Symbiodinium Functional Diversity in the Coral Siderastrea siderea Is Influenced by Thermal Stress and Reef Environment, but Not Ocean Acidification

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Coral bleaching events are increasing in frequency, demanding examination of the physiological and molecular responses of scleractinian corals and their algal symbionts (Symbiodinium sp.) to stressors associated with bleaching. Here, we quantify the effects of long-term (95-day) thermal and CO_2-acidification stress on photochemical efficiency of in hospite Symbiodinium within the coral Siderastrea siderea, along with corresponding coral color intensity, for corals from two reef zones (forereef, nearshore) on the Mesoamerican Barrier Reef System. We then explore the molecular responses of in hospite Symbiodinium to these stressors via genome-wide gene expression profiling. Elevated temperatures reduced symbiont photochemical efficiencies and were highly correlated with coral color loss. However, photochemical efficiencies of forereef symbionts were more negatively affected by thermal stress than nearshore symbionts, suggesting greater thermal tolerance and/or reduced photodamage in nearshore corals. At control temperatures, CO_2-acidification had little effect on symbiont physiology, although forereef symbionts exhibited constitutively higher photochemical efficiencies than nearshore symbionts. Gene expression profiling revealed that S. siderea were dominated by Symbiodinium goreaui (C1), except under thermal stress, which caused shifts to thermotolerant Symbiodinium trenchii (D1a). Comparative transcriptomics of conserved genes across the host and symbiont revealed few differentially expressed S. goreaui genes when compared to S. siderea, highlighting the host's role in the coral's response to environmental stress. Although S. goreaui transcriptomes did not vary in response to acidification stress, their gene expression was strongly dependent on reef zone, with forereef S. goreaui exhibiting enrichment of genes associated with photosynthesis. This finding, coupled with constitutively higher forereef S. goreaui photochemical efficiencies, suggests that functional differences in genes associated with primary production exist between reef zones.

Keywords: symbiodinium, climate change, transcriptomics, coral, thermal stress, thermal tolerance, ocean acidification

INTRODUCTION

Dinoflagellates are ubiquitous unicellular algae that can occur as free-living cells, endosymbionts, or parasites across a wide variety of marine organisms. The most recognized among endosymbionts is the genus *Symbiodinium*, which establish obligate relationships with many reef-building corals (Trench and Blank, 1987). Photosynthetically-derived nutrients translocated from *Symbiodinium* to the host can yield up to 100% of the coral energy budget (Muscatine and Cernichiari, 1969; Muscatine, 1990), allowing for prolific coral growth in shallow oligotrophic tropical waters. In turn, *Symbiodinium* gain a protected microenvironment with access to light, inorganic nutrients, and dissolved inorganic carbon provided by the host (Muscatine and Cernichiari, 1969; Yellowlees et al., 2008; Wangpraseurt et al., 2014; Barott et al., 2015; Enríquez et al., 2017; Scheufen et al., 2017). Although initially considered a single species, advancements in genetics have uncovered nine divergent clades within *Symbiodinium*, designated A–I (Coffroth and Santos, 2005; Pochon and Gates, 2010; Thornhill et al., 2014), with additional evidence for significant within-clade genetic diversity (Howells et al., 2012; Pettay and Lajeunesse, 2013; Thornhill et al., 2017).

Increasing atmospheric pCO_2 has reduced seawater pH, which impairs reef accretion (Hoegh-Guldberg et al., 2007), and has increased sea surface temperatures, which can cause a breakdown of the coral-algal symbiosis—a process termed "coral bleaching" (Hoegh-Guldberg, 1999; Baker, 2003; Weis, 2008; Hoegh-Guldberg and Bruno, 2010). During bleaching, the coral is deprived of symbiont-derived organic carbon, which can lead to reduced growth, infection, and mortality. Bleaching events have been recognized as the primary driver of recent global coral reef degradation and have been increasing in frequency and severity over the past century (Pandolfi et al., 2011; Hughes et al., 2017). However, the rich genetic diversity of *Symbiodinium* often yields functional diversity, which may confer resilience to environmental change. For example, the composition of *in hospite Symbiodinium* communities can shift after bleaching disturbances (Baker, 2001; Thornhill et al., 2006; Jones et al., 2008; Kemp et al., 2014; Silverstein et al., 2015), which can strongly influence coral gene expression and future bleaching susceptibility (Desalvo et al., 2010a; Jones and Berkelmans, 2010; Howells et al., 2012). A more comprehensive understanding of how coral-*Symbiodinium* associations respond to ocean warming and acidification is needed to predict coral reef responses to global change.

Studies of *Symbiodinium* physiology document a wide array of stress responses to environmental change, including impairment/inactivation of photosynthesis at high temperatures (Iglesias-Prieto et al., 1992; Iglesias-Prieto and Trench, 1994), increased production of reactive oxygen species (ROS) and antioxidant activity in response to thermal and UV exposure (Lesser, 1996; Suggett et al., 2008; Gardner et al., 2017), and reduced symbiont pigment concentrations at low pH (Tremblay et al., 2013). In contrast to these strong physiological responses, there is mounting evidence that *Symbiodinium* lack strong transcriptional responses to global change stressors, which

starkly contrasts their host's transcriptional response (Leggat et al., 2011; Barshis et al., 2014; Davies et al., 2016). Leggat et al. (2011) found that *Symbiodinium* exhibit few changes in expression of stress response genes under thermal stress when compared to these same genes in their coral host. Minimal transcriptomic responses have also been observed in both *Symbiodinium* types (ITS1, ITS2, and cp23-determined) D2 and C3K following heat exposure (Barshis et al., 2014), which contrasts widespread transcriptomic shifts observed in the coral host exposed to the same heat stress (Barshis et al., 2013).

Despite the unresponsiveness of *Symbiodinium* gene expression to stressors, transcriptome profiles were found to be highly divergent among *Symbiodinium* clades D2 and C3K, regardless of experimental treatment, providing evidence for functional differences amongst *Symbiodinium* lineages (Barshis et al., 2014). Functional differences inferred from transcriptomics have also been observed within *Symbiodinium* lineages. For example, comparative transcriptomics of a variety of clade B strains revealed strain-specific differences in expression (Parkinson et al., 2016), providing additional evidence that substantial transcriptomic functional variation exists within and across *Symbiodinium* lineages.

Clade C strains are the most derived *Symbiodinium* lineage and exhibit higher within-clade diversity when compared to other, more basal clades (Pochon et al., 2006; Pochon and Gates, 2010; Lesser et al., 2013; Thornhill et al., 2014, 2017). There is therefore great interest in understanding how this diversity within clade C *Symbiodinium* influences the coral's response to global change stressors. Transcriptomes of two divergent C1 lineages previously shown to exhibit distinct responses to thermal stress, both *in hospite* and *in culture* (Howells et al., 2012), revealed divergent expression patterns in response to heat stress, providing insights into how symbiont functional variation could lead to variation in coral thermal tolerance (Levin et al., 2016). Given the important role that coral host-*Symbiodinium* interactions play in a corals' response to environmental stress (Parkinson et al., 2015), it is critical to determine how each symbiotic partner responds to multiple stressors. However, few studies have explored whole transcriptome responses of different *in hospite Symbiodinium* populations to ocean warming and acidification, and even fewer have compared the responses of both symbiotic partners in parallel.

Here, we exposed the resilient and ubiquitous Caribbean reef-building coral *Siderastrea siderea* and its *in hospite Symbiodinium* from two different reef zones (nearshore and forereef) to thermal stress (Temperature = 25, 28, 32°C) and CO_2-acidification stress (pCO_2 = 324, 477, 604, 2553 μatm) for 95 days. Our previous work has shown that both elevated temperature and pCO_2 elicited strong but divergent responses of the host's transcriptome (Davies et al., 2016), with increased temperatures substantially reducing calcification rates, with smaller declines observed in response to elevated pCO_2 (Castillo et al., 2014). Here, we build on these studies by quantifying changes in coral color intensity, *Symbiodinium* photochemical efficiencies of photosystem II (F_v/F_m), and transcriptomic responses of *Symbiodinium in hospite*. We combine these physiological and transcriptomic observations of the symbiont with previously

published observations of the host to advance our understanding of their combined response to thermal and acidification stress, thereby improving our ability to predict a corals' ecological trajectory in response to these global change stressors.

MATERIALS AND METHODS

Experimental Design

Physiological measurements, transcriptomes, and gene expression analyses presented here build upon experiments previously published by Castillo et al. (2014) and Davies et al. (2016). Briefly, whole *S. siderea* colonies from nearshore ($N = 6$) and forereef ($N = 6$) reef zones were collected from the Belize Mesoamerican Barrier Reef (total colonies = 12) in July 2011 and transported to the University of North Carolina at Chapel Hill (UNC) Aquarium Research Center. At UNC, whole coral colonies were sectioned into 18 fragments, each of which were maintained in one of six experimental treatments (3 tanks per treatment; 18 tanks total). Treatments tested the effects of temperature and pCO$_2$ independently. The pCO$_2$ treatments corresponded to near-pre-industrial (P324: $324 \pm 89\,\mu$atm, $28.14 \pm 0.27°$C), predicted end-of-century (P604: $604 \pm 107\,\mu$atm, $28.04 \pm 0.28°$C), and an extreme mid-millennium scenario (P2553: $2553 \pm 506\,\mu$atm, $27.93 \pm 0.19°$C). Temperature treatments spanned the monthly minimum and maximum seawater temperatures (T25: $25.01 \pm 0.14°$C, $515 \pm 92\,\mu$atm; T28: $28.14 \pm 0.27°$C, $324 \pm 89\,\mu$atm, T32: $32.01 \pm 0.17°$C, pCO$_2$ $472 \pm 86\,\mu$atm) over the 2002–2014 interval (Castillo and Helmuth, 2005; Castillo and Lima, 2010; Castillo et al., 2012). The same treatment condition was used for pCO$_2$ and temperature controls, which represented near-present-day pCO$_2$ and annual mean temperature (Control: $477 \pm 83\,\mu$atm, $28.16 \pm 0.24°$C). Tanks were illuminated with 250 μmol photons m^{-2} s^{-1} on a 12-h light-dark cycle for 95 days. Upon completion of the experiment, tissue (coral + symbiont) was extracted by water pick, immediately preserved in RNAlater, and stored at $-80°$C until RNA was extracted.

Symbiodinium Physiology: Coral Color and Photochemical Efficiency

Coral color from standardized photographs has been historically used as an indicator of chlorophyll A concentration and symbiont density, and more generally as an indicator of coral bleaching (i.e., Winters et al., 2009; Kenkel et al., 2013; Chakravarti et al., 2017). Coral fragments were photographed with a standardized color scale at the end of the 95-day experiment prior to tissue extraction. Adobe Photoshop was first used to balance exposures, which were standardized across photographs using a white standard. Total red, green, blue, and sums of all color channel intensities (red, green, blue) were normalized relative to the standardized color scale in each photo and calculated for 10 quadrats of 25×25 pixels within each coral fragment as a measure of brightness, with higher brightness indicating a reduction in coral color (i.e., reduced symbiont density and chlorophyll a concentration). This analysis was performed following Winters et al. (2009) using the MATLAB macro "AnalyzeIntensity." Resulting data were inverted so that higher values represent increased coral pigmentation (i.e., increased symbiont density and chlorophyll a concentration).

One-time measurements of the maximum photosynthetic efficiency of photosystem II (F$_v$/F$_m$; where F$_v$ = F$_m$ – F$_o$; and F$_v$, F$_m$, and F$_o$ are variable, maximum, and minimum fluorescence, respectively) were obtained on day 94 of the experiments with an underwater PAM fluorometer (saturation width 0.80 s of >5,000 μmol photon m^{-2} s^{-1} saturation light pulse; *Diving-PAM, Walz*, Germany) at 20:00 h, 2 h after daytime illumination ended to ensure that non-photochemical quenching was suppressed and that the corals were adequately dark-adapted (three measurements per coral fragment). Reductions in F$_v$/F$_m$ values are indicative of sustained damage to the photosystem.

All statistical analyses were implemented in R (R Development Core Team, 2015) using the ANOVA function based on log-transformed sum of all color channels and F$_v$/F$_m$ data. The sum of all channels was chosen as the color metric proxy here since these data correlated best with F$_v$/F$_m$ (**Figure 1B**; Adjusted r^2: 0.7366, $p < 0.001$). Experimental treatment and reef zone were modeled as fixed effects and significant differences across levels within factors were evaluated using *post-hoc* Tukey's HSD tests. All assumptions of parametric testing were explored using diagnostic plots in R.

Transcriptome Assembly, Annotation, and *Symbiodinium* Identification

Detailed descriptions of sequence library preparations, transcriptome assembly, and separation of host and symbiont contigs can be found in Davies et al. (2016). Briefly, RNA was isolated from 94 coral fragments and pooled by reef zone within each experimental treatment, yielding a total of 12 sequencing libraries (**Table 1**), each of which contained RNA from at least six fragments. Since each coral can host a community of *Symbiodinium*, RNA pooling is not expected to yield any additional complications compared to preparing each coral independently. Pooled libraries were prepared and sequencing was performed using four lanes of *Illumina HiSeq* 2000 at the UNC—Chapel Hill High Throughput Sequencing Facility, which yielded paired-end (PE) 100 bp reads (**Table 1**).

Transcriptome *de novo* assembly of over 770 million PE reads was completed using Trinity (Grabherr et al., 2011) and resulting contigs were assigned as either coral- or *Symbiodinium*-derived contigs using a translated blast search (tblastx) against host and symbiont specific databases (Davies et al., 2016). *Symbiodinium*-specific contigs were annotated by BLAST sequence homology searches against UniProt and Swiss-Prot NCBI NR protein databases with an *e*-value cutoff of e^{-5} (UniProt Consortium, 2015). Annotated sequences were then assigned to Gene Ontology (GO) categories. Transcriptome contiguity analysis (Martin and Wang, 2011) and Benchmarking Universal Single-Copy Orthologs v2 (BUSCO; Simão et al., 2015) were used to assess *Symbiodinium* transcriptome quality and completeness.

Symbiodinium Community Composition

To compare *Symbiodinium* expression across reef zones and experimental treatments, it was first necessary to determine
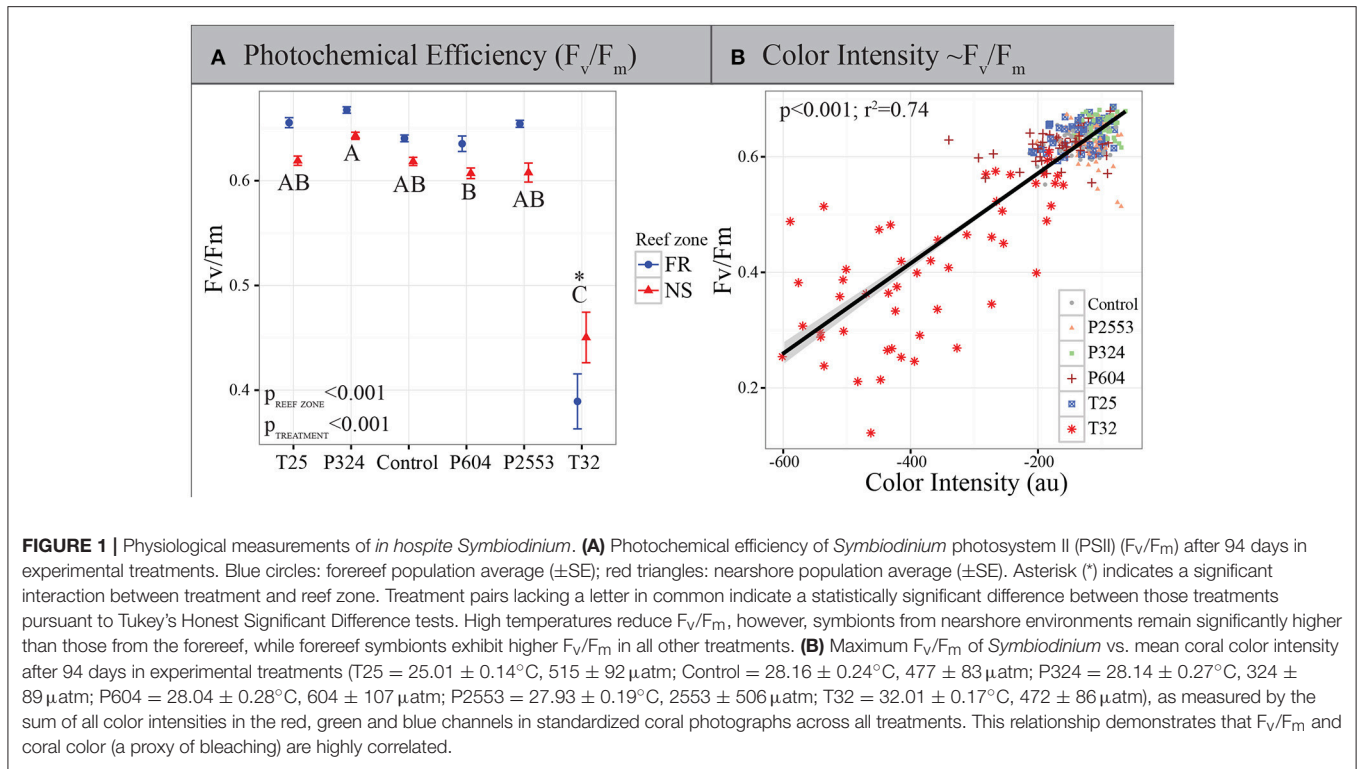
FIGURE 1 | Physiological measurements of *in hospite Symbiodinium*. **(A)** Photochemical efficiency of *Symbiodinium* photosystem II (PSII) (F_v/F_m) after 94 days in experimental treatments. Blue circles: forereef population average (±SE); red triangles: nearshore population average (±SE). Asterisk (*) indicates a significant interaction between treatment and reef zone. Treatment pairs lacking a letter in common indicate a statistically significant difference between those treatments pursuant to Tukey's Honest Significant Difference tests. High temperatures reduce F_v/F_m, however, symbionts from nearshore environments remain significantly higher than those from the forereef, while forereef symbionts exhibit higher F_v/F_m in all other treatments. **(B)** Maximum F_v/F_m of *Symbiodinium* vs. mean coral color intensity after 94 days in experimental treatments (T25 = 25.01 ± 0.14°C, 515 ± 92 μatm; Control = 28.16 ± 0.24°C, 477 ± 83 μatm; P324 = 28.14 ± 0.27°C, 324 ± 89 μatm; P604 = 28.04 ± 0.28°C, 604 ± 107 μatm; P2553 = 27.93 ± 0.19°C, 2553 ± 506 μatm; T32 = 32.01 ± 0.17°C, 472 ± 86 μatm), as measured by the sum of all color intensities in the red, green and blue channels in standardized coral photographs across all treatments. This relationship demonstrates that F_v/F_m and coral color (a proxy of bleaching) are highly correlated.

TABLE 1 | Summary of RNA libraries, including the number of corals from which RNA was pooled ("Pool"), reef zone where corals were collected (forereef = "FR" and nearshore = "NS"), temperature (°C) ±SD, pCO_2 (μatm) ±SD, raw 100 bp paired-end reads ("PE Reads"), mapped reads to the coral host, and symbiont ("Mapped") and total number of *Symbiodinium goreaui*-specific read counts ("Symbiont").

Sample	Pool	Reef zone	Temp (°C)	pCO_2 (μatm)	PE reads (10^6)	Mapped (10^6)	Symbiont (10^6)
FR_P324	6	FR	28.14 ± 0.27	324 ± 89	63.6	59.2	24.1
FR_P604	6	FR	28.04 ± 0.28	604 ± 107	77.1	72.3	16.8
FR_P2553	7	FR	27.93 ± 0.19	2553 ± 506	48.1	44.7	17.1
NS_P324	6	NS	28.14 ± 0.27	324 ± 89	88.3	82.4	35.0
NS_P604	8	NS	28.04 ± 0.28	604 ± 107	48.5	45.3	10.5
NS_P2553	6	NS	27.93 ± 0.19	2553 ± 506	69.2	64.6	23.3
FR_T25	11	FR	25.01 ± 0.17	515 ± 92	50.9	47.4	20.5
FR_Control	14	FR	28.16 ± 0.24	477 ± 83	63.2	58.8	24.3
FR_T32	9	FR	32.01 ± 0.17	472 ± 86	50.0	47.2	1.2
NS_T25	7	NS	25.01 ± 0.17	515 ± 92	100.0	93.1	39.9
NS_Control	7	NS	28.16 ± 0.24	477 ± 83	56.7	52.7	23.6
NS_T32	7	NS	32.01 ± 0.17	472 ± 86	54.7	51.5	3.9
				TOTAL	770.3	719.2	235.1

Shaded samples were excluded from analyses due to low read counts arising from low RNA yields, resulting in the availability of 235.1 million reads for downstream analyses.

which *Symbiodinium* lineages were present in each library. Reads were trimmed with Fastx_toolkit (<20 bp length cutoff and bp quality score >20) and resulting quality filtered reads were then mapped to an ITS2-specific database (Franklin et al., 2012) using Bowtie 2.2.0 with the—a flag to search all possible alignments (Langmead and Salzberg, 2012). All possible alignments were identified and then the read was quantified in the analysis if all possible alignments for that read mapped to a single reference lineage. This analysis determined that 96.7–100% of

mapped reads from 10 of the 12 libraries exclusively aligned to a single *Symbiodinium* lineage (*Symbiodinium goreaui* C1), with remaining libraries [nearshore 32°C (NS_T32) and forereef 32°C (FR_T32)] hosting divergent communities consisting of *S. goreaui* (C1) and *Symbiodinium trenchii* (D1a). However, these two libraries hosting divergent communities were excluded from downstream expression analysis due to temperature-induced symbiont loss causing low symbiont concentrations, which ultimately led to insufficient quantities of mapped *Symbiodinium*

reads (1.2 and 3.9 million) relative to other libraries (10.5–39.9 million).

Mapping and Differential Expression Analysis for *Symbiodinium Goreaui*

Raw reads across samples ranged from 48.1 to 100.0 million PE 100 bp sequences. Quality filtered reads were mapped to the combined host-symbiont transcriptome (*S. siderea* + *S. goreaui*) and *S. goreaui* mapped reads ranged from 10.5 (NS_P604) to 39.9 (NS_T25) million (**Table 1**). Differential gene expression analyses were performed on raw counts with *DESeq2* [v. 1.6.3; (Love et al., 2014) in R (v. 3.1.1; R Development Core Team, 2015)] using the model: design ∼ reef zone + treatment. Raw counts are available in **Supplementary Table 1**. Counts were normalized for size factor differences and pairwise contrasts were computed for each treatment relative to the control and between the two reef zones. A contig was considered significantly differentially expressed if it had an average basemean expression >5 and an FDR adjusted $p < 0.05$ (Benjamini and Hochberg, 1995). Raw counts were then *rlog* normalized, a principle coordinate analysis was performed, and the *adonis* function tested for overall expression differences across treatments and reef zones (Oksanen et al., 2018). Gene expression heatmaps with hierarchical clustering of expression profiles were created with the *pheatmap* package in R (Kolde, 2012). Gene ontology (GO) enrichment analysis was performed using the GO_MWU method, which uses adaptive clustering of GO categories and Mann–Whitney *U*-tests (Voolstra et al., 2011) based on a ranking of signed log *p*-values (Dixon et al., 2015). Results were plotted as a dendrogram, which traces the level of gene sharing between significant categories and lists the proportion of genes in the dataset with raw $p < 0.05$ relative to the total number of genes within the entire expression dataset.

Expression Comparison Across Symbiotic Partners

To compare expression across symbiotic partners, a subset of highly conserved genes (HCG) from the coral host (*S. siderea*) and *S. goreaui* were mined based on conserved gene annotation, which were determined based on BLAST sequence homology searches against UniProt and Swiss-Prot NCBI NR protein databases ($e < e^{-5}$; UniProt Consortium, 2015 ($N = 2,862$ genes; **Supplementary Table 2**). Differential gene expression analyses were performed with *DESeq2* using only count data from the HCG set (design ∼ reef zone + partner.treatment) (**Supplementary Table 3**). Counts were normalized for size factor differences and pairwise contrasts were computed for each treatment relative to the control for each symbiotic partner. A contig was considered a significantly differentially expressed gene (DEG) if it had an average basemean expression >5 and an FDR adjusted $p < 0.05$ (Benjamini and Hochberg, 1995). Significant DEGs were then compared across symbiotic partners. In order to confirm that *S. goreaui* gene expression results were not confounded by strong reef zone differences in expression, heatmaps were generated on the HCG panel that

was significantly differentially expressed in the host but not the symbiont.

RESULTS

Reef Zone Variation in *Symbiodinium* Photophysiology

Long-term thermal stress treatments resulted in significantly reduced F_v/F_m of *Symbiodinium* ($p < 0.001$; **Figure 1A**), which was highly correlated with reductions in coral color intensity across all treatments ($p < 0.001$, $r^2 = 0.74$; **Figure 1B**), but particularly in the high-temperature treatment (Adjusted r^2: 0.6183, $p < 0.001$; **Supplementary Figure 1A**). Interestingly, *Symbiodinium* originating from nearshore habitats had significantly higher F_v/F_m under thermal stress when compared to forereef *Symbiodinium* (Tukey's HSD $p = 0.008$), suggesting increased photoacclimation or local adaptation of nearshore symbionts to warmer temperatures. However, nearshore *Symbiodinium* only exhibited significantly higher F_v/F_m compared to forereef symbionts in the 32°C treatment, although coral color intensity did not differ between reef zones under control conditions or under thermal stress (**Supplementary Figure 1B**). In all other experimental treatments, forereef symbionts had constitutively higher photochemical efficiencies (**Figure 1A**; $p < 0.001$).

Symbiodinium Goreaui Transcriptome

Sequencing data suggest that *S. siderea* predominantly hosted *S. goreaui* (C1), which corroborates previous ITS2 sequencing work that found *S. siderea* from Belize hosts primarily C1 (Baumann et al., 2018). Although several clade C *Symbiodinium* transcriptomes are publicly available, we assembled a novel transcriptome since our samples were derived from *S. siderea* and clade C *Symbiodinium* are known to exhibit host specificity (Thornhill et al., 2014) and may therefore be divergent from previously assembled transcriptomes of clade C *Symbiodinium* hosted by the Pacific coral *Acropora hyacinthus* (Barshis et al., 2014) and the Caribbean anemone *Discosoma sanctithomae* (Rosic et al., 2015). A total of 1,255,626,250 reads were retained after adapter trimming and quality filtering (81.5%; 69.6% paired, 11.9% unpaired). The resulting metatranscriptome contained 333,835 contigs (N50 = 1,673), of which 65,838 were unambiguously assigned as *Symbiodinium* specific contigs, with an average length of 1,482 bp and an N50 of 1,746. Among symbiont contigs, 45,947 unique isogroups were obtained, of which 22,239 (48.4%) had gene annotations based on sequence homology. Thirty-nine percent of *Symbiodinium* contigs had protein coverage exceeding 0.75 (**Supplementary Figure 1**) and results from BUSCO suggest that 81.9% of complete and fragmented BUSCOs were present (77.6% complete, 4.3% fragmented), indicating that the transcriptome was fairly comprehensive. All raw reads are archived in the National Center for Biotechnology Information (NCBI) Short Read Archive (SRA) under accession number PRJNA307543, with transcriptome assembly and annotation files available at www.bco-dmo.org/project/635863.

Altered *Symbiodinium* Communities Under Thermal Stress

Analysis of sequencing libraries for *Symbiodinium* maintained at the low (25°C) and control (28°C) temperature treatments (for all pCO_2 treatments) revealed that 96.7–100% of reads mapped exclusively to one clade ($N = 30$–436) aligned to *S. goreaui* (C1; **Figure 2**). Analysis of sequencing libraries for *Symbiodinium* maintained at high temperature treatments [nearshore 32°C (NS_T32); forereef 32°C (FR_T32)] consisted of divergent *Symbiodinium* communities hosting 50–75% *Symbiodinium* D1a (**Figure 2**). However, both libraries had very few mapped reads (NS_T32: 16, FR_T32: 4). Therefore, these two libraries were removed from all downstream gene expression analyses due to their divergent ITS2-derived communities, which have been shown to exhibit strong transcriptomic differences (Barshis et al., 2014). These libraries also had the lowest mapped *Symbiodinium* reads (**Table 1**), most likely due to reduced symbiont RNA available under thermal stress.

Minimal Response to Acidification Stress, With Population-Specific Differences in Expression of Genes Associated With Photosynthesis

Without consideration of the high temperature treatment libraries that were excluded due to low mapped reads, transcriptomic analysis of *S. goreaui* gene expression across the remaining treatments exhibited few DEGs relative to the control treatment, with 92 (25°C), 40 (324 μatm), 576 (604 μatm), and 23 (2,553 μatm) DEGs observed for the respective treatments relative to co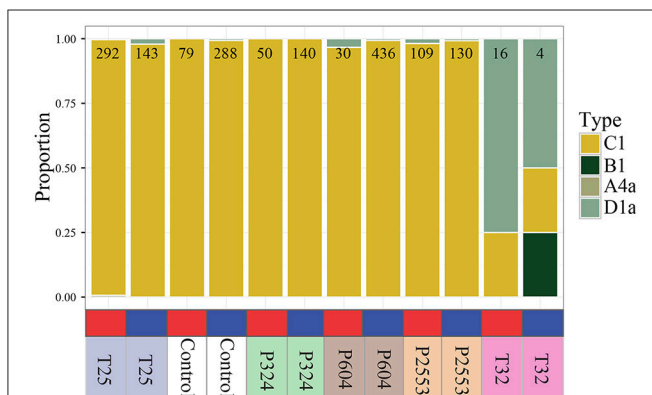ntrol treatments, representing a total of 1.69% of the entire transcriptome responding to any treatment (**Figure 3A**; **Supplementary Table 4**). In contrast, 24.45% of genes were differentially expressed with respect to reef zone, with 3,792 upregulated in nearshore specimens and 6,441 upregulated in forereef specimens, regardless of treatment condition—demonstrating a strong whole-transcriptomic response to reef zone (**Figures 3B**, **4A**, $p = 0.016$; **Supplementary Table 4**). No significant differences in whole-transcriptome response were detected across experimental treatments ($p = 0.33$; **Figure 3B**). Gene ontology enrichment analysis across reef zones revealed many significantly enriched GO terms within cellular components, which were dominated by GO terms associated with photosynthesis in forereef *S. goreaui* [e.g., *thylakoid part* (GO:0044436), *photosystem* (GO:0009521), *light-harvesting complex* (GO:0030076), *plastid part* (GO:0044435)], suggesting fundamentally different regulation of photosynthetic genes across reef zones (**Figure 4B**). Genes associated with these GO categories can be found in **Supplementary Table 5**.
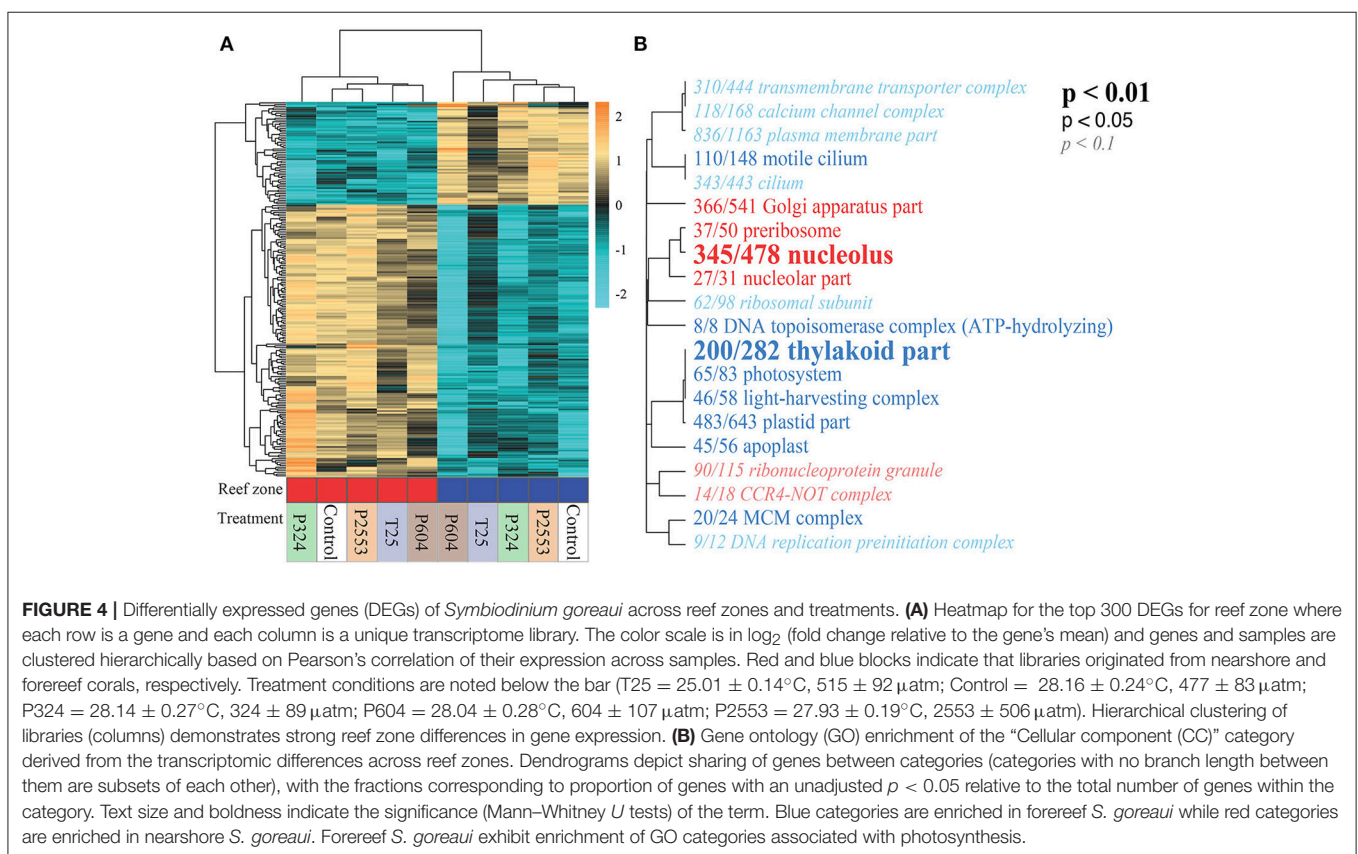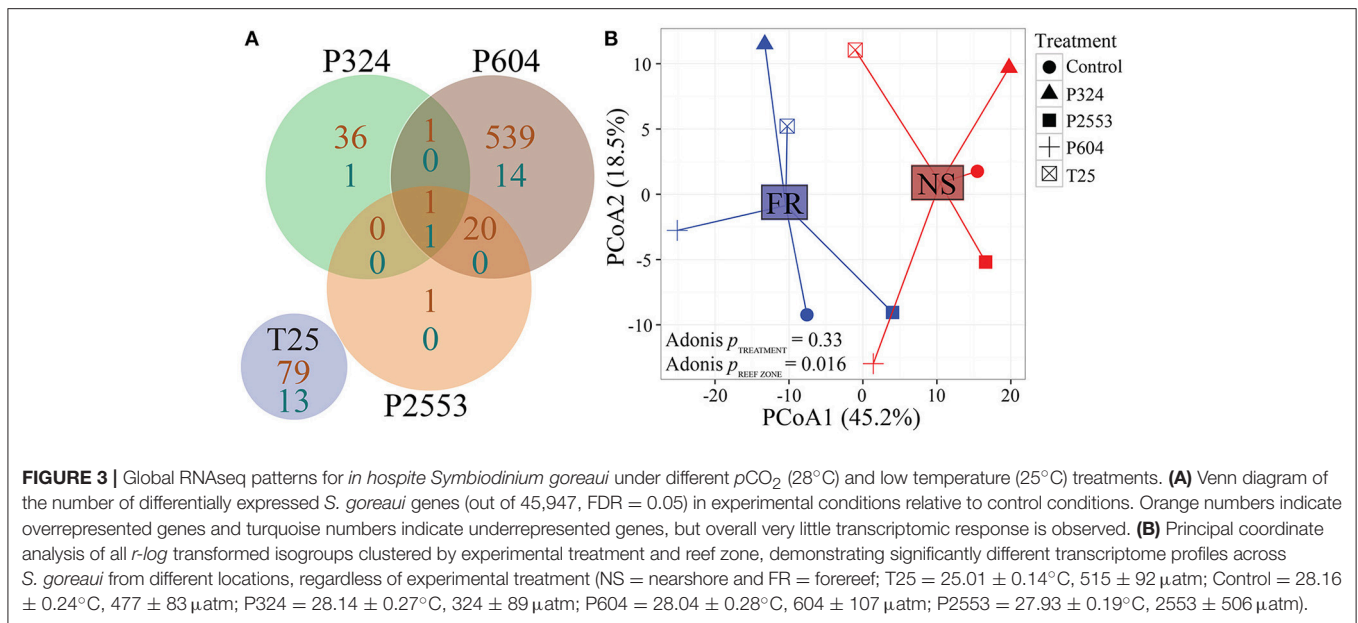
Coral Host Elicits Stronger Transcriptomic Response Than *Symbiodinium Goreaui*

Contrasting gene expression patterns of the HCG sets of the coral host (*S. siderea*) and *in hospite S. goreaui* ($N = 2,862$; **Supplementary Table 2**) reveal that coral hosts were far more transcriptionally responsive to experimental treatments than their *Symbiodinium* partners (**Figure 5**). Among these HCGs, coral hosts modified expression by 3.7–15.7% across experimental treatments, while *S. goreaui* only modified expression by 0.05–2.8% across the same treatments, demonstrating that coral hosts were far more responsive to low temperature and acidification stress when compared to their algal symbionts (**Figure 5**). Heatmaps also confirmed that the lack of differential expression of HCG within *S. goreaui* was not driven by high levels of within-treatment variance arising from strong differences in *S. goreaui* gene expression across reef zones. Instead, genes that were highly differentially expressed in the host showed no differences in expression across reef zones for *S. goreaui* (**Supplementary Figures 2A–D**), although other genes within *S. goreaui* were differentially expressed across reef zone.

DISCUSSION

Increased Thermotolerance of *Siderastrea siderea* From More Thermally Variable Environments
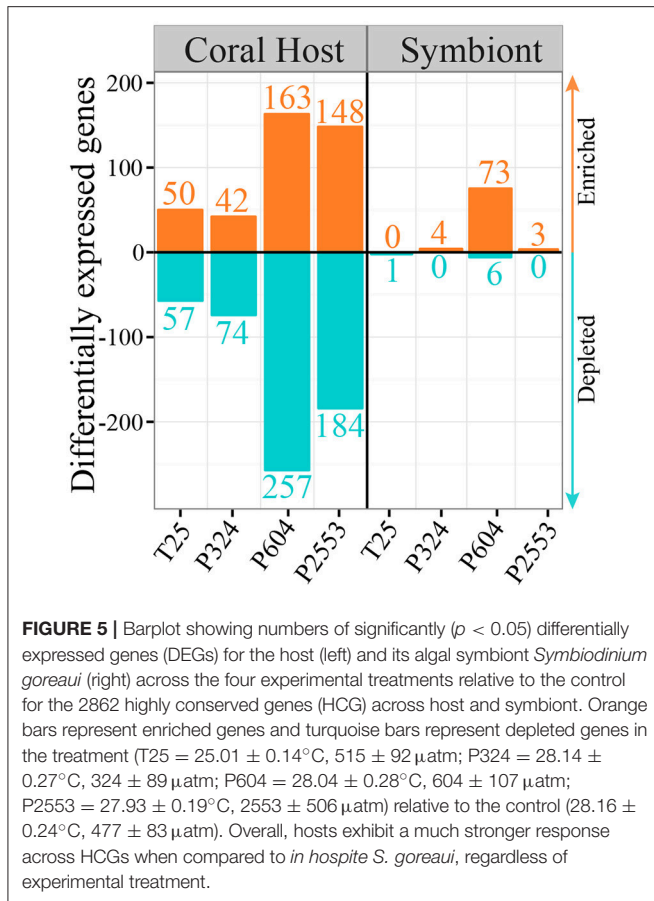
Reductions in *Symbiodinium* photosynthetic ability have been strongly correlated with a lack of thermal tolerance and increased susceptibility to bleaching (Warner et al., 1999; Takahashi et al., 2009; Howells et al., 2012). Here, we observed strong reductions in photochemical efficiency in response to elevated temperatures (**Figure 1A**), which has been consistently observed for *Symbiodinium* exposed to thermal stress (Warner et al., 1996; Roth, 2014). Strong reductions in calcification rate (Castillo et al., 2014) and overall host gene expression reflecting disruption of homeostasis (Davies et al., 2016) was previously observed for these same coral specimens in response to the prescribed thermal

FIGURE 2 | Proportion of RNAseq reads mapping exclusively to one *Symbiodinium* lineage (A4a, B1, C1, D1a) for each transcriptome library, with independent columns representing a unique transcriptome library. Number at top of bar indicates total number of reads mapping exclusively to one clade within that library. Red and blue blocks indicate that the library originated from nearshore and forereef corals, respectively. Treatment conditions are noted below the bar (T25 = 25.01 ± 0.14°C, 515 ± 92 μatm; Control = 28.16 ± 0.24°C, 477 ± 83 μatm; P324 = 28.14 ± 0.27°C, 324 ± 89 μatm; P604 = 28.04 ± 0.28°C, 604 ± 107 μatm; P2553 = 27.93 ± 0.19°C, 2553 ± 506 μatm; T32 = 32.01 ± 0.17°C, 472 ± 86 μatm). All libraries were dominated by *Symbiodinium goreaui* (C1), excepting the T32 treatments that were dominated by *Symbiodinium trenchii* (D1a).

FIGURE 3 | Global RNAseq patterns for *in hospite Symbiodinium goreaui* under different pCO_2 (28°C) and low temperature (25°C) treatments. **(A)** Venn diagram of the number of differentially expressed *S. goreaui* genes (out of 45,947, FDR = 0.05) in experimental conditions relative to control conditions. Orange numbers indicate overrepresented genes and turquoise numbers indicate underrepresented genes, but overall very little transcriptomic response is observed. **(B)** Principal coordinate analysis of all *r-log* transformed isogroups clustered by experimental treatment and reef zone, demonstrating significantly different transcriptome profiles across *S. goreaui* from different locations, regardless of experimental treatment (NS = nearshore and FR = forereef; T25 = 25.01 ± 0.14°C, 515 ± 92 μatm; Control = 28.16 ± 0.24°C, 477 ± 83 μatm; P324 = 28.14 ± 0.27°C, 324 ± 89 μatm; P604 = 28.04 ± 0.28°C, 604 ± 107 μatm; P2553 = 27.93 ± 0.19°C, 2553 ± 506 μatm).

FIGURE 4 | Differentially expressed genes (DEGs) of *Symbiodinium goreaui* across reef zones and treatments. **(A)** Heatmap for the top 300 DEGs for reef zone where each row is a gene and each column is a unique transcriptome library. The color scale is in log$_2$ (fold change relative to the gene's mean) and genes and samples are clustered hierarchically based on Pearson's correlation of their expression across samples. Red and blue blocks indicate that libraries originated from nearshore and forereef corals, respectively. Treatment conditions are noted below the bar (T25 = 25.01 ± 0.14°C, 515 ± 92 μatm; Control = 28.16 ± 0.24°C, 477 ± 83 μatm; P324 = 28.14 ± 0.27°C, 324 ± 89 μatm; P604 = 28.04 ± 0.28°C, 604 ± 107 μatm; P2553 = 27.93 ± 0.19°C, 2553 ± 506 μatm). Hierarchical clustering of libraries (columns) demonstrates strong reef zone differences in gene expression. **(B)** Gene ontology (GO) enrichment of the "Cellular component (CC)" category derived from the transcriptomic differences across reef zones. Dendrograms depict sharing of genes between categories (categories with no branch length between them are subsets of each other), with the fractions corresponding to proportion of genes with an unadjusted $p < 0.05$ relative to the total number of genes within the category. Text size and boldness indicate the significance (Mann–Whitney U tests) of the term. Blue categories are enriched in forereef *S. goreaui* while red categories are enriched in nearshore *S. goreaui*. Forereef *S. goreaui* exhibit enrichment of GO categories associated with photosynthesis.

stress. These severe impairments in physiological measures of the host in response to chronic high temperatures might be driven, in part, by the observed reductions in symbiont photochemical efficiency (**Figure 1A**).

Notably, we observed that reductions in *S. siderea* symbiont photophysiology at 32°C were dependent on a coral's natal reef location, where the photochemical efficiency (**Figure 1A**) and coral color intensity (**Figure 1B**) of nearshore *Symbiodinium* were significantly higher under thermal stress than those of forereef *Symbiodinium*. Since *Symbiodinium* photophysiology is often associated with thermal tolerance (Warner et al., 1996, 1999; Ragni et al., 2010; Wang et al., 2012), these fluctuating

FIGURE 5 | Barplot showing numbers of significantly ($p < 0.05$) differentially expressed genes (DEGs) for the host (left) and its algal symbiont *Symbiodinium goreaui* (right) across the four experimental treatments relative to the control for the 2862 highly conserved genes (HCG) across host and symbiont. Orange bars represent enriched genes and turquoise bars represent depleted genes in the treatment (T25 = 25.01 ± 0.14°C, 515 ± 92 μatm; P324 = 28.14 ± 0.27°C, 324 ± 89 μatm; P604 = 28.04 ± 0.28°C, 604 ± 107 μatm; P2553 = 27.93 ± 0.19°C, 2553 ± 506 μatm) relative to the control (28.16 ± 0.24°C, 477 ± 83 μatm). Overall, hosts exhibit a much stronger response across HCGs when compared to *in hospite S. goreaui*, regardless of experimental treatment.

responses may be due to photoacclimation or adaptation of *Symbiodinium* to more thermally variable nearshore environments (Castillo and Helmuth, 2005; Baumann et al., 2016) or to variation in light availability across reef environments. Indeed, Castillo et al. (2012) observed reductions in linear extension rates over the past three decades of anthropogenic warming within cores of forereef *S. siderea* colonies, while cores of nearshore, and backreef colonies exhibited constant (nearshore) or increasing (backreef) extension rates over the same interval. Castillo et al. (2012) attributed these differential trends in linear extension to thermal preconditioning of backreef and nearshore corals, to the extent that nearshore and backreef environments experience more extreme baseline diurnal and seasonal fluctuations in seawater temperature compared to more thermally stable forereef environments. However, differences in light levels across reef environments and seasons have also been shown to influence cross-reef differences in a coral's response to anthropogenic warming and bleaching (Iglesias-Prieto et al., 2004) and may also contribute to *Symbiodinium* performance.

The potential for local adaption of *Symbiodinium* is thought to be high given their haploid genomes and short generation times (Santos and Coffroth, 2003; Correa and Baker, 2011). Howells et al. (2012) observed increased thermotolerance of *Symbiodinium* C1 originating from warmer reef locations, both *in hospite* and in culture, and Hume et al. (2016) detected strong

selection for thermal tolerance in *S. thermophilium*, a lineage found in extreme temperatures of the Persian Gulf. Recent work has also revealed that *Symbiodinium* can rapidly evolve thermal tolerance in culture (Chakravarti et al., 2017), providing further evidence of *Symbiodinium*'s potential for local adaptation to thermal stress. However, these variations in photochemical efficiencies in nearshore *Symbiodinium* may also be attributed to the relative changes in ITS2-determined symbiont communities [from *S. goreaui* (C1) to *S. trenchii* (D1a): **Figure 2**], which warrant future exploration.

Chronic High Temperatures Cause Loss of *Symbiodinium Goreaui*

Symbiodinium genetic diversity has been reported to vary not only among different coral host species and environments, but also within a single species of coral (Baker, 2003; Coffroth and Santos, 2005; Thornhill et al., 2014, 2017). This genetic diversity has been shown to drive physiological variation within *Symbiodinium* (Iglesias-Prieto and Trench, 1994; Warner et al., 1999; Iglesias-Prieto et al., 2004) and numerous studies have demonstrated that thermo-tolerance varies amongst lineages of *Symbiodinium* (Robison and Warner, 2006; Suggett et al., 2008). Consequently, some lineages of *Symbiodinium* appear less affected by thermal stress, while others are impacted by even small changes in temperature (Rowan, 2004; Berkelmans and Van Oppen, 2006; Jones and Berkelmans, 2010; Hume et al., 2016).

Here, we observed that long-term thermal stress induced compositional shifts in *Symbiodinium* communities from *S. goreaui* (C1) to *S. trenchii* (D1a) in *S. siderea*, regardless of natal reef zone (**Figure 2**). However, this result is at least partially due to a loss of symbiosis with *S. goreaui*, rather than *S. trenchii* becoming more successful *in hospite* under increased temperatures since corals were exhibiting color loss evident of bleaching (**Figure 1B**). These results are consistent with prior work showing that *S. siderea* from southern Belize primarily host *S. goreaui* (C1) and *S. trenchii* (D1a) (Baumann et al., 2018), although Finney et al. (2010) also observed that shallow water *S. siderea* from Carrie Bow Caye (>100 km north of the sites sampled here) hosted *Symbiodinium* C3, which was not found here.

Symbiodinium trenchii (D1a) are generally associated with hosts from marginal reef environments exposed to increased thermal stress (Pettay et al., 2011, 2015; Pettay and Lajeunesse, 2013). Correspondingly, Pacific *Acropora millepora* corals hosting predominantly clade D *Symbiodinium* have been shown to exhibit greater thermal tolerance when compared to those corals hosting *Symbiodinium* C2 (Berkelmans and Van Oppen, 2006; Jones et al., 2008). However, there appears to be a trade-off associated with hosting more thermally tolerant symbionts, as *A. millepora* hosting clade D *Symbiodinium* were observed to grow significantly slower than corals hosting C2 under both control and field conditions (Jones and Berkelmans, 2010). Although it is unclear from the data at hand if the *Symbiodinium* communities tested here would shift back to being dominated by *S. goreaui* after the alleviation of thermal stress (Sampayo et al., 2016), these shifts could represent acclimation potential for corals exposed

to future ocean warming (Cunning et al., 2015; Silverstein et al., 2015).

Symbiodinium Gene Expression and Photochemical Efficiency Are Unresponsive to CO_2-Induced Acidification

Increases in atmospheric pCO_2 have been shown to reduce calcification rates of reef-building corals (Kleypas et al., 1999; Hoegh-Guldberg et al., 2007; Chan and Connolly, 2013; Castillo et al., 2014), but the effects of pCO_2 on corals' algal symbionts are less clear. Some studies report that elevated pCO_2 enhances *Symbiodinium* primary production, suggesting that the concentration of dissolved inorganic carbon in seawater—elevated under high pCO_2–is the limiting substrate for photosynthesis (Crawley et al., 2010; Brading et al., 2011). In contrast, others have observed negative effects of increased pCO_2 on *Symbiodinium* physiology, including reduced productivity, photochemical efficiency, and calcification (Anthony et al., 2008; Zhou et al., 2016). Here, we observed minimal effects of elevated pCO_2 on *Symbiodinium* photochemical efficiencies across a wide range of pCO_2 conditions (**Figure 1A**). This lack of response starkly contrasts the strong physiological response (i.e., impaired photophysiology) and community shifts (i.e., C1–D1a) of *Symbiodinium* under increased temperatures (**Figures 1**, **2**). One explanation for this lack of physiological response to pCO_2 could be that the specific *S. goreaui* populations investigated here are simply not stressed by elevated pCO_2, which has been observed for other *Symbiodinium* strains (Brading et al., 2011).

This lack of *S. goreaui* physiological response to acidification stress was also evident at the whole-transcriptome level, where no significant differences in overall gene expression were observed across low temperature and variable pCO_2 treatments (**Figure 3**), even when reef zone-specific responses were considered (**Supplementary Figure 2**). This paucity of a transcriptional stress response in *Symbiodinium* in response to stress is consistent with previous studies, which generally observe little to no transcriptional responses to environmental stressors (Leggat et al., 2011; Putnam et al., 2013; Barshis et al., 2014), with the exception of extreme heat stress (Baumgarten et al., 2013; Levin et al., 2016; Gierz et al., 2017)—which we were unable to investigate here due to a combination of low symbiont RNA yield (**Figure 1A**) and compositional changes in *Symbiodinium* communities under elevated temperature (**Figure 2**).

Instead of responding transcriptionally, it has been proposed that *Symbiodinium* use post-transcriptional regulatory mechanisms, including translational regulation, and post-translational modifications, to drive molecular responses. Evidence suggests that very few transcription factors are present in *Symbiodinium* transcriptomes and genomes (Bayer et al., 2012; Shoguchi et al., 2013). Instead, it has been proposed that these algae utilize small RNAs and microRNAs, (Baumgarten et al., 2013; Lin et al., 2015), RNA-editing (Liew et al., 2017), and *trans*-splicing of spliced leader sequences (Zhang et al., 2007; Lin et al., 2010; Lin, 2011) to regulate their environmental stress responses. Another possibility is that the timescale of this study (95 days) was insufficient to trigger physiological and

molecular responses in *Symbiodinium*. Although the stability of dinoflagellate mRNA is known to be considerably longer than for other organisms (Morey and Van Dolah, 2013), it is possible that physiological and transcriptomic responses were minimized by long-term acclimatization of *Symbiodinium* via phenotypic buffering (Reusch, 2014).

Lastly, and perhaps most likely, it could be that *in hospite Symbiodinium* simply do not respond to changes in pCO_2 because their positions within host-derived tissue-bound spaces buffer the algae from external changes in pH (Rands et al., 1993; Venn et al., 2009; Barott et al., 2015). However, determination of the specific factor(s) that account for these results requires further examination, as they cannot be assessed with the data at hand.

Reef Zone Differences in Photochemical Efficiency and Genes Related to Photosynthesis

Symbiodinium exist endosymbiotically across a variety of hosts and habitats, which presents these algae with diverse challenges with respect to photosynthesis and survival. Photochemical efficiency of photosystem II (F_v/F_m) is widely used as an indicator of photosynthetic performance and stress (Murchie and Lawson, 2013). Here, higher F_v/F_m values observed in forereef *Symbiodinium* relative to nearshore symbionts (**Figure 1A**) may indicate photoadaptation between the two populations of symbionts, since nearshore environments experience increased temperatures and nutrients along with reduced light levels relative to forereef habitats (Castillo and Lima, 2010; Baumann et al., 2016). These environmental differences may stimulate nearshore corals to rely more heavily on heterotrophy and dissolved/particulate organic matter relative to corals in light-replete forereef habitats (Grottoli et al., 2006; Tremblay et al., 2014).

It is important to note, however, that both nearshore and forereef *Symbiodinium* exhibited F_v/F_m > 0.6, which is generally considered healthy. Therefore, these data do not necessarily indicate that forereef hosts are receiving increased photo-assimilates from their *Symbiodinium*, which corroborates the comparable calcification rates for coral specimens from different reef zones within each experimental pCO_2-temperature treatment (Castillo et al., 2014). Instead, because the photochemical efficiencies of symbionts from forereef and nearshore corals were divergent even after 95 days in the same experimental treatments, we speculate that the environment could have selected for distinct nearshore and forereef *Symbiodinium* "ecotypes" that reflect photoadaptation to their natal reef zone (Iglesias-Prieto et al., 2004; Howells et al., 2012; Chakravarti et al., 2017)—although further work is required to test this hypothesis. Another possibility is that *S. goreaui* populations from the nearshore and forereef reef zones represent distinct species, although analyses of higher resolution loci are required to test this hypothesis.

Notably, reef-zone-specific photochemical efficiencies were also reflected at the transcriptomic level, where consistent differences in gene expression were observed (**Figures 3B**, **4A**). Divergent stable-state gene expression is perhaps not surprising

given that transcriptomic differences across clades (Barshis et al., 2014; Rosic et al., 2015) and across strains within a clade (Parkinson et al., 2016) have been previously observed. Gene ontology enrichment analysis of differential gene expression across reef zones detected strong upregulation of genes related to photosynthesis in forereef symbionts (**Figure 4B**), corroborating our observation of higher photochemical efficiencies for forereef *S. goreaui*. These differences in regulation of photosynthesis-related genes across reef zone could facilitate the cross-reef-zone differences in physiological responses to thermal stress observed in the present study (**Figure 1A**).

Thermally sensitive *Symbiodinium* have been shown to exhibit increased disruption of PSII photochemistry (Warner et al., 1999; Robison and Warner, 2006), which has been associated with variations in the regulation of genes involved in photosynthesis (Mcginley et al., 2012). Given that investigations into the differences in gene expression amongst *Symbiodinium* lineages consistently observe enrichment of photosynthesis-related genes (Baumgarten et al., 2013; Barshis et al., 2014; Rosic et al., 2015; Parkinson et al., 2016; this study), we propose the evolution of unique photoadaptive *Symbiodinium* "ecotypes" that can be differentiated by their transcriptomic signatures of photosynthesis-related genes.

Coral Hosts Elicit Stronger Transcriptomic Responses Than *Symbiodinium Goreaui*

Understanding how each partner of the coral–*Symbiodinium* symbiosis responds to environmental stress is required to accurately predict the susceptibility of corals reefs to future global change (Weis, 2008). Although both partners exhibit a wide array of physiological stress responses, *Symbiodinium* are assumed to initiate symbiosis breakdown (Berkelmans and Van Oppen, 2006; Stat et al., 2006; Stat and Gates, 2011) due to their production of ROS, which can damage the host (Lesser, 1996; Weis, 2008). In contrast, *S. siderea* exhibit stronger transcriptomic responses to thermal and CO_2-acidification stress than their *Symbiodinium* symbionts across a HCG (**Supplementary Table 2**) set (**Figure 5**), suggesting that symbiosis breakdown, or the process of "bleaching" in *S. siderea*, is initiated by the host instead of the symbiont. In addition, the observation that this color loss is associated with a shift from *S. goreaui* (C1) to the more thermotolerant *S. trenchii* (D1a) suggests that symbiont loss influences functional diversity of the symbiont community in a manner that supports thermal tolerance.

Strong transcriptomic responses of coral hosts are well-documented (Desalvo et al., 2010b; Meyer et al., 2011; Moya et al., 2012; Seneca and Palumbi, 2015; Davies et al., 2016), while *Symbiodinium* transcriptomic responses are generally more subtle (Leggat et al., 2011; Baumgarten et al., 2013; Barshis et al., 2014). For example, Barshis et al. (2014) similarly observed few transcriptional changes across >50,000 genes in response to thermal stress across two *Symbiodinium* lineages involved in symbiosis (D2, C3K), starkly contrasting the broad transcriptomic shifts observed in the symbiont's host when exposed to identical conditions (Barshis et al., 2013). As discussed

above, *S. goreaui* in our study may be unresponsive to the stressors investigated, or the stressors may not have been applied long enough to elicit a transcriptomic response. Alternatively, transcriptomic stability of *S. goreaui* could result from *in hospite* buffering of the symbiosome under pCO_2 stress, which has been observed in corals exposed to variable seawater pH (Rands et al., 1993; Venn et al., 2009; Barott et al., 2015) and in response to cold stress (Parkinson et al., 2015). Although potentially costly to the host, manipulation of *Symbiodinium* responses to stress through active regulation of the symbiont's environment might be favored over the potential chemical toxicity resulting from the release of reactive molecules by stressed *Symbiodinium* (e.g., Lesser, 1996).

CONCLUSION

Acclimation and adaptation play critical roles in determining an organism's ability to tolerate environmental variability (Schlichting and Pigliucci, 1996; Reusch, 2014). Here, we demonstrate that coral-associated *Symbiodinium* exhibit the potential for photoadaptation and/or photoacclimation to thermal and acidification stress. Photochemical efficiencies of *S. goreaui* from nearshore locations were more resilient to thermal stress when compared to forereef symbionts, suggestive of local adaptation across reef zones. We also observed differences in gene expression between *S. goreaui* from nearshore and forereef environments that were coupled with differences in photochemical efficiencies, irrespective of treatment condition. These transcriptomic differences suggest that photosynthesis-related gene expression varies by habitat, which may reflect photoadaptation to unique environments over potentially long timescales. Host retention of a more thermotolerant *S. trenchii* (D1a) under thermal stress was also documented, providing evidence for a potential mechanism of coral acclimation to thermal stress. Acclimation to pCO_2 was also observed at both physiological and transcriptomic levels, the mechanism(s) of which have not yet been identified, although pH-buffering by the host remains a viable hypothesis. Thus, we find evidence for both photoadaptation and photoacclimation in the *S. siderea-Symbiodinium* symbiotic relationship, which may explain the relative resilience of this coral species to global change stressors.

AUTHOR CONTRIBUTIONS

FUNDING

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2018.00150/full#supplementary-material

Supplementary Figure 1 | **(A)** Maximum F_V/F_m of *Symbiodinium* vs. mean coral color intensity after 94 days under experimental heat stress (T32 = 32.01 ± 0.17°C, 472 ± 86 μatm), as measured by the sum of all color intensities in the red, green and blue channels in standardized coral photographs across all treatments. This relationship demonstrates that F_V/F_m and coral color are highly correlated under thermal stress. **(B)** Coral color intensity after 94 days in control and thermal stress conditions. Blue circles: forereef population average (±SE); red triangles: nearshore population average (±SE). High temperatures reduce coral color intensity, however, symbionts from nearshore and forereef corals do not differ significantly within a treatment.

Supplementary Figure 2 | Heatmaps for the expression of highly conserved genes (HCG) found to be differentially expressed in the coral host for each experimental treatment. The left four columns represent expression of these genes in the coral host (orange blocks) and the right four columns represent expression of these same genes in *Symbiodinium goreaui* (green blocks) across the experimental treatments relative to the control (28.16 ± 0.24°C, 477 ± 83 μatm): **(A)** low temperature (T25 = 25.01 ± 0.14°C, 515 ± 92 μatm), **(B)** pre-industrial pCO_2 (P324 = 28.14 ± 0.27°C, 324 ± 89 μatm), **(C)** next-century pCO_2 (P604 = 28.04 ± 0.28°C, 604 ± 107 μatm), and **(D)** extreme-high pCO_2 (P2553 = 27.93 ± 0.19°C, 2553 ± 506 μatm). Red and blue blocks indicate that the library originated from nearshore and forereef reef zones, respectively. Each row is a gene and each column is a unique transcriptome library. The color scale is in $\log_2$ (fold change relative to the gene's mean) and genes are clustered hierarchically based on Pearson's correlation of their expression across samples. Results reveal lack of differential gene expression amongst experimental treatments relative to the control in *S. goreaui*, compared to strong differential gene expression amongst treatments in the host, and that this result is not due to differential gene expression amongst reef zones in *S. goreaui*.

Supplementary Table 1 | Raw Symbiont Counts.

Supplementary Table 2 | Normalized Counts of Highly Conserved Gene Set for Coral and Symbiont and their p values from DESeq2.

Supplementary Table 3 | Raw Counts Highly Conserved Gene Set for Coral and Symbiont.

Supplementary Table 4 | Symbiont normalized counts and gene expression results from DESeq2.

Supplementary Table 5 | Genes associated with photosynthetic GO categories in symbiont.

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