Coral Reefs
https://doi.org/10.1007/s00338-022-02236-9

REPORT

Nitrate enrichment has lineage specific effects on *Pocillopora acuta* adults, but no transgenerational effects in planulae

Marie E. Strader[1] · Lauren I. Howe-Kerr[2] · Jordan A. Sims[2,5] · Kelly E. Speare[3] · Amanda N. Shore[2,4] · Deron E. Burkepile[3] · Adrienne M. S. Correa[2]

Received: 16 April 2021 / Accepted: 6 February 2022

Abstract Local-scale nutrient pollution can alter coral growth and reproductive output, as well as their resident communities of microorganisms (dinoflagellates in the family Symbiodiniaceae, bacteria). Yet, the ways in which nutrient pollution alters coral interactions with their microorganisms are not fully understood, and no studies have tested for transgenerational impacts of nutrient stress on coral holobionts. To investigate this, colonies of *Pocillopora acuta* were enriched with nitrate in situ for up to one year and monitored for planulation. Gene expression, resident microbial communities and holobiont traits were characterized in adults, as well as in planulae. Although separated by > 5 m, clonality and chimerism were observed in the majority of coral colonies. Lineage- and treatment-specific effects of nitrate treatment were detected in adults and planulae. Nitrate-enriched adults contained higher densities of Symbiodiniaceae and exhibited downregulation of genes involved in the synthesis of nitrogenous compounds. Planulae harbored higher Symbiodiniaceae and bacteria diversity than adults; this study constitutes the first assessment of these microorganisms from individual planulae. Coral-associated bacteria communities were *Endozoicomonas*-dominated and were not altered by nutrient treatment. Planula-associated bacteria communities differed from their parents but not from parental exposure to nutrients, and no changes in fecundity or settlement success resulted from enrichment. Taken together, these findings suggest that adult corals acclimate to chronic nutrient pollution by harboring higher Symbiodiniaceae densities, with no observed negative effects on the subsequent generation.

Keywords Nitrate · *Pocillopora* · Transgenerational effects · Gene expression · Symbiodiniaceae · Bacteria

Background

In many coastal ecosystems, anthropogenically derived inorganic nutrient inputs now dwarf nutrients from natural sources, particularly in terms of nitrogen and phosphorus (Vitousek et al. 1997; Bennett et al. 2001). Coral reefs are particularly sensitive to nutrient pollution because reef organisms evolved to tightly recycle nutrients in an oligotrophic setting (Fabricius 2005; Furnas et al. 2011). Within coral colonies, excess inorganic nutrients can shift the nature of the relationship between coral hosts and their resident symbiotic microorganisms (endosymbiotic dinoflagellates in the family Symbiodiniaceae, bacteria, Shaver et al. 2017; Wang et al. 2018; Maher et al. 2019; Morris et al. 2019)—collectively termed the holobiont—from mutualistic to antagonistic (Zaneveld et al. 2016; Allgeier et al. 2020). Downstream effects of these disrupted relationships can include slower colony growth,

Marie E. Strader and Lauren I. Howe-Kerr have contributed equally to this work.

✉ Marie E. Strader
stradermarie@gmail.com

[1] Department of Biological Sciences, Auburn University, Auburn, AL, USA

[2] BioSciences at Rice, Rice University, Houston, TX, USA

[3] Department of Ecology, Evolution and Marine Biology, University of California Santa Barbara, Santa Barbara, CA, USA

[4] Department of Biology, Farmingdale State College, New York, NY, USA

[5] Department of Environmental Science and Policy, George Mason University, Fairfax, VA, USA

Published online: 24 February 2022

reduced reproductive success, increased susceptibility to bleaching and disease, and reduced survivorship (Bruno et al. 2003; D'Angelo and Wiedenmann 2014; Shantz and Burkepile 2014; Vega Thurber et al. 2014; Burkepile et al. 2020; Donovan et al. 2020). However, the molecular mechanisms underpinning shifts in host-microbe interactions, especially in an ecologically relevant context, remain unknown.

Impacts of eutrophication on reef-building corals are challenging to quantify because nutrients from natural sources (e.g., fish-derived ammonium) are present in conjunction with anthropogenic sources (e.g., land-based runoff high in nitrate), and these two source categories differ in how they affect the coral holobiont (Shantz and Burkepile 2014; Ezzat et al. 2015; Morris et al. 2019; Marangoni et al. 2020). For example, effects of ammonium can vary, sometimes reducing coral growth (Stambler et al. 1991; Ferrier-Pagès et al. 2000) and often enhancing coral growth (Shantz et al. 2015; Allgeier et al. 2020). Nitrate is generally associated with declines in coral health such as significantly reduced growth, and increased bleaching severity and mortality (Wiedenmann et al. 2013; Burkepile et al. 2020). Net negative effects on coral colonies enriched with nitrate arise because this form of nitrogen is energetically costly for Symbiodiniaceae to utilize (Dagenais-Bellefeuille and Morse 2013) and can drive symbionts into phosphate starvation, rendering them more susceptible to heat and light stress (Wiedenmann et al. 2013; Rosset et al. 2017). As a result, photosynthesis is impaired and Symbiodiniaceae transfer less carbon to corals (Ezzat et al. 2015; Morris et al. 2019). Despite this, nitrogen enrichment can increase the density of Symbiodiniaceae *in hospite*, depending on the coral species and form of nitrogen (Stambler et al. 1991; Snidvongs and Kinzie 1994; Morris et al. 2019; Marangoni et al. 2020; Fox et al. 2021). When holobionts exhibit increased Symbiodiniaceae densities in responses to nutrient enrichment, two (non-mutually exclusive) scenarios may be occurring: Changes in Symbiodiniaceae densities following nutrient enrichment could either indicate (1) Symbiodiniaceae have increased access to nutrients; and/or (2) the host has relaxed its regulation of symbiont densities. Ultimately, how coral hosts respond to nitrate remains unclear as net negative, neutral and positive responses of corals to nutrient enrichment have been observed (e.g., Becker and Silbiger 2020; Becker et al. 2021). These different responses may partly be explained by complex responses to skewed nutrient stoichiometry (Wiedenmann et al. 2013; Rosset et al. 2017; Lapointe et al. 2019) and/or differences in background nutrient availability or other abiotic factors among study sites and reef zones. No study has examined coral host gene expression under chronic nutrient enrichment, although the connection between altered nutrient cycling and bleaching is becoming increasingly well understood (Rädecker et al. 2021).

Varied responses to nutrient enrichment have additionally been documented in early coral life stages; these responses also vary according to nutrient stoichiometry, other abiotic environmental factors (such as heat, Humanes et al. 2017; Serrano et al. 2018), and life history (i.e., broadcast spawners vs brooders; Kitchen et al. 2020). However, the extent to which impacts of nutrient enrichment carry-over to the next generation through transgenerational effects has not been tested (but see Harrison and Ward 2001). In other marine systems, transgenerational effects have been observed in diverse taxa in response to temperature and pCO_2, but not under nutrient enrichment conditions (Donelson et al. 2012; Miller et al. 2012; Parker et al. 2012; Jensen et al. 2014; Wong et al. 2018). One study in corals examined larval responses to elevated pCO_2 after adult conditioning and found changes in larval body size and respiration in a brooding *Pocillopora* (Putnam and Gates 2015), suggesting that stressors that impact corals may also impact their offspring through transgenerational effects. In addition, corals may transmit beneficial microbial communities from adults to offspring. Approximately 90% of brooding coral species acquire Symbiodiniaceae from their parents via vertical transmission (Baird et al. 2009). Coral parents may also transmit bacteria to their offspring (Ceh et al. 2013; Webster and Reusch 2017; Quigley et al. 2019), although coral-associated bacteria communities are understudied in planulae (Damjanovic et al. 2020a). These results highlight the possibility for parental or transgenerational effects in brooding *Pocillopora* and the need for additional studies under various abiotic conditions, particularly in situ.

Anthropogenic nutrient inputs have been linked to shifts in the bacteria (Dinsdale et al. 2008; Garren et al. 2009) and Symbiodiniaceae (Pogoreutz et al. 2018) community composition of adult corals. Such shifts may reflect dysbiosis within the holobiont (McDevitt-Irwin et al. 2017; Zaneveld et al. 2017; Claar et al. 2020; Zhang et al. 2021) and/or directly contribute to disease and declines in coral health (Morrow et al. 2012; Zaneveld et al. 2016). Claar et al. (2020) observed increased variability of bacteria and symbiont communities with increased levels of chronic disturbance (including land-based runoff) on reefs. Nutrient enrichment may permit the proliferation of opportunistic and pathogenic bacteria, especially in conjunction with thermal stress (Zaneveld et al. 2016; Maher et al. 2019, but see Maher et al. 2020), increasing overall bacteria richness. Increases in richness can be accompanied by declines in beneficial coral bacteria, including *Endozoicomonas* spp. which are considered bacterial endosymbionts of some corals (Morrow et al. 2012; Ziegler et al. 2016; Leite et al. 2018). Decreased abundance of

Endozoicomonadaceae in corals has been linked with signs of coral disease (Neave et al. 2016), possibly because these bacterial symbionts contribute to nitrogen and sulfur cycling and the production of antimicrobials (Morrow et al. 2015). The effects of long-term nitrate-only enrichment on coral microbial communities, in combination with host responses, such as gene expression, have not been explicitly studied in situ.

To investigate the impact of chronic nitrate enrichment on adult coral holobionts, as well as the potential for transgenerational effects, we established an in situ enrichment experiment in Mo'orea, French Polynesia and examined host gene expression, microbial community composition and holobiont traits of *Pocillopora acuta* adults and planulae. Clones, cryptic species, and/or chimeras are known to occur within the genus *Pocillopora* (Stoddart 1983; Torda et al. 2013; Schmidt-Roach et al. 2014; Rinkevich et al. 2016; Johnston et al. 2017; Burgess et al. 2021). We therefore controlled for colony genotype, since this could have significant effects on holobiont responses to treatments, as documented in other studies (Dixon et al. 2015). We hypothesized that nitrate enrichment causes: (1) changes in holobiont gene expression related to altered micronutrient exchange, dysbiosis and/or decreased growth/reproductive investment; (2) increased diversity and variability in Symbiodiniaceae and bacteria communities; (3) transgenerational shifts in Symbiodiniaceae and bacteria communities (i.e., changes in parents are also observed in the microbial communities of their respective planulae); and (4) reduced coral reproductive success.

Methods

Experimental setup and chronic nitrate enrichment

Ten colonies of *P. acuta* were collected from a shallow (< 2 m) lagoon reef in Mo'orea, French Polynesia on April 16, 2018 (Fig. 1A) and divided into four 10–15 cm fragments. Colonies were collected from > 5 m apart to reduce the potential for collection of clones. Two fragments from each colony were attached to a 'control' cinder block (no nutrients added) and two were attached to a 'nutrient' cinder block (Fig. 1B) containing a diffuser with slow-release nitrate-only fertilizer (polymer-coated potassium nitrate, Multicote 12-0-44, Haifa Chemicals Ltd., Electronic Supplementary Methods). Cinder blocks were then deployed back in the lagoon reef. Nutrient diffusers were replaced every 8–12 weeks. Our past work using this method has shown that nitrate concentrations in the surrounding seawater are 3–7 uM above ambient concentrations for at least 10 weeks (Burkepile et al. 2020). This approach has been similarly effective with other fertilizers as well (Vega Thurber et al. 2014; Zaneveld et al. 2016; Dougan et al. 2020). After four months, two of the colonies had experienced mortality in either both controls or both treatments, and one colony could not be located. Since this mortality was not driven by the nutrient enrichment, all fragments of those colonies were removed from the experiment and replaced with three new colonies on August 18, 2018 that were fragmented as described above (four fragments, two in each treatment, control and nitrate-enriched). Thus, all fragments analyzed in this study were enriched for 8–12 months before planulae collection in the austral fall of 2019.

Pocillopora acuta adult and planulae sampling

In *P. acuta,* planulation generally starts 3–4 days following the new moon (Smith et al. 2019). Therefore, on April 5, 2019 (the day of the new moon), the largest control and enriched fragments from each colony were transferred to individual 75-L, flow-through seawater tanks under shade cloth at the Richard B. Gump South Pacific Research station on Mo'orea. The largest of each of the two replicate fragments was selected in order to maximize the likelihood of collecting sufficient planulae for downstream analyses. Samples from parent colonies were collected immediately to characterize microbial community compositions resulting from in situ treatment (a $\sim$ 0.5 cm^2 branch in DNA/RNA Shield, hereafter referred to as 'pre-planulation' samples). Total planulae output was monitored from each fragment to inform when gene expression and microbial community sampling should occur, targeting peak release (Fig. 1C, Electronic Supplemental Methods).

Planulae from every colony (n = 10) were sampled for gene expression at 06:00 h on April 9, 2019, which corresponded to peak planulation for the majority of the fragments (Fig. 1C). Microbial samples were also collected from a subset of the colonies (n = 4) when control and nitrate-enriched fragments from the same colony had high planulation on the same night (Fig. 1C). Specifically, for each type of sample (gene expression and microbial), ten actively swimming planulae from each fragment were transferred to a holding container filled with seawater. Individual planulae were then rinsed three times in 0.02 µm filtered seawater and preserved in individual collection tubes filled with 100% ethanol for gene expression or with DNA/RNA shield (Zymo Research, Irvine, CA) for microbial analysis. When additional planulae were available during a sampling event, they were also preserved to calculate planula volume and Symbiodiniaceae density. Two pieces of each parent fragment were preserved once planulation had ended (April 13th): a $\sim$ 3 $\times$ 2 cm fragment per parent was fixed in DNA/

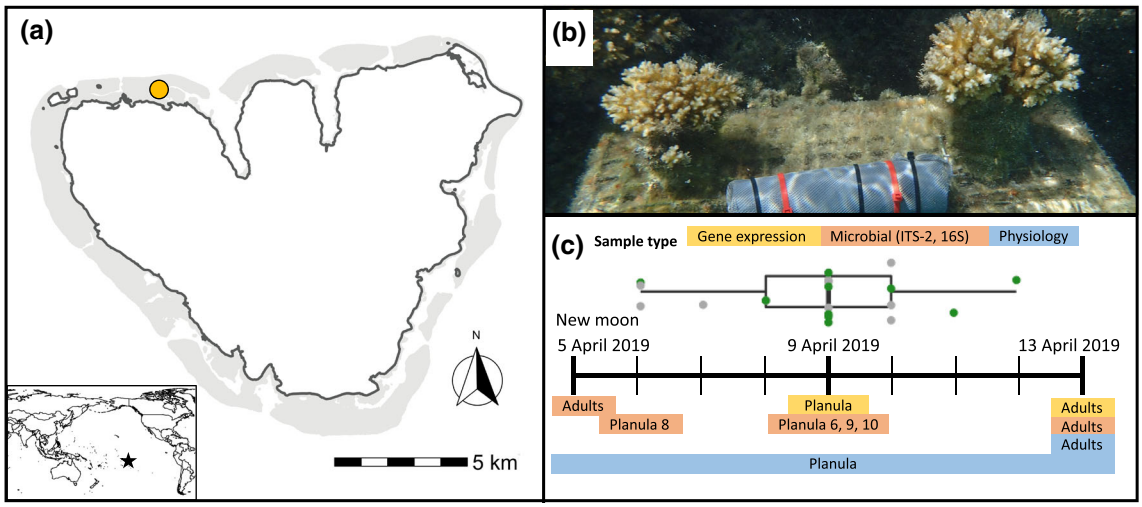

Fig. 1 Experimental design and sampling timeline. (**A**) An in situ nitrate-enrichment experiment was established in the north shore lagoon (yellow dot, S17° 29.323' W149° 52.852') of Mo'orea, French Polynesia (black star on inset world map). (**B**) Representative image of *Pocillopora acuta* fragments that were nitrate-enriched. (**C**) Timeline shows dates of the new moon (when colonies were retrieved from the in situ experiment and placed in aquaria), planulation and sampling. Boxplot shows the distribution of peak planulation days (i.e., when the highest numbers of planula were released for each colony). The 10 nitrate fragments are in green; associated control fragments are in gray. Colored boxes correspond to the sample type collected (e.g., Planula 8-microbial samples from individual planula of colony 8 control and nitrate paired fragments were collected April 6, 2019). Planula from colonies 6, 8, 9, and 10 were sampled for microbial analyses; planula from all colonies were sampled for gene expression

RNA shield and vortexed with lysing beads to disrupt tissues for gene expression and microbial analysis (referred to as 'post-planulation' samples), and a $\sim$ 3 cm^3 cm piece per parent was preserved for measurement of holobiont traits (Symbiodiniaceae density, chlorophyll concentrations, and total protein, Supplementary methods).

RNA sequencing and differential gene expression

Total RNA was extracted from adult and planula tissue samples (Electronic Supplementary Methods) and sent to the University of Texas at Austin Genome Sequencing and Analysis Facility for 3' Tag-Seq library prep following Meyer et al. (2011) and Lohman et al. (2016) and sequenced on the Novaseq 6000 with 100 bp SE reads. Reads were trimmed of adapter sequences, de-duplicated and filtered for quality using cutadapt (Martin 2011) following wrapper scripts available at https://github.com/z0on/tag-based_RNAseq. The adult Tag-Seq sequences were evaluated for the presence of clones, six of which were identified from experimental colonies, and given the identifiers "A-F" (Electronic Supplementary Methods). Cleaned reads were mapped to a holobiont transcriptome containing the *Pocillopora damicornis* (Traylor-Knowles et al. 2011) and *Durusdinium trenchii* (Bellantuono et al. 2019) transcriptomes using Bowtie2 (Langmead and Salzberg 2012). Gene expression was analyzed using DESeq2 (Love et al. 2014). Adult and planulae datasets were analyzed separately due to the use of different preservation methods that may have biased measured gene expression. Only isogroups with $>$ 10 mean count across samples were retained. For each dataset, two separate models were run, one that specified effects of the treatment and clone group ($\sim$ treatment + clone) and another that grouped treatment + cloneID to identify differential treatment effects for each clone, or interactions ($\sim$ group). Significantly differentially expressed genes (DEGs) were defined by a false discovery rate adjusted p-value of < 0.1. Stat values from each comparison were used for GO enrichment analysis using the GO_MWU package (Wright et al. 2015). This test involved a two-sided Mann–Whitney U test to identify functional enrichment of GO terms without having to set arbitrary cutoffs. PCoA analysis was conducted using regularized log-transformed count data, and significance among factors was assessed using the adonis function in the package *vegan* (Oksanen et al. 2020).

Symbiodiniaceae and bacteria community profiling

DNA was extracted from vortexed tissue slurry of adults and planulae using ZymoBIOMICS DNA/RNA Miniprep Kits. All samples were amplified using two sets of primers to target the Internal Transcribed Spacer-2 (ITS-2) region (Sym_VAR_5.8SII and Sym_VAR_REV, Hume et al. 2018) of Symbiodiniaceae rDNA and the hypervariable region 4 (V4, 515f and 806rB, Caporaso et al. 2011) of the bacteria 16S rRNA gene and sequenced on an Illumina

Miseq instrument using PE250 (Supplemental Methods). Symbiodiniaceae reads were processed with Symportal (Hume et al. 2019), and differential abundance analyses were conducted on the pre-MED (minimum entropy decomposition, Eren et al. 2015) output file using DESeq2 (version 1.36.0; Love et al. 2014), after collapsing total sequence abundances within each genus. Bacteria reads were processed in RStudio (version 1.1.456) through the DADA2 pipeline (version 1.11.0, Callahan et al. 2016). The DADA2 pipeline generated a table of amplicon sequence variants (ASVs), and bacteria taxonomy was assigned using the SILVA rRNA database (version 132, Quast et al. 2013), and reads were maintained at ASV-level resolution.

Alpha diversity of bacteria communities in the parents and planulae from both treatments was assessed using Shannon's and Simpson's indices at the ASV level. Differences in alpha diversity indices between treatment conditions and life stages were assessed using either a Wilcoxon rank-sum test or t test, depending on whether the assumption of normality was met. Multivariate tests for variance and dispersion were conducted using a weighted UniFrac distance matrix after rarefying to 21,000 reads, unless otherwise noted. Permutational multivariate analysis of variance (PERMANOVA) using the adonis function was used to separately assess differences in bacteria community composition between life stages, treatments, and parental clone groups. Permutation tests for homogeneity in multivariate dispersion (PERMDISP) using the betadisper function in *vegan* tested for dispersion or distance-to-centroid differences in bacteria communities between treatment conditions and life stages. Differential abundance analysis was conducted with DESeq2 (version 1.36.0; Love et al. 2014) at the family and genus level.

Quantification of planula traits

To assess planula condition, we measured their Symbiodiniaceae densities and recorded the proportion of planulae that underwent metamorphosis (settlement). Individual planulae (n = 56 from clone A, n = 16 from clone D) were preserved for symbiont density counts (Electronic Supplementary Methods). Settlement assays were set up on April 10, 2019, using planulae that were collected at 06:00 AM that day. First, planulae were collected from all parents that released planulae on April 10 and pooled by treatment (i.e., control versus nutrient). One fragment of one colony (1C) did not release any planulae on April 10, so we did not include any planulae from colony 1 (either 1C or 1 N) in settlement assays. Then, 15 planulae were counted from each pool and released into settlement chambers (44 mL plastic cups) that contained ~ 40 mL of seawater. There were 20 replicates with planulae from control parents, and 21 replicates with planulae from enriched parents. Terracotta tiles were preconditioned for one year in the lagoon prior to starting the settlement assays and a piece of terracotta tile ~ 1 × 1 × 1 cm was placed at the bottom of each settlement chamber as settlement substrate. Settlement assays ran for four days, and the water in settlement chambers was changed each day. Settlement was assessed using microscopy after two days (April 13) and again after four days (April 15) and all individuals that were settled on the piece of terracotta and on the inside of the cup were counted. Settlement and densities of planulae from enriched parents and control parents were compared using a Wilcoxon rank-sum test.

Results

Clones and chimeras of *Pocillopora acuta* are prevalent in Mo'orea

To test whether all experimental colonies were distinct genetic individuals, we extracted SNPs from tag-seq data generated from paired (nitrate-enriched and control) samples of each adult colony. After filtering, 16,651 high-quality SNPs were retained. Analysis using GenoDive determined that five of the ten colonies were the same clone and two of the colonies were chimeras of unique genotypes or clonal fragments of other colonies. Identity-by-state analysis confirmed this pattern of genetic relatedness among our samples (Fig. 2A). A total of six clone groups were assigned identifying letters (A–F) that will be used throughout the rest of this work. Principal Coordinate Analysis illustrated the variance between these samples and confirmed the tight clustering of the five colonies, clone "A" (Fig. 2B).

Some holobiont traits differed or exhibited trends by life stage or nutrient treatment

Symbiont densities as well as chlorophyll *a* and *c* concentrations (Fig. 2C) were higher in the nitrate-enriched colony fragments (paired t-tests; df = 7, p = 0.028, d = 0.977; df = 7, p = 0.027, d = 0.988; df = 5, $p < 0.001$, d = 2.29, respectively, with the two chimeric colony pairs excluded). All adult colonies and planulae were dominated by Symbiodiniaceae in the genus *Durusdinium* (Electronic Supplementary Material Fig. S1). Two samples from colony 3 (3C-post-planulation and 3 N-post-planulation) additionally contained *Cladocopium* sequences in abundances of 3.8 and 8.7% of reads per sample, and ten other samples contained *Symbiodinium, Breviolum*, and/or *Cladocopium* sequences at abundances of < 0.1% of reads per sample (Electronic Supplementary Material Fig. S1). Although

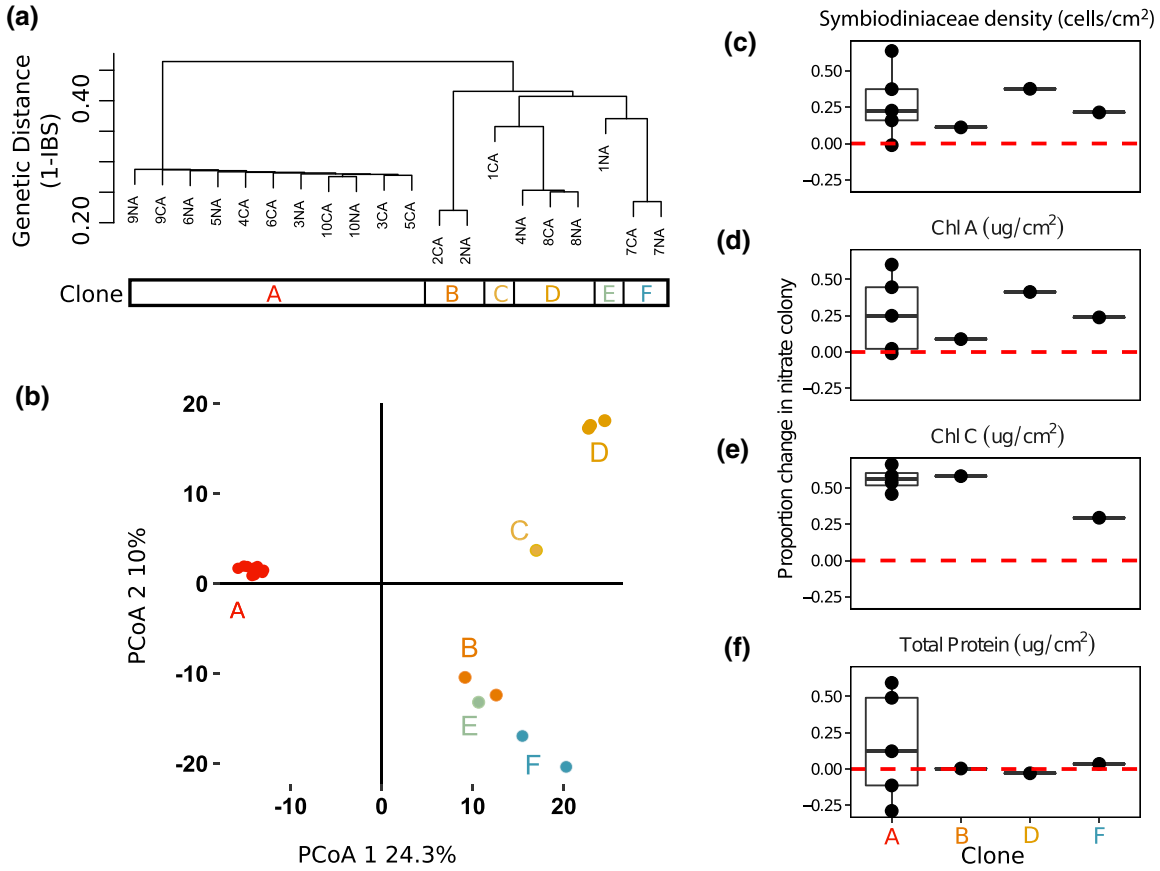

Fig. 2 Clonal identity of adult colonies and their holobiont traits by nutrient treatment. (**A**) Identity-by-state analysis delineated clonal groups within sampled colonies, which were confirmed by a principal coordinates analysis (**B**). (**C**) Proportion change in holobiont traits after one year of in situ nitrate enrichment, relative to control fragments, including Symbiodiniaceae densities (Sym. density), Chlorophyll *a*, Chlorophyll *a*, and total soluble coral protein. Each measurement was standardized to units per square centimeter of coral tissue. Samples are grouped by clonal group, and pairs of control and nitrate fragments (derived from the same colony) that were identified as belonging to separate clone groups were excluded

also dominated by *Durusdinium*, planulae samples had higher abundances of other genera (*Symbiodinium, Breviolum,* and *Cladocopium*) relative to their respective adults, based on a significant decrease in *Durusdinium* (DESeq2, log2fold change = −1.141, $p = 0.002$, Electronic Supplementary Material Fig. S3). SymPortal designated Symbiodiniaceae ITS-2-type profiles for parent- and planula-associated Symbiodiniaceae sequences that were sufficiently abundant in our sequence dataset; these type profiles (based on the post-MED output) are provided in Supp. Mat. Figures S2 and S4, with the caveat that these graphs do not depict all Symbiodiniaceae genetic diversity detected in a given sample. Control and enriched colonies did not differ in terms of total protein (Fig. 2C, paired Wilcoxon rank-sum test, $p = 0.547$).

Pocillopora acuta gene expression varied by clone group and nitrate treatment

Removing reads with mean counts < 10 resulted in 8322 highly expressed host genes for downstream analysis. *Durusdinium trenchii*-associated genes made up very few of the mapped reads and only 550 genes had mean counts > 3, and thus we focused the analysis on host gene expression responses. For host gene expression, clone group significantly contributed to overall patterns of gene expression in adults (adonis, $p = 0.001$, Fig. 3), but significant treatment effects were not detected (adonis, $p = 0.129$ for adults, Fig. 3). However, modeling adult gene expression using the model ~ treatment + clone resulted in 2 differentially expressed genes (DEGs) (pFDR < 0.1, LFC > 0) between control and nitrate-enriched fragments, both of which are coral-specific and unannotated. In testing the same model design with planula samples, 0 DEGs were identified. Gene Ontology (GO) using a ranked-based Mann–Whitney U test revealed

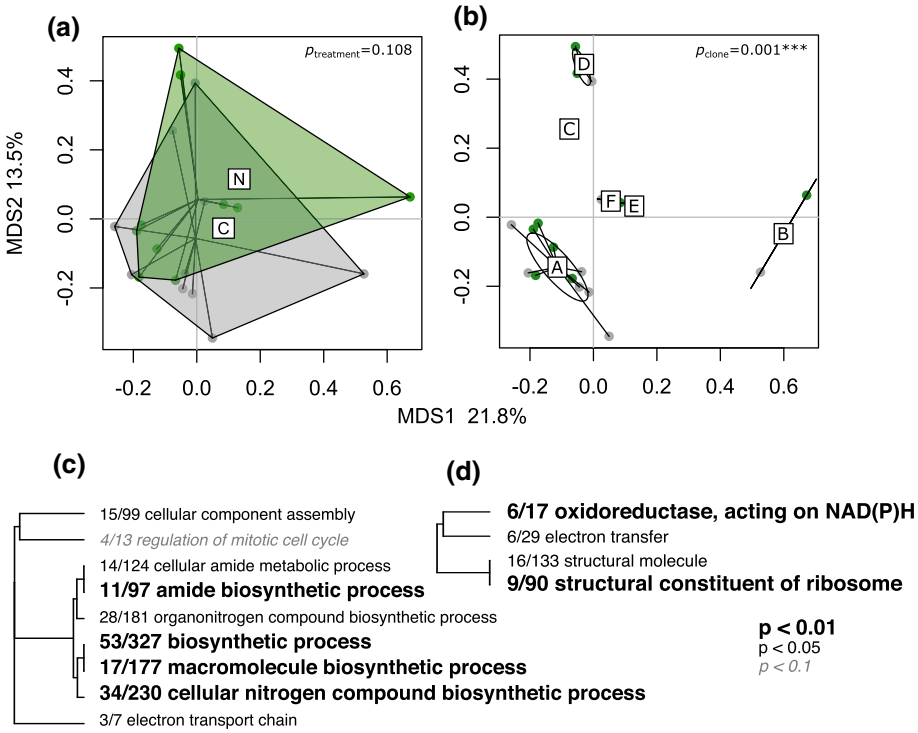

Fig. 3 Clone identity and nitrate exposure influence adult *Pocillopora acuta* gene expression. (**A**) Principal Coordinates Analysis of *P. acuta* gene expression in situ on the back reef in control (C, grey shading) and nitrate (N, green shading) treatments; and (**B**) by clone group. Significantly enriched GO terms in downregulated genes under nitrate enrichment compared to control for both (**C**) Biological Processes and (**D**) Molecular Function. For each GO term, the preceding fraction indicates the number of genes annotated with the term that pass an unadjusted p-value threshold of 0.05 out of all genes annotated with the term

significantly enriched GO terms for the nitrate treatment in both adult and planulae datasets (Fig. 3, Electronic Supplementary Material TableS1). Despite the low numbers of DEGs in our analysis, we performed a GO enrichment using a Mann–Whitney U rank-based approach, which eliminates the need for setting arbitrary p-value cutoffs (Wright et al. 2015). In adults, GO terms including "amide biosynthetic process," "organonitrogen compound biosynthetic process," "cellular nitrogen compound biosynthetic process," "electron transfer," "oxidoreductase, acting on NAD(P)H," and "regulation of mitotic cell cycle" were significantly enriched in genes that were downregulated under nitrate enrichment (Electronic Supplementary Material TableS1). In planulae, we observed GO enrichment of the following categories: "cellular response to stimulus," "nucleic acid metabolic process," and "carbohydrate binding" in genes downregulated in response to nitrate enrichment (Electronic Supplementary Material TableS1). Clone-specific patterns of gene expression by nitrate treatment are provided in the Electronic Supplementary Material.

Bacteria communities were influenced by clone group and life stage with no effects of nitrate treatment

Sequencing of the bacteria 16S rRNA gene of all parent coral fragments yielded 4,798,612 total reads. After quality filtering and merging, 1,903,433 paired reads remained, corresponding to a total of 1,085 unique ASVs. ASVs that were identified as mitochondrial DNA (n = 209) or found in the negative control (n = 210) were removed, leaving 666 ASVs for community analysis. The fragments sampled pre-planulation had higher richness than those sampled post-planulation, with 433 versus 263 unique ASVs, respectively. Bacteria ASVs were identified to the family level, and 204 families were represented in total: 177 in the pre-planulation adults and 133 in the post-planulation adults. All adults were dominated by members of Endozoicomonadaceae, which was present in all samples at 99.2 ± 0.3% relative abundance (mean ± SEM). Additionally, parent samples contained diverse low abundance bacteria types (Fig. 4).

Control and nitrate samples were compared in pre- and post-planulation samples and did not differ in terms of alpha diversity (Wilcoxon rank-sum test, Shannon and Simspon diversity, $p > 0.1$ for all), beta dispersion (betadisper, df = 1, $p > 0.1$), or community composition (adonis, df = 1, $p = 0.086$ pre-planulation; $p > 0.1$ post-planulation). Bacteria community composition of pre-planulation communities was influenced significantly by clone group (adonis, df = 5, $p = 0.023$), whereas composition of post-planulation communities did not differ by clone group (adonis, df = 5, $p > 0.1$). Both bacteria community composition (adonis, df = 1, $p = 0.028$) and alpha diversity (Wilcoxon rank-sum test, Shannon diversity, $p = 0.019$; Simpson diversity, $p = 0.105$) of control fragments were significantly different between the pre-

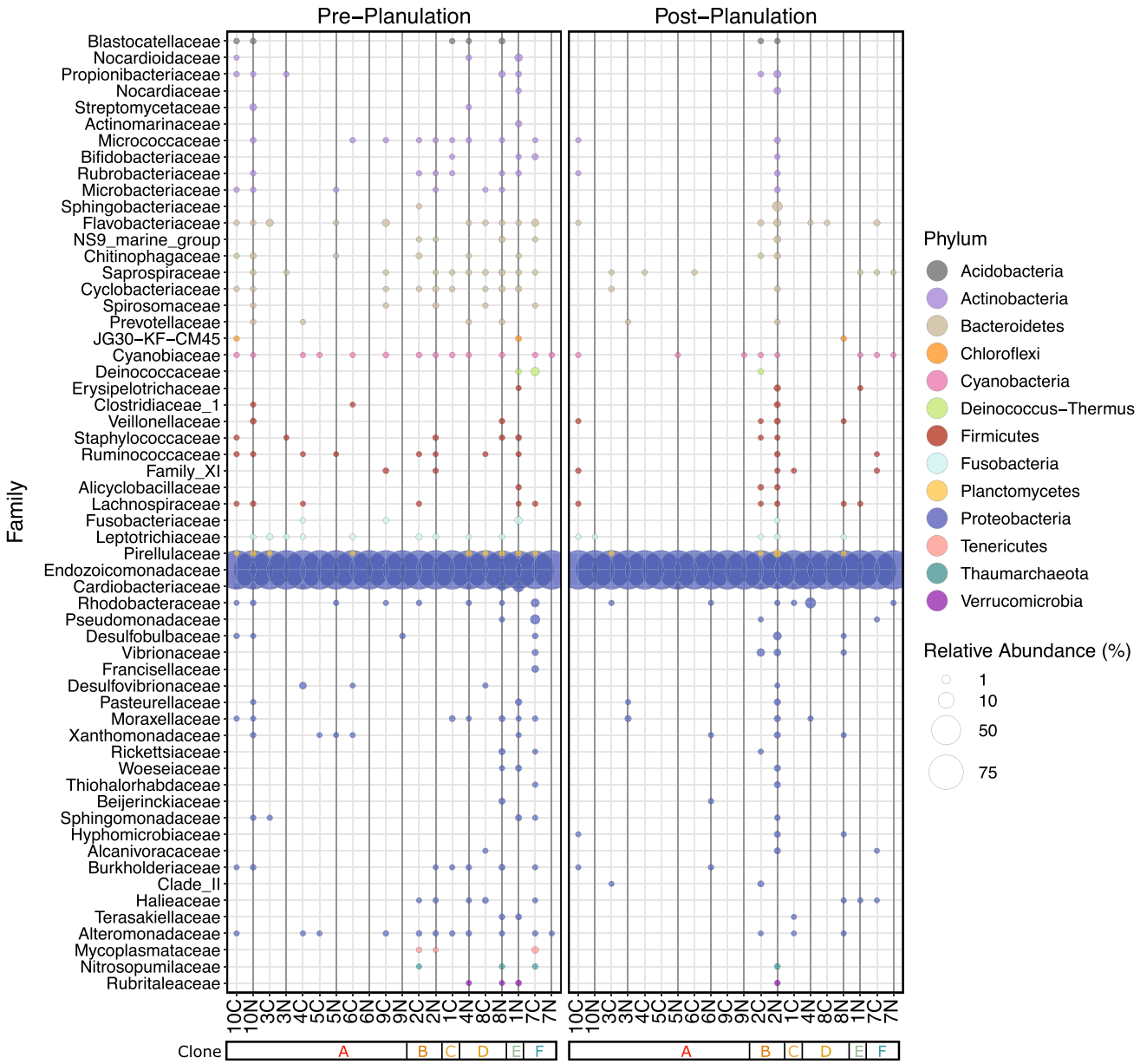

Fig. 4 Bacteria families present in each adult coral fragment, based on the V4 region of the 16S rRNA gene. Samples are grouped by time sampled (pre- or post-planulation), and labels on the x-axis reflect colony ID, treatment (control, C, or nitrate, N) and clone group (colored **A**–**F**). Dark vertical gridlines correspond to nitrate samples (N); light gridlines are control (C) samples. Bacteria community members present at > 0.1% abundance in each sample are shown. Dot sizes scale to relative abundance; representative sizes are provided for 1, 10, 50 and 75% relative abundance

planulation and post-planulation sampling timepoints, but no difference in beta dispersion between the timepoints was observed (betadisper, df = 1, $p > 0.1$). There were no significantly differentially abundant families or genera between control and nitrate samples pre- or post-planulation, nor between pre- and post-planulation samples (DESeq2, $p > 0.05$). Planulae bacteria communities did not differ by treatment but were more diverse and variable than adults (Electronic Supplementary Results).

Nitrate enrichment in adults does not impact planulae traits

P. acuta colonies were monitored for planula output from April 6–12, 2019. Each night planulae were released from some fragments, but not every fragment released planulae every night. Control fragments released between 0 and 1,414 planulae each night (mean = 184 planulae fragment^{-1} night^{-1}), whereas fragments that were enriched with nitrate released between 0–1,498 planulae each night

(mean = 135 planulae fragment^{-1} night^{-1}, Fig. 5A). The total number of planulae released ranged from 17 to 4978 planulae (mean = 1287 planulae) and 35–2835 (mean = 943 planulae) for control and nitrate-enriched fragments, respectively, but the total number of planulae released by each fragment (standardized to the planar surface area of each fragment) did not differ between control and enriched fragments (Wilcoxon rank-sum test, $p = 0.436$, Fig. 5A). Symbiodiniaceae densities of planulae from control and enriched colonies also did not differ (Wilcoxon rank-sum test, $p = 0.381$, Fig. 5B) and ranged from 1.24e^6–12.604e^6 (mean = 6.519e^6) and 1.999e^6–12.144e^6 (mean = 6.135e^6) cells per mm^3, respectively. The total percent settlement in each replicate ranged from 0 to 20.0% (mean = 5%) for planulae from control fragments and 0–26.7% (mean = 4%) for planulae from enriched fragments. Mean percent settlement of planulae from control (5%) versus enriched (4%) fragments was not significantly different (Wilcoxon rank-sum test, $p = 0.538$, Fig. 5C).

Discussion

Here, we report lineage (clone) specific molecular and microbial responses of a common fringing reef coral (*Pocillopora acuta*) in response to long-term in situ nitrate enrichment. We found that although Symbiodiniaceae densities and chlorophyll concentrations were elevated in nitrate-enriched colonies and coral hosts modulated genes involved in host-symbiont interactions, bacterial communities did not differ in nitrate-enriched colonies. This study is the first to test for transgenerational impacts of chronic enrichment on metrics of coral reproductive success and on the microbiota of individual coral planulae. Although adults exhibited subtle responses to nitrate, importantly, few to no transgenerational effects of enrichment were detected in parental fecundity, planula Symbiodiniaceae densities, molecular responses, or settlement. Given this, coral offspring may need little to no 'recovery time' to establish healthy microbiota and growth rates on reefs where point sources of nutrient pollution are mitigated.

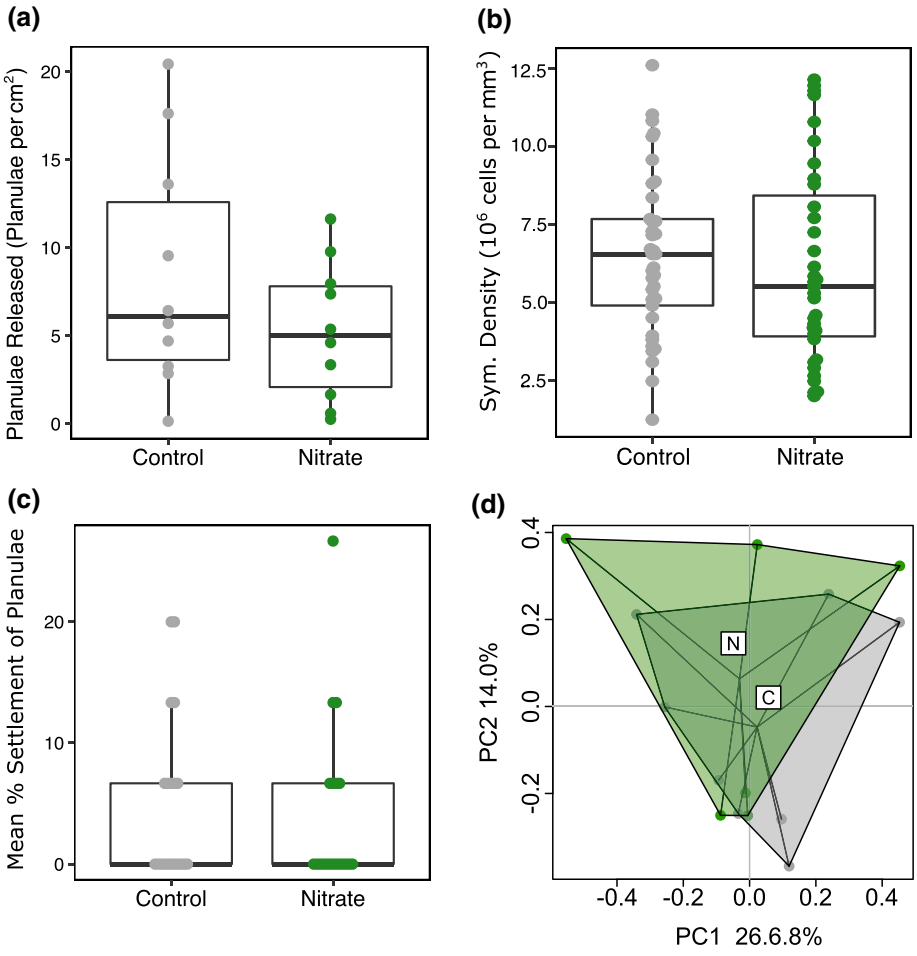

Fig. 5 Reproductive and planula traits from enriched and control *Pocillopora acuta* colony fragments. (**A**) Total planulae released, standardized to the planar surface area of each coral fragment. (**B**) Symbiodiniaceae densities of individual planulae from a subset of control and enriched fragments, normalized to 10^6 cells per mm^3. (**C**) Mean percent settlement of planulae. (**D**) Principal Coordinates Analysis of planulae gene expression in control (C, grey shading) and nitrate (N, green shading) treatments

Nitrate enrichment increases Symbiodiniaceae densities and alters host-symbiont nutrient exchange

In cnidarian-Symbiodiniaceae partnerships, corals generate ammonium as a by-product of amino-acid catabolism; the controlled transfer of this ammonium to Symbiodiniaceae limits their densities *in hospite* (Pernice et al. 2012; Xiang et al. 2020). Since corals lack the enzymes to metabolize nitrate (Grover et al. 2003; Kopp et al. 2013; Rädecker et al. 2021), we propose that the Symbiodiniaceae in our study directly accessed and utilized nitrate in the enriched treatment. This additional source of N likely released Symbiodiniaceae from nutrient limitation and contributed to their increased densities in enriched fragments (Fig. 2C). However, conversion of nitrate to ammonium is an energetically costly process (Dagenais-Bellefeuille and Morse 2013; Ezzat et al. 2015), and consequently, net carbon per symbiont cell transferred to the coral host may have been reduced. If this was the case, carbon exchange could still have been maintained or balanced by the higher symbiont densities (Morris et al. 2019; Krueger et al. 2020). We note that increased Symbiodiniaceae density has not frequently been observed in nitrate-enrichment experiments (Shantz and Burkepile 2014; Marangoni et al. 2020, but see Marubini and Davies 1996; Chumun et al. 2013; Ezzat et al. 2015); this effect of nitrate enrichment may depend on simultaneous availability of phosphorus (Ezzat et al. 2016; Morris et al. 2019) and light intensity (Wiedenmann et al. 2013).

We observed that in nitrate-enriched corals, downregulated genes exhibited enrichment of the GO term: "cellular nitrogen compound biosynthetic process," among others, potentially signifying that hosts modified their gene expression to minimize transfer of nitrogenous compounds (Fig. 3, Electronic Supplementary Material Table S1), although up and down regulation of specific genes within these GO categories could signify contrasting responses. Further, clone group had a stronger effect than nitrate enrichment on host gene expression, and total host protein was indistinguishable across treatments (Fig. 2). These lines of evidence, in concert with no detection of a holobiont stress response, suggest our long-term nutrient enrichment led to a new equilibrium between host and symbiont characterized by higher Symbiodiniaceae densities *in hospite*. Elevated symbiont densities are sometimes associated with higher susceptibility to bleaching (e.g., Cunning and Baker 2013; Kenkel and Bay 2018), and nutrient stress shows interactive effects of temperature on bleaching severity (Donovan et al. 2020). Therefore, although the adults in our experiment appear physiologically stable, they could be more susceptible to bleaching under different thermal stress regimes (Wiedenmann et al. 2013). Additionally, although differential survival of the nitrate-enriched and control fragments was not detected in this study, since we selected the largest fragment from each control and nitrate pair for analysis (to ensure sufficient planula availability), our results may overestimate the performance of small colonies under the treatment conditions.

While we did not directly measure growth, our gene expression results that nitrate-enriched fragments exhibit downregulation of GO terms "mitotic cell cycle," "electron transport chain" and "ribosomal activity" in nitrate-enriched corals (Electronic Supplementary Material Table S1) indicate these fragments potentially experienced reduced growth and metabolic weakening. Downregulation of genes associated with the cell cycle and ribosomal activity is commonly observed in organismal responses to stress, reflecting a shift from cellular maintenance to stress mitigation within the cell (López-Maury et al. 2008). Previous work has shown reductions in calcification in response to nitrate due to an imbalance in coral redox status (Shantz and Burkepile 2014; Marangoni et al. 2020). In another study, coral growth increased in two Caribbean species with exposure to fish-mediated nutrients (ammonium) only, as opposed to anthropogenic sources of nutrients (i.e., nitrate with ammonium, Allgeier et al. 2020). These responses were driven by differences in carbon acquisition and exchange (Ezzat et al. 2015). Quantification of Symbiodiniaceae densities, host gene expression, and holobiont metabolic physiology in a multi-year nitrate-enrichment experiment constitutes an important next test of the mechanistic holobiont responses proposed above.

Endozoicomonas-dominated bacteria communities are resistant to nitrate pollution

All adult samples were dominated by a bacteria symbiont in the family Endozoicomonadaceae that is commonly associated with some coral species (Bourne et al. 2016; Neave et al. 2016) and especially dominant in pocilloporids (Brener-Raffalli et al. 2018; Pogoreutz et al. 2018). Although the dominance of this family likely obscured our ability to detect significant nitrate-driven shifts in lower abundance bacteria taxa in these corals, the apparent stability of the bacteria communities agrees with past findings that *Pocillopora verrucosa* bacteria communities remain consistent during exposure to nutrient stress (Pogoreutz et al. 2018). Interestingly, there was a significant decrease in bacterial diversity after planulation, driven by a loss of lower abundance taxa. While this loss of taxa could be a post-planulation response (though the opposite pattern—increased bacterial diversity after planulation—was documented in *Pocillopora damicornis* colonies sampled in situ, Ceh et al. 2012), it is also possible that the artificial (aquaria) environment where corals were maintained

during planulation drove the observed changes in bacteria community structure (Kooperman et al. 2007; Bergman et al. 2021). Future studies should investigate whether tipping points exist in pocilloporid microbiota resistance to nutrient enrichment and confirm that transgenerational effects do not arise under more concentrated nutrient enrichment and/or multi-year exposure duration.

Parental nitrate enrichment does not affect microbial diversity or reproductive traits in individual planulae

Symbiodiniaceae and bacteria communities in planulae were more diverse than those in parent fragments (Electronic Supplementary Material Figs. S2 and S3). This matches observations in Epstein et al. (2019) and Damjanovic et al. (2020a; b), in which bacteria communities profiled from young *P. damicornis* and *P. acuta* recruits had more variable bacteria communities than adults, including low abundance taxa not detected in adult fragments. Consistent with past reports of *P. acuta*, Symbiodiniaceae in the genus *Durusdinium* dominated all adult fragments, regardless of treatment (Poquita-Du et al. 2020), with additional genera detected at low relative read abundances (Electronic Supplementary Material Fig. S1). Most planulae were also dominated by *Durusdinium*, but contained higher relative proportions of Symbiodiniaceae in *Symbiodinium, Breviolum,* and *Cladocopium* (Electronic Supplementary Material Fig. S3). Although we were not able to adjust for differences in rDNA copy number among Symbiodiniaceae species in different genera in this study, published data suggest that species in *Durusdinium* have a much lower rDNA copy number than species in *Symbiodinium* and *Cladocopium* (Saad et al. 2020). Thus, it is unlikely that such high proportions of *Durusdinium* ITS2 sequence reads would be detected in this study if cells of this symbiont genus were not dominant in sampled colonies.

Since *P. acuta* vertically transmits Symbiodiniaceae to planulae, *Symbiodinium, Breviolum,* and *Cladocopium* symbionts may have been disproportionately passed to their offspring, or these symbiont taxa may have proliferated more rapidly in the planula at this stage. This is possible given that these genera were detected in both parents (albeit at low abundance) and planulae (Electronic Supplementary Material Figs. S1 and S3). While it has been suggested that *P. acuta* planulae may acquire Symbiodiniaceae through mixed mode transmission (Epstein et al. 2019 with *P. damicornis* and Quigley et al. 2018; Damjanovic et al. 2020b with *P. acuta*), planulae were sampled from aquaria hours after being released from the parent fragment in this study. Taken together, we interpret that the low abundance Symbiodiniaceae types detected in planulae in this study (Electronic Supplementary Fig.S1, Fig.S3) represent *in hospite* symbionts acquired from parent colonies. The functional contribution of these low abundance symbionts to the coral holobiont (if any) is outside the scope of this study but constitutes a promising future research direction. The diversity in both Symbiodiniaceae and bacteria communities suggests that the pocilloporid microbiota may be mutable as corals develop (Epstein et al. 2019).

No effect of parental enrichment was detected on reproductive output per fragment, planula Symbiodiniaceae densities, microbial communities, or percent settlement; minimal effects were detected on planula gene expression (Fig. 5, Electronic Supplementary Material Figs. S2, S3 & Table S1). Fish-farm derived nutrients have been documented to increase gonad abundance in coral tissues (Bongiorni et al. 2003) but decrease overall successful planula production (Loya et al. 2004); decreases in reproductive output have also been observed during experimental nutrient enrichment (Ward and Harrison 2000; Harrison and Ward 2001). These studies did not explicitly test for the impact of nitrate, though, which we find does not impact viable planula production in *P. acuta*. Whereas some studies have observed transgenerational effects of pCO_2 in pocilloporid corals (Putnam and Gates 2015), the capacity for transgenerational effects in the context of other stressors is not well understood, especially in situ. Encouragingly, our findings suggest subtle but net neutral impacts of chronic nutrient enrichment in adults. Although no impact of parental nutrient environment was detected on bacteria or Symbiodiniaceae community patterns in planulae, clone group did influence planula bacteria communities, suggesting that host factors do have some influence (Dunphy et al. 2019; Epstein et al. 2019; Glasl et al. 2019) and highlighting the importance of incorporating host genetics in studies of coral microbial communities.

Conclusions

Nutrient pollution constitutes a chronic stress on many reefs around the world (Carpenter et al. 1998; Bennett et al. 2001; Lapointe et al. 2019; Adam et al. 2021). This study demonstrates that long-term nitrate enrichment shifts holobionts toward tolerating higher densities of Symbiodiniaceae, which has previously been shown to increase bleaching susceptibility. Despite this, transgenerational effects of chronic nutrient enrichment were not detected in terms of coral reproductive success and/or the microbiota of individual coral planulae. Therefore, our data suggests that impacts of nutrient runoff can be marginal for *P. acuta*; this coral species is likely to benefit most from management efforts focusing on mitigating climate change.

However, *P. acuta* is a common inshore coral potentially adapted to higher nutrient conditions; nutrient impacts to holobionts in situ and across generations should be further investigated in additional species and reefs to gain a more complete picture of coral sensitivity to nutrients.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00338-022-02236-9.

Acknowledgements We thank Anna Knochel, Hailee Clover, and Jake Emmert (Moody Gardens Aquarium) for field support and Olivia Simon for laboratory support. The Alabama SuperComputer Authority provided computational resources. A Lewis and Clark Grant from the American Philosophical Society to LHK, a John E. Parish Fellowship from Wiess College and a Clara Carter Higgins Scholarship from the Garden Club of America to JAS, and a NSF Graduate Research Fellowship to KES supported this project. This project was also supported by the US National Science Foundation (OCE #1935308 to MES, OCE #1547952 to DEB, and OCE #1635798 to AMSC), an Early-Career Research Fellowship (#2000009651 to AMSC) from the Gulf Research Program of the National Academies of Sciences, and start-up funds to AMSC from Rice University. Samples were collected under research permit https://doi.org/10.21973/N3R94R. Research was completed under permits issued by the Territorial Government of French Polynesia (Délégation à la Recherche) and the Haut-Commissariat de la République en Polynésie Francaise (DTRT) (Protocole d'Accueil 2013–2019), and we thank the Délégation à la Recherche and DTRT for their continued support.

Data availability Raw gene expression and microbial data are archived as an NCBI SRA via bioproject PRJNA694169. Holobiont data and R code for microbial analysis are available at https://github.com/LaurenHK/Pacu_nitrate_Moorea_microbe.git. R code for tag-seq analysis is available at https://github.com/mariestrader/Pacu_nitrate_Moorea.git.

Declarations

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

References

Adam TC, Burkepile DE, Holbrook SJ, Carpenter RC, Claudet J, Loiseau C, Thiault L, Brooks AJ, Washburn L, Schmitt RJ (2021) Landscape-scale patterns of nutrient enrichment in a coral reef ecosystem: implications for coral to algae phase shifts. Ecol Appl 31:e02227

Allgeier JE, Andskog MA, Hensel E, Appaldo R, Layman C, Kemp DW (2020) Rewiring coral: anthropogenic nutrients shift diverse coral–symbiont nutrient and carbon interactions toward symbiotic algal dominance. Glob Change Biol 26:5588–5601

Baird AH, Guest JR, Willis BL (2009) Systematic and biogeographical patterns in the reproductive biology of scleractinian corals. Ann Rev Ecol Evol Syst 40:551–571

Becker DM, Putnam HM, Burkepile DE, Adam TC, Vega Thurber R, Silbiger NJ (2021) Chronic low-level nutrient enrichment benefits coral thermal performance in a fore reef habitat. Coral Reefs 40:1637–1655

Becker DM, Silbiger NJ (2020) Nutrient and sediment loading affect multiple facets of functionality in a tropical branching coral. J Exp Biol 223

Bellantuono AJ, Dougan KE, Granados-Cifuentes C, Rodriguez-Lanetty M (2019) Free-living and symbiotic lifestyles of a thermotolerant coral endosymbiont display profoundly distinct transcriptomes under both stable and heat stress conditions. Mol Ecol 28:5265–5281

Bennett EM, Carpenter SR, Caraco NF (2001) Human impact on erodable phosphorus and eutrophication: a global perspective. Bioscience 51:227–234

Bergman JL, Leggat W, Ainsworth TD (2021) The meta-organism response of the environmental generalist *Pocillopora damicornis* exposed to differential accumulation of heat stress. Front Mar Sci 8:664063

Bongiorni L, Shafir S, Angel D, Rinkevich B (2003) Survival, growth and gonad development of two hermatypic corals subjected to in situ fish-farm nutrient enrichment. Mar Ecol Prog Ser 253:137–144

Bourne DG, Morrow KM, Webster NS (2016) Insights into the coral microbiome: underpinning the health and resilience of reef ecosystems. Annu Rev Microbiol 70:317–340

Brener-Raffalli K, Clerissi C, Vidal-Dupiol J, Adjeroud M, Bonhomme F, Pratlong M, Aurelle D, Mitta G, Toulza E (2018) Thermal regime and host clade, rather than geography, drive Symbiodinium and bacterial assemblages in the scleractinian coral *Pocillopora damicornis sensu lato*. Microbiome 6:39

Bruno JF, Petes LE, Harvell CD, Hettinger A (2003) Nutrient enrichment can increase the severity of coral diseases. Ecol Lett 6:1056–1061

Burgess SC, Johnston EC, Wyatt ASJ, Leichter JJ, Edmunds PJ (2021) Response diversity in corals: hidden differences in bleaching mortality among cryptic *Pocillopora species*. Ecology 102:e03324

Burkepile DE, Shantz AA, Adam TC, Munsterman KS, Speare KE, Ladd MC, Rice MM, Ezzat L, McIlroy S, Wong JCY, Baker DM, Brooks AJ, Schmitt RJ, Holbrook SJ (2020) Nitrogen identity drives differential impacts of nutrients on coral bleaching and mortality. Ecosystems 23:798–811

Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP (2016) DADA2: high-resolution sample inference from Illumina amplicon data. Nat Methods 13:581–583

Caporaso JG, Lauber CL, Walters WA, Berg-Lyons D, Lozupone CA, Turnbaugh PJ, Fierer N, Knight R (2011) Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. Proc Natl Acad Sci USA 108:4516–4522

Carpenter SR, Caraco NF, Correll DL, Howarth RW, Sharpley AN, Smith VH (1998) Nonpoint pollution of surface waters with phosphorus and nitrogen. Ecol Appl 8:559–568

Ceh J, van Keulen M, Bourne DG (2013) Intergenerational transfer of specific bacteria in corals and possible implications for offspring fitness. Microb Ecol 65:227–231

Ceh J, Raina JB, Soo RM, van Keulen M, Bourne DG (2012) Coral-bacterial communities before and after a coral mass spawning event on Ningaloo Reef. PLoS ONE 7:e36920

Chumun P, Casareto B, Higuchi T, Irikawa A, Bhagooli R, Ishikawa Y, Suzuki Y (2013) High nitrate levels exacerbate thermal photo-physiological stress of Zooxanthellae in the reef-building coral *Pocillopora damicornis*. Eco-Eng 25:75–83

Claar DC, McDevitt-Irwin JM, Garren M, Vega Thurber R, Gates RD, Baum JK (2020) Concordant shifts in the diversity and structure of Symbiodiniaceae and bacterial communities subjected to chronic anthropogenic disturbance. Mol Ecol 0–3

Cunning R, Baker AC (2013) Excess algal symbionts increase the susceptibility of reef corals to bleaching. Nat Clim Chang 3:259–262

Dagenais-Bellefeuille S, Morse D (2013) Putting the N in dinoflagellates. Front Microbiol 4:369

Damjanovic K, Menéndez P, Blackall LL, van Oppen MJH (2020a) Early life stages of a common broadcast spawning coral associate with specific bacterial communities despite lack of internalized bacteria. Microb Ecol 79:706–719

Damjanovic K, Menéndez P, Blackall LL, van Oppen MJH (2020b) Mixed-mode bacterial transmission in the common brooding coral *Pocillopora acuta*. Environ Microbiol 22:397–412

D'Angelo C, Wiedenmann J (2014) Impacts of nutrient enrichment on coral reefs: new perspectives and implications for coastal management and reef survival. Curr Op Environ Sustain 7:82–93

Dinsdale EA, Pantos O, Smriga S, Edwards RA, Angly F, Wegley L, Hatay M, Hall D, Brown E, Haynes M, Krause L, Sala E, Sandin SA, Thurber RV, Willis BL, Azam F, Knowlton N, Rohwer F (2008) Microbial ecology of four coral atolls in the Northern Line Islands. PLoS ONE 3:e1584

Dixon G, Davies S, Aglyamova G, Meyer E, Bay L, Matz M (2015) Genomic determinants of coral heat tolerance across latitudes. Science 348:1460–1462

Donelson JM, Munday PL, McCormick MI (2012) Climate change may affect fish through an interaction of parental and juvenile environments. Coral Reefs 31:753–762

Donovan MK, Adam TC, Shantz AA, Speare KE, Munsterman KS, Rice MM, Schmitt RJ, Holbrook SJ, Burkepile DE (2020) Nitrogen pollution interacts with heat stress to increase coral bleaching across the seascape. Proc Natl Acad Sci USA 117:5351–5357

Dougan KE, Ladd MC, Fuchs C, Vega Thurber R, Burkepile DE, Rodriguez-Lanetty M (2020) Nutrient pollution and predation differentially affect innate immune pathways in the coral *Porites porites*. Front Mar Sci 7:563865

Dunphy CM, Gouhier TC, Chu ND, Vollmer SV (2019) Structure and stability of the coral microbiome in space and time. Sci Rep 9:6785

Epstein HE, Torda G, Munday PL, van Oppen MJH (2019) Parental and early life stage environments drive establishment of bacterial and dinoflagellate communities in a common coral. ISME J 13:1635–1638

Eren AM, Morrison HG, Lescault PJ, Reveillaud J, Vineis JH, Sogin ML (2015) Minimum entropy decomposition: unsupervised oligotyping for sensitive partitioning of high-throughput marker gene sequences. ISME J 9:968–979

Ezzat L, Maguer JF, Grover R, Ferrier-Pagés C (2015) New insights into carbon acquisition and exchanges within the coral–dinoflagellate symbiosis under NH4+ and NO3- supply. Proc Royal Soc b Biol Sci 282:20150610

Ezzat L, Maguer JF, Grover R, Ferrier-Pagès C (2016) Limited phosphorus availability is the Achilles heel of tropical reef corals in a warming ocean. Sci Rep 6:1–11

Fabricius KE (2005) Effects of terrestrial runoff on the ecology of corals and coral reefs: review and synthesis. Mar Pollut Bull 50:125–146

Marangoni L, Ferrier-Pagès C, Rottier C, Bianchini A, Grover R (2020) Unravelling the different causes of nitrate and ammonium effects on coral bleaching. Sci Rep 10:11975

Ferrier-Pagès C, Gattuso JP, Dallot S, Jaubert J (2000) Effect of nutrient enrichment on growth and photosynthesis of the zooxanthellate coral Stylophora pistillata. Coral Reefs 19:103–113

Fox MD, Nelson CE, Oliver TA, Quinlan ZA, Remple K, Glanz J, Smith JE, Putnam HM (2021) Differential resistance and acclimation of two coral species to chronic nutrient enrichment reflect life-history traits. Funct Ecol 35:1081–1093

Furnas M, Alongi D, McKinnon D, Trott L, Skuza M (2011) Regional-scale nitrogen and phosphorus budgets for the northern (14°S) and central (17°S) Great Barrier Reef shelf ecosystem. Cont Shelf Res 31:1967–1990

Garren M, Raymundo L, Guest J, Harvell CD, Azam F (2009) Resilience of coral-associated bacterial communities exposed to fish farm effluent. PLoS ONE 4:e7319

Glasl B, Smith CE, Bourne DG, Webster NS (2019) Disentangling the effect of host-genotype and environment on the microbiome of the coral Acropora tenuis. Peer J

Grover R, Maguer JF, Allemand D, Ferrier-Pagès C (2003) Nitrate uptake in the scleractinian coral *Stylophora pistillata*. Limnol Oceanogr 48:2266–2274

Harrison PL, Ward S (2001) Elevated levels of nitrogen and phosphorus reduce fertilisation success of gametes from scleractinian reef corals. Mar Biol 139:1057–1068

Humanes A, Ricardo GF, Willis BL, Fabricius KE, Negri AP (2017) Cumulative effects of suspended sediments, organic nutrients and temperature stress on early life history stages of the coral *Acropora tenuis*. Sci Rep 7:e0161616

Hume BCC, Smith EG, Ziegler M, Warrington HJM, Burt JA, LaJeunesse TC, Wiedenmann J, Voolstra CR (2019) SymPortal: A novel analytical framework and platform for coral algal symbiont next-generation sequencing ITS2 profiling. Mol Ecol Resour 19:1063–1080

Hume BCC, Ziegler M, Poulain J, Pochon X, Romac S, Boissin E, de Vargas C, Planes S, Wincker P, Voolstra CR (2018) An improved primer set and amplification protocol with increased specificity and sensitivity targeting the *Symbiodinium* ITS2 region. Peer J 6:e4816

Jensen N, Allen RM, Marshall DJ (2014) Adaptive maternal and paternal effects: gamete plasticity in response to parental stress. Funct Ecol 28:724–733

Johnston EC, Forsman ZH, Flot JF, Schmidt-Roach S, Pinzón JH, Knapp ISS, Toonen RJ (2017) A genomic glance through the fog of plasticity and diversification in Pocillopora. Sci Rep 7:1–11

Kenkel CD, Bay LK (2018) Exploring mechanisms that affect coral cooperation: symbiont transmission mode, cell density and community composition. Peer J 6:e6047

Kitchen RM, Piscetta M, de Souza MR, Lenz EA, Schar DWH, Gates RD, Wall CB (2020) Symbiont transmission and reproductive mode influence responses of three Hawaiian coral larvae to elevated temperature and nutrients. Coral Reefs 39:419–431

Kooperman N, Ben-Dov E, Kramarsky-Winter E, Barak Z, Kushmaro A (2007) Coral mucus-associated bacterial communities from natural and aquarium environments. FEMS Microbiol Lett 276:106–113

Kopp C, Pernice M, Domart-Coulon I, Djediat C, Spangenberg JE, Alexander DTL, Hignette M, Meziane T, Meibom A (2013) Highly dynamic cellular-level response of symbiotic coral to a sudden increase in environmental nitrogen. Mbio 4:e00052-e113

Krueger T, Horwitz N, Bodin J, Giovani ME, Escrig S, Fine M, Meibom A (2020) Intracellular competition for nitrogen controls dinoflagellate population density in corals. Proc Royal Soc b Biol Sci 287:20200049

Langmead B, Salzberg SL (2012) Fast gapped-read alignment with Bowtie 2. Nat Methods 9:357–359

Lapointe BE, Brewton RA, Herren LW, Porter JW, Hu C (2019) Nitrogen enrichment, altered stoichiometry, and coral reef decline at Looe Key, Florida Keys, USA: a 3-decade study

Leite DCA, Salles JF, Calderon EN, Castro CB, Bianchini A, Marques JA, van Elsas JD, Peixoto RS (2018) Coral bacterial-core abundance and network complexity as proxies for anthropogenic pollution. Front Microbiol 9:833

Lohman BK, Weber JN, Bolnick DI (2016) Evaluation of TagSeq, a reliable low-cost alternative for RNAseq. Mol Ecol Resour 16:1315–1321

López-Maury L, Marguerat S, Bähler J (2008) Tuning gene expression to changing environments: from rapid responses to evolutionary adaptation. Nat Rev Gen 9:583–593

Love MI, Huber W, Anders S (2014) Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol 15:550

Loya Y, Lubinevsky H, Rosenfeld M, Kramarsky-Winter E (2004) Nutrient enrichment caused by in situ fish farms at Eilat, Red Sea is detrimental to coral reproduction. Mar Pollut Bull 49:344–353

Maher RL, Rice MM, McMinds R, Burkepile DE, Vega Thurber R (2019) Multiple stressors interact primarily through antagonism to drive changes in the coral microbiome. Sci Rep 9:1–12

Maher RL, Schmeltzer ER, Meiling S, McMinds R, Ezzat L, Shantz AA, Adam TC, Schmitt RJ, Holbrook SJ, Burkepile DE, Vega Thurber R (2020) Coral microbiomes demonstrate flexibility and resilience through a reduction in community diversity following a thermal stress event. Front Ecol Evolut 8:555698

Marangoni LF de B, Ferrier-Pagès C, Rottier C, Bianchini A, Grover R (2020) Unravelling the different causes of nitrate and ammonium effects on coral bleaching. Sci Rep 10:11975

Martin M (2011) Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet.journal 17:10–12

Marubini F, Davies PS (1996) Nitrate increases zooxanthellae population density and reduces skeletogenesis in corals. Mar Biol 127:319–328

McDevitt-Irwin JM, Baum JK, Garren M, Vega Thurber RL (2017) Responses of coral-associated bacterial communities to local and global stressors. Front Mar Sci 4:262

Meyer E, Aglyamova G, v., Matz M v. (2011) Profiling gene expression responses of coral larvae (Acropora millepora) to elevated temperature and settlement inducers using a novel RNA-Seq procedure. Mol Ecol 20:3599–3616

Miller GM, Watson SA, Donelson JM, McCormick MI, Munday PL (2012) Parental environment mediates impacts of increased carbon dioxide on a coral reef fish. Nat Clim Chang 2:858–861

Morris LA, Voolstra CR, Quigley KM, Bourne DG, Bay LK (2019) Nutrient availability and metabolism affect the stability of coral-symbiodiniaceae symbioses. Trends Microbiol 27:678–689

Morrow KM, Bourne DG, Humphrey C, Botté ES, Laffy P, Zaneveld J, Uthicke S, Fabricius KE, Webster NS (2015) Natural volcanic CO2 seeps reveal future trajectories for host-microbial associations in corals and sponges. ISME J 9:894–908

Morrow KM, Moss AG, Chadwick NE, Liles MR (2012) Bacterial associates of two caribbean coral species reveal species-specific distribution and geographic variability. Appl Environ Microbiol 78:6438–6449

Neave MJ, Apprill A, Ferrier-Pagès C, Voolstra CR (2016) Diversity and function of prevalent symbiotic marine bacteria in the genus Endozoicomonas. Appl Microbiol Biotechnol 100:8315–8324

Oksanen J, Blanchet F, Friendly M, Kindt R, Legendre P, McGlinn D, Minchin P, O'Hara R, Simpson G, Solymos P, Stevens M, Szoecs E, Wagner H (2020) vegan: Community Ecology Package

Parker LM, Ross PM, O'Connor WA, Borysko L, Raftos DA, Pörtner HO (2012) Adult exposure influences offspring response to ocean acidification in oysters. Glob Change Biol 18:82–92

Pernice M, Meibom A, van den Heuvel A, Kopp C, Domart-Coulon I, Hoegh-Guldberg O, Dove S (2012) A single-cell view of ammonium assimilation in coral-dinoflagellate symbiosis. ISME J 6:1314–1324

Pogoreutz C, Rädecker N, Cárdenas A, Gärdes A, Wild C, Voolstra CR (2018) Dominance of Endozoicomonas bacteria throughout coral bleaching and mortality suggests structural inflexibility of the Pocillopora verrucosa microbiome. Ecol Evol 8:2240–2252

Poquita-Du RC, Huang D, Chou LM, Todd PA (2020) The contribution of stress-tolerant endosymbiotic dinoflagellate *Durusdinium* to *Pocillopora acuta* survival in a highly urbanized reef system. Coral Reefs 39:745–755

Putnam HM, Gates RD (2015) Preconditioning in the reef-building coral Pocillopora damicornis and the potential for trans-generational acclimatization in coral larvae under future climate change conditions. J Exp Biol 218:2365–2372

Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, Peplies J, Glöckner FO (2013) The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucl Acids Res 41

Quigley KM, Warner PA, Bay LK, Willis BL (2018) Unexpected mixed-mode transmission and moderate genetic regulation of *Symbiodinium* communities in a brooding coral. Heredity 121:524–536

Quigley KM, Willis BL, Kenkel CD (2019) Transgenerational inheritance of shuffled symbiont communities in the coral *Montipora digitata*. Sci Rep 9:13328

Rädecker N, Pogoreutz C, Gegner HM, Cárdenas A, Roth F, Bougoure J, Guagliardo P, Wild C, Pernice M, Raina JB, Meibom A, Voolstra CR (2021) Heat stress destabilizes symbiotic nutrient cycling in corals. Proc Natl Acad Sci USA 118:e2022653118

Rädecker N, Pogoreutz C, Voolstra CR, Wiedenmann J, Wild C (2015) Nitrogen cycling in corals: the key to understanding holobiont functioning? Trends Microbiol 23:490–497

Rinkevich B, Shaish L, Douek J, Ben-Shlomo R (2016) Venturing in coral larval chimerism: a compact functional domain with fostered genotypic diversity. Sci Rep 6:1–7

Rosset S, Wiedenmann J, Reed AJ, D'Angelo C (2017) Phosphate deficiency promotes coral bleaching and is reflected by the ultrastructure of symbiotic dinoflagellates. Mar Pollut Bull 118:180–187

Saad OS, Lin X, Ng TY, Li L, Ang P, Lin S (2020) Genome size, rDNA copy, and qPCR assays for symbiodiniaceae. Front Microbiol 11:847

Schmidt-Roach S, Miller KJ, Lundgren P, Andreakis N (2014) With eyes wide open: a revision of species within and closely related to the Pocillopora damicornis species complex (Scleractinia; Pocilloporidae) using morphology and genetics. Zool J Linn Soc 170:1–33

Serrano XM, Miller MW, Hendee JC, Jensen BA, Gapayao JZ, Pasparakis C, Grosell M, Baker AC (2018) Effects of thermal stress and nitrate enrichment on the larval performance of two Caribbean reef corals. Coral Reefs 37:173–182

Shantz AA, Burkepile DE (2014) Context-dependent effects of nutrient loading on the coral-algal mutualism. Ecology 95:1995–2005

Shantz AA, Ladd MC, Schrack E, Burkepile DE (2015) Fish-derived nutrient hotspots shape coral reef benthic communities. Ecol Appl 25:2142–2152

Shaver EC, Shantz AA, McMinds R, Burkepile DE, Thurber RLV, Silliman BR (2017) Effects of predation and nutrient enrichment on the success and microbiome of a foundational coral. Ecology 98:830–839

Smith HA, Moya A, Cantin NE, van Oppen MJH, Torda G (2019) Observations of simultaneous sperm release and larval planulation suggest reproductive assurance in the coral *Pocillopora acuta*. Front Mar Sci 6:362

Snidvongs A, Kinzie RA (1994) Effects of nitrogen and phosphorus enrichement on in vivo symbiotic zooxanthellae of *Pocillopora damicornis*. Mar Biol 118:705–711

Stambler N, Popper N, Dubinsky Z, Stimson J (1991) Effects of nutrient enrichment and water motion on the coral *Pocillopora damicornis*. Pac Sci 45:299–307

Stoddart JA (1983) Asexual production of planulae in the coral Pocillopora damicornis. Mar Biol 76:279–284

Torda G, Lundgren P, Willis BL, van Oppen MJH (2013) Genetic assignment of recruits reveals short- And long-distance larval dispersal in *Pocillopora damicornis* on the Great Barrier Reef. Mol Ecol 22:5821–5834

Traylor-Knowles N, Granger BR, Lubinski TJ, Parikh JR, Garamszegi S, Xia Y, Marto JA, Kaufman L, Finnerty JR (2011) Production of a reference transcriptome and transcriptomic database (PocilloporaBase) for the cauliflower coral. Pocillopora Damicornis BMC Genom 12:585

Vega Thurber RL, Burkepile DE, Fuchs C, Shantz AA, Mcminds R, Zaneveld JR (2014) Chronic nutrient enrichment increases prevalence and severity of coral disease and bleaching. Glob Change Biol 20:544–554

Vitousek PM, Aber JD, Howarth RW, Likens GE, Matson PA, Schindler DW, Schlesinger WH, Tilman DG (1997) Human alteration of the global nitrogen cycle: sources and consequences. Ecol Appl 7:737–750

Wang L, Shantz AA, Payet JP, Sharpton TJ, Foster A, Burkepile DE, Thurber RV (2018) Corals and their microbiomes are differentially affected by exposure to elevated nutrients and a natural thermal anomaly. Front Mar Sci 5:101

Ward S, Harrison P (2000) Changes in gametogenesis and fecundity of acroporid corals that were exposed to elevated nitrogen and phosphorus during the ENCORE experiment. J Exp Mar Biol Ecol 246:179–221

Webster NS, Reusch TBH (2017) Microbial contributions to the persistence of coral reefs. ISME J 11:2167–2174

Wiedenmann J, D'Angelo C, Smith EG, Hunt AN, Legiret FE, Postle AD, Achterberg EP (2013) Nutrient enrichment can increase the susceptibility of reef corals to bleaching. Nat Clim Chang 3:160–164

Wong JM, Johnson KM, Kelly MW, Hofmann GE (2018) Transcriptomics reveal transgenerational effects in purple sea urchin embryos: Adult acclimation to upwelling conditions alters the response of their progeny to differential pCO_2 levels. Mol Ecol 27:1120–1137

Wright RM, Aglyamova GV, Meyer E, Matz MV (2015) Gene expression associated with white syndromes in a reef building coral, Acropora hyacinthus. BMC Genom 16

Xiang T, Lehnert E, Jinkerson RE, Clowez S, Kim RG, DeNofrio JC, Pringle JR, Grossman AR (2020) Symbiont population control by host-symbiont metabolic interaction in Symbiodiniaceae-cnidarian associations. Nat Commun 11:108

Zaneveld JR, Burkepile DE, Shantz AA, Pritchard CE, McMinds R, Payet JP, Welsh R, Correa AMS, Lemoine NP, Rosales S, Fuchs C, Maynard JA, Thurber RV (2016) Overfishing and nutrient pollution interact with temperature to disrupt coral reefs down to microbial scales. Nat Commun 7:11833

Zaneveld JR, McMinds R, Thurber RV (2017) Stress and stability: applying the Anna Karenina principle to animal microbiomes. Nat Microbiol 2:17121

Zhang Y, Yang Q, Zhang Y, Ahmad M, Ling J, Tang X, Dong J (2021) Shifts in abundance and network complexity of coral bacteria in response to elevated ammonium stress. Sci Total Environ 768:144631

Ziegler M, Roik A, Porter A, Zubier K, Mudarris MS, Ormond R, Voolstra CR (2016) Coral microbial community dynamics in response to anthropogenic impacts near a major city in the central Red Sea. Mar Pollut Bull 105:629–640