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Microbiome dynamics in early life stages of the scleractinian coral *Acropora gemmifera* in response to elevated pCO_2

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Summary

Reef-building corals are complex holobionts, harbouring diverse microorganisms that play essential roles in maintaining coral health. However, microbiome development in early life stages of corals remains poorly understood. Here, microbiomes of *Acropora gemmifera* were analysed during spawning and early developmental stages, and also under different seawater partial pressure of CO_2 (pCO_2) conditions, using amplicon sequencing of 16S rRNA gene for bacteria and archaea and of ITS2 for *Symbiodinium*. No remarkable microbiome shift was observed in adults before and after spawning. Moreover, microbiomes in eggs were highly similar to those in spawned adults, possibly suggesting a vertical transmission from parents to offspring. However, significant stage-specific changes were found in coral microbiome during development, indicating that host development played a dominant role in shaping coral microbiome. Specifically, Cyanobacteria were particularly abundant in 6-day-old juveniles, but decreased largely in 31-day-old juveniles with a possible subclade shift in *Symbiodinium* dominance from C2r to D17. Larval microbiome showed changes in elevated pCO_2, while juvenile microbiomes remained rather stable in response to higher pCO_2. This study provides novel insights into the microbiome development during the critical life stages of coral.

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Introduction

Animals can be viewed as specific habitats that harbour diverse and complex microbes. Symbionts can be acquired by the host in each new generation horizontally (from environments to offspring), vertically (from parents to offspring) or through a combination of both (Bright and Bulgheresi, 2010). Mounting evidences suggest that microbial colonization is essential for host development, growth, survival, and behaviour therefore playing an important role within the hosts and ecosystems (Bosch, 2013; Funkhouser and Bordenstein, 2013; McFall-Ngai *et al.*, 2013; Hosokawa *et al.*, 2016). However, microbial colonization is dependent on biotic (e.g., host and microbes) and abiotic (e.g., pH and temperature) factors, most of which are poorly understood.

Reef-building corals harbour a variety of microbial communities, including endosymbiotic dinoflagellates, bacteria, archaea and viruses. Such multipartite symbioses make up coral holobionts, which influence the coral health thus forming a critical part of the reef ecosystems (Knowlton and Rohwer, 2003; Krediet *et al.*, 2013). Previous studies have suggested that the composition of *Symbiodinium* in juvenile corals is more flexible than that in adult corals (Little *et al.*, 2004; Abrego *et al.*, 2009). The transmission patterns of bacterial communities in corals are likely to be determined by reproductive modes (Apprill *et al.*, 2009; 2012 Sharp *et al.*, 2010; 2012), and those host-microbial associations are dynamic in early life stages, as shown in recent studies (Sharp *et al.*, 2012; Lema *et al.*, 2014). Although the mechanisms of microbiome development have been well established in some model animal systems such as the squid-vibrio symbiosis (Nyholm and Mcfall-Ngai, 2004; McFall-Ngai *et al.*, 2013), cnidarian Hydra (Bosch, 2013; Franzenburg *et al.*, 2013), and the human gut (Yatsunenko *et al.*, 2012), our understanding of

microbial symbionts in terms of their acquisition, development and maintenance in corals is rather limited.

The dramatic increase in atmospheric carbon dioxide (CO_2) results in increased partial pressure of CO_2 (pCO_2) in seawater and the concomitant ocean acidification (OA) has been proposed as one of the greatest threats to the viability of coral reefs globally (Hoegh-Guldberg *et al*., 2007; Pandolfi *et al*., 2011; Andersson and Gledhill, 2013). Physiological studies have demonstrated that OA can influence coral photosynthesis, calcification, growth and survival (Albright and Langdon, 2011; Huang *et al*., 2014). Additionally, OA has been demonstrated to impact free-living and host-associated microbial communities at different levels (O'Brien *et al*., 2016). Mounting evidence suggests that OA and reduced pH can affect microbiome of adult corals in natural CO_2-rich environments (Meron *et al*., 2012; Morrow *et al*., 2015) and aquaria systems (Thurber *et al*., 2009; Meron *et al*., 2011; Webster *et al*., 2013; 2016). Microbiome is crucial for coral holobionts to adapt to future changing environments thus playing a major role in determining which coral species will be the 'winner' in response to global climate change (Ainsworth and Gates, 2016).

Coral recruitment is critical for the persistence and resilience of coral reefs, particularly under local and global disturbances (Albright *et al*., 2010). The early life stages of corals and other invertebrates are more likely to be sensitive to environmental changes. OA adversely affects these animals in early life stages by disrupting embryogenesis, decreasing larval survivorship and competency, or promoting premature metamorphosis (Albright *et al*., 2010; Albright and Langdon, 2011; Chua *et al*., 2013). However, it is not clear how coral microbiome would respond to reduced pH or increased pCO_2 during the early developmental stages of coral life cycle. Understanding microbiome changes during the early developmental stages of coral can help us predict how coral recruitment and resilience can alter in response to future climate change.

In this study, we have characterized the microbiome assemblages of scleractinian coral *Acropora gemmifera* (Brook, 1892) at early life developmental stages with three pCO_2 levels (Fig. 1), using amplicon sequencing and analysis of 16S ribosomal RNA (rRNA) gene for bacteria and archaea and internal transcribed spacer 2 (ITS2) for *Symbiodinium*. Two fundamental questions have been addressed: (i) How does microbiome change over the early life stages of *A. gemmifera*? (ii) How does the coral microbiome respond to elevated pCO_2 in early life development of *A. gemmifera*?

Results

Overview of microbial communities in coral and seawater samples

To characterize the microbiome of *A. gemmifera*, we used amplicon sequencing to analyse 16S rRNA gene from

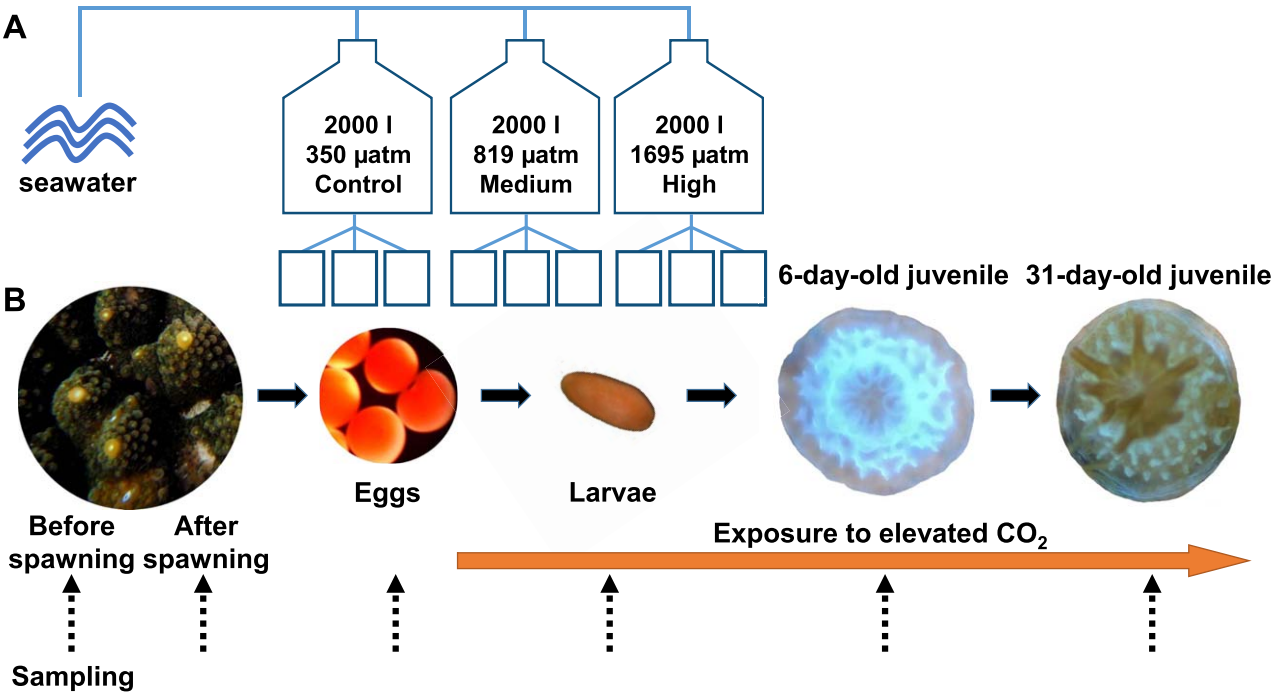

Fig. 1. An illustration of experimental design.
A. Experimental culture system.
B. Sampling points during the course of the study. [Colour figure can be viewed at wileyonlinelibrary.com]

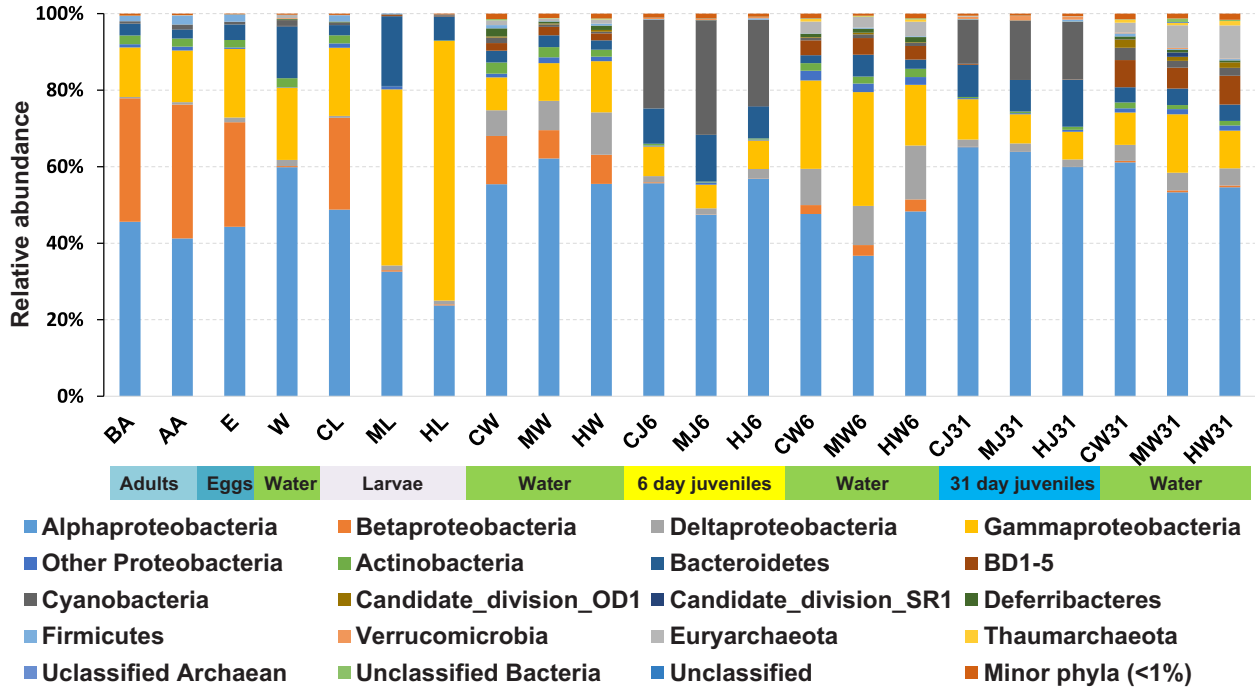

Fig. 2. Relative abundance of microbial communities at phylum level (except for Proteobacteria which is represented at class level). 'Minor phyla' represents the sum of all phyla with relative abundances less than 1%. The letters BA and AA refer to samples collected before and after adult spawning. C, M and H refers to samples from the control, medium and high pCO_2 treatments respectively. E, L, J and W refers to eggs, larvae, juveniles and water, respectively. The numbers 6 and 31 indicate 6- and 31-day-old juveniles, respectively. [Colour figure can be viewed at wileyonlinelibrary.com]

bacteria and archaea. For the purpose of comparison, seawater samples were also analysed. In total, amplicon sequencing generated 504,498 high-quality 16S rRNA gene reads (2,299–22,827 reads per sample). The number of 16S rRNA gene reads, number of OTUs and alpha diversity estimate calculated from OTUs at 3% dissimilarity (Shannon and Chao1 index) are listed in Supporting Information Table S1. The rarefaction curves for the 16S rRNA gene reads showed that the microbial diversity (Shannon index) reached a plateau (Supporting Information Fig. S1). The most abundant phylum for both *A. gemmifera* and seawater samples was Proteobacteria. In contrast, the representation of archaea was low for all the samples studied (Fig. 2). However, archaea were found to be better represented in seawater samples than in corals. The rank-abundance curves showed rich microbial communities in coral and seawater samples, but most microbial species were rarely represented (Supporting Information Fig. S2).

Shift in microbial communities throughout the host early life stages

The alpha diversity of the microbial community in adult *A. gemmifera* showed no statistically significant difference before and after spawning (Kruskal–Wallis test, $P > 0.05$), but exhibited increased patterns in early life stages including newly released eggs, planula larvae, 6- and 31-day-old settled juveniles at the control pCO_2 (Supporting Information Fig. S3 and Table S1). Alphaproteobacteria was the dominant class associated with corals in all life stages at the control pCO_2 (Fig. 2), with sequences mostly affiliated to Rhizobiales and Caulobacterales in both adults and eggs and Rhodobacterales in larvae and juveniles (Supporting Information Fig. S4). The increase in the abundance of Cyanobacteria, Bacteroidetes and Alphaproteobacteria correspond to a decrease or even absence of Betaproteobacteria and Gammaproteobacteria in the juvenile stages compared to adults, eggs and larvae. Interestingly, the abundance of Cyanobacteria decreased markedly in the 31-day-old juveniles (Fig. 2 and Supporting Information Fig. S4).

Comparing the entire communities at the species level, microbial compositions of corals before and after spawning were found to be similar (PERMANOVA, $P = 0.0637$). Microbial communities in newly released eggs shared similar abundance patterns with maternal corals, which gradually became similar to the surrounding seawater during the course of coral development (Fig. 3, Supporting Information Fig. S5). As shown in Fig. 3, we found variations in the microbial community compositions at different developmental stages of the coral. Principal coordinates analysis (PCoA) using OTU data for the coral and the seawater samples revealed that they were grouped by developmental stage (Fig. 4). Microbial communities of

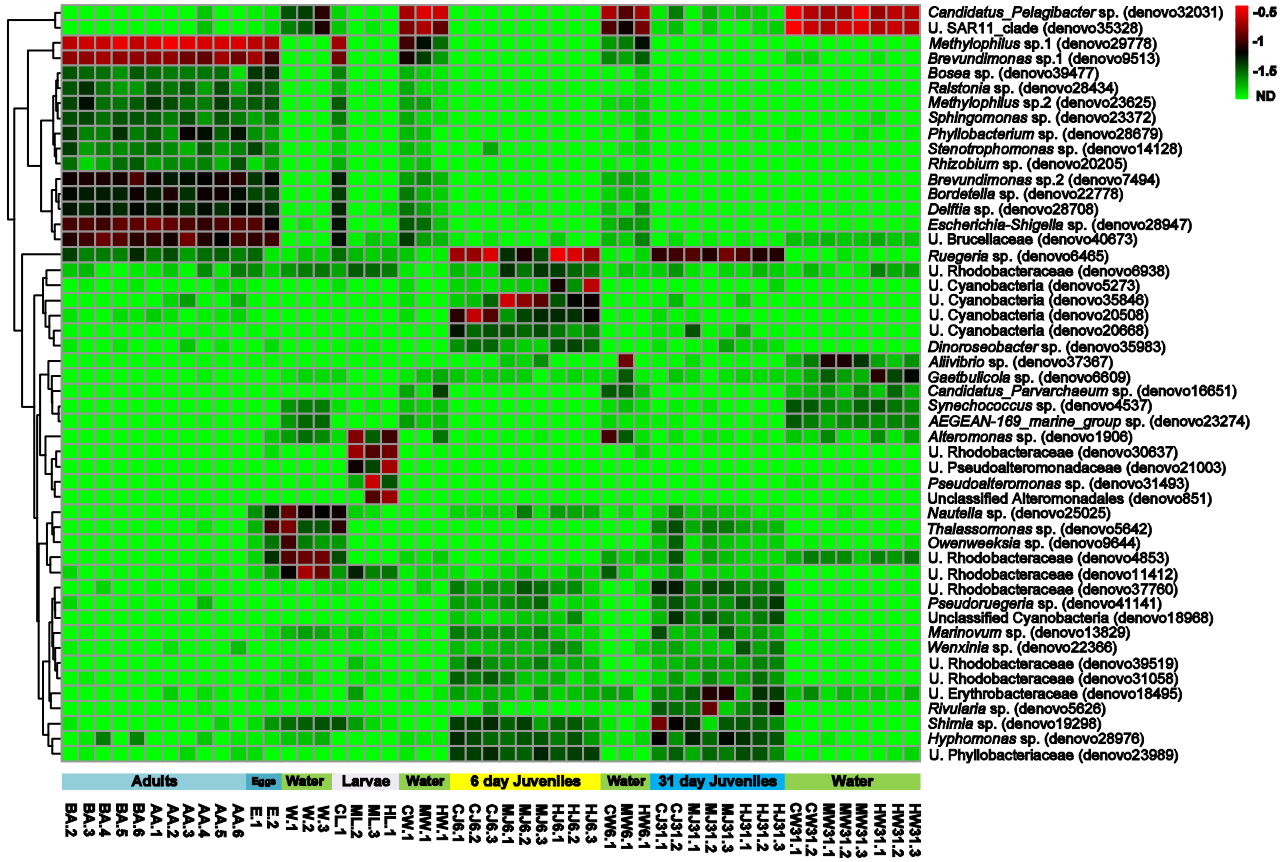

Fig. 3. Log-scaled percentage heat map of the 50 most abundant OTUs of samples. The scale 'ND, −1.5, −1, and −0.5' shows the relative abundance at '0, 2%, 9% and 31%' respectively. The letters BA and AA refer to samples collected before and after adult spawning. C, M and H refers to samples from the control, medium and high pCO_2 treatments, respectively. E, L, J and W refers to eggs, larvae, juveniles and water respectively. The numbers 6 and 31 indicate 6- and 31-day-juveniles respectively. Numbers after the dots represent replicates. [Colour figure can be viewed at wileyonlinelibrary.com]

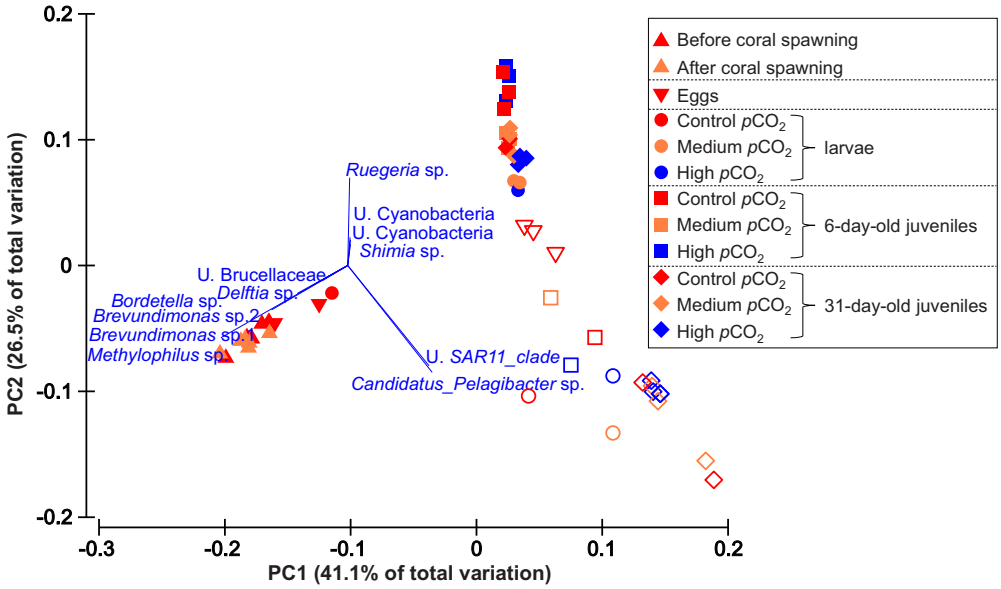

Fig. 4. Principal coordinates analysis of microbial communities associated with coral life stages. Vector overlays represent correlations (0.1) based on multiple rankings. The unfilled circles, squares and triangles indicate corresponding seawater samples. [Colour figure can be viewed at wileyonlinelibrary.com]

parental adults and 6-day-old juveniles were significantly different (PERMANOVA, $P = 0.0186$). Multiple ranking vectors associated with PCoA also indicated that unclassified Cyanobacteria and *Ruegeria* sp. were abundant in juveniles, whereas *Brevundimonas* sp., *Methylophilus* sp., *Delftia* sp., and unclassified species of Brucellaceae were abundant in the adults, eggs and untreated larvae. These dynamic patterns were further supported by accumulation of specific OTUs at certain developmental stages, as demonstrated by SIMPER analysis (Supporting Information Fig. S5). Compared to the microbial communities in adults, eggs and larvae, an eight-fold increase in relative abundance of *Ruegeria* sp. was observed in 6-day-old juveniles. However, the relative abundance of *Ruegeria* sp. sharply dropped in the microbiome of the 31-day-old juveniles. Unclassified Cyanobacterial species were prevalent in the juveniles, with a higher abundance observed in the 6-day-old juveniles in comparison to the 31-day-old juveniles. Interestingly, only a small group of microbes was shared among the samples from different developmental stages (Supporting Information Fig. S5, Table S2). For instance, an OTU cluster affiliated with the family Rhodobacteraceae including *Ruegeria* spp. and *Roseobacter* spp. found in the adult, egg, larval, and juvenile stages of *A. gemmifera*.

Changes in the microbial community during exposure to increasing pCO_2

The larval settlement was found to decrease with elevated levels of pCO_2 (Tukey's HSD, $P < 0.05$), but the survival rate of 6-day-old juveniles showed no significant difference between three pCO_2 treatments (Tukey's HSD, $P > 0.05$) under experimental conditions (Supporting Information Fig. S6). As shown in Fig. 3, variations in microbial community compositions were observed in larval samples exposed to different pCO_2 levels. Of the microbes that showed differences in response to pCO_2, dramatic decrease in the abundance of classes like Alphaproteobacteria, Betaproteobacteria and Deltaproteobacteria was observed with increasing pCO_2. Specifically, approximately 24% Betaproteobacteria was present at the control pCO_2, while less than 0.5% was shown at the medium and high pCO_2. Moreover, the increase in Gammaproteobacteria from 18% to 68% in larvae was mostly due to an increase in microbial species affiliated with the genera *Vibrio* (0.3–3%), *Pseudoalteromonas* (0.3–18%) and *Alteromonas* (0.2–6%) at the medium and high pCO_2 (Fig. 4). No significant difference in microbial community structure was detected in either 6-day-old (PERMANOVA, $P > 0.05$) or 31-day-old juveniles under various pCO_2 treatments (PERMANOVA, $P > 0.05$). As evident from Figs 3 and 4, it can be assumed that coral development plays a more profound role in comparison to varied pCO_2 conditions in shaping coral microbiomes.

Symbiodinium diversity in coral juvenile stages exposed to increasing pCO_2 treatment

To characterize the diversity of *Symbiodinium* in early life stages of *A. gemmifera*, we performed amplicon sequencing of the ITS2 region of *Symbiodinium*. The ITS2 sequences could be successfully amplified for all the 31-day-old and only one of 6-day-old (CJ6.2) juvenile samples. All the juvenile samples contained several *Symbiodinium* clades with different ITS2 subclades (Supporting Information Fig. S7). The 6-day-old juveniles were dominated by *Symbiodinium* ITS2 subclade C2r (73.2%), in contrast, the 31-day-old juveniles were overwhelmingly dominated by the subclade D17 (13.5% in 6-day-old juveniles and 70.6% in 31-day-old juveniles, Fig. 5). However, there was no significant difference in *Symbiodinium* assemblages in 31-day-old juveniles exposed to various pCO_2 conditions (PERMANOVA, $P > 0.05$).

Discussion

The dynamics of coral microbiome over different developmental stages

Our results showed that microbial communities in adult coral *A. gemmifera* did not change much over the spawning process, consistent with a previous study (Ceh *et al.*, 2012). Taken together, these observations further confirmed that the spawning event does not affect the adult coral-microbial associations, although such event often has considerable impact on the reef environment (Wild *et al.*, 2008). Furthermore, the microbial communities in the eggs of *A. gemmifera* were highly similar to those in parental colonies, and some microbes even persisted till the larval and subsequent juvenile stages, possibly indicating a transmission from coral parents to eggs, larvae and juveniles. It has been speculated that microbial vertical transmission in corals may occur either by coating the eggs with maternally-derived microbes or through uptake of microbes from the surrounding seawater released by the parental colony (Ceh *et al.*, 2013a; Thompson *et al.*, 2015; Leite *et al.*, 2017). More work on microbial transmission in spawning corals is required to gain a clear picture of the transmission of microbial symbionts in reef-building corals.

In this study, a gradual increase in alpha diversity of microbial communities was observed in *A. gemmifera* offspring over time. Furthermore, the microbial communities in juveniles were distinct from the parental adults, eggs and larvae, suggesting that the *A. gemmifera* microbiome undergoes a winnowing process over the developmental stages. This finding is supported by growing evidences that coral microbial communities are dynamic and flexible

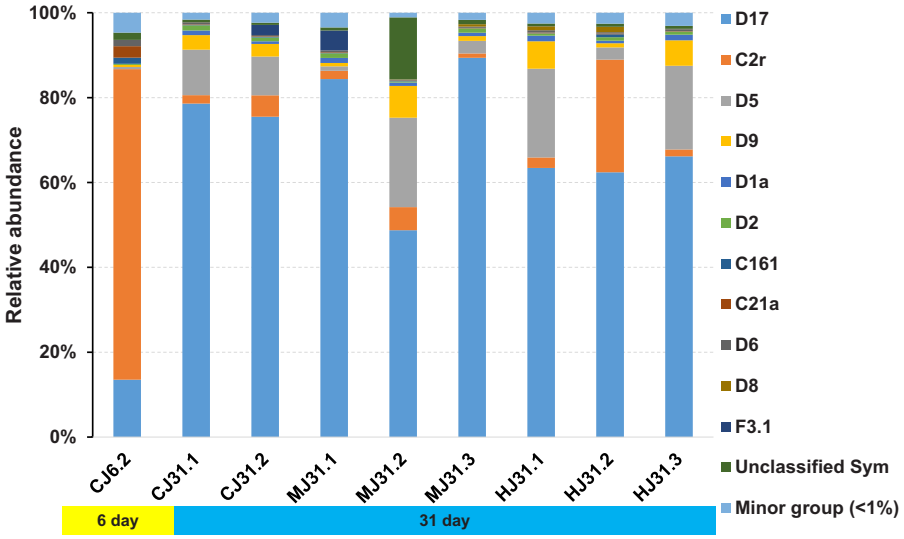

Fig. 5. *Symbiodinium* diversity in coral early life stages. Minor group represents the sum of all *Symbiodinium* subclades with relative abundances of less than 1%. The letters CJ, MJ and HJ refer to juvenile coral samples from the control, medium and high pCO_2 treatments respectively. The numbers 6 and 31 indicate 6- and 31-day-juveniles respectively. The numbers after the dots represent replicates. [Colour figure can be viewed at wileyonlinelibrary.com]

throughout the early life stages of coral development (Apprill *et al.*, 2009; Littman *et al.*, 2009; Sharp *et al.*, 2012; Lema *et al.*, 2014; Williams *et al.*, 2015). In this study, the microbial communities of *A. gemmifera* were found to be quite different at various developmental stages.

Ontogenetic alterations such as changes in morphology, physiology and immunology may contribute to changes in the microbial community structure. Coral undergoes significant metamorphic and developmental changes from a larva to a settled polyp, which provides a highly complex niche for microbial colonization (Sharp *et al.*, 2012). The physiological changes and immunological development in corals at different developmental stages (Puill-Stephan *et al.*, 2012; Hamada *et al.*, 2013) may also contribute to the microbiome development. The carbon, nitrogen, and energy requirements of the host at different developmental stages may favour specific microbial partners while suppressing others (Little *et al.*, 2004). For example, *Brevundimonas* spp. and *Methylophilus* sp. are consistently dominant in parental adults, eggs and untreated larvae of *A. gemmifera*, suggesting they may play a key role in the holobiont at different life stages. It is highly likely that such microbes are vertically transmitted from the parents to the offspring. Surprisingly, *Methylophilus* sp. was rarely detected in reef-building corals (Lins-de-Barros *et al.*, 2010; Li *et al.*, 2014) but was present in sponges (Kennedy *et al.*, 2008) and hydra (Franzenburg *et al.*, 2013). The dominance of Rhodobacterales, particularly *Ruegeria* sp. and other *Roseobacter* spp. in juveniles suggests specific roles of these microbes at this particular developmental stage. *Ruegeria* sp. might participate in the degradation of dimethylsulphoniopropionate (DMSP), which has been retrieved with high abundance in juveniles of *A. millepora* and *A. tenuis* (Raina *et al.*, 2013). Importantly, the Roseobacter clade has always been detected in coral early life stages, adults and seawater during coral reproduction (Apprill *et al.*, 2009; 2012; Sharp *et al.*, 2012; Ceh *et al.*, 2013a; Lema *et al.*, 2014). Some members of the Roseobacter clade can provide fixed organic nitrogen to *Symbiodinium* (Ceh *et al.*, 2013b) and defend against pathogenic bacteria (Nissimov *et al.*, 2009), indicating that Roseobacter clade potentially performs versatile functions at different life stages of coral. Immune system components in cnidarian Hydra, such as antimicrobial peptides (AMPs), can shape microbial compositions (Franzenburg *et al.*, 2013). Such AMPs have also been found in coral (Vidal-Dupiol *et al.*, 2011), but their potential functions in coral holobionts remain unclear.

In addition, microbial succession can be influenced by interactions among different microbial partners. Pioneer colonizers, such as the microbes associated with *A. gemmifera* eggs, can be replaced by some other secondary colonizers. Some species, such as r-strategists may have a greater competitive ability under the optimal conditions. For instance, certain unclassified Alphaproteobacterial and Gammaproteobacterial species are demonstrated to inhibit *Vibrio* growth (Rypien *et al.*, 2010). Furthermore, the organic sulphur compounds produced by photosymbiotic *Symbiodinium* are implicated to affect coral microbiome (Littman *et al.*, 2009; Bourne *et al.*, 2013). Variations in physiological function among different *Symbiodinium* subclades may also influence microbial compositions (Littman *et al.*, 2010). In *A. gemmifera*, endosymbiotic *Symbiodinium* is horizontally transmitted across generations. In this study, Cyanobacteria were particularly abundant in juveniles, with an observed higher abundance in 6-day-old juveniles than of 31-day-old juveniles. The abundant Cyanobacteria might contribute to the high capacity of carbon fixation required to fulfil the energy requirements for the 6-day-old juveniles. Additionally, it has been demonstrated

by amplification of PCR and observation of the low density of *Symbiodinium* in transparent coral tissue at this developmental stage. This is consistent with the finding that the decrease in Cyanobacteria corresponds to an increase in *Symbiodinium* at the 31-day-old juveniles. Moreover, a shift of *Symbiodinium* from clade C to D may reflect the host fitness during early life stages of coral (Little *et al*., 2004).

Effects of elevated pCO_2 on coral microbiome in larvae and juveniles

Changes in microbial communities in the early life stages of *A. gemmifera* under different pCO_2 treatments may be explained by several possible factors. First, it is presumed that differences in microbial function drive the assembly of coral microbiome. Microbes which are less competitive in adjusting to elevated pCO_2 conditions may be eliminated during environmental shifts. For instance, Betaproteobacteria disappeared completely in larvae exposed to the medium and high pCO_2 treatments. Similarly, field studies showed a decreased abundance of Gammaproteobacteria in *A. millepora* and *Porites cylindrica* at CO_2 seeps (Morrow *et al*., 2015). An increase in Bacteroidetes has been shown in corals exposed to low pH conditions (Thurber *et al*., 2009; Meron *et al*., 2011; Morrow *et al*., 2015) and in diseased coral (Bourne *et al*., 2009). Decreased pH also leads to an increase in functional genes associated with stress response (e.g., antibiotics and toxins) and a decrease of functional genes involved in adhesion in *Porites compressa* (Thurber *et al*., 2009). Second, the host physiological response to increased pCO_2 may contribute to microbial variations through an indirect effect. For example, OA has deleterious effects on survival, growth and calcification of early life stages of coral (Albright and Langdon, 2011). Gene expression involved in metabolism and calcification in the early life stages of *Acropora* showed differential responses to elevated pCO_2 (Moya *et al*., 2012; Moya *et al*., 2015). Larvae were found to be more sensitive to elevated pCO_2 stress than juveniles. This is corroborated by a pronounced shift in the microbial communities associated with larvae under elevated pCO_2 stress, which can also be correlated with the observed higher mortality and lower settlement in our experiment. *Vibrio*, *Pseudoalteromonas* and *Alteromonas*, considered as potential coral pathogens (Krediet *et al*., 2013), were found to be sharply increased in the larval stage under elevated pCO_2 and might have led to the low larval survival rate. These potentially pathogenic microbes were not persistent in juvenile coral, which could be partially attributed to the death of infected larvae.

Although changes in microbial diversity and assemblages in reef-building corals in response to OA have been increasingly reported, there is no consistency in the changing pattern (Thurber *et al*., 2009; Meron *et al*., 2011; 2012; Webster *et al*., 2013; 2016; O'Brien *et al*., 2016). The experimental duration, species specificity and other undetermined factors may contribute towards potential inconsistencies. For the first time, we report here that microbiome is stable in the juvenile stage of coral exposed to elevated pCO_2, whereas the larval microbiome changes under pCO_2 stress. It should be noted, however, that the larval microbiome can also be influenced by other factors such as tank effects. Hence, long-term experiments are needed to confirm how coral microbiomes will respond to higher pCO_2.

The acquisition and maintenance of beneficial microbes play a critical role in coral holobiont function under environmental perturbations. Emerging evidences indicate that the winnowing process in early developmental stages of coral occurs not only for symbiotic algae (Little *et al*., 2004; Abrego *et al*., 2009) but also for microbes (Apprill *et al*., 2009; Littman *et al*., 2009; Sharp *et al*., 2012; Lema *et al*., 2014). This process allows the coral to acquire different microbes during the course of its development, thus leading to the observed microbial variations. In this study, a significant shift in the coral microbiome has been noted during development. Several possible factors may drive this shift in microbial communities in the early life stages of coral but it remains unknown whether these microbes persist in later developmental stages as well. Also, very little is known about the potential functions of the resident microbes. Moreover, the cellular and molecular mechanisms underlying both dynamics and homeostasis of the coral microbiome also remain unclear. Further studies of coral microbiome throughout all life cycles are required to address these issues.

Conclusions

In this study, we have found the stability of microbial communities in *A. gemmifera* before and after spawning and showed that eggs and larvae contained a highly diverse, yet parent-like microbiome. The structures and dynamics of microbial communities in early life stages of *A. gemmifera* are likely to be driven primarily by host development, and affected by undetermined factors including the conditions of the surrounding seawater and tank effects. In addition, the microbial communities in juvenile stages were found to be rather stable under increasing pCO_2. These findings provide the first insights into microbial colonization and assembly in early life stages of *A. gemmifera* under an acidic ocean environment.

Experimental procedures

Experimental setup

OA experiments were conducted from May to June 2013, at the Tropical Marine Biological Research Station (TMBRS) in Hainan, China, at the outdoor flow-through aquarium facility. There were three treatments: control pCO_2 (pH 8.14, 350

Table 1. Values of pH, total alkalinity, dissolved inorganic carbon (DIC), pCO_2 and aragonite saturation state (Ω_A) in treatment aquaria.

Treatment	pH_T	Alkalinity (μmol/kg)	DIC (μmol/kg)	HCO_3^- (μmol/kg)	CO_3^{2-} (μmol/kg)	pCO_2 (μatm)	Ωara
Control	8.14 ± 0.03	2203 ± 28	1869 ± 38	1623 ± 45	235 ± 12	350 ± 30	3.85 ± 0.20
Medium CO_2	7.83 ± 0.04	2194 ± 22	2023 ± 27	1869 ± 33	133 ± 11	819 ± 76	2.17 ± 0.18
High CO_2	7.54 ± 0.03	2204 ± 24	2140 ± 24	2021 ± 24	75 ± 5	1695 ± 106	1.23 ± 0.08

All values are represented as means ± SE from three replicate aquaria.

μatm), medium pCO_2 (pH 7.83, 819 μatm) and high pCO_2 (pH 7.54, 1695 μatm), which were designed to mimic the future ocean CO_2 concentrations (Table 1) based on the worst-case stabilization levels of scenario RCP 8.5, as predicted for 2100 and beyond (IPCC 2014).

The outdoor aquarium facility was supplied with running seawater drawn at a depth of 6 m on the reef flat. Seawater was pumped through a pipeline, filtered through sand filtration and stored in three 2000-l tanks. The seawater was bubbled with CO_2 gas from a high-pressure CO_2 cylinder. CO_2 gas flow rates were regulated to achieve three target CO_2 levels. Triplicate aquaria per pH treatment were set up using 9 independent aquaria in total. All aquaria were maintained in natural light–dark cycles.

The inflow rate of seawater was adjusted to ~1.5-l min^{-1}, ensuring that the water in each aquarium was completely renewed every 45 min. The pH measurements were conducted daily in each treatment tank using a potentiometric pH Probe (Orion Star™, Thermo Scientific, MA, USA). Alkalinity replicates within each sample were also analysed using an automated titration system (Metrohm 877 Titrino plus, Herisau, Switzerland). The carbonate system parameters including pCO_2, bicarbonate, carbonate, and the aragonite saturation state (Ω_A) were calculated from the measured pH and total alkalinity values using CO2SYS (Table 1).

Coral collection, spawning and larval settlement

Ten colonies of *A. gemmifera* were collected from Luhuitou Bay (18.21°N, 109.47°E), Sanya, Hainan Island, China and transported to the reef-flat near the TMBRS, two weeks before the predicted spawning period in April-May 2013. Six colonies were moved to tanks immediately before they released egg-sperm bundles on May 5, 2013. Gametes were collected from the water surface immediately after spawning and mixed with gametes from other colonies. Fertilized eggs were then transferred to 1000-l experimental tanks with different pCO_2 aeration levels. Approximately 3 days after spawning, preconditioned terracotta tiles were placed in the rearing tanks to promote larval settlement. Larval settlement assays were conducted on those tiles by randomly transferring 300 larvae to 70-l aquaria with treated seawater and inspected for settlement under a dissecting microscope. When most larvae were settled and metamorphosed (approx. 4 days after spawning), tiles with newly formed juveniles (approx. 30 recruits per tile) were randomly removed from the rearing tanks, and placed in the experimental aquaria (10 tiles per aquaria) with running water for 31 days of CO_2 exposure. The survival rate of juveniles was also assessed after 6 days by recording the number of live juveniles on tiles at different pCO_2 treatments under a dissecting microscope.

Sample collection and total DNA extraction

Samples at different life stages, including adult parents, eggs, larvae, 6-day-old juveniles and 31-day-old juveniles (Fig. 1), were collected and thoroughly washed with autoclaved artificial seawater. Except for adults, each developmental sample consisted of 20 individuals to provide sufficient biomass for DNA extraction. For seawater sample collections, one litre of seawater per sample was also filtered through 0.2-μm polycarbonate (PC) membrane filters. All samples were preserved in 0.8 ml of DNA extraction buffer (100 mM Tris-HCl, 100 mM Na_2-EDTA, 100 mM Na_2HPO_4, 1.5 M NaCl, 1% CTAB [cetyltrimethylammonium bromide], pH 8) for subsequent total DNA extraction.

DNA extraction and amplicon sequencing

Total DNA was extracted from each sample using the Fast DNA® SPIN Kit for Soil (MP Biomedicals, Irvine, CA, USA), following the manufacturer's instructions. The quality and quantity of the DNA were checked with a NanoDrop spectrophotometer (ND-1000; NanoDrop, Thermo Scientific, MA, USA). The extracted DNA samples were preserved at −20°C for future use.

All DNA samples were PCR-amplified using the barcoded primer: 341F (5′-CCTAYGGGRBGCASCAG-3′) and 802R (5′-TACNVGGGTATCTAATCC-3′) (Cai *et al.*, 2013) to target the hypervariable regions V3-V4 of the 16S ribosomal RNA gene for Bacteria and Archaea. To detect *Symbiodinium* in eggs, larvae and juveniles, a specific primer set was used to amplify the ITS2 region of *Symbiodinum*: ITS2F (5′-GAATTGCAGAA CTCCGTG-3′) and ITS2R (5′-GGGATCCATATGCTTAAGTT CAGCGGGT-3′) (LaJeunesse and Trench, 2000). PCR amplification was carried out on a thermocycle controller (MJ Research, Bio-Rad) with the following program: initial denaturation at 94°C for 5 min; 35 cycles at 94°C for 30 s, 50°C for 30 s (16S) or 51°C for 30 s (ITS2), 72°C for 60 s; and final extension at 72°C for 5 min. All PCR products were purified using the Qiagen Agarose Gel DNA Purification Kit (Qiagen, Germany) and quantified with the NanoDrop device. All amplicon products were mixed in equal concentrations, followed by sequencing on an Illumina Miseq platform using 2 × 300 bp mode at Novogene (Beijing, China). The raw sequencing reads were submitted to the NCBI Sequence Read Archive under accession numbers SRR2917917 (16S) and SRR2919180 (ITS2).

16S sequencing data processing

Overlapping paired-end reads were merged to obtain 16S V3-V4 fragments using PEAR (Zhang *et al.*, 2014). After de-

multiplexing and quality control, the downstream processing of 16S rRNA gene amplicon sequencing data was analysed using the QIIME 1.6.0 pipelines (Caporaso *et al*., 2010). Reads were assigned to respective samples according to the barcodes and were clustered using UCLUST. Operational taxonomic units (OTUs) were picked at 97% identity. The most abundant reads for each OTU were selected as representatives and aligned using PyNAST against the SILVA 108 database (Pruesse *et al*., 2007). Representatives that were annotated as chloroplast, mitochondria and eukaryotes, were filtered out of the data set. The generated OTU table was resampled to 2,299 reads per data set before subsequent taxonomic annotation and quantitative analysis. Taxonomic abundance was summarized at the phylum, class, order, family and genus levels.

ITS2 sequencing data processing

The ITS2 amplicon sequencing data sets were quality-filtered and trimmed to remove bases with a quality score <20, followed by the merging of reads. A custom BLAST database of *Symbiodinium* ITS2 subclades that contains 408 ITS2 sequences had been constructed by Arif and his/her colleagues (2014). Samples were randomly subsampled to 2000 sequences which were subsequently batch-searched against the database using the BLASTN. ITS2 sequences were assigned to the ITS2 subclades that represented the best identity in the BLASTN hits (Tong *et al*., 2017).

Statistical data analysis

Rarefaction curves, rank abundance curves and alpha diversity (Shannon and Chao1 index) statistics of the samples were generated under QIIME. Alpha diversity was compared among coral life stages by analysis of variance or Kruskal–Wallis tests. Normalized OTU table was square-root-transformed and similarity percentage (SIMPER) analysis was performed to examine which OTU contributed most to the dissimilarity between different coral life stages. Bray-Curtis distance matrices were built to examine additional patterns of community structure and were visualized using principal coordinates analysis. Pearson correlation vectors were overlaid to demonstrate which OTU have strong positive or negative correlations with either PCO axis, which is indicative of stage differences. PERMANOVA (permutational multivariate analysis of variance) was used to determine whether the stage separation was statistically significant, and to compare distances among different coral life stages and between controls and treatments. Tukey's HSD multiple comparisons were performed as post hoc tests when ANOVAs detected significant differences in larval settlement and juvenile survival between treatments. All multidimensional statistical analyses were performed in R using the package Vegan. The heat map was also plotted under the R platform.

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Supporting information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Table S1. Sample information including number of sequences, total number of OTUs and diversity indicated by the Shannon and Chao 1 index with normalized number of 16S sequences.

Table S2. 21 Shared OTUs among coral samples at different life stages with control pCO_2 treatment. Rhodobacteraceae is highlighted in green.

Fig. S1. Rarefaction curve of the 16S rRNA gene reads from each sample showing the diversity of the bacterial and archaea community in coral and seawater samples. The *x*-axis represents the number of rarified reads, and the *y*-axis represents the diversity indicated by the Shannon index. The letters BA and AA refer to samples collected before and after adult spawning. C, M and H refers to samples from the control, medium and high pCO_2 treatments respectively. E, L, J and W refers to eggs, larvae, juveniles and water, respectively. The numbers 6 and 31 indicate 6- and 31-day-juveniles respectively. Numbers after the dots are replicate numbers.

Fig. S2. Rank-abundance curves based on bacterial and archaea operational taxonomic units (OTUs) at a 97% sequence similarity threshold.

Fig. S3. Changes in bacterial and archaea community at different life stages of coral. (A) Shannon evenness. (B) Chao1 evenness. Shannon evenness and Chao1 evenness were calculated using QIIME. The letters BA and AA refer to samples collected before and after spawning. C, M and H refers to samples from the control, medium and high pCO_2 treatments respectively. E, L, J and W refers to eggs, larvae, juveniles and water, respectively. The numbers 6 and 31 indicate 6- and 31-day-old juveniles respectively.

Fig. S4. The microbial community composition of coral samples at the order level. The *y*-axis shows the relative abundance of the taxa, and the *x*-axis shows the samples. The letters BA and AA refer to samples collected before and after adult spawning. C, M and H refers to samples from the control, medium and high pCO_2 treatments respectively. E, L, J and W refers to eggs, larvae, juveniles and water, respectively. The numbers 6 and 31 indicate 6- and 31-day-old juveniles respectively.

Fig. S5. (A)The similarity of microbial communities at eggs, larvae, 6-day-old juveniles and 6-day-old juveniles to the adult corals and seawater. (B) Similarity Percentage Analysis (SIMPER) for 10 most OTUs driving differences in microbial community composition of coral samples at different life stages with control pCO_2 treatment. (C) Venn diagram illustrating the number of unique and shared OTUs (97% sequence similarity) at different life stages with control pCO_2 treatment.

Fig. S6. (A) The larval settlement at different pCO_2 treatments. (B) Juveniles survivorship after 6 day at different pCO_2 treatments. Asterisks indicate significant differences (*, $P < 0.05$; **, $P < 0.01$).

Fig. S7. (A) Rarefaction curve of the ITS2 reads from each sample showing the diversity of the *Symbiodinum* community. (B) Species accumulation curves based on the progressive addition of samples calculated using 'Random' methods.