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Changes in microbial communities, photosynthesis and calcification of the coral *Acropora gemmifera* in response to ocean acidification

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With the increasing anthropogenic CO₂ concentration, ocean acidification (OA) can have dramatic effects on coral reefs. However, the effects of OA on coral physiology and the associated microbes remain largely unknown. In the present study, reef-building coral *Acropora gemmifera* collected from a reef flat with highly fluctuating environmental condition in the South China Sea were exposed to three levels of partial pressure of carbon dioxide ($p\text{CO}_2$) (i.e., 421, 923, and 2070 μatm) for four weeks. The microbial community structures associated with *A. gemmifera* under these treatments were analyzed using 16S rRNA gene barcode sequencing. The results revealed that the microbial community associated with *A. gemmifera* was highly diverse at the genus level and dominated by Alphaproteobacteria. More importantly, the microbial community structure remained rather stable under different $p\text{CO}_2$ treatments. Photosynthesis and calcification in *A. gemmifera*, as indicated by enrichment of $\delta^{18}\text{O}$ and increased depletion of $\delta^{13}\text{C}$ in the coral skeleton, were significantly impaired only at the high $p\text{CO}_2$ (2070 μatm). These results suggest that *A. gemmifera* can maintain a high degree of stable microbial communities despite of significant physiological changes in response to extremely high $p\text{CO}_2$.

Rising CO₂ in the atmosphere elevates the partial pressure of carbon dioxide ($p\text{CO}_2$) in seawater and reduces the global oceanic pH and carbonate ion concentrations, which is called ocean acidification (OA). It has been suggested that OA has profound effects on marine organisms and ecosystems, particularly calcifying organisms such as reef-building corals^{1–3}. With the increasing OA associated with ocean warming due to the rising global CO₂ emissions², there is an urgent need to understand and predict the tolerance and response of corals to future climate change.

Reef-building corals are commonly referred to as holobionts, which comprise coral host and associated micro-organisms including endosymbiotic photosynthetic algae, bacteria, and archaea, among others. These complex microbial partners play pivotal roles in coral health and holobiont function in carbon, nitrogen and sulfur cycles. The future fate of coral reefs largely depends on the capacity of corals and their symbionts to acclimatize or adapt to climate change^{2,4–7}. There is emerging evidence that corals can adapt to climate change⁸, although coral photosynthesis and growth can be negatively impacted by OA⁹. Moreover, flexible coral-algal symbiosis may facilitate the acclimatization/adaptation of the holobiont through algal shuffling or switching^{10,11}.

Shifting in the composition of coral-associated microbiota has been observed following environmental disturbances (e.g., elevated temperature) and has often been linked to impaired host health^{12–14}, although it has also been hypothesized to mediate holobiont resistance to environmental perturbations^{6,15}. Coral-associated microbial communities may be affected directly or indirectly by OA and would subsequently compromise holobiont fitness (i.e., changes in photosynthesis or calcification) and survival, possibly due to a shift in the functional roles of microbial associations^{12,16–18}. However, to date it is not clear how microbial communities change in coral in

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Sample ID	Treatment	Number of qualified reads	Total OTUs	Chao1 Ave.	Observed species Ave.	Shannon Ave.
HA.1	High $p\text{CO}_2$ treatment	37600	1301	1678.248	707.2	5.420
HA.2		19098	1115	1826.916	874.8	5.494
HA.3		29468	1687	2102.160	1087	6.333
MA.1	Medium $p\text{CO}_2$ treatment	27489	1108	1545.223	707.2	5.360
MA.2		27946	1295	1780.889	822.9	5.316
MA.3		26650	1074	1528.017	709.3	5.431
CA.1	Control $p\text{CO}_2$ treatment	24616	1065	1629.961	716.8	5.258
CA.2		21224	989	1576.258	727.8	5.527
CA.3		28163	1244	1688.842	783.5	5.450
HW.1	High $p\text{CO}_2$ treatment	14358	1237	1901.201	1152.6	5.252
MW.1	Medium $p\text{CO}_2$ treatment	15688	1203	1795.261	1075.2	4.860
CW.1	Control $p\text{CO}_2$ treatment	12463	1127	1886.261	1126.7	5.908

Table 1. Sample information and summary of microbial communities in corals and seawaters. “H”, “M” and “C” refer to high, medium and control $p\text{CO}_2$ treatment, respectively. “A” and “W” refer to coral and seawater samples, respectively. The number following the letter indicates a replicate. Chao1, Observed species and Shannon index were determined at 3% dissimilarity after normalizing the full 16S dataset (including bacterial and archaeal sequences) to 12,459 sequences per sample.

response to OA, or whether these changes also alter host physiology. Preliminary laboratory-based investigations have revealed a remarkable impact of increased $p\text{CO}_2$ or reduced pH on coral microbial communities^{12,16,18}. In contrast, no significant changes were observed in the microbial communities of transplanted corals in natural CO_2 vents¹⁹ and associated with two Pacific corals after 8 weeks of exposure to increased $p\text{CO}_2$ ²⁰. These contradictory findings underscore the need for further research. Most recently, microbial communities associated with coral and sponge from natural CO_2 seeps have demonstrated species-specific acclimatization to their habitats²¹. Therefore, the potential of natural microbial communities in corals to acclimatize/adapt to OA cannot be overlooked.

Natural fluctuations in seawater pH/ $p\text{CO}_2$ are common, especially diel pH/ $p\text{CO}_2$ fluctuations in shallow water coral reefs²² and these fluctuations affect the abundance and distribution of marine organisms²³. The fauna have been suggested to be locally acclimatized/adapted to the variable pH environment as an evolutionary mechanism to cope with future acidification^{24–26}. However, the adaptation of the coral holobionts to OA remains largely unexplored and is worth careful investigation.

The Luhuitou fringing reef (18°12'N, 109°28'E) is located in the southern Hainan Island, South China Sea (see Supplementary Fig. S1) and used to have a high coverage of living coral, which has declined by 80% since the 1960s²⁷. The diurnal and seasonal variation of the reef flat seawater pH/ $p\text{CO}_2$ is high^{28–30}, and the recorded extreme level is even lower than the value currently predicted at the end of this century (see Supplementary Table S1). The rapidly-growing branching coral *Acropora*, which is distributed worldwide, is an ecologically important genus in this reef flat. In the present study, *A. gemmifera* colonies collected from this reef flat were exposed to three $p\text{CO}_2$ levels to test our hypothesis that both the coral physiology and the microbial communities associated with this coral species are stable and resistant to OA exposure.

Results and Discussion

Overview of the microbial communities. After quality filtering, 308,591 reads were used for the downstream analyses. The number of operational taxonomic units (OTUs), Chao1 estimation of species richness, and Shannon index were obtained at a dissimilarity of 3% (Table 1). The rarefaction analyses revealed that the sequencing effort for each sample was sufficient to reflect the microbial diversity, and the rank-abundance curve showed that most OTUs had an abundance lower than 0.1%, which demonstrated that the microbial communities were occupied by rare species (see Supplementary Fig. S2). The prevalence of rare species has been widely demonstrated elsewhere, yet the ecological and functional roles of these rare species remain unknown³¹. There were no significant differences in beta diversity among the $p\text{CO}_2$ treatments, which is in contrast to the findings of a previous report showing an increase in coral microbial diversity with decreasing pH, possibly caused by an intermediate disturbance¹⁶.

In total, 24 bacterial and 2 archaeal phyla were detected in the coral and seawater samples, including Proteobacteria, Actinobacteria, Bacteroidetes, Cyanobacteria, Firmicutes, Thaumarchaeota and Euryarchaeota (see Supplementary Fig. S3). The relative abundance of archaea made up less than 0.1% of the seawater samples, with most OTUs belonging to autotrophic ammonia-oxidizing archaea (AOA) within the phylum Thaumarchaeota, which was even less abundant in the coral samples. It has been suggested that archaea may play prominent roles in corals and reefs⁵, although their abundance in both corals and reef water is much lower than that of bacteria³². Notably, the bacterial communities in both *A. gemmifera* and seawater were dominated by Proteobacteria and the most abundant class was Alphaproteobacteria (56–80%), among which the majority were assigned to the family Brucellaceae in the order Rhizobiales (Fig. 1) followed by Gammaproteobacteria (9–26%). Both Alphaproteobacteria and Gammaproteobacteria are commonly highly abundant in corals, but their relative abundance varies among species³³.

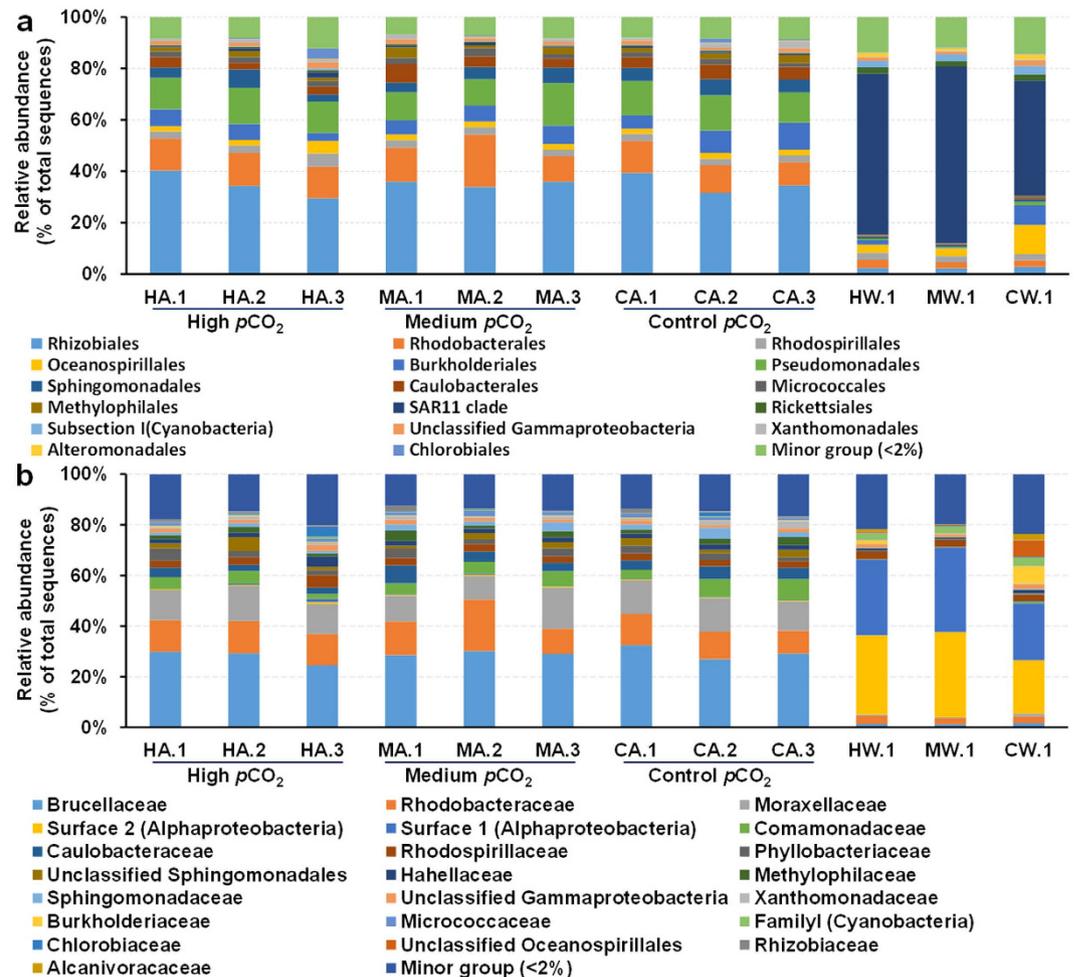


Figure 1. Coral and seawater microbial communities at the order (a) and family (b) level. The minor group represents the sum of all orders or families representing <2% in all samples. “H”, “M” and “C” refer to high, medium and control $p\text{CO}_2$ treatment, respectively. “A” and “W” refer to coral and seawater samples, respectively. The number following the letter indicates a replicate.

Taxonomic assignment at the genus level was summarized, and genera with an abundance of greater than 1% in at least one sample are shown in a heat map (Fig. 2). In the present study, the unclassified Brucellaceae (>24%), *Acinetobacter* (>9%) and *Pannonibacter* (>5%) were the most abundant genera in coral, regardless of the $p\text{CO}_2$ treatment. Diazotrophs within the order Rhizobiales have been found in other coral species and were considered to be important for coral holobiont in nitrogen-limited waters⁵. It has been shown that copiotrophic taxa including Brucellaceae were enriched in algal-dominated environment³⁴. Diverse algal communities on the Luhuitou fringing reef³⁵ might contribute to the dominance of unclassified Brucellaceae in *A. gemmifera*. *Acinetobacter* spp. have also been commonly reported in bleached and healthy corals³⁶. Therefore, it is reasonable to suggest that the dominant genera, including *Acinetobacter* and the unclassified Brucellaceae, play critical roles in *A. gemmifera*. Interestingly, the putatively endosymbiotic *Endozoicomonas*³⁷ was detected at a very low level in all coral samples (<2%). The photosynthetic Cyanobacteria assigned to the genus *Synechococcus* have been reported in sponge and coral²¹ and were also detected at a very low abundance (0.2%) in *A. gemmifera*. However, the functions of bacteria and archaea and their interactions in the coral holobiont remain largely unclear.

Stability of microbial communities in *A. gemmifera*. As estimated by Adonis analysis at all taxonomic levels (Adonis test, $p > 0.05$) and nMDS ordination (see Supplementary Fig. S4), there were no significant differences in microbial community compositions in *A. gemmifera* among the different $p\text{CO}_2$ treatments even after a 4-week exposure. Additionally, results from the SIMPER analysis showed that the dissimilarity of microbial communities among $p\text{CO}_2$ treatments was very small (see Supplementary Fig. S5). Taken together, these findings suggest that the *A. gemmifera* microbiome was not significantly affected by elevated $p\text{CO}_2$ and could remain relatively stable (Fig. 2). This result is inconsistent with the findings of some previous studies in which the coral microbiome shifted under higher $p\text{CO}_2$ or lower pH treatments over treatment periods ranging from days to months^{16,38}. However, our finding is consistent with some other studies. For example, there were no differences in the microbial community structure in coral between pH 7.7 ($p\text{CO}_2 = 1187 \mu\text{atm}$) and 7.5 ($p\text{CO}_2 = 1638 \mu\text{atm}$) whereas a significant difference was observed between pH 8.1 ($p\text{CO}_2 = 464 \mu\text{atm}$) and 7.9 ($p\text{CO}_2 = 822 \mu\text{atm}$)

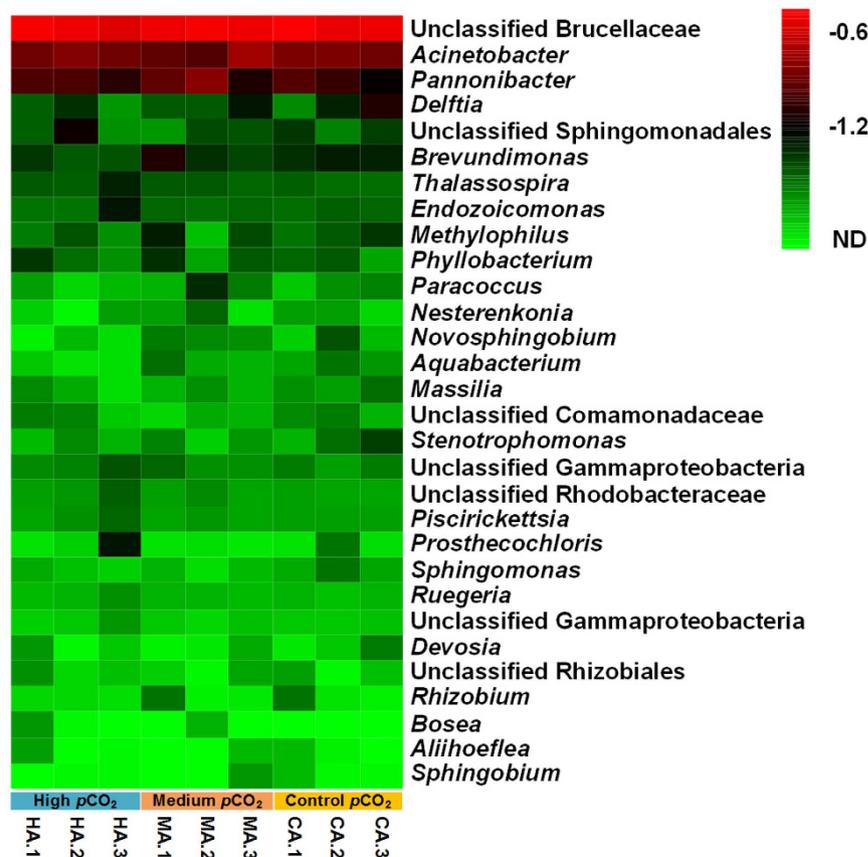


Figure 2. Heat map showing the abundance of microbial assignments in coral at the genus level. Genera abundance >1% at least in one sample are shown. The abundance values are $\log_{10}(x + 0.01)$ -transformed for plotting. For the heat map scale, “ND”, -1.2 , and -0.6 indicate relative abundance “0, 5%, and 24%”, respectively. The heat map was generated with R (version 3.1.3, R Development Core Team, 2015). “H”, “M” and “C” refer to high, medium and control pCO₂ treatment, respectively. “A” refer to coral samples. The number following the letter indicates a replicate.

after 6 weeks of CO₂ exposure¹⁸. Moreover, Meron *et al.*¹⁹ observed no significant changes in microbial communities associated with two Mediterranean coral species that were transplanted along natural pH gradients. A recent study reported that the microbial communities of two Pacific coral species were tolerant to reduced pH 7.9 (pCO₂ 738–835 μatm)²⁰. These inconsistent results might reflect that some coral-microbial associations are more resistant to increases in pCO₂/decreases in pH than others, but these findings could also be partially attributed to differences in the experimental conditions (e.g., field vs laboratory, pCO₂ or pH level, among others), and the exposure duration.

In most cases, microbial communities are dynamic and can rapidly respond to OA in seawater³⁹, biofilms⁴⁰ and other associated systems⁴¹. The genus *Acropora* is among the most sensitive coral to environmental change⁴². The potential for coral acclimatization or adaptation to climate change has been studied⁴³, and the physiological and molecular mechanisms responsible for OA resistance have recently been proposed⁸. Although there is limited evidence for biological adaptation to climate change in coral microbial symbionts, the adaptive power to climate change has been well documented in the photosymbiotic *Symbiodinium*^{10,11,44}. The shallow habitat of the coral *A. gemmifera* sampled in the present study has been experiencing regular large diurnal and seasonal variations in pH/pCO₂ (see Supplementary Table S1), which are mainly driven by biological activities of the reef^{28–30}. Therefore, it is most likely that microbial communities harbored by the natural population of *A. gemmifera* are resistant to the increased pCO₂, due to long-term acclimatization/adaptation to the highly dynamic pH conditions within the reef flat. Thus, there may be a resilient relationship between coral and microbial partners that can help corals overcome the fluctuations in seawater pH/pCO₂. However, we note that the stability of the coral microbiome is based on only one species collecting from a fluctuating environment. The application of variable pCO₂ conditions and controls from stable pH/pCO₂ environments in highly replicated culture experiments with consideration of tank effects could further confirm this assumption in future studies.

A recent study supports this interpretation. Morrow *et al.*²¹ found that microbial communities associated with coral and sponge originally from natural volcanic CO₂ seeps were distinct from the nearby control sites, reflecting the acclimatization of the host-symbiont to the high pCO₂ environment. Local acclimatization/adaptation to environmental variations in pCO₂, temperature and nutrients, among others, has revealed the capabilities of marine organisms including reef-building corals and symbiotic algae, to adapt to future climate change^{8,24,25,44,45}.

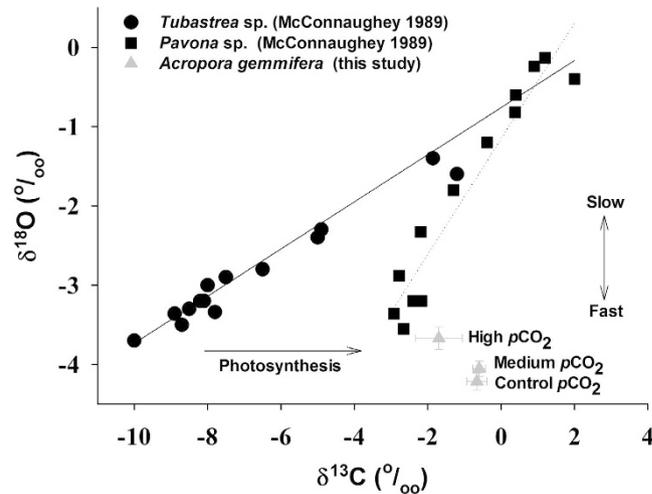


Figure 3. Skeletal isotopic response of *Acropora gemmifera* cultured under the control (421 μatm) and increased (923 μatm and 2070 μatm) $p\text{CO}_2$ conditions. Skeletal isotopic composition of non-photosynthetic coral *Tubastrea* sp. and photosynthetic coral *Pavona* sp. reported in a previous study (McConnaughey 1989) are plotted for comparison. Photosynthesis indicated the carbon isotopic offset due to respiration and photosynthesis. “Slow” and “Fast” indicated slow and fast coral calcification rates. Fast growing *Acropora* corals are expected to have more enriched $\delta^{13}\text{C}$ but depleted $\delta^{18}\text{O}$ values.

However, in general, the species-specific response of marine organisms to OA remains poorly understood^{1,23,46}. Thus, it is rather premature to conclude whether we can extrapolate the adaptive power of coral and its associated microbes documented in the present study to other coral species living in highly fluctuating reef environments.

Skeletal isotopic response to ocean acidification. During our experiments, all blocks of *A. gemmifera* exposed to the different $p\text{CO}_2$ treatments grew, survived and formed new skeleton (see Supplementary Fig. S6), even at the high $p\text{CO}_2$ (pH reduced to 7.47). When the fast-growing coral *A. gemmifera* skeletal $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ were compared among the different $p\text{CO}_2$ treatments, the skeletal $\delta^{13}\text{C}$ values in *A. gemmifera* were significantly different between any two $p\text{CO}_2$ treatments except between the control and the medium (Fig. 3). Skeletal $\delta^{13}\text{C}$ values were depleted by 1.10‰ and 1.04‰ for the control vs. high $p\text{CO}_2$ and for the medium vs. high $p\text{CO}_2$, respectively (one-way ANOVA, Tukey test, $p < 0.05$). Skeletal $\delta^{18}\text{O}$ values in *A. gemmifera* were enriched with increased $p\text{CO}_2$; they were 0.55‰ and 0.38‰ heavier in response to high $p\text{CO}_2$ than those in the control and medium, respectively (one-way ANOVA, Tukey test, $p < 0.05$). Compared with the previous data⁴⁷, skeletal $\delta^{18}\text{O}$ values revealed greater depletion in fast-growing coral *A. gemmifera*, while the skeletal $\delta^{13}\text{C}$ values remained within range. In addition, the relationship between skeletal $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ in the non-photosynthetic coral *Tubastrea* sp. deviated the most from those both in the photosynthetic corals *A. gemmifera* and *Pavona* sp. (Fig. 3).

The isotopic composition of the coral skeleton can be affected by metabolic isotope effects (e.g., photosynthesis and respiration) and kinetic isotope effects (e.g., the calcification process)⁴⁸. The coral skeletal $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ were generally used as an effective proxy to study photosynthesis, respiration and calcification processes^{48,49}. In general, photosynthesis and calcification can enrich skeletal $\delta^{13}\text{C}$ but deplete skeletal $\delta^{18}\text{O}$ due to isotope fractionation^{47,49}. Compared non-photosynthetic (*Tubastrea* sp.) and photosynthetic (*Pavona* sp.) corals⁴⁷, the relationship of $\delta^{13}\text{C}$ vs. $\delta^{18}\text{O}$ in *Pavona* sp. and *A. gemmifera* was different from that in *Tubastrea* sp. (non-photosynthetic coral) due to active photosynthesis. In addition, more $\delta^{18}\text{O}$ deviation was observed in *A. gemmifera* than *Tubastrea* sp. and *Pavona* sp., mostly due to the highest growth rate in *A. gemmifera* (Fig. 3). Skeletal $\delta^{13}\text{C}$ values in *A. gemmifera* were significantly depleted at the high $p\text{CO}_2$, suggesting that the photosynthetic rates were much lower at the high $p\text{CO}_2$ than at the control $p\text{CO}_2$. The variation in skeletal $\delta^{18}\text{O}$ values of *A. gemmifera* was consistent with the findings of a previous study demonstrating an enrichment of $\delta^{18}\text{O}$ in the coral skeleton in response to elevated $p\text{CO}_2$ ⁴⁹. The coral calcification rate decreases under reduced pH conditions⁹, which corresponds to heavier skeletal $\delta^{18}\text{O}$, whereas low $p\text{CO}_2$ and higher pH lead to species with lighter $\delta^{18}\text{O}$ because HCO_3^- is isotopically heavier than CO_3^{2-} ⁴⁹. Consequently, the significantly enriched $\delta^{18}\text{O}$ and more depleted $\delta^{13}\text{C}$ in *A. gemmifera* observed herein may reflect slight reductions in photosynthesis and calcification at the high $p\text{CO}_2$. It should be noted that *A. gemmifera* skeletal $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values did not vary significantly at the medium $p\text{CO}_2$, potentially because this stress level did not exceed its acclimatization range. These findings indicate that the coral *A. gemmifera* is able to acclimate to an acidifying ocean, even in the presence of a dramatically increasing atmospheric CO_2 concentration.

Although the mechanisms by which extremely high $p\text{CO}_2$ induces decreased photosynthesis and calcification efficiencies in *A. gemmifera* are unknown, several potential mechanisms have been proposed, such as photoinhibition and suppression of the carbon concentrating process^{3,9}. Photosynthesis, calcification and other physiological processes in reef-building corals can be influenced by their microbial partners or vice versa under OA¹⁷. However, the microbial communities associated with *A. gemmifera* remained unchanged as a consequence

of host physiological changes, further supporting our hypothesis that highly stable microbial associations are likely driven by local acclimatization/adaptation to the fluctuating environment. Alternatively, host physiological costs might result from a potentially increasing energy demand to maintain stable microbial assemblages at the extremely high $p\text{CO}_2$ that exceeds its tolerance level.

It has also been proposed that physiological differences among symbiotic algal phylotypes may influence the stable isotopic composition of coral skeleton⁵⁰. Furthermore, the distinct mechanisms used to concentrate carbon by different *Symbiodinium* phylotypes and their physiological responses to OA are phylotype-specific⁵¹. For example, *Symbiodinium* community shifts may occur in response to environmental stresses^{10,11}. In the present study, we did not investigate *Symbiodinium* phylotypes associated with *A. gemmifera*. A recent study found no changes in *Symbiodinium* phylotypes associated with corals among different pH conditions¹⁹, suggesting the presence of stable *Symbiodinium* assemblages in corals in response to OA. In general, a stable microbial partnership to maintain key metabolic functions can improve coral holobiont acclimatization or adaptation to environmental stresses⁵. However, the interactions between microbial communities and coral physiology remain far from clarified.

Conclusions

In this study, the tropical fast-growing coral *A. gemmifera* from a shallow habitat with natural pH/ $p\text{CO}_2$ fluctuations was selected as a representative species and was exposed to a 4-week CO_2 treatment. The microbial communities and skeletal isotopic compositions were examined simultaneously. We found that the microbial communities in *A. gemmifera* remained remarkably stable. In contrast, neither photosynthesis nor calcification in the coral were impacted under medium $p\text{CO}_2$ but were both negatively affected under extremely high $p\text{CO}_2$, as demonstrated by an enrichment of $\delta^{18}\text{O}$ and increased depletion of $\delta^{13}\text{C}$ in the skeleton under extremely high CO_2 stress. The present findings indicate that some reef-building corals may be more tolerant to OA in pH/ $p\text{CO}_2$ fluctuating environments and have a high degree of host-symbiont fidelity, despite the observed impairment of host physiological processes in response to high CO_2 stress. This study also contributes to our understanding of the variability of OA resistance among coral-microbial associations. Because coral reefs are facing other environmental stresses in addition to OA, the synergistic effects of multiple stressors on the coral microbiome must be carefully examined to understand the persistence of the coral holobiont and coral reefs in the future ocean.

Methods

Experimental design and sample collection. The experiment was conducted in an outdoor seawater flow-through system at the Tropical Marine Biological Research Station in Hainan (TMBRS) near the Luhuitou fringing reef. Seawater was pumped directly from a depth of 6 m in the front of the TMBRS into three 2000-L header tanks in which the designated $p\text{CO}_2$ level was adjusted with CO_2 gas. Three $p\text{CO}_2$ treatments were projected current $p\text{CO}_2$ levels, those at the end of the present century, and double the end of the present century: 421 μatm , 923 μatm , and 2070 μatm with pH values of 8.07, 7.76 and 7.47, respectively. The well-mixed seawater from the header tank continually flowed into three aquaria at a rate of 0.51 min^{-1} . Each aquaria was equipped with a submerged pump to drive the water flow, and all aquaria were maintained under a natural light–dark cycle to mimic the field condition.

Six healthy colonies of *A. gemmifera* were collected from the Luhuitou fringing reef flat at a depth of ~2 m in May 2014 and divided into small pieces. After acclimation for two weeks in large aquaria with running water, one coral nubbin was randomly selected and was suspended using fine nylon strings in three aquaria for exposure to each of the three $p\text{CO}_2$ treatments. The coral nubbins from same colony were evenly distributed among the $p\text{CO}_2$ treatments to avoid any possible sampling bias. A total of 9 coral nubbins were maintained for further analysis during the experiment.

At the end of the experiment (i.e., 4-week exposure), 1 L of seawater from each treatment was filtered through 0.2 μm polycarbonate (PC) membrane filters and stored at -20°C for further analyses. One coral nubbin from each treatment aquarium was sampled and divided into two aliquots. One was rinsed three times and then preserved in 70% ethanol and stored at -20°C for DNA extraction; the other was used for stable isotope analyses.

Determination of environmental parameters. Photosynthetically active radiation (PAR) in each aquarium was recorded every 30 min below the seawater surface using the Hobo[®] logger (Onset, USA). The average diurnal variations in PAR during the 4-week period are shown in Fig. S7. The seawater pH and temperature were measured daily in each aquarium using a pH meter (Orion Star[™]) and the total alkalinity was also determined weekly using an automated titration system (Metrohm 877 Titrino plus, Switzerland). Carbonate system parameters, including $[\text{HCO}_3^-]$, $[\text{CO}_3^{2-}]$, $p\text{CO}_2$ and aragonite saturation state (Ω_A), were calculated from the measured pH and total alkalinity values using the CO2SYS program⁵² (Table 2).

DNA extraction and 16S rDNA amplicon sequencing. A preserved piece of coral was homogenized in liquid nitrogen and then the total DNA from the resulting coral powder and filtered seawater samples was subsequently extracted using the Fast DNA[®] SPIN Kit for Soil (MP Biomedicals, Irvine, CA) according to the company's protocol. The DNA samples were amplified by PCR using barcoded primers targeting the hypervariable region V3-V4 of the 16S rRNA gene of Bacteria and Archaea: 341F (5'-CCTAYGGGRBGCASCAG-3') and 802R (5'-TACNVGGGTATCTAATCC-3')⁵³. The PCR amplification was performed using a thermocycler controller (MJ Research Inc., Bio-Rad) with the following program: an initial denaturation at 94°C for 5 min, followed by 35 cycles at 94°C for 30 s, 50°C for 30 s, 72°C for 30 s, and a 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 using a NanoDrop device (Thermo Scientific, USA). All amplicon products were mixed at equal concentrations and

Treatment	pH _{NBS}	Alkalinity (μmol kg ⁻¹)	HCO ₃ ⁻ (μmol kg ⁻¹)	CO ₃ ²⁻ (μmol kg ⁻¹)	pCO ₂ (μatm)	Ω _{ara}
Control pCO ₂	8.07 ± 0.02	2233 ± 22	1701 ± 35	214 ± 15	421 ± 49	3.6 ± 0.25
Medium pCO ₂	7.76 ± 0.02	2223 ± 11	1912 ± 36	125 ± 11	923 ± 113	2.1 ± 0.19
High pCO ₂	7.47 ± 0.02	2230 ± 13	2089 ± 25	67 ± 6	2070 ± 259	1.1 ± 0.01

Table 2. Carbonate chemistry parameters of seawater for each treatment. Values are means ± SE. Seawater pH (NBS scale), and salinity (31.5) were measured daily during the experiment (n = 28). Total alkalinity (TA) was measured at a specific time point every week (n = 4). The remaining parameters for carbonate seawater chemistry were calculated using the CO₂SYS program.

sequenced on an Illumina Miseq platform using 2 × 300 bp mode at Novogene (Beijing, China). The raw reads were submitted to the NCBI Sequence Read Archive under accession number SRP066229 (SRR2917919).

Sequence data processing. Overlapping paired-end reads were merged to obtain full-length 16S V3-V4 fragments using PEAR⁵⁴. After de-multiplexing and quality control, the downstream bioinformatics analysis was performed with QIIME1.5.0 pipelines⁵⁵. Briefly, OTUs with 97% similarity were defined after the qualified reads were clustered using Uclust⁵⁶. Representative sequences for each OTU were assigned to different taxa using the Ribosomal Database Project (RDP) classifier version 2.2⁵⁷ against the SILVA108 database⁵⁸ with a 50% cut-off threshold. Representatives assigned to eukaryotes, chloroplasts and mitochondria were filtered out. The taxon and abundance were summarized at the phylum, class, order, family, and genus levels. The species diversity, Shannon index, rarefaction curves and rank-abundance curves were determined using the QIIME pipeline.

Stable isotope analyses. Skeleton fragments of *A. gemmifera* were soaked in 30% hydrogen peroxide to remove coral tissue and then sonicated for 4 min at 20 °C. Skeletons were subsequently washed several times with double-distilled water and dried overnight at 50 °C. The newly grown part was scalped and ground into powder. Coral skeletal δ¹³C and δ¹⁸O data were obtained using a Finnigan MAT 253 Isotope Ratio Mass Spectrometer coupled to a Kiel Carbonate Device IV at the South China Sea Institute of Oceanology, Chinese Academy of Sciences, China. δ¹³C and δ¹⁸O were determined by repeated measurements of the international reference standard NBS-18. δ¹³C and δ¹⁸O values were presented as the per mil (‰) deviation of ¹³C/¹²C and ¹⁸O/¹⁶O, respectively.

Statistical analyses. To test the effect of pCO₂ treatments on microbial community compositions of coral samples, after normalizing all sequence reads for taxonomic analysis to the lowest sequencing depth (19,098 reads), pairwise dissimilarities among coral samples were calculated based on the Bray–Curtis index for ‘Adonis’, which is a non-parametric multivariate analysis of variance. In the Adonis analysis, the distance matrix was the response variable with pCO₂ treatment as independent variable. Non-metric multidimensional scaling (nMDS) was also performed to visualize the dissimilarities. Genera making the greatest contribution to dissimilarity among the pCO₂ treatments were further investigated through similarity percentage (SIMPER) analysis. To compare coral skeletal δ¹³C and δ¹⁸O among pCO₂ treatments, one-way ANOVA and Tukey’s test were employed. All analyses were conducted using the package vegan within the R statistical environment (version 3.1.3, R Development Core Team, 2015).

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Author Contributions

G.W.Z. wrote the manuscript with all authors commenting. G.W.Z., T.Y., L.C. and L.J. performed the experiment and undertook carbonate analyses. W.P.Z., R.M.T., H.Y.T. and X.C.Y. participated in data analyses. G.W.Z., S.L., H.H. and P.Y.Q. designed the research.

Additional Information

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