


ORIGINAL ARTICLE

Co-dynamics of Symbiodiniaceae and bacterial populations during the first year of symbiosis with *Acropora tenuis* juveniles

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Abstract

Interactions between corals and their associated microbial communities (Symbiodiniaceae and prokaryotes) are key to understanding corals' potential for and rate of acclimatory and adaptive responses. However, the establishment of microalgal and bacterial communities is poorly understood during coral ontogeny in the wild. We examined the establishment and co-occurrence between multiple microbial communities using 16S rRNA (bacterial) and ITS2 rDNA (Symbiodiniaceae) gene amplicon sequencing in juveniles of the common coral, *Acropora tenuis*, across the first year of development. Symbiodiniaceae communities in juveniles were dominated by *Durusdinium trenchii* and *glynnii* (D1 and D1a), with lower abundances of *Cladocopium* (C1, C1d, C50, and Cspc). Bacterial communities were more diverse and dominated by taxa within Proteobacteria, Cyanobacteria, and Planctomycetes. Both communities were characterized by significant changes in relative abundance and diversity of taxa throughout the year. D1, D1a, and C1 were significantly correlated with multiple bacterial taxa, including Alpha-, Delta-, and Gammaproteobacteria, Planctomycetacia, Oxyphotobacteria, Phycisphaerae, and Rhizobiales. Specifically, D1a tended to associate with Oxyphotobacteria and D1 with Alphaproteobacteria, although these associations may represent correlational and not causal relationships. Bioenergetic modeling combined with physiological measurements of coral juveniles (surface area and Symbiodiniaceae cell densities) identified key periods of carbon limitation and nitrogen assimilation, potentially coinciding with shifts in microbial community composition. These results demonstrate that Symbiodiniaceae and bacterial communities are dynamic throughout the first year of ontology and may vary in tandem, with important fitness effects on host juveniles.

KEYWORDS

16S rRNA gene, co-occurrence, coral, coral-associated bacteria, ITS2 rDNA gene, juvenile, Symbiodiniaceae

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1 | INTRODUCTION

Scleractinian corals are key habitat engineers of one of the most ecologically diverse, economically valuable, but climatically vulnerable ecosystems on the planet (Bellwood, Hughes, Folke, & Nystrom, 2004). The sensitivity of reef-building corals to contemporary rates of environmental change in an era of ever-increasing anthropogenic influence on coastal ecosystems has led to alarming declines in coral reefs globally (De'ath & Fabricius, 2010; Hoegh-Guldberg & Bruno, 2010; Hughes et al., 2003; Thompson & Dolman, 2010). A key contributor to coral health and survival under rapid environmental change is the corals' microbial community, particularly Symbiodiniaceae (Baird, Bhagooli, Ralph, & Takahashi, 2009) and potentially bacterial (Bourne, Morrow, & Webster, 2016) communities that respond more rapidly to change than their coral hosts (Torda et al., 2017). Although coral-Symbiodiniaceae symbioses and coral-bacterial associations have been extensively studied, few have focussed on the potential tripartite linkages among all three of these components of the coral holobiont. Documenting early stages in the development of coral-microbe symbioses is an important step in identifying potential linkages in these complex symbioses. Such knowledge will lay the foundations for understanding how the corals' microbiome contributes to whole organism fitness and potential resilience when faced with the cumulative effects of ocean warming and other anthropogenic stressors.

Metazoans host large numbers of microbial associates that influence their host's phenotype in associations that range from mutualistic to parasitic (Salvucci, 2016). These associations are integral to host health and performance, although they may also influence each partner's evolutionary trajectories and adaptive potential (Hoang, Morran, & Gerardo, 2016; Torda et al., 2017). Increases in seawater temperatures of as little as 1–2°C above summer means disrupt the symbioses between corals and their microbes, resulting in mass coral bleaching (Hughes et al., 2018, 2017) and coral disease outbreaks (Brodnicke et al., 2019). Correlations between bacterial and Symbiodiniaceae community structures in some adult corals are likely due to coupled nutrient cycling (Bourne et al., 2013), for example, changes in carbon and nitrogen ratios in the environment (Cooper et al., 2011), which may influence symbiont community population dynamics within corals (Morris, Voolstra, Quigley, Bourne, & Bay, 2019). Thus, even fine-scale changes in the diversity or abundance of symbionts can impact the coral host's physiology (Cunning, Silverstein, & Baker, 2015) due to variability in nutrient sharing and thermal tolerances across various partners (Karim, Nakaema, & Hidaka, 2015; Swain, Chandler, Backman, & Marcelino, 2016). In some cases, the co-occurrence of some symbionts appears to be unrelated to host phylogenetic history or biogeographical area (Bernasconi, Stat, Koenders, & Huggett, 2018). In other cases, host/bacterial communities demonstrate phylosymbiotic patterns (e.g., members from *Endozoicomonas*) (O'Brien, Webster, Miller, & Bourne, 2019; Pollock et al., 2018). Overall, the intimacy of these associations suggests that the holobiont's biology can only be understood when the interactions of all members are considered (Bordenstein

& Theis, 2015; Bosch & McFall-Ngai, 2011; Gordon, Knowlton, Relman, Rohwer, & Youle, 2013).

The acquisition and development of bacterial and Symbiodiniaceae communities in juvenile varies throughout early stages of coral ontogeny (Abrego, van Oppen, & Willis, 2009a, 2009b; Fieth, Gauthier, Bayes, Green, & Degnan, 2016; Lema, Bourne, & Willis, 2014; Quigley, Bay, & Willis, 2017; Quigley, Willis, & Bay, 2017) likely regulated by both host and environmental factors (Poland & Coffroth, 2017; Quigley, Bay, et al., 2017; Quigley, Willis, et al., 2017; Souza et al., 2016). In the few species examined to date, the composition of bacterial communities is largely influenced by acquisition from the environmental pool and to a lesser extent by vertical, parental transmission (Apprill, Marlow, Martindale, & Rappé, 2009; Epstein, Torda, Munday, & van Oppen, 2019; Leite et al., 2017; Lema et al., 2014; Sharp, Distel, & Paul, 2012; Zhou et al., 2017). Once acquired, community shifts are common throughout ontogeny across a range of invertebrate groups (Douglas & Werren, 2016). For example, in corals, both Symbiodiniaceae (Abrego, van Oppen, & Willis, 2009b) and bacterial community shifts (Lema et al., 2014) have been demonstrated to be triggered by changes in immunocompetency or environmental conditions (Littman, Willis, & Bourne, 2009). Immune system development (Hoang et al., 2016) and host factors (e.g., quorum quenching; Grandclément, Tannières, Moréra, Dessaux, & Faure, 2016; Kuniya et al., 2015; Souza et al., 2016) also regulate endosymbiotic community flux in addition to environmental factors such as temperature, $p\text{CO}_2$, or salinity (Glasl, Smith, Bourne, & Webster, 2019). The regulation of the microbiome's organization by the host during development (Davy, Allemand, & Weis, 2012) or inter-symbiont competition (also partly host regulated) (Cunning et al., 2015) may also influence these shifts. Although a model for "holobiont" functioning and feedback loops has been proposed (Rohwer, Seguritan, Azam, & Knowlton, 2002), it has not been utilized for coral juvenile development. Despite the importance of these microbial communities and their capacity to enhance host acclimation and adaptation potential, there is limited understanding of how bacterial and Symbiodiniaceae communities potentially influence each other and co-develop in association with host ontogeny in the wild.

In this study, we determined co-occurrence patterns and population dynamics of Symbiodiniaceae and bacterial communities during the first year of life in juveniles of the hard coral species, *Acropora tenuis*, at an inshore location on the Great Barrier Reef. These data provide key baseline information of changes in microbial communities associated with juvenile coral ontogeny and shed light on key stages of symbiosis establishment and maintenance, with implications for host functioning and potential for adaptation.

2 | MATERIALS AND METHODS

2.1 | Spawning and larval rearing

Fourteen colonies of *A. tenuis* were collected from Geoffrey Bay, Magnetic Island (S 19°09.326', E 146°51.861') on the full moon in November 2014 (Great Barrier Reef Marine Park Authority Permit

Number: G13/36318.1) and transported to the National Sea Simulator Facility (SeaSim) at the Australian Institute of Marine Sciences (AIMS). Corals were maintained in outside aquaria with constant flow through 0.1- μ m filtered seawater (FSW) and checked nightly for signs of imminent spawning. Gametes were collected on the fourth night after the full moon from 14 colonies. Eggs and sperm from each colony were mixed in equal proportions and left to fertilize for two hours, with periodic checks of fertilization. After >85% of the eggs had cleaved, fertilized eggs were gently collected and moved into 0.1- μ m FSW for a total of two washes to remove residual sperm. Once past the early gastrula stage, embryos were moved into three 500-L culture tanks with a continuous, gentle flow of 26.5°C FSW, and low aeration. Aeration was then increased once embryos had matured into larvae.

2.2 | Settlement

Approximately 3 months prior to spawning, 660 terracotta tiles were placed in large aquaria at the SeaSim for conditioning, that is, development of crustose coralline algae (CCA) communities and bacterial biofilms. Two days prior to larval settlement, the tiles were autoclaved at 2,000 kPa for 20 min at 180°C (Sabac T63) for sterilization. To help induce settlement and metamorphosis, CCA chips were removed from live rock, autoclaved, as described above, and kept at 4°C until ready for use. Once larvae were competent to settle (7 days postspawning), tiles were strung vertically on new flexible PVC aeration tubing in batches of 20, with 2.5-cm PVC spacers used to separate tiles. Nine to ten strings of tiles were then hung vertically in each of the three 500-L culture tanks. One milliliter of CCA slurry, composed of $\sim 1 \times 1$ cm pieces of autoclaved CCA in FSW, was pipetted onto each tile to attract larvae and induce settlement. Larvae were left to settle for 11 days, during which time water flow (0.1- μ m FSW) was kept constant with low aeration to ensure optimal water conditions.

2.3 | Field deployment

Settlement on each tile was assessed visually, and tiles containing juveniles were strung onto stainless steel rods in groups of 15 separated by PVC spacers. Nineteen days postspawning, 240 tiles, each with 40–200 juveniles, were transported to Magnetic Island in FSW. Nine rods (135 tiles) and 7 rods (105 tiles) were deployed at two sites at Magnetic Island (Geoffrey Bay and Nelly Bay), respectively, at a depth of approximately 6 m and secured with zip ties between pairs of vertical pickets hammered into the substratum. Fluorescent microscopy was used to confirm the absence of Symbiodiniaceae cells prior to field deployment. Juveniles were sampled from tiles on the 19th of each month from 8:30 to 10:30 a.m. The age of juveniles at each sampling time point was as follows: 40 days (T_1), 71 (T_2), 111 (T_3), 129 (T_4), 167 (T_5), 194 (T_6), 316 (T_7), and 380 (T_8). Individual juveniles were placed into 100% ethanol or FSW. No samples were collected during the months of June, July, and August due to repeated sightings of a saltwater crocodile at the field site.

2.4 | Surface area determination

Between 6 and 17 juveniles were photographed at each time point using a Leica microscope with a Leica MC170 HD lens (Switzerland) and the Leica Application Suite (v.4.4.0, Switzerland) for surface area determination. This method was adequate while the juveniles were flat. However, juveniles sampled in April began to present more complex branching patterns that could not be captured by the Leica Application Suite or were partially obscured while taking the photograph. 3D modeling, as used to estimate surface area of adult corals (Jones, Berkelmans, Berkelmans, van Oppen, Mieog, & Sinclair, 2008; Jones, Cantin, Berkelmans, Sinclair, & Negri, 2008), was then employed to capture the geometries of these more complex juvenile corals. Juveniles were measured using 3D scanning with the NextEngine scanner (NextEngine) and processed using NextEngine ScanStudioPro (v. 2.0.2). Each juvenile was scanned twice using the 360° horizontal scan with 14 divisions and two bracket scans at 160,000 points per inch (HD resolution). Resulting.STL files were then imported into Geomagic Verify Viewer software (v. 2.0) to calculate surface area after trimming, polishing to fill holes, and then fusing. As described further below, surface area measurements were matched with Symbiodiniaceae cell counts and DNA extractions for each juvenile.

2.5 | Symbiodiniaceae cell counts

Live juveniles were transported back to the SeaSim on tiles for size determination through either microscopy or 3D scanning. After size determination, juveniles were removed from tiles, and then spun down at 16,000 rcf for 90 s in seawater without fixative, after which the seawater was replaced with 5% formic acid and samples left for two hours for digestion. Eppendorf tubes containing juvenile tissue were then centrifuged again on the same settings, and the formic acid replaced with filtered seawater, well mixed, and then aliquoted into a hemocytometer for cell counts. Three hemocytometer plates were counted for each juvenile, corresponding to six-well counts. The number of Symbiodiniaceae cells for each individual juvenile was then standardized to the surface area that had been measured for that exact juvenile (cells per mm^2).

2.6 | Symbiodiniaceae and bacterial community genotyping

Ten individual juveniles per time point were sequenced using Illumina technology targeting the Symbiodiniaceae ITS2 locus ($n = 80$). Within each time point, DNA was extracted from whole, single juveniles following SDS extraction (Wilson et al., 2002), including a bead-beating step that was performed before water-bath incubation using 1-mm silica spheres (MPBio) on a FastPrep-24 (MPBio) for three cycles of 30 s at 4.0 m/s, with the same DNA extract, sourced from the same individual used for cell counts, ITS2, and 16S rRNA gene sequencing. Library preparation and paired-end Miseq sequencing (Illumina) were performed at the University of Texas at Austin's

Genomics Sequencing and Analysis Facility (USA) using the following primers: ITS2alg-F (5'-TCGTCGGCAGCGTCAGATGTGATAAGAGACAGGTGAATTGCAGAACTCCGTG) and ITS2alg-R (3'-TTCGTATATTCATTTCGCTCCGACAGAGAATATGTGTAGAGGCTCGGGTGCTCTG-5') (Pochon, Pawlowski, Zaninetti, & Rowan, 2001).

Bacterial sequencing was performed on the same DNA extracts and sequenced with the 16S_V1-V3 (27f-519r) primers (Bioplatforms Australia Marine Microbes primers): 27f (5'-AGAGTTTGATCMTGGCTCAG) and 519R (5'-GWATTACCGCGGCKGCTG) at the Ramaciotti Centre for Genomics (Australia). Raw reads were analyzed using the DADA2 pipeline (Callahan et al., 2016). Full details and scripts can be found in Quigley, Willis, and Kenkel (2019). Cleaned and mapped sequence data were variance normalized, using the "DESeq2" package in R (Love, Huber, & Anders, 2014). Differential relative abundance analysis was performed with the same package using the contrast function to extract specific comparisons from the negative binomial generalized linear model. Nonmetric multidimensional scaling (NMDS) was performed on variance normalized ASVs (amplicon sequence variants) relative abundances using the packages "Phyloseq," "vegan," and "ellipse" with a Bray-Curtis dissimilarity matrix (McMurdie & Holmes, 2013; Murdoch, Chow, & Celayeta, 2007; Oksanen et al., 2013). NMDS analysis does not assume linear relationships between underlying variables and ASVs, resulting in distances between samples that are indicative of their similarity, with samples positioned closer in space being more similar (Ramette, 2007). The use of a Bray-Curtis matrix allows for the incorporation of the presence/absence of ASVs as well as their relative abundance. Permutational multivariate analysis of variance was used to determine if Symbiodiniaceae communities differed significantly between time points using the "adonis" function in "vegan." Explanation of variance was calculated using the "stressplot" function in "vegan." To collapse taxonomic complexity, mean percentage values were calculated at the level of bacterial Order and Symbiodiniaceae Class. Pearson's product-moment correlation coefficients and respective *p* values were calculated per time point to test for co-occurrence between each pairwise combination of bacterial and Symbiodiniaceae ASVs using the "Hmisc" package with the "rcorr" function (Harrell, 2007). Correlations were filtered to retain only those that were significant (*p* < .05).

2.7 | Juvenile-Symbiodiniaceae bioenergetics modeling

Estimating juvenile-Symbiodiniaceae bioenergetics was calculated using the "coRal" package (Cunning, Muller, Gates, & Nisbet, 2017). This model incorporates carbon and nitrogen budgets empirically measured across a range of coral taxa and Symbiodiniaceae associations. Assumptions include preferential sharing of nutrients and direct access to carbon by Symbiodiniaceae via host and nitrogen fluxes to the host via the environment. Time for model run was set from 0 to 380 days (T_0 : 9/11/2014; T_8 : 24/11/2015) with light varying from 0.3 to 42 $\mu\text{mol photons m}^{-2} \text{d}^{-1}$ to estimate changing light environments as juveniles grow from shaded crevices (Doropoulos

et al., 2016) to more exposed, high-light positions over time (Stimson, 1997). The final ratio of symbiont cells to host surface area for day 380 was used as a starting value (3,526.5 cells per mm^2). Dissolved inorganic nitrogen (DIN) and heterotrophy and initial parameters were set to default values (Cunning et al., 2017).

2.8 | Networks

Networks were constructed using the packages "igraph" and "phyloseq" (Csardi & Nepusz, 2006; McMurdie & Holmes, 2013). Igraph constructs networks by calculating Euclidean distances between points using nodes (ASVs in this case) and edges (line connections) derived from the relative abundance datasets. Relative abundances were converted to Bray-Curtis distances, with a maximum distance set at $\text{max.dist} = 0.7-0.9$, where separation was undirected.

3 | RESULTS

3.1 | Physiology

Juveniles exhibited exponential growth over the 380 days of sampling (Figure 1a), growing in size from $1.2 \text{ mm}^2 \pm 0.07$ ($n = 6$) in the first month to $635 \text{ mm}^2 \pm 243.9$ ($n = 9$) by the final time point. Symbiodiniaceae density was initially low, and variable in the first month (789 ± 624 cells per mm^2) increased rapidly to $\sim 2.2 \times 10^4$ cells per mm^2 over the next 3 months, but then decreased to densities just below 10^4 cells per mm^2 and appeared to stabilize at lower densities for the last two time points until juveniles were 380-days old (Figure 1a).

Bioenergetic modeling revealed that juveniles experienced an initial, brief period of carbon limitation during Symbiodiniaceae establishment (which is common at low symbiont densities typically seen during bleaching), but then intracellular nitrogen rapidly became limited as algal densities increased (Figure 1b). Initial carbon limitation was driven by CO_2 limitation (relative units of the ratio of substrate input fluxes), which dissipated after ~ 130 days, potentially when symbiont densities equilibrated at around 10^4 cells per mm^2 . Photosynthesis increased rapidly with cell proliferation and then reached an asymptote at a "healthy" level of $\sim 1.5 \text{ mol C C}^{-1} \text{ mol S d}^{-1}$ (Figure 1c), after about 200 - 250 days of proliferation, coinciding with the point when growth became exponential in coral juveniles.

3.2 | Symbiodiniaceae population shifts across ontogeny

Multiple Symbiodiniaceae from *Cladocopium* and *Durussidium* associated with tissues of *A. tenuis* were highly abundant in the first year of juvenile development (Figure 2). At day 40 (T_1), Symbiodiniaceae communities were variable among juveniles (light orange hull, Figure 2 NMDS inset 1), but converged across individuals throughout the remaining months, becoming the most conserved at the final time point (yellow hull, Figure 2). In general, Symbiodiniaceae communities associated with juveniles were dominated by D1 and D1a,

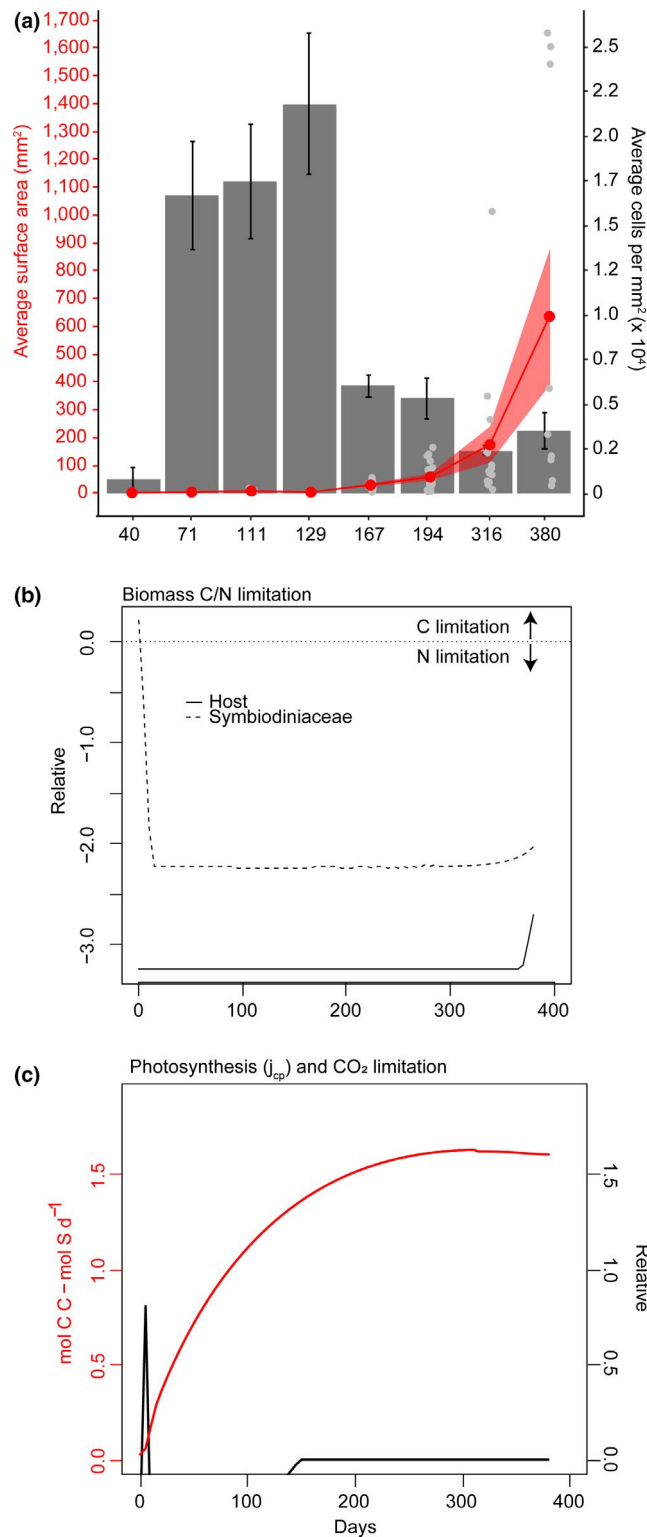


FIGURE 1 Physiological (a) and bioenergetic model outputs (b, c) for *Acropora tenuis* juveniles outplanted in the field for 1 year. (a) Average surface area of juveniles (red line; mm², red point means ± SE) and Symbiodiniaceae cell densities (average cells in ×10⁴, grey barplots ± SE). (b) Relative ratio of carbon to nitrogen limitation in the host and Symbiodiniaceae populations modelled over 0–400 days of growth in the field. Solid and dashed lines represent host and Symbiodiniaceae values, respectively. Relative ratios are limitation coefficients that are unbounded by zero, where negative values are indicative of formation rates that are lower than maximum production rates in either the host or symbiont (negative: nitrogen limitation over carbon; positive: carbon limitation over nitrogen; zero: neither is limiting). (c) Estimated CO₂ limitation (black line) (relative units as this is a ratio of substrate input fluxes) and photosynthesis (red line; mol C C⁻¹ mol S d⁻¹) for juveniles modelled over 400 days. Relative values represent limitation coefficients, with zero signifying that neither carbon nor nitrogen are limited (Cunning et al., 2017)

(permutational multivariate analysis of variance (PERMANOVA): Df₇, F = 1.73, R² = 0.14, p = .01), with time explaining 14% of the variance in community structure. These changes were driven by the varying dominant abundances of D1 and D1a as well as changes to the diversity in background Symbiodiniaceae through time. For example, juveniles were initially dominated at day 40 (T₁) by D1 (31.5%) and lower abundances of D1a (8.5%). This was followed by a significant increase and co-dominance of D1a with D1 until the last time points in which D1 re-established dominance within the coral juveniles.

3.3 | Bacterial population shifts across ontogeny

Bacterial communities varied significantly among juveniles over time (PERMANOVA: Df₇, F = 1.23, R² = 0.16, p = .001), with time explaining 16% of the variance in community structure. In comparison to Symbiodiniaceae communities, bacterial communities at the first and second time points were distinct relative to all other time points (Figure 2 NMDS inset 2, light blue and teal hulls), converged in diversity and abundance in T₃ through T₇, before diverging again at the final time point (T₈) (dark pink hull).

A diverse bacterial community was associated with the early life stages of *A. tenuis* juveniles, with sequences affiliated across multiple taxa from the phyla Proteobacteria, Cyanobacteria, and Planctomycetes present at high relative abundances, although other key phyla were also detected, including Firmicutes and Bacteroidetes at lower abundances (Figure 2). Within the Proteobacteria, the dominant sequences were affiliated with both Alphaproteobacteria (43.5%, e.g., Rhodobacteria, Rhizobiales, Rickettsiales, Rhodovibrionales, Rhodospirillales, Caulobacterales, and Kordiimonadales) and Gammaproteobacteria (0.5%, e.g., Alteromonadales and Oceanospirillales). Specifically, 22, 39, and three ASVs were detected for Oceanospirillales (potential *Endozoicomonas*), *Roseobacter*, and *Burkholderia*. Sequences belonging to *Litoribrevibacter*, *Balneatrix*, *Oleiphilus*, and *Kiloniellales* (NCBI accession: KU688966.1) within Oceanospirillales (and related to the *Endozoicomonas* genus) were also commonly retrieved from the *A. tenuis* juveniles through the study

across all time points sampled, with these taxa making up 25% and 17% of all Symbiodiniaceae ASVs. Other taxa from *Durusdinium* were also detected, but at lower relative abundances, including D5 and D9, followed by D6 and D2.2 (4.8–0.3% of ASVs). Taxa belonging to C1 (4.5%) were also prevalent over the sampling period, with C3, C15, C1d, C50, Cspc, and taxa from clades F and I detected in low but consistent abundances associated with *A. tenuis* juveniles. The Symbiodiniaceae community structure varied significantly over time

period. ASVs from Oceanospirillales were associated with juveniles across sampling time points from T_3 to T_8 , increasing in relative abundance from 0.03% to 0.2% at the final time point. Alphabacterial sequences belonging to the *Roseobacter* were detected in all time points at background abundances (0.2%–0.9%), while *Burkholderia* affiliated sequences were retrieved from two juveniles only at T_6 and T_7 (0.03%–0.08% relative abundance, respectively). Oxyphotobacteria (Cyanobacteria) was one of the most dominant overall (30%) ASVs associated with *A. tenuis* juveniles, becoming increasingly abundant throughout the year (T_1 : 3.4 to T_8 : 6.1%). Conversely, other taxa decreased in relative abundance throughout the year, including Pirellulales–Planctomycetes (T_1 : 0.04 to T_8 : 0.01%), Caulobacteriales (T_1 : 0.3 to T_8 : 0.02%), and Rhodospirillales affiliated sequences (T_1 : 0.5 to T_8 : 0.2%).

3.4 | Co-occurrence

The first month of development for *A. tenuis* juveniles was typified by slow growth and initially low and variable Symbiodiniaceae cell densities. At this time point, *Durusdinium* taxa were more frequently correlated with specific bacterial taxa (e.g., Proteobacteria) compared to other Symbiodiniaceae genera (Figure 3). For example, D1 was highly positively correlated with Rhodobacterales (Alphaproteobacteria), but D1 was negatively correlated with D9. Co-occurrence frequently occurred between D1, D1a, D9, D5, and sequences within the classes Alphaproteobacteria, Oxyphotobacteria, Gammaproteobacteria, Planctomycetacia, and Phycisphaerae (Figure 4a, co-occurring nodes 1–3). Leptolyngbyales (Cyanobacteria) were also highly positively

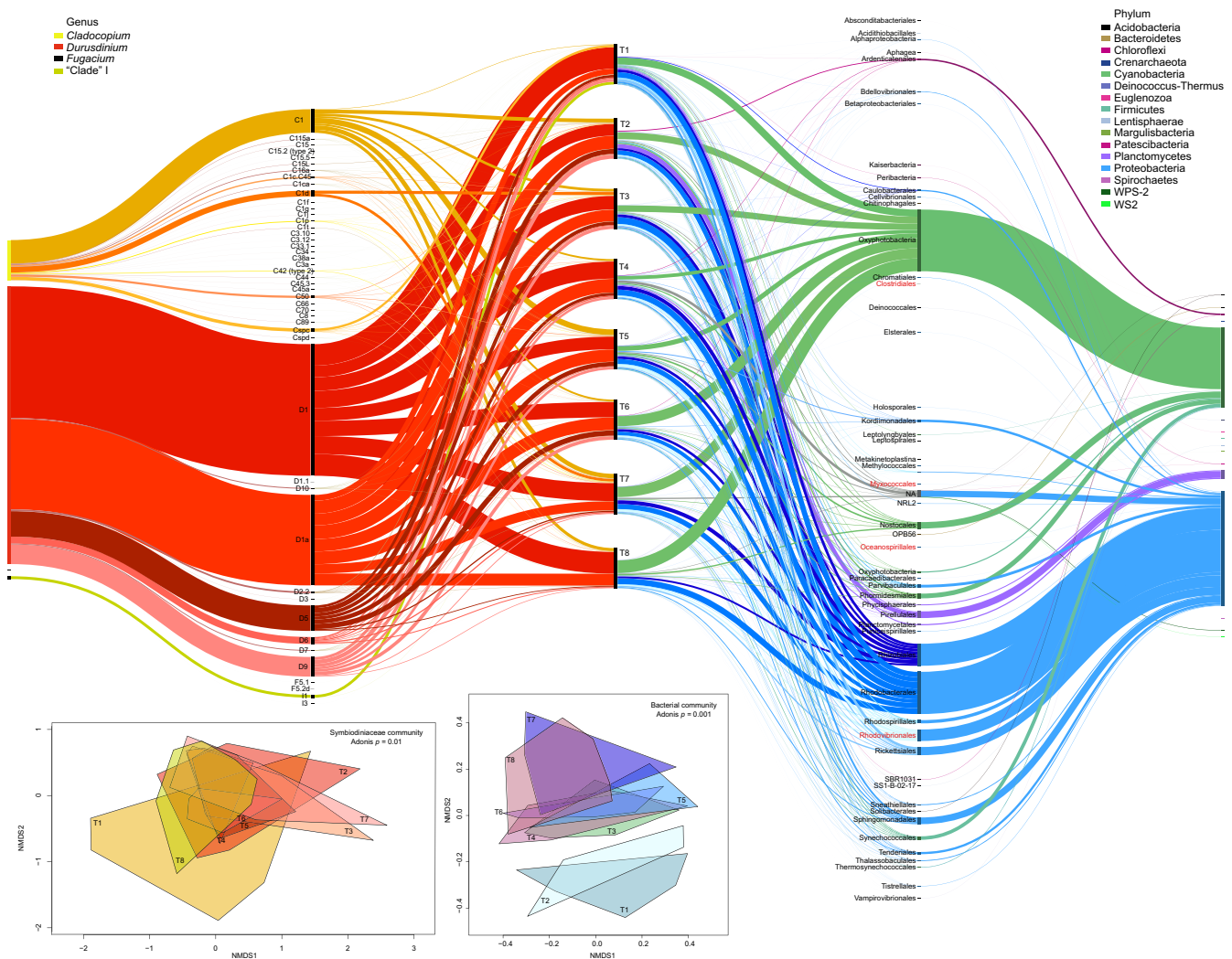


FIGURE 2 Alluvial plot of variance-normalized relative abundances of Symbiodiniaceae (warm colors) and bacterial ASVs (cool colors) across one year in *Acropora tenuis* juveniles outplanted to Magnetic Island. Timepoints (T_1 – T_8) are represented in the middle section with black bars (time axis), where taxa are split to show the relative abundances of each at each of the time points. Abundances for Symbiodiniaceae taxa feed to the time axis from left and bacterial taxa feed in from the right, increasing in taxonomic resolution. Insets: Nonmetric multidimensional scaling (NMDS) of variance-normalized abundances of Symbiodiniaceae (warm colors) and bacterial ASVs (cool colors) constructed using Bray-Curtis distances. Only taxa greater than 0.01% are shown (hence rare taxa are excluded), with cut-offs defined (Galand, Casamayor, Kirchman, & Lovejoy, 2009; Logares et al., 2014; Pedrós-Alió, 2012). Bacterial taxa in red are those that have been identified as key mutualistic and parasitic mutualists in corals (Neave, Rachmawati, et al., 2016; Pollock et al., 2018)

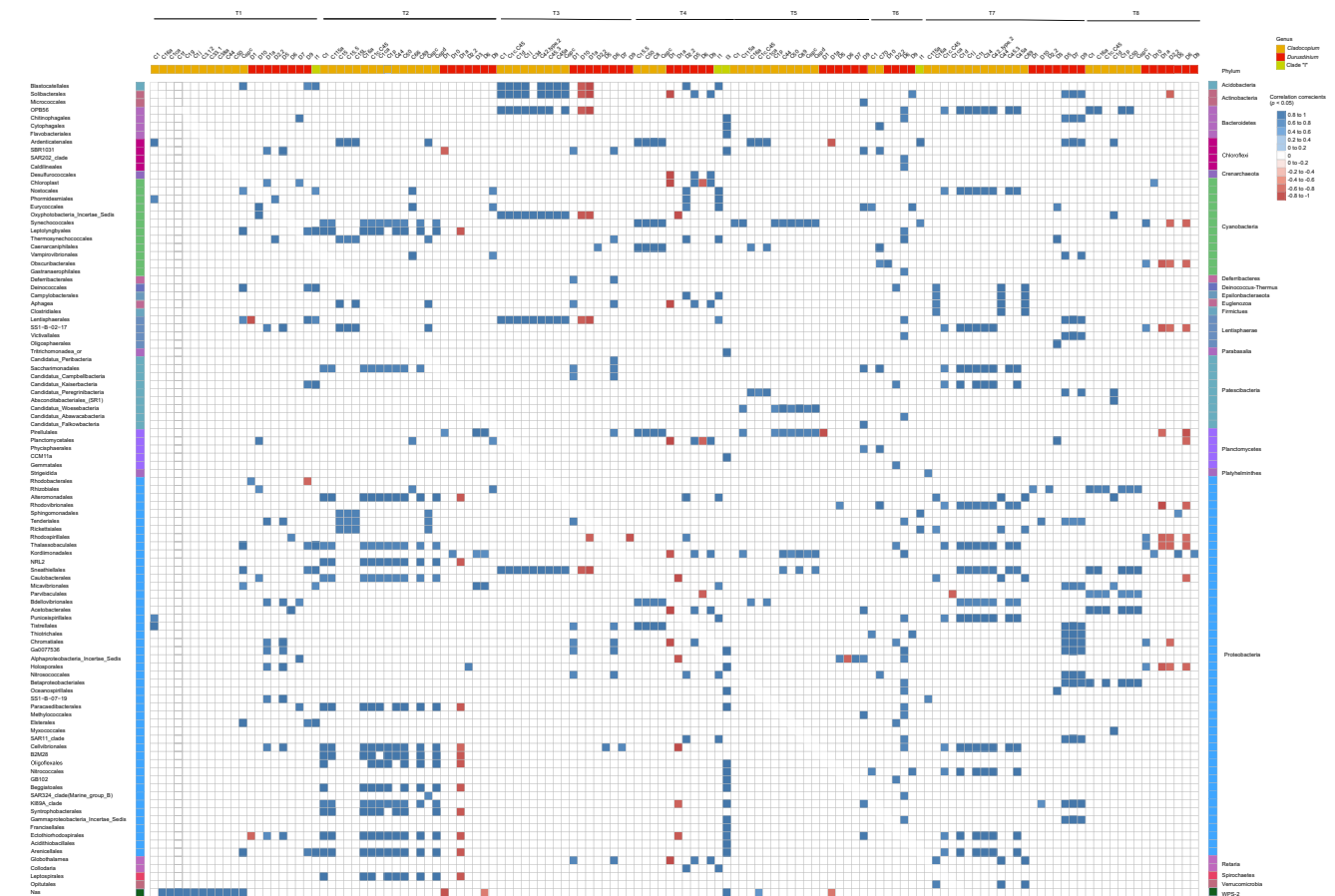


FIGURE 3 Heatmap of significant Pearson's product-moment correlation coefficients ($p < .05$) between Symbiodiniaceae and bacterial taxa

correlated with multiple *Durusdinium* sampled in T_1 and T_2 . The most connected taxa were Sphingomonadales (degree = 635 connections), indicating many first-degree connections to other taxa. Other important hubs included Ruegeria (Proteobacteria–Alphaproteobacteria) and Synechococcus (Oxyphotobacteria–Cyanobacteria). Similar to the correlation analysis, very few *Cladocopium* co-occurred with bacterial taxa at this earliest time point.

Juveniles remained small over the subsequent months, while Symbiodiniaceae cell densities increased rapidly. During these time points, connectedness between taxa was greater at T_2 (71 days) and T_3 (111 days) compared to T_1 , with the Rhizobiaceae (Proteobacteria) being the most connected taxa at both time points (Degree = 497 and 415, respectively). The diversity of correlations also increased after T_1 , with both *Cladocopium* and *Durusdinium* associating with many bacterial taxa at the later time points T_2 and T_3 (Figure 3). For example, Alphaproteobacteria, Planctomycetacia, and Oxyphotobacteria typically associated with *Cladocopium* (C1, C115a, C1c.C45, C1ca, C44, Cspd, and C50) (Figure 4b node 1) or D2.2, D5, and D1a (node 2) or D9 and D1 (node 3). While many more bacterial and Symbiodiniaceae taxa co-occurred in T_2 and T_3 samples (Figure 4c), they were still centered around the same taxa detected in T_1 samples (i.e., D1, D1a,

Alphaproteobacteria, Planctomycetacia, Gammaproteobacteria, and Oxyphotobacteria) though with some additional taxa (C15.5). Co-occurrence appeared to be more diverse for *Cladocopium* compared to *Durusdinium* (Figure 4c node 1). For example, multiple *Cladocopium* (e.g., C1cC45, C1j, C1, and C45.3) associated with many bacterial taxa (Alphaproteobacteria, Deltaproteobacteria, Gammaproteobacteria, Planctomycetacia, Oxyphotobacteria, and Phycisphaerae), whereas *Durusdinium* (D1, D1a, D2.2, D5, D9, and D10) associated with fewer bacteria (Alphaproteobacteria, Gammaproteobacteria, Phycisphaerae, and Oxyphotobacteria) (nodes 2 and 3).

Peak Symbiodiniaceae cell densities were reached by T_4 (129 days) and corresponded to the beginning of the exponential growth phase in *A. tenuis* juveniles. During this time, the co-occurrence of *Cladocopium* with bacterial taxa increased relative to earlier time points (e.g., C1, C1ca, C1p, C89, and Alphaproteobacteria, Gammaproteobacteria, Planctomycetacia) (Figure 4d node 1). Associations between D1, D1a, and Alphaproteobacteria remained abundant (Figure 4d node 2). Oxyphotobacteria (Cyanobacteria) exhibited the highest connectedness (Degree = 575).

A decrease and stabilization of Symbiodiniaceae densities associated with juveniles corresponded with accelerated growth rate at T_5 (167 days) and T_6 (194 days). During this time, associations

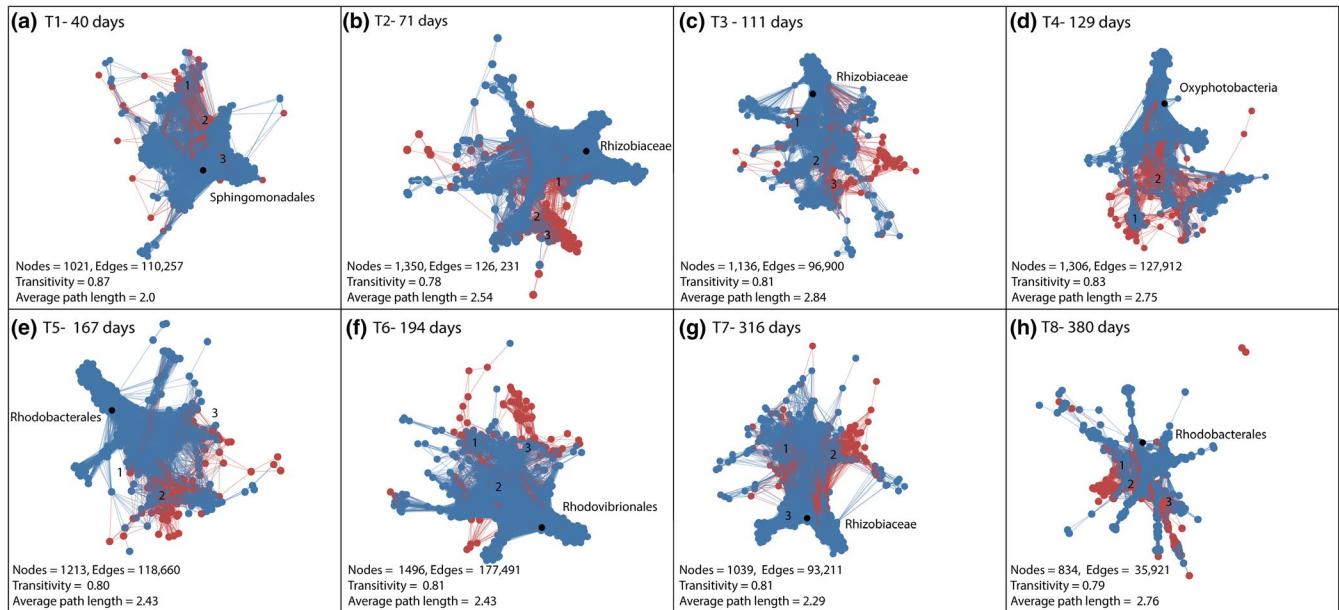


FIGURE 4 Network analysis of Symbiodiniaceae and bacterial co-occurrence across 1 year of growth for *Acropora tenuis* juveniles

between D1, D1a, multiple *Cladocopium* and Alphaproteobacteria, and Oxyphotobacteria were abundant (Figure 4e nodes 1 and 2, 4F node 1, node 3). Gammaproteobacteria and Oxyphotobacteria associated with a greater diversity of *Durusdinium*, including D1a, D3, D5, D6, and D9 (Figure 4f nodes 2 and 3). Rhodobacterales and Rhodovibrionales (Proteobacteria—Alphaproteobacteria) were the most connected nodes in T_5 and T_6 samples, respectively (Degree = 463, 568). Multiple Proteobacteria were also significantly positively correlated with Symbiodiniaceae I1 and I3 (e.g., Thalassobaculales, Sneathiellales, Micavibrionales, and Gammaproteobacteria, Figure 3).

Symbiodiniaceae cell densities decreased further as growth rates increased in T_7 (316 days) and T_8 (380 days), in which networks exhibited higher connectedness between bacterial and Symbiodiniaceae ASV taxa compared to other time points (Figure 4g across nodes 1–2, 4H node 1–2). These dense clusters of connectedness between ASVs consisted of a higher diversity of *Cladocopium* and *Durusdinium* (e.g., C1, C1p, C45, C1c, C115a, D1, D1a, D3, D2.2, D10 among others) associated with the same bacterial taxa represented at previous time points. However, some node clusters remained tightly associated with either *Durusdinium* (Figure 4g node 2, 4H node 1) or *Cladocopium* (4G node 3, 4H node 2). Additional bacterial diversity (Phycisphaerae and Deltaproteobacteria) was also associated with these clusters. Rhizobiaceae was the most connected taxon in T_7 samples (Degree = 523), although connectedness decreased by almost half by the final sampling time point (Rhodobacterales, Degree = 268). Finally, the total number of nodes and edges was substantially lower at this final time point (nodes 834, edges = 35,921, Figure 4a–h), corresponding to reduced multidimensional space in the Symbiodiniaceae community (Figure 2 insets).

4 | DISCUSSION

Changes in the relative abundances of constituent Symbiodiniaceae taxa allow corals to increase their thermal tolerance (Baker, 2014), where shifts to more heat-tolerant communities significantly increase survival probability under various climate change scenarios (Logan, Dunne, Eakin, & Donner, 2014). However, the contribution of corals' bacterial partners and their influence on the Symbiodiniaceae community remains unclear. Elucidating symbiont establishment, maintenance, and community composition dynamics are critical as microbial partners may promote physiological resilience of the host; even as climate change is expected to cause the breakdown or disruption of symbioses (Kikuchi et al., 2016). For example, host fitness can improve if the faster-growing microbial (e.g., bacterial, fungal, eukaryotic) community becomes more mutualistic (Pillai, Gouhier, & Vollmer, 2016); where net benefits to the host can be achieved through changes in background, cryptic microbes (Pillai, Gouhier, & Vollmer, 2014).

Understanding how host-associated microbial communities develop will elucidate potential acclimatory and adaptive routes for the holobiont (Theis et al., 2016). Here, we show that bacterial and Symbiodiniaceae communities associated with the coral *A. tenuis* vary concurrently during the first year of ontogeny. Correlation and network analysis revealed key co-occurrence groups within and across both Symbiodiniaceae and bacterial taxa during development. Acroporid juveniles at this site are known to be dominated by *Durusdinium* (Abrego et al., 2009b) and Proteobacteria (Alphaproteobacteria) (Lema et al., 2014). More specifically, we found *A. tenuis* juveniles were dominated by D1 and D1a over the year of sampling, with other taxa from *Durusdinium* and *Cladocopium*, including *C. goreau*, detected at lower and persistent abundances.

Proteobacteria (e.g., Alphaproteobacteria inclusive of Rhodobacteria, Rhizobiales, Rickettsiales, Rhodovibrionales, and Rhodospirillales; Gammaproteobacteria inclusive of Oceanospirillales), Cyanobacteria, and Planctomycetes were also highly abundant and prevalent across sampled juveniles.

4.1 | The functional role of *Durusdinium* during juvenile *A. tenuis* development

Development in the first month for *A. tenuis* juveniles was typified by low Symbiodiniaceae cell densities, the dominance of *Durusdinium* compared to *Cladocopium* taxa, slow growth, and initial carbon limitation. Combined, these results are consistent with reports of slower growth in juveniles dominated by *Durusdinium* due to reduced nutrient sharing (Cantin, van Oppen, Willis, Mieog, & Negri, 2009). Later time points were typified by large increases in Symbiodiniaceae cell densities, which may explain the increased diversity and connectedness between *Durusdinium* and numerous bacterial taxa. The increased relative abundances of *Durusdinium* and *Cladocopium* may be linked to growth trade-offs, in which juveniles benefit from increased photosynthate incorporation to achieve fast “escape size” growth during the transition from horizontal to vertical accretion (Abrego, Willis, & van Oppen, 2012; Cantin et al., 2009). The final time points (i.e., T_7 and T_8) suggest a consolidation of community diversity, as evidenced by increased network connectedness and decreased diversity, which potentially signals increased nutrient sharing (i.e., the dissipation of carbon limitation and stabilization of photosynthesis in our models) and consolidation of key symbiosis partnerships, which leads to exponential growth in juveniles.

Multiple *Durusdinium* types were dominant in *A. tenuis* juveniles, especially at the earliest time point. This initial dominance may be linked to the stress-tolerance associated with this genus (Abrego, van Oppen, & Willis, 2009a, 2009b; reviewed in Quigley, Baker, Coffroth, Willis, & van Oppen, 2018). *Durusdinium* are commonly detected at higher abundances in marginal environments (Baums, Johnson, Devlin-Durante, & Miller, 2010) and opportunistically increase in abundance during bleaching events (Guest et al., 2016; LaJeunesse, Smith, Finney, & Oxenford, 2009), with free-living taxa detected at higher abundances in warmer-inshore reefs (Quigley, Bay, et al., 2017; Quigley, Willis, et al., 2017). Adult corals that are *Durusdinium*-dominated generally survive better under thermal stress events (Baker, Starger, McClanahan, & Glynn, 2004; Jones, Berkelmans, et al., 2008; Jones, Cantin, et al., 2008). Therefore, juveniles that establish symbiosis with *Durusdinium* may develop into more “resilient” adults by retaining the option to shuffle their symbiont community to one that performs better under more stressful environmental conditions (Jones, Berkelmans, et al., 2008; Jones, Cantin, et al., 2008). It may therefore be preferable for juveniles to establish symbiosis with this taxon under lower light, turbid environments (Finney et al., 2010; Reynolds, Bruns, Fitt, & Schmidt, 2008) as *Durusdinium* perform poorly in response to highlight and temperature in juveniles (Abrego, Ulstrup, Willis, & van Oppen, 2008). Alternatively, *Durusdinium* may be able to out-compete other

Symbiodiniaceae, though further work is needed to establish these functional competitive networks during early coral development.

4.2 | Bioenergetics and physiology identify key periods of growth, carbon limitation, and nitrogen assimilation

Juveniles grew exponentially over the course of the year, on par with previously reported juvenile and adult growth rates (Bak, 1976; Cunning et al., 2017; Mieog, Olsen, et al., 2009; Mieog, van Oppen, Berkelmans, Stam, & Olsen, 2009). We also found that algal cell densities in juveniles were consistent with previous estimates ($\sim 1 \times 10^4$ cells/mm², Abrego et al., 2012) and expectedly lower compared to adults ($\sim 1.4 \times 10^6$ cells/mm², Mieog, Olsen, et al., 2009; Mieog, van Oppen, et al., 2009). Interestingly, Symbiodiniaceae densities increased rapidly between 40 and 71 days to $\sim 2.2 \times 10^4$ cells per mm² and then decreased to $\sim 1 \times 10^4$. Bioenergetics indicate that photosynthesis reached “healthy” levels after this time point, suggesting that carrying-capacity for juveniles may have been reached for this time of year (April), when water temperatures become cooler. This time point (T_2) also corresponded with a large shift in the bacterial community structure and an increase in the number of significant positive correlations between Symbiodiniaceae and bacterial taxa. A previous study also found shifts within 2-week-old compared to 3-month-old juveniles, especially in taxa belonging to *Roseobacter* and *Sphingomonadales* sp. (Lema et al., 2014). Therefore, changes in the Symbiodiniaceae community and their abundances may drive changes in nutrient dynamics with flow-on effects to bacterial community structure or vice versa, all of which have important implications for regulating host physiology and stress responses (Cunning & Baker, 2014).

4.3 | Symbiodiniaceae and bacterial co-occurrence networks associated with *A. tenuis* juvenile ontogeny

Overall, we found specific associations between Symbiodiniaceae *Cladocopium goreau*, *Durusdinium trenchii*, and *Durusdinium glynnii* (hereafter referred to as C1, D1, and D1a for brevity; LaJeunesse et al., 2018), across multiple bacterial taxa, including Alphaproteobacteria, Deltaproteobacteria, Gammaproteobacteria, Planctomycetacia, Oxyphotobacteria, Phycisphaerae, and Rhizobiales. In particular, D1a tended to associate with Oxyphotobacteria and D1 with Alphaproteobacteria. *Cladocopium* were consistently highly correlated with Synechococcales and Thermosynechococcales (Cyanobacteria), Thalassobaculales, Sneathiellales, and Proteobacteria. Coral-bacterial associations are known to be diverse and dynamic (Bourne et al., 2016) with previous studies also demonstrating some taxa such as Gammaproteobacteria associated with marine taxa that also establish partnerships with Symbiodiniaceae (Bourne et al., 2013). Recently, more specific associations have been reported across multiple coral species, including Rhodospirillaceae and C3/C1; *Endozoicomonas* (Gammaproteobacteria; Oceanospirillales) and C1, C1c, C43, C3u, C3, C43a, D1, and D1a; *Pseudomonas/Alteromonadaceae/Flavobacteriaceae* and C1, C3,

D1, and D1a; Cyanobacteria/Pleurocapsa and C1, C3, A3, and B1; and finally, *Vibrio* spp. and C1, D1, and D1a (Bernasconi et al., 2018). Many of the bacterial taxa detected in this current study are considered part of a “healthy” community, including high abundances of Cyanobacteria and Proteobacteria (including Firmicutes and Bacteroidetes) (Littman, Willis, & Bourne, 2011).

We found evidence for the presence of three potentially key taxonomic groups in corals, *Endozoicomonas*-related sequences (Oceanospirillales), *Roseobacter*, and *Burkholderia*. *Endozoicomonas* affiliated sequences have been found in adult corals and are in high abundance in healthy adults at this specific site on the GBR (Bourne, Iida, Uthicke, & Smith-Keune, 2008; Neave, Apprill, Ferrier-Pagès, & Voolstra, 2016), and large-scale cophylogenetic analyses have identified *Endozoicomonas* as a key taxon in host evolution (Pollock et al., 2018). Tissue-specific sequencing and fluorescence in situ hybridization have also found *Endozoicomonas* within the coral tissue, indicative of a true coral symbiont (Bourne et al., 2013; Neave et al., 2016; Wada et al., 2019). Generally, Alphaproteobacteria have been shown to be more dominant in the early life stages of corals, giving way to Gammaproteobacteria (including *Endozoicomonas*) later in life (Lema et al., 2014). This is consistent with patterns seen here, where multiple Alphaproteobacteria were retrieved at high relative abundance (Rhodobacteria, Rhizobiales, Rickettsiales, Rhodovibrionales, and Rhodospirillales), suggesting this is a key bacterial class for acquisition during coral ontogeny. Furthermore, we did not recover *Endozoicomonas*-related sequences until T₃, with the highest relative abundance in the final time point (T₈). *Roseobacter* and *Burkholderia*–*Paraburkholderia* may also be important for juvenile health by way of nitrogen fixation (Damjanovic, Blackall, Webster, & van Oppen, 2017; Epstein et al., 2019; Leite et al., 2017), although more targeted sequencing is needed to determine if these represent true coral symbionts (e.g., Ainsworth et al., 2015).

Host-microbiome interactions may also play key roles in influencing host evolution in marine ecosystems. Kiloniellales (mutualist), Myxococcales, and Clostridiaceae (parasitic) have all been implicated in host co-evolution (Pollock et al., 2018). These taxa were also recovered in *A. tenuis* juveniles, including 300 ASVs identified as Kiloniellaceae (Proteobacteria–Alphaproteobacteria–Rhodovibrionales), and three within both Clostridiales (Family: Clostridiaceae) and Myxococcales. Other taxa detected here (Sphingomonadales, Rhizobiaceae, Rhodobacterales, Rhodovibrionales, and Rhodospirillaceae) have previously been identified as key taxa associated with C1, C3, D1, and D1a in adult corals globally (Bernasconi et al., 2018) and implicated in promoting host productivity and regulating symbiont community evenness in other aquatic and terrestrial ecosystems (Banerjee, Schlaeppli, & Heijden, 2018). Diazotrophic bacteria (*Rhizobiales*) were found to be key “hub” taxa associated with juvenile *A. tenuis*. Diazotrophs are key coral symbionts that potentially fix nitrogen for the host (Lema et al., 2014) and may contribute significantly to host nitrogen cycling (McDevitt-Irwin, Baum, Garren, & Vega Thurber, 2017).

Changes in Symbiodiniaceae taxa may also lead to corresponding shifts in bacterial communities. For example, changes in

nutrients produced or excreted by symbionts may induce changes in other symbiotic communities. There is some evidence of the influence of Symbiodiniaceae on bacterial communities, where increases in *Symbiodinium* (originally termed “clade A”) and decreases in *Durusdinium* (“clade D”) were correlated with increases in *Vibrio* spp. (Rouzé, Lecellier, Saulnier, & Berteaux-Lecellier, 2016; but see Littman et al., 2009). However, co-occurrence of Symbiodiniaceae and bacteria may not be present in all coral species (Bonthond et al., 2018). We also found that *Durusdinium*–bacterial co-occurrence networks exhibited less bacterial diversity compared to networks with *Cladocopium*. *Acropora tenuis* juveniles infected with *Durusdinium* also harbored a reduced diversity of bacterial taxa compared to those infected with C1 (seven taxonomic groups vs. ten) (Littman et al., 2009). Interestingly, the flexibility of Symbiodiniaceae taxa to associate with different bacterial groups has been observed in adults as well, in which C1 associated with the greatest bacterial diversity, followed by D1a, C3, and D1 (Bonthond et al., 2018).

Concurrently sequencing the bacterial and Symbiodiniaceae communities from single juveniles allows predictions for how respective partners may influence each other and potential roles in host development (Bourne et al., 2013). However, it must be acknowledged that the associations detected here may represent secondary, indirect effects. For example, bacterial community shifts may be driven by mucus production derived from photosynthates or community patterns may reflect early colonists to the substrates around the juvenile corals (e.g., Leptolyngbyales–Cyanobacteria) and therefore not symbionts. Indeed, some of the taxa retrieved here (both bacterial and Symbiodiniaceae) may represent transient members of the community, contamination (*Propionibacterium*, *Acinetobacter*), or pathogens (*Vibrio* spp.) (Hammer, Sanders, & Fierer, 2019). The physical location of these symbionts is also important in understanding their role in the host coral, where Symbiodiniaceae reside within gastrodermal host cells (Davy et al., 2012), and the bacterial community is found throughout the outer mucosal layer, within tissues, and partitioned in the skeleton (Ainsworth et al., 2015; Wada et al., 2019). Given that the results presented here represent community patterns derived from whole juvenile sequencing, we are not able to partition where different bacterial taxa originate and can therefore not exclude the possibility that some members may be transient versus obligate symbionts (e.g., potentially those found exclusively in the host tissue). Targeted sequencing of Symbiodiniaceae cells themselves would elucidate co-occurrence networks more clearly, in which bacterial taxa associated within or outside symbiont cells would suggest a direct symbiosis between the two (e.g., *Ralstonia*, Actinobacteria, and Proteobacteria; Ainsworth et al., 2015). Understanding the direct versus indirect interactions between Symbiodiniaceae and bacteria is important to elucidating the function behind these associations and remains to be resolved.

In summary, these data provide key insights into the establishment, maintenance, and potential interactions between bacterial and Symbiodiniaceae communities associated with coral juveniles in the wild. Co-occurrence and network analysis identified novel

associations between bacterial and Symbiodiniaceae taxa associated with the first year of *A. tenuis* development, including D1, D1a, C1, Alphaproteobacteria, Gammaproteobacteria, Oxyphotobacteria, and Rhizobiales. Together, these results elucidate stages in Symbiodiniaceae and bacterial community development that are potentially linked to early carbon and nitrogen dynamics associated with symbiosis establishment. Efforts should also be placed on assigning function to symbiont community structure (e.g., communities associated with heat tolerance or nutrient transport/cycling) and predicting how communities shift under projected climate change scenarios. Characterizing these communities is essential given the substantial acclimatory and adaptive potential Symbiodiniaceae and possibly bacterial taxa provide to their hosts.

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CONFLICT OF INTEREST

None declared.

AUTHOR CONTRIBUTIONS

KQ and BW: conceived the experiment; KQ, CAR, and GT: performed the fieldwork; DB and BW: contributed reagents and materials; KQ: analyzed the data and wrote the first draft. All authors edited and reviewed the manuscript.

ETHICAL APPROVAL

This study was carried out with permission and in accordance with the recommendations from the Great Barrier Reef Marine Park Authority (Great Barrier Reef Marine Park Authority Permit Number: G13/36318.1 to BW).

DATA AVAILABILITY STATEMENT

Sequencing data will be made available from Github (<https://github.com/LaserKate>).

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