Nutrient availability affects the response of juvenile corals and the endosymbionts to ocean acidification

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Abstract

   The interactive effects of nutrient availability and ocean acidification on coral calcification were investigated using post-settlement juvenile corals of *Acropora digitifera* cultured in nutrient-sufficient or nutrient-depleted seawater for 4 d and then exposed to seawater with different partial pressure of carbon dioxide (P_{CO_2}) conditions (38.8 or 92.5 Pa) for 10 d. After the nutrient pretreatment, corals in the high nutrient condition (HN corals) had a significantly higher abundance of endosymbiotic algae than did those in the low nutrient condition (LN corals). The high abundance of endosymbionts in HN corals was reduced as a result of subsequent seawater acidification, and the chlorophyll *a* per algal cell increased. The photosynthetic oxygen production rate by endosymbionts was enhanced by the acidified seawater regardless of the nutrient treatment, indicating that the reduction in endosymbiont density in HN corals due to acidification was compensated for by the increase in chlorophyll *a* per cell. Though the photosynthetic rate increased in the acidified conditions for both LN and HN corals, the calcification rate significantly decreased for LN corals but not for HN corals. The acquisition of nutrients from seawater, rather than the increase in alkalinity caused by photosynthesis, might effectively alleviate the negative response of coral calcification to seawater acidification, suggesting that the response of corals and their endosymbionts to ocean acidification can be influenced by nutrient conditions.

   Increasing atmospheric carbon dioxide (CO_2) dissolves in the ocean and lowers pH in the seawater. It is estimated that the partial pressure of carbon dioxide (P_{CO_2}) in seawater might more than double by the end of this century; concomitantly, the oceanic pH will lower by as much as 0.4 pH units (Feely et al. 2009). This ocean acidification (OA) decreases the saturation state of aragonite in seawater, which chemically prevents the production of calcium carbonate by marine organisms (Ries et al. 2009). Scleractinian corals are one of the most threatened calcifiers, and many previous studies have reported that OA decreases coral calcification rates (Gattuso et al. 1998; Leclercq et al. 2002; Jokiel et al. 2008). However, the interactive effect of the endosymbiotic dinoflagellates (genus *Symbiodinium*) in corals has been little documented (Krief et al. 2010; Iguchi et al. 2012; Kaniewska et al. 2012).

   The endosymbiotic algae, zooxanthellae, reside in animal host-derived vacuoles in the endodermal layer of the coral (Wakefield and Kempf 2001). They perform photosynthesis and produce organic matter and it is largely translocated to the coral animal host (Tanaka et al. 2006; Tremblay et al. 2012). The photosynthetic products support the energetic demand of both the coral animal host and endosymbionts (Tremblay et al. 2012). Moreover, the endosymbiont photosynthesis makes the carbonate equilibrium chemically favorable for the production of coral carbonate skeletons (Furla et al. 2000; Tanaka et al. 2007). Thus, the metabolic activity of endosymbionts influences the animal host calcification.

   The physiological condition of endosymbionts changes with various environmental factors. For example, high seawater temperature decreases the density of endosymbionts (Fagoonee et al. 1999) and sometimes leads to devastating loss (i.e., bleaching). In contrast, the incorporation of inorganic nutrients and heterotrophic organic matter such as zooplankton increases the abundance of endosymbionts (Titlyanov et al. 2000; Tanaka et al. 2007; Houlbrèque et al. 2003). Lower light intensity increases the chlorophyll *a* (Chl *a*) of endosymbionts (Titlyanov et al. 2000; Houlbrèque et al. 2003). Consequently, these environmental factors change the photosynthetic rate of endosymbionts and the calcification rate of the coral animal host (Marubini and Davies 1996; Ferrier-Pagès et al. 2003; Sawall et al. 2011).

   OA affects not only coral calcification but also the physiological condition of endosymbionts. Under high P_{CO_2} conditions, Chl *a* per endosymbiont cell in the coral *Acropora formosa* increased, and the photosynthetic rate per Chl *a* decreased (Crawley et al. 2010). Reynaud et al. (2003) found that the average number of endosymbionts per animal host cell increased as a result of OA for the coral *Stylophora pistillata*. These studies suggest that OA can

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indirectly affect coral calcification by changing the endosymbiont photosynthetic activity. Although many experiments have been conducted on the effect of OA on marine phytoplankton (Hurd et al. 2009), few data are available concerning the endosymbionts in corals.

The present study investigates how nutrient availability affects the response of endosymbiont physiology and coral calcification to high P_{CO_2} seawater. It is widely recognized that inorganic nutrients promote the proliferation of endosymbionts in corals (Yellowlees et al. 2005). Post-settlement juvenile corals were pre-incubated in nutrient-depleted or nutrient-sufficient seawater to create differences in endosymbiont physiological condition, and then the corals were exposed to acidified seawater. The interactive effects of nutrients and OA have been studied for some adult corals (Langdon and Atkinson 2005; Holcomb et al. 2010; Chauvin et al. 2011) but never for juvenile corals. Studying post-settlement corals should minimize the effect of animal host heterotrophy and isolate the direct effects of nutrient incorporation. Additionally, juvenile corals may be more sensitive to OA than are adults, as has been shown for other marine invertebrates (Talmage and Gobler 2009).

Methods

Preparation of post-settlement juvenile corals—The preparation of juvenile corals was conducted according to our previous methods (Inoue et al. 2012; Tanaka et al. 2013). The scleractinian coral *Acropora digitifera*, which is one of the most common species in the Ryukyu Islands, Japan, was collected from a fringing reef of Sesoko Island (26°37–39′N, 127°51–52′E) on 21 May 2013. The specimens were maintained in outdoor continuous-flow aquaria at Sesoko Station, University of the Ryukyus. Gametes spawned from six colonies of the *A. digitifera* on 01 June 2013 were collected and thoroughly mixed in buckets. The gametes were then transferred to the indoor laboratory and cultured in plastic containers filled with sufficient reef seawater. The seawater was filtered through a cartridge-type filter (pore size, 1 μm) in advance and was replaced every day until the ensuing settlement treatment. The fertilized eggs developed into planula larvae approximately 4 d after spawning. Light was provided by fluorescent lamps at an intensity of 40 μmol m^{-2} s^{-1} for 12 h d^{-1}. The seawater temperature in the containers was controlled by maintaining the room temperature at 27°C, which is the typical seawater temperature during the coral spawning season in the Ryukyu Islands (Negri et al. 2007).

Nine days after spawning, the larvae settled onto eight replicate plastic culture plates (six-well type, 16.4 mL in each well) with the coral metamorphosis inducer peptide Hym-248 (Hirose et al. 2008). Approximately 50 larvae settled onto each plate within 6 h of the addition of the peptide. Twenty-four hours after settling, a solution containing zooxanthellae (*Symbiodinium*, clade A, obtained from the giant clam *Tridacna crocea*) that easily infects young *A. digitifera* polyps (Hirose et al. 2008) was added to each culture well (final concentration, 2.7 × 10⁴ cells mL^{-1}) for symbiont infection. The culture plates were supplied with fresh filtered seawater every day. Three days after the

infection treatment, endosymbionts were confirmed present in the juvenile corals with a microscope, and then the culture plates were submerged in two closed tanks (four plates in each) filled with fresh filtered seawater of 10 liters. The tanks were illuminated with metal-halide lamps for 12 h a day (07:00 h to 19:00 h), and the average irradiance was 110 μmol m^{-2} s^{-1} at the depth of the corals. The seawater temperature was maintained with a thermostat and a heater and was 27.2°C ± 0.4°C (mean ± standard deviation [SD], n = 805), which was measured every 30 min during the following experiments using a logger (Thermochron SL, KN Laboratories). The seawater was replaced every 1–2 d, and the juvenile corals were incubated for 7 d, until the start of the acidification experiment. During the latter 4 d in this pre-incubation period, KNO$_3$ and NaH$_2$PO$_4$ were added to one of the two tanks to prepare juvenile corals growing in high nutrient (HN) or low nutrient (LN) conditions. Nutrient concentrations in the original seawater, which was pumped up from the reef in front of the research station, were measured throughout the experimental period and were 0.55 ± 0.04, 0.21 ± 0.02, 0.04 ± 0.02, and 0.02 ± 0.01 μmol L^{-1} for NO$_3^-$, NO$_2^-$, NH$_4^+$, and PO$_4^{3-}$ (mean ± SD, n = 6), respectively. KNO$_3$ and NaH$_2$PO$_4$ were added to raise the concentrations of NO$_3^-$ and PO$_4^{3-}$ by 3 and 0.2 μmol L^{-1}, respectively, after the tank seawater was freshened.

After the pretreatment, 10 juvenile corals were randomly chosen from each nutrient treatment and photographed with a digital camera (C-4040ZOOM, Olympus) under a microscope (SZH10, Olympus). The organic tissue of the corals was removed from the skeleton with a water-pik method and the tissue solution was immediately filtered onto a membrane filter (pore size, 0.8 μm). The filters were stored at −30°C in the dark until analysis of the endosymbiont abundance. The coral skeleton was collected from the plate, dried at room temperature, and stored until analysis of the skeletal weight.

OA experiment in flow-through aquaria—Four flow-through tanks were set up under the same temperature and light conditions as during the pretreatment period and were assigned to either a high P_{CO_2} (HC) or a control P_{CO_2} (CC) condition (duplicate tanks for each P_{CO_2} condition). The juvenile corals pre-incubated in LN and HN conditions (LN and HN corals, respectively) were distributed to each tank (i.e., one plate from LN and one plate from HN to each tank). Thus, as a whole, four treatments were included in this experimental design: LN corals were exposed to HC and CC, and HN corals were exposed to HC and CC. P_{CO_2} in the supplied seawater was adjusted with a high-precision P_{CO_2} control system (Kimoto Electric), which was described in Hikami et al. (2011). Briefly, fresh filtered seawater (pore size, 1 μm) is exposed to a gas mixture of CO_2 and dilution air in a bubbling tank. P_{CO_2} of the seawater flowing out from the bubbling tank was directly measured and maintained at the desired level by continuously regulating P_{CO_2} in the gas mixture. Two types of seawater (HC and CC) were prepared with this system (Table 1) and supplied at the rate of 120 mL min^{-1} to each tank. The pH, HCO$_3^-$, CO$_3^{2-}$, and aragonite

Table 1. Summary of seawater chemical conditions during the acidification experiment. All values are shown as the mean ± standard deviation (SD; $n = 244$), and the deviation is derived from measured P_{CO_2} values. pH_T, pH in total hydrogen ion concentration scale; P_{CO_2}, partial pressure of CO_2; Ω_{arg}, aragonite saturation state.

Treatment	pH_T	P_{CO_2} (Pa)	HCO_3^- ($\mu mol\ kg^{-1}$)	CO_3^{2-} ($\mu mol\ kg^{-1}$)	Ω_{arg}
Control	8.03±0.01	38.8±0.9	1629±9	202±3	3.24±0.05
High P_{CO_2}	7.71±0.07	92.5±13.4	1853±43	112±17	1.80±0.28

saturation state (Ω_{arg}) were estimated from P_{CO_2}, temperature, total alkalinity of 2131 $\mu mol\ kg^{-1}$, and salinity of 34.5, using the computer program CO2SYS (Lewis and Wallace 1998). The total alkalinity and salinity were not measured during the present experiment, and the values represent the averages previously measured in the same laboratory (Hayashi et al. 2013). The OA experiment was conducted for 10 d, and the tank position was regularly changed to minimize the position effect. On the fifth day of the OA experiment, the juvenile corals were temporarily taken out of the OA tanks and put in closed tanks for 3 h (in the same way as the nutrient pretreatment) to further facilitate nutrient incorporation by HN corals.

On the seventh day of the OA experiment, photosynthetic O_2 production rates by the juvenile corals were measured. After the culture plate was filled with the corresponding treatment seawater, two randomly selected wells were tightly covered with transparent plastic plates, and the culture plate was placed in the seawater under the same light and temperature conditions as the P_{CO_2} treatment tank. The O_2 concentration in the well was measured every 5 min for 15 min using a fiber optical oxygen sensor (Fibox 3, Presens Precision Sensing). The sensor was calibrated with a zero solution (0.08 mol L^{-1} sodium sulfite) and air-saturated filtered seawater before the analysis (Okubo et al. 2008). The seawater in the well was stirred with a magnetic stirrer (1 cm long), with care taken not to touch corals, and the water flow rate in the well was approximately 3 cm s^{-1}. The photosynthetic measurement was conducted during the 13:00–16:00 h period.

After the OA experiment, the corals were collected and similarly processed for the analysis of endosymbiont abundance, skeletal weight, and planar skeletal area (*see* below). The dried skeletons were stored until the analysis of oxygen (O) and carbon (C) stable isotopes in the calcium carbonate ($\delta^{18}O$ and $\delta^{13}C$, respectively). Additionally, 25 corals were sampled from each treatment and stored in five polypropylene tubes (five corals each) with pure methanol to extract Chl *a*.

Analyses and calculations—The carbonate skeletons of the corals were completely dried and the weight was measured with an ultramicrobalance (Thermo Cahn C-35). The calcification rate ($\mu g\ d^{-1}$) of corals during the OA experiment was calculated by subtracting the initial weight from the final weight. The initial weight was determined from the average value at the end of the pretreatment period for each nutrient condition. The photographs of juvenile corals showed the planar circular area of coral

skeletons. The circular area was measured using ImageJ software (National Institutes of Health) with scale calibration, and the growth rate of the planar area was calculated in the same way as the growth rate of weight.

The abundance of endosymbionts on the filters was observed with a fluorescent microscope (BX53, Olympus) at a magnification of ×100–200. For each filter, 20 fields were observed, and the average and standard error (SE) were determined. The Chl *a* concentration in the methanol solution was measured using a spectrophotometer (UV-1800, Shimadzu) and was calculated according to Ritchie (2006). The dissolved inorganic N (DIN: NO_3^-, NO_2^-, NH_4^+) and PO_4^{3-} concentrations in the seawater were quantified using a nutrient analyzer (AACS-II, Bran+-Luebbe). The photosynthetic O_2 production rate by corals (R: pmol μg^{-1} min^{-1}) was calculated as follows:

$$R = \frac{S \times V}{W} \qquad (1)$$

where S is the slope between time (min) and O_2 concentrations (pmol mL^{-1}) during the measurement period of coral incubation. V is a seawater volume in each well (16.4 mL), and W (μg) is the total weight of coral skeletons in the well. The skeletal weight on the day of O_2 measurement was estimated from the final weight and the growth rate, assuming that the growth rate was constant during the OA experiment.

The dried coral skeletons were reacted with 104% H_3PO_4 at 25°C in a custom-made carbonate preparation device (Ishimura et al. 2008). $\delta^{18}O$ and $\delta^{13}C$ in the generated CO_2 were determined with a mass spectrometer (Micromass Isoprime) relative to Vienna Peedee Belemnite, adopting the consensus values of −2.20‰ and 1.95‰, respectively, for the international reference material NBS 19, which was distributed by the National Institute of Standards and Technology, formerly known as the National Bureau of Standards (NBS). The analytical precision was < 0.1‰ and < 0.2‰ for $\delta^{18}O$ and $\delta^{13}C$, respectively.

Data from four replicate plates during the pretreatment were combined, and the effect of nutrient addition was evaluated using a two-tailed Student's *t*-test. Data from duplicate plates during the OA experiment were also combined, and the effects of P_{CO_2} on calcification and endosymbiont parameters were examined by a two-tailed Student's *t*-test for each nutrient treatment. Because CO_2 was artificially supplied to the HC condition, $\delta^{18}O$ and $\delta^{13}C$ values for the coral skeleton should be compared between samples in the same P_{CO_2} condition. The effect of nutrients on $\delta^{18}O$ and $\delta^{13}C$ was evaluated by *t*-test in each P_{CO_2} treatment. All data were checked for normal

Table 2. The conditions of coral skeleton and endosymbionts of the juvenile coral *Acropora digitifera* at the start of the acidification experiment. *p*-values evaluate the results of the two-tailed Student's *t*-test between the low and high nutrient pretreatments (LN and HN, respectively). Data are shown as the mean ± standard error (SE; *n* = 8–10).

	LN	HN	*p*
Skeletal weight (μg)	109±9	135±9	0.06
Planar skeletal area (mm²)	0.82±0.04	1.01±0.05	<0.01
Endosymbiont abundance (cells polyp^{-1})	3860±30	7370±20	<0.0001
Endosymbiont density (cells μg^{-1})	37±2	56±5	<0.01
Endosymbiont density (cells mm^{-2})	4740±470	7540±510	<0.001

distribution of the residual values for each variable (Shapiro–Wilk test) and were log-transformed when required. All statistical analyses were performed using the JMP software (SAS Institute). Comparisons with $p < 0.05$ were considered statistically significant. All data are shown as the mean ± SE, unless otherwise mentioned.

Results

At the start of the OA experiment, HN corals had a significantly higher abundance of endosymbionts than did LN ones (*t*-test, $t = 6.3$, degrees of freedom [df] = 17, $p < 0.0001$; Table 2). The endosymbiont density was also higher in HN than in LN corals when it was normalized to the skeletal weight (*t*-test, $t = 2.1$, df = 15, $p < 0.01$) and to the skeletal surface area (*t*-test, $t = 2.1$, df = 17, $p < 0.001$). The average skeletal weight of HN corals was higher than that of LN corals (135 and 109 μg, respectively), though the difference was not statistically significant ($p = 0.06$).

During the OA experiment, the calcification rate of LN corals was reduced by 25% under the HC conditions compared to CC (*t*-test, $t = 1.9$, df = 42, $p < 0.05$; Fig. 1).
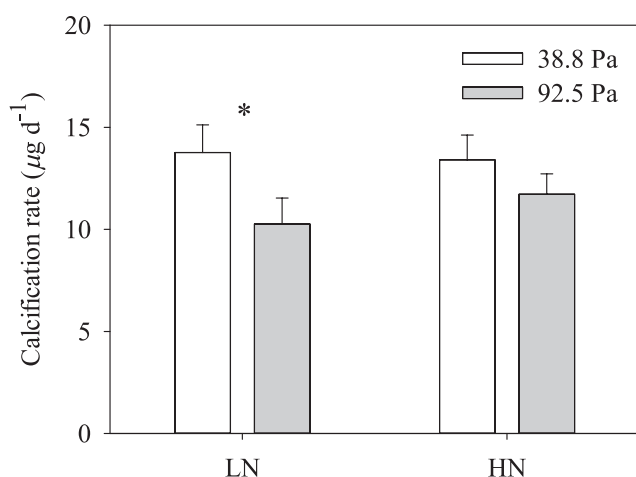

Fig. 1. The response of coral calcification rates to the different P_{CO_2} seawater of 38.8 Pa and 92.5 Pa. LN and HN show that the corals were pre-incubated in low and high nutrient conditions, respectively, before they were exposed to the acidified seawater. The asterisk indicates a significant difference between the low and high P_{CO_2} conditions. Each bar shows mean ± SE (*n* = 21–26).

The average calcification rate of HN corals decreased under the HC conditions, but the reduction was not significant (*t*-test, $t = 1.1$, df = 45, $p = 0.29$). The photographed planar area of the corals ranged from 1.1 to 1.3 mm² after the OA experiment, and the growth rate calculated from the planar area was not different between the acidification treatments (0.030–0.031 mm² d^{-1}).

Chl *a* content normalized to the endosymbiont cell largely increased, from 3.9 to 6.6 pg cell^{-1}, with the acidified seawater for HN corals (*t*-test, $t = 5.4$, df = 18, $p < 0.0001$) but not for LN corals (4.3 to 4.8 pg cell^{-1}; Fig. 2A). Chl *a* content normalized to the skeletal weight was not significantly affected by the HC seawater for both LN and HN corals (Fig. 2B). The endosymbiont density normalized to the skeletal weight was reduced from 44 to 30 cells μg^{-1} in the HC seawater for HN corals (*t*-test, $t = 2.1$, df = 16, $p = 0.0001$), while it was not significantly affected for LN corals (Fig. 2C). When the endosymbiont density was normalized to the skeletal planar area, the pattern was similar: the density decreased from 8700 to 5700 cells mm^{-2} in the HC seawater for HN corals (*t*-test, $t = 2.1$, df = 18, $p < 0.0001$), while it was not significantly affected for LN (average 6400 cells mm^{-2}). The photosynthetic O_2 production rate significantly increased in the HC conditions for both LN (*t*-test, $t = 9.3$, df = 6, $p < 0.0001$; Fig. 2D) and HN (*t*-test, $t = 3.1$, df = 6, $p < 0.05$) corals.

The nutrient addition tended to make skeletal $\delta^{18}O$ heavier, regardless of the P_{CO_2} conditions, but the effect was significant only under the high P_{CO_2} condition (*t*-test, $t = 2.4$, df = 10, $p < 0.05$; Fig. 3A). Skeletal $\delta^{13}C$ was higher by 0.5‰ for HN than LN corals under the P_{CO_2} control conditions (*t*-test, $t = 4.4$, df = 9, $p < 0.01$; Fig. 3B).

Discussion

This is the first study to show that nutrient availability changes the response of post-settlement juvenile corals to OA. The first important result is that HN corals were less sensitive to OA than were LN ones (Fig. 1). Similar results have been reported for adult corals in some studies (Langdon and Atkinson 2005; Holcomb et al. 2010; Chauvin et al. 2011), while not in the study by Renegar and Riegl (2005). Holcomb et al. (2010) proposed that nutrient-sufficient corals experience limited dissolved inorganic carbon (DIC) as a result of the increased endosymbiont density and therefore can benefit from higher DIC levels, which increase photosynthesis and
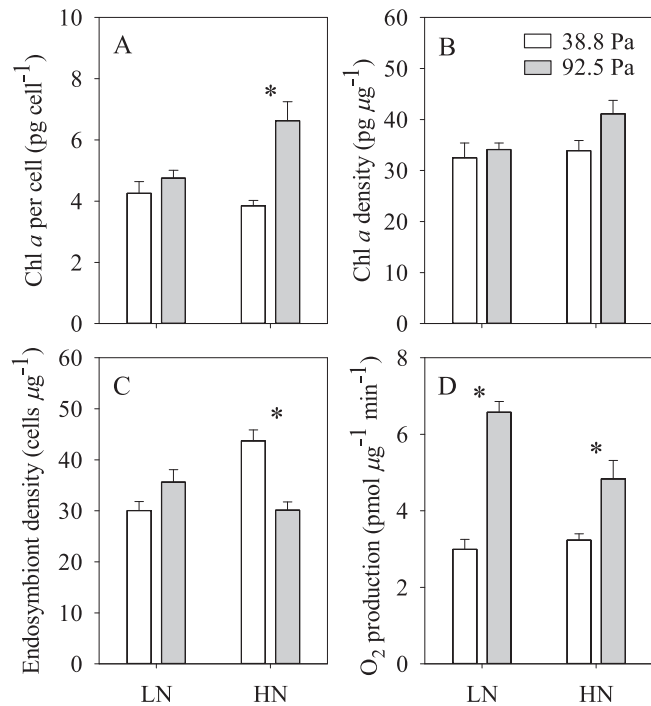
Fig. 2.   The response of endosymbiont physiological parameters to the different P_{CO_2} seawater of 38.8 Pa and 92.5 Pa. (A) Chl *a* per endosymbiont cell; (B) Chl *a* normalized to the skeletal weight; (C) The abundance of endosymbionts normalized to the skeletal weight; and (D) Oxygen production rate normalized to the skeletal weight. The meaning of LN, HN, and asterisks was explained in the legend for Fig. 1. Each bar shows mean ± SE: (A, C) $n = 7$–10; (B) $n = 5$; and (D) $n = 4$.

Fig. 3.   Skeletal (A) $\delta^{18}O$ and (B) $\delta^{13}C$ values for the juvenile corals after the acidification experiment. Asterisks indicate a significant difference between the LN and HN conditions. Each plot is shown as the mean ± SE ($n = 5$ or 6).

provide alkalinity and energy to drive the animal host calcification. While their hypothesis is based on observations of calcification responses, the present study measured both calcification and the endosymbiont parameters, allowing us to discuss the interaction between nutrient availability and P_{CO_2} in more detail.

Chl *a* content per algal cell significantly increased as a result of OA for HN corals (Fig. 2A). This suggests that the photosynthesis of endosymbionts in HN corals was limited by DIC (Crawley et al. 2010). Crawley et al. (2010) similarly reported that endosymbionts in the coral *Acropora formosa* had a higher Chl *a* per cell content when exposed to acidified seawater (P_{CO_2}: 60.8–152 Pa). They considered that the DIC limitation for endosymbionts was supported by the increased rate of light-enhanced dark respiration (LEDR) with CO_2 enrichment and the failure to observe such an increase in the dark acclimated respiration rate. The increase in the rate of LEDR is typically attributed to an increased concentration of immediate products of the Calvin cycle (Crawley et al. 2010). The increase in Chl *a* per algal cell due to OA was also observed for the other corals (*Stylophora pistillata* and *Porites* sp.; Krief et al. 2010) under the P_{CO_2} of 402 Pa.

The endosymbiont density in HN corals decreased by 31% as a result of OA (Fig. 2C). Reduction of endosymbiont density has been reported for adult corals in previous
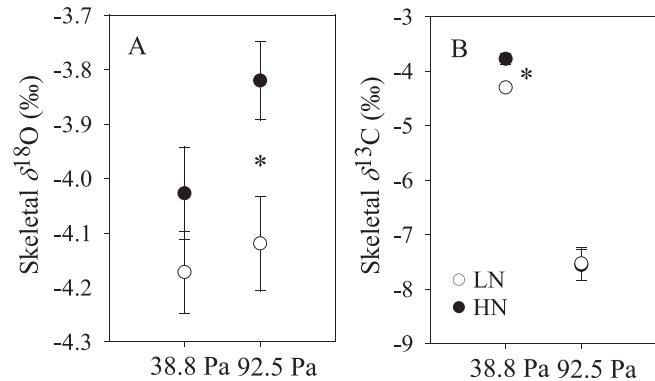
OA experiments (Krief et al. 2010; Kaniewska et al. 2012; Tremblay et al. 2013), though not in the work of Reynaud et al. (2003). Krief et al. (2010) suggested that a lower density of endosymbionts is sufficient to sustain the energy demand for the symbiotic colony under high P_{CO_2} conditions because the animal host cannot actively perform calcification, spends less energy on skeletal growth, and instead allocates the energy to produce animal host tissue. In a subsequent study, Kaniewska et al. (2012) explained that high P_{CO_2} seawater affects acid–base regulation and cell membrane transporters in the coral animal host and increases the stress of reactive oxygen species (ROS) in the endosymbiont cell and the animal host mitochondrion, which consequently leads to the loss of endosymbiont abundance. In the present study, only HN corals, which had a higher endosymbiont density than LN corals at the start of the OA experiment, exhibited a decrease in the density of endosymbionts due to OA. This observation supported the latter hypothesis because a higher endosymbiont density could induce more ROS stresses (Cunning and Baker 2012) and thus might have been more stressful for the coral colony. It was also suggested by Kaniewska et al. (2012) that the reduced abundance of endosymbionts might decrease the translocation of photosynthetic products from the endosymbiont to the animal host. Meanwhile, Tremblay et al. (2013) showed with a ^{13}C labeling technique that the coral animal host of *S. pistillata* acquired the same amount of translocated autotrophic carbon under pH values of both 8.1 and 7.2 (P_{CO_2}: 39.2 and 395 Pa, respectively). They calculated that the decrease in endosymbiont density due to the lower pH seawater was compensated for by the increase in photosynthates translocated from each endosymbiont cell.

Even though the endosymbiont density decreased in HN corals, the photosynthetic rate normalized to the skeletal weight was clearly enhanced by the higher P_{CO_2}, and enhancement was also observed for LN corals (Fig. 2D). Calculating the photosynthetic rate per endosymbiont cell from the average density (Fig. 2C), the rates under high P_{CO_2} were very similar between LN and HN corals (0.18 ± 0.01 and 0.16 ± 0.02 pmol cell^{-1} min^{-1}, respectively) and

were twice as high as those under control P_{CO_2} (0.10 ± 0.01 and 0.07 ± 0.01 pmol cell^{-1} min^{-1} for LN and HN, respectively). These data show that the photosynthetic rate per endosymbiont was also elevated along with the increased P_{CO_2} level. A similar enhancement of photosynthetic rate per cell was observed for the adult coral *S. pistillata* (P_{CO_2}: 395 Pa; Tremblay et al. 2013). They explained that this enhancement could be due to the direct effect of increased CO_2 and bicarbonate in seawater caused by seawater acidification or the indirect 'bleaching effect,' which decreased areal endosymbiont density in the animal host tissue and therefore increased the availability of light and DIC for each endosymbiont cell. Both of these mechanisms are based on the idea that the photosynthesis of endosymbionts is more or less limited by the availability of DIC. Such limitation was already observed in a previous study (Marubini et al. 2008), in which areal rates of gross photosynthesis of *S. pistillata* increased when bicarbonate concentration was elevated in seawater, regardless of the pH conditions. Unlike these observations, photosynthetic rates per cell in the coral *Acropora millepora* decreased by 60% after 28 d exposure to the high P_{CO_2} seawater (102–137 Pa), simultaneously decreasing the endosymbiont density by 56% (Kaniewska et al. 2012). The inconsistent results among studies might be a result of differences in experimental conditions. Anthony et al. (2008) reported that net photosynthetic rates normalized to coral surface area increased for the coral *Acropora intermedia* under P_{CO_2} levels of 52.7–71.4 Pa, while rates decreased for *Porites lobata*. Moreover, the coral *A. intermedia* experienced a decreased photosynthetic rate under higher P_{CO_2} levels of 103–138 Pa. These results show that the response of endosymbiont photosynthesis is variable depending on the coral species and the P_{CO_2} level.

Heavier skeletal $\delta^{18}O$ caused by high P_{CO_2} (193–402 Pa) has been observed for *S. pistillata* and *Porites* sp. by Krief et al. (2010). They inferred that reduction in seawater CO_3^{2-} due to acidification might cause the higher skeletal $\delta^{18}O$ because $\delta^{18}O$ in HCO_3^- is heavier than that in CO_3^{2-} (Zhang et al. 1995). However, the present study showed that HN corals had a significantly higher skeletal $\delta^{18}O$ and $\delta^{13}C$ than did LN corals under the same P_{CO_2} conditions (Fig. 3A,B), indicating that the increased nutrient availability for endosymbionts made the skeletal isotope ratios heavier, as observed in previous studies using adult corals (Reynaud-Vaganay et al. 2001; Grottoli 2002). HN corals had a higher abundance of endosymbionts at the start of the OA experiment (Table 2), and the endosymbionts affected by nutrient supply could efficiently and actively incorporate DIC into the algal cell. Though the photosynthetic rates per cell were similar between HN and LN corals in the same P_{CO_2} conditions (*see* above), this might be due to the low sample number (*n* = 4). Endosymbionts perform photosynthesis using DIC derived mainly from the animal host respiration and partially from external seawater (Furla et al. 2000; Reynaud-Vaganay et al. 2001). Enhanced photosynthetic processes would reduce isotope fractionation of C and O during the DIC absorption and, moreover, facilitate the uptake of seawater-derived DIC, which generally has a heavier C isotope
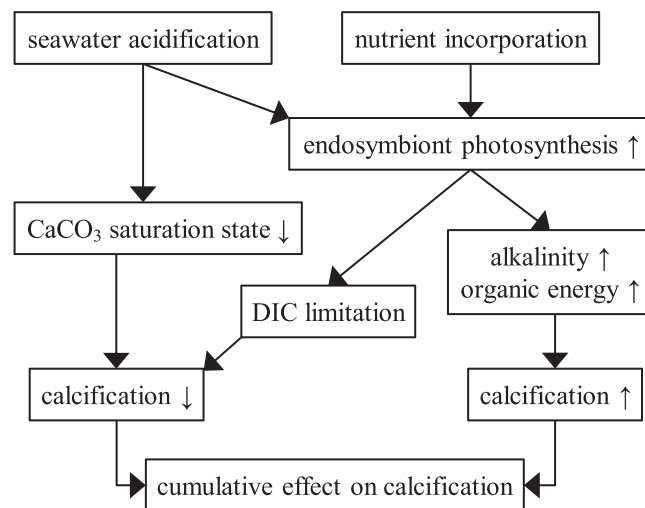
ratio than does host-derived DIC. Therefore, endosymbionts with higher nutrient availability might produce organic matter and O_2 with heavier $\delta^{18}O$ and $\delta^{13}C$ (Reynaud-Vaganay et al. 2001; Eisenstadt et al. 2010). The organic matter and O_2 are consumed by animal host and endosymbiont respiration and are finally used for the carbonate skeleton. Because the skeletal $\delta^{18}O$ of corals is an important proxy for past environmental changes, such as seawater temperature and salinity, specific interactions between nutrients and P_{CO_2} should be investigated in future studies.

To summarize, the effect of OA on juvenile corals largely differed depending on the nutrient conditions of the seawater. In the present P_{CO_2} seawater, corals in nutrient-sufficient conditions have a higher abundance of endosymbionts than do those in nutrient-depleted conditions. When the P_{CO_2} level increases, some sort of stress tends to reduce the excess number of endosymbionts (Kaniewska et al. 2012). This reduction relieves the DIC limitation for the endosymbionts and increases Chl *a* per cell, helping perform efficient photosynthesis. Moreover, higher P_{CO_2} in seawater promotes CO_2 diffusion into the endosymbiont cell and, thus, has the potential to increase their photosynthesis, regardless of the nutrient conditions (Fig. 4). Nutrient assimilation basically plays a role in increasing the endosymbiont density and photosynthetic rate because inorganic nutrients are generally depleted in coral reef seawaters (Sawall et al. 2011). The endosymbiont photosynthesis increases the alkalinity in the coral animal host and produces organic matter, which is subsequently

Fig. 4. Schematic overview of the chemical and biological factors that affect coral calcification rates under different conditions of P_{CO_2} and nutrient availability. Seawater acidification reduces the aragonite saturation state in seawater and functions as a negative effect on coral calcification. Meanwhile, higher P_{CO_2} has the potential to increase endosymbiont photosynthesis, and nutrient incorporation from seawater also enhances the photosynthetic rate. The increased photosynthesis has a positive effect on coral calcification, but excess photosynthesis can cause DIC limitation. The cumulative effect on calcification could be determined by the intensity of these positive and negative effects.

translocated to the animal host and used as an energy source for the host metabolisms (Furla et al. 2000; Tanaka et al. 2007). Both of these processes function as positive effects on coral calcification.

Excess photosynthesis leads to competition for DIC between endosymbiont photosynthesis and animal host calcification and consequently results in a decrease in calcification, which has been proposed in nutrient enrichment experiments on adult corals (Marubini and Davies 1996). Therefore, not only a decrease in aragonite saturation state due to OA but also the intensifying competition for DIC might negatively affect coral calcification, if excess nutrient incorporation occurred. In practice, calcification rates could be determined by the intensity of these positive and negative effects (Fig. 4). Our results show that the photosynthetic rate was enhanced in the acidified seawater for both LN and HN corals (Fig. 2D), but the calcification rate was significantly reduced only for the former (Fig. 1). This suggests that incorporation of nutrients by HN corals might have helped coral calcification, likely because inorganic nutrients are used for the production of new organic tissue, such as proteins, covering the carbonate skeleton. Thus, rather than increasing alkalinity due to photosynthesis, acquisition of inorganic nutrients from seawater might more effectively alleviate the negative response of juvenile coral calcification to seawater acidification, at least in the present experiment. Drenkard et al. (2013) recently reported the effect of heterotrophy and OA on juvenile corals of *Favia fragum* and concluded that increased heterotrophy mitigated the negative response of the coral calcification to OA. Because heterotrophy-derived nutrients are utilized not only by an animal host but also by the endosymbionts (Houlbrèque et al. 2003), increased heterotrophy could have an effect on the endosymbionts that is similar to that of the incorporation of inorganic nutrients from seawater.

Whether enhanced photosynthesis positively or negatively affects the host calcification may depend on the density of endosymbionts and Chl *a*, and these algal parameters would be affected not only by inorganic nutrients but also by the other habitat conditions, such as heterotrophy (Houlbrèque et al. 2003; Drenkard et al. 2013) and light intensity (Titlyanov et al. 2000). However, even if some nutrients were supplied, the present and previous experiments have shown that coral calcification rates were more or less reduced in the acidified seawater (Langdon and Atkinson 2005; Holcomb et al. 2010; Chauvin et al. 2011). It is inevitable that seawater acidification decreases the calcification rate of juvenile corals, but the present study has confirmed that moderate nutrient incorporation by corals has the potential to ameliorate that negative effect.

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