



Effects of Ocean Acidification on Carbon and Nitrogen Fixation in the Hermatypic Coral *Galaxea fascicularis*

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The supply of metabolites from symbionts to scleractinian corals is crucial to coral health. Members of the Symbiodiniaceae can enhance coral calcification by providing photosynthetically fixed carbon (PFC) and energy, whereas dinitrogen (N₂)-fixing bacteria can provide additional nutrients such as diazotrophically-derived nitrogen (DDN) that sustain coral productivity especially when alternative external nitrogen sources are scarce. How these mutualistic associations benefit corals in the future acidifying ocean is not well understood. In this study, we investigated the possible effects of ocean acidification (OA; pHs 7.7 and 7.4 vs. 8.1) on calcification in the hermatypic coral *Galaxea fascicularis* with respect to PFC and DDN assimilation. Our measurements based on isotopic tracing showed no significant differences in the assimilation of PFC among different pH treatments, but the assimilation of DDN decreased significantly after 28 days of stress at pH 7.4. The decreased DDN assimilation suggests a nitrogenous nutrient deficiency in the coral holiont, potentially leading to reduced coral calcification and resilience to bleaching and other stressful events. This contrasting impact of OA on carbon and N flux demonstrates the flexibility of *G. fascicularis* in coping with OA, apparently by sustaining a largely undamaged photosystem at the expense of N₂ fixation machinery, which competes with coral calcification for energy from photosynthesis. These findings shed new light on the critically important but more vulnerable N cycling *in hospite*, and on the trade-off between coral hosts and symbionts in response to future climate change.

Keywords: ocean acidification, coral calcification, carbon fixation, nitrogen fixation, symbiosis, photosynthesis

INTRODUCTION

Nutritional interactions are a key reason why scleractinian corals, the main reef builders in the oceans, thrive in the oligotrophic tropical waters where nitrogen (N) sources are particularly scarce (Muscatine and Porter, 1977; Cardini et al., 2014; Peixoto et al., 2017). The obligate relationship between corals and Symbiodiniaceae is the basis of a functioning reef ecosystem. The coral host

provides the endosymbionts with inorganic nutrients for photosynthesis, and in turn acquires photosynthetically fixed carbon (PFC) from the symbionts in the form of glycerol, glucose, amino acids, and other organic compounds that the host cannot synthesize independently (Rowan, 1998). Symbiodiniaceae, hence, play a central role in the trophic foundation of coral reef ecosystems (Silveira et al., 2017) and in the formation of the physical reef structure (Tambutté et al., 2011).

Other microbes are increasingly being recognized as important to the health of the corals and in coral reef biogeochemistry, particularly diazotrophs including dinitrogen (N_2)-fixing bacteria and archaea (Lema et al., 2014; Peixoto et al., 2017). N_2 fixation is one of the main sources of new N in the ocean necessary to sustain marine primary productivity, particularly in coral reef ecosystems where the availability of nitrogenous nutrients can be low (Benavides et al., 2017). Diazotrophs are able to fix N_2 utilizing the nitrogenase enzyme, which cleaves the triple bond of the N_2 molecule to form bioavailable ammonium (NH_4^+ ; Zehr and Kudela, 2011). In this context, endosymbiotic photosynthesis-dependent diazotrophic cyanobacteria were discovered in the coral tissue of *Montastraea cavernosa* (Lesser et al., 2004), and N_2 fixation activity has been detected in several coral species (Lesser et al., 2007). High throughput sequencing has subsequently identified diverse communities of non-cyanobacterial diazotrophs associated with numerous scleractinian corals from varying geographical regions (Fiore et al., 2010; Olson and Lesser, 2013), exemplifying their importance in meeting nutritional demand of corals (Fiore et al., 2010; Cardini et al., 2014). At the ecosystem level, up to 11% of the N used in coral reef primary production is provided by diazotrophs in supporting the productivity of that ecosystem (Cardini et al., 2014; Lema et al., 2016). The ability of corals to overcome N limitation may determine their success in oligotrophic waters, ultimately influencing the distribution and abundance of coral reefs (Fiore et al., 2010). Additionally, Symbiodiniaceae and associated bacteria form calcifying structures termed symbiolites (Frommlet et al., 2015). This bacterial–algal calcification, induced by Symbiodiniaceae photosynthesis through the assimilation of CO_2 and HCO_3^- and the release of hydroxyl ion from carbon (C) concentrating mechanism, implies that symbiotic bacteria might also facilitate coral calcification (Frommlet et al., 2018).

As a result of anthropogenically-driven increase in the ocean concentration of carbon dioxide (CO_2), tropical coral reefs are highly vulnerable to ocean acidification (OA). This is because the ecosystem structure depends on calcium carbonate-secreting organisms, which are subject to negative impacts of changing ocean carbonate chemistry associated with OA (Byrne et al., 2013; Leung et al., 2017, 2020; Mollica et al., 2018). It has been estimated that coral reefs may transition from net calcium carbonate accretion to net dissolution by the end of this century (Hoegh-Guldberg et al., 2007; Dove et al., 2013; Enochs et al., 2016; Eyre et al., 2018). OA not only inhibits calcification, reduces photosynthesis and species diversity directly, but also indirectly impacts coral-associated microbes, thereby potentially disrupting the normal function of the coral holobiont. This loss of function may in turn impact coral reef ecosystems as a

whole (Moya et al., 2012; Kaniewska et al., 2015). However, N_2 fixation activities in the coral holobiont may moderate the host's response to stress (Rädecker et al., 2015). The increased transfer of diazotrophically-derived nitrogen (DDN) to endosymbiotic Symbiodiniaceae occurs particularly under conditions of low availability of external nutrients or during stress (Bednarz et al., 2017, 2019). Moreover, N_2 fixation may be particularly important for providing nutrients to the host under stressful conditions, especially when Symbiodiniaceae are lost from host tissues (Fine and Loya, 2002). Therefore, N_2 fixation may play a key role in regulating coral–Symbiodiniaceae symbiosis.

However, investigations into the coral nutrient were mainly focused on C fixation (Tremblay et al., 2012; Hoadley et al., 2015), and few on N_2 fixation (Bednarz et al., 2017; Lesser et al., 2019). The effects of OA on these processes remain largely unknown and there is simply one study on N_2 fixation response to OA according to the best of our current knowledge (Rädecker et al., 2014). N_2 fixation rates can be detected through the acetylene reduction assay (Capone, 1993) and the $^{15}N_2$ -tracer method (Montoya et al., 1996). The former is an indirect method, converting acetylene produced to overall rates of N_2 fixation but usually overestimating the rates. Instead, the latter allows measurement of the net N_2 fixation directly, simply by adding filtered seawater enriched with $^{15}N_2$ gas to the incubation to ensure consistency of $^{15}N_2$ concentrations (White et al., 2020). In this study, we used $H^{13}CO_3^-$ and $^{15}N_2$ as tracers and performed indoor experiments to mimic OA conditions (pHs 7.7 and 7.4) relative to the ambient condition in the field (pH 8.1 as control) to investigate: (i) Whether OA would affect PFC and DDN assimilation in the ecologically important scleractinian coral *G. fascicularis*; (ii) Is there a correlation between the C/N flux and coral calcification and how they interact with each other to cope with OA.

MATERIALS AND METHODS

Experimental Setup

Twelve colonies of healthy, adult *G. fascicularis* with surface areas ranging from 10 to 15 cm² were collected from Changjiang (110°65'E, 19°25'N), Hainan, China, at noon in July 2015. The corals were cultivated in an aquarium with approximately 1,000 L of recirculated artificial seawater for up to 3 months of acclimation. The coral genotype was identified as the mt-L1 type of *G. fascicularis* (Lin et al., 2017). A detailed description of the aquarium setup and water quality has been reported elsewhere (Zheng et al., 2018a). Photosynthetically active radiation (PAR) of $150 \pm 25 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ was provided on a 12 h/12 h photoperiod using metal halide lamps (Phillips, Amsterdam, Netherlands). The live rock containing denitrifying bacteria was used to stabilize the water quality by removing dissolved inorganic nitrogen (DIN; Li et al., 2017). The DIN and dissolved inorganic phosphorus (DIP) concentrations were all $< 0.05 \mu\text{M}$.

Fragments (~3 cm in diameter) of *G. fascicularis* were grown in the aquarium until coral tissue entirely covered the skeleton. Ninety coral fragments were randomly and equally assigned to three 100-L experimental tanks (30 fragments per tank), each

maintained at a specific pH (8.1, 7.7, and 7.4). The carbonate chemistry was manipulated by bubbling pure CO₂ gas (99% purity) into the artificial seawater in the experimental tanks to maintain the pH (total scale, pH_T) levels (**Supplementary Table 1**) with a pH-stat system (CO₂SYN ZB-LS 3.0; Xiamen, China), which monitors and regulates the pH every second with an accuracy of ± 0.02 pH unit (Zheng et al., 2018b). The pH in each tank was recorded every 5 min by the CO₂SYN ZB-LS 3.0 (**Supplementary Figure 1**).

The three tanks were maintained at a salinity of approximately 34 ppt and a temperature of 27°C, and irradiated on a 12 h/12 h photoperiod with 150 μmol photons m⁻² s⁻¹ of PAR provided by T5HO lamps (ATI, Hamm, Germany). Submersible pumps (EHEIM, Deizisau, Germany) were used to ensure a high level of seawater circulation and easy dissolution of the CO₂ gas into the seawater. A protein skimmer (Bubble Magus, Jiangmen, China) was used to maintain the inorganic nutrients at low levels (**Supplementary Table 1**).

While parallel, independent replicate tanks were not used per each pH treatment, the water quality was well maintained over the course of experiment and no statistical differences were found among the tanks for all the environmental parameters measured except the pH (**Supplementary Table 1**). Given that the number of replicate coral fragments in each tank was high and comparable to those in previous studies (Rädecker et al., 2014; Kurman et al., 2017), the resulting data should be regarded as reliable as in other OA studies that also adopted a single tank for each pH treatment (Barkley et al., 2017; Comeau et al., 2017; Kurman et al., 2017; Coronado et al., 2019). Additionally, the tanks were rigorously cleaned weekly to eliminate “tank” effects and ensure stable water quality throughout the experiment.

Monitoring of Water Quality and Carbonate Chemistry

A total of 300 mL seawater was sampled weekly for the measurement of inorganic nutrient concentrations (PO₄³⁻, NO₃⁻, NH₄⁺, and SiO₃²⁻) and carbonate chemistry (pH and total alkalinity: TA). Saturated mercuric chloride (7 mg mL⁻¹) was added to preserve the water samples. An aliquot of 100 mL seawater was used for the measurement of inorganic nutrients using a 7,230 Spectrophotometer (Jingmi, Shanghai, China). The pH was measured using a 3,430 portable meter (WTW, Munich, Germany) that was calibrated daily, and TA was measured by potentiometric titration following the standard procedure (Dickson et al., 2007). Changes in seawater carbonate chemistry were calculated from pH_T and TA using the CO₂ Sys Excel Macro (Lewis et al., 1998).

Measurement of Photochemical Efficiency

The photochemical efficiency of the algal symbiont was assessed using an underwater chlorophyll fluorometer (Diving-PAM; Walz, Effeltrich, Germany) that measured the quantum yield of chlorophyll *a* fluorescence. Measurements of the maximum quantum yield (F_v/F_m) and effective quantum yield ($\Delta F/F_m'$) of

photosystem II (PSII) were conducted 2 h into the dark and light periods, respectively, in every 14 days ($n = 6$).

Measurement of PFC and DDN Assimilation

The ¹³C and ¹⁵N stable isotopic tracing experiment was carried out to quantify the rates of PFC and DDN assimilation in the coral holobiont. The DDN and PFC assimilation rates were measured using the dissolution method (Großkopf et al., 2012). In summary, ¹⁵N₂ pre-dissolved seawater was made with ¹⁵N₂ gas (98.9 atom%, Cambridge Isotope Laboratories) following the procedure of Shiozaki et al. (2015). Seawater was filtered through 0.2 μm membrane, degassed using Sterapore membrane unit (20 M 1,500 A: Mitsubishi Rayon Co., Ltd., Tokyo, Japan), and filled into 2-L Tedlar bags. Twenty milliliter, of ¹⁵N₂ was injected into each seawater-filled bag, which was tapped slightly until complete dissolution of the gas. The coral fragments were incubated, with one fragment per 1-L polycarbonate bottle (Nalgene, Rochester, NY, United States, the actual volume is ~1,225 mL) filled with 100 mL of the ¹⁵N₂ pre-dissolved seawater and 1,125 mL seawater from the corresponding experimental tanks (pHs 8.1, 7.7, and 7.4). The ¹³C-labeled sodium bicarbonate (99 atom% ¹³C; Cambridge Isotope Laboratories) was added in parallel with ¹⁵N₂ at a final tracer concentration of 70 μmol L⁻¹. The incubation lasted 24 h (12 h light/12 h dark; 150 μmol photons m⁻² s⁻¹). Coral fragments were sampled at the start and end of the isotope tracer experiment to assess the PFC and DDN assimilation rates. Since OA has negligible effect on coral respiration (Comeau et al., 2017; Van der Zande et al., 2020), a 24-h incubation represents the net assimilation of PFC, which has subtracted the respiration of consumed metabolites. The samples collected prior to the incubation were also used to assess the natural isotope ratios δ¹³C and δ¹⁵N. Samples were immediately snap-frozen in liquid nitrogen followed by storage at -80°C until further processing.

Coral tissue was detached from the skeleton using a WaterPik, filtered onto pre-combusted (450°C, 4 h) 0.3 μm GF-75 filters (Advantec, Taiwan, China) and dried at 60°C overnight. The isotopic values were determined using a Delta V plus isotope ratio mass spectrometer (Thermo Fisher Corporation, Carthage, MO, United States) interfaced with a Flash HT 2000 elemental analyzer (Thermo Fisher Corporation, Carthage, MO, United States). International reference material (USGS40) with a different amount of C/N and certified δ¹³C and δ¹⁵N values of -4.5 and -26.2‰, respectively, was inserted every 5 samples to check the drift and to ensure the accuracy of the measurements. The reproducibility for δ¹³C and δ¹⁵N measurements were both better than 0.3‰. The PFC and DDN assimilation rates were calculated by using equations proposed by Hama et al. (1983) and Montoya et al. (1996), respectively.

Measurement of Coral Calcification

The coral calcification rates were determined using the buoyant weight method measuring increase in skeletal mass, as reported previously (Davies, 1989). Briefly, the coral fragments were

suspended in seawater beneath an analytical balance that weighs to an accuracy of 0.01 mg. Optimal weighing conditions were reached when the air temperature was stable and close to that of the seawater. A total of eight replicates ($n = 8$) of coral fragments were measured at two timepoints (day = 14 and 28).

Statistical Analysis

Differences in water quality (pH, TA, $p\text{CO}_2$, and inorganic nutrients) and all parameters among the pH treatments were compared using one-way analysis of variance (ANOVA). The effects of pH and time on the physiological parameters ($\Delta F/F_m'$, F_v/F_m , calcification) were tested using two-way ANOVA. The post-erori Tukey's test was followed when the differences were significant ($p < 0.05$). Data were checked for normality and homogeneity (Shapiro-Wilk and Levene tests, respectively), and when necessary were transformed to achieve ANOVA assumptions. All data were expressed as an average value \pm standard error (SE). Statistical analysis was performed using IBM SPSS Statistics 23 (IBM Corp., Armonk, NY, United States).

RESULTS

Water Quality and Carbonate Chemistry of Seawater

The results of the water quality and carbonate chemistry analyses of seawater are shown in **Supplementary Table 1**. Over the 28-day experimental period, temperature and salinity were kept at $27 \pm 0.3^\circ\text{C}$ and 34 ± 0.1 ppt, respectively. No differences in the water temperature and salinity were found among the pH treatments. The inorganic nutrient concentrations were low and comparable to those in the field, with approximate concentrations of $0.03\text{--}0.05 \mu\text{M}$ for phosphate (PO_4^{3+}), $0.45\text{--}0.58 \mu\text{M}$ for nitrite (NO_2^-), < 0.05 for nitrate (NO_3^-), $0.18\text{--}0.51 \mu\text{M}$ for silicate (SiO_3^{2-}), and $0.35\text{--}0.84 \mu\text{M}$ for NH_4^+ . Although the average values were higher at pH 7.4, there were no significant differences among the three pH treatments (**Supplementary Table 1**; $p = 0.287$ for PO_4^{3+} , $p = 0.964$ for NO_2^- , $p = 0.563$ for SiO_3^{2-} , and $p = 0.665$ for NH_4^+).

Data from a total of 8,064 measurements recorded in each tank by the CO2SYS ZB-LS 3.0 system indicated that each treatment pH was very stable during the entire experimental period (**Supplementary Table 1** and **Supplementary Figure 1**). The standard error was ± 0.01 at pHs 7.7 and 8.1, and ± 0.02 at pH 7.4 (**Supplementary Table 1**). The TA was approximately $2,600 \mu\text{M}$, and there were no significant differences among treatments (**Supplementary Table 1**; $df = 2$, $F = 1.655$, $p = 0.244$). Because of the relatively high TA (relative to $2,300 \mu\text{M}$ in the field), the aragonite saturation state (Ω_{arag}) value at pH 7.7 reached 3.29, but rapidly reduced to 1.79 at pH 7.4. The concentration of carbonate (CO_3^{2-}) declined under OA conditions (**Supplementary Table 2**; $df = 2$, $F = 3,337$, $p < 0.001$) but other carbonate parameters, including $p\text{CO}_2$, CO_2 , and HCO_3^- , increased ($p < 0.001$).

TABLE 1 | Two-way ANOVA for physiological parameters as a function of pH and time.

Dependent variable	Effect	SS	df	MS	F	p
$\Delta F/F_m'$	pH	0.013	2	0.006	3.351	0.043
	Time	0.006	2	0.003	1.478	0.238
	pH*Time	0.13	4	0.003	1.643	0.178
F_v/F_m	pH	0.003	2	0.001	1.403	0.257
	Time	0.003	2	0.002	1.775	0.182
	pH*Time	0.003	4	0.001	0.726	0.579
Calcification	pH	0.443	2	0.221	5.968	0.005
	Time	0.096	1	0.096	2.586	0.115
	pH*Time	0.032	2	0.016	0.426	0.656

Significant differences ($p < 0.05$) are highlighted in bold. SS, type III sum of squares; df, degree of freedom; MS, mean square; F, F-ratio; p, p-value.

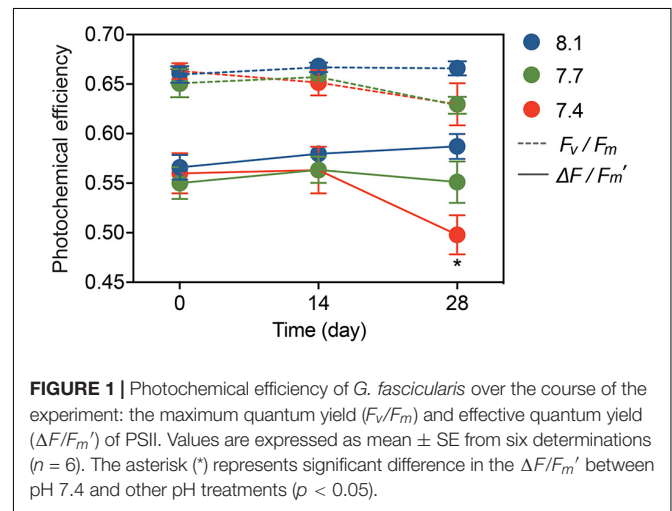


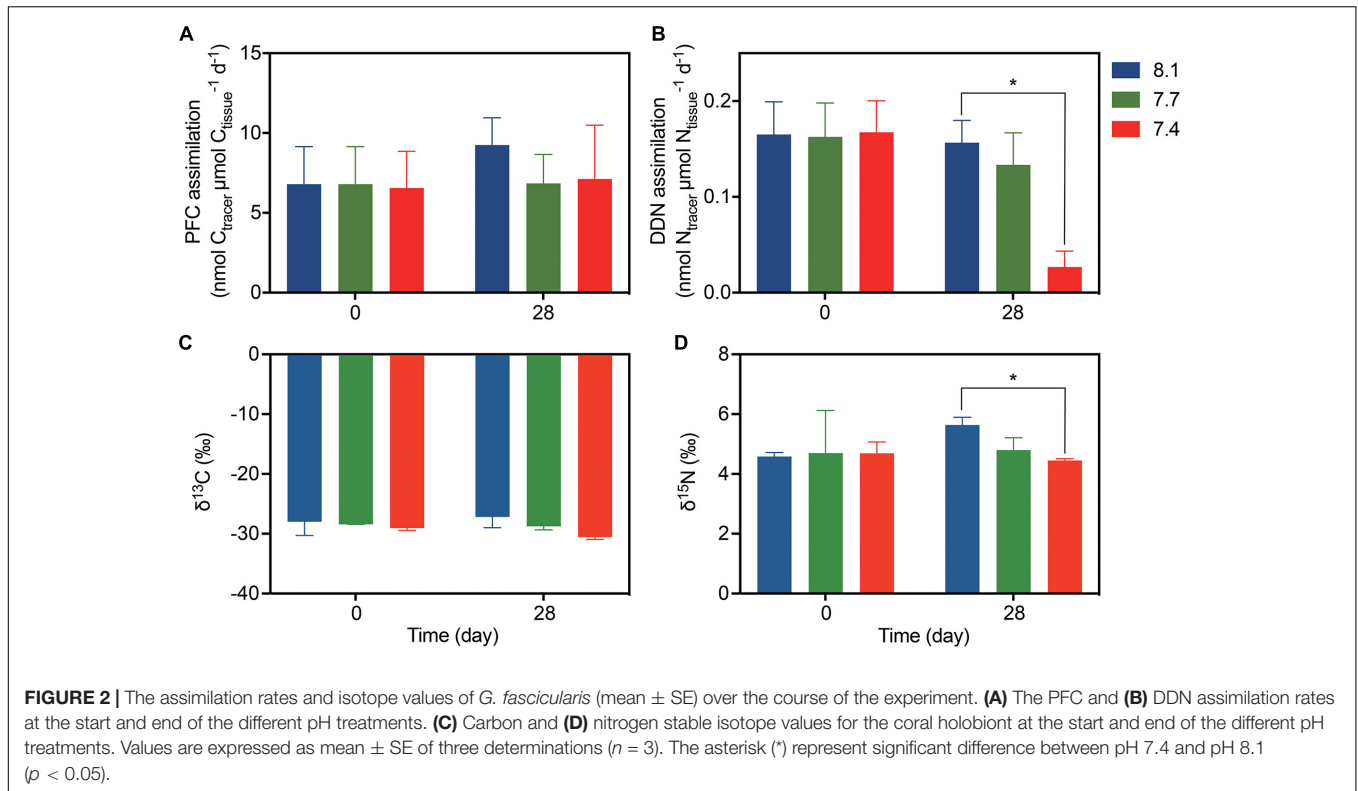
FIGURE 1 | Photochemical efficiency of *G. fascicularis* over the course of the experiment: the maximum quantum yield (F_v/F_m) and effective quantum yield ($\Delta F/F_m'$) of PSII. Values are expressed as mean \pm SE from six determinations ($n = 6$). The asterisk (*) represents significant difference in the $\Delta F/F_m'$ between pH 7.4 and other pH treatments ($p < 0.05$).

Photochemical Efficiency of the Algal Symbiont

Statistical analysis showed no significant pH \times time interactions for F_v/F_m (**Table 1**; $df = 4$, $F = 0.726$, $p = 0.579$), and no significant differences among the pH treatments (**Table 1**; $df = 2$, $F = 1.403$, $p = 0.257$) or times (**Table 1**; $df = 2$, $F = 1.775$, $p = 0.182$). The trend for $\Delta F/F_m'$ was similar to that for F_v/F_m , but the values of $\Delta F/F_m'$ declined significantly from 0.58 ± 0.01 at pH 8.1 to 0.49 ± 0.02 at pH 7.4 after 28-day stress (**Figure 1**, **Table 1**, and **Supplementary Table 3**; $df = 2$, $F = 3.351$, $p = 0.043$).

PFC and DDN Assimilation Rates

Galaxea fascicularis exhibited detectable gross PFC and DDN assimilation under control and OA conditions (**Figure 2**). At day 0, the assimilation of PFC and DDN were similar among all treatments ($6.55\text{--}6.80 \text{ nmol } C_{\text{tracer}} \mu\text{mol } C_{\text{tissue}}^{-1} \text{ d}^{-1}$ for PFC assimilation, and $0.16\text{--}0.17 \text{ nmol } N_{\text{tracer}} \mu\text{mol } N_{\text{tissue}}^{-1} \text{ d}^{-1}$ for DDN assimilation; **Supplementary Table 3** and **Figure 2**). The OA treatments did not affect PFC assimilation rates (**Figure 2A** and **Table 2**; $F = 0.884$, $p = 0.461$), but the DDN assimilation rates decreased at pH 7.4 ($0.03 \pm 0.02 \text{ nmol } N_{\text{tracer}} \mu\text{mol } N_{\text{tissue}}^{-1} \text{ d}^{-1}$) compared with those at pH 8.1 after 28-day stress (**Figure 2B**



and **Table 2**; $F = 7.454$, $p = 0.024$). For natural isotope ratio, no significant effect was found for $\delta^{13}\text{C}$ values (**Figure 2C** and **Table 2**; $F = 2.053$, $p = 0.223$), but the $\delta^{15}\text{N}$ values decreased significantly at pH 7.4 compared with those at pH 8.1 after 28-day stress (**Figure 2C** and **Table 2**; $F = 7.313$, $p = 0.033$).

TABLE 2 | One-way ANOVA for physiological parameters as a function of time.

Dependent variable		SS	df	MS	F	p
$\Delta F/F_m'$	0 day	0.001	2	0.000	0.215	0.809
	14 day	0.001	2	0.001	0.402	0.674
	28 day	0.022	2	0.011	4.697	0.025
F_v/F_m	0 day	0.000	2	0.000	0.384	0.689
	14 day	0.000	2	0.000	0.929	0.430
	28 day	0.007	2	0.004	2.336	0.121
PFC assimilation	0 day	0.343	2	0.171	0.032	0.969
	28 day	10.4	2	5.2	0.884	0.461
DDN assimilation	0 day	0.000	2	0.000	0.005	0.995
	28 day	0.029	2	0.014	7.454	0.024
$\delta^{13}\text{C}$ (‰)	0 day	1.799	2	0.899	0.162	0.854
	28 day	17.146	2	8.573	2.053	0.223
$\delta^{15}\text{N}$ (‰)	0 day	0.019	2	0.010	0.009	0.991
	28 day	2.216	2	1.108	7.313	0.033
Calcification	14 day	0.179	2	0.09	6.392	0.008
	28 day	0.414	2	0.207	3.747	0.041

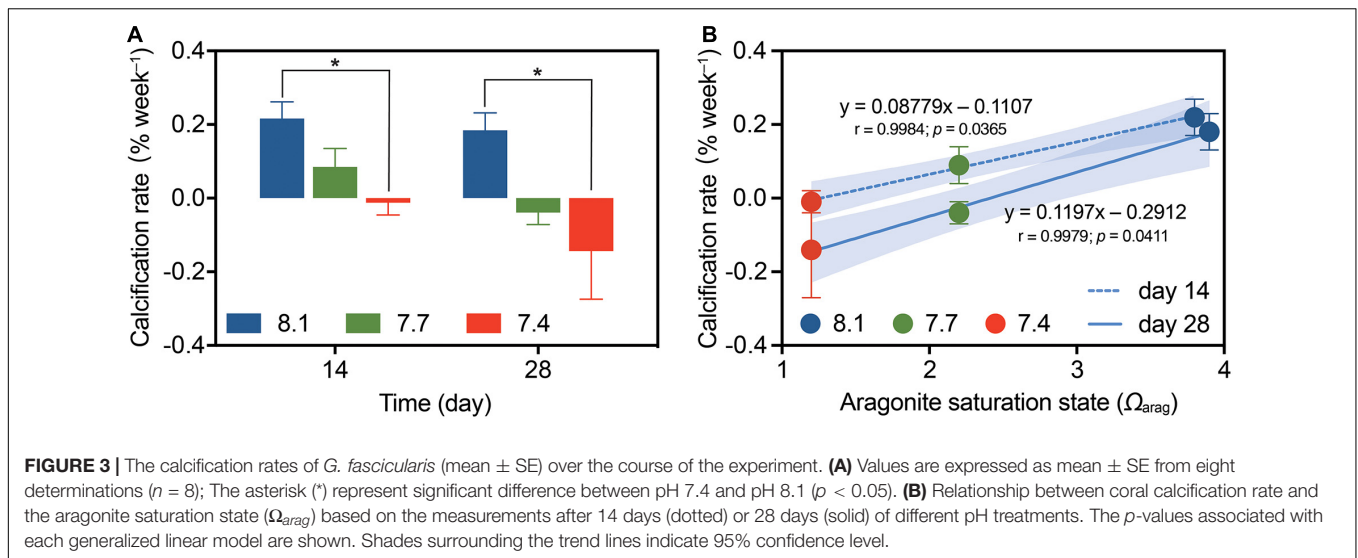
Significant differences ($p < 0.05$) are highlighted in bold. SS, type III sum of squares; df, degrees of freedom; MS, mean square; F, F-ratio; p, p-value.

Calcification Rate

There were no significant pH \times time interactions for coral calcification (**Table 1**; $df = 2$, $F = 0.426$, $p = 0.656$), and no significant differences among times (**Table 2**; $df = 2$, $F = 2.586$, $p = 0.115$), but significant differences among pH treatments were found (**Table 1**; $df = 2$, $F = 5.968$, $p = 0.005$). At pH 8.1, *G. fascicularis* maintained stable calcification rates, averaging $0.22 \pm 0.05\%$ week $^{-1}$ and $0.18 \pm 0.05\%$ week $^{-1}$ during the first and the second 14 days of growth, respectively (**Supplementary Table 3** and **Figure 3A**). The calcification rates at pH 8.1 were significantly higher than those at pH 7.4 during the first ($-0.01 \pm 0.03\%$ week $^{-1}$) and the second 14 days ($-0.14 \pm 0.13\%$ week $^{-1}$), and significant carbonate dissolution was found at pH 7.4 (**Figure 3A**). The coral calcification rate decreased at pH 7.7, but was not significantly different from that at pH 8.1 (**Figure 3A**). Generalized linear models showed a significant positive relationship between the calcification rate and Ω_{arag} over the experimental period (**Figure 3B**).

DISCUSSION

Our measurements revealed that the effective quantum yield of the algal symbiont in *G. fascicularis* decreased significantly at pH 7.4 after 28 days of OA stress, but the algal maximum quantum yield and the coral assimilation of PFC did not appear to be affected by OA (**Figures 1, 2A**). Conflicting results of OA impact on algal photosynthesis have been reported, ranging from decreased photochemical efficiency in *Acropora millepora*

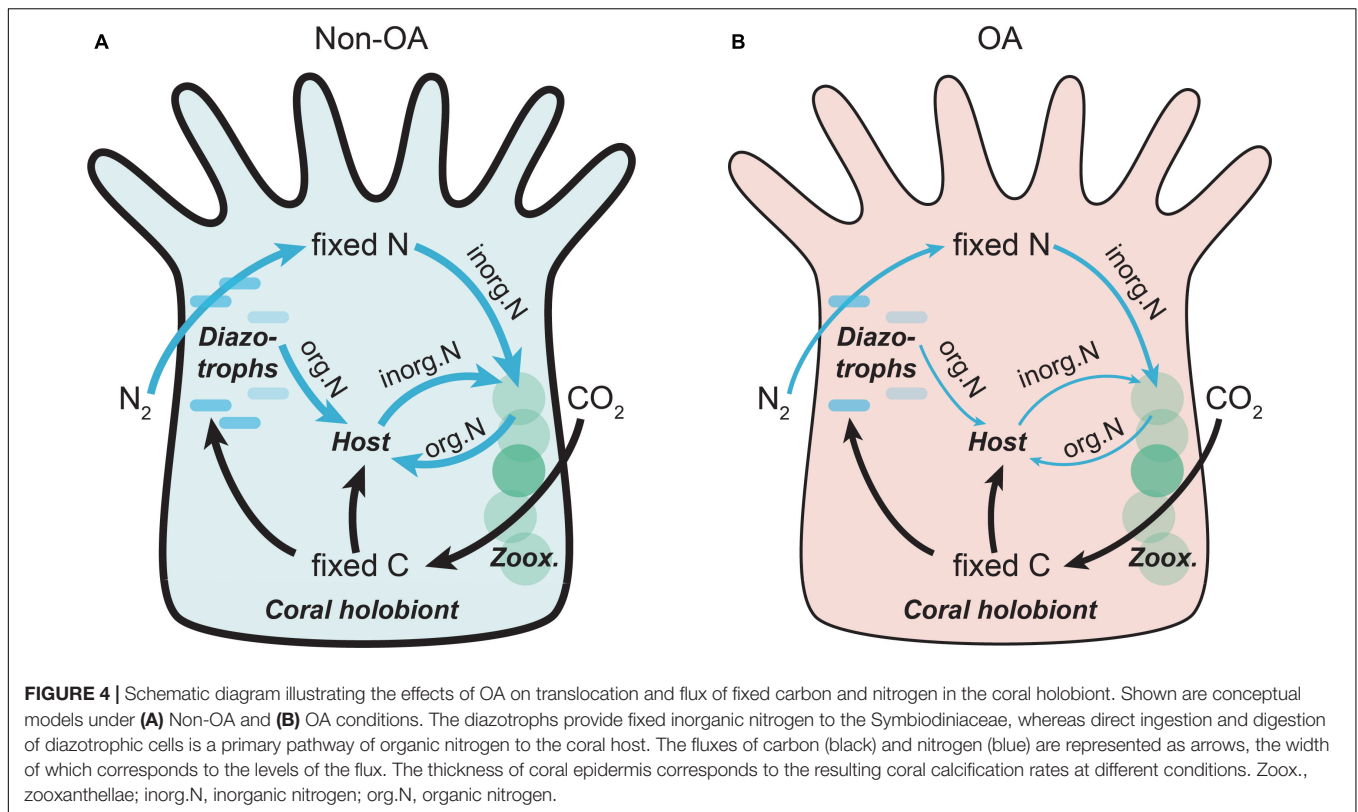


at pH 7.6–7.7 and pH 7.8–7.9 (Kaniewska et al., 2012) and negative effects of long-term OA on coral productivity (Anthony et al., 2008), unaffected photosynthesis in *A. digitifera* at pH 7.56 (Takahashi and Kurihara, 2013), to enhanced net photosynthesis in *Porites* spp., *A. millepora*, and *Pocillopora damicornis* around volcanic CO_2 seeps (pH 7.8) (Strahl et al., 2015). For many plants, acidification has a “fertilizing effect” on photosynthesis, as high $p\text{CO}_2$ alleviates C limitation in the Calvin cycle, and facilitates greater rates of photosynthesis compared with those under ambient conditions (Vogel et al., 2015). The absence of the “fertilizing effect” of OA on photosynthesis in *G. fascicularis* suggests that this phenomena might be species-specific and maximum carbon fixation in the coral may have already attained under normal ambient pH condition. This could also be due to the fact that Symbiodiniaceae are located within the gastroderm of the coral host (i.e., not in direct contact with seawater) and thereby cannot efficiently sequester CO_2 in acidified seawater. As a result, host respiration probably provides some CO_2 that is easier to access than the external dissolved inorganic carbon to Symbiodiniaceae for photosynthesis (Furla et al., 2000). Moreover, the virtually unaffected assimilation of PFC in corals could be tied to the stable Symbiodiniaceae community. Symbiodiniaceae have been shown to be able to thrive in low pH and a stable Symbiodiniaceae community under OA may be expected to provide PFC to the coral holobiont (Noonan et al., 2013). This was further confirmed by the results of $\delta^{13}\text{C}$ values showing no difference among different pH treatments (Figure 2C). The natural C isotope abundance has been used to understand the balance of autotrophy and heterotrophy in hermatypic corals (Wall et al., 2020), with the $\delta^{13}\text{C}$ values positively relating to the photosynthate or autotrophic input (Tremblay et al., 2015; Allgeier et al., 2020). Therefore, the similar $\delta^{13}\text{C}$ values among different pH treatments suggest stable PFC assimilation.

Microbes involved in N cycling may be fundamental to coral resilience to climate change (Rädecker et al., 2014, 2015). Rapid and significant decrease in DDN assimilation rates under OA (pH

7.71 with $p\text{CO}_2$ 1,080 μatm) has been reported in *Seriatopora hystrix* based on the acetylene reduction assay (Rädecker et al., 2014). In this study, the rates of DDN assimilation were differentially affected depending on the severity of the OA stress, with a non-significant decrease at pH 7.7 and a significant sharp decline at pH 7.4 (Figure 2B). The unaffected DDN assimilation at pH 7.7 may be attributed to the homeostasis of microbial, particularly diazotrophic community, whereas the marked decrease of DDN assimilation at pH 7.4 may be related to the disordered symbiosis with diazotrophs. Evidences that corals maintain high bacterial compositional stability under mild OA conditions (pH 7.9) have been reported in *A. millepora* and *S. hystrix* (Webster et al., 2016; Glasl et al., 2019). In contrast, a break in the coral–bacterial symbiosis and marked changes in bacterial abundance and diversity were found under severe OA (Morrow et al., 2015; Zaneveld et al., 2017). The abundance of cyanobacterial symbionts and *Endozoicomonas* sp., which were proposed to contribute to N cycling, was significantly reduced in the coral microbiome at a CO_2 seep (Morrow et al., 2015). These findings imply that OA may disrupt the community structure of microbes involved in N cycling, which poses a threat to coral growth and calcification. The significant decrease in coral calcification rates under OA in the present study (Figure 3A) may also be attributed to the disordered processes associated with calcifying bacteria, such as the recently discovered photosynthesis-induced, bacterial–algal calcification (Frommlet et al., 2015, 2018), or changes in the community structure of some symbiotic cyanobacteria that promote calcium carbonate formation (Durak et al., 2019; Nitschke et al., 2020).

The $\delta^{15}\text{N}$ values is positively correlated with the availability of N nutrient or heterotrophic input in coral holobiont (Donovan et al., 2020), therefore, the declined $\delta^{15}\text{N}$ values at lowed pH in the present study (Figure 2D) suggest reduced N utilization and heterotrophy in *G. fascicularis* under OA conditions. In N-replete corals, acquisition and translocation of PFC were significantly higher and thus resulted in enhanced host calcification (Langdon and Atkinson, 2005; Béraud et al., 2013). Conversely, N-deficient



corals showed reduced metabolism and nutrient limitation, leading to reduced calcification rates and decreased symbiont growth and density (Wiedenmann et al., 2013; Ezzat et al., 2015). The combined reduction of DDN assimilation rates and $\delta^{15}\text{N}$ natural isotope abundance under OA in *G. fascicularis* (Figure 2) potentially may have compromised the coral calcification, although interpreting this in symbiotic corals is difficult because calcification is affected by various processes (Karcher et al., 2020).

Corals have the capacity to maintain elevated pH levels in calcifying fluids despite decreased seawater pH (McCulloch et al., 2012). However, the upregulation of calcifying fluid pH is believed to be an energy-requiring process that involves removal of protons from the calcifying fluid by Ca^{2+} ATPases (Tambutté et al., 2011). In this study, the coral calcification rates decreased linearly with prolonged acidification and were negatively correlated with the corresponding Ω_{arag} of the seawater. Hence, a lowering of the Ω_{arag} makes the calcification process more energy consuming (Hohn and Merico, 2012). Furthermore, the N cycle is driven by complex and unique microbial transformations that are energetically expensive. For example, N_2 fixation requires at least 16 ATPs to reduce N_2 to NH_3 (Benavides et al., 2017). It has been found in corals that N_2 fixation was inhibited when photosynthesis was blocked, but could be recovered if glucose was added (Shashar et al., 1994), suggesting that N_2 fixation strongly depends on photosynthesis to meet its energetic demands (Garcia et al., 2013). As calcification and N_2 fixation are both energy-intensive processes, they probably compete for energy within the coral

holobiont. Because PFC assimilation was not increased under OA condition, the increased energy demand to cope with OA stress must create an energy deficit and result in decreased rates of DDN assimilation and calcification.

Based on our measurements and published modeling data (Benavides et al., 2017), we propose a conceptual model of C and N fluxes in the coral holobiont under non-OA vs. OA conditions (Figure 4). Under non-OA control condition, fixed inorganic nitrogen, mostly in the form of NH_4^+ , is primarily available to the Symbiodiniaceae, whereas the ingestion and digestion of diazotrophic cells provides organic nitrogen (e.g., in the form of amino acids) to the coral host. The release of organic carbon and energetic substrates from Symbiodiniaceae thus enhances diazotrophic growth and subsequent DDN assimilation, actively promoting coral calcification. On the contrary, OA triggers decreases in the overall assimilation of DDN, leading to insufficient organic nutrient supply to the growth and metabolism of the coral host, further exacerbating the coral calcification. To cope with this limited nutrient availability and imbalance between organic and inorganic nutrients, the coral appears to have evolved a trade-off mechanism to conserve and recycle the remaining fixed inorganic nitrogen to temporarily satisfy the demand of Symbiodiniaceae for barely unaffected assimilation of PFC and energy supply to the holobiont. The contrasting impacts of OA on PFC and DDN assimilation strongly suggests the flexibility of corals to sustain a largely undamaged photosystem and coral-algal symbiosis at the expense of N_2 fixation machinery and/or change

in the coral–bacterial symbiosis. While this strategic trade-off might work temporarily, it should be noted that long-term loss of bioavailable N would limit Symbiodiniaceae growth and photosynthesis, ultimately suppressing coral resilience to OA (Anthony et al., 2008). Taken together, the findings and proof-of-concept model presented in our study highlight the importance of N cycling and nutrient dynamics for calcifying organisms such as corals to adapt and acclimatize to future acidifying oceans (Thomsen et al., 2013; Leung et al., 2019). In this light, we emphasize the need for research focusing on other essential elements, such as N, which have important roles in addition to C in shaping marine symbioses.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

Corals were collected under permit number 2014003 for the Domestication and Breeding of Aquatic Wild Animals, issued by Department of Ocean and Fisheries of Hainan Province of the People's Republic of China.

AUTHOR CONTRIBUTIONS

XZ, TS, and LY conceived and designed the study. CW performed the experiments, with the help from HS for isotopic

measurements, XD for water quality assessment, and GN for statistical analysis. XZ, CW, and TS interpreted the data and wrote the manuscript. All authors read and approved the final version of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fmars.2021.644965/full#supplementary-material>

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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