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Complex dynamics of coral gene expression responses to low pH across species

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Abstract

Coral capacity to tolerate low pH affects coral community composition and, ultimately, reef ecosystem function. Low pH submarine discharges ('Ojo'; Yucatán, México) represent a natural laboratory to study plasticity and acclimatization to low pH in relation to ocean acidification. A previous >2-year coral transplant experiment to ambient and low pH common garden sites revealed differential survivorship across species and sites, providing a framework to compare mechanistic responses to differential pH exposures. Here, we examined gene expression responses of transplants of three species of reef-building corals (*Porites astreoides*, *Porites porites* and *Siderastrea siderea*) and their algal endosymbiont communities (Symbiodiniaceae) originating from low pH (Ojo) and ambient pH native origins (Lagoon or Reef). Transplant pH environment had the greatest effect on gene expression of *Porites astreoides* hosts and symbionts and *P. porites* hosts. Host *P. astreoides* Ojo natives transplanted to ambient pH showed a similar gene expression profile to Lagoon natives remaining in ambient pH, providing evidence of plasticity in response to ambient pH conditions. Although origin had a larger effect on host *S. siderea* gene expression due to differences in symbiont genera within Reef and Lagoon/Ojo natives, subtle effects of low pH on all origins demonstrated acclimatization potential. All corals responded to low pH by differentially expressing genes related to pH regulation, ion transport, calcification, cell adhesion and stress/immune response. This study demonstrates that the magnitude of coral gene expression responses to pH varies considerably among populations, species and holobionts, which could differentially affect acclimatization to and impacts of ocean acidification.

KEYWORDS
acclimatization, common garden transplantation, coral transcriptome, gene expression, ocean acidification, plasticity

1 | INTRODUCTION

A large proportion of human-derived atmospheric CO_2 is absorbed by the ocean, causing a decrease in aragonite saturation ($\Omega_{aragonite}$) and a reduction in seawater pH (termed ocean acidification [OA]; Keeling et al., 2005; Kleypas et al., 1999). Ocean acidification is a major threat to marine organisms, particularly marine calcifiers including reef-building corals (Kroeker et al., 2010). Coral calcium carbonate skeletons are vulnerable to OA due to a reduction in coral calcification rate and/or skeletal density under elevated CO_2 concentrations (Crook et al., 2013; Gattuso et al., 1999; Marubini et al., 2008). To investigate the impacts of OA on corals, aquaria-based experiments with controlled manipulation of CO_2 or field-based studies in naturally low pH environments are employed. There is abundant evidence of the negative impacts of OA on adult and juvenile coral physiology, skeletal growth and structure, and recruit settlement (Albright et al., 2008; Cohen et al., 2009; Mollica et al., 2018; van der Zande et al., 2020; Wall et al., 2017). It has also been shown that OA results in changes in algal symbiont density (Martinez et al., 2019; Mason, 2018), which can have consequences for coral holobiont metabolism and growth.

The influence of OA on natural systems often leads to diminished coral diversity that can affect coral reef ecosystem function (Barkley et al., 2015; Crook et al., 2012; Edmunds et al., 2016; Fabricius et al., 2011; Inoue et al., 2013). Based on physiological performance, it has been suggested that some coral species may be able to acclimatize to natural sources of high pCO_2 (Strahl et al., 2015). In particular, species of the genus *Porites* are consistently present at naturally acidified sites such as volcanic CO_2 seeps and low pH bays in the Pacific Ocean and submarine springs in the Caribbean Sea (Barkley et al., 2015; Crook et al., 2012; Fabricius et al., 2011). *Porites* species may either acclimatize to natural acidification by maintaining net calcification (Strahl et al., 2015) or otherwise persist despite reduced calcification rates and impaired growth (Crook et al., 2013; McCulloch et al., 2012). Reports from naturally acidic, tropical coral reef environments include volcanic CO_2 seeps and submarine discharge sites (e.g., Crook et al., 2012; Fabricius et al., 2011). At our study site in México only three species of reef-building coral, including two *Porites* species, occur in the low pH waters of localized submarine springs (Ojo) while nine species occur in nearby supersaturated seawater ($\Omega_{aragonite}$ >2.5) where colony sizes are significantly larger (Crook et al., 2012). The occurrence of only three species at the low pH Ojo sites suggests that these species either are locally adapted to low pH where other species cannot survive, have an overall broad tolerance, and/or a stronger acclimatization ability. Coral species may have different capacities to acclimatize to low pH because of differences in reef environment, calcification, growth and associated trade-offs (Barott, Perez, et al., 2015; Brown et al., 2022; Kenkel et al., 2018; Moya et al., 2012).

To study the organismal response to environmental stress, such as OA, transcriptomes can be used to investigate plasticity and acclimatization (Hofmann et al., 2008; Rivera et al., 2021; Strader et al., 2020). A month-long aquaria experiment exposing coral (*Acropora millepora*) to low pH treatments found differential expression among genes related to binding, cytoskeleton, metabolism, signalling and transport (Kaniewska et al., 2012). In the same coral species (*A. millepora*), juvenile primary polyps subject to OA conditions showed a differential gene expression response in metabolism, skeletal organic matrix components and ion transporters including calcium transporters and channels (Moya et al., 2012). Other coral transcriptomic responses (*Siderastrea siderea*) to low pH include overrepresentation of genes relation to proton regulation and respiration (Davies et al., 2016). In situ work comparing coral hosts and symbionts (*A. millepora*) from low pH CO_2 seep environments to control sites showed a consistent host gene expression response to OA including genes related to lipid metabolism and stress response (Kenkel et al., 2018). Leveraging natural pH variability, our study used an in situ common garden experiment to test whether three species of coral from ambient pH environments demonstrate gene expression plasticity when transplanted to low pH for >2 years and vice-versa.

In Puerto Morelos, México near the Mesoamerican Reef, naturally low aragonite saturation submarine springs ('Ojo') have discharged high pCO_2 water in the back-reef lagoon for millennia (Beddows et al., 2007). These Ojo sites are considered 'extreme' because of the continuous discharge of low pH (6.70–7.30) and low saturation ($\Omega_{aragonite}$ ~ 0.5) groundwater (Crook et al., 2012; Hofmann et al., 2011). Importantly, groundwater-driven salinity at the Ojo sites is within the range of natural variability in coastal areas and not lower than 30 salinity units (Crook et al., 2013). In our study, we use naturally low pH Ojo sites to examine gene expression plasticity in response to pH differences of two coral species that grow at the Ojos (*Porites astreoides*, *Siderastrea siderea*) and one species found only on the reefs adjacent (<500 m) to the Ojos (*Porites porites*) during a >2-year, multi-site, common garden transplantation experiment (Martinez et al., 2019). Corals were transplanted from their different origins (Lagoon, Ojo or Reef) to ambient pH and low pH (Ojo) common garden sites. Each species had a survival rate of 80%–100% when transplanted to ambient pH (Martinez et al., 2019), indicating a negligible effect of transplantation. In contrast, corals transplanted to low pH showed a species-specific survival response that suggests variable capacity for phenotypic plasticity (Figure 1). Using coral survivorship as a framework for investigating plasticity to low pH, we examine stress tolerant and sensitive populations (Rivera et al., 2021). Whole transcriptome gene expression profiles (mRNA-sequencing) were compared among the three species of coral hosts and their algal symbionts to investigate the mechanisms of and potential for acclimatization to OA. Pairing transcriptome data with coral traits (skeletal density and extension, protein, symbiont density, and chlorophyll-a; Table 1) measured in the same samples (Martinez et al., 2019) is important to link gene expression responses to holobiont phenotypes following >2 years in different pH environments.

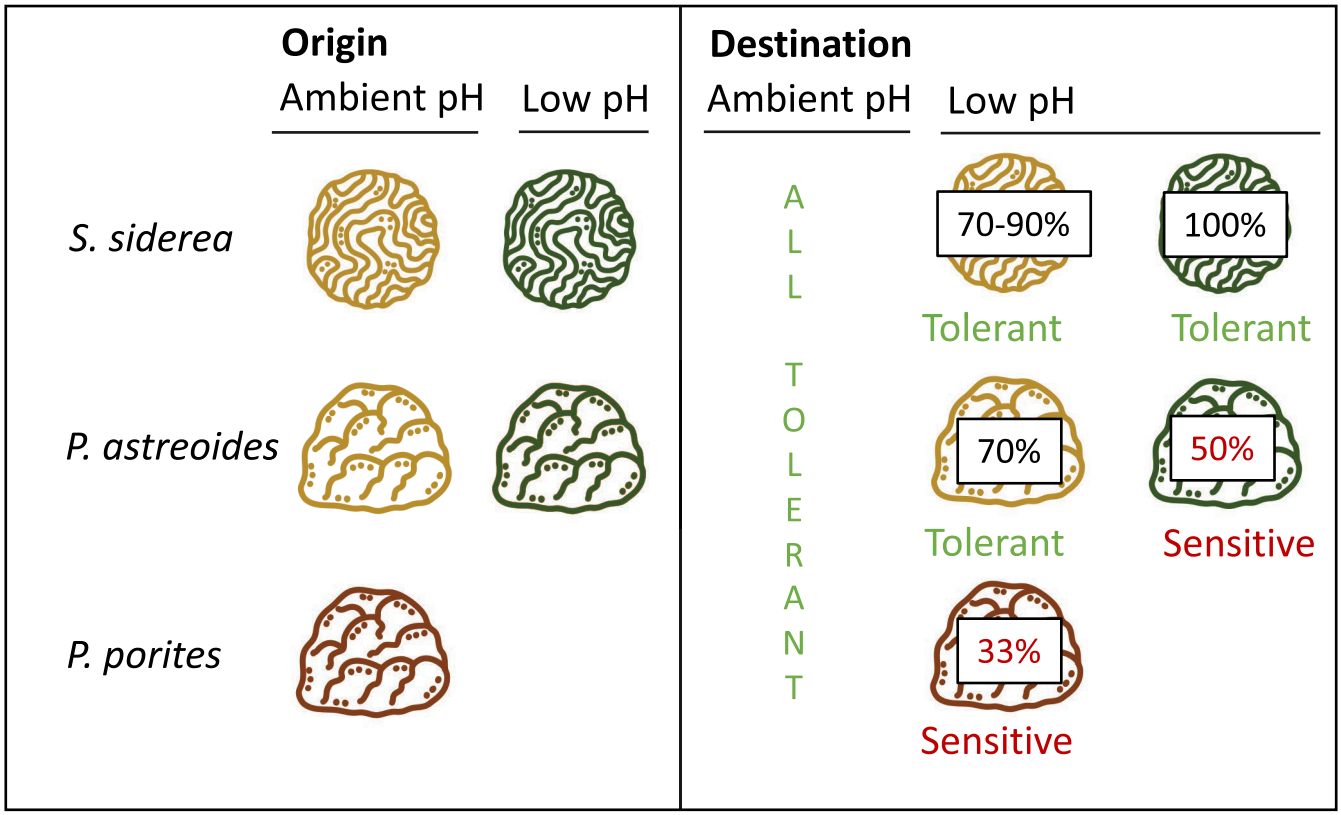

FIGURE 1 Overview of the experimental design and transplant survival rates. Coral fragments from three species (*Siderastrea siderea*, *Porites astreoides*, and *Porites porites*) were transplanted from ambient (Lagoon, Reef) and low (Ojo) pH sites to ambient and low pH conditions for >2 years in Puerto Morelos, México. Corals transplanted to the ambient pH site had a high survival rate (80%–100%; all three species) indicating a negligible effect of transplantation (Martinez et al., 2019). Corals transplanted to the low pH destination showed differential rates of survival. Corals with ≥70% survival during the >2-year transplant are considered stress tolerant populations while corals with ≤50% survival are considered stress sensitive populations. Note that *P. porites* only exists in the ambient pH Reef site and is absent near low pH Ojos (Crook et al., 2012) (CC licence coral icons: Hanna-Vernydub).

2 | MATERIALS AND METHODS

2.1 | Sample collection and transplantation experiment

Nineteen colonies of *Porites astreoides* were collected from an ambient pH ($n=9$, Norte lagoon) and a low pH site ($n=10$, Ojo Norte) (20.886° N, 86.857° W; 12–13 December 2012; Figure S1). A total of 22 colonies of *Siderastrea siderea* were collected from an exposed forereef site ($n=10$, Reef), an ambient pH site ($n=6$, Pargos lagoon) and a low pH site ($n=6$, Ojo Pargos; 20.880° N, 86.861° W; 14–15 December 2012). For *Porites porites*, 10 colonies were collected from an exposed forereef ('Reef', 20.883° N, 86.851° W; 15 December 2012; pH range 8.28 to 8.31); this species does not occur near the low pH Ojos.

From each site, replicate fragments of each colony (~3 cm diameter) were transplanted to two common garden sites: a low pH site (pH 7.70 ± 0.14; Ω_{arag} 2.10 ± 0.35, Ojo Laja), and an adjacent (<3 m away) ambient pH site (pH 8.17 ± 0.07; Ω_{arag} 3.53 ± 0.43) (20.879° N, 86.861° W; 17 December 2012; for full experimental details see Martinez et al., 2019; Table S1). The Ojo Laja has continuous groundwater discharge while the adjacent, ambient pH site (a few meters away) is outside the influence of the spring. After >2 years of transplantation (25 months; 15 January 2015), the surviving coral fragments were collected and preserved in an RNA stabilization buffer and flash frozen (−80°C). Sample sizes were (Native origin–Destination): Lagoon-Ambient pH ($n=9$), Lagoon-Low pH ($n=8$), Ojo-Low pH ($n=8$), and Ojo-Ambient pH ($n=8$) for *P. astreoides*; Reef-Ambient pH ($n=8$) and Reef-Low pH ($n=5$) for *P. porites*; Lagoon-Ambient pH ($n=6$), Lagoon-Low pH ($n=5$), Ojo-Low pH ($n=6$), Ojo-Ambient pH ($n=6$), Reef-Ambient pH ($n=9$), and Reef-Low pH ($n=7$) for *S. siderea*. These same experimental fragments were previously analysed for physiological responses following transplantation (Martinez et al., 2019).

2.2 | RNA extraction, library preparation and sequencing

Each sample was crushed with liquid nitrogen in a mortar and pestle and total RNA was extracted with TRIzol (Invitrogen, Life Technologies) using a modified protocol described in

TABLE 1 Physiological metrics of corals following >2 years in situ transplantation (mean ± standard error).

Origin – destination	*n*	Skeletal extension (mm)	Skeletal density (g/cm^3)	Symbiont density (cells/cm^2)	Chlorophyll (μg/cm^2)	Chlorophyll (μg/cell)	Protein (mg/cm^2)
Porites astreoides							
Lagoon native-Ambient pH	9	8.17 ± 0.76	1.60 ± 0.03	1.13E+07 ± 3.98E+06	59.10 ± 23.99	5.44E-06 ± 1.39E-06	1.33 ± 0.21
Lagoon native-Low pH	5	9.68 ± 2.04	1.11 ± 0.03	2.95E+07 ± 8.62E+06	266.07 ± 86.84	8.30E-06 ± 8.89E-07	3.06 ± 1.04
Ojo native-Ambient pH	8	7.81 ± 0.39	1.58 ± 0.02	1.04E+07 ± 2.03E+06	49.08 ± 11.12	4.43E-06 ± 4.69E-07	1.15 ± 0.19
Ojo native-Low pH	7	13.55 ± 1.17	1.13 ± 0.04	2.98E+07 ± 1.21E+07	202.65 ± 72.41	7.84E-06 ± 1.10E-06	1.72 ± 0.70
Siderastrea siderea							
Lagoon native-Ambient pH	6	2.97 ± 0.60	1.72 ± 0.03	1.16E+07 ± 6.62E+06	32.64 ± 19.08	2.59E-06 ± 4.40E-07	2.23 ± 1.00
Lagoon native-Low pH	5	4.50 ± 0.98	1.40 ± 0.06	1.48E+07 ± 6.17E+06	83.65 ± 43.75	6.33E-06 ± 1.61E-06	1.37 ± 0.46
Ojo native-Ambient pH	5	2.80 ± 0.32	1.48 ± 0.02	1.37E+06 ± 4.59E+05	5.12 ± 1.65	3.82E-06 ± 3.35E-07	0.36 ± 0.21
Ojo native-Low pH	6	3.94 ± 0.48	1.31 ± 0.03	9.47E+06 ± 2.20E+06	59.15 ± 13.63	6.10E-06 ± 7.08E-07	0.88 ± 0.19
Reef native-Ambient pH	7	6.19 ± 0.72	1.68 ± 0.02	1.99E+06 ± 3.94E+05	6.80 ± 1.64	3.09E-06 ± 2.70E-07	0.32 ± 0.08
Reef native-Low pH	7	6.43 ± 0.91	1.42 ± 0.07	2.33E+07 ± 9.35E+06	81.21 ± 26.81	4.23E-06 ± 5.06E-07	1.27 ± 0.27

Note: Data are shown for the specific corals that were analysed for gene expression, as presented in Figure 5 (full physiology dataset published in Martinez et al., 2019).

Barshis et al. (2013). RNA yield was quantified (Qubit 2.0 fluorometer, Life Technologies) and libraries prepared with the Illumina TruSeq RNA Sample Prep Kit v2 following manufacturer protocols but using half reaction volumes at each step to increase the number of libraries constructed. Unique adapters/indexes were ligated to identify each sample. The quantity and quality of cDNA libraries were determined with a Qubit 2.0 fluorometer and a Fragment Analyzer CE system (Agilent Technologies), respectively. A total of 32 libraries were constructed for sequencing of *P. astreoides*, 13 libraries for *P. porites* and 39 libraries for *S. siderea*. Libraries were multiplexed across seven lanes of 50 bp single-end sequencing on an Illumina HiSeq2500 Rapid platform at the Vincent J. Coates Genomics Sequencing Laboratory (University of California, Berkeley). One Reef-Ambient pH and two Reef-Low pH *P. porites* libraries and one Ojo-Ambient pH *S. siderea* library were not sequenced due to poor quality RNA.

2.3 | De novo transcriptome assembly

A de novo transcriptome was assembled for *S. siderea* because mapping to existing transcriptomes yielded very low alignment (<10% singly aligned). An additional subset of Lagoon origin samples were sequenced across three lanes to acquire four samples of paired-end 150 bp reads and five samples of single-end 150 bp reads (Illumina HiSeq2500 Rapid platform; University of California, Berkeley) for transcriptome assembly. FastQC v0.11.9 was used to assess raw read quality of each library (Andrews, 2010). Reads were trimmed (fastq_quality_trimmer) and adapter clipped (fastx_clipper) with a 20 bp minimum length threshold and a minimum quality score threshold of 33 followed by quality filtering (fastx_quality_filter -q 20 -p 90) using the FASTX-Toolkit v0.0.13 (Gordon, 2013). The de novo transcriptome assembly was constructed using Trinity v2.11.0 with default parameters including in silico normalization (200 maximum read coverage; Grabherr et al., 2011; Haas et al., 2013). The longest isoforms/transcript were selected from the Trinity assembly via a custom python script, and the assembly was further filtered by a minimum length threshold (500 bp). The assembly was compared via BLASTn against SILVA rRNA (138.1, downloaded on 5 November 2020; LSU and SSU) databases (Altschul et al., 1990; Quast et al., 2013) and matches (minimum 78% match over at least 100 bp) were removed from the assembly leaving 146,151 host or symbiont contigs. Next, the assembly was divided into host or algal symbiont assemblies. Briefly, the de novo assembly was compared via BLASTn against four aggregated databases: cnidarian, Symbiodiniaceae, aposymbiotic cnidarian and cultured Symbiodiniaceae (Aichelman & Barshis, 2020; Davies et al., 2016; Ladner et al., 2012). A 70% identity across 100 bp or greater cut-off was applied to assign host or symbiont identity to each contig. For the host, Symbiodiniaceae and cultured Symbiodiniaceae database matches were removed from the cnidarian and aposymbiotic cnidarian database matches resulting in 19,222 contigs in the final *S. siderea* host assembly. For the symbionts, cnidarian and aposymbiotic cnidarian database matches were

removed from the Symbiodiniaceae and cultured Symbiodiniaceae database matches resulting in 3063 contigs in the final symbiont assembly. However, the majority of the final *S. siderea* symbiont assembly reads mapped to the more robust *Cladocopium goreaui* reference (Davies et al., 2018) and thus our limited de novo symbiont assembly was not used. The combined de novo host and *Cladocopium goreaui* references were annotated via the total annotation protocol outlined in De Wit et al. (2012). Briefly, each transcript was compared via BLASTx against the NCBI non-redundant protein database (nr; downloaded on 26 March 2021), uniprot sprot database (sprot; downloaded on 16 March 2021), and uniprot TrEMBL database (TrEMBL; downloaded on 16 March 2021). Annotation information was compiled for each match (e-value < 1e-4) to nr and in a hierarchical fashion for sprot and TrEMBL, where matches to TrEMBL were only used for sequences that had no match in sprot, the more curated database. Gene Ontology and other functional information was extracted for each match from the UniprotKB repository. For host *S. siderea* 14% of genes had no annotation, and 32% did not have GO terms. For host *P. astreoides* 18% of genes had no annotation, and 33% did not have GO terms.

2.4 | Symbiont community identification

Holobiont reads were mapped to an ITS2 database at the individual sample level using Bowtie 2.2.4 (default parameters, -local sensitivity) to estimate the proportion of symbiont genera per host species (Arif et al., 2019; Langmead et al., 2009). Samples with <100 mapped reads were not used for symbiont identification. After determining the dominant genus from ITS2 mapping, we tested alignments against multiple symbiont transcriptomes of each genus and species (Avila-Magaña et al., 2021; Chen et al., 2019, 2020; Davies et al., 2018; González-Pech, Ragan, et al., 2021; González-Pech, Stephens, et al., 2021; Rivera & Davies, 2021) and used the symbiont reference with the highest proportion of uniquely mapped reads for analysis (Table S2). All symbiont references were similarly filtered (same as the *S. siderea* de novo assembly) for a length threshold (500 bp) and longest isoforms/transcript.

2.5 | Sequence mapping and gene expression analysis

All libraries were quality checked and reads were quality trimmed and filtered with adapters clipped as above. The *P. astreoides* reads were initially mapped against several host *P. astreoides* references (Bhattacharya et al., 2016; Kenkel et al., 2013; Liew et al., 2016) in order to select the host reference with the highest proportion of singly aligned reads (past.fasta; Kenkel et al., 2013). The same host *P. astreoides* reference was also used for *P. porites* mapping since no other Caribbean *Porites* references were available. For each species, a compiled hybrid reference (host plus symbiont reference(s)) was assembled for mapping to avoid potentially assigning reads to the wrong taxon. Reads were mapped to the respective hybrid references with Bowtie 2.2.4 (default parameters, -local; Langmead et al., 2009) and read counts were assembled by isogroup.

All host- and symbiont-specific statistical analyses and visualization were conducted in the R environment (v4.0.3; R Core Team, 2020; Wickham, 2016; Wickham et al., 2021). Data were filtered to exclude genes with low expression (mean count < 3) and samples with >85% null counts were removed, resulting in ~7400–8100 genes among coral host species and ~ 7000–10,800 genes among symbiont groups. An outlier individual was removed for host *S. siderea* based on the lowest number of raw counts, very small size factor, and visualization via cluster dendrogram and PCA (final sample sizes in Table S3). Gene counts were normalized and log-transformed (variance stabilizing transformation, 'DESeq2') for subsequent analyses (Love et al., 2014). Transformed counts were visualized by PCA (plotPCA, 'DESeq2') and species-specific variance of normalized counts was analysed by PERMANOVA for the factors native origin, transplant destination and their interaction (adonis, 'vegan'; pairwise. adonis, 'pairwiseAdonis'; Martinez Arbizu, 2020; Oksanen et al., 2020).

Generalized linear models were fit for the differential expression analysis of each group (each species, host or symbiont) with default DESeq2 gene-level filtering used to replace outlier genes and refit genes during model fitting (parametric fit type, Wald test) and a local regression fit automatically substituted only for symbiont *Cladocopium* (*S. siderea* and *P. porites*). Differentially expressed genes (DEGs) were identified using an FDR *p*-adjusted value $\alpha < 0.1$. DEGs shared among species-specific groups (Native origin–Destination) were visualized using 'VennDiagram' (Chen, 2011). To compare gene expression among groups for each coral species and taxon (host or symbiont; groups defined a priori), a discriminant analysis of principal components (DAPC; 'adegenet') was used with cross-validation (xvalDapc) determining the optimal number of PCs associated with the lowest Mean Squared Error (Jombart, 2008; Jombart et al., 2010). The PCs retained were 8, 4 and 16 for *P. astreoides* host, symbionts, and *S. siderea* host, respectively. Gene expression plasticity in response to pH destination was tested by examining the magnitude of the shift in genome-wide gene expression following transplantation using Markov chain Monte Carlo (MCMC; 'MCMCglmm', Hadfield, 2010) linear mixed models with individual coral fragments as the random effect following Kenkel and Matz (2016). The *p*-value was derived for group-specific differences by analysing 2800 MCMC samples of parameter estimates.

A weighted gene co-expression network analysis (WGCNA) was used to find clusters of highly correlated genes (signed network; softPower = 14 *P. astreoides* host, softPower = 9 *S. siderea* host, softPower = 22 *P. astreoides* symbiont), merge modules with highly similar expression profiles, and then relate the modules to site (origin and pH transplant destination) and post-transplantation trait data from the same samples (method = 'hybrid' hierarchical clustering; Langfelder & Horvath, 2008, 2012; Martinez et al., 2019). Functional enrichment analysis was conducted to identify functionally enriched Gene Ontology categories

associated with differential gene expression response to transplantation and WGCNA modules ('GOSeq'; FDR *p*-adjusted value $\alpha < 0.1$; Young et al., 2010).

To investigate the potential of a cross-species DEG response, orthologs were assigned (4435 hierarchical orthogroups) based on comparison of the de novo host *S. siderea* assembly and the longest isoform host *P. astreoides* reference (as above, same Kenkel reference used for both *Porites* species; 'OrthoFinder2', all-versus-all algorithm; Emms & Kelly, 2015, 2019). Differentially expressed orthogroups (1:1 and multi-orthogroups) were compared between the same pairwise contrasts and were visualized by PCA. Coral DEG responses were compared between all species transplanted to low versus ambient pH using a euKaryotic Orthologous Group (KOG) analysis ('KOGMWU'; Dixon et al., 2015).

3 | RESULTS

3.1 | Sequencing overview

Sequencing of nine samples (Lagoon natives) for de novo assembly of *S. siderea* resulted in ~160 million total reads across all samples (PE-150 and SE-150). There were 9.3–15 million raw reads per library (mean 12.3 million), and 8.7–13.6 million reads (mean 11.4 million) after adapter clipping and quality trimming. The final de novo *S. siderea* host assembly consisted of 19,222 contigs after assembly, quality control, and screening for high confidence coral host transcripts. For gene expression analyses, sequencing of 38 samples of *S. siderea* (16 Reef, 11 Ojo, and 11 Lagoon natives) resulted in ~785 million total reads mapped (SE-50). Per *S. siderea* holobiont library, 5–32 million raw reads were generated (mean 20.7 million) and 3.6–14.9 million unique reads per sample (mean 9.3 million) mapped to the hybrid reference assembly. Sequencing of 32 samples of *P. astreoides* (15 Ojo and 17 Lagoon natives) resulted in ~873 million total reads mapped (SE-50). Per *P. astreoides* holobiont library, 5.4–62.5 million raw reads were generated (mean 27.3 million) and 2–56.9 million unique reads per sample (mean 23.5 million) mapped to the hybrid reference assembly. Sequencing of 10 samples of *P. porites* (Reef natives) resulted in ~320 million total reads mapped (SE-50). Per *P. porites* holobiont library, 5–69.7 million raw reads were generated (mean 32 million) and 3–63 million unique reads per sample (mean 27.1 million) mapped to the hybrid reference assembly.

3.2 | Ortholog identification

OrthoFinder analyses identified 4435 hierarchical orthogroups among *P. astreoides* and *S. siderea* host assemblies, including 1562 1:1 orthogroups and 558 multi-orthogroups, with the remaining orthogroups belonging to only one species. No shared response was found between species at the orthogroup level among the differentially expressed genes (DEGs) for any pairwise group comparison. For each pairwise contrast, PCA was applied for all DEG orthogroups from both species but a clear cross-species shared response was not observed (Figure S2). However, some separation was observed at the orthogroup level for *S. siderea* and *P. porites* Reef natives transplanted to low versus ambient pH although the latter had a small sample size (Figure S2a-b).

3.3 | *Porites astreoides* – Gene expression plasticity to contrasting pH environments

Transplantation to different pH environments had the largest effect on differential expression in host *P. astreoides* originating from low pH Ojos (342 DEGs; Figure 2a); 87% of DEGs were unique to this comparison (299; Figure S3). Host *P. astreoides* transplanted to ambient pH had only one DEG when comparing between origins. The majority of DEGs (29; 88%), mostly upregulated, in the Ojo-Low pH versus Lagoon-Low pH contrast were shared with Ojo native corals transplanted to low versus ambient pH destinations (Figure S3). These shared, upregulated DEGs comprised the GO biological processes of chromosome condensation, mRNA processing and transport, autophagy, and endosomal vesicle and protein transport; GO molecular functions included histone, mRNA and RNA binding, ferric ion binding, and ferroxidase activity.

Corals originating from the ambient pH lagoon transplanted to low versus ambient pH environments shared 16 DEGs (43%) with Ojo native corals transplanted to low versus ambient pH; each gene showed the same response direction (up- or downregulated; Figure S3). The shared DEGs included cell adhesion, actin cytoskeleton organization, actin filament processing, cell population proliferation and plasma membrane repair among the top GO biological processes while protein, actin filament, and mRNA binding, ATPase activity, and microfilament motor activity were among the top molecular functions. DEGs of host *P. astreoides* originating from the ambient pH Lagoon and transplanted to low versus ambient pH had top GO terms of cell adhesion plus calcium ion, actin, and cytoskeletal protein binding among others that represents potential acclimatization to low pH (Table S4).

Total gene expression of host *P. astreoides* was affected by the transplant destination (ambient vs. low pH; PERMANOVA, $p < .001$). Similarly, discriminant analysis of principal components (DAPC) differentiated most strongly between pH transplant environments, though there was a more subtle distinction between origins within the same destination (Figure 3a). Coral host *P. astreoides* gene expression was differentiated by low versus ambient pH transplant environment (Figure 4a). To quantify genome-wide gene expression plasticity, native pH site axes (Lagoon or Ojo) were used as a scale to score transplantation to contrasting pH environments to quantify shifts in gene expression. Ojo native (low pH) corals transplanted to ambient pH (grey) resulted in similar expression profiles to Lagoon native corals remaining in ambient pH (blue; $P_{MCMC} = 0.106$), demonstrating strong plasticity of Ojo native corals in response to >2 years transplant to an ambient

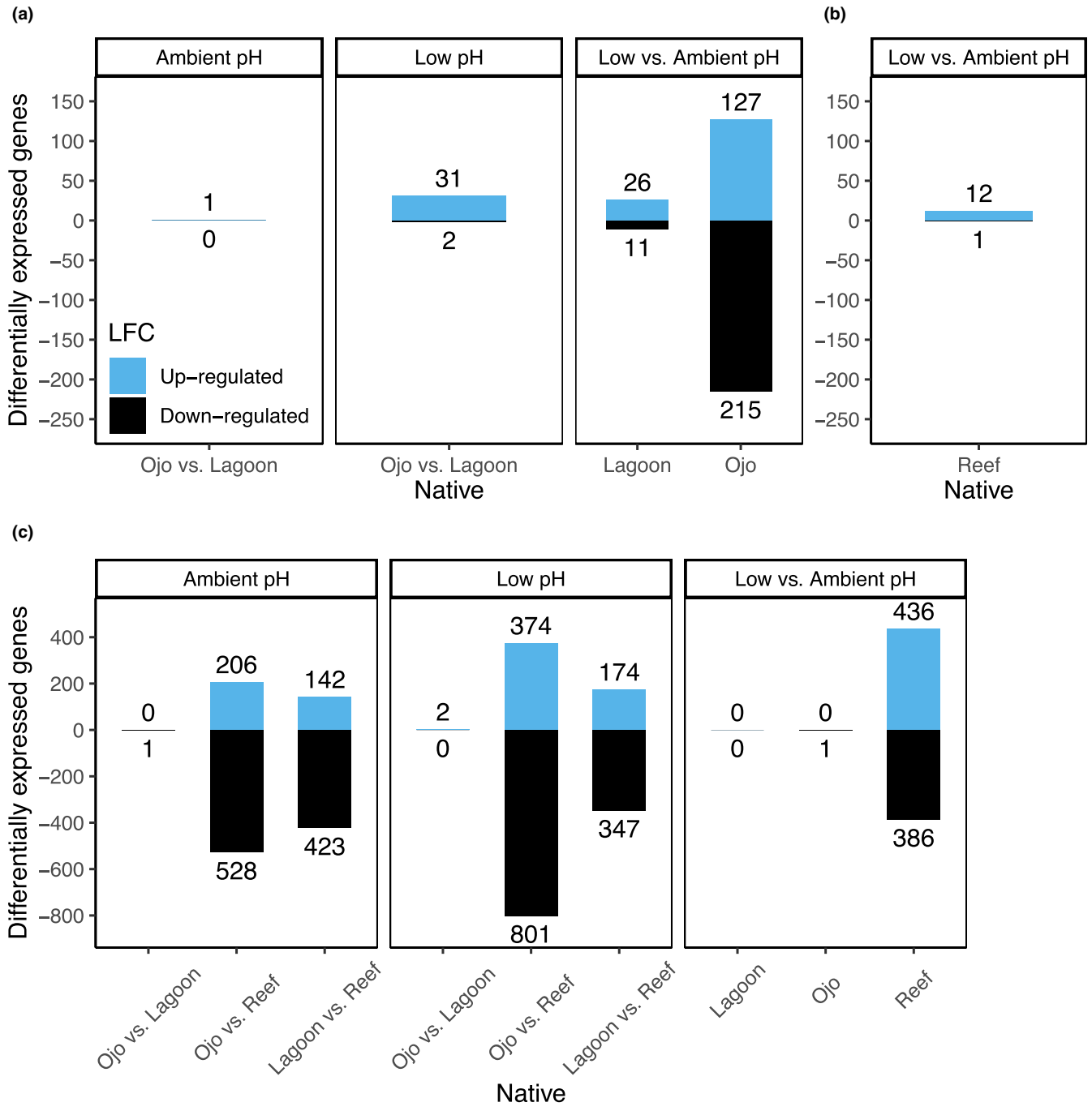

FIGURE 2 Differentially expressed genes of coral hosts following >2 years transplantation from Lagoon, Ojo and Reef native origin sites to common garden ambient pH or low pH (Ojo) sites. Panels show the common garden transplant destinations of each pairwise contrast for (a) *Porites astreoides*, (b) *Porites porites* and (c) *Siderastrea siderea*.

pH environment. Lagoon native (ambient pH) corals transplanted to low pH (yellow) showed a partial gene expression shift; this stress tolerant population had a more similar expression profile to the stress sensitive Ojo native (low pH) corals remaining in low pH (red; $P_{MCMC} = 0.264$) while showing differentiation to the Lagoon native (ambient pH) corals remaining in ambient pH (blue; $P_{MCMC} < 0.001$; Figure 4a). Ojo native corals transplanted to low pH had a more variable gene expression response compared to those transplanted to ambient pH (Figure S4).

3.4 | *Porites astreoides* – Common response genes

Host *P. astreoides* Ojo natives in low versus ambient pH transplant environments showed upregulation of a heat shock protein and heat shock factor, a carbonic anhydrase 2-like gene and a Bcl-2-like apoptosis regulator gene (Table S4). Lagoon natives in low versus ambient pH transplant environments also had upregulated expression of the same carbonic anhydrase 2-like gene. Both contrasts had DEGs related to calcium ion binding (GO:0005509), cellular calcium

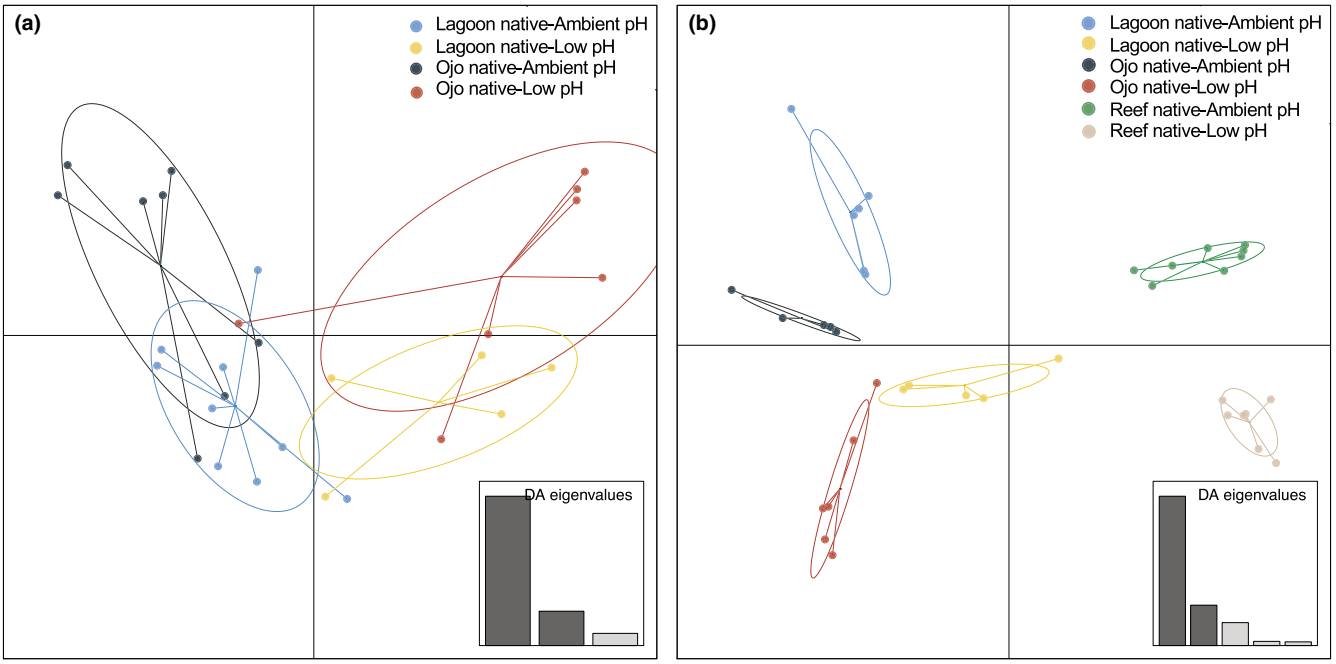

FIGURE 3 Variation in total gene expression among (a) host *Porites astreoides* and (b) host *Siderastrea siderea* individual corals (circles) and groups (ellipses). The first two discriminant axes represent the relative relationships among the groups from Lagoon, Ojo and Reef native origins transplanted for >2 years to common garden ambient pH or low pH sites.

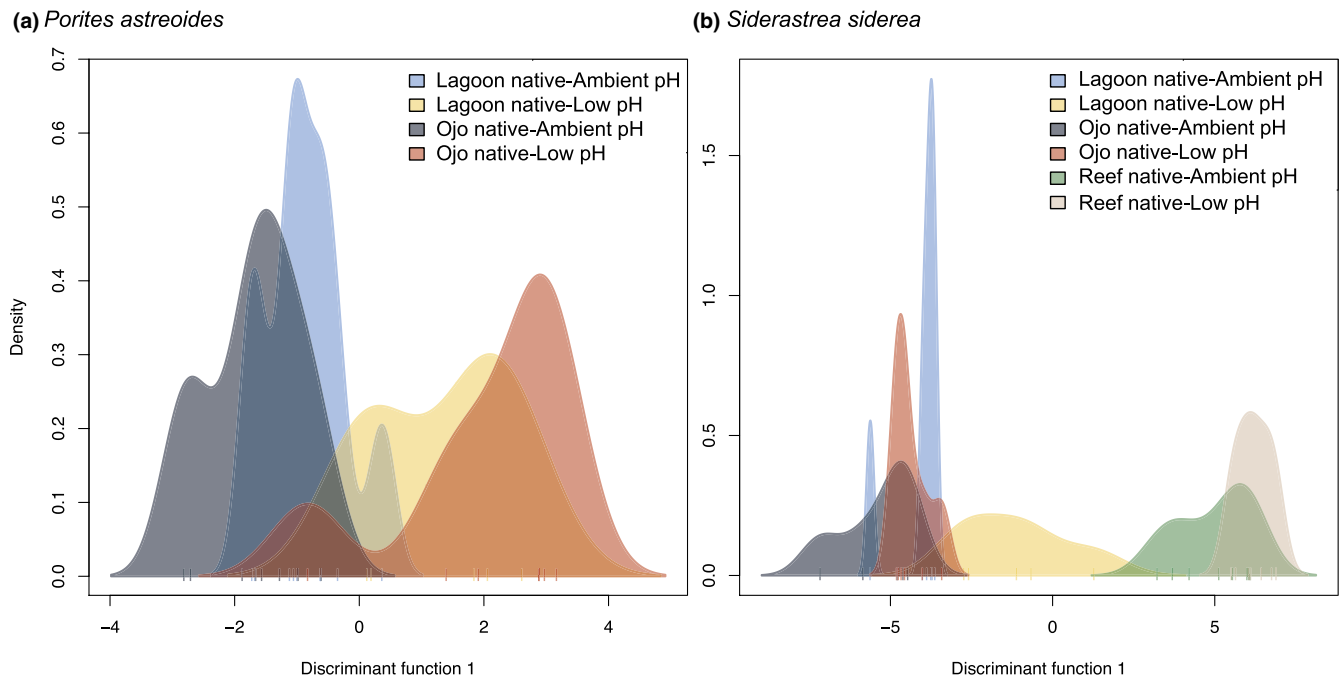

FIGURE 4 Genome-wide gene expression plasticity in corals transplanted to different pH common garden sites. (a) Differentiation in coral host *Porites astreoides* due to pH transplant environment when comparing ambient and low pH native origins. (b) Corals originating from the Reef versus Lagoon or Ojo environments strongly influenced gene expression in host *Siderastrea siderea*.

ion homeostasis (GO:0006874) and P-type calcium transporter activity (GO:0005388) plus various other calcium ion transport activities. Further, both contrasts had DEGs related to cell adhesion (GO:0007155), actin binding (GO:0003779), actin cytoskeleton (GO:0015629), focal adhesion, adherens junction (GO:0005912) and upregulation of a protocadherin-like protein. One gene related to β-catenin/vinculin binding was upregulated in corals originating from low pH Ojos transplanted to low versus ambient pH. Genes (12) related to ATPases were differentially expressed, including an upregulated plasma membrane calcium-transporting ATPase and

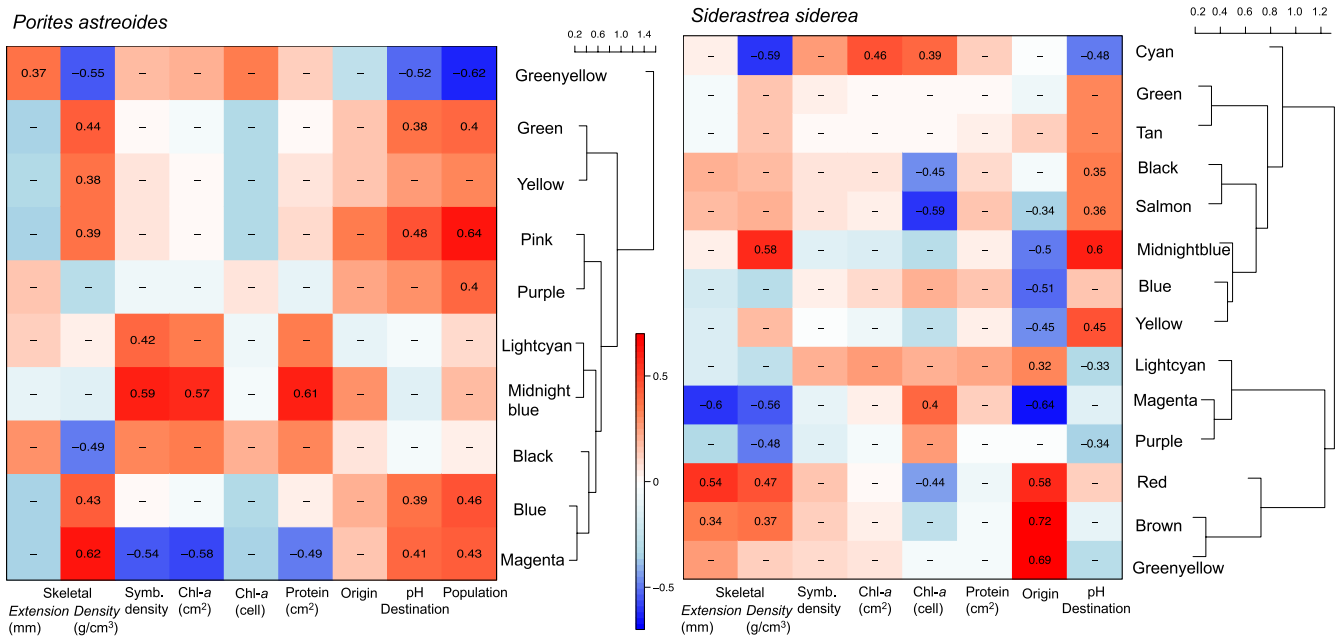

FIGURE 5 Eigengene module-trait correlation heatmap with dendrogram for coral hosts *Porites astreoides* and *Siderastrea siderea*. Red indicates a positive correlation while blue indicates a negative correlation, with Pearson's *R* for significant correlations reported ($p < .05$).

downregulated sodium/potassium transporting ATPase subunit and V-type proton ATPase in Ojo natives in low versus ambient pH. Several DEGs related to transposable elements including Retrovirus-related Pol polyprotein were observed.

3.5 | *Porites astreoides* – Gene module correlation with physiological responses

A weighted gene co-expression network analysis (WGCNA) investigated gene expression in relation to physiological traits published previously (Martinez et al., 2019). Coral host *P. astreoides* genes (8118) were assigned to 10 gene co-expression modules; nine modules were significantly correlated with one or more traits. Four modules were positively correlated (green, pink, blue, magenta) and one module negatively correlated (greenyellow) for the traits skeletal density, pH destination and population (tolerant vs. sensitive based on survival outcomes; Figure 5). The greenyellow module (153 genes) showed significant but contrasting correlations with skeletal traits (skeleton extension vs. density; skeletal density is the gross density of intact skeleton with its pores) (Figure 5, Table S5). The midnight blue module (60 genes) was positively correlated with symbiont density, chlorophyll-a concentration (normalized to surface area), and protein content. Conversely, the magenta module (304 genes) was negatively correlated with symbiont density, chlorophyll-a concentration, and protein content and positively correlated with skeletal density, pH destination and population. Of note, the magenta module contained several GO terms related to proton-transporting ATPase activity (GO:0046961, GO:0008553, GO:0016471), ATPase activity and binding (GO:0016887, GO:0051117), and voltage-gated ion channel activity (GO:0005244, GO:0005248) that may relate to carbon-concentrating mechanisms. A functional enrichment analysis showed zero significant GO categories for host *P. astreoides* DEG contrasts but identified one enriched gene in the yellow WGCNA module ('isogroup17202_Past'; centrosomal protein; no assigned GO category). Top GO terms for each contrast's DEGs are in the supplement (Table S4). Seven modules were correlated with skeletal density, which was higher in corals from either origin transplanted to ambient pH (Figure 6a).

At the DEG level, host *P. astreoides* Ojo-Low pH versus Ojo-Ambient pH contrast shared 10 DEGs (one down- and nine upregulated) with host *P. porites* Reef-Low pH versus Reef-Ambient pH contrast; most DEGs were not annotated except for two related to rRNA processing and RNA-directed DNA polymerase. Although *P. porites* originated only from the Reef and *P. astreoides* originated only from Lagoon or Ojo sites, Lagoon native *P. astreoides* and Reef native *P. porites* (both ambient pH origins) transplanted to low versus ambient pH shared one DEG that was upregulated 4–8 fold ('isogroup03915_Past'; hypothetical protein, UniProt match A0A4Y7JIL6).

3.6 | *Porites astreoides* – Symbiont gene expression affected by pH environment

For *P. astreoides* symbionts (majority *Symbiodinium* spp.), Ojo natives transplanted to low versus ambient pH had 89 DEGs while low pH transplants from either origin had 31 DEGs (Figure S5a), of which 22 upregulated genes were shared. Symbionts of corals originating from the Lagoon had only one DEG when comparing between pH transplant destinations. No DEGs were found for symbionts of ambient pH transplants from either origin. No downregulated genes were

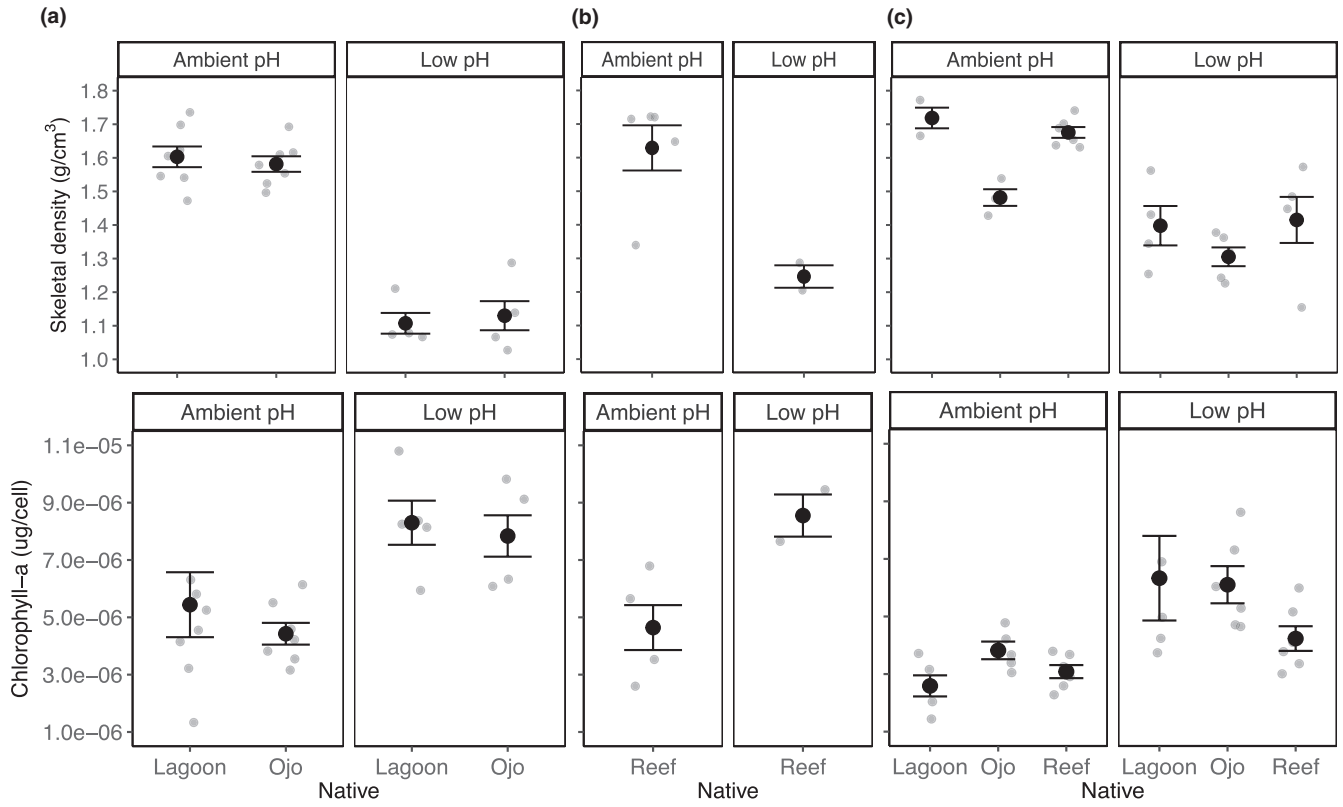

FIGURE 6 Skeletal density (g/cm^3) and symbiont chlorophyll-a (μg/cell) of three coral species (a) *Porites astreoides*, (b) *Porites porites* and (c) *Siderastrea siderea* Lagoon, Ojo, or Reef natives transplanted to ambient and low pH common gardens for >2 years (panel label depicts transplant destination).

shared among the symbiont pairwise contrasts, and no DEGs had significantly enriched GO terms.

The total gene expression of *P. astreoides* symbionts was affected by native origin and pH transplant environment, respectively (PERMANOVA, $p = .0499$ and $p = .017$). Pairwise PERMANOVA showed a significant difference between ambient and low pH destinations ($p = .023$) while Lagoon versus Ojo native origins were marginally significant ($p = .0585$). Lagoon natives showed a differential response to ambient versus low pH as well as unique response patterns compared to Ojo natives transplanted to ambient pH (Figure S6a-b). A WGCNA of *P. astreoides* symbiont genes (7098) identified 12 gene co-expression modules including six modules significantly correlated with one or more traits; five modules were positively correlated with skeletal density while one module (grey, 36 genes) was positively correlated with symbiont density, chlorophyll-a concentration (normalized to surface area) and protein content (Figure S10).

3.7 | *Porites porites* – Muted gene expression response to pH

Reef native host *P. porites* had very few DEGs (13) between low versus ambient pH transplant environments (Figure 2b), of which 10 DEGs were in common with host *P. astreoides* Ojo natives transplanted to low versus ambient pH. One upregulated DEG (lin-54 homologue; 'isogroup03915_Past') was in common among hosts Reef native *P. porites*, Ojo native *P. astreoides*, and Lagoon native *P. astreoides* transplanted to low versus ambient pH. Although the majority of DEGs of host *P. porites* were uncharacterized proteins, the annotated DEGs for the Reef-Low pH versus Reef-Ambient pH contrast included an upregulated matrix metalloproteinase related to cadherin-mediated cell–cell adhesion, an upregulated cytosolic phospholipase A2-like related to phospholipid catabolism and calcium ion binding, and an upregulated rRNA-processing protein (Table S4).

Total gene expression of Reef native host *P. porites* was affected by the pH transplant environment ($p = .026$). A PCA of the genes of Reef natives transplanted to ambient versus low pH showed differentiation between the groups ($p < .05$) although there were only three low pH samples due to 50% mortality following >2-year transplantation and two samples that failed sequencing (Figure S7). There were zero DEGs in Reef native *P. porites* symbionts, and no effect of pH on gene expression ($p = .095$). The lower sample size of *P. porites* may limit power to investigate a response.

3.8 | *Siderastrea siderea* – Differential gene expression primarily due to origin, with subtle effects of pH environment

For host *S. siderea*, there were large DEG responses overall especially between Reef natives versus Ojo/Lagoon natives for both pH

environments, with the highest number of DEGs (1175) between Ojo and Reef natives transplanted to low pH (Figure 2c). Although the majority of DEGs were unique (603) in Ojo-Low pH versus Reef-Low pH host *S. siderea*, 347 DEGs were shared with the contrast Lagoon-Low pH versus Reef-Low pH (Figure S8). In contrast, there were few DEGs (0–2) for *S. siderea* Lagoon and Ojo natives when comparing pH destinations. Top GO terms for DEGs of each pairwise contrast are presented in the supplement (Table S4), with nearly 30 GO terms enriched (Table S6). Total gene expression was affected by the native origin of host *S. siderea* (PERMANOVA, $p = .007$), though this was likely driven by Reef natives because a pairwise comparison indicated differences between Reef and Lagoon natives ($p = .012$) and Reef and Ojo natives ($p = .015$) but not between Lagoon and Ojo natives ($p = .876$).

Despite the lack of DEGs between Lagoon and Ojo natives and within Lagoon and Ojo natives in either pH environment (low vs. ambient pH; Figure 2c), discriminant analysis of principal components (DAPC) showed separation among all *S. siderea* groups, in particular between Reef natives versus Lagoon and Ojo natives (Figures 3b and 4b). However, *S. siderea* Lagoon and Ojo natives were grouped more closely based on their pH transplant environment (Figure 3b). Host *S. siderea* Ojo native-Low pH (red) shared a similar gene expression profile with Ojo native-Ambient pH (grey; $P_{MCMC} = 0.110$) and Lagoon native-Ambient pH corals (blue; $P_{MCMC} = 0.659$; Figure 4b). Further, Reef native-Low pH (tan) shared a similar gene expression profile with Reef native-Ambient pH (green; $P_{MCMC} = 0.027$). The gene expression profile of *S. siderea* Lagoon native-Low pH (yellow) was significantly different from all other contrasts ($P_{MCMC} < 0.001$).

3.9 | *Siderastrea siderea* – Common response genes

Low pH transplants (both Lagoon vs. Reef natives and Ojo vs. Reef natives) had upregulated expression of the same carbonic anhydrase 2 gene and two genes related to heat shock proteins (Table S4). Corals originating from the Reef transplanted to low versus ambient pH environments showed downregulation of one of the same genes related to the heat shock protein family and upregulation of a heat shock factor gene. Further, Lagoon versus Reef native low pH transplants had an upregulated gene for a Bcl-2 protein. Various host *S. siderea* contrasts had DEGs related to TNF receptors and NFκB signalling including upregulation of TNF receptor-associated factor 3-like in Reef-Low pH versus Reef-Ambient pH and Ojo-Ambient pH versus Reef-Ambient pH contrasts (Table S4). DEGs related to regulation of MAP kinase cascade and serine/threonine protein kinase were identified in five of the contrasts. Among all five host *S. siderea* main pairwise contrasts, four putative solute carrier family genes (SLC13, SLC23, SLC39, SLC40) were all downregulated in Lagoon/Ojo natives in ambient and low pH versus Reef natives in ambient and low pH (Table S4).

3.10 | *Siderastrea siderea* – Calcification gene responses

Many DEGs related to transmembrane transport and calcium ion binding and activity were identified in host *S. siderea*. Various DEGs related to voltage-dependent calcium channel activity were all downregulated in four host *S. siderea* contrasts including a downregulated sodium/calcium exchanger in Reef native low versus ambient pH corals. In contrast, DEGs related to voltage-gated chloride or sodium channel activity were upregulated only in the host *S. siderea* Ojo versus Reef native low pH transplants. A plasma membrane calcium-transporting ATPase and a sarcoplasmic/endoplasmic reticulum calcium ATPase were upregulated in the same contrast. There were numerous DEGs related to cell adhesion, including protocadherins and genes related to β-catenin binding, β-catenin-TCF complex, vinculin binding, focal adhesion, actin binding, actin cytoskeleton, adherens junction and tyrosine kinase or tyrosine phosphatase. Several DEGs related to biomineralization (collagen, von Willebrand factor type A domain) were consistently found plus DEGs related to guanine nucleotide binding and exchange. Many DEGs related to transposable elements, including genes related to Retrovirus-related Pol polyprotein, retrotransposons, and histone activity, were observed in host *S. siderea*.

3.11 | *Siderastrea siderea* – Gene module correlation with physiological responses

For host *S. siderea*, WGCNA identified 14 gene co-expression modules (8000 genes), with 12 modules significantly correlated with one or more traits and a functional enrichment analysis identified many enriched GO terms (>2700) in all 14 modules (Figure 5, Table S7). The brown module (1443 genes) was the most significantly, positively correlated module with origin. Brown and red (434 genes) modules both had positive correlations with the traits skeletal extension, skeletal density and origin; they shared many enriched GO terms including DNA binding, nucleic acid binding, DNA integration and recombination, DNA polymerase activity, zinc ion binding, actin binding, extracellular matrix structural constituent and retrotransposon nucleocapsid. Skeletal density of *S. siderea* was higher in corals transplanted to ambient pH compared to low pH but density was reduced in Ojo natives (Figure 6c).

3.12 | *Siderastrea siderea* – Symbiont gene expression differed by symbiont genus

Interestingly, *Breviolum* spp. dominated the *S. siderea* Lagoon native (93%–100%) and Ojo native corals (86%–99%), although one individual Lagoon colony (from which replicate fragments were cut) was ~95% *Cladocopium* spp. In contrast, Reef native *S. siderea* were dominated by *Cladocopium* (78%–96%); excluding two individual colonies

cut into replicate fragments that were dominated by *Durusdinium* spp. (73%–100%). The very high DEG responses (~3000–6000) of *S. siderea* symbionts are likely due to different symbiont genera between Reef versus Ojo or Lagoon natives (i.e., differential mapping rate of transcripts among taxa) rather than differences in within-gene expression; contrasts of the same symbiont genus showed a lower DEG response (Figure S5b-c). *Cladocopium* spp. Ojo versus Lagoon natives transplanted to low pH had 72 DEGs. Total gene expression of *S. siderea Breviolum* spp. was affected by coral origin (PERMANOVA, $p = .006$), specifically for Reef versus Ojo natives ($p = .005$) but not Reef versus Lagoon natives ($p = .109$) nor Lagoon versus Ojo natives ($p = .220$; Figure S9a). Further, native origin affected total gene expression of *S. siderea Cladocopium* spp. (PERMANOVA, $p < .001$), specifically Reef native versus Lagoon or Ojo native symbionts ($p = .001$ and $p < .001$, respectively; Figure S9b).

3.13 | Comparing gene expression responses among species

Co-expression gene modules that significantly correlated with physiological traits were compared at the orthogroup level between *P. astreoides* and *S. siderea* hosts. For the trait chlorophyll-a (cm^2), *S. siderea* and *P. astreoides* hosts shared only one orthogroup (transcriptional coactivator YAP1-like; 'Siderastrea_Mexico_Barshis-Radice_Host_contig16395', 'isogroup01493_Past') although the associated gene module correlations were positive and negative, respectively. For the trait skeletal extension, hosts *S. siderea* and *P. astreoides* shared only one orthogroup (zinc finger MYM-type protein 2-like/ uncharacterized protein; 'Siderastrea_Mexico_Barshis-Radice_Host_contig03263', 'isogroup03131_Past'). The trait skeletal density had the most module-trait correlations; hosts *S. siderea* and *P. astreoides* shared 163 orthogroups (Table S8).

Coral host gene expression responses were compared between species transplanted to low versus ambient pH using a KOG analysis. The *P. astreoides* contrast Ojo-Low pH versus Ojo-Ambient pH shared 13 KOG terms with *S. siderea* Reef-Low pH versus Reef-Ambient pH, whereas Ojo and Lagoon *P. astreoides* natives transplanted to low versus ambient pH shared 5 KOG terms (Table S9). Two KOG terms related to 'Cytoskeleton' and 'Posttranslational modification, protein turnover, chaperones' were enriched in the *P. astreoides* Ojo-Low pH versus Ojo-Ambient pH contrast ($p = .05$, Fisher's exact test). One KOG term ('Cytoskeleton', KOG0161 Myosin class II heavy chain) was shared among three low versus ambient pH contrasts (*P. astreoides* and *S. siderea*).

4 | DISCUSSION

Following transplantation to a low pH environment, the coral host *P. astreoides* stress tolerant population (Lagoon native-Low pH) had similar gene expression profiles to the stress sensitive population that remained in low pH (Ojo native-Low pH). This suggests that the stress tolerant population was able to acclimatize to the low pH conditions, yet maintained higher survival rates likely related to environmental history of an ambient pH origin. Further, host *P. astreoides* Ojo native (low pH) corals transplanted to ambient pH showed similar expression profiles to Lagoon native-Ambient pH corals, which demonstrates plasticity in Ojo native corals when returned to an ambient pH environment. Host gene expression of *P. astreoides* was primarily affected by pH transplant destination, with Ojo native corals exhibiting a much larger response to different pH environments (342 DEGs) compared to Lagoon natives (37 DEGs). This could indicate a greater capacity for plasticity or acclimatization in *P. astreoides* Ojo natives, a shorter lag-time for reaching homeostasis in Lagoon natives (i.e., Lagoon origin corals have fully acclimated to the differing conditions after >2 years), or potential utilization of different genes/pathways for dealing with the stresses of the low pH common garden (though the relative lack of DEGs between origins within destinations suggest some similarity in response; sensu Rivera et al., 2021). Host gene co-expression modules (*P. astreoides* and *S. siderea*) most frequently correlated with skeletal density, which was reduced in all species transplanted to low pH, although skeletal extension was sometimes higher in low pH transplants (Martinez et al., 2019). Although the DEG response was low for host *P. porites*, pH transplant environment had a subtle effect on host gene expression, though we acknowledge the smaller sample size for this species. In the ambient pH environment, Reef native host *P. porites* had the highest calcification, linear extension and skeletal density but the highest mortality rate among all species in low pH (Martinez et al., 2019), indicating limited acclimatization potential.

In contrast, coral native origin had the strongest effect on host *S. siderea* gene expression, largely due to differences among Reef versus Lagoon or Ojo natives. This was likely related to effects of native environmental variability and/or different host-symbiont combinations (Brown et al., 2022). Interestingly, Reef native *S. siderea* responded strongest to low versus ambient pH environments (822 DEGs) while Ojo native gene expression was similar between pH environments (1 DEG), supporting long-term acclimatization in host *S. siderea* Ojo natives. When comparing the same contrast of low pH Ojo natives transplanted to low versus ambient pH, host *P. astreoides* (342 DEGs) had a more plastic response. Nevertheless, despite a lack of DEGs in the Lagoon and Ojo natives in low versus ambient pH environments, all host *S. siderea* corals transplanted to low pH were separated from their respective ambient pH transplants along the LD2 axis in the DAPC analysis, suggesting subtle effects of the low pH common garden on all origins (Figure 3b). Overall, corals transplanted to low pH showed differential expression of many genes related to pH regulation, calcification, biomineralization, cell adhesion and stress/immune responses among others, revealing potential conservation in the pathways needed to survive in a low pH environment. As a whole, the multi-species data present a complex picture of shared, contrasting and unique responses to survival in a low pH environment which we discuss in detail below.

4.1 | Low pH impact on coral cell adhesion and biomineralization pathways

Our data indicates that under low pH stress, the disruption of calcium homeostasis appears to affect the expression of calcium-dependent proteins integral to cell adhesion in corals. Host *S. siderea* and *P. astreoides* had many DEGs related to coral biomineralization, including cadherins, collagens, actins, catenins and von Willebrand factor. Cell–cell interactions are important for cell spatial organization and function. Calcium-dependent cell adhesion molecules known as cadherins have a key role in animal tissue development and cell–cell interactions (Takeichi, 1988). As in other multicellular organisms, the role of cadherins in cell adhesion is conserved in invertebrates (Oda et al., 1994) with cadherins ubiquitous in coral tissue development and organization (Mass et al., 2016; Takeuchi et al., 2016). Various structural/adhesion proteins involved in cnidarian cell–cell adhesion and biomineralization include cadherins, protocadherins, catenins and von Willebrand factor among others (Clarke et al., 2016; Drake et al., 2013).

Cadherin activity is dependent on catenins, proteins that anchor the transmembrane cadherin to the actin cytoskeleton at sites of intercellular adhesion. We observed differential expression of genes related to β-catenin binding and TCF complex (putative signalling role) in response to low pH whereas differential expression of an α-catenin was observed in other coral species from low pH sites (volcanic seep; Kenkel et al., 2018). Cadherin-catenin complexes are regulated by kinases (Nelson, 2008), including tyrosine kinases which were found differentially expressed in host *S. siderea* in our study (12; and 1 in host *P. astreoides*). Lagoon and Ojo host *P. astreoides* transplanted to low versus ambient pH shared many DEGs (same response direction) that were related to cell adhesion, actin cytoskeleton organization and actin filament processing including upregulation of a protocadherin-like protein; this demonstrates a shared response to pH transplant environment. Further, *P. astreoides* low pH Ojo natives in low versus ambient pH showed upregulation of various components related to cell-extracellular matrix adhesions (e.g., paxillin, focal adhesions). In a transcriptome analysis of primary coral polyps subjected to low pH in aquaria, many genes related to components of both the extracellular and skeletal organic matrices were differentially expressed (Moya et al., 2012).

A disruption of calcium homeostasis and intracellular pH regulation was also evident based on various DEGs. Only Ojo native host *P. astreoides* and *S. siderea* transplanted to low pH (compared to ambient pH counterparts) showed upregulation of a gene encoding a plasma membrane calcium-transporting ATPase 3, potentially a compensation for ion transport to sites of calcification (Barott, Perez, et al., 2015). Plasma membrane calcium-transporting ATPase genes were also upregulated in other coral species subject to experimental low pH (*Acropora digitifera*, Kurihara et al., 2018; *Acropora austera*, Yuan et al., 2019), suggesting corals may need to upregulate ion transporters in low pH environments. For example, corals from naturally oscillating pH environments such as reef flat habitats show the capacity to maintain pH homeostasis when exposed to acidification stress (Brown et al., 2022). In our study, host *P. astreoides* low pH Ojo natives transplanted to low versus ambient pH sites showed downregulation of a V-type proton ATPase ('isogroup01789_Past'), which are important for regulating the microenvironment and ultimately enhancing photosynthesis (Barott, Venn, et al., 2015). The upregulation of genes related to calcium and carbonate transport and organic matrix has also been observed in other coral species (*Pocillopora damicornis*) subject to low pH conditions (Vidal-Dupiol et al., 2013). Increased calcium ATPase activity has been demonstrated in calcifying organisms including coral (Ip et al., 1991) and benthic foraminifera subject to low pH conditions (Prazeres et al., 2015). It is likely that the increased gene expression of ATPase activity observed in our study and in other coral species following exposure to low pH (*Acropora millepora*; Kaniewska et al., 2012) relates to ion transport that is critical to maintaining pH homeostasis, as well as other membrane transport functions. Similarly, differential expression of genes related to ion transport (in particular, hydrogen ion transmembrane transport) was also observed in *S. siderea* exposed to low pH (Davies et al., 2016).

Corals tightly regulate intracellular pH via various transporters (Capasso et al., 2021). When comparing native origins, many genes related to calcium ion binding were differentially expressed in low pH conditions for both host *P. astreoides* and *S. siderea*. Interestingly in our study, only host *S. siderea* (but not the other two species) originating from the Ojo and Reef sites and transplanted to low pH showed consistent downregulation of DEGs (9) related to voltage-dependent or -gated calcium channel activity, including an L-type voltage-dependent calcium channel involved in calcium uptake (Yuan et al., 2019; Zoccola et al., 1999). Conversely, genes (3) related to voltage-gated chloride or sodium activity were upregulated in host *S. siderea* Ojo versus Reef natives transplanted to a low pH environment, suggesting acclimatization to regulate chloride exchange/channel activity in Ojo natives living in low pH over longer time scales. Acid–base transport mechanisms, including transport proteins such as solute carrier (SLC) families, have an important role in intracellular pH regulation in corals (Capasso et al., 2021). Plasma membrane bicarbonate transporters, including SLC genes, are key to coral calcification (Zoccola et al., 2015). Four DEGs encoding for SLC membrane transporters (SLC13, SLC23, SLC39 and SLC40) were consistently downregulated in both Ojo natives and low pH transplants across multiple *S. siderea* pairwise contrasts in our study. Various bicarbonate ion transporters have been reported for different coral species (Zoccola et al., 2015), including downregulation of a SLC4 member under low pH conditions (Yuan et al., 2018); however, the DEGs in our study matched different SLC families. Recently, the role of SLC13 and SLC23 in coral calcification were described for the non-symbiotic coral *Tubastraea* (Capasso et al., 2022). The consistent downregulation of various SLC family proteins in corals subject to low pH in our study suggests an impairment of intracellular pH regulation.

4.2 | Apparent energetic costs to survival in low pH conditions

Differential gene expression related to calcium ion activity and calcification in host *P. astreoides* is consistent with reduced calcification in low pH relative to ambient pH sites (driven by a decrease in skeletal density; Crook et al., 2013). In reefs across the Pacific, *Porites* skeletal density but not extension was also negatively affected by low pH (Mollica et al., 2018); likewise, skeletal porosity of massive *Porites* was higher in low pH sites (Prada et al., 2021). Interestingly, *P. astreoides* Ojo natives that remained in low pH had the highest calcification and linear extension rates (albeit with lower density) but had the lowest survivorship (50%) within the species (Martinez et al., 2019). The trade-off to maintain linear extension or calcification at the expense of skeletal density in the long term may result in the loss of carbonate frameworks (Dove et al., 2020). Despite Reef natives having the greatest DEG responses overall to pH environment (number of DEGs), *S. siderea* had the lowest calcification and linear extension rates among the three species (Martinez et al., 2019). For both host *P. astreoides* and *S. siderea*, more co-expression gene modules were correlated with skeletal density rather than skeletal extension suggesting density is regulated as a trade-off to growth extension under low pH (Rathbone et al., 2022). The lower skeletal density of *P. astreoides* and *S. siderea* transplanted to low pH was related to higher chlorophyll-*a* content per symbiont cell, as well as higher symbiont density overall (Martinez et al., 2019). When comparing between pH transplant destinations among *S. siderea* groups, there was a trend of higher calcification rates in Reef natives that is likely due to inherently higher calcification rates potentially related to a genetic factor and/or environment. Although skeletal density was depressed for *S. siderea* Ojo natives transplanted to ambient pH, the equivalent *P. astreoides* corals had the same skeletal density and similar gene expression profile as Lagoon-Ambient pH corals suggesting long-term effects of low pH may potentially be reconciled when returned to ambient pH conditions for some species but not others, at least on timescales of 2 years.

4.3 | Differential expression of genes related to cnidarian immunity and stress response

Transposon activity is hypothesized to increase in an effort to acclimatize to stressors or novel environmental conditions (Maor-Landaw & Levy, 2016). For example, a sea anemone under low pH conditions showed differential gene expression of various transposable elements, including retrotransposons (Urbarova et al., 2019). In our study, there were numerous DEGs related to transposable elements and transposons in host *S. siderea* Ojo natives, low pH transplants or both (corals remaining in low pH) compared to Reef natives in low versus ambient pH. For example, retrotransposon DEGs (3; e.g. Transposon Ty3 Gag-Pol polyproteins) were downregulated in low pH corals but other DEGs (5) related to transposable and retrotransposable elements were upregulated in Lagoon versus Reef native low pH transplants. Transposable element activity as well as histone regulation has also been observed in diatoms grown in low pH for multiple generations (Huang et al., 2019). Although transposable element DEGs in host *S. siderea* Reef natives may indicate a response to pH environment, it is also possible that species with larger gene inventories may use transposable elements to expand gene functions (Shumaker et al., 2019), including symbiotic Symbiodiniaceae (González-Pech, Stephens, et al., 2021). Alternatively, the expression of transposon-derived transcripts could point to the stimulation of the coral immune system and/or DNA damage. Various genes related to heat shock proteins were differentially regulated, particularly in *P. astreoides* and *S. siderea* originating from and transplanted to low pH. Many genes related to putative NFκB targets (e.g. TNF receptor-associated factors, cyclic AMP, guanyl binding proteins) were differentially expressed in host *S. siderea*, suggesting an immune stress response (Cleves et al., 2020; Herrera et al., 2021).

4.4 | Habitat-specific symbiont gene expression

Gene expression of host *S. siderea* was primarily affected by native origin, mostly due to differences between Reef natives versus Ojo/Lagoon natives which aligned with differences in symbiont genera. Holobiont populations may thus be locally adapted to native environments, with signatures maintained in both low and ambient pH sites. Of note, the majority of Reef native *S. siderea* were associated with *Cladocopium* (except a couple of colonies associated with *Durusdinium*) while Lagoon and Ojo native colonies were associated with *Breviolum*. Another study also found unique symbiont DEGs in reef origin versus low pH origin corals from a volcanic seep site (Kenkel et al., 2018). When comparing species-specific pairwise contrasts of symbionts of the same genus (i.e., Lagoon vs. Ojo, or within Reef native origin corals), there were fewer symbiont DEGs (up to 89) than in contrasts comparing different native origins (Lagoon/Ojo vs. Reef). Symbionts associated with Reef native *P. porites* transplanted to low versus ambient pH had no DEGs. Similarly, unaltered gene expression was observed in an aquaria study of symbionts associated with *Montipora capitata* exposed to low pH (González-Pech et al., 2017). It is more challenging to discern a symbiont gene expression response because symbiont communities may be dynamic over spatial and temporal scales, making direct comparison difficult. However, pairing gene expression data with coral host and symbiont physiology as in our study provides robust evidence of the longer term, potentially adaptive response to low pH conditions in the natural environment.

5 | CONCLUSIONS

Each coral species responded differently to pH environment, and there was no shared response of host gene expression at the orthogroup level although several KOG terms were in common across species DEGs between low versus ambient pH transplants. Overall,

S. siderea had a more fixed response that differed primarily by native origins and symbiont genera except for the large DEG response of Reef natives transplanted to low versus ambient pH. In contrast, *P. astreoides* had a more plastic response to pH with contrasting effects of low versus ambient pH for the host. Host *P. astreoides* Ojo versus Lagoon natives transplanted to ambient pH showed only one DEG while Ojo natives transplanted to low versus ambient pH had 10 times the DEG response compared to Lagoon natives, suggesting a high acclimatization capacity or longer lag-time in achieving homeostasis, although the potential mechanism underlying this difference is unknown. Finally, *S. siderea* hosted different symbiont genera across reef habitats providing evidence of tight host-symbiont coupling and possibly local holobiont adaptation. As a whole, these data highlight the complex and differential response dynamics of opposing pH exposures, suggesting that different populations, species and holobionts may vary considerably in their ability to respond to the impacts associated with ocean acidification.

AUTHOR CONTRIBUTIONS

AP, DCP and DJB conceived this project and designed the experiment. AM, AP and DCP participated in the fieldwork and AM conducted the laboratory analyses. VZR analysed the data, including bioinformatics and statistical analyses. All authors contributed to the interpretation of the data. VZR wrote the paper with contribution from all authors.

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CONFLICT OF INTEREST STATEMENT

None.

OPEN RESEARCH BADGES

This article has earned an Open Data and Open Materials badge for making publically available the digitally-sharable data and materials, as per data availability details.

DATA AVAILABILITY STATEMENT

The de novo *Siderastrea siderea* holobiont transcriptome is accessible at doi.org/10.17605/OSF.IO/U47CN, with custom scripts for assembly and annotation and the concatenated holobiont *Porites astreoides* transcriptome accessible at github.com/barshislab. Scripts used to generate analyses are accessible at github.com/veronicar239. Raw sequence reads were deposited in the NCBI SRA (PRJNA865460).

BENEFIT SHARING STATEMENT

Benefits from this research accrue from the sharing of our data and results on public databases as described above.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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