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Authors for correspondence:

Leïla Ezzat

e-mail: leila@centrescientifique.mc

Christine Ferrier-Pagès

e-mail: ferrier@centrescientifique.mc

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New insights into carbon acquisition and exchanges within the coral–dinoflagellate symbiosis under NH_4^+ and NO_3^- supply

Leïla Ezzat¹, Jean-François Maguer², Renaud Grover¹ and Christine Ferrier-Pagès¹

¹Marine Department, Centre Scientifique de Monaco, 8 Quai Antoine 1er, MC-98000 Monaco, Principality of Monaco

²LEMAR - UMR 6539 UBO/CNRS/IRD, Institut Universitaire Européen de la Mer, Place Nicolas Copernic, Plouzané 29280, France

Anthropogenic nutrient enrichment affects the biogeochemical cycles and nutrient stoichiometry of coastal ecosystems and is often associated with coral reef decline. However, the mechanisms by which dissolved inorganic nutrients, and especially nitrogen forms (ammonium versus nitrate) can disturb the association between corals and their symbiotic algae are subject to controversial debate. Here, we investigated the coral response to varying N:P ratios, with nitrate or ammonium as a nitrogen source. We showed significant differences in the carbon acquisition by the symbionts and its allocation within the symbiosis according to nutrient abundance, type and stoichiometry. In particular, under low phosphate concentration (0.05 μM), a 3 μM nitrate enrichment induced a significant decrease in carbon fixation rate and low values of carbon translocation, compared with control conditions (N:P = 0.5:0.05), while these processes were significantly enhanced when nitrate was replaced by ammonium. A combined enrichment in ammonium and phosphorus (N:P = 3:1) induced a shift in nutrient allocation to the symbionts, at the detriment of the host. Altogether, these results shed light into the effect of nutrient enrichment on reef corals. More broadly, they improve our understanding of the consequences of nutrient loading on reef ecosystems, which is urgently required to refine risk management strategies.

1. Introduction

Mutualistic symbioses between animals and algae are common in marine habitats [1]. Prominent examples include the associations of cyanobacteria with sponges and ascidians [2], algae with ciliates [3], dinoflagellates with sea anemones, sponges, clams, hydra and reef corals [4,5]. Those associations allow the animal host to exploit otherwise unsuitable food sources by acquiring or synthesizing nutrients lacking in its diet. In particular, algal symbionts fix inorganic carbon and transfer their photosynthates to the host for its own nutritional needs [6]. They can acquire other inorganic nutrients from the environment and recycle the host nitrogenous waste products as a substrate for the synthesis of high-value compounds, which are transferred back to the animal [7,8]. These symbiotic relations, however, rely on a fragile equilibrium, which can be disrupted by many stressors, such as ocean warming or acidification [9], and pollution and eutrophication (i.e. increased nutrient and sedimentation levels) [10].

The most well-known host–algal association in tropical ecosystems is formed by corals and dinoflagellates of the genus *Symbiodinium*. The ecological relevance of this mutualistic interaction is brought to light by two considerations: it structures reef food webs [11] and the benefits exchanged convey ecological advantages to the partners [1,12]. Coral reefs indeed rank among the most productive ecosystems, although they usually thrive in oligotrophic

waters with low nutrient concentrations. Their success is due to the 'tight recycling of nutrients' both within the ecosystem and the coral–dinoflagellate association. Yet, the nutritional ecology of such symbiosis and in particular the role of inorganic nutrient availability on the coral performances is still poorly understood [13,14]. Investigating those aspects will allow for informed conservation actions to efficiently protect coral reefs which face severe threats through climate change and human activities, including profound modifications in the seawater chemistry and nutrient concentrations [10]. On one hand, global climate change will probably result in higher water temperatures, stronger stratification and severe nutrient shortage in surface waters, limiting growth of all primary producers [15]. On the other hand, many inshore fringing reefs experience increased sedimentation and nutrient levels in response to fertilizer use and land clearing [10]. Consequently, at certain periods of the year, inorganic nitrogen and phosphorus concentrations can be much higher [16–19] than the usual levels measured on reefs (less than $0.5 \mu\text{M}$ for nitrogen [20–22], less than $0.1 \mu\text{M}$ for phosphate [23–25]). Furthermore, the geomorphology and the different sources and types of pollution, will also change the abundance of nitrogen sources (nitrate versus ammonium) and the N : P ratios of coastal ecosystems [26,27].

Although it is known that eutrophication can lead to coral overgrowth by macroalgae through the algae's fast nutrient uptake and use [28,29], the impact of nutrient availability on the functioning of the coral–dinoflagellate symbiosis is still poorly understood. To our knowledge, only one study has shown a loss of symbionts under nutrient shortage [13], so that further work is needed to fully understand the minimum nutrient requirements of corals and other comparable symbiotic associations. Conversely, the physiological effects of nutrient enrichment have been more intensively investigated. It is clear now that the supply of phosphorus induces a decrease in calcification rates or skeletal density [23,30,31], while results are more controversial for nitrogen addition. Nitrate reduced calcification and photosynthesis in some coral species [30,32], but had no effect or a positive one on other species [32–34]. The same was observed with ammonium enrichment [30,35]. Furthermore, it has been suggested that ammonium may have a different effect than nitrate, because nitrate use requires an energetically costly reduction [36,37], potentially lowering the amount of photosynthates transferred by symbionts to the host [38]. Altogether, these results suggest that there is a need to better understand the physiological processes and nutrient fluxes within the coral–dinoflagellate association, which will shed light on the functioning of other nutrient-sharing symbioses also affected by nutrient enrichments, such as the plant–mycorrhizae, or chlorella–mycobacterium associations [39,40]. Therefore, the first aim of this study was to test the effect of nitrogen enrichment, either supplied in the form of ammonium or nitrate, on the acquisition of carbon and allocation of photosynthates in the tropical coral *Stylophora pistillata* in symbiosis with *Symbiodinium* clade A1. In addition, independently of the nitrogen source considered, a recent study has demonstrated that the response of corals to nutrient enrichment also depends on the balance between N and P availability, as a lack of phosphorus, coupled to nitrate enrichment, increased bleaching susceptibility [13]. The second aim of this study was to test the effect of different ammonium–phosphorus ratios on *S. pistillata* metabolism and photosynthates translocation,

to investigate the potential role of nutrient stoichiometry on coral physiology.

2. Material and methods

(a) Experimental set-up

Six colonies of the scleractinian coral *S. pistillata* (Esper 1797) from the Red Sea (Gulf of Aqaba, Jordan; CITES DCI/89/32) were used to generate 240 nubbins (40 colony^{-1}). Nubbins were suspended on a nylon thread, equally distributed into eight 20 l aquaria ($5 \text{ nubbins colony}^{-1} \text{ aquaria}^{-1}$) and maintained for six weeks under the following controlled conditions: aquaria were continuously supplied with oligotrophic seawater at a flow rate of 20 l h^{-1} , and metal halide lamps (Philips, HPIT 400 W, Distrilamp, France) provided a constant irradiance of $150 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$. Seawater temperature was maintained constant at $25 \pm 1^\circ\text{C}$ using temperature controllers (Toshniwal N6100, West Instruments, Brighton, UK) and submersible resistance heaters (Aquarium Systems, France). Nubbins were kept unfed to avoid any interaction with the nutrient enrichments.

After this first period, four inorganic nutrient conditions were generated in duplicated tanks: (i) a control condition (called 'C') with $0.5 \mu\text{M}$ nitrogen (N- mainly nitrate) and $0.05 \mu\text{M}$ phosphorus (P); (ii) a $0.05 \mu\text{M}$ P and $3 \mu\text{M}$ N, with addition of $2.5 \mu\text{M}$ ammonium (called ' NH_4 ' condition); (iii) a $0.05 \mu\text{M}$ P and $3 \mu\text{M}$ N, with addition of $2.5 \mu\text{M}$ nitrate (called ' NO_3 '); and (iv) a $1 \mu\text{M}$ P and $3 \mu\text{M}$ N (with addition of $2.5 \mu\text{M}$ NH_4) enriched condition, called ' $\text{NH}_4\text{-PO}_4$ '. For this purpose, the control tanks received only natural seawater (N : P = $0.5 : 0.05 \mu\text{M}$). For the three enriched conditions, tanks were continuously supplied with ammonium chloride (NH_4Cl) or sodium nitrate (NaNO_3) pumped from a stock solution via a peristaltic pump at a flow rate of 0.31 l h^{-1} . The $\text{NH}_4\text{-PO}_4$ tank was also continuously supplied with sodium dihydrogen phosphate (NaH_2PO_4), delivered at the same flow rate as for nitrogen. Nutrient concentrations were monitored twice to three times a week using an autoanalyzer (Alliance Instrument, AMS, France). The total nitrogen concentration varied between 0.4 and 0.55 in the control tank, and between 3.1 and 3.5 in the other enriched conditions. Phosphorus levels varied from 0.03 to 0.05 in the control and nitrogen-enriched tanks, and from 0.8 to 1.2 in the $\text{NH}_4\text{-PO}_4$ tanks. Nubbins were incubated for three weeks under these conditions before analyses.

(b) Measurements

To assess the acquisition and exchange of photosynthates under the different conditions, we used the model fully described in Tremblay *et al.* [41], which follows the acquisition of external ^{13}C -labelled bicarbonate and its allocation into the different compartments of the symbiotic association as well as the carbon lost as mucus (particulate organic carbon (POC) and dissolved organic carbon (DOC)). Carbon fluxes were calculated using the rates of photosynthesis, of host and symbiont respiration and of calcification. Symbionts density and animal tissue growth were also evaluated to complement the model.

(i) Physiological measurements

All measurements were performed on six nubbins per treatment (one per colony). Net photosynthesis (P_n) and respiration (R) rates were assessed at 0 and $150 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ on nubbins incubated in glass chambers filled with filtered seawater (FSW) containing the respective amount of inorganic nutrients. The water was homogenized using a stirring bar and each chamber was equipped with a Unisense optode connected to a computer with Oxy-4 software (Chanel fiber-optic oxygen meter, PreSens, Regensburg, Germany). Optodes were calibrated against

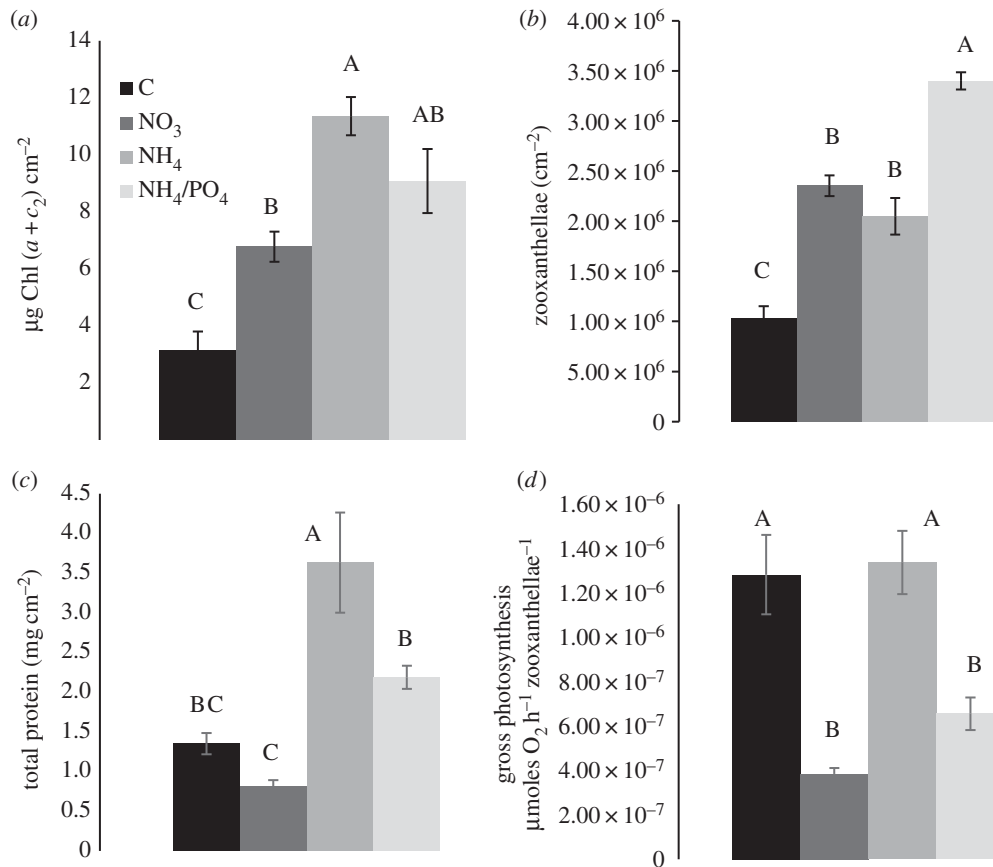


Figure 1. (a) Total chlorophyll ($\mu\text{g Chl } (a + c_2) \text{ cm}^{-2}$), (b) symbiont (no. zoox. cm^{-2}), (c) protein (mg cm^{-2}) content, and (d) gross photosynthesis rate ($\mu\text{moles O}_2 \text{ h}^{-1} \text{ zoox.}^{-1}$) for the different treatments. Data are expressed as mean \pm s.e.

nitrogen-saturated and air-saturated seawater for the 0% and 100% oxygen, respectively. P_n and R rates were estimated by regressing oxygen data against time. Gross photosynthetic (P_g) rates were calculated by adding R to P_n . After incubation, nubbins were frozen for the determination of symbiont density, total chlorophyll and protein concentrations according to Hoogenboom *et al.* [42]. Data were normalized to the skeletal surface area (cm^2) of each nubbin, using the wax-dipping technique [43]. Oxygen fluxes were converted to carbon equivalent based on molar weights according to Anthony & Fabricius [44].

Respiration rates of freshly isolated symbionts, as well as their concentration, were also measured according to Tremblay *et al.* [41]. Rates of calcification were determined using the buoyant weight technique [45], and normalized to the nubbin surface area and the incubation time. Carbon allocation to calcification (C_c) was calculated with the following formula: $C_c = \text{Msk} \times 12/100$, where Msk represents the amount (μg) of CaCO_3 produced and 12/100 is the ratio of molecular masses of C (12 g mol^{-1}) and CaCO_3 (100 g mol^{-1}).

(ii) H^{13}CO_3 labelling

Incubations were performed according to Tremblay *et al.* [41]. For each condition, 18 nubbins (three nubbins per colony) were placed in individual beakers containing 200 ml FSW with the right amount of nutrients, and enriched with 0.6 mM $\text{NaH}^{13}\text{CO}_3$ (98 atom % ^{13}C , no. 372382, Sigma-Aldrich, St Louis, MO, USA). They were incubated for 5 h and then transferred into non-enriched seawater for two chase periods (0 and 24 h). One nubbin per colony was removed after each period and directly frozen at -20°C for ^{13}C measurements in the animal tissue and symbionts. Eighteen control nubbins (three nubbins per colony) were incubated in parallel in non ^{13}C -enriched seawater. The % ^{13}C enrichment was measured using a Delta Plus Mass

spectrometer coupled to a C/N analyser (ThermoFisher Scientific, Bremen, Germany). The equations are described in Tremblay *et al.* [41]. As the results from the two chase periods (0 and 24 h) were very similar, we only report the carbon budget obtained after 24 h in the following results, for clarity.

(iii) Statistics

Statistical analyses were conducted with R statistical software v. 3.2.0 [46] by the use of the 'stats' package. Normality and homoscedasticity of the data residuals were tested using Kolmogorov–Smirnov (using Lilliefors corrections) and Levene tests, and data were log-transformed when required. One-way ANOVAs were performed on all response variables with 'nutrient treatment' as factor except for the carbon incorporation rates (ρ) and percentage of fixed carbon remaining (C_r), which were tested using a two-way ANOVA, with 'nutrient treatment' and 'coral compartment' as factors (compartments are symbionts and host tissue; see the electronic supplementary material, S6). When there were significant differences between treatments, an *a posteriori* test was performed (Tukey's test). The Bonferroni correction was used to account for multiple testing in the ^{13}C labelling part of the experiment. p -values were considered significant for $p < 0.05$.

3. Results

(a) Physiological measurements

Control corals contained lower concentrations of chlorophyll (Chl) and symbionts than the nutrient-enriched nubbins (figure 1 and the electronic supplementary material, S5, Tukey HSD, $p < 0.0001$). Symbiont density was not different between NO_3 and NH_4 conditions (Tukey HSD, $p = 0.39$),

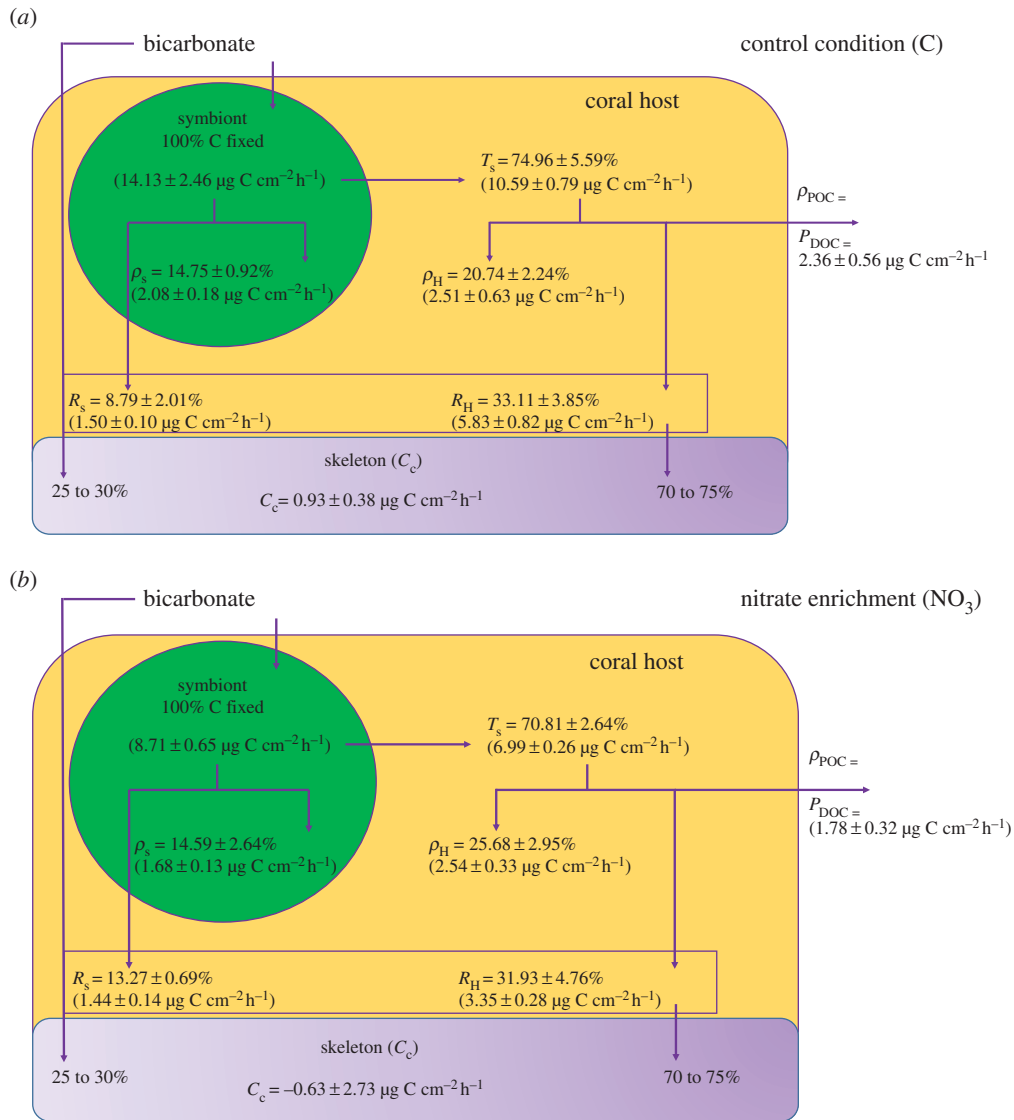


Figure 2. Carbon budgets for (a) control corals, N:P = 0.5:0.05 and (b) corals maintained under N- NO_3 :P = 3:0.05 enrichment. Data are expressed as mean \pm s.e.

but higher in the NH_4 - PO_4 one (figure 3b and the electronic supplementary material, S5, Tukey HSD, $p < 0.001$). The amount of total chlorophyll content per symbiont remained equivalent in all conditions except for NO_3 nubbins, which showed a lower amount (Tukey HSD, $p < 0.001$). NH_4 corals synthesized the highest amount of total protein compared with the other conditions (figure 3a and the electronic supplementary material, S5, Tukey HSD, $p < 0.02$). For the other physiological parameters, control and NO_3 -conditions were different from NH_4 and NH_4 - PO_4 conditions. Indeed, control and NO_3 groups presented lower calcification rates (electronic supplementary material, S5, Tukey HSD, $p < 0.003$), as well as lower net (P_n) and gross (P_g) photosynthesis per skeletal surface area (electronic supplementary material, S5, Tukey HSD, $p < 0.01$ and $p < 0.001$) than the NH_4 groups. Moreover, NO_3 corals showed significantly lower respiration rates (R) than the NH_4 nubbins (Tukey HSD, $p < 0.0001$). Mean P_n values normalized to skeletal surface area were equal to 0.35–0.41 $\mu\text{mol O}_2 \text{cm}^{-2} \text{h}^{-1}$ in control and NO_3 corals versus two to three times more (0.94–1.25 $\mu\text{mol O}_2 \text{cm}^{-2} \text{h}^{-1}$) for NH_4 and NH_4 - PO_4 nubbins. There was no significant difference in the above parameters between the NH_4 and NH_4 - PO_4 treatments (electronic supplementary material, S5, ANOVA, $p > 0.05$). P_g per symbiont cell was above $1 \times 10^{-6} \mu\text{mol O}_2 \text{h}^{-1}$

symbiont $^{-1}$ in control and NH_4 -enriched corals, whereas it was three times lower in NO_3 and NH_4 - PO_4 corals (electronic supplementary material, S5, Tukey HSD, $p < 0.001$).

(b) Carbon budget

The natural atom $\%^{13}\text{C}$ measured in non-enriched corals ranged between 1.128 and 1.133% in symbionts, and between 1.125 and 1.130% in coral tissue. After 5 h incubation in ^{13}C -bicarbonate, all nubbins were significantly enriched in ^{13}C compared with control corals (atom% ^{13}C between 1.157 and 1.180% in symbionts, and between 1.136 and 1.152% in host tissues). Only the final carbon budgets under the different conditions are reported in the results, intermediate calculations are shown in the electronic supplementary material.

For control corals, the initial carbon fixed by symbionts (gross photosynthesis, P_g) was equal to $14.13 \pm 2.46 \mu\text{g C cm}^{-2} \text{h}^{-1}$. More than two-thirds ($74.96 \pm 5.59\%$ or $10.59 \pm 0.79 \mu\text{g C cm}^{-2} \text{h}^{-1}$) of this carbon was translocated to the host after 24 h (figure 2a) and half was lost as respiration (*ca* 55%) and mucus (dissolved and particulate organic carbon, *ca* 23%; figure 2a). Only 20 to 25% remained both in the host and symbionts after 24 h. The final incorporation rates were thus equivalent in symbionts and

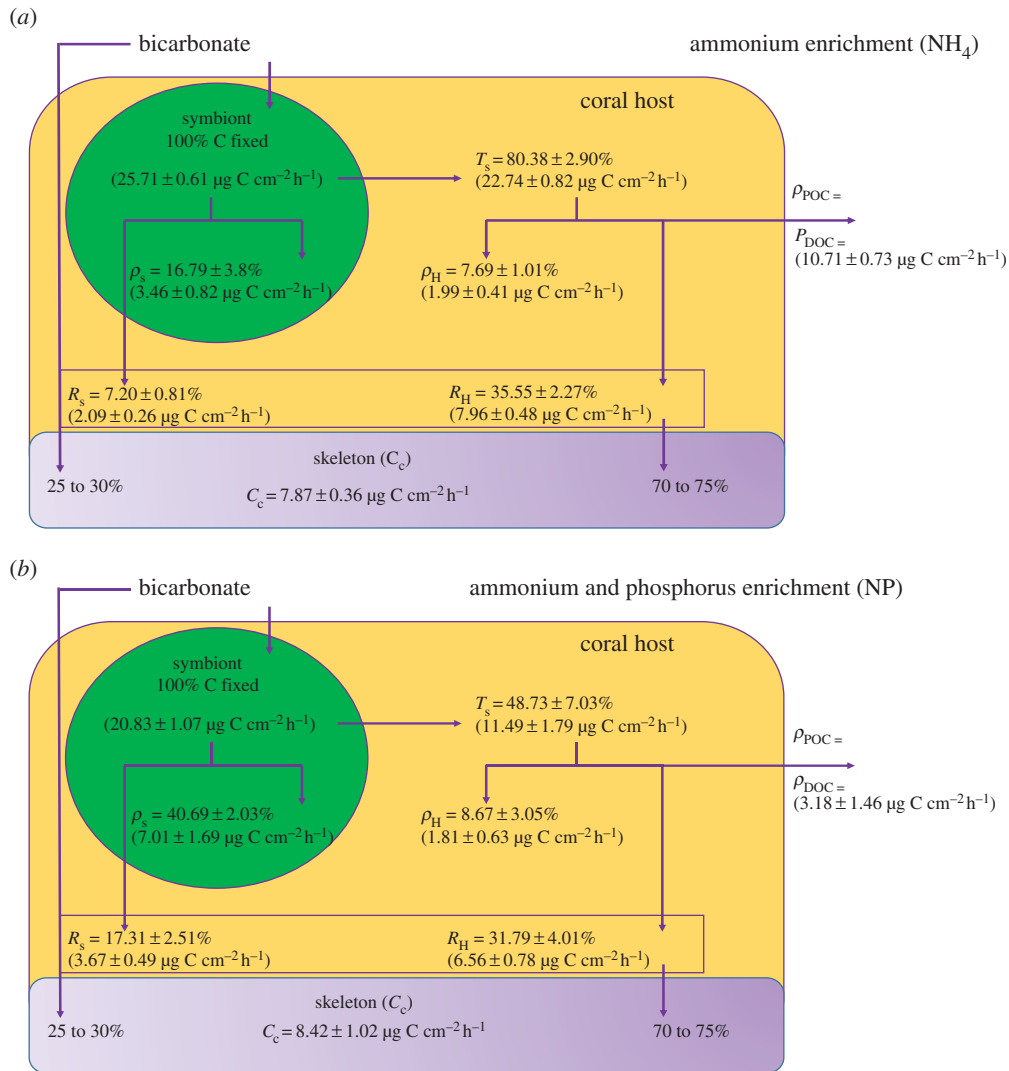


Figure 3. Carbon budgets for corals maintained under (a) N-NH₄:P = 3:0.05 supply and (b) N-NH₄:P = 3:1 addition. Data are expressed as mean \pm s.e.

host tissue (ANOVA, $p > 0.05$), 2.08 ± 0.18 and $2.51 \pm 0.63 \mu\text{g C cm}^{-2} \text{h}^{-1}$, respectively.

Nutrient enrichment had significant effects on the fate of the autotrophically acquired carbon, with significant differences between NH₄ or NO₃ enrichments, or when ammonium was combined with PO₄. NH₄ alone significantly increased the amount of carbon initially fixed by the symbionts ($P_g = 25.71 \pm 0.61 \mu\text{g C cm}^{-2} \text{h}^{-1}$) compared with control nubbins (Tukey HSD, $p = 0.001$; figure 3a). It also enhanced the amount of carbon translocated after 24 h (Tukey HSD, $p < 0.0001$; figure 3a) in terms of total quantity (double amount, or $22.74 \pm 0.82 \mu\text{g C cm}^{-2} \text{h}^{-1}$) and percentage ($80.38 \pm 2.90\%$). Eighty per cent of the translocated carbon was lost after 24 h through respiration and mucus release (*ca* $10.71 \pm 0.73 \mu\text{g C cm}^{-2} \text{h}^{-1}$ significantly higher than in control corals, Tukey HSD, $p < 0.001$; figure 3a). However, the amount of carbon remaining in the host and symbionts ($5.5 \mu\text{g C cm}^{-2} \text{h}^{-1}$) was still significantly higher than in the control condition.

The NH₄-PO₄ enrichment again changed the carbon budget compared with control corals. The initial carbon fixation rate in symbionts was intermediate between control and NH₄ corals ($P_g = 20.83 \pm 1.07 \mu\text{g C cm}^{-2} \text{h}^{-1}$ Tukey HSD, $p_1 = 0.5$ and $p_2 = 0.2$). Carbon translocation rate was significantly lower than in control corals, as only $48.73 \pm 7.03\%$ were translocated after 24 h (Tukey HSD, $p < 0.05$), but the amount of

translocated carbon stayed similar (Tukey HSD, $p > 0.05$). Half of the photosynthesized carbon was lost as respiration and 'mucus' (figure 3b). The carbon incorporation rate in symbionts ($40.69 \pm 2.03\%$ of the total photosynthesized carbon or $7.01 \pm 1.69 \mu\text{g C cm}^{-2} \text{h}^{-1}$) was significantly higher compared with control and nitrogen-enriched corals (Tukey HSD, $p < 0.002$).

NO₃ nubbins presented a significant lower initial carbon fixation rate compared with control corals (Tukey HSD, $p < 0.03$; figure 2b), with only $8.71 \pm 0.65 \mu\text{g C cm}^{-2} \text{h}^{-1}$. Carbon translocation rates also tended to be lower, although not significantly. More than half of the photosynthesized carbon (52%) was lost as respiration and mucus (figure 2b). Symbiont respiration and carbon incorporation rates were similar to control conditions (Tukey HSD, $p > 0.05$). Compared with NH₄-enriched corals, initial carbon fixation rates, as well as the percentage and amount of carbon translocated were significantly lower (Tukey HSD, $p < 0.002$).

4. Discussion

Global climate change and other anthropogenic perturbations affect the biogeochemical cycles and the nutrient stoichiometry of marine ecosystems [47]. As organisms need stable nutrient cycles to flourish in a particular environment [48], any alteration leading to an imbalanced N:P stoichiometry will impact

the organisms' health. Our study shows that the coral–dinoflagellate association is very responsive to changes in the nitrogen and phosphorus availability. We found evidence for significant differences in the acquisition of autotrophic carbon and its allocation within the symbiosis depending on the inorganic N : P ratio in seawater as well as the type of nitrogen source available. In addition, our results support the general view that the coral–algal symbiosis is nutrient limited when grown under the low dissolved N and P concentrations observed in oligotrophic environments.

After three weeks of culture at low N-P concentrations (control condition; N : P = 0.5 : 0.05), colonies showed a reduced metabolism and primary signs of nutrient limitation compared with NH_4 -enriched corals: i.e. lower biomass (protein concentration) and calcification rates, and a reduced symbiont growth, which is comparable to what is observed in other nutrient-limited algae [49,50]. A pronounced decrease in the symbiont density of several coral species was also previously observed under similarly low nutrient levels [13]. Conversely, in the Gulf of Eilat (Israel), colonies of *S. pistillata* present 30% more symbionts and from identical up to three times more areal chlorophyll content [51]. Although *in situ* inorganic nutrient concentrations are equivalent to those supplied in this experiment, corals benefit from heterotrophic feeding, which can constitute a significant nutrient source [52]. Interestingly, in this control poor-nutrient condition, a large amount of autotrophic carbon was still transferred to sustain the host respiratory needs. However, corals implemented a nutrient conservation strategy as little of this newly produced carbon was lost as dissolved and particulate material. These observations suggest that recovery from thermal stress-induced bleaching may be more difficult in very oligotrophic environments. Indeed, the lack of nutrient availability may limit symbiont growth and photosynthesis, and in turn the resilience of coral colonies.

The carbon budget comparison under nitrate and ammonium enrichment sheds new light into how both forms of nitrogen affect the symbiosis and helps explaining their different effects on coral metabolism. The general belief is that nitrogen enrichment alleviates the symbiont dependency on the host for nitrogen supply, resulting in higher symbiont density, higher retention of photosynthates in symbionts for their own development, at the expense of host metabolism [53,54] and in a competition between symbiont photosynthesis and host calcification for inorganic carbon use [55,56]. That theory was however challenged by studies showing a positive effect of nutrient enrichment on coral growth [35,57,58] and by the fact that some corals can thrive in high-nutrient waters [58,59]. Our results highlight major differences in the carbon budget of the symbiosis between the two forms of nitrogen enrichment. Although symbiont density under both nitrogen forms doubled compared with control corals, total carbon acquisition and translocation were significantly higher in NH_4 -enriched corals, inducing an enhancement in host calcification. These results are in agreement with previous studies showing that short-term ammonium enrichment in low concentration tends to enhance coral growth [35,57,58,60] and suggest that inorganic carbon supply was sufficient to cover the needs for symbiont photosynthesis and host calcification. Conversely, nitrate supplementation induced a significant decrease in the amount of carbon acquired compared with control corals, owing to lower rates of photosynthesis per symbiont cell. Compared with ammonium, both carbon acquisition

and translocation were significantly and largely decreased. This is explained by the fact that nitrate reduction into ammonium is an energy and electron-consuming process [37]. Indeed, nitrite reductase, which reduces nitrite into ammonium in the chloroplasts of photosynthetic cells, uses the reduced ferredoxin from the photosynthetic chain as an electron donor and consumes six electrons to catalyse the reduction. Thus, in this reaction, electrons are lost for the photosynthetic process. On the long term, or under stress, the reduced carbon fixation under nitrate enrichment may induce a nutrient shortage and may weaken the symbiosis, as observed in corals but also in other dinoflagellate–host associations [61]. Although the detrimental effect of nitrate on photosynthesis has to be confirmed with different coral species and nitrate concentrations, excess nitrate in reef waters, which is mainly brought by industry and agriculture runoff, will probably severely affect the entire coral primary productivity. On the contrary, ammonium is the principal form of recycled nitrogen from fish excretion [60,62], although rain can also be a significant source in some reef environments [27]. Enhancing fish populations above reefs will probably be beneficial for corals, as already observed with resident fish schools [63].

The last treatment ($\text{NH}_4\text{-PO}_4$) reveals how a change in nutrient ratio in seawater can affect the animal–algal symbiosis by shifting nutrient limitation from one partner to the other. Compared to the NH_4 -treatment, in which P level was kept low, the $\text{NH}_4\text{-PO}_4$ enrichment induced a significant increase in symbiont density (above 3×10^6 cells cm^{-2}). The allocation of autotrophic nutrient thus shifted from the host to the symbionts, which decreased their photosynthate translocation down to 48% and kept a large amount of photosynthetic products for their own use. In addition, owing to their high density in the host tissue, each symbiont cell was less efficient in acquiring carbon owing to a self-shading and light limitation [64,65], and/or owing to a CO_2 limitation [53]. Those results suggest that differences in symbiont genotype and/or density within the host tissue may shape the response of corals to nutrient enrichment. Coral species, which host low symbiont densities and/or slow-growing symbiont genotypes are likely to be more resilient than others to eutrophication stress and also to bleaching [66]. Soft corals such as *Heteroxenia fuscescens*, which harbour rhythmic pulsating tentacles, can represent another potential resilient group. Pulsations indeed maintain an active water movement, which enhances photosynthesis via the fast removal of excess oxygen and the fast supply of inorganic carbon [67]. We therefore suggest that eutrophication may cause a shift in the coral species composition of the reefs, by favouring species, which can avoid carbon limitation of their symbionts.

Taken all together, our results suggest that both the nitrogen source and the N : P ratio in seawater modify the symbiotic relationship between host and algae, by affecting the autotrophic carbon acquisition and allocation. The relevance of seawater N : P ratio stoichiometry for coral health was already pointed out by Wiedenmann *et al.* [13]. They observed, during thermal stress, a higher decrease in photosynthetic efficiency of the symbionts under imbalanced N(nitrate) : P ratio (3 : 0.07) than when phosphorus was available. Our data bring another dimension to the conclusions of these authors, as in both N : P imbalanced ratios presented in this study ($\text{NH}_4\text{:PO}_4$ or $\text{NO}_3\text{:PO}_4$ ratios: 3 : 0.05), only the nitrate-supplied condition weakened the symbiosis after three weeks. Additionally, we also showed that balanced

N(ammonium) : P ratio significantly decreased the percentage of carbon translocation in *S. pistillata* at the detriment of the coral host, compared with an imbalanced N(ammonium) : P ratio, suggesting that the effect of nutrient enrichment on the coral symbiosis is more complex than previously thought. Finally, the carbon budget analysis suggests that not only the N : P but also the C : N : P ratio and the symbiont density have to be taken into account to understand the nutritional relationship between the symbionts and their host.

5. Conclusion

Nutrient-induced changes in host–symbiont interactions have been well studied in terrestrial plant–fungal associations [68], but much less is known about it in aquatic systems. There are still large gaps in our understanding on how nutrients affect marine symbioses. This study showed that nutrient concentrations and ratios differently impact the carbon acquisition and allocation within the coral–dinoflagellate symbiotic association. Future studies should further investigate nutrient combinations and ratios, as well as their synergistic effects with other stressors, such as thermal, light and acidification stresses, which all affect the acquisition and allocation of carbon between the two partners [13]. More, these experiments were run with the coral holobiont *S. pistillata*, in symbiosis with clade A. As symbionts from different clades present various thermal sensitivity in nitrogen metabolism [69], further research is required to assess the relevance and the effect of

variation in N : P stoichiometry on coral health. Finally, our study sheds new light into how marine symbioses face eutrophication compared with other terrestrial and marine species, and is thus essential to predict the responses of communities to anthropogenic disturbances.

Ethics. The care of animals used in experimentation was in accordance with institutional guidelines under the following CITES number: DCI/89/32.

Data accessibility. Additional data supporting this article have been uploaded as part of the electronic supplementary material. All data are accessible via the following Dryad Digital Repository: <http://dx.doi.org/10.5061/dryad.361gs> [70].

Authors' contributions. Conceived and designed the experiments: C.F.P., L.E. and R.G. Performed the experiments: L.E. and J.F.M. Analysed the data: L.E. Contributed reagents/materials/analysis tools: C.F.P., R.G. and J.F.M. Wrote the paper: L.E., C.F.P. and R.G.

Competing interests. We declare we have no competing interests

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