frontiers | Frontiers in Marine Science

TYPE Original Research
PUBLISHED 29 August 2024
DOI 10.3389/fmars.2024.1433382

OPEN ACCESS

EDITED BY
Wei Jiang,
Guangxi University, China

REVIEWED BY
Manuel Aranda,
King Abdullah University of Science and Technology, Saudi Arabia
Jin-Yu T. Yang,
Xiamen University, China

*CORRESPONDENCE
Xingchen Tony Wang
xingchen.wang@bc.edu

†These authors have contributed equally to this work and share first authorship

RECEIVED 15 May 2024
ACCEPTED 07 August 2024
PUBLISHED 29 August 2024

CITATION
Donnelly HA, Valadez-Ingersoll M, Lin M, Rivera HE, Tramonte CA, Davies SW and Wang XT (2024) Groundtruthing nitrogen isotopes as a symbiosis proxy using the facultatively symbiotic coral *Oculina arbuscula*. *Front. Mar. Sci.* 11:1433382. doi: 10.3389/fmars.2024.1433382

Groundtruthing nitrogen isotopes as a symbiosis proxy using the facultatively symbiotic coral *Oculina arbuscula*

Heather A. Donnelly[1†], Maria Valadez-Ingersoll[2†], Marcus Lin[1,3], Hanny E. Rivera[2], Carlos A. Tramonte[1,2,4], Sarah W. Davies[2] and Xingchen Tony Wang[1*]

[1]Department of Earth and Environmental Sciences, Boston College, Chestnut Hill, MA, United States, [2]Department of Biology, Boston University, Boston, MA, United States, [3]Department of Earth and Planetary Sciences, McGill University, Montreal, QC, Canada, [4]School of Ocean and Earth Science and Technology, University of Hawai'i at Mānoa, Mānoa, HI, United States

The resilience of coral reefs in oligotrophic, (sub)tropical oceans is largely due to the symbiotic relationship between scleractinian corals and Symbiodiniaceae algae, which enables efficient internal nutrient recycling. Investigating the history of this coral symbiosis can provide insights into its role in sustaining the health of both present and future coral reefs. The isotopic composition of organic nitrogen ($^{15}N/^{14}N$ or $\delta^{15}N$) bound within coral skeletons has been utilized to trace the existence of symbiosis in fossil corals, suggesting that coral symbiosis dates back to at least 210 million years ago. The basis of this proxy is that symbiotic corals are expected to exhibit lower $\delta^{15}N$ compared to their non-symbiotic (aposymbiotic) counterparts within the same environments, owing to internal nitrogen recycling between the coral host and algal symbiont, and reduced leakage of low-$\delta^{15}N$ ammonium into seawater. However, this hypothesis has not been adequately tested in contemporary settings. In a laboratory experiment, we examined the $\delta^{15}N$ differences between the symbiotic and aposymbiotic branches within the same genetic backgrounds of the facultatively symbiotic coral *Oculina arbuscula* under well-fed conditions. Across five different genotypes in two separate experiments, symbiotic branches consistently showed lower $\delta^{15}N$ than their aposymbiotic counterparts. These findings corroborate the use of $\delta^{15}N$ as a proxy for identifying coral symbiosis in the past, particularly when multiple species of corals coexisted in the same environments.

KEYWORDS

coral symbiosis, *Oculina arbuscula*, nitrogen isotope, coral evolution, climate change

1 Introduction

Coral reef habitats predominantly exist in oligotrophic (low nutrient) environments in tropical and subtropical oceans (Capone et al., 2008; Dubinsky and Stambler, 2011). These habitats comprise a diverse array of organisms, with scleractinian (stony) corals serving as the primary reef builders. These ecosystems formed by corals are biodiversity hotspots, offering a range of ecosystem services including serving as nursery habitats for juvenile fish, facilitating carbon sequestration, and providing coastal protection against storms (Rosenberg et al., 2007; Woodhead et al., 2019). Remarkably, coral reef tourism alone generates up to $35.8 billion globally per year (Woodhead et al., 2019). However, these vital organisms and the ecosystems they support are under severe threats from rising sea surface temperatures and ocean acidification due to anthropogenic CO_2 emissions (Goldberg and Wilkinson, 2004; Dubinsky and Stambler, 2011; Hughes et al., 2018). In addition, local anthropogenic stressors, such as nitrogen (N) pollution, further imperil coral reefs and their ecosystem services by adversely affecting coral health, including increased disease susceptibility and bleaching, loss of coral diversity, and high mortality rates (Rosenberg et al., 2007; D'Angelo and Wiedenmann, 2014; Burkepile et al., 2020). Over the past few decades, coral coverage in tropical and subtropical waters has decreased by approximately 50% (Eddy et al., 2021).

The success of tropical and subtropical coral reefs in oligotrophic waters hinges on their symbiosis with Symbiodiniaceae algae within their gastrodermal cells. The recent decline of coral reefs is largely attributed to the loss of Symbiodiniaceae under conditions of rising water temperatures and other adverse factors, leading to "coral bleaching" (Li et al., 2023). In healthy symbiotic conditions, this symbiosis facilitates the transfer of photosynthetic products from the algae to the coral (Rädecker et al., 2015; Gustafsson et al., 2013; Houlbrèque and Ferrier-Pagès, 2009). In return, the algae assimilates metabolic waste (e.g., N) excreted by the coral, preventing its loss to the ambient ocean (Cui et al., 2019). This symbiosis fosters an "internal nutrient recycling" mechanism, enabling corals to conserve nutrients and thrive in oligotrophic waters. In the absence of these algal symbionts, corals would struggle to thrive due to nutrient scarcity (Grover et al., 2008; Yellowlees et al., 2008; Falkowski et al., 1984; D'Elia et al., 1983). As a result, the once-healthy symbiotic relationship deteriorates, resulting in reduced coral growth rates, increased mortality rates, and ultimately, coral reef degradation.

Numerous studies have investigated the interactions between the host coral and their algal symbionts (Davy et al., 2012; Kopp et al., 2013; Weis et al., 2008; Yellowlees et al., 2008), though most have focused on present-day coral symbioses. The response of coral symbiosis to a warmer climate in the past, however, might also provide important insights into the future of coral symbiosis under global warming (Frankowiak et al., 2016; Weis et al., 2008; Pochon et al., 2006). While corals leave behind skeletal fossils composed of calcium carbonate, Symbiodiniaceae do not, complicating the study of historical coral symbiosis. The molecular clock technique has been used to study the origin of symbiosis in contemporary scleractinian corals, placing it between approximately 140 Ma to 200 Ma (LaJeunesse et al., 2018). However, this method is based on assumptions regarding genetic mutation rates and can lack precision without corroboration from fossil evidence. Moreover, this late origin of symbiosis in stony corals is at odds with the rapid expansion of ancient coral reefs around 200–251 Ma during the Triassic period (Stanley, 2003).

In addition to the molecular clock approach, indirect "proxies" derived from coral skeleton records have also been employed to infer the origin and evolution of the coral-algal symbiosis. These proxies include skeletal microstructures and the isotopic composition of carbon (C), oxygen (O), and nitrogen (N) (Stanley and Swart, 1995; Frankowiak et al., 2016). Notably, the N isotopic composition ($^{15}N/^{14}N$ or $\delta^{15}N$) of skeleton-bound organic matter has been recently utilized to suggest the presence of symbiosis in fossil corals dating back to approximately 210 million years (Frankowiak et al., 2016; Tornabene et al., 2017). These studies have also suggested that these corals lived in oligotrophic water and that symbiosis with photosynthetic algae was crucial for the evolution and expansion of scleractinian corals (Frankowiak et al., 2016).

The coral $\delta^{15}N$ symbiosis proxy is predicated on the differences in skeletal $\delta^{15}N$ values between symbiotic and aposymbiotic corals within identical environmental settings (Frankowiak et al., 2016). Nitrogen has two stable isotopes, ^{14}N and ^{15}N. ^{14}N constitutes ~99.63% of naturally occurring N, while ^{15}N accounts for the remaining ~0.37% (Reddy and DeLaune, 2008). The ratio of ^{15}N to ^{14}N in a sample is expressed with the $\delta^{15}N$ notation, defined as $\delta^{15}N = [(^{15}N/^{14}N_{sample})/(^{15}N/^{14}N_{air})\text{-}1] \times 1{,}000‰$. In a typical ecosystem, a trophic enrichment of approximately ~3‰ in $\delta^{15}N$ is observed at each successive trophic level, which is due to the preferential breakdown of ^{14}N-rich proteins during catabolism and the excretion of ^{14}N-rich ammonium or urea (Deniro and Epstein, 1981; Minagawa and Wada, 1984). In symbiotic corals, however, the ^{14}N-rich ammonium is recycled within the symbiotic system, reducing its excretion to the surrounding seawater and consequently limiting isotopic fractionation. As a result, when residing in identical environments, symbiotic corals are expected to exhibit lower $\delta^{15}N$ values compared to their non-symbiotic counterparts. However, this hypothesis has not been sufficiently tested in contemporary settings using extant coral species as most coral $\delta^{15}N$ studies have focused on its use as a trophic level indicator in modern corals (Conti-Jerpe et al., 2020; Wiedenmann et al., 2023).

In this study, we investigate the impact of symbiosis on coral $\delta^{15}N$ using a facultatively symbiotic coral species, *Oculina arbuscula*. Unlike most extant symbiotic corals that harbor obligate symbionts, facultatively symbiotic corals exhibit a range of symbiotic states—from hosting high to low densities of algal symbionts—and can even be manipulated to achieve aposymbiosis (functionally devoid of symbionts) in laboratory settings similar to other closely related cnidarian clades (Madin et al., 2016; Matthews et al., 2016). This flexibility allows for a detailed examination of the differences between symbiotic states. We explored the $\delta^{15}N$

differences between aposymbiotic and symbiotic branches of *Oculina arbuscula* under consistent laboratory conditions. Our experimental design focuses on a single species while also considering the influence of genetic background on $\delta^{15}N$, thereby isolating the presence or absence of symbionts as the sole variable. The results could enhance our understanding of $\delta^{15}N$ as a proxy for symbiosis in fossil corals and provide valuable information on how contemporary corals may respond to global warming.

2 Materials and methods

2.1 Sample collection

All facultatively symbiotic coral colonies, *Oculina arbuscula* Agassiz, 1864, were collected on May 25, 2018 from Radio Island, North Carolina (34° 42.520′ N, 76° 40.796′ W; Supplementary Figure 1) from depths of 4.5–6 m using a hammer and chisel (NC Division of Marine Fisheries Permit #1627488; Rivera and Davies, 2021). Five genetically distinct parent colonies (genets) of *O. arbuscula* were collected and transported overnight to the Davies Marine Population Genomics Lab at Boston University. Parent colonies were used to generate two nubbins per colony for each experiment, creating genotypic replicates, and resulting in a total of 8 nubbins used in Experiment 1 and 10 nubbins used in Experiment 2. These coral fragments were then attached to ceramic tiles and acclimated in a common tank until used for the experiments. Although *O. arbuscula* used in our experiment does not inhabit the same environmental conditions as tropical reef-building corals, they are able to calcify (Rivera and Davies, 2021), making an effective model species for groundtruthing the use of $\delta^{15}N$ as a proxy for symbiosis in extant corals.

2.2 Laboratory experiments

Corals were maintained under common garden conditions, with a temperature of 25 ± 0.17°C, salinity of 34.6 ± 0.76, and pH of 8.0 ± 0.08 for 3–4 years leading up to and throughout the sampling period. Three months prior to each sampling date, one nubbin from each genet was menthol-bleached for 7 days in a 20% weight/volume menthol solution (Matthews et al., 2016). Following menthol bleaching, aposymbiotic fragments were housed in a separate tank from symbiotic fragments (Figure 1; Matthews et al., 2016; Wang et al., 2012). Each experimental tank was equipped with a powerhead (1321 gallons hr^{-1}) to ensure water circulation within the tank. Additionally, a large pump was used to recirculate water (4000 gallons hr^{-1}) among all experimental tanks. Water changes were conducted every other week, during which turf algal growth on the tank walls was scrubbed, and about 1/3 of the total tank volume was replaced. Two experiments, each lasting approximately 3 months, were conducted. Aposymbiotic and symbiotic fragments from the same genet were paired for multiple coral colonies (Experiment 1: 4 genets; Experiment 2: 5 genets). In both experiments, corals were fed the same diet (combined *Artemia nauplii* and commercial coral food) three times a week for the duration of each experiment.

2.3 Host/symbiont separation

Three months following menthol bleaching of aposymbiotic fragments, tissue samples were taken from a symbiotic and aposymbiotic nubbin from each genet using the same process outlined below (n=8 coral fragments for Experiment 1 and n=10

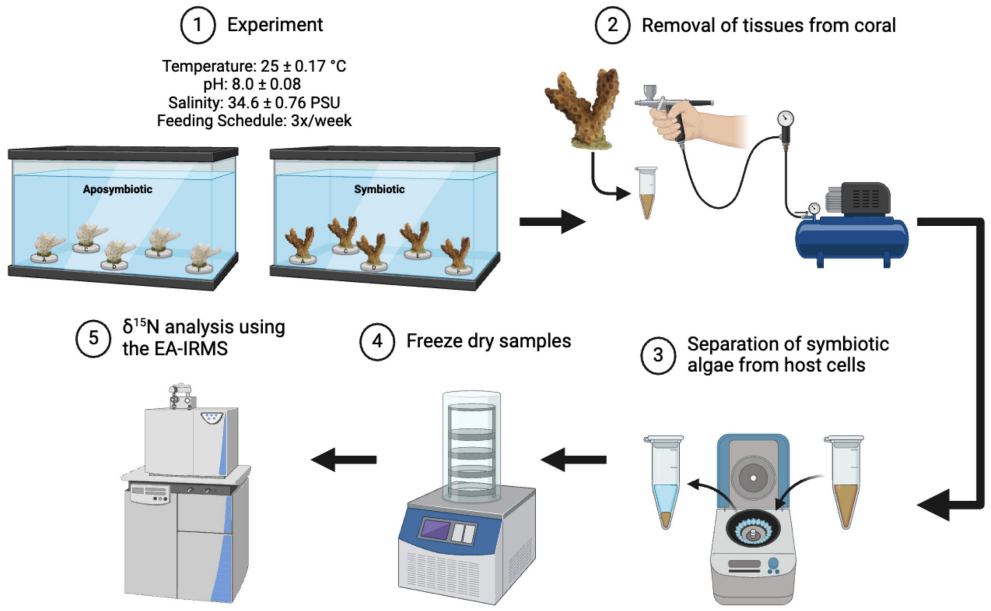

FIGURE 1
Illustration of experimental flow in the study.

coral fragments for Experiment 2). Briefly, a section of coral was detached and the surface of the coral tissue was removed via airbrushing using 0.2 micron filter sterilized artificial seawater (FSW, Instant Ocean) (Figure 1; Rivera et al., 2023; Aichelman et al., 2021). The tissue slurry was homogenized using a handheld tissue homogenizer for 3 minutes (Tissue-Tearor Model 985370, BioSpec Products, Inc.). A subsample of the slurry was preserved at -20°C as the 'Host + Symbiont' sample, which included the animal and algal symbiont cells for further analyses (detailed below). The remaining slurry underwent centrifugation at 3300 rpm for 10 minutes to separate the animal cells 'Host' (supernatant) from the Symbiodiniaceae 'Symbiont' (pellet) components. The 'Host' fraction was decanted, further purified via centrifugation at 12,000 rpm at 4°C for 10 minutes, transferred to a new tube, and stored at -80°C. The 'Symbiont' fraction was treated with 2 mL of 2N HCl to dissolve calcium carbonate particles. This pellet was then rinsed three times with FSW, each time followed by centrifugation at 3000 rpm for 3 minutes. After the final rinse, the supernatant was discarded, and the pellet was resuspended in 1.5 mL FSW and sieved through a 40-micron mesh. The filtrate was subjected to a final centrifugation at 12,000 rpm at 4 °C for 10 minutes. After this, the supernatant was discarded, and the remaining pellet was stored at -80°C.

2.4 Symbiont density analyses

The 'Host + Symbiont' aliquots were used to determine symbiont cell densities (cells mm^{-2}). Three replicates per coral genet and symbiosis type were measured using 10 µL of tissue slurry aliquots with a hemocytometer under a light microscope (Rodrigues and Grottoli, 2007). Counts were averaged, extrapolated to the total slurry volume, and normalized to coral tissue surface area (as measured by an Einscan-SE scanner and MeshLab software) to quantify symbiont densities for each coral fragment.

2.5 $\delta^{15}N$ analysis

All coral tissue samples were freeze-dried overnight (Figure 1). Samples were then homogenized and crushed to form a powder. These powdered samples were weighed into tin capsules (Kolasinski et al., 2008). Their nitrogen isotopic composition was analyzed on an isotope ratio mass spectrometer interfaced with a Flash*Smart*™ Elemental Analyzer (EA-IRMS; ThermoFisher Scientific; Figure 1). Although coral samples were separated into 'Host', 'Host + Symbiont', and 'Symbiont' fractions, only the 'Host' and 'Host + Symbiont' samples yielded sufficient mass for precise isotopic data. The 'Symbiont' fraction produced a peak intensity on the EA-IRMS that was only marginally higher than the blanks, rendering the data unusable. Consequently, we report only the $\delta^{15}N$ values of the 'Host' and 'Host + Symbiont' fractions in this paper. The precision of the method, based on replicates and standards, was approximately 0.2‰. The $\delta^{15}N$ of the food mixture was also measured alongside the coral samples.

2.6 Nitrate concentration and $\delta^{15}N$ analysis

To analyze the nitrate (NO_3^-) concentrations and $\delta^{15}N$ values of the water, samples were collected from the tank at the onset and conclusion of Experiment 2. Both NO_3^- concentration and $\delta^{15}N$ were determined using the denitrifier method (Sigman et al., 2001). Briefly, NO_3^- in the water sample was converted into N_2O using *Pseudomonas aureofaciens* in pre-purged 20 mL gas-tight vials. The concentration and $\delta^{15}N$ of the resulting N_2O were then analyzed on a customized Gas Bench coupled to an IRMS (GB-IRMS). NO_3^- isotope standards with known concentrations (IAEA-NO3 and USGS 34) were used to calibrate the NO_3^- $\delta^{15}N$ and concentrations of the samples. The precision of this method for NO_3^- $\delta^{15}N$ and concentration are better than 0.2‰ and 2%, respectively.

2.7 Statistical analysis

$\delta^{15}N$ for the 'Host' and 'Host + Symbiont' were assessed using a series of linear mixed effects models (*lmer*) for each (including fixed effects of experiment and symbiosis type) using a forward model selection method as described in Aichelman et al. (2021) using R Version 1.3.959 (R Core Team, 2017). A random effect of genet was included in all models to account for variation across genets. Assumptions of normality were assessed using a Shapiro–Wilk Test. Assumptions for $\delta^{15}N$ for the 'Host' and 'Host + Symbiont' were both met. The best fit model was derived by starting with the intercept-only model and then using forward-selection to incorporate additional parameters, starting with the most significant parameter, until further addition of parameters did not significantly improve the model fit. Additional parameters were retained in the model if they were significant ($p<0.05$) and produced smaller AIC values (Akaike, 1974). Comparisons between the $\delta^{15}N$ averages of the aposymbiotic 'Host' and the symbiotic 'Host' and 'Host + Symbiont' were performed using a Tukey's HSD *post-hoc* pairwise comparison for each experiment (*lsmeans*). A one-way ANOVA with a Tukey's HSD posthoc pairwise comparison were performed for significant effects from the linear mixed model for each genet in Prism GraphPad Version 10.2.3.

3 Results

3.1 Cell density

Symbiont densities were highest in symbiotic coral and lowest for aposymbiotic coral for both experiments, which was expected. Three abnormal symbiont densities were observed in aposymbiotic corals across all experiments, where the density was not the expected 0 cells mm^{-2}. In Experiment 1, the aposymbiotic coral for genet A showed a density of 65 cells mm^{-2}. In Experiment 2, the aposymbiotic corals for genet A and F had a cell density of 48 cells mm^{-2} and 124 cells mm^{-2}, respectively (Table 1). The cell densities for genet D and E in symbiotic corals in Experiment 2 were also lower, registering at 3,125 cells mm^{-2} and 5,626 cells mm^{-2}, respectively, compared to

10.3389/fmars.2024.1433382

TABLE 1 Aposymbiotic and symbiotic coral tissue symbiont count (cells mm^{-2}) for all genets in Experiments 1 and 2.

Genet	Experiment 1				Experiment 2				
	A	C	E	F	A	C	D	E	F
Aposymbiotic Coral	65	0	0	0	48	0	0	0	124
Symbiotic Coral	17,202	16,943	13,611	22,915	12,212	15,763	3,125	5,626	12,178

other symbiotic coral for all experiments that had an average cell density of 13,286 cells mm^{-2} (Table 1).

3.2 Nitrogen isotopes

Overall, both the $\delta^{15}N$ of the 'Host' and 'Host + Symbiont' for the corals exhibited distinct differences between the two types (i.e., symbiotic coral and aposymbiotic coral) in the first experiment ($p_{1,\ Apo\ 'Host'\ -\ Sym\ 'Host'} = 0.014$, $p_{1,\ Apo\ 'Host'\ -\ Sym\ 'Host+Sym'} = 0.0001$; Supplementary Table 1). Experiment 2 revealed a significant difference only between the aposymbiotic 'Host' and symbiotic 'Host + Symbiont' ($p_{2,\ Apo\ 'Host'\ -\ Sym\ 'Host+Sym'} = 0.0014$; Supplementary Table 1) and no differences between the aposymbiotic 'Host' and symbiotic 'Host' ($p_{2,\ Apo\ 'Host'\ -\ Sym\ 'Host'} = 0.36$; Supplementary Table 1). In each experiment, the $\delta^{15}N$ values of the symbiotic branches were consistently lower than those of the corresponding aposymbiotic branches (Figure 2). Between the two experiments (1 and 2), significant differences were observed for the 'Host' and 'Host + Symbiont' $\delta^{15}N$ ($p_{\ Apo\ 'Host'\ -\ Sym\ 'Host'} = 0.0023$, $p_{\ Apo\ 'Host'\ -\ Sym\ 'Host+Sym'} < 0.0001$; Table 2).

In Experiment 1, the $\delta^{15}N$ of the aposymbiotic 'Host' ranged from 10.3–11.4‰, while that of the symbiotic 'Host' ranged from 9.4–10.1‰ and the symbiotic 'Host + Symbiont' ranged from 6.4–8.5‰ (Figure 2A). Across all genets in the first experiment, there was a significant difference between the aposymbiotic 'Host', symbiotic 'Host', and the symbiotic 'Host + Symbiont' (Figure 2A; Supplementary Table 2). In Experiment 2, the $\delta^{15}N$ of all coral samples were lower than in Experiment 1, with the aposymbiotic 'Host' ranging from 8.1–10.4‰, the symbiotic 'Host' from 7.7–9.4‰, and the symbiotic 'Host + Symbiont' from 6.8–8.0‰ (Figure 2B). For all genets in Experiment 2, there was no significant difference between the aposymbiotic 'Host' and the symbiotic 'Host' except for genet F ($p_{F,\ Apo\ 'Host'\ -\ Sym\ 'Host'} = 0.029$; Supplementary Table 2). However, there was a significant difference between the aposymbiotic 'Host' and the symbiotic 'Host + Symbiont' as well as the symbiotic 'Host' and the symbiotic 'Host + Symbiont' (Figure 2B; Supplementary Table 2).

In both experiments, the $\delta^{15}N$ of the symbiotic 'Host + Symbiont' was >2‰ lower than the aposymbiotic 'Host'. When averaging the $\delta^{15}N$ of all genets for each experiment, the offset between the aposymbiotic 'Host' and the symbiotic 'Host + Symbiont' was 2.9‰ for Experiment 1 and 2.0‰ for Experiment 2. The difference for both experiments was significantly different (Experiment 1: $p = 0.0001$, t-ratio = 7.6, df = 10.0; Experiment 2: $p = 0.0014$, t-ratio = 5.4, df = 10.0; Supplementary Table 1; Figure 3). For Experiment 1, there was also a significant difference between the averages of the aposymbiotic 'Host' and symbiotic 'Host' ($p = 0.013$, t-ratio = 3.9, df = 10.0; Supplementary Table 1), and between the symbiotic 'Host' and symbiotic 'Host + Symbiont' ($p < 0.0001$, t-ratio = 7.9, df = 11.0; Supplementary Table 1). For Experiment 2, there was only an additional significant difference between the symbiotic 'Host' and symbiotic 'Host + Symbiont' ($p < 0.0001$, t-ratio = 7.9, df = 11.0; Supplementary Table 1). The $\delta^{15}N$ of the food mixture used during the duration of the study averaged to be 8.0 ± 2.0‰. In Experiment 2, water samples were collected at both the start and end for nitrate concentration and $\delta^{15}N$ measurements. The nitrate concentration increased from 2.3 ± 0.02 µM in the beginning to 14.2 ± 0.24 µM at the end. Nitrate $\delta^{15}N$ decreased from 14.5 ± 0.1‰ to 10.3 ± 0.1‰.

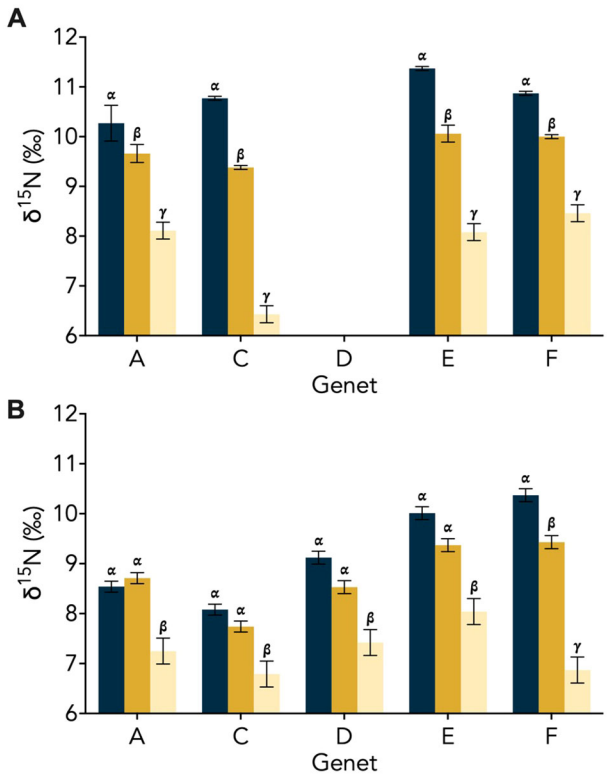

FIGURE 2
Genet $\delta^{15}N$ (± SD) for the aposymbiotic 'Host' (blue), symbiotic 'Host' (dark yellow), and symbiotic 'Host + Symbiont' (light yellow) for the *Oculina arbuscula* corals from **(A)** Experiment 1 and **(B)** Experiment 2. Different Greek letters (α, β, γ) indicate a significant difference ($p < 0.05$). For all variables with the same Greek letter, the difference between the means is not statistically significant.

10.3389/fmars.2024.1433382

TABLE 2 Model results for $\delta^{15}N$ for the aposymbiotic and symbiotic 'Host' as well as aposymbiotic 'Host' and symbiotic 'Host + Symbiont' (experiment and type).

Factor	Estimate	SE	DF	T-value	P-Value
$\delta^{15}N$ Aposymbiotic 'Host' and Symbiotic 'Host'					
Model = lmer(Host ~ experiment * type + 1|genet)					
Experiment (1 or 2)	-1.56	0.29	10.44	-5.35	0.00028
Type (Apo or Sym)	-1.88	0.66	10.21	-2.84	0.017
Type: Experiment	0.70	0.41	10.21	1.74	0.11
$\delta^{15}N$ Aposymbiotic 'Host' and Symbiotic 'Host + Sym'					
Model = lmer(Host + Sym ~ experiment * type + 1|genet)					
Experiment (1 or 2)	-1.60	0.39	10.74	-4.11	0.0018
Type (Apo or Sym)	-4.15	0.88	10.38	-4.70	0.00077
Type: Experiment	1.10	0.54	10.38	2.03	0.069

Models were determined from forward model selection and are written under to the metric being considered. SE, standard error; DF, degrees of freedom.

4 Discussion

4.1 Influence of symbiosis on coral $\delta^{15}N$

While coral $\delta^{15}N$ has been used as a proxy to determine the presence of coral symbiosis in fossil corals (Frankowiak et al., 2016; Tornabene et al., 2017), its efficacy has not been sufficiently groundtruthed in contemporary settings with extant corals. Using a facultatively symbiotic coral species under well-fed conditions in a controlled laboratory experiment, we observed that the $\delta^{15}N$ of the symbiotic fragments were lower than that of the aposymbiotic fragments while controlling for genetic background (Figure 2). This difference can be attributed to the internal N cycling between the coral host and its symbionts (Rädecker et al., 2015; Gustafsson et al., 2013; Houlbrèque and Ferrier-Pagès, 2009; Bednarz et al., 2017), which results in reduced or minimal low-$\delta^{15}N$ ammonium excretion and less ^{15}N enrichment in corals. Although the overall average $\delta^{15}N$ values were higher in the aposymbiotic 'Host' compared to both the symbiotic 'Host' and 'Host + Symbiont' (Figure 3), the differences between the aposymbiotic 'Host' and symbiotic 'Host' were not statistically significant in Experiment 2 (Figure 3). This may be attributed to the high frequency of feeding during the experiment, which will be discussed further in Section 4.3.

In the experiments, the average $\delta^{15}N$ of coral food was 8.0 ± 2.0‰. Given aposymbiotic corals lack symbionts, their excretion of low-$\delta^{15}N$ ammonium would lead to an increase of 3–4‰ in $\delta^{15}N$ relative to that of the food source under steady-state conditions (Deniro and Epstein, 1981; Minagawa and Wada, 1984; Glibert et al., 2019). The $\delta^{15}N$ differences between the aposymbiotic corals and their food averaged 2.7‰ in Experiment 1 and 1.2‰ in Experiment 2. The smaller offset in Experiment 2 may be attributed to the experimental duration. Specifically, the aposymbiotic corals had been stripped of their algal symbionts about 3 months prior and might require more time to acclimate and exhibit the 3‰ increase.

4.2 N heterotrophy vs. autotrophy in symbiotic corals

Aposymbiotic corals obtain N through heterotrophic means, despite recent research indicating that aposymbiotic coral have the potential to assimilate ammonium at a much lower rate than

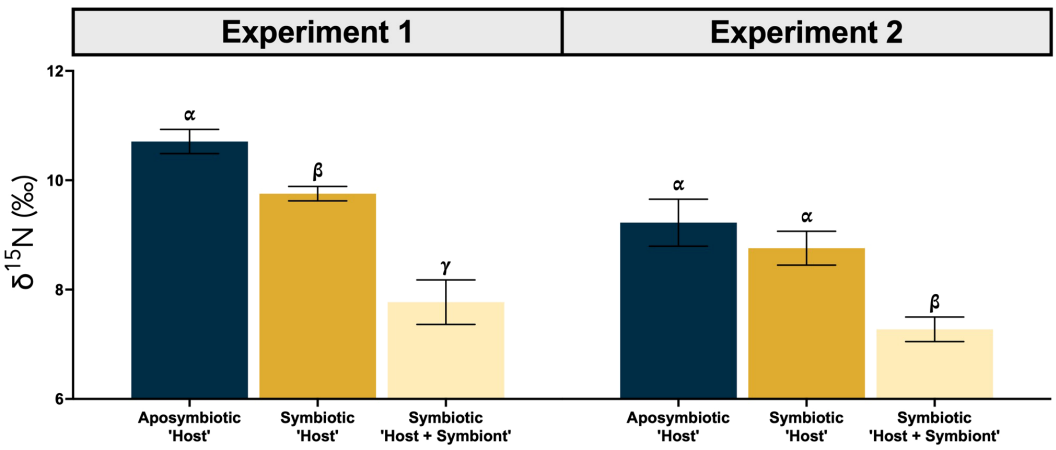

FIGURE 3
Average $\delta^{15}N$ (± SE) for the aposymbiotic 'Host' (blue), symbiotic 'Host' (dark yellow), and symbiotic 'Host + Symbiont' (light yellow) for the *Oculina arbuscula* corals from Experiment 1 (left) and 2 (right). Different Greek letters (α, β, γ) indicate a significant difference ($p < 0.05$). For all variables with the same Greek letter, the difference between the means is not statistically significant.

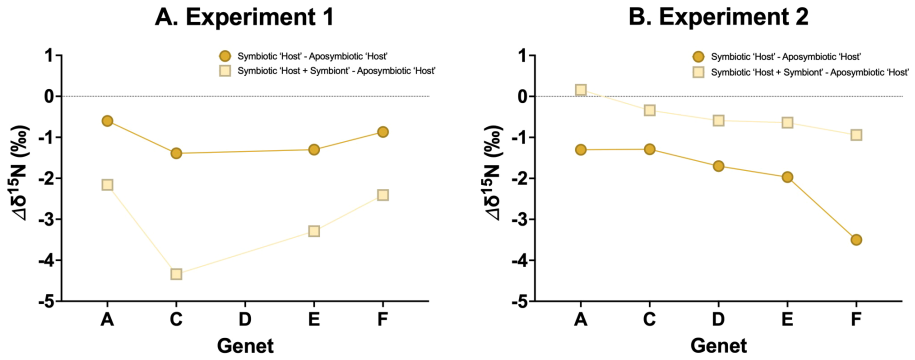

FIGURE 4
$\delta^{15}N$ differences ($\Delta\delta^{15}N$) between the aposymbiotic 'Host' and symbiotic 'Host' (dark yellow circles) and the aposymbiotic 'Host' and symbiotic 'Host + Symbiont' (light yellow squares) for **(A)** Experiment 1 and **(B)** Experiment 2.

symbiotic coral in high ambient DIN conditions (Cui et al., 2019). In contrast, symbiotic corals have been reported to obtain N through both heterotrophic feeding on particulate organic N (PON) and plankton (N heterotrophy; Gustafsson et al., 2013; Houlbrèque and Ferrier-Pagès, 2009) and autotrophic assimilation of dissolved inorganic N (DIN) from seawater via their intracellular algae (N autotrophy; Gustafsson et al., 2013; Cui et al., 2019). However, most experiments that explore DIN assimilation are conducted under little to no feeding and/or high DIN concentrations (Bythell, 1990; Hoegh-Guldberg and Williamson, 1999; Grover et al., 2002; 2003; Tanaka et al., 2006; Pernice et al., 2012; Kopp et al., 2013; Tanaka et al., 2015; Bednarz et al., 2017). Discrepancies exist in the literature regarding food availability and its influence on DIN assimilation (DiRoberts et al., 2021), although starvation (i.e., no feeding) could potentially increase DIN assimilation (Szmant-Froelich and Pilson, 1984; Grover et al., 2002). It is still unclear whether symbiotic corals rely on N heterotrophy vs. autotrophy in natural environments.

To minimize potential assimilation of DIN by corals, corals were purposely fed three times per week in our experiments, mimicking highly productive ocean environments where corals have access to plenty of food such as those of the central and eastern equatorial Pacific (Wang et al., 2016). This approach also reflected the natural habitat of *O. arbuscula*, which thrives in subtropical to temperate regions-environments that are nutrient-richer than that of oligotrophic waters (Gleason et al., 2018). This frequent feeding led to a build-up of nitrate in the tank and turf algae growth due to the degradation of coral food, which increased from 2.3 ± 0.02 µM to 14.0 ± 0.28 µM over the course of Experiment 2, despite periodic water replacement and turf algae removal. Nitrate $\delta^{15}N$ decreased from 14.5 ± 0.1‰ to 10.3 ± 0.1‰ in Experiment 2, inconsistent with assimilation of seawater nitrate by coral. In such nutrient-rich environments, it is energetically more favorable for symbionts to utilize ammonium excreted by the coral host rather than assimilating seawater nitrate, which must cross multiple membrane layers before being reduced to ammonium and taken up by the symbionts. Thus, in high-productivity ocean environments, coral N heterotrophy likely dominates over N autotrophy.

4.3 Ammonium leakage in well-fed symbiotic coral and its impact on coral $\delta^{15}N$

Most natural conditions in which corals live are oligotrophic in nature, leading to highly efficient N recycling within symbiotic corals with little to no ammonium leakage (O'Neil and Capone, 2008). As a result, symbiotic corals are expected to exhibit a ~3‰ lower $\delta^{15}N$ than co-existing aposymbiotic corals. However, in our experiments, the observed $\delta^{15}N$ offset between aposymbiotic coral and symbiotic coral generally falls below this 3‰ (Figures 4A, B), suggesting an underlying mechanism that warrants further investigation.

Previous studies have suggested that well-fed corals may exhibit increased ammonium leakage because the symbionts are unable to fully assimilate the ammonium excreted by the coral host (Szmant-Froelich and Pilson, 1984; Piniak et al., 2003; Piniak and Lipschultz, 2004; Erler et al., 2015; Wang et al., 2015), with the rate of ammonium leakage increasing with the frequency of feeding. This implies that an abundant food supply could lead to a rise in coral $\delta^{15}N$ even in symbiotic corals (Erler et al., 2015; Wang et al., 2015), which may explain the observations from our experiments. While aposymbiotic coral will remain consistent in their $\delta^{15}N$ regardless of food supply (Figure 5A), symbiotic corals may display a range in their $\delta^{15}N$ (Figures 5B, C). Specifically, in Experiment 2, in which both the aposymbiotic 'Host' and symbiotic 'Host' displayed more similar $\delta^{15}N$ values (Figure 4B), suggests ammonium leakage occurred in both coral types. Depending on the extent of this leakage, symbiotic corals could show a range of $\delta^{15}N$ elevations relative to their food source (Figure 5B). In scenarios with no leakage (i.e., in oligotrophic waters), coral $\delta^{15}N$ might not display any trophic enrichment relative to their food. In high leakage scenario (i.e., in highly productive environments), coral $\delta^{15}N$ could be up to ~3‰ higher than the $\delta^{15}N$ of their food.

4.4 Implications for coral $\delta^{15}N$ as a symbiosis proxy

To assess the utility of $\delta^{15}N$ as a proxy for coral symbiosis, we calculated the $\delta^{15}N$ offset ($\Delta\delta^{15}N$) between symbiotic and

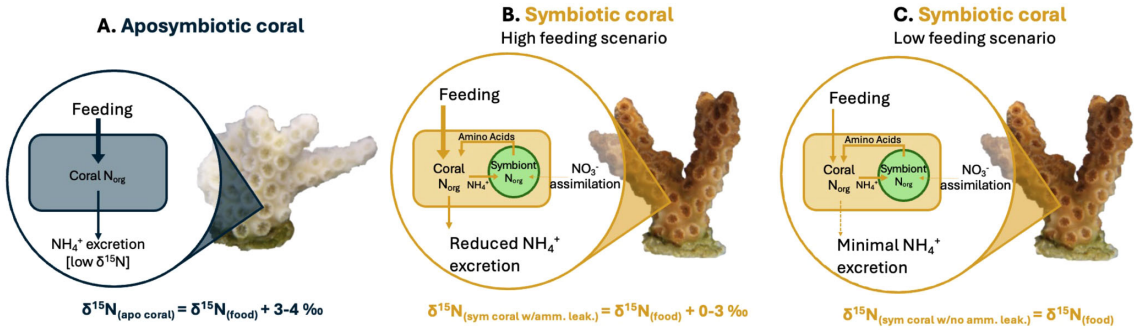

FIGURE 5
Cartoons showing the causes of $\delta^{15}N$ differences between **(A)** aposymbiotic coral, **(B)** symbiotic coral with reduced ammonium leakage, and **(C)** symbiotic coral with minimal ammonium leakage. The $\delta^{15}N$ value of a symbiotic coral with ammonium leakage will fall between the $\delta^{15}N$ of symbiotic coral with minimal ammonium leakage and the $\delta^{15}N$ of aposymbiotic coral. Note: coral growth is not included in this illustration. As most corals inhabit oligotrophic environments, the assimilation of inorganic nitrogen [i.e., nitrate (NO_3^-) or ammonium ($NH4^+$)] from seawater by the algal symbiont, Symbiodiniaceae, inside the symbiotic coral is considered negligible in this illustration. In all 3 scenarios the input arrow is thicker than the output arrow as a portion of the N is used for coral growth.

aposymbiotic corals. This offset can be used to determine the symbiotic state of the coral, with the $\Delta\delta^{15}N$ values ranged from -4 to 1‰ (Figure 6A). This range can be attributed to the degree of ammonium leakage, with a $\Delta\delta^{15}N$ close to 0‰ indicating full aposymbiosis and -4‰ suggesting full symbiosis.

Applying this framework to fossil corals requires analyzing multiple coral samples from a specific region. The highest $\delta^{15}N$ value within a fossil group can be designated as representing the 'fully aposymbiotic' state. The $\Delta\delta^{15}N$ from this baseline then helps determine the symbiotic state of the corals. To validate this framework, $\delta^{15}N$ data from a previous study using multiple species of Triassic corals from Antalya, Turkey was used (Frankowiak et al., 2016), which showed a similar $\Delta\delta^{15}N$ range as our experiments (Figure 6B), supporting the conclusion that that some of these fossil corals were indeed symbiotic.

5 Conclusion

As climate change impacts marine ecosystems, corals are increasingly expelling their symbiotic algae, leading to mass bleaching events and elevated mortality rates. Understanding the historical evolution between corals and their intracellular algal symbionts is crucial for the health of both present and future coral reefs. A primary challenge in tracing the evolution of coral symbiosis is that symbiotic algae are not preserved in the fossil record. Recent studies have used the $\delta^{15}N$ of fossil-bound organic matter as a proxy for symbiosis in Scleractinian corals as old as 210-million-years-old, uncovering evidence of early symbioses (Tornabene et al., 2017; Frankowiak et al., 2016). In our study, we employed the modern facultatively symbiotic coral, *Oculina*

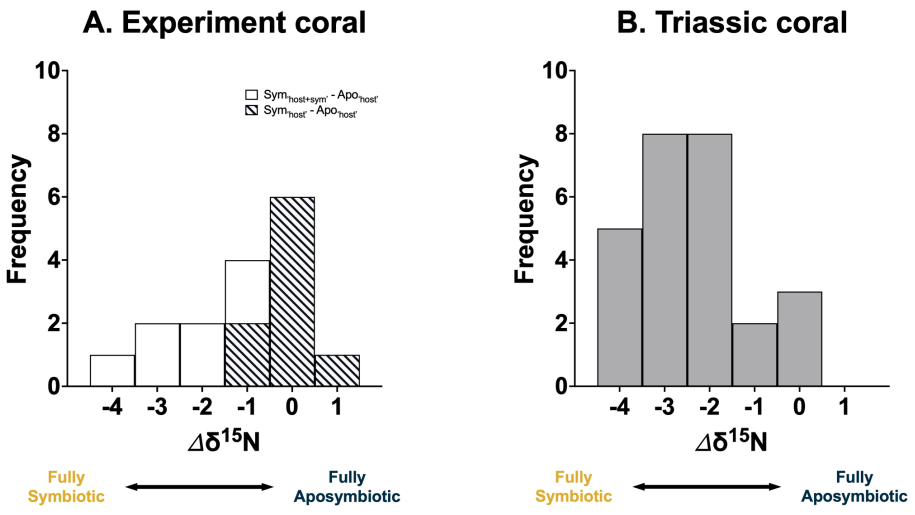

FIGURE 6
Frequency distribution of the observed coral $\delta^{15}N$ offset ($\Delta\delta^{15}N$) from the **(A)** 'Host + Symbiont' (white) and 'Host' (diagonal slash) of the facultatively symbiotic coral *Oculina arbuscula* under laboratory conditions from this study and **(B)** Triassic coral (grey) from Antalya, Turkey from a previous study (Frankowiak et al., 2016). Fully aposymbiotic corals release low-$\delta^{15}N$ ammonium and exhibit a higher $\delta^{15}N$. In contrast, fully symbiotic corals demonstrate high internal N recycling efficiency, minimal ammonium leakage, and lower $\delta^{15}N$.

arbuscula, to validate the $\delta^{15}N$ differences between aposymbiotic and symbiotic corals. We found that the $\delta^{15}N$ of the aposymbiotic corals was overall higher than that of the symbiotic corals, most likely due to internal N cycling facilitated by the symbiont. These experimental results support the use of $\delta^{15}N$ as an indirect proxy for coral symbiosis, applicable to both historical and contemporary settings. However, one limitation of our study is that the corals were maintained under well-fed conditions, which may not accurately represent the nutrient-poor environments typical of most coral reefs. Future research should aim to replicate these experiments under oligotrophic conditions to better simulate natural reef conditions.

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.

Ethics statement

The manuscript presents research on animals that do not require ethical approval for their study.

Author contributions

HD: Data curation, Formal analysis, Investigation, Methodology, Resources, Validation, Visualization, Writing – original draft, Writing – review & editing. MV: Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Resources, Writing – original draft, Writing – review & editing. ML: Data curation, Formal analysis, Investigation, Methodology, Writing – review & editing. HR: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – review & editing. CT: Data curation, Formal analysis, Investigation, Methodology, Writing – review & editing. SD: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing – review & editing. XW: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – review & editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. Funding was provided by the U.S. Army Engineer Research and Development Center to XW (W912HZ2020061-RA2), Boston College Start-up funds to XW, Boston University Start-up funds to SD, NSF funds to SD (IOS-SDS 1937650), BU URBAN (NSF NRT DGE 1735087), and NSF GRFP to MV.

Acknowledgments

We thank Linh Ta for helping in freeze drying the coral tissue samples.

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.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2024.1433382/full#supplementary-material

References

Aichelman, H. E., Bove, C. B., Castillo, K. D., Boulton, J. M., Knowlton, A. C., Nieves, O. C., et al. (2021). Exposure duration modulates the response of Caribbean corals to global change stressors. *Limnology Oceanography* 66, 3100–31155. doi: 10.1002/lno.11863

Akaike, H. (1974). A new look at the statistical model identification. *IEEE Trans. Automatic Control* 19, 716–723. doi: 10.1109/TAC.1974.1100705

Bednarz, V. N., Grover, R., Maguer, J. F., Fine, M., and Ferrier-Pagès, C. (2017). The assimilation of diazotroph-derived nitrogen by scleractinian corals depends on their metabolic status. *mBio* 8, 105. doi: 10.1128/mbio.02058-16

Burkepile, D. E., Shantz, A. A., Adam, T. C., Munsterman, K. S., Speare, K. E., Ladd, M. C., et al. (2020). Nitrogen identity drives differential impacts of nutrients on coral bleaching and mortality. *Ecosystems* 23, 798–811. doi: 10.1007/s10021-019-00433-2

Bythell, J. C. (1990). Nutrient uptake in the reef-building coral acropora palmata at natural environmental concentrations. *Mar. Ecol. Prog. Ser.* 68, 65–69. doi: 10.3354/meps068065

Capone, D. G., Bronk, D. A., Mulholland, M. R., and Carpenter, E. J. (2008). *Nitrogen in the Marine Environment. 2nd ed* (Burlington, MA: Academic Press).

Conti-Jerpe, I. E., Thompson, P. D., Wong, C. W. M., Oliveira, N. L., Duprey, N. N., Moynihan, M. A., et al. (2020). Trophic strategy and bleaching resistance in reef-building corals. *Sci. Adv.* 6, eaaz5443. doi: 10.1126/sciadv.aaz5443

Cui, G., Liew, Y. J., Li, Y., Kharbatia, N., Zahran, N. I., Emwas, A.-H., et al. (2019). Host-dependent nitrogen recycling as a mechanism of symbiont control in aiptasia. *PloS Genet.* 15, e10081895. doi: 10.1371/journal.pgen.1008189

D'Angelo, C., and Wiedenmann, J. (2014). Impacts of nutrient enrichment on coral reefs: new perspectives and implications for coastal management and reef survival. *Curr. Opin. Environ. Sustainability* 7, 82–93. doi: 10.1016/j.cosust.2013.11.029

D'Elia, C. F., Domotor, S. L., and Webb, K. L. (1983). Nutrient uptake kinetics of freshly isolated zooxanthellae. *Mar. Biol.* 75, 157–167. doi: 10.1007/BF00405998

Davy, S. K., Allemand, D., and Weis, V. M. (2012). Cell biology of cnidarian-dinoflagellate symbiosis. *Microbiol. Mol. Biol. Rev.* 76, 229–615. doi: 10.1128/mmbr.05014-11

Deniro, M. J., and Epstein, S. (1981). Influence of diet on the distribution of nitrogen isotopes in animals. *Geochimica Cosmochimica Acta* 45, 341–515. doi: 10.1016/0016-7037(81)90244-1

DiRoberts, L. E., Dudek, A., Ray, N. E., Fulweiler, R. W., and Rotjan, R. D. (2021). Testing assumptions of nitrogen cycling between a temperate, model coral host and its facultative symbiont: symbiotic contributions to dissolved inorganic nitrogen assimilation. *Mar. Ecol. Prog. Ser.* 670, 61–74. doi: 10.3354/meps13731

Z. Dubinsky and N. Stambler (Eds.) (2011). *Coral Reefs: An Ecosystem in Transition* (Dordrecht: Springer Netherlands). doi: 10.1007/978-94-007-0114-4

Eddy, T. D., Lam, V. W.Y., Reygondeau, G., Cisneros-Montemayor, A. M., Greer, K., Palomares, M. L. D., et al. (2021). Global decline in capacity of coral reefs to provide ecosystem services. *One Earth* 4, 1278–1855. doi: 10.1016/j.oneear.2021.08.016

Erler, D. V., Wang, X. T., Sigman, D. M., Scheffers, S. R., and Shepherd, B. O. (2015). Controls on the nitrogen isotopic composition of shallow water corals across a tropical reef flat transect. *Coral Reefs* 34, 329–385. doi: 10.1007/s00338-014-1215-5

Falkowski, P. G., Dubinsky, Z., Muscatine, L., and Porter, J. W. (1984). Light and the bioenergetics of a symbiotic coral. *BioScience* 34, 705–795. doi: 10.2307/1309663

Frankowiak, K., Wang, X. T., Sigman, D. M., Gothmann, A. M., Kitahara, M. V., Mazur, M., et al. (2016). Photosymbiosis and the expansion of shallow-water corals. *Sci. Adv.* 2, e16011225. doi: 10.1126/sciadv.1601122

Gleason, D. F., Harbin, L. R., Divine, L. M., and Matterson, K. O. (2018). The role of larval supply and competition in controlling recruitment of the temperate coral *oculina arbuscula*. *J. Exp. Mar. Biol. Ecol.* 506, 107–114. doi: 10.1016/j.jembe.2018.06.006

Glibert, P. M., Middelburg, J. J., McClelland, J. W., and Zanden, M.J. V. (2019). Stable isotope tracers: enriching our perspectives and questions on sources, fates, rates, and pathways of major elements in aquatic systems. *Limnology Oceanography* 64, 950–815. doi: 10.1002/lno.11087

Goldberg, J., and Wilkinson, C. (2004). Global threats to coral reefs: coral bleaching, global climate change, disease, predator plagues, and invasive species. *Status of coral reefs of the world* 26, 67–92.

Grover, R., Maguer, J. F., Allemand, D., and Ferrier-Pagés, C. (2003). Nitrate uptake in the scleractinian coral stylophora pistillata. *Limnology Oceanography* 48, 2266–2745. doi: 10.4319/lo.2003.48.6.2266

Grover, R., Maguer, J. F., Allemand, D., and Ferrier-Pagès, C. (2008). Uptake of dissolved free amino acids by the scleractinian coral stylophora pistillata. *J. Exp. Biol.* 211, 860–655. doi: 10.1242/jeb.012807

Grover, R., Maguer, J. F., Reynaud-Vaganay, S., and Ferrier-Pagès, C. (2002). Uptake of ammonium by the scleractinian coral stylophora pistillata: effect of feeding, light, and ammonium concentrations. *Limnology Oceanography* 47, 782–905. doi: 10.4319/lo.2002.47.3.0782

Gustafsson, M. S. M., Baird, M. E., and Ralph, P. J. (2013). The interchangeability of autotrophic and heterotrophic nitrogen sources in scleractinian coral symbiotic relationships: A numerical study. *Ecol. Model.* 250, 183–194. doi: 10.1016/j.ecolmodel.2012.11.003

Hoegh-Guldberg, O., and Williamson, J. (1999). Availability of two forms of dissolved nitrogen to the coral pocillopora damicornis and its symbiotic zooxanthellae. *Mar. Biol.* 133, 561–570. doi: 10.1007/s002270050496

Houlbrèque, F., and Ferrier-Pagès, C. (2009). Heterotrophy in tropical scleractinian corals. *Biol. Rev.* 84, 1–175. doi: 10.1111/j.1469-185X.2008.00058.x

Hughes, T. P., Kerry, J. T., Baird, A. H., Connolly, S. R., Dietzel, A., Eakin, C.M., et al. (2018). Global warming transforms coral reef assemblages. *Nature* 556, 492–496. doi: 10.1038/s41586-018-0041-2

Kolasinski, J., Rogers, K., and Frouin, P. (2008). Effects of acidification on carbon and nitrogen stable isotopes of benthic macrofauna from a tropical coral reef. *Rapid Commun. Mass Spectrometry* 22, 2955–2605. doi: 10.1002/rcm.3694

Kopp, C., Pernice, M., Domart-Coulon, I., Djediat, C., Spangenberg, J. E., Alexander, D. T. L., et al. (2013). Highly dynamic cellular-level response of symbiotic coral to a sudden increase in environmental nitrogen. *mBio* 4. doi: 10.1128/mbio.00052-13

LaJeunesse, T. C., Parkinson, J. E., Gabrielson, P. W., Jeong, H. J., Reimer, J. D., Voolstra, C. R., et al. (2018). Systematic revision of symbiodiniaceae highlights the antiquity and diversity of coral endosymbionts. *Curr. Biol.* 28, 2570–25805.e6. doi: 10.1016/j.cub.2018.07.008

Li, M., Sheng, H.-X., Dai, M., and Kao, S.-J. (2023). Understanding nitrogen dynamics in coral holobionts: comprehensive review of processes, advancements, gaps, and future directions. *Front. Mar. Sci*. 10 (2023), 1203399. doi: 10.3389/fmars.2023.1203399

Madin, J. S., Anderson, K. D., Andreasen, M. H., Bridge, T. C.L., Cairns, S. D., Connolly, S. R., et al. (2016). The coral trait database, a curated database of trait information for coral species from the global oceans. *Sci. Data* 3, 160017. doi: 10.1038/sdata.2016.17

Matthews, J. L., Sproles, A. E., Oakley, C. A., Grossman, A. R., Weis, V. M., and Davy, S. K. (2016). Menthol-induced bleaching rapidly and effectively provides experimental aposymbiotic sea anemones (Aiptasia sp.) for symbiosis investigations. *J. Exp. Biol.* 219, 306–105. doi: 10.1242/jeb.128934

Minagawa, M., and Wada, E. (1984). Stepwise enrichment of 15N along food chains: further evidence and the relation between δ15N and animal age. *Geochimica Cosmochimica Acta* 48, 1135–1405. doi: 10.1016/0016-7037(84)90204-7

O'Neil, J. M., and Capone, D. G. (2008). "Nitrogen cycling in coral reef environments," in *Nitrogen in the Marine Environment* (30 Corporate Drive, Suite 400, Burlington, MA 01803, USA; Radarweg 29, PO Box 211, 1000 AE Amsterdam, The Netherlands; 525 B Street, Suite 1900, San Diego, California 92101-4495, USA; 84 Theobald's Road, London WC1X 8RR, UK: Elsevier), 949–989. doi: 10.1016/B978-0-12-372522-6.00021-9

Pernice, M., Meibom, A., Heuvel, A. V. D., Kopp, C., Domart-Coulon, I., Hoegh-Guldberg, O., et al. (2012). A single-cell view of ammonium assimilation in coral–dinoflagellate symbiosis. *ISME J.* 6, 1314–1245. doi: 10.1038/ismej.2011.196

Piniak, G. A., and Lipschultz, F. (2004). Effects of nutritional history on nitrogen assimilation in congeneric temperate and tropical scleractinian corals. *Mar. Biol.* 145, 1085–1965. doi: 10.1007/s00227-004-1410-y

Piniak, G. A., Lipschultz, F., and McClelland, J. (2003). Assimilation and partitioning of prey nitrogen within two anthozoans and their endosymbiotic zooxanthellae. *Mar. Ecol. Prog. Ser.* 262, 125–136. doi: 10.3354/meps262125

Pochon, X., Montoya-Burgos, J. I., Stadelmann, B., and Pawlowski, J. (2006). Molecular phylogeny, evolutionary rates, and divergence timing of the symbiotic dinoflagellate genus symbiodinium. *Mol. Phylogenet. Evol.* 38, 20–305. doi: 10.1016/j.ympev.2005.04.028

R Core Team. (2017). "R: A Language and Environment for Statistical Computing." *Vienna, Austria: R Foundation for Statistical Computing*. https://www.R-project.org/.

Rädecker, N., Pogoreutz, C., Voolstra, C. R., Wiedenmann, J., and Wild, C. (2015). Nitrogen cycling in corals: the key to understanding holobiont functioning? *Trends Microbiol.* 23, 490–975. doi: 10.1016/j.tim.2015.03.008

Reddy, K. R., and DeLaune, R. D. (2008). *Biogeochemistry of Wetlands: Science and Applications* (Boca Raton: CRC Press).

Rivera, H. E., and Davies, S. W. (2021). Symbiosis maintenance in the facultative coral, oculina arbuscula, relies on nitrogen cycling, cell cycle modulation, and immunity. *Sci. Rep.* 11, 21226. doi: 10.1038/s41598-021-00697-6

Rivera, H. E., Tramonte, C. A., Samaroo, J., Dickerson, H., and Davies, S. W. (2023). Heat challenge elicits stronger physiological and gene expression responses than starvation in symbiotic oculina arbuscula. *J. Heredity* 114, 312–255. doi: 10.1093/jhered/esac068

Rodrigues, L. J., and Grottoli, A. G. (2007). Energy reserves and metabolism as indicators of coral recovery from bleaching. *Limnology Oceanography* 52, 1874–1825. doi: 10.4319/lo.2007.52.5.1874

Rosenberg, E., Koren, O., Reshef, L., Efrony, R., and Zilber-Rosenberg, I. (2007). The role of microorganisms in coral health, disease and evolution. *Nat. Rev. Microbiol.* 5, 355–625. doi: 10.1038/nrmicro1635

Sigman, D. M., Casciotti, K. L., Andreani, M., Barford, C., Galanter, M., and Böhlke, J. K. (2001). A bacterial method for the nitrogen isotopic analysis of nitrate in seawater and freshwater. *Analytical Chem.* 73, 4145–4153. doi: 10.1021/ac010088e

Stanley, G. D. (2003). The evolution of modern corals and their early history. *Earth-Science Rev.* 60, 195–225. doi: 10.1016/S0012-8252(02)00104-6

Stanley, G. D., and Swart, P. K. (1995). Evolution of the coral-zooxanthellae symbiosis during the triassic: A geochemical approach. *Paleobiology* 21, 179–995. doi: 10.1017/S0094837300013191

Szmant-Froelich, A., and Pilson, M. E. Q. (1984). Effects of feeding frequency and symbiosis with zooxanthellae on nitrogen metabolism and respiration of the coral astrangia danae. *Mar. Biol.* 81, 153–162. doi: 10.1007/BF00393114

Tanaka, Y., Grottoli, A. G., Matsui, Y., Suzuki, A., and Sakai, K. (2015). Partitioning of nitrogen sources to algal endosymbionts of corals with long-term 15N-labelling and a mixing model. *Ecol. Model.* 309–310, 163–169. doi: 10.1016/j.ecolmodel.2015.04.017

Tanaka, Y., Miyajima, T., Koike, I., Hayashibara, T., and Ogawa, H. (2006). Translocation and conservation of organic nitrogen within the coral-zooxanthella symbiotic system of acropora pulchra, as demonstrated by dual isotope-labeling techniques. *J. Exp. Mar. Biol. Ecol.* 336, 110–195. doi: 10.1016/j.jembe.2006.04.011

Tornabene, C., Martindale, R. C., Wang, X. T., and Schaller, M. F. (2017). Detecting photosymbiosis in fossil scleractinian corals. *Sci. Rep.* 7, 94655. doi: 10.1038/s41598-017-09008-4

Wang, J.-T., Chen, Y.-Y., Tew, K. S., Meng, P.-J., and Chen, C. A. (2012). Physiological and biochemical performances of menthol-induced aposymbiotic corals. *PloS One* 7, e464065. doi: 10.1371/journal.pone.0046406

Wang, X. T., Sigman, D. M., Cohen, A. L., Sinclair, D. J., Sherrell, R. M., Cobb, K. M., et al. (2016). Influence of open ocean nitrogen supply on the skeletal δ15N of modern shallow-water scleractinian corals. *Earth Planetary Sci. Lett.* 8, 125–132. doi: 10.1016/j.epsl.2016.02.032

Wang, X. T., Sigman, D. M., Cohen, A. L., Sinclair, D. J., Sherrell, R. M., Weigand, M. A., et al. (2015). Isotopic composition of skeleton-bound organic nitrogen in reef-building symbiotic corals: A new method and proxy evaluation at Bermuda. *Geochimica Cosmochimica Acta* 148, 179–190. doi: 10.1016/j.gca.2014.09.017

Weis, V. M., Davy, S. K., Hoegh-Guldberg, O., Rodriguez-Lanetty, M., and Pringle, J. R. (2008). Cell biology in model systems as the key to understanding corals. *Trends Ecol. Evol.* 23, 369–765. doi: 10.1016/j.tree.2008.03.004

Wiedenmann, J., D'Angelo, C., Loreto Mardones, M., Moore, S., Benkwitt, C. E., Graham, N. A.J., et al. (2023). Reef-building corals farm and feed on their photosynthetic symbionts. *Nature* 620, 1018–1024. doi: 10.1038/s41586-023-06442-5

Woodhead, A. J., Hicks, C. C., Norström, A. V., Williams, G. J., and Graham, N. A. J. (2019). Coral reef ecosystem services in the anthropocene. *Funct. Ecol.* 33, 1023–1034. doi: 10.1111/1365-2435.13331

Yellowlees, D., Rees, T.A. V., and Leggat, W. (2008). Metabolic interactions between algal symbionts and invertebrate hosts. *Plant Cell Environ.* 31, 679–945. doi: 10.1111/j.1365-3040.2008.01802.x