Nitrogen Isotope Analysis of Nitrogen Cycling in Coral Host Tissue and Algal Symbionts: A Study of Acropora Spp. and Stylophora Spp. near the Fulong Reefs in Taiwan

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Plain Language Summary

This study investigated how nitrogen levels vary in different parts of coral and the algae that live within their coral cells. Samples were collected from nine coral heads of two coral types, Acropora spp. and Stylophora spp., near the Fulong coral reefs in Northern Taiwan in August 2024. Each sample was separated into three different parts: the coral host tissue, the algae living in the coral, and the whole coral, which includes other microorganisms involved in nitrogen exchange. Nitrogen levels were measured using a method that turns nitrogen within each sample into a gas for more precise analysis. The study found that the nitrogen levels in the coral tissue and the algae were different from each other, indicating that corals feed their symbionts indirectly. The nitrogen levels in the algae were closely linked to the levels in the whole coral, suggesting algae exert a larger influence on the whole coral's nitrogen levels than the coral host. The study also found that Acropora spp. corals had higher nitrogen levels compared to Stylophora spp. This likely happens because Acropora spp. have different feeding habits and energy needs compared to Stylophora spp. This study is important because it adds to the growing body of knowledge of how nitrogen moves between coral and the algae, something that needs to be fully understood to help save corals from climate change.

Abstract

This study investigated the differences in $\delta^{15}N$ values across Coral Host Tissue, Algal Symbionts, and Whole Tissue Sample in two coral genera, Acropora spp. and Stylophora spp., collected from a harbor structure at the National Taiwan Ocean University Aquatic Biological Research and Conservation Center near Fulong, Northern Taiwan in August 2024. Nine coral heads were sampled, subdivided into tissue fractions, and analyzed for stable nitrogen isotope composition using the denitrifier method, which reduces the sample's nitrate (NO_3^-) to nitrous oxide (N_2O). Using nitrogen isotopes ($\delta^{15}N$) as a nutrient tracer, this study explored three hypotheses regarding nitrogen cycling within the coral holobiont: the Host Coral provides nitrogen to the Algal Symbionts directly through the system, the Host Coral provides nitrogen to the Algal Symbionts indirectly through the system, or the Algal Symbionts do not receive nitrogen from the Host Coral but instead fix dissolved inorganic nitrogen from the environment. Significant differences in $\delta^{15}N$ were found between Coral Host Tissue and Algal Symbionts, suggesting that corals feed their symbionts indirectly. Genus-specific differences were also observed, with Acropora spp. exhibiting higher $\delta^{15}N$ values across tissue types compared to Stylophora spp., likely due to variations in trophic strategies, metabolic demands, and morphological characteristics. Additionally, this study found that Algal Symbionts have a greater influence on Whole Tissue Samples $\delta^{15}N$ than Coral Host Tissue, reflecting their varying diet and nutrient uptake patterns. This study contributes to the growing body of knowledge on coral nitrogen cycling, emphasizing how nitrogen isotopes can illuminate the complex interplay between coral hosts and their algal symbionts.

Introduction

Coral reefs are the cornerstone of a crucial marine ecosystem that keeps our ocean healthy (NOAA, 2019). Corals are able to thrive in nutrient poor environments (oligotrophic waters) because of their symbiotic relationship with Zooxanthellae, a type of microalgae that resides within coral cells. These zooxanthellae capture solar energy through photosynthesis, providing corals with over 95% of their metabolic needs, which supports coral growth and the expansion of reef colonies (Hoegh-Guldberg et al., 2007). Coral reefs contribute heavily to the ecosystem by excreting ammonium, an important nutrient that supports other organisms (Lema et al., 2012) . They also provide crucial shelter to support a diverse range of species, who thrive in oligotrophic waters where life might not otherwise flourish due to nutrient scarcity (NOAA, 2019).

Corals face immense threats as environmental stressors, such as ocean acidification and rising temperatures, continue to worsen. These pressures have caused widespread coral bleaching, leading to significant ecological and economic consequences. Fisheries have collapsed due to the loss of living coral, and coastal erosion has intensified as corals lose their ability to buffer waves (NOAA, 2019). This weakening is caused by a reduction in calcium carbonate density, driven by elevated CO_2 levels as the ocean absorbs the excess greenhouse gasses produced by human activity (Hoegh-Guldberg et al., 2007).

Coral resilience, the ability of coral to withstand, recover from, or adapt to environmental stressors, is a complex concept that needs more research. Genetic factors, symbiotic relationships (Bay et al., 2017), and physiological plasticity (Oppen et al., 2017) all play a role in determining how corals cope with stress. Nitrogen cycling within the coral holobiont is increasingly gaining attention due to it being a critical factor that affects coral health and resilience (Fujii et al., 2020). Nitrogen plays an essential role in coral metabolism, and understanding the mechanisms by which nitrogen is cycled within the coral-algal partnership remains poorly understood even though it is fundamental to understanding coral health.

The process of nitrogen recycling within the coral holobiont is driven by the mutualistic relationship between coral hosts and their symbiotic zooxanthellae. The holobiont consists not only of the coral and zooxanthellae but also other microorganisms, such as fungi, diazotrophs, nitrifiers, and denitrifiers which all participate in the nitrogen exchange (Fujii et al., 2020). Corals primarily obtain nitrogen from organic sources, such as prey, and dissolved inorganic nitrogen from surrounding seawater (Badgley et al., 2006). The zooxanthellae require nitrogen, mainly in the form of ammonium, which is excreted by the coral as waste (Lema et al., 2012). The coral also provides zooxanthellae with a protected environment and compounds necessary for photosynthesis. In return for this protection, the coral benefits from the zooxanthellae symbionts, which supply the coral with photosynthetically-derived carbon, a crucial food source for the coral (Lema et al., 2012). Additionally, in nitrogen-poor conditions, diazotrophs which are microorganisms (bacteria or archaea) that may also be part of the coral holobiont can fix atmospheric nitrogen by converting it into ammonium, a biologically usable form (Rädecker et

al., 2015). This process provides an extra nitrogen source for the holobiont, which is especially important in nutrient-limited environments like coral reefs (Fujii et al., 2020). Finally, the role of nitrifiers and denitrifiers are also essential for maintaining holobiont function. Nitrifiers are microbes that convert ammonium, a byproduct of coral and zooxanthellae metabolism, into nitrate, which is the form of nitrogen most easily uptaken by the coral host (Rädecker et al., 2015). On the other hand, denitrifiers are a microbe that regulates nitrogen levels by removing excess nitrate and converting it into nitrogen gas, which is then released into the surrounding seawater (Rädecker et al., 2015).

The efficiency of nitrogen recycling within the coral holobiont can be tracked by the system's nitrogen isotopic signature ($\delta^{15}N$). Variations in $\delta^{15}N$ between the coral host and its symbionts can reveal differences in food sources, thereby providing insights into the efficiency of nitrogen recycling within the holobiont (Ren et al., 2017). This study tests three hypotheses related to nitrogen cycling within the coral holobiont. The first hypothesis proposes the host coral provides nitrogen directly to the algal symbionts, indicated by similar $\delta^{15}N$ values, suggesting a shared food system and balanced nitrogen exchange. The second hypothesis suggests the host coral provides nitrogen indirectly to the algal symbionts, evident by significant differences in $\delta^{15}N$ values, implying that the symbionts are utilizing nitrogen excreted by bacteria within the holobiont. The third hypothesis proposes that the algal symbionts do not receive nitrogen from the host coral but instead fix dissolved inorganic nitrogen from the environment.

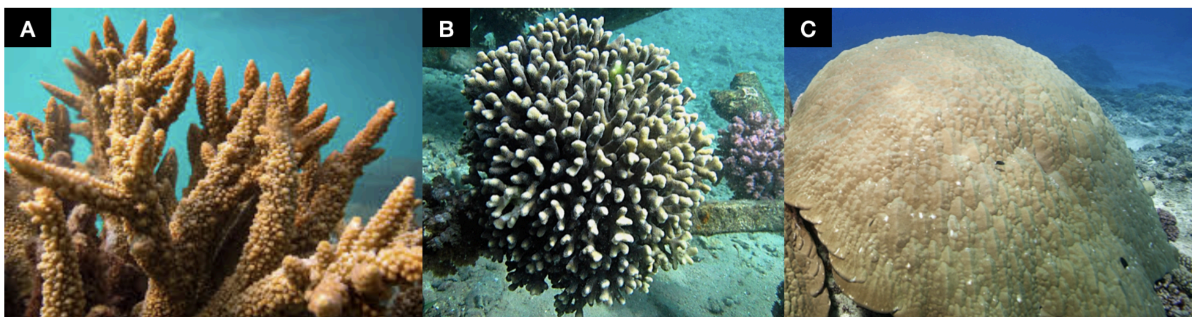

Figure 1. Images of different coral genera from *Corals of the World* Genus Identification (Corals of the World, 2010). **(A)** Acropora spp. coral. **(B)** Stylophora spp. coral. **(C)** Porites spp. coral.

In addition to examining Coral Host to Algal Symbiont dynamics, this study also considers genus-specific differences in $\delta^{15}N$, which reflect variations in trophic strategies, metabolic demands, and structural characteristics. Previous studies have suggested that the coral's microenvironment can further influence the efficiency of nitrogen exchange. Surrounding the coral is a thin layer of seawater called the diffusive boundary layer (DBL), whose thickness can affect the coral's ability to exchange gases and nutrients with the surrounding seawater (Fujii et al., 2020). Branched corals like Acropora spp. (Figure 1A) have a reduced DBL due to their increased surface area, which enhances nutrient exchange and leads to more efficient uptake of inorganic nitrogen from the surrounding seawater. This can result in lower $\delta^{15}N$ values in their tissues. In contrast, massive corals like Porites spp. (Figure 1C) has a thicker DBL, which limits

nutrient exchange and could cause higher $\delta^{15}N$ values (Fujii et al., 2020). While some research has explored the influence of the DBL on $\delta^{15}N$, there is even less research on nitrogen recycling between trophic levels (from Coral Host Tissue to Algal Symbionts), particularly regarding whether this process occurs directly or indirectly and if it is influenced by nitrogen trophic fractionation. Nitrogen trophic fractionation refers to the isotopic difference in nitrogen between the different trophic levels, which typically results in higher $\delta^{15}N$ values at higher trophic levels due to preferential incorporation of lighter nitrogen isotopes ($\delta^{14}N$) during metabolism (Denk et al., 2017). Additionally, there is a lack of research examining potential differences in nitrogen cycling across different coral genera.

This research aims to close these gaps by investigating the nitrogen isotopic signatures in coral host tissues, algal symbionts, and whole tissue samples from two distinct coral genera, Acropora spp. and Stylophora spp., collected near the Fulong Reefs at the northern tip of Taiwan. In addition to testing the Coral Host to Algal Symbiont hypothesis, distinct differences in $\delta^{15}N$ values are expected to be observed between coral host tissue, symbiotic algae, and whole coral tissue across different genera, reflecting the varied nitrogen dynamics and nutritional strategies used by each genus. To gain further insight into nitrogen utilization in these genera, nine coral heads were sampled, subdivided into tissue fractions (Coral Host Tissue, Algal Symbionts, and Whole Tissue Samples), and analyzed using the Denitrifier Method (Wang et al., 2015) to extract the nitrogen isotopic signature ($\delta^{15}N$). By exploring this research gap, this study seeks to contribute to the growing body of knowledge on coral nitrogen cycling, with a focus on how nitrogen isotopes can shed light on the interplay between coral hosts and their corresponding algal symbionts. This is more crucial than ever as climate change intensifies, making the understanding of nitrogen dynamics essential for assessing coral health, ensuring the sustainability of coral reef ecosystems, and identifying strategies to enhance coral resilience in the face of environmental stressors.

Method

Hydrographic Setting

Northern Taiwan is a subtropical region characterized by diverse coral reef ecosystems (Chen et al., 2021). In the summer, southwesterly winds produce a rainy season in the North, while in the winter, northeasterly winds prevail creating drier conditions (Britannica, 2019). Typhoons are frequent in the late summer to early fall (Britannica, 2019). On average, Taiwan gets 3.5 Typhoons per year (Chen et al., 2021). Average summer rainfall is around 328 mm with 17 to 19 rainy days each month (Chen et al., 2021). Winter rainfall is considerably lower, averaging at 35 mm (Central Weather Administration, 2023). Summer temperatures peak at 36.8°C, with lows around 22.9°C, while winter temperatures typically range from 10°C to 28°C (Chen et al., 2021). Sea surface temperatures range between 27.7°C and 29.5°C in the summer. In 2024, Taiwan recorded its warmest year on record, with an average annual temperature of

24.97°C, surpassing the previous high of 24.91°C set in 2020 (Taiwannews.com.tw, 2024). In addition, the year 2024 marked a mass coral bleaching event, as sea surface temperatures peaked at 31°C (NOAA, 2024).

Field Sample Collection

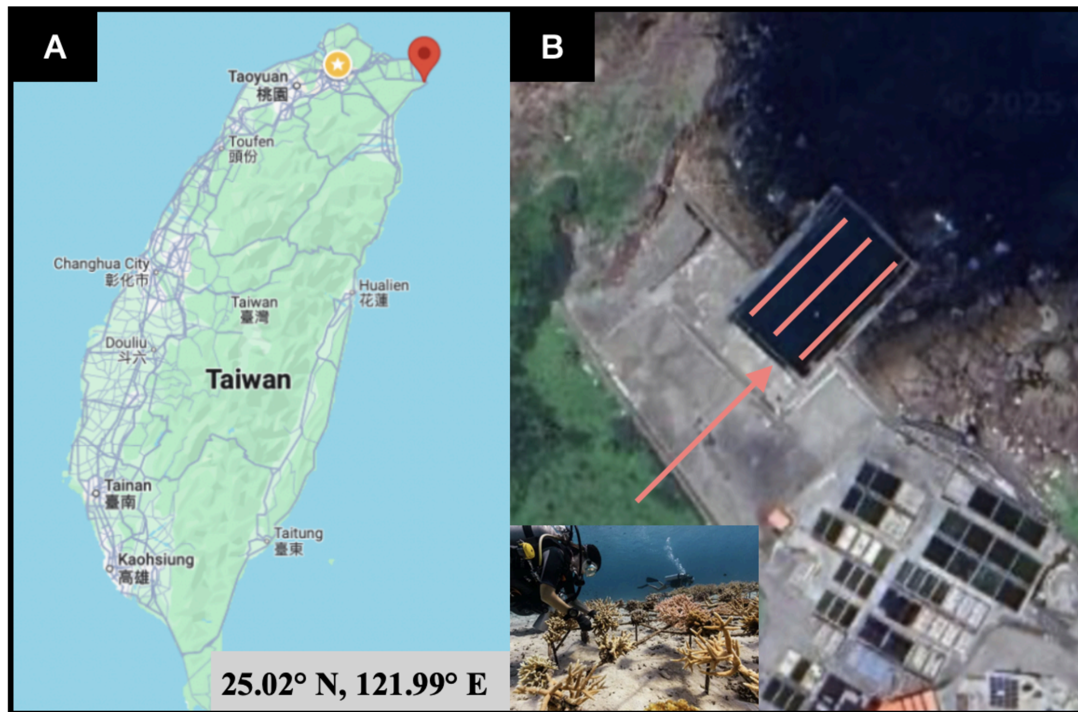

Figure 2. (A) Map showing the harbor's location in Northern Taiwan. **(B)** Aerial view of the National Taiwan Ocean University Aquatic Biological Research and Conservation Center's old harbor structure, where nine non-bleached coral heads were collected.

On August 5th, 2024, nine non-bleached coral heads were collected from an old harbor structure at National Taiwan Ocean University Aquatic Biological Research and Conservation Center (Figure 2). Corals were collected at 3.5 meters deep in 29°C seawater. Since 2024 was a mass bleaching year, several corals in the structure were bleached by August 5th. However, effort was made to ensure that only non-bleached coral heads were collected. Two-inch piece cuts were made from six Acropora spp., three Stylophora spp., and one Porites spp. A high pressure spray gun filled with low nitrogen sea water (LNSW) was immediately used to remove all the coral tissue from the coral skeleton. The tissue slurry was then collected into sample tubes, while the coral skeleton was wrapped in plastic wrap and placed into sample bags.

Sample Preparation

Coral samples were processed using a homogenizer processor. Each sample was blended twice for 30 seconds, with the vial shaken between blends to ensure thorough mixing. After blending, the samples were filtered through a 40 μm cell strainer into a new vial. 5 mL of the resulting blended and filtered whole tissue sample (Wtbf) was then used for symbiont and coral cell separation. 1 mL of the whole blended tissue was diluted with1 mL of LNSW to form Wtbf. The resulting whole blended tissue (Wtbf) was then used for symbiont and coral cell separation.

Coral cells and symbiont cells have different sizes (coral cells have a diameter of 10-25 μm while symbiont cells are around 40-50 μm) and different masses (Roger et al., 2021). Therefore, a centrifuge was used to divide the coral tissue slurry into these two fractions. To prepare for centrifugation, two racks were prepared containing the necessary labeled symbiotic (S) and tissue (T) vials for each sample. 5 mL from each filtered sample was pipetted into the S vials, and then centrifuged at 1000 RPM for 5 minutes to separate the larger symbiont cells from the smaller coral cells (Nuber et al., in prep). The supernatant containing tissue cells was then transferred into the T vials, and 5 mL of LNSW was added to the S vials before the vials were centrifuged again for 5 minutes to ensure complete separation. The supernatant was discarded and 4.5 mL of LNSW was added to the S vials, completing the preparation of S vials (Nuber et al., in prep).

For the T vials, the centrifugation process was repeated four times: each time, the supernatant was transferred to a new vial and the old vial, containing the cell clumps, was discarded (Nuber et al., in prep). After four rounds of centrifugation, the final supernatant was checked for clarity. If the liquid appeared yellowish or cloudy, additional centrifugation was performed until the liquid was clear. 4.5 mL of LNSW was added to the final T vials (Nuber et al., in prep). All vials were then sealed with parafilm and placed in separate labeled bags according to sample type (S, T, and Wtbf) before being stored at -80°C.

Oxidation

Over the course of four weeks, I analyzed 170 samples, including 22 Coral Host Tissue samples, 22 Algal Symbionts samples, 22 Whole Tissue samples, 18 standards for training, and 86 blanks used for method validation. The five-day Denitrifier method was used to convert nitrogen to nitrous oxide in the samples, allowing for precise analysis of their nitrogen isotope signatures. Coral Host Tissue, Algal Symbionts, and Whole Tissue Samples splits were thawed and each biological sample had one technical replicate to verify analytical accuracy and limited contamination of external nitrogen within the samples. The process began with chemical oxidation, a step that converts all available nitrogen in the sample into nitrate. Each sample was mixed with 3 grams of persulfate oxidizing reagent (POR) and sodium hydroxide solution in 100 mL of 18.3 MΩ MilliQ Water. The samples were then autoclaved at 121°C and 107kPa (1 bar/psi) for 1.5 hours to ensure complete oxidation of organic nitrogen to nitrate (Wang et al.,

2015; Nuber et al., in prep). To verify the accuracy of the oxidation, an L-glutamic acid standard (USGS 40, $\delta^{15}N$ = -4.52‰) with known nitrogen isotopic values and POR blanks were included in analysis (Wang et al. 2016). Following oxidation, the pH of the sample was adjusted to neutral (pH 5-7) using 4N HCl and 2N NaOH.

Samples were then run through a Teledyne NOx analyzer model T200 to verify that nitrate levels were within acceptable limits. High nitrate levels could indicate contamination from external nitrogen, which could affect the validity of the results and potentially damage the mass spectrometer, leading to skewed $\delta^{15}N$ values (Wang et al., 2015; Nuber et al., in prep). More importantly, the NOx analyzer measures both nitrate and nitrite concentrations, which allows for the calculation of the required sample volume needed to yield 5 nanomoles of nitrogen—the optimal amount to minimize plasma spikes and reduce errors during the mass spectrometry analysis (Wang et al., 2015; Nuber et al., in prep).

Harvest

The next step was harvesting, during which nitrate is converted into nitrous oxide gas using a bacterial solution of Pseudomonas *chlororaphis*. First, the bacteria was evenly distributed into 12 vials, which were then centrifuged at 18°C, 7600 RPM for 10 minutes. The bacterial solution was tested to confirm the absence of nitrate, and the supernatant was discarded. Each vial was resuspended with 9 mL of resuspension medium, and one drop of antifoam was added to prevent foaming. The vials were shaken to dislodge any bacteria from the walls and placed on a mixer to ensure thorough mixing. All vials were then transferred into a jug for homogenization, which was swirled to ensure even distribution.

Next, 1.5 mL of the homogenized liquid was transferred into 20-mL vials, which were sealed with Teflon-backed silicone septa and crimp seals to ensure airtightness and prevent the introduction of external nitrogen (Sigman et al., 2001). Harvesting blanks, consisting of 1 mL of Milli-Q water and bacteria, were also prepared to assess bacterial function. To create anaerobic conditions and ensure that no nitrous oxide was present when the samples were added to the bacteria, each vial was purged with dinitrogen (N_2) gas (Wang et al., 2016). Dinitrogen gas was introduced through a 26-gauge needle to bubble through the medium, replacing the air in the vial with pure dinitrogen gas over 3 hours. The air was vented out through a 22-gauge needle. After the purging process, the vent needle was removed first, followed immediately by the bubbling needle to minimize the risk of outside air from entering (Sigman et al., 2001). Samples were then added to the vented vials, allowing *Pseudomonas chlororaphis*, a bacterium that lacks N_2O-reductase activity, to convert all the nitrate in each sample into nitrous oxide, which allows for more precise analysis of isotopic ratios (Wang et al., 2015; Nuber et al., in prep).

In addition to these sample vials, two additional standards (USGS 34, $\delta^{15}N$ = -3.0‰ and IAEA N3, $\delta^{15}N$ = 4.7‰) were included in six vials. These standards have known nitrogen isotopic ratios, which were added to a few vials to test whether there were any inconsistencies in the harvest method step. (Wang et al., 2015; Nuber et al., in prep). Every vial was then kept in

the dark for a minimum of 12 hours to allow ample time for the bacteria to consume all the nitrate and nitrogen dioxide, leaving only nitrous oxide. In the morning, the bacterial activity was terminated by adding concentrated 12N NaOH (Wang et al., 2015; Nuber et al., in prep).

Mass Spectrometer

To calibrate and verify the performance of the mass spectrometer, nitrous oxide reference gas was used. This standard accounts for potential changes in analysis factors, such as changes in the size of the bacterial N_2O blank (Sigman et al., 2001). The samples were analysed on Mass Spectrometer MAT253 at Professor Ren Nitrogen Isotope Laboratory at the Department of Geosciences, National Taiwan University. Prior to analysis, the sample was taken up by a gas autosampler, and sent through an individualised gas bench including multiple gas traps to purify the N_2O gas sample (Wang et al., 2015; Nuber et al., in prep).

Method Validation

This method demonstrates high sensitivity, as only 5 nanomoles of N_2O–N was introduced into the mass spectrometer (Wang et al., 2015). This level of sensitivity required minimizing nitrogen contamination throughout the entire process. Therefore, standards during oxidation, harvest, and mass spectrometer were essential to ensure that both the chemical and biological processes occurred correctly and with minimal external contamination. The measured $\delta^{15}N$ result for the oxidation USGS 40 standard was -4.53‰. This was determined by calculating the linear relationship between the $\delta^{15}N$ values of the standards and their respective total nitrogen amounts. The true $\delta^{15}N$ value for L-glutamic acid is -4.52‰, indicating that the oxidation process was carried out accurately.

For the harvest standards, USGS 34 ($\delta^{15}N$ = -3.0‰) and IAEA N3 ($\delta^{15}N$ = 4.7‰) were used. Additionally, the mass spectrometer was calibrated with a nitrous oxide reference gas standard. Both harvest and mass spectrometer standards were graphed over the time of measurements to check that the isotopic composition was stable over time. The stability of these standards was confirmed by low standard deviation values: USGS 34 (± 0.0711‰), IAEA N3 (± 0.0378‰), and the 5 nanomole N2O–N standard (± 0.0465‰).

Stable Nitrogen Isotope Measurements

Nitrogen isotope ($\delta^{15}N$) measurements were conducted for Coral Host Tissue, Algal Symbionts, and Whole Tissue Samples using a mass spectrometer. These measurements were corrected for nitrogen added from the POR used to oxidize nitrogen into nitrate. As indicated earlier, POR blanks were included throughout the method and measured by the mass spectrometer to know how much nitrogen they added to each sample.

To process the raw $\delta^{15}N$ data from the mass spectrometer, the following math equation was applied:

$$\text{Corrected } \delta^{15}N = \frac{\delta^{15}N_{\text{raw}} - \left(\frac{\text{Concentration of the Blank}}{\text{Concentration of the Sample}} \times \text{Average } \delta^{15}N_{\text{of the POR blanks}}\right)}{1 - \frac{\text{Concentration of the Blank}}{\text{Concentration of the Sample}}}$$

The corrected $\delta^{15}N$ values was then reported in delta notation $\delta^{15}N$:

$$\delta^{15}N = \left(\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1\right) \times 1000$$

where R equals $^{15}N/^{14}N$. The values were reported as the amount of $\delta^{15}N$ in each sample per mille (‰) against atmospheric dinitrogen, which is the air standard referenced against.

Statistical Methods

Statistical analyses were performed using both Microsoft Excel and R. A two-way Analysis of Variance (ANOVA) was used to determine whether the $\delta^{15}N$ signatures in Coral Host Tissue and Algal Symbionts, Coral Host Tissue and Whole Tissue Samples, and Algal Symbionts and Whole Tissue Samples significantly influenced each other and/or varied by genus. The null hypothesis assumed that the relationship between the variables was consistent across all genera. A significance threshold of 0.05 was set as the p-values, with values less than 0.05 indicating statistical significance and rejection of the null hypothesis. Conversely, p-values greater than 0.05 indicated that the observed differences were not statistically significant, and the null hypothesis was not rejected.

To further support the relationship findings from the two-way ANOVA, Pearson's Correlation test was applied to assess the strength of the relationships. The correlation coefficient (r) ranges from -1 to 1, with values closer to 1 indicating a strong positive correlation, and values closer to -1 indicating a strong negative correlation. The R^2 coefficient represents the percentage of variance in the dependent variable's $\delta^{15}N$ explained by the independent variable's $\delta^{15}N$. Additionally, a Post-Hoc Tukey's Honest Significant Difference (HSD) test was applied to simultaneously test all pairwise comparisons between subgroups (Coral Host Tissue, Algal Symbionts, and Whole Tissue Samples) to determine if their $\delta^{15}N$ were statistically different or comparable within the dataset. These statistical methods were chosen to provide robust results into how nitrogen moves, exchanges, and interacts across the different components of the coral holobiont.

Results

Analytical Averages

To ensure the reliability of the results and account for potential external contamination of nitrogen, each sample was technical replicated once, and analytical averages and standard deviations were calculated for each (Table 1). The sample Fu-POR6-S was excluded from analyses because it had an extremely high standard deviation (1.96 ± 2.04) and was the only Porites spp. sample. In addition to examining the reliability of results based on analytical averages, samples were also checked to see if the blank's $\delta^{15}N$ made up a large percentage (greater than 7 percent) of the overall sample's $\delta^{15}N$ (Table 1). The corals with a blank contribution greater than 7% were Af7-T1, Af7-T2, Af9-S1, Af9-S2, S6-S1, S6-S2, S7-S1, S7-S2, S8-S1, and S8-S2, with blank percentages ranging from 10.6% to 18.0%. This indicates that between $1/10^{th}$ and $1/5^{th}$ of the $\delta^{15}N$ signal in these samples could be influenced by or potentially contaminated by the blank's $\delta^{15}N$. However, the resulting $\delta^{15}N$ values have been corrected for the blank contribution, meaning the data should still be valid and reliable, although it is important to consider this potential contamination when interpreting the results.

Table 1. Analytical averages with standard deviations from two technical replicates for each sample. The table also displays the proportion of the blank's $\delta^{15}N$ contribution to the overall sample's $\delta^{15}N$, noting whether it exceeds 7%. "Fu" refers to Fulong, Taiwan, where the coral heads were extracted. "Af," "S," and "POR" represent the coral genera, with "Af" for Acropora spp., "S" for Stylophora spp., and "POR" for Porites spp. Additionally, "S," "T," or "Wtbf" denote Algal Symbionts (S), Coral Host Tissue (T), and Whole Blended and Filtered Tissue (Wtbf). For example, "Fu-Af7-T" refers to the Coral Host Tissue from Fulong Acropora spp. Sample 7.

Sample ID	$\delta^{15}N$ (‰ vs. N_2)	% of Blank's $\delta^{15}N$ Contribution to Overall Sample's $\delta^{15}N$ (exceeding 7%)
Fu-Af6-S	5.11 ± 0.04	0
Fu-Af6-T	6.3 ± 0.05	0
Fu-Af6-Wtbf	5.95 ± 0.01	0
Fu-Af7-S	5.19 ± 0.04	0
Fu-Af7-T	6.97 ± 0.48	13.9%
Fu-Af7-Wtbf	6.65 ± 0.14	0
Fu-Af8-S	4.85 ± 0.15	0
Fu-Af8-T	6.12 ± 0.02	0
Fu-Af8-Wtbf	5.89 ± 0.06	0
Fu-Af9-S	4.94 ± 0.44	10.6%
Fu-Af9-T	6.23 ± 0.01	0
Fu-Af9-Wtbf	3.88 ± 0.01	0
Fu-Af10-S	3.88 ± 0.01	0
Fu-Af10-T	6.3 ± 0.36	0
Fu-Af10-Wtbf	5.63 ± 0.27	0
Fu-S6-S	3.47 ± 0.49	18.0%
Fu-S6-T	4.55 ± 0.15	0
Fu-S6-Wtbf	4.40 ± 0.05	0
Fu-S7-S	1.87 ± 0.39	15.4%
Fu-S7-T	3.87 ± 0.06	0
Fu-S7-Wtbf	3.25 ± 0.01	0
Fu-S8-S	4.73 ± 0.36	15.3%
Fu-S8-T	5.02 ± 0.07	0
Fu-S8-Wtbf	5.13 ± 0.13	0
Fu-POR6-S	1.96 ± 2.04	0
Fu-POR6-T	6.00 ± 0.11	0
Fu-POR6-Wtbf	5.36 ± 0.02	0

Coral Results

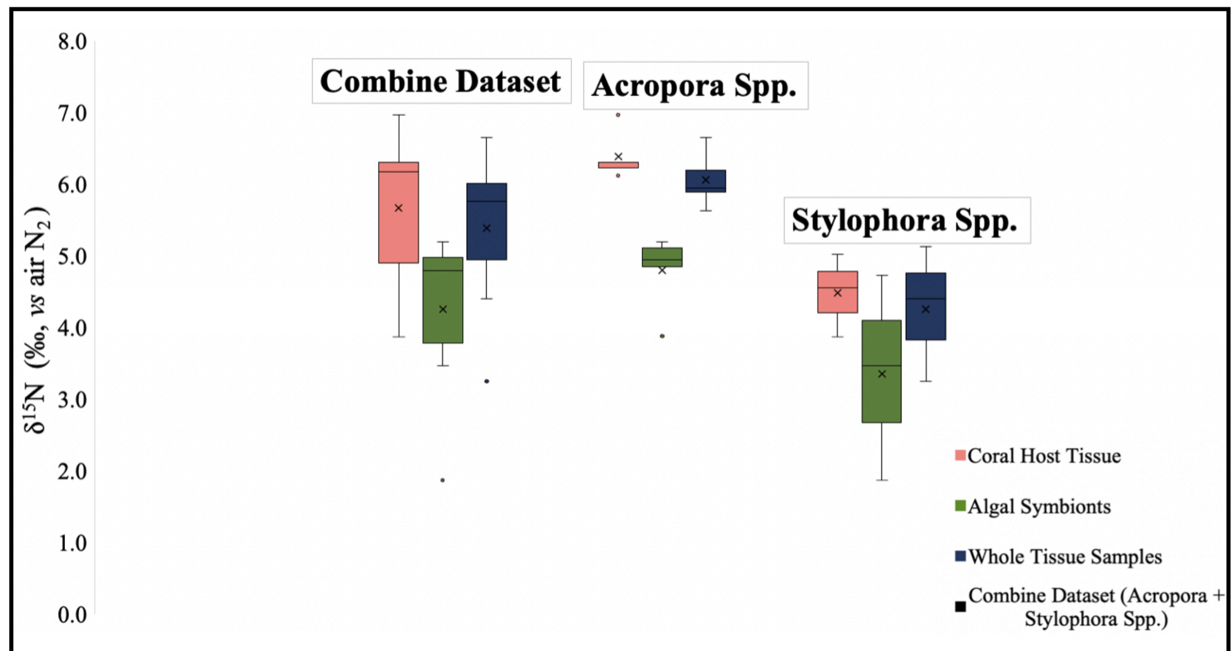

Figure 3. Box plot comparison of $\delta^{15}N$ in Coral Host Tissue, Algal Symbionts, and Whole Tissue Samples across combined and genus-specific datasets, displaying the mean (X), median (horizontal line), outliers (⚫) and whiskers (error bars). Long whiskers suggest a wider range of values in the data, while shorter whiskers indicate that the data is more concentrated around the median. Outliers are those that fall outside the whisker lines. The mean (X) represents the average value of the dataset.

To obtain robust results for $\delta^{15}N$ comparison, the dataset was plotted not only for the combined dataset, which included all Acropora spp. and Stylophora spp. data, but also to examine whether the trends held true for genus-specific data. In both the combined dataset and individual genera, $\delta^{15}N$ in Coral Host Tissue were consistently higher than in Algal Symbionts (Figure 3). $\delta^{15}N$ levels in Whole Tissue Samples were intermediate, exhibiting values higher than those in Algal Symbionts but lower than those in Coral Host Tissue. Notably, the genus Acropora spp. displayed higher $\delta^{15}N$ values across all tissue types (Coral Host Tissue, Algal Symbionts, and Whole Tissue Samples) compared to Stylophora spp. (Figure 3).

An ANOVA test further supported these trends by evaluating statistical differences across the tissue types. The results showed a statistically significant difference ($p = 0.0422$) in $\delta^{15}N$ among all three tissue types. To explore these differences further, a Post-hoc Tukey's High Significant Difference test was conducted. Significant differences were found between Coral Host Tissue and Algal Symbionts ($p = 0.0453$), with Coral Host Tissue exhibiting higher $\delta^{15}N$ values. However, the p-value was just below the threshold for significance, which brings caution to a full interpretation of this result. No statistically significant differences were observed between Coral Host Tissue and Whole Tissue ($p = 0.124$), nor between Algal Symbionts and

Whole Tissues ($p = 0.866$). These results suggest that not only were their $\delta^{15}N$ values comparable within the dataset, but also that the Whole Tissue Sample reflects a mixture of isotopic signatures from both.

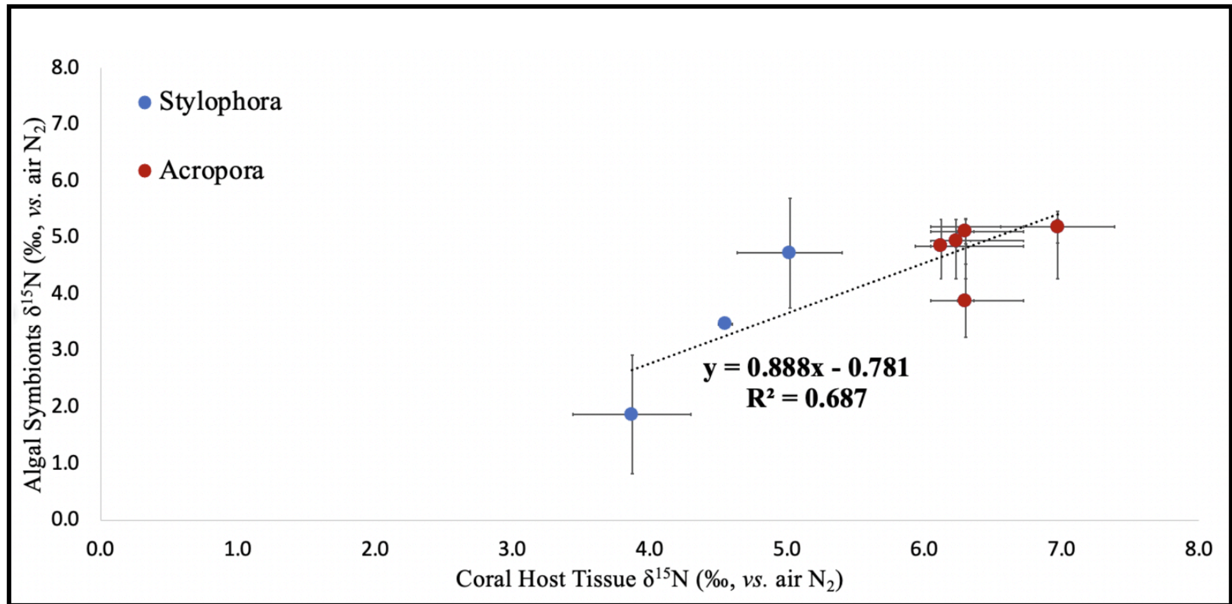

Figure 4. Pearson Correlation analysis of $\delta^{15}N$ in Coral Host Tissue and $\delta^{15}N$ in Algal Symbionts. Error bars represent the standard deviation derived from the analytical average of replicated samples of $\delta^{15}N$. Horizontal error bars indicate variation in $\delta^{15}N$ within replicated samples of Coral Host Tissue, while vertical error bars represent variation in $\delta^{15}N$ within replicated samples of Algal Symbionts.

Further analysis was conducted using a two-way ANOVA to examine the relationships between $\delta^{15}N$ in Coral Host Tissue and $\delta^{15}N$ in Algal Symbionts, $\delta^{15}N$ in Coral Host Tissue and $\delta^{15}N$ in Whole Tissue Samples, and $\delta^{15}N$ in Algal Symbionts and $\delta^{15}N$ in Whole Tissue Samples. A statistically significant effect of Coral Host Tissue on the $\delta^{15}N$ signature of Algal Symbionts was found ($p = 0.0108$). To assess potential differences in the nitrogen exchange between Coral Host Tissue and Whole Tissue Samples for Acropora spp. and Stylophora spp., the effect of Genus was tested and found to be not significant ($p = 0.203$). This suggests that the relationship between Coral Host Tissue and Algal Symbiont $\delta^{15}N$ signatures does not vary significantly across genera. In addition, a Pearson correlation analysis revealed a strong correlation ($r = 0.829$), with 68.7% of the variance ($R^2 = 0.687$) in Algal Symbiont $\delta^{15}N$ explained by Coral Host Tissue $\delta^{15}N$ (Figure 4).

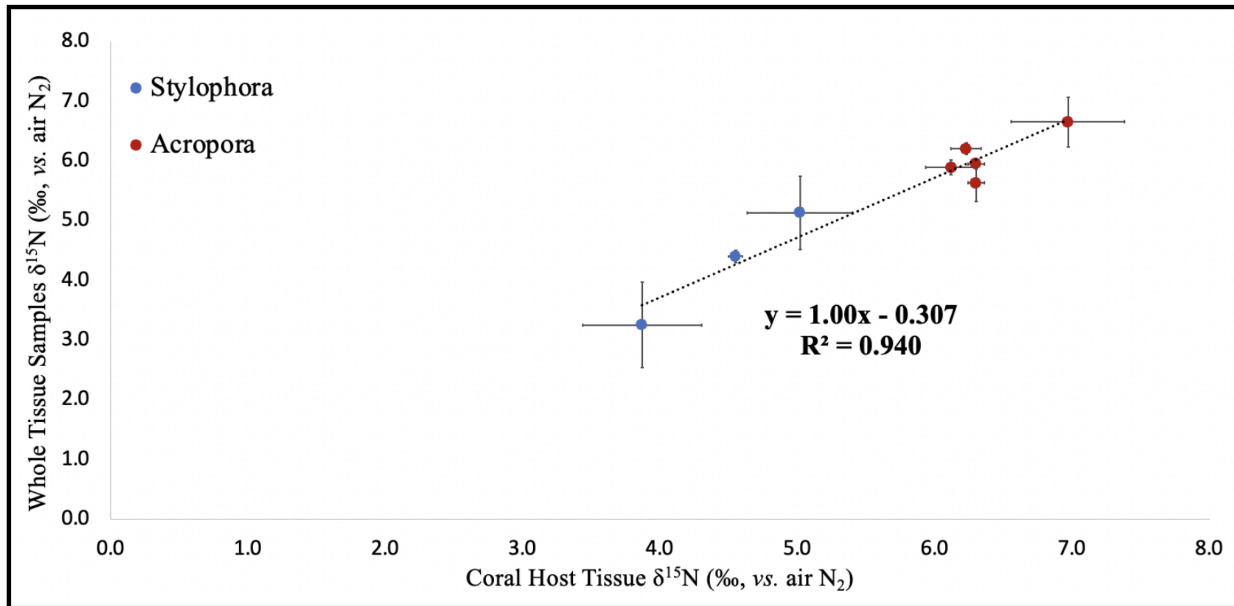

Figure 5. Pearson Correlation analysis of $\delta^{15}N$ in Coral Host Tissue and $\delta^{15}N$ in Whole Tissue Samples. Error bars represent the standard deviation derived from the analytical average of replicated samples of $\delta^{15}N$. Horizontal error bars indicate variation in $\delta^{15}N$ within replicated samples of Coral Host Tissue, while vertical error bars represent variation in $\delta^{15}N$ within replicated samples of Whole Tissue Samples.

Additionally, a highly statistically significant effect of Coral Host Tissue on Whole Tissue Sample $\delta^{15}N$ signature was found ($p = 1.27e-4$), indicating a strong relationship between the $\delta^{15}N$ signatures in these tissues. Like the previous analyses, the effect of genus was not statistically significant ($p = 0.199$), indicating that the influence of Coral Host Tissue on Whole Tissue $\delta^{15}N$ signatures is consistent across species. A strong correlation between $\delta^{15}N$ in Coral Host Tissue and $\delta^{15}N$ in Whole Tissue was also found ($r = 0.970$), with 94.0% of the variability in $\delta^{15}N$ in Whole Tissue Samples explained by Coral Host Tissue $\delta^{15}N$ (Figure 5).

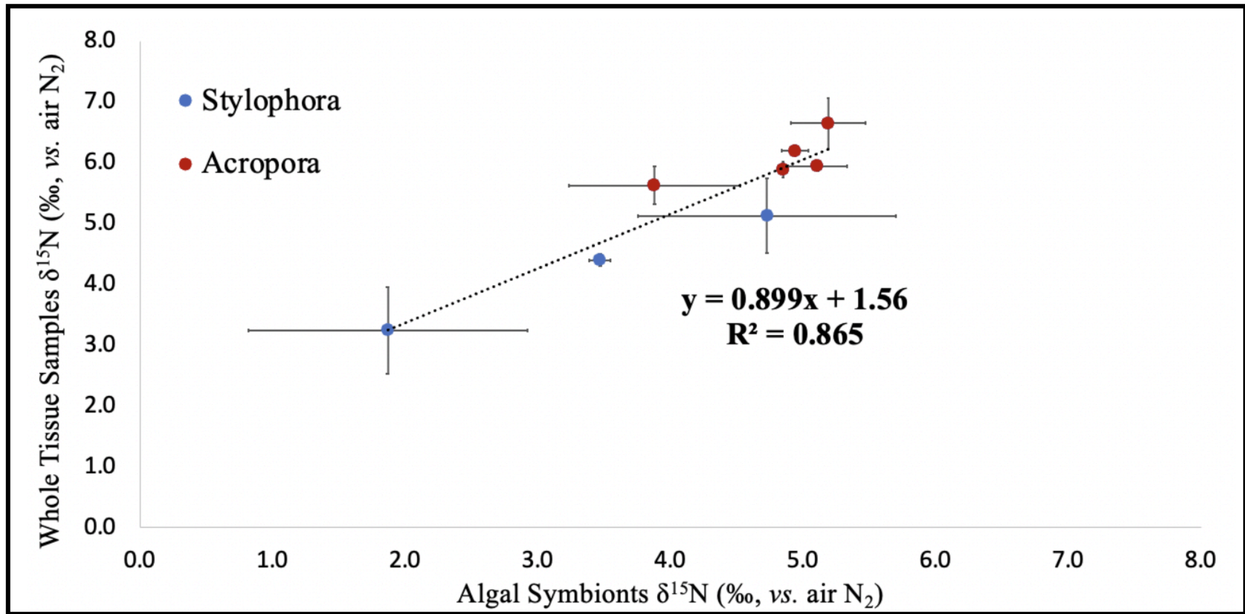

Figure 6. Pearson Correlation analysis of $\delta^{15}N$ in Algal Symbionts and $\delta^{15}N$ in Whole Tissue Samples. Error bars represent the standard deviation derived from the analytical average of replicated samples of $\delta^{15}N$. Horizontal error bars indicate variation in $\delta^{15}N$ within replicated samples of Algal Symbionts, while vertical error bars represent variation in $\delta^{15}N$ within replicated samples of Whole Tissue Samples.

A statistically significant effect of Algal Symbionts on Whole Tissue ($p = 9.44e-5$) and a statistically significant genus effect ($p = 0.0118$) was found. This not only indicates a strong relationship between the signatures but also suggests the influence of Algal Symbionts on Whole Tissue $\delta^{15}N$ signatures varies across species. A similarly strong correlation between $\delta^{15}N$ in Algal Symbionts and $\delta^{15}N$ in Whole Tissue was found ($r = 0.930$), with 86.5% of the variability in $\delta^{15}N$ in Whole Tissue Samples explained by Algal Symbionts $\delta^{15}N$ (Figure 5). Furthermore, a visual inspection of the data revealed a greater degree of spread in both the x- and y-directions, compared to the more linear trend observed between $\delta^{15}N$ in Coral Host Tissue and $\delta^{15}N$ in Whole Tissue Sample (Figure 6).

Table 2. Comparison of $\delta^{15}N$ differences within genera's tissue types using a Post-Hoc Tukey's High Significant Difference test.

Type	**P Value**	**Significant Difference between the Groups**
Acropora spp. Coral Host Tissue vs. Stylophora spp. Coral Host Tissue	0.0158	Yes
Acropora spp. Algal Symbionts vs. Stylophora spp. Algal Symbionts	0.0984	No
Acropora spp. Whole Tissue Samples vs. Stylophora spp. Whole Tissue Samples	0.0238	Yes

Finally, to assess whether there was a statistical difference between genera and one tissue type, a Post-Hoc Tukey's High Significant Difference test was performed. It is important to note that the results of this test were less robust due to the limited data points from Stylophora spp. (n=3). However, they still provide valuable insights into key differences between the coral genera. Acropora's spp. Coral Host Tissue and Stylophora's spp. Coral Host Tissue $\delta^{15}N$ values were statistically different ($p < 0.05$), with Acropora spp. having significantly higher $\delta^{15}N$ Coral Host Tissue values. Acropora's spp. Whole Tissue Sample and Stylophora's spp. Whole Tissue Sample $\delta^{15}N$ values were also statistically different ($p < 0.05$). In contrast, $\delta^{15}N$ values for Algal Symbionts were statistically similar between Acropora spp. and Stylophora spp., indicating that they were comparable across the two genera in this study.

Overall, these findings highlight a distinct pattern in nitrogen isotopic composition across the tissue types. In our study, Coral Host Tissue consistently has higher $\delta^{15}N$ than Algal Symbionts. Additionally, Algal Symbionts were found to have a more significant influence on the amount of $\delta^{15}N$ in Whole Tissue Samples compared to Coral Host Tissue. A genus effect was observed only in the influence of Algal Symbionts on Whole Tissue $\delta^{15}N$ signatures. Finally, there were two tissue types, Coral Host Tissue and Whole Tissue Samples, that showed a statistically significant difference between Acropora spp. and Stylophora spp.

Discussion

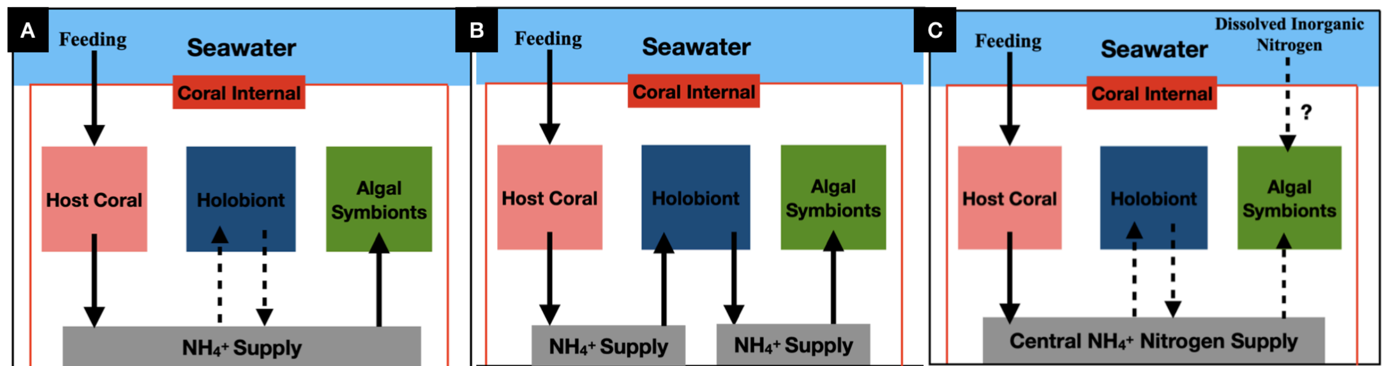

Figure 7. The 3 hypotheses of this study. **(A)** The Host Coral provides nitrogen to the Algal Symbionts directly through the system. **(B)** The Host Coral provides nitrogen to the Algal Symbionts indirectly through the system. **(C)** The Algal Symbionts do not receive nitrogen from the Host Coral but instead fix dissolved inorganic nitrogen from the environment.

The observed trend in our study, where Coral Host Tissue consistently exhibits higher $\delta^{15}N$ values compared to Algal Symbionts, contrasts with previous research which shows that during the summer, Coral Host Tissue and Algal Symbionts exhibit similar $\delta^{15}N$ signatures (Figure 3). For example, Ferrier-Pages et al. (2012) found that in their study, which involved corals sampled under similar temperate conditions, the $\delta^{15}N$ signatures of coral hosts and their algal symbionts were the same. Although the corals studied in their research belong to different genera than those in this study, their findings remain relevant, as both studies focus on the internal processes within corals that generate isotopic signatures in both Coral Host Tissue and Algal Symbionts, rather than genus-specific climate adaptation influences. Their study observed similarities in $\delta^{15}N$ values between the tissues, which suggests the corals are directly providing nitrogen to the symbionts (Figure 7A).

In contrast, our study found distinct $\delta^{15}N$ signatures between coral host and algal symbionts, which suggests the coral may feed the symbionts indirectly through the system, or that the symbionts fix nitrogen from the environment. The more likely explanation is that the coral indirectly provides nitrogen to the symbionts, as a symbiotic relationship would be unnecessary if the symbionts were capable of fixing enough nitrogen to sustain themselves (Figure 7C). This finding supports Hypothesis 2 (Figure 7B), which suggests that corals excrete nitrogen, which is then taken up by the holobiont. The rest of the holobiont may excrete nitrogenous waste, typically in the form of urea, which is utilized as food by the symbionts (Lema et al., 2012). This indirect nitrogen transfer could explain the observed light isotopic fractionation, where $\delta^{14}N$ is preferentially released in ammonium, leading to a nitrogen isotope loss between the host and symbiont tissues (Figure 3).

The genus-specific differences in $\delta^{15}N$ between Acropora spp. and Stylophora spp. further support the idea that nitrogen isotopic signatures vary across different coral genera and coral host tissues, algal symbionts, and whole tissue samples. Acropora spp. exhibited higher

$\delta^{15}N$ values across tissue types, while Stylophora spp. displayed lower $\delta^{15}N$ values overall (Figure 3). This difference may be reflected from variations in trophic strategies, metabolic demands, and structural characteristics between the two species. Fujii et al. (2020) suggested that Acropora spp. typically exhibits lower $\delta^{15}N$ values due to its branched morphology that increases the surface area, which reduces the diffusive boundary layer, a thin layer of seawater whose thickness can affect the coral's ability to exchange gases and nutrients with the surrounding seawater (Fujii et al., 2020). Therefore, the branched morphology limits dissolved inorganic nitrogen (DIN) uptake and results in lower $\delta^{15}N$ values. However, this explanation does not hold in our study, as Stylophora spp., which is also a branched coral, but not as extensively branched as Acropora spp., exhibited lower $\delta^{15}N$ values than Acropora spp.

Genus-specific differences in $\delta^{15}N$ have also been attributed to the use of different nitrogen sources by the corals. Conti-Jerpe et al., (2020) concluded that when there is little to no difference in $\delta^{15}N$ values between coral host tissue and algal symbionts, it suggests that both share a common nitrogen source, likely through nitrogen recycling within the holobiont. Specifically, Conti-Jerpe et al., (2020) found that Acropora spp. exhibited similar $\delta^{15}N$ values in both the host and symbionts, suggesting direct nutrient transfer from the coral to the symbionts or that high levels of nitrogen recycling occur within the holobiont, reducing or eliminating most of the nitrogen trophic fractionation between the niches (Figure 7A). In contrast, our study found significant isotopic differences between host and symbiont tissues, which cannot be explained by direct transfer of nitrogen from coral feeding. Therefore, our data supports the hypothesis that Acropora spp. symbionts obtain a significant portion of their nitrogen indirectly through the coral's recycling process (Figure 7B). On the other hand, Stylophora spp. did not show a distinct isotopic separation between host and symbiont, which may indicate a more direct feeding relationship between the coral and its symbionts, consistent with previous studies (Fujii et al., 2020). While the ANOVA found no significant genus effect of Coral Tissue $\delta^{15}N$ on Algal Symbiont $\delta^{15}N$, the overall strong correlation between $\delta^{15}N$ in Coral Host Tissue and Algal Symbionts suggests that limited sample sizes were likely a limiting factor in our analysis. Further research with larger sample sizes across different coral species is needed to determine if genera-specific differences in trophic exchange strategies exist.

In addition to genera-genera insights, this study reveals insights into Whole Tissue nitrogen isotopic composition. The ANOVA results indicated a statistically significant difference in $\delta^{15}N$ among the three tissue types, with Coral Host Tissue showing higher $\delta^{15}N$ than Algal Symbionts ($p = 0.0422$). Post-hoc Tukey's HSD test confirmed significant differences between Coral Host Tissue and Algal Symbionts ($p = 0.0453$), but no significant differences were observed between Coral Host Tissue and Whole Tissue ($p = 0.124$), nor between Algal Symbionts and Whole Tissue ($p = 0.866$). These results suggest that our method was effective and accurate, as we expected that both Coral Host Tissue and Algal Symbionts would influence Whole Tissue $\delta^{15}N$ values. Moreover, these findings align with previous studies that have emphasized the dynamic interactions between coral hosts and their symbiotic algae, where

nutrient exchange plays a pivotal role in determining the overall Whole Tissue isotopic composition (Ferrier-Pages et al., 2012).

Furthermore, the strength of the correlation ($R^2 = 0.865$), along with the greater variability observed in the data [a higher range of values for Algal Symbionts and Whole Tissue Samples (3.88 to 6.65) compared to the narrower range for Coral Host Tissue and Whole Tissue samples (6.12 to 6.65)], supports the conclusion that Algal Symbionts exert a more significant influence on $\delta^{15}N$ in Whole Tissue Sample compared to Coral Host Tissue (Figure 5). This is logical given that corals are sedentary, feeding the same each day, which would result in less variability in their nitrogen isotopic composition. In contrast, symbionts have more variation in their nitrogen uptake patterns. Algal Symbionts may receive nitrogen in varying amounts from the host or environmental sources, or their associated holobiont may exhibit fluctuations in activity. This variability is further evidenced by the higher standard deviation (2SD = ± 1.06) in $\delta^{15}N$ for Algal Symbionts compared to Coral Host Tissue (2SD = ± 0.672), suggesting that the nitrogen isotopic signatures in symbionts are more variable than those in the coral host.

A genus-specific effect was observed only in the influence of Algal Symbionts on Whole Tissue $\delta^{15}N$, further supporting the idea that differences in morphology or metabolic activity between genera can influence nitrogen cycling within the holobiont. Acropora spp., known for its branched morphology, has been suggested to have higher metabolic rates compared to massive corals like Porites spp. (Figure 1C) (Fuji et al., 2020). These differences in metabolic activity likely explain the higher $\delta^{15}N$ values observed in Acropora spp., as the holobiont may engage in more efficient nitrogen recycling or exhibit different interactions with its algal symbionts compared to Stylophora spp. (Fujii et al., 2020). Although Coral Host Tissue have a less pronounced influence on Whole Tissue compared to Algal Symbionts, it is still important to note the strong effect of Coral Host Tissue on Whole Tissue $\delta^{15}N$ values (Figure 5), where both Coral Host Tissue and Whole Tissue appear in roughly the same $\delta^{15}N$ space (Figure 3). This highlights the clear role of coral within the holobionts nitrogen cycling system. Specifically, nitrogen internally released by the coral may be taken up by bacteria or other microbial symbionts, which are then consumed by the algae, reflecting in the whole tissue samples $\delta^{15}N$ (Fujii et al., 2020).

Further research should increase the sample size, particularly within the genera Stylophora spp. and Porites spp., to allow for a more robust comparison with Acropora spp. Expanding the sampling size to include a broader range of coral species with varying morphologies and metabolic rates would help clarify how these traits influence nitrogen cycling dynamics within the holobiont. Additionally, more research is needed to explore nitrogen cycling across the entire coral holobiont, including the breakdown of nitrogen isotopic units within the holobiont like the components of bacteria or denitrifiers. Nitrogen isotopic analysis of amino acids would also provide valuable insights into metabolic processes at the molecular level, shedding light on trophic differences, nitrogen partitioning between coral hosts and their algal symbionts, and how $\delta^{15}N$ is cycled throughout the system. While this study focused on nitrogen cycling within the holobiont, it did not consider the coral's external food sources, such as plankton, which may indirectly contribute to the nitrogen pool. Future research should

investigate the nitrogen isotopic composition of coral food sources to gain a more comprehensive understanding of coral metabolism and its role in the nitrogen cycle. Finally, hypothesis 3 (Figure 7C) was not tested, as seawater samples near the coral were not collected. Future studies should include these samples to explore the broader environmental context of dissolved inorganic nitrogen (DIN) in seawater and its impact on corals and their algal symbionts. Specifically, it will be important to examine how ambient nitrogen levels influence $\delta^{15}N$ values in corals, their algal symbionts, and whole tissues, and how this contributes to the nitrogen cycle

Conclusion

In conclusion, this study provides valuable insights into nitrogen cycling within the coral holobiont, highlighting genus-specific differences and challenging previous understandings of the interactions between Coral Host Tissues and Algal Symbionts. This study found that corals feed their symbionts indirectly through the system, rather than directly as previously thought. This indirect process is likely explained by coral's internal release of nitrogen, which is then taken up by the associate holobiont. The bacteria within the holobiont releases nitrogen in the form of urea, which is subsequently utilized by the symbionts. Additionally, genus-specific differences in nitrogen cycling were found, with Acropora spp. exhibiting higher $\delta^{15}N$ across all tissue types compared to Stylophora spp. This could be due to variations in trophic strategies, metabolic demands, and morphological characteristics, although limited sample sizes prevented further conclusions. The variability in nitrogen isotopic composition between tissue types also emphasized the dynamic nature of nutrient exchange between coral hosts and their symbionts, particularly with Algal Symbionts exerting more influence on Whole Tissue Sample than Coral Host Tissue due to their varying diet. While this study helps to bridge the research gap on nitrogen isotopic composition within the coral holobiont, it also underscores the need for further research into how broader environmental factors, such as the role of external food sources and dissolved inorganic nitrogen in seawater, influences nitrogen cycling. Expanding this research to include a broader range of coral species and examining molecular-level processes could provide a deeper understanding of coral metabolism and nitrogen dynamics within coral reef ecosystems, ultimately helping to inform strategies for enhancing coral resilience in the face of climate change.

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References

Bay, R. A., Rose, N. H., Logan, C. A., & Palumbi, S. R. (2017). Genomic models predict successful coral adaptation if future ocean warming rates are reduced. *Science Advances*, *3*(11). https://doi.org/10.1126/sciadv.1701413

Britannica (2019). Taiwan - Climate. In: *Encyclopædia Britannica*. [online] Available at: https://www.britannica.com/place/Taiwan/Climate.

Conti-Jerpe I.E., Thompson, P. D., Martin, W., Oliveira, N. L., Duprey, N., Moynihan, M. A., & Baker, D. M. (2020). Trophic strategy and bleaching resistance in reef-building corals. *Science Advances*, *6*(15). https://doi.org/10.1126/sciadv.aaz5443

Corals of the World. (2010). *Species factsheets*. Retrieved January 15, 2025, from https://www.coralsoftheworld.org/species_factsheets/

Denk, T., Mohn, J., Decock, C., Dominika Lewicka-Szczebak, E Clare Harris, Klaus Butterbach-Bahl, Ralf Kiese, & Wolf, B. (2017). The nitrogen cycle: A review of isotope effects and isotope modeling approaches. *Soil Biology & Biochemistry*, *105*, 121–137. https://doi.org/10.1016/j.soilbio.2016.11.015

Ferrier-Pagès, C., Peirano, A., Abbate, M., Cocito, S., Negri, A., Rottier, C., Riera, P., Rodolfo-Metalpa, R., & Reynaud, S. (2011). Summer autotrophy and winter heterotrophy in the temperate symbiotic coral Cladocora caespitosa. *Limnology and Oceanography*, *56*(4), 1429–1438. https://doi.org/10.4319/lo.2011.56.4.1429

Fujii, T., Tanaka, Y., Maki, K., Saotome, N., Morimoto, N., Watanabe, A., & Miyajima, T. (2020). Organic Carbon and Nitrogen Isoscapes of Reef Corals and Algal Symbionts: Relative Influences of Environmental Gradients and Heterotrophy. *Microorganisms*, *8*(8), 1221. https://doi.org/10.3390/microorganisms8081221

Hoegh-Guldberg, O., Mumby, P. J., Hooten, A. J., Steneck, R. S., Greenfield, P., Gomez, E., Harvell, C. D., Sale, P. F., Edwards, A. J., Caldeira, K., Knowlton, N., Eakin, C. M., Iglesias-Prieto, R., Muthiga, N., Bradbury, R. H., Dubi, A., & Hatziolos, M. E. (2007). Coral reefs under rapid climate change and ocean acidification. *Science*, *318*(5857), 1737–1742.

La, S. (2011). Coral Reef Biodiversity in the Face of Climatic Changes. InTech. doi: 10.5772/25049

Lema, K. A., Willis, B. L., & Bourne, D. G. (2012). Corals Form Characteristic Associations with Symbiotic Nitrogen-Fixing Bacteria. *Applied and Environmental Microbiology*, *78*(9), 3136–3144. https://doi.org/10.1128/AEM.07800-11

Moran, M. (2017, November 20). Checking for Influential Data Points in Regression Analyses. Statistics Solutions. https://www.statisticssolutions.com/checking-for-influential-data-points-in-regression-analyses/

NOAA. (2019, February 1). *Coral Reef Ecosystems*. Www.noaa.gov; National Oceanic and Atmospheric Administration. https://www.noaa.gov/education/resource-collections/marine-life/coral-reef-ecosystems

Nuber, S., Tanuputri, J., Faye, E.A., Lichter, A., Shikina, S., & Ren, H. A. (in prep.). A new method for the analysis of nitrogen isotopes in symbionts from bleached coral tissue: Combining flow cytometry with the denitrifier method.

Oppen, M. J. H., Gates, R. D., Blackall, L. L., Cantin, N., Chakravarti, L. J., Chan, W. Y., Cormick, C., Crean, A., Damjanovic, K., Epstein, H., Harrison, P. L., Jones, T. A., Miller, M., Pears, R. J., Peplow, L. M., Raftos, D. A., Schaffelke, B., Stewart, K., Torda, G., & Wachenfeld, D. (2017). Shifting paradigms in restoration of the world's coral reefs. *Global Change Biology*, *23*(9), 3437–3448. https://doi.org/10.1111/gcb.13647

Rädecker, N., Pogoreutz, C., Voolstra, C. R., Wiedenmann, J., & Wild, C. (2015). Nitrogen Cycling in Corals: the Key to Understanding Holobiont Functioning? *Trends in Microbiology*, *23*(8), 490–497. https://doi.org/10.1016/j.tim.2015.03.008

Ren, H., Chen, Y.-C., Wang, X. T., Wong, G. T. F., Cohen, A. L., DeCarlo, T. M., Weigand, M. A., Mii, H.-S., & Sigman, D. M. (2017). 21st-century rise in anthropogenic nitrogen deposition on a remote coral reef. *Science*, *356*(6339), 749–752. https://doi.org/10.1126/science.aal3869

Rodrigues, L. J., & Grottoli, A. G. (2006). Calcification rate and the stable carbon, oxygen, and nitrogen isotopes in the skeleton, host tissue, and zooxanthellae of bleached and recovering Hawaiian corals. *Geochimica et Cosmochimica Acta*, *70*(11), 2781–2789. https://doi.org/10.1016/j.gca.2006.02.014

Roger, L. M., Reich, H. G., Lawrence, E., Li, S., Vizgaudis, W., Brenner, N., Kumar, L., Klein-Seetharaman, J., Yang, J., Putnam, H. M., & Lewinski, N. A. (2021). Applying model approaches in non-model systems: A review and case study on coral cell culture. *PLOS ONE*, *16*(4), e0248953. https://doi.org/10.1371/journal.pone.0248953

Sigman, D. M., Casciotti, K. L., Andreani, M., Barford, C., Galanter, M., & Böhlke, J. K. (2001). A Bacterial Method for the Nitrogen Isotopic Analysis of Nitrate in Seawater and Freshwater. *Analytical Chemistry*, *73*(17), 4145–4153. https://doi.org/10.1021/ac010088e

Taiwannews.com.tw. (2024). *Abnormally high sea temperatures lead to Taiwan's scorching heat | Taiwan News | Jul. 2, 2024 18:12*. [online] Available at: https://www.taiwannews.com.tw/news/5897690 [Accessed 31 Jan. 2025].

Wall, C. B., Ritson‐Williams, R., Popp, B. N., & Gates, R. D. (2019). Spatial variation in the biochemical and isotopic composition of corals during bleaching and recovery. *Limnology and Oceanography*, *64*(5), 2011–2028. https://doi.org/10.1002/lno.11166

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