have demonstrated a coral's tolerance to future temperature stress correlates with environmental variability of their natal habitat on both daily and annual scales (Oliver & Palumbi, 2011; Soto et al., 2011). For example, corals experiencing moderate environmental variability may have increased resilience relative to those exposed to high environmental variability (Soto et al., 2011). Additionally, short- or long-term exposure to local environmental variability may result in species-specific adaptation and acclimatization, that may increase the capacity for corals to survive future climate scenarios (Oliver & Palumbi, 2011). In light of future climate conditions, the impact of environmental variability on the structure of coral's symbiotic communities may influence corals' capacity to either adapt or succumb to climate change, and therefore warrants further examination.

Here, we investigate the relative importance of coral host species and environmental variability in shaping coral-associated BCC and the algal component of the coral holobiont. We collected coral samples from three reef sites on the Belize Mesoamerican Barrier Reef System (MBRS). These sites were characterized as exhibiting either high (Placencia and Sapodilla inshore) or low (Placencia offshore) environmental variability, based on a previous assessment of thermal and nutrient variability across the Belize MBRS (Baumann et al., 2016). Samples were retrieved from two corals with distinct life history strategies: *Siderastrea siderea*, a massive, stress-tolerant reef-builder and *Siderastrea radians*, a weedy short-lived congener (Darling et al., 2012). Analysis of 16S rRNA amplicons revealed that *Siderastrea* spp. microbial symbionts are structured by deterministic forces. In particular, alpha and beta diversity, the core bacterial community, and algal diversity exhibit differences between coral host species of the same genus. We propose that the mode with which corals respond to environmental variability is microbial partner-specific. These findings further our understanding of how components of the coral microbiome are shaped by environmental variability and shed light on the manner in which corals may adapt to future environmental conditions.

2 | MATERIALS AND METHODS

2.1 | Site selection and sample collection

To determine how environmental variability affects coral BCC, we collected coral tissue samples from sites along the MBRS. All corals sampled were in approximately three to five meters of water. Coral tissue samples of approximately two to four cm diameter were collected from the outer edge of each coral using a hammer and chisel. Coral tissue samples were collected from corals with no visible signs of bleaching or disease. We collected samples from three sites along the Belize MBRS (Table S1; Figure S1) where differences in coral species diversity and richness were previously observed (Baumann et al., 2016). Two of these sites, Placencia inshore and Sapodilla

inshore, showed high annual temperature variation, annual maximum temperatures, annual chlorophyll-a concentrations, and frequent exposure to temperatures above the regional bleaching threshold of 29.7°C (Aronson, Macintyre, Precht, Murdoch, & Wapnick, 2002; Baumann et al., 2016). The third site, Placencia offshore, was farther from shore and showed less overall thermal variability than the other two sites (Aronson et al., 2002; Baumann et al., 2016). All three sites were populated with large, mounding, stress tolerant corals, including *Siderastrea siderea* and *Pseudodiploria strigosa*, and smaller weedy coral species, such as *Siderastrea radians* and *Porites asteroides* (Baumann et al., 2016; Darling et al., 2012). At each site, coral tissue samples were collected from each of two coral species (*S. siderea* and *S. radians*; Table S1) and placed immediately on ice, preserved in 96% ethanol, stored at −20°C, and transported to the University of North Carolina at Chapel Hill. Previous work in other systems has shown preservation of coral samples in ethanol rather than flash-freezing can result in alterations in coral BCC (Rocha, Coelho, Peixe, Gomes, & Calado, 2014); however, because all samples from this study were treated with the same methodology, they can be directly compared. Additional sample collection details have been previously described (Baumann, Davies, Aichelman, & Castillo, 2018).

2.2 | DNA extraction, PCR amplification and metabarcoding

Coral holobiont DNA was isolated from each sample following a modified phenol-chloroform method (Davies et al., 2013). Briefly, DNA was isolated by immersing the tissue in digest buffer (100 mM NaCl, 10 mM Tris-Cl pH 8.0, 25 mM EDTA pH 9.0, 0.5% SDS, 0.1 mg/ml Proteinase K, and 1 μg/ml RNase A) for 1 hr at 42°C followed by a standard phenol-chloroform extraction (Chomczynski & Sacchi, 2006). Isolated DNA was confirmed on agarose gels and quantified using a Nanodrop 2000 Spectrophotometer (Thermo Scientific). The bacterial component of the coral holobiont was examined by amplifying a 444 bp section of the V3–V4 hypervariable regions of the 16S gene for each sample using primers modified from another study (Klindworth et al., 2013). Negative controls were included for each PCR to ensure PCR reagents contained minimal contaminant DNA. The algal component of the holobiont was examined by amplifying the ITS-2 locus; a detailed description of gene amplification, Illumina adapter construction, and the bioinformatics pipeline for the algal community has been described in previous work (Baumann et al., 2018).

Samples were submitted for sequencing to the University of North Carolina at Chapel Hill High Throughput Sequencing Facility, and sequenced in one run using the Illumina MiSeq platform. The forward primer (Bact-0341-F) was 5′-TCGTCGGCAGCGTC + *AGATG TGTATAAGAGACAG* +NNNN + **CCTACGGGNGGCWGCAG**-3′ where the 5′-universal linker is underlined, spacer sequences are italicized, degenerative bases are indicated by N's, and primer is bolded. The reverse primer (Bact-0785-R) was 5′-GTCTCGTGGGCTCGG + *AGA TGT GTA TAA GAG ACAG* NNNN + **GACTACHVGGGTATCTAATCC**-3′.

2.3 | Bacterial community bioinformatics pipeline

The bioinformatics pipeline incorporated a previous metabarcoding data analysis pipeline (Green, Davies, Matz, & Medina, 2014) and used the Quantitative Insights Into Microbial Ecology (QIIME) Illumina workflow (Caporaso et al., 2010). Barcodes were trimmed, and all forward and reverse reads were concatenated into two files having a mean sequence length and standard deviation of 250 bp ± 3, and 249 bp ± 4 for forward and reverse reads, respectively. Because only 10% of reads had overlap between forward and reverse reads, the paired end sequences were not joined; instead, only the forward sequences were examined in this analysis. This analysis was carried out with both a 97% and 99% similarity cutoff for identification of operational taxonomic units (OTUs); however, no significant differences were observed between the two analyses. Therefore, only the analysis using 97% similarity is shown. OTUs were picked using the pick_open_reference_otu.py command in the QIIME software package, selecting uclust as the OTU picking method and Greengenes version 13_8 (DeSantis et al., 2006) as the OTU reference database for assigning taxonomy. Our analysis produced 2,483,469 raw reads, of which 2,291,920 (92.8%) clustered to 59,453 total OTUs at 97% sequence identity (Tables S2 and S3).

2.4 | Statistical analysis

Data were analysed using several descriptive and statistical methods using the QIIME package (Caporaso et al., 2010) and the MCMC. OTU package in R. OTU abundance analysis used the R package MCMC.OTU (Green et al., 2014). OTUs that were classified as nonbacterial (Unassigned, Archaea, Chloroplast, Mitochondria) were removed. OTUs belonging to taxa commonly associated with human or reagent "contaminants" (*Staphylococcus*, *Corynebacterium*, *Propionibacterium*, and *Streptococcus*; Salter et al., 2014) made up less than 0.29% of OTUs in the data set and were consistently present across all samples. Outlier samples with low sequence coverage (total log counts ≥2.5 standard deviations below the mean of all samples) were identified and also removed ($n = 1$). Because the number of reads per sample for the remaining 63 samples ranged from 1,103 to 272,211, the data set was rarefied to permit alpha diversity comparisons. The data set was rarefied at 11,000 sequences per sample, and 14 samples were discarded due to low sequence counts. After rarefaction, the data set contained 40,343 OTUs at the 97% sequence similarity cutoff (Tables S2 and S3). The downstream analyses described below were performed on the remaining 49 samples, with at least four representatives from each coral host species (*S. radians* and *S. siderea*) at each site.

Next, Shannon-Wiener index (H′) and species richness—presented here as observed OTUs (OTUs)—were calculated to determine alpha diversity (Shannon, Weaver, & Burks, 1951). The vegan package in R was used to perform principal coordinates analysis (PCoA) using the Bray-Curtis-based dissimilarity index, PerMANOVA using the Adonis

function, and beta-diversity using the permutest.betadisper function (Oksanen et al., 2013). PCoA and PerMANOVA were performed on: (a) the unrarefied bacterial data set; (b) bacterial data set rarefied at 11,000 sequences per sample; (c) core bacterial community; and (d) algal data set. A contingency table that was generated by comparing average OTU frequencies between sampling groups using a student's *t* test and corrected for multiple comparisons using the Benjamini–Hochberg procedure (Benjamini & Hochberg, 1995) was used to generate volcano plots. Differential abundance analysis was used to identify the taxa driving the dissimilarity between BCC of the two coral host species and visualized as volcano plots (Figure 3), as described previously (Speare, Smith, Salvato, Kleiner, & Septer, 2020). Finally, the core microbiome (OTU present in at least 70% of samples) was identified for each host species from each site (six cores total) using the compute_core_microbiome.py command in the QIIME software package (Ainsworth et al., 2015; Hernandez-Agreda, Leggat, Bongaerts, & Ainsworth, 2016).

2.5 | Nearest taxon index (NTI)

To investigate the phylogenetic structure of bacterial communities, we calculated the nearest taxon index (NTI) based on the mean nearest taxon distance (MNTD = −NTI) and compared these to null model communities (Stegen, Lin, Konopka, & Fredrickson, 2012). Phylogenetic distances were calculated from the FastTree output in the QIIME analysis using the "cophenetic" distance function in R. As calculating distances for the entire rarefied data set (11,000 sequences per sample) is computationally intensive, the data set was further rarefied to 2,000 sequences per sample for the NTI analysis. The ses.mntd function in the R package Picante was used to calculate NTI and its standardized effect size compared to null models, created with the abundance-weighted taxa.labels algorithm and iterated over 999 permutations to obtain statistical significance values (Kembel, 2010). Bacterial communities characterized by NTI values above +2 or below −2 and with $p < .05$ indicate phylogenetic clustering or phylogenetic overdispersion, respectively, compared to expectations by chance (null models). NTI values that deviate from null model (stochastic) expectations suggest deterministic community assembly processes. The deterministic interpretations of phylogenetic clustering, in particular, are based on expectations that closely-related taxa occupy similar ecological niches, and that strong environmental filtering should result in phylogenetically clustered communities (Stegen et al., 2012; Webb, Ackerly, McPeek, & Donoghue, 2002).

3 | RESULTS

3.1 | Host species and other deterministic factors influence coral bacterial community composition

Principal coordinates analysis (PCoA) performed on both the unrarefied and rarefied bacterial data sets revealed BCC was influenced by host species (Figures 1a and S2a, ANOVA: $p < .001$) and site (Figures 1c and S2b, ANOVA: $p < .02$), although host species accounted for a notably larger percentage of the variation in BCC (Table S4). Within-host beta diversity of bacterial communities also differed between host species, with *Siderastrea radians* samples showing lower β-diversity than *Siderastrea siderea* samples (Table S4, permutest: $p < .037$); beta diversity did not differ between sites. This same trend was observed for the unrarefied data set (Table S4). *S. radians* communities had statistically higher richness and Shannon Index (H′) values relative to *S. siderea* (Figure 1b). These data indicate that *S. radians* and *S. siderea* communities differed in community composition, alpha diversity, and within-host beta diversity. Alpha and beta diversity measures were not statistically different between sites (Figure 1d; Table S4).

Within each site, BCC was distinct between host species (Figures S2e–g, S3a, ANOVA: $p < .02$), and for each coral host, BCC exhibited distinct patterns across sites. For *S. radians*, BCC from Placencia offshore was distinct from those at the other sites (Table S4; Figure S3b, ANOVA: $p < .006$). For *S. siderea*, BCC from Sapodilla inshore was distinct from those at the Placencia sites (Table S4; Figure S3b, ANOVA: $p < .004$). However, these differences explain less than 11% of the variation in the data set (Table S4).

Given that host species and site explained only a small percentage of the variation in the data set, other factors probably structured BCC. To determine whether these structuring forces were deterministic we conducted an analysis of the NTI. For all bacterial communities analysed and irrespective of host species, NTI values were >+2 and $p \leq .001$ (Figure 2), indicating phylogenetic clustering compared to expectations by randomly-assembled (stochastic) null model communities. This finding suggests that strong deterministic forces shape community structure, but does not preclude minor influences of stochasticity in the assembly process. Phylogenetic clustering specifically suggests ecological niche filtering—based on host species and other environmental factors unaccounted for in this study—plays a dominant role on community assembly.

3.2 | Relative abundance of bacterial phyla

Proteobacteria was the dominant phylum in the sequence data sets, detected at an average relative proportion of 53.5% ± 17.6, followed by *Cyanobacteria* at 16.0% ± 18.0, and *Bacteriodetes* at 10.0% ± 6.5 (Figure S4). Phyla individually detected at proportions below 1% in each sample accounted for 0.009% to 12.4% of the community for a given sample and an average of 4.0% ± 3.0, throughout all communities in the data set. On average, all communities were dominated by *Alphaproteobacteria* (ca. 30.2% ± 12.2), *Gammaproteobacteria* (ca. 18.1% ± 11.7), and *Deltaproteobacteria* (ca. 8.5% ± 6.6) (Figure S4). Volcano plots displaying comparative analyses of OTU frequencies between host species showed *S. radians* had over 14 times the number of differentially abundant OTUs relative to *S. siderea*, 320 and 21 OTUs, respectively (Figure 3). The majority of differentially abundant OTUs from both hosts were

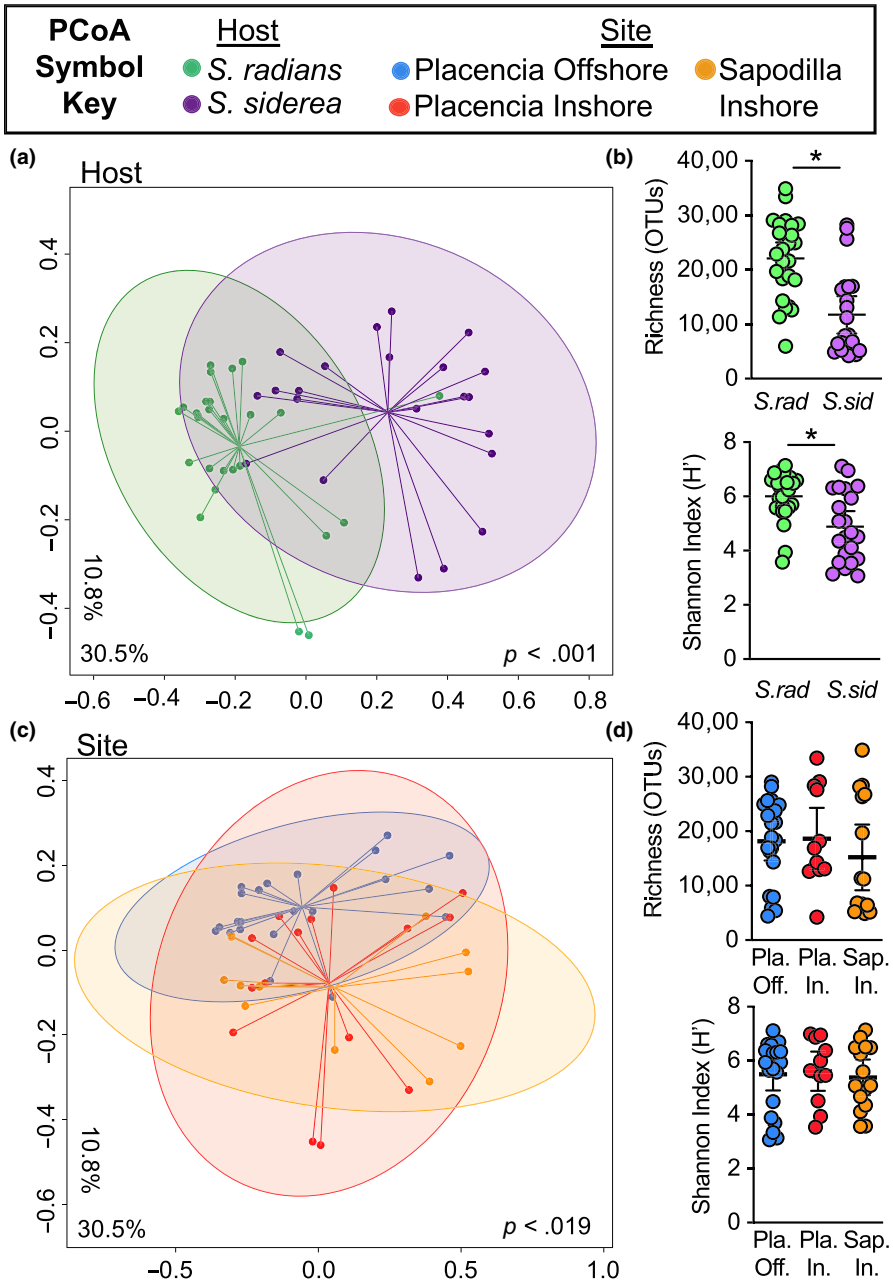

FIGURE 1 Bacterial community composition (BCC) clusters by host species. Principal coordinates analysis (PCoA) based on Bray-Curtis dissimilarities of BCC by host species (a) and site (c). Percentages on each axis indicate the amount of variation explained by each axis. *p*-values indicate significant results of PERMANOVA tests. PCoA symbol key is shown above panel (a). Alpha-diversity of bacterial communities based on OTU richness and Shannon index are shown by host species (b) and site (d). Asterisks indicate alpha diversity was significantly different between host species (Student's *t* test: $p < .001$)

from the phylum *Proteobacteria*: ca. 77% in *S. radians* and ca. 67% in *S. siderea* communities. Six deltaproteobacterial orders were statistically more abundant in *S. radians* communities, specifically *Desulfobacterales*, *Desulfarculales*, *NB1-j*, *Sva0853*, *Myxococcales*, and *Entotheonellales* (Figure 3). The gammaproteobacterial orders *Chromatiales* and *Thiotrichales*, as well as the alphaproteobacterial order *Rhodospirillales*, were also statistically more abundant in *S. radians* communities.

3.3 | The core microbiome drives differences between host species

The core microbiome (bacterial OTUs shared among 70% of the same host species from a single site) was determined for each host species from each site. PCoA of core microbiomes using Bray-Curtis dissimilarity index revealed BCC clustered by host species, which explained 26.9% of the variation in community composition (Figure 4a; Table S4, ANOVA: $p < .001$). Statistical analysis via Sørensen's index showed host species explained 38.3% of the variation in the data set (ANOVA: $p < .001$). This observation indicates that the presence/absence of OTUs in the core (Sørensen) rather than the relative abundance combined with presence/absence of these OTUs (Bray-Curtis) explained more variation in the data set. Similar to observations for the entire microbiome, species richness was statistically higher (Figure 4b), and beta-diversity was statistically lower for *S. radians* core microbiomes relative to those for *S. siderea* (Table S4). PCoA showed core microbiomes also clustered by site; however, site explained a smaller percentage of the variation in the data set relative to host species (Table S4, ANOVA: $p < .001$). At each site, core

microbiomes clustered by host species, which explained a substantial percentage of the variation in the data set at Placencia offshore (73.2%) and Sapodilla inshore (35.4%) sites (Figure 4c; Table S4, ANOVA: $p < .001$). Using pairwise PERMANOVAs, we found that core microbiomes differed by site for both *S. radians* and *S. siderea* (Figure 4d; Table S4, ANOVA: $p < .003$). *S. radians* core microbiome from Placencia offshore had statistically higher OTU richness than all other core microbiomes, and those from *S. siderea* from Placencia offshore had statistically lower richness (Figure 4e, ANOVA: $p < .003$). There was no difference in OTU richness between the other four core microbiomes. Taken together, these data yield two important findings: (a) core microbiomes are distinct between hosts, as well as across sites for a given host; and (b) host core microbiomes differ more significantly by the presence/absence of a given OTU rather than its relative abundance combined with presence/absence.

The majority of core OTUs were exclusive to one core microbiome and not shared between core microbiomes (Figure 4f). Taxonomic analysis of core microbiomes showed class-level differences between host species, and order-level differences between sites for a given host species (Figure 4f). *S. radians* core microbiome contained six phyla (*Acidobacteria*, *Actinobacteria*, *Bacteriodetes*, *Cyanobacteria*, *Gemmatimonadetes*, and *Planctomycetes*), and several alphaproteobacterial orders (*Kordiimonadales* and orders making up less than 1% of the community) that were absent in *S. siderea* core microbiome (Figure 4f). *SBR1093* and several gammaproteobacterial orders—e.g., *Enterobacteriales*, *Oceanospirillales*, *Pseudomonadales*, which comprise less than 1% of the community—were present only in the *S. siderea* core microbiome. Although the gammaproteobacterial order *Thiotrichales* was present in the core microbiome of both host species, it made up over half of the core microbiome in *S. siderea* (ca. 54.6%) and a small percentage of the core in *S. radians* (ca. 2.1%). Notably, the deltaproteobacterial orders *Desulfobacterales*, *Myxococcales*, and *Sva0853* were found in the core microbiome for both *S. radians* and *S. siderea*.

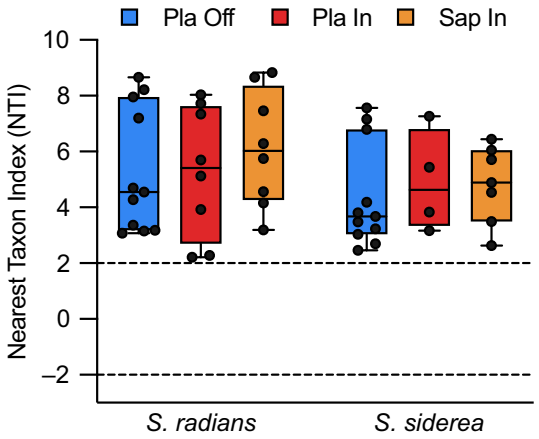

FIGURE 2 Nearest taxon index (NTI) of coral bacterial communities. Within-community nearest taxon index (-MNTD; mean nearest taxon distance) indicating phylogenetic clustering (NTI > +2). NTI values between +2 and −2 (within the dashed lines) indicate no statistically significant difference between null model communities. All *p*-values are ≤.001 (data not shown), and are based on comparisons of NTI for the coral bacterial communities versus those for null model communities over 999 permutations. Pla, Placencia; Sap, Sapodilla; Off, offshore; In, inshore

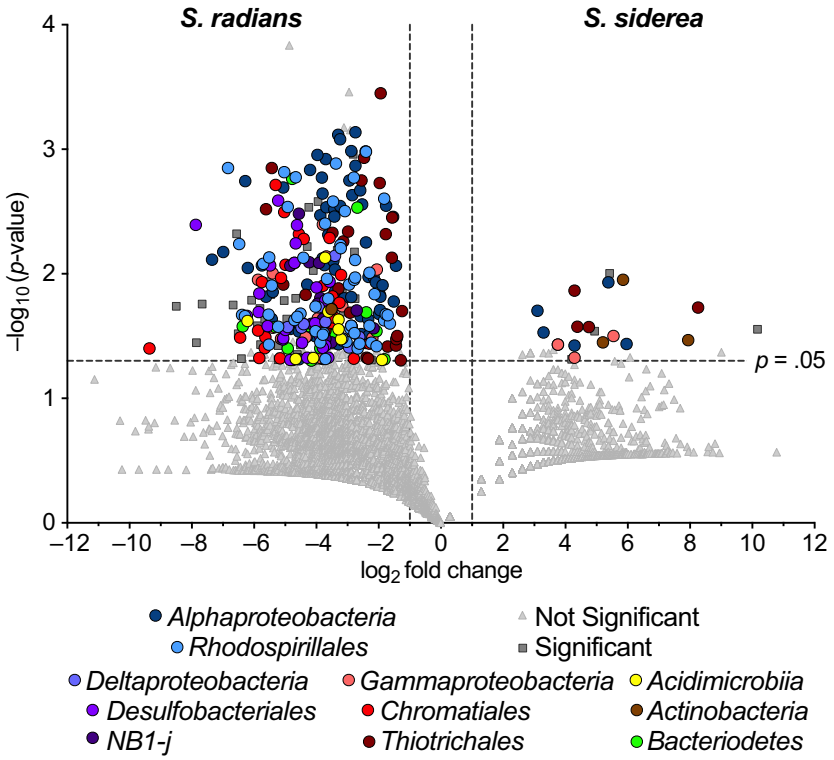

FIGURE 3 Differentially abundant OTUs between *S. radians* and *S. siderea* communities. Volcano plot showing comparative analysis of OTU frequencies between coral hosts for all three sites. Grey squares and coloured circles indicate OTUs with a magnitude fold-change>|1| $\log_2$ (*x*-axis) and *p*-value < .05 corrected for multiple comparisons with a Benjamini-Hochberg correction. Data points (OTUs) with positive $\log_2$ fold change values were more abundant in *S. siderea* communities, whereas those with negative $\log_2$ fold change values were more abundant in *S. radians* communities; light grey triangles indicate OTUs that are not significantly differentially abundant between host species. Taxa of interest are shown as coloured circles. Horizontal dashed lines show $p = .05$ and vertical dashed lines show where $\log_2 = |1|$

When compared between sites for a single host species, *S. radians* core microbiome contained only two orders that were statistically different between sites: *Rhizobiales*, which was more abundant in Placencia inshore relative to the other sites, and *Chromatiales*, which was more abundant in Placencia offshore relative to the other sites (Figure 4f). *S. siderea* core microbiome, on the other hand, showed more variation between sites, with three alphaproteobacterial orders and three gammaproteobacterial orders that were statistically differentially abundant between sites (Figure 4f). The Placencia inshore site was the most diverse of the *S. siderea* core microbiome and contained a statistically larger proportion of *Rhodobacterales*, *Rhodospirillales*, *Chromatiales* and smaller proportion of *Thiotrichales* relative to the other sites (Figure 4f).

3.4 | The algal community is distinct between hosts at inshore sites

We analysed the algal component of the coral holobiont to determine whether patterns similar to those observed for the BCC emerged. Previous analysis of these communities, along with those from seven additional sites that were classified as having either high or low thermal variability, revealed that algal communities clustered in a species-specific manner: *S. siderea* algal communities clustered based on thermal variation of the environment, whereas those for *S. radians* did not separate based on thermal variation (Baumann et al., 2018). PCoA was performed on the algal communities for the three sites in which bacterial communities were examined (Placencia and Sapodilla inshore and Placencia offshore) to determine whether algal communities also clustered by host species. Host species explained 27% of the variation in the data set (Figure 5a, ANOVA: $p < .001$), and algal communities clustered by host species at the Placencia and Sapodilla inshore sites (Figure 5c,e, ANOVA: $p < .001$), yet did not cluster by host species at the Placencia offshore site (Figure 5d; Table S4). When analysed separately by host species, *S. radians* algal communities did not cluster by site (Figure 5b; Table S4), whereas those for *S. siderea* clustered by site (ANOVA: $p < .04$; Figure 5c; Table S4). Similar to what was observed for the BCC, site explained <5% of the variation among *S. siderea* algal communities, suggesting other biotic, abiotic, or stochastic factors are important in structuring these communities. Taken together, these data indicate host species is an important factor in determining algal community composition exclusively at the inshore sites, and does not shape algal community structure at the offshore site on the Belize MBRS.

4 | DISCUSSION

4.1 | BCC is partially structured by host species and other deterministic forces

Our findings demonstrate that the BCC of two Caribbean coral species in the genus *Sidereastrea* are shaped by deterministic factors, including host species (Figure 1; Table S4). Similar phylogenetic-dependence has been observed for more distantly related corals that do not belong to the same family, including two species of Caribbean corals, *Orbicella* (previously *Montastrea*) *faveolata* and *Porites astreoides* (Morrow et al., 2012), and phylogenetically diverse Scleractinian corals (Pollock et al., 2018). Future work should examine the BCC of corals with both similar and distinct phylogenies simultaneously to determine the relative importance of host evolutionary history in shaping coral BCC.

Although host species is the main driver of BCC of the variables examined in the current study and site explained a small portion of the variation in coral BCC (Table S4), a substantial portion of the variation remains unexplained. Recent work has highlighted the importance of stochastic community assembly processes (Hubbell, 2001), such as ecological drift (Lankau, Hong, & Mackie, 2012) due to dispersal limitation (Sul, Oliver, Ducklow, Amaral-Zettler, & Sogin, 2013), in microbiome assembly. Ecological drift due to geographic isolation has shown to be significant in shaping BCC of other coral communities (Morrow et al., 2012). However, as BCC differed between host species within the same site, we interpret this to mean that different coral species differentially recruit bacterial taxa from the same regional species pool. This deterministic community assembly is reflected by the phylogenetic clustering of all analysed coral bacterial communities (Figure 2). That a large portion of bacterial community dissimilarity remains unexplained hints at the deterministic influences of other unmeasured environmental variables (Stegen et al., 2013). Abiotic factors, such as levels of stress associated with oceanic warming and acidification (Grottoli et al., 2018), are possible structuring forces. In addition, factors such as the proximity of the coral to other corals, sponges, or other co-evolutionary relationships (Kelly et al., 2014), host genotype (Neave et al., 2017), host species and within-coral anatomy (Pollock et al., 2018), age and stage of development (Sweet & Bulling, 2017) should be examined in future work to determine their contribution to the complexity and plasticity of the bacterial component of the coral holobiont.

4.2 | Differentially abundant *Alpha-* and *Deltaproteobacteria* drive differences between host species

Our analysis found differences at the bacterial order and class level as well as high diversity of *Siderastrea radians* communities partially explain dissimilarities between host species BCC (Figures 3 and S3). Members within the alphaproteobacterial order *Rhodospirillales*, six deltaproteobacterial orders (*Desulfarculales*, *Desulfobacterales*, *Entheonellales*, *Myxococcales*, *NB1-j*, and *Sva0853*), and gammaproteobacterial order *Chromatiales* were among the taxa contributing to differences between host communities. Recent work has shown several of these taxa are overrepresented during stress events and in the face of chronic stressors such as climate change, water pollution, and overfishing (McDevitt-Irwin, Baum, Garren, & Vega Thurber, 2017). OTUs belonging to orders of known

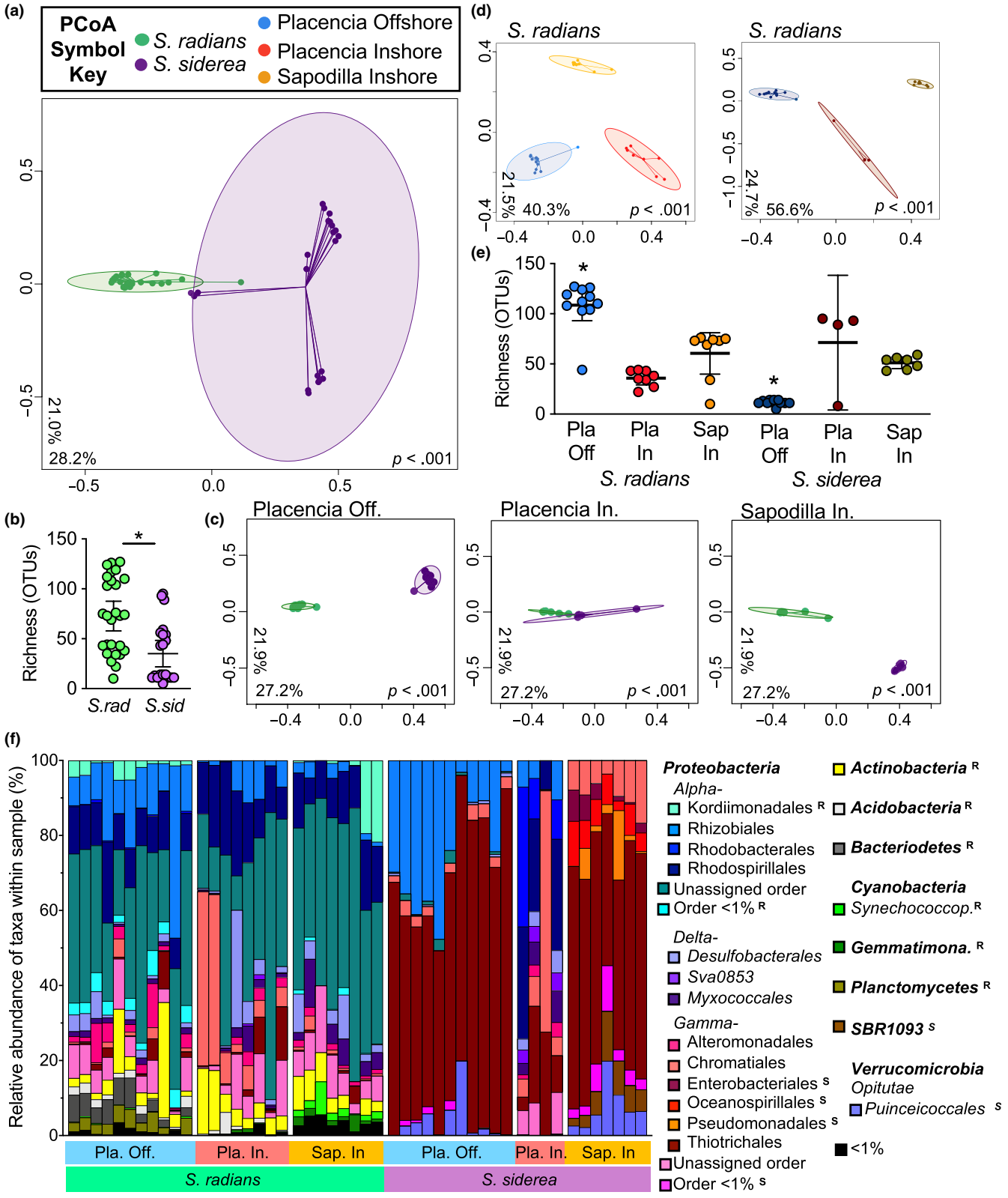

FIGURE 4 Core microbiomes are distinct from one another. Principal coordinates analysis (PCoA) based on Bray-Curtis dissimilarity index of core microbiomes by host species (a), by host species at each site (c), by site for each host (d). Percentages on each axis indicate the amount of variation explained by each axis. *p*-values indicate significant results of PERMANOVA tests. PCoA symbol key is shown in panel (a). Alpha-diversity based on OTU richness of core microbiomes by host species (b) and site for *S. radians* and *S. siderea* (e). Asterisks indicate alpha diversity was significantly different between host species or sites for a given host species (Student's *t* test: $p < .001$). (f) Relative abundance (%) of each taxon by sample; taxa individually detected below 1% relative abundance on average are grouped and displayed as <1%. Taxa with 'R' were statistically more abundant in *S. radians* relative to *S. siderea*, and taxa with 'S' were statistically more abundant in *S. siderea* (Student's *t* test, $p < .05$)

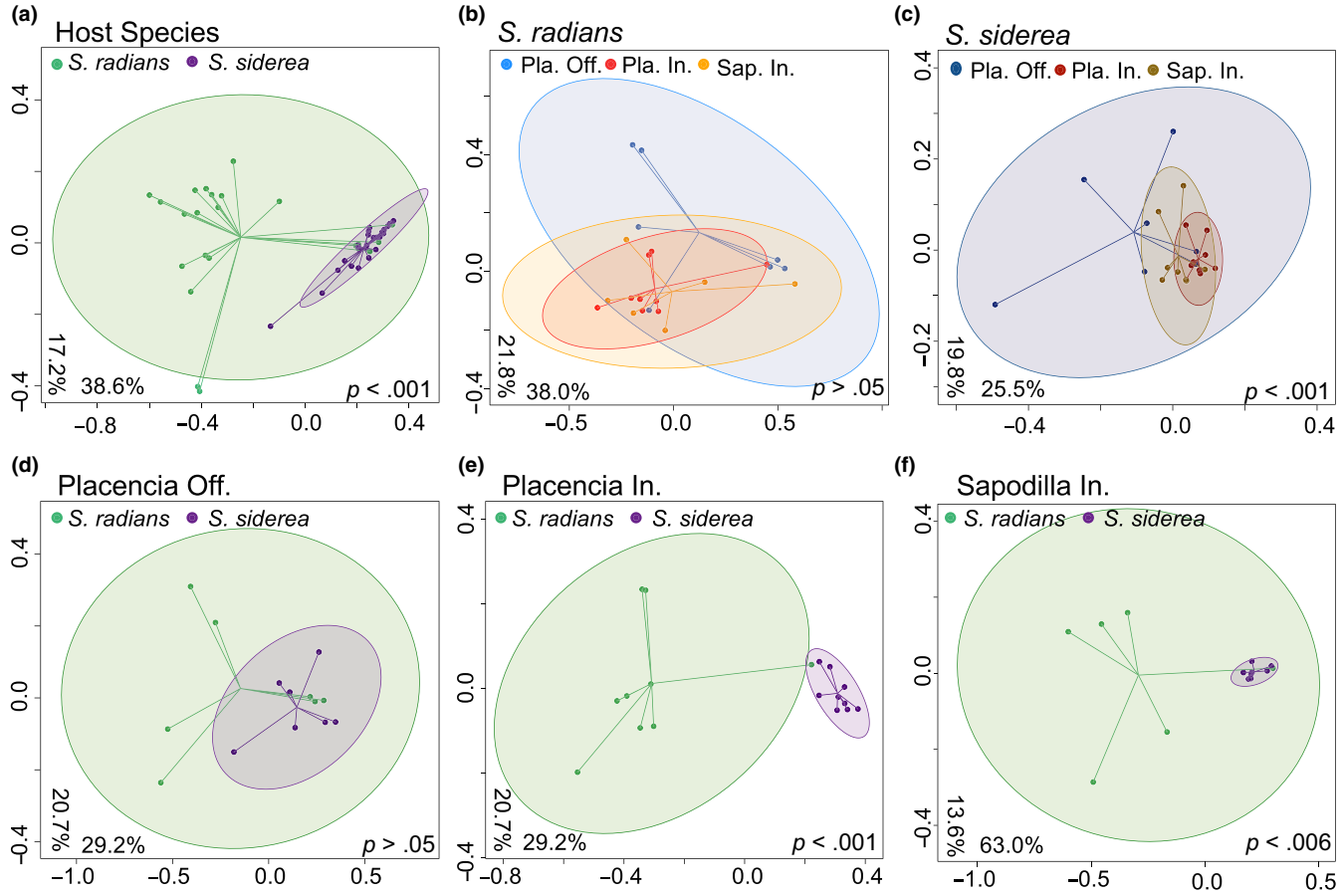

FIGURE 5 Algal communities cluster by host species. Principal coordinates analysis (PCoA) based on Bray-Curtis dissimilarity index of algal communities by host species (a), by site for *S. radians* (b) or *S. siderea* corals (c), and host species at each site (d–f). Percentages on each axis indicate the amount of variation explained by each axis. *p*-values indicate significant results of PERMANOVA tests. Pla., Placencia; Sap, Sapodilla; Off., offshore; In, inshore

sulphate-reducing *Deltaproteobacteria* (Giovannelli et al., 2016; Sato et al., 2017) were differentially abundant in *S. radians* relative to *Siderastrea siderea* communities and have been linked to black band disease (BBD) in corals (Ducklow & Mitchell, 1979; Richardson, 1997; Sekar, Kaczmarsky, & Richardson, 2008), as these microbes produce sulfide that can be toxic to coral tissue (Barneah, Ben-Dov, Kramarsky-Winter, & Kushmaro, 2007; Richardson, 1997; Sato et al., 2017; Sekar, Mills, Remily, Voss, & Richardson, 2006; Viehman, Mills, Meichel, & Richardson, 2006). Alternatively, an increase in taxa commonly associated with disease in general, or specifically BBD, may suggest the *S. radians* host or entire holobiont is less sensitive to opportunistic taxa or their potentially toxic byproducts. For example, sulphide can provide benefits to other members of the holobiont, such as endosymbiotic algae, where it can function as an antioxidant (Steinke, Brading, Kerrison, Warner, & Suggett, 2011) and potentially promote the presence of sulphate-reducers within the microbial community.

Although bacterial communities contained specific taxa that are commonly found in stressed corals (McDevitt-Irwin et al., 2017), the coral samples collected for this study had no visible signs of bleaching or disease. There is evidence that bacteria linked to disease outbreaks are also common members of the healthy microbiome (Bourne et al., 2009; Thurber et al., 2009); therefore, it is unclear whether the presence of sulphate-reducers or other opportunistic taxa are indicative of thermal stress, disease, or are simply members of the commensal microbiome. Because individual species in the bacterial communities of stressed and diseased corals can vary and community function can change with the alteration of only a few taxa (McDevitt-Irwin et al., 2017; Sekar et al., 2008), future studies are required to elucidate how BCC variations can affect interspecies dynamics within the coral-associated communities and the overall health of the entire holobiont.

4.3 | The core microbiome is distinct between host species and across sites for a given host species

The core microbiome, which consists of members of the community with a shared presence across two or more microbial assemblages (Hamady & Knight, 2009; Shade & Handelsman, 2012; Sweet & Bulling, 2017; Turnbaugh et al., 2007), showed distinct clustering by host species within each site and for all sites combined (Figure 4a,c).

Consistent with other studies, very few OTUs were shared between core microbiomes of the two hosts (Ainsworth et al., 2015; Hernandez-Agreda, Leggat, Bongaerts, Herrera, & Ainsworth, 2018). Host species explained over 70% of the variation in core microbiomes at the Placencia offshore site (Table S4), that was previously characterized as having low overall thermal variability relative to the other two sites (Baumann et al., 2016). Core microbiomes also showed opposite patterns at Placencia offshore: OTU richness of *S. radians* core microbiomes were statistically higher than those for *S. siderea* (Figure 4d,e). These observations suggest the core microbiomes of *S. radians* and *S. siderea* vary by geographic location and level of environmental variability. Core microbiomes are most distinct between host species based on community composition and OTU richness when thermal variability is low (Placencia offshore) and are more similar when thermal variability is higher (Placencia and Sapodilla inshore). Future work examining additional sites is required to determine whether this trend is observed consistently across the Belize MBRS and for other reef systems.

Taxonomy of core microbiomes varied widely between host species (Figure 4f). Notably, the taxa that drove these differences represent key constituents of *S. radians* core microbiomes. Specifically, *Rhodospirillales*, *Desulfobacterales*, and *Myxococcales* were present in nearly all *S. radians* core microbiomes, and absent from those of *S. siderea*. *Thiotrichales* was found in all core microbiomes although it made up nearly half of the core microbiome for *S. siderea*, but only 2% of the *S. radians* core microbiomes; however, little is known about the function of this taxa in the coral microbiome. Although OTUs within the core microbiome made up only 3.3% of OTUs found in the entire sequence data set, the core made up ca. 18.9% of the reads for the entire BCC, and probably serve important ecological and functional roles for the coral host (Brener-Raffalli et al., 2018). Furthermore, similar trends in relative abundance of taxa observed in the entire BCC were also observed among the core component, suggesting differences between core microbiomes probably drive the differences observed between the entire microbial community.

4.4 | Interactions between life history strategies and trends in BCC may dictate how corals will cope with stress

Exposure to environmental variability is predicted to have different impacts on corals that employ different life history strategies (Darling et al., 2012), and several studies have shown the impact of life history strategy on coral growth (Castillo, Ries, Weiss, & Lima, 2012), response to bleaching events (Pineda et al., 2013), and thermal tolerance (Baumann et al., 2016; Darling, McClanahan, & Côté, 2013). Coral-associated BCC is likely important for maintaining holobiont health during periods of thermal stress (Grottoli et al., 2018; Reshef et al., 2006) through alterations in community structure (Grottoli et al., 2018; Leggat et al., 2011; Tanner et al., 1996) and accompanying alterations in function. Therefore, corals that employ different life history strategies may uniquely alter their microbiomes as a response to environmental variability (Neave et al., 2017). Although *S. radians* and *S. siderea* are of the same genus, they employ distinct life history strategies that are both predicted to persist in areas impacted by environmental and anthropogenic disturbance (Darling et al., 2012).

Siderastrea radians is classified as a weedy species that has smaller colony sizes, faster maturity and reproduction through brooding (Darling et al., 2012), and shows remarkable resistance and resilience (Lirman & Manzello, 2009). Weedy species also display more variation in their species traits relative to species employing other life history strategies; this allows them to succeed in high stress environments by rapidly colonizing recently disturbed habitats (Darling et al., 2012; Green et al., 2014). *S. radians* had rich and diverse BCC (Figure 1b; Table S4), which, according to the insurance hypothesis, may prevent deterioration of holobiont function through either redundant or complementary functioning (Yachi & Loreau, 1999). *S. radians* core communities showed higher richness in the offshore relative to inshore sites (Figure 4d) that further reflects the highly dynamic nature of *S. radians* microbiome (Hernandez-Agreda et al., 2018). A diverse bacterial community may play a role in *S. radians*' ability to quickly adapt to new and variable environments, as microbiome plasticity is predicted to facilitate adaptation to environmental stress (Ziegler, Seneca, Yum, Palumbi, & Voolstra, 2017). In the core and entire microbiome, *S. radians* had significantly lower beta-diversity than *S. siderea* (Table S4), indicating the *S. radians* communities showed less variation between individuals. Stable microbiomes in the face of temperature stress have been found in other scleractinian corals (Grottoli et al., 2018; Hadaidi et al., 2017; Ziegler et al., 2017), which may indicate the host's role in preadaptation (acclimation) to stress (Grottoli et al., 2018).

Siderastrea siderea are classified as stress-tolerant corals (Davies, Marchetti, Ries, & Castillo, 2016) that are large and domed, slow growing, reproduce by broadcast spawning, and have high fecundity that may be advantageous during stress events (Darling et al., 2012). These corals can also withstand recruitment failure for decades, that may allow them to persist in stressful environments (Darling et al., 2012; Hughes & Tanner, 2000). In addition, they generally have higher energy reserves, that have been shown as a key component to resilience against temperature stress (Anthony, Hoogenboom, Maynard, Grottoli, & Middlebrook, 2009; Grottoli et al., 2014). The high variation of the *S. siderea* microbiome (Table S4) may have functional consequences or, if functional redundancy is high, these microbial communities may perform the same or similar roles for each individual animal (Allison & Martiny, 2008; Brener-Raffalli et al., 2018; Yachi & Loreau, 1999). The *S. siderea* core microbiome at the Placencia offshore site had lower diversity than the other two sites (Figure 4e), that could be indicative of an overall less dynamic microbiome (Hernandez-Agreda et al., 2018). This lower diversity could also indicate a stress response, as lower diversity and higher variation has been observed in Pacific corals prior to showing signs of disease (Pollock et al., 2018). *S. siderea* from sites with different thermal variability can have different thermal tolerances (Davies, Ries, Marchetti, & Castillo, 2018). However, *S. siderea* were abundant

in all three sites (Baumann et al., 2016), and the corals that were examined in this work showed no apparent signs of disease. Therefore, further research is required to determine the impact of bacterial diversity and thermal variability on *S. siderea* fitness.

4.5 | The mode through which a coral responds to environmental variability is microbial partner-specific

Our data reveal that microbial communities of *Siderastrea* spp. exhibit statistically significant differences between sites with distinct thermal variability. The identity and richness of specific microbial partners characterize these differences: algal communities are distinct between hosts in a thermally variable environment (Placencia and Sapodilla inshore), whereas community composition and richness for the entire bacterial community and the core are distinct between hosts in more thermally stable environment (Placencia offshore; Figure 6). These findings suggest that the factors governing community structure are distinct for algae and bacterial partners. Given these observations, we propose that *Siderastrea* corals cope with environmental variability by modulating interactions with different microbial partners: in thermally variable environments it may be through an altered interaction with the algal community, whereas in more thermally stable environments it may be through an altered interaction with bacteria, specifically those in the core microbiome (Figure 6). Future work examining these communities at additional sites along the Belize MBRS as well as sites following a similar thermally variable/stable gradient will reveal whether this trend is observed throughout coral reef ecosystems or is specific to the Belize MBRS sites investigated here.

Previous work has shown coral resilience can come from interactions with its symbiotic partners (Davies et al., 2018), and several hypotheses may describe how *Siderastrea* microbial communities could impact coral resilience. The unique algal or bacterial communities of each congeneric coral species at inshore or offshore sites observed here could suggest potential disparate community function and interaction with the host (Brener-Raffalli et al., 2018; Yachi & Loreau, 1999) and therefore provide distinct benefits in response to environmental variability. An alternative hypothesis is that these unique microbiomes may be functionally redundant, performing the same or similar roles for the coral host in each environment (Allison & Martiny, 2008; Brener-Raffalli et al., 2018; Yachi & Loreau, 1999), that is widespread in microbial systems (Louca et al., 2018) and may serve to buffer disturbances (Shade & Handelsman, 2012). This theory is particularly attractive for predicting the function of the core microbiomes, which are hypothesized to play critical roles for the entire microbiome (Shade & Handelsman, 2012), and may therefore perform similar functions for the coral holobiont in diverse environments (Allison & Martiny, 2008; Brener-Raffalli et al., 2018; Yachi & Loreau, 1999). It is also possible that unique microbial community composition indicates differing sensitivities to environmental variability and stress (Gajigan et al., 2017) where holobionts with stable microbiomes are less sensitive to environmental change, more resilient, and more likely to persist in the future (Grottoli et al., 2018). However, patterns of variability remain undefined for a normal, healthy coral microbiome (Zaneveld, McMinds, & Thurber, 2017) and may be host species-specific. Taking into account the high abundance of each host species in sites with both low and high thermal variability (Baumann et al., 2016), it is possible both species have reduced sensitivity to environmental variability. The potential for

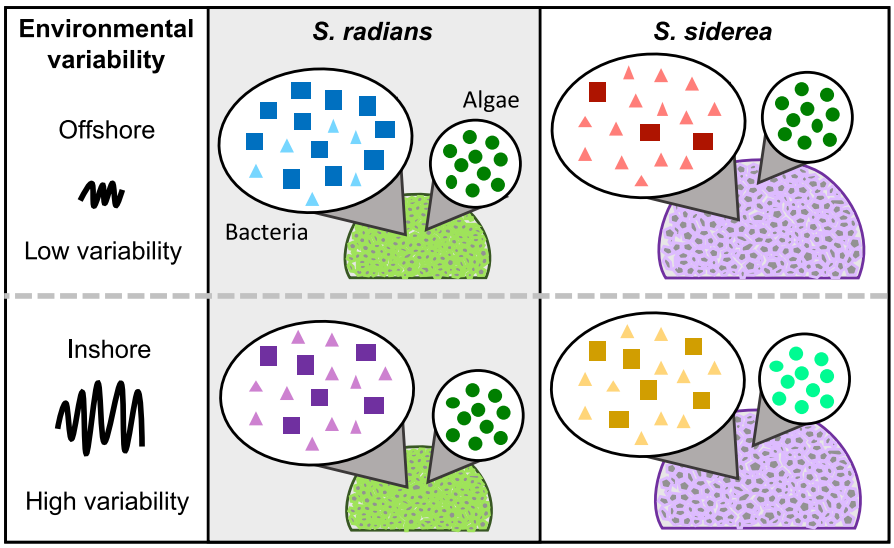

FIGURE 6 Conceptual model for the influence of environmental variability on the composition of algal and bacterial communities associated with *Siderastrea* spp. on the MBRS. Each *Siderastrea* spp. harbours distinct symbionts, where colours indicate different communities. Our data suggest corals host unique bacterial communities made up of core (squares) and accessory (triangles) microbiomes, and an algal community (circles). When environmental variability is low, *S. radians* has increased diversity of the core microbiome, whereas *S. siderea* shows decreased core microbiome diversity. We hypothesize that both *Siderastrea* spp. alter the composition and diversity of their bacterial communities, whereas only *S. siderea* adjusts its algal communities to cope with environmental variability

Siderastrea spp. to cope with environmental variability by interacting with unique microbial partners, particularly in habitats with decreasing coral abundance and species richness (Baumann et al., 2016; Gardner, Côté, Gill, Grant, & Watkinson, 2003), sheds light on the dynamics of symbiont communities across domains and diverse modes of adaptation available to the coral holobiont.

ACKNOWLEDGEMENTS

We thank H. Aichelman and A.N. Septer for field and laboratory assistance, respectively. We also thank M. Kanke, S. Brown, and A. Hyde for assistance with coding, and the Marchetti Laboratory for use of laboratory equipment. We would like to thank the reviewers and editor for their helpful feedback. We thank the Belize Fisheries Department for issuing research and collection permits, and Garbutt's Marine for providing local expert guides and boats for field research. This work was supported by the National Science Foundation Biological Oceanography grant to KDC (OCE 1459522), Rufford Foundation (http://www.rufford.org) Small Grant to JHB (15802-1); Department of Defense NDSEG fellowship to JHB; S.W.D. was a Simons Family Foundation Fellow of the Life Sciences Research Foundation (LSRF). J.P.B was funded by the UNC Dissertation Completion Fellowship and the Carl Tryggers Foundation.

CONFLICT OF INTEREST

The authors declare that there are no conflict of interest.

AUTHOR CONTRIBUTIONS

L.S., S.W.D., J.B., and K.D.C. designed research; L.S., S.W.D., and J.B. performed research; L.S., S.W.D., J.P.B., and J.B. analysed data; and L.S., J.P.B., and J.B. wrote the paper and all authors read and provided feedback on the manuscript.

DATA AVAILABILITY STATEMENT

Sequences are available in the NCBI Sequence Read Archive (SRA) database under the accession number PRJNA628692.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Speare L, Davies SW, Balmonte JP, Baumann J, Castillo KD. Patterns of environmental variability influence coral-associated bacterial and algal communities on the Mesoamerican Barrier Reef. *Mol Ecol*. 2020;29:2334–2348. https://doi.org/10.1111/mec.15497