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Trophic plasticity in a common reef-building coral: Insights from $\delta^{13}C$ analysis of essential amino acids

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Abstract

1. Reef-building corals are mixotrophic organisms that can obtain nutrition from endosymbiotic microalgae (autotrophy) and particle capture (heterotrophy). Heterotrophic nutrition is highly beneficial to many corals, particularly in times of stress. Yet, the extent to which different coral species rely on heterotrophic nutrition remains largely unknown because it is challenging to quantify.
2. We developed a quantitative approach to investigate coral nutrition using carbon isotope ($\delta^{13}C$) analysis of six essential amino acids (AA_{ESS}) in a common Indo-Pacific coral (*Pocillopora meandrina*) from the fore reef habitat of Palmyra Atoll. We sampled particulate organic matter (POM) and zooplankton as the dominant heterotrophic food sources in addition to the coral host and endosymbionts. We also measured bulk tissue carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope values of each sample type.
3. Patterns among $\delta^{13}C$ values of individual AA_{ESS} provided complete separation between the autotrophic (endosymbionts) and heterotrophic nutritional sources. In contrast, bulk tissue $\delta^{13}C$ and $\delta^{15}N$ values were highly variable across the putative food sources and among the coral and endosymbiont fractions, preventing accurate estimates of coral nutrition on Palmyra.
4. We used linear discriminant analysis to quantify differences among patterns of AA_{ESS} $\delta^{13}C$ values, or 'fingerprints', of the food resources available to corals. This allowed for the development of a quantitative continuum of coral nutrition that can identify the relative contribution of autotrophic and heterotopic nutrition to individual colonies. Our approach revealed exceptional variation in conspecific colonies at scales of metres to kilometres. On average, 41% of AA_{ESS} in *P. meandrina* on Palmyra are acquired via heterotrophy, but some colonies appear capable of obtaining the majority of AA_{ESS} from one source or the other.
5. The use of AA_{ESS} $\delta^{13}C$ fingerprinting analysis offers a significant improvement on the current methods for quantitatively assessing coral trophic ecology. We anticipate that this approach will facilitate studies of coral nutrition in the field, which are essential for comparing coral trophic ecology across taxa and multiple spatial scales. Such information will be critical for understanding the role of heterotrophic nutrition in coral resistance and/or resilience to ongoing environmental change.

KEYWORDS
amino acids, compound-specific stable isotope analysis, coral reef, heterotrophy, mixotrophy, Palmyra Atoll, symbiosis, zooplankton

1 | INTRODUCTION

Due to dietary flexibility, mixotrophs have become some of the most widespread organisms on the planet (Selosse, Charpin, & Not, 2016). Mixotrophy has evolved repeatedly in vascular plants and marine organisms, underscoring the value of trophic plasticity at the individual and population levels. This dietary flexibility allows mixotrophs to play important roles in energy flow by creating novel linkages between trophic levels (Stoecker, Hansen, Caron, & Mitra, 2017). Some vascular plants, for example, rely on heterotrophic nutrition from mycorrhizal fungi to sustain metabolic demands in association with seasonal variability (Matsuda, Shimizu, Mori, Ito, & Selosse, 2012) or use carnivory to enhance productivity under nutrient-poor conditions (Ellison & Gotelli, 2009). Despite their integral role in ecosystem functioning, our knowledge of the trophic ecology of mixotrophs and how they are likely to respond to environmental change remains limited because accurately disentangling the relative contributions of autotrophic versus heterotrophic nutrition is challenging.

Reef-building corals are globally distributed and ecologically important mixotrophs, yet the importance of trophic plasticity in coral growth and survival at the population and ecosystem scales remains unknown (Ferrier-Pagès, Hoogenboom, & Houlbrèque, 2011). Corals are often considered to be principally autotrophic (Muscatine & Porter, 1977); however, they are also voracious carnivores (Lewis & Price, 1975) and heterotrophy can supply up to 60% of daily metabolic carbon demands in healthy corals and >100% in bleached colonies (Grottoli, Rodrigues, & Palardy, 2006; Palardy, Rodrigues, & Grottoli, 2008). Experimental work has shown that heterotrophic nutrition can enhance coral growth and fecundity, reduce mortality during bleaching and accelerate post-bleaching recovery (Anthony, Hoogenboom, Maynard, Grottoli, & Middlebrook, 2009; Baumann, Grottoli, Hughes, & Matsui, 2014; Cox, 2007; Ferrier-Pagès, Witting, Tambutte, & Sebens, 2003; Tremblay, Gori, Maguer, Hoogenboom, & Ferrier-Pagès, 2016). In aquaria, corals increase feeding rates as a function of food availability (Ferrier-Pagès et al., 2003; Wijgerde, Diantari, Lewaru, Verreth, & Osinga, 2011), which can drive nonlinear increases in growth and survivorship (Petersen, Wietheger, & Laterveer, 2008; Toh, Ng, Peh, Toh, & Chou, 2014). Thus, coral trophic ecology is likely an important driver of coral population dynamics and recovery from disturbances, but few studies have assessed the ecological relevance of coral heterotrophy at broader spatiotemporal scales (Grottoli et al., 2006; Palardy, Grottoli, & Matthews, 2005) due to the difficulties of quantifying variation in coral nutrition in the field.

While informative, visual observations and polyp dissections (Porter, 1976) are not tractable methods to quantitatively study coral heterotrophy at broad scales. To date, analysis of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotopes has been the primary tool used to address this problem (Muscatine, Porter, & Kaplan, 1989); however, interpreting the high degree of variability in the $\delta^{13}C$ and $\delta^{15}N$ values of coral and endosymbiont tissues is challenging (Hoogenboom, Rottier, Sikorski, & Ferrier-Pagès, 2015). In spite of this uncertainty, estimates of coral heterotrophy based on bulk tissue $\delta^{13}C$ analysis are strongly correlated with patterns of primary production across the tropics (Fox et al., 2018), suggesting that corals increase feeding as a function of food availability in situ. Food supply on coral reefs is highly variable within and among islands (Gove et al., 2016; Williams et al., 2018), which underscores the need for improved methods to quantify the contribution of heterotrophy to coral nutrition.

Recent advancements in compound-specific stable isotope analysis provide a powerful tool for tracing the flow of key compounds through complex food webs (McMahon, Thorrold, Houghton, & Berumen, 2016). Carbon isotope ($\delta^{13}C$) analysis of individual essential (AA_{ESS}) and non-essential (AA_{NESS}) amino acids (collectively referred to as AA $\delta^{13}C$ analysis) is particularly powerful for partitioning resource contributions to consumer diets, as these compounds are the building blocks of proteins critical for metabolism. Most animals cannot synthesize AA_{ESS} de novo and must acquire them directly from diet, which results in little to no carbon isotopic fractionation across trophic levels (Hare, Fogel, Stafford, Mitchell, & Hoering, 1991; Jim, Jones, Ambrose, & Evershed, 2006; McMahon, Fogel, Elsdon, & Thorrold, 2010). Thus, AA_{ESS} $\delta^{13}C$ analysis can trace the relative contribution of basal sources of production to consumers at different trophic levels (Howland et al., 2003; Whiteman, Elliott Smith, Besser, & Newsome, 2019). Perhaps most importantly, taxa that can synthesize AA_{ESS} de novo can use different biochemical pathways to do so, resulting in unique patterns among AA_{ESS} $\delta^{13}C$ values, or 'fingerprints' sensu Larsen, Taylor, Leigh, and O'Brien (2009) that are characteristic of each taxon (e.g. bacteria, fungi, microalgae or macroalgae; Elliott Smith, Harrod, & Newsome, 2018; Larsen et al., 2013; McMahon et al., 2016). Because AA_{ESS} $\delta^{13}C$ fingerprints are typically multivariate representations of at least six different AA_{ESS}, they can accurately track sources of AA_{ESS} contribution to animal diets even when there is substantial overlap in $\delta^{13}C$ values of bulk tissue or individual AA_{ESS} among sources (Larsen et al., 2009).

Unlike most metazoans, there is some evidence that corals and sea anemones are capable of synthesizing some AA_{ESS} de novo (Fitzgerald & Szmant, 1997; Wang & Douglas, 1999). However, recent genomic investigations have failed to identify synthesis pathways for the six AA_{ESS} that are commonly used to characterize $\delta^{13}C$ fingerprints, and also suggest the capacity for de novo AA_{ESS} synthesis is not present in all corals and is likely limited to specific AA_{ESS} (Lin et al., 2015; Ying et al., 2018). One remaining question is if coral-associated microbes contribute AA_{ESS} to the coral host. The current

evidence shows that AA_{ESS} putatively synthesized by the coral host and/or coral-associated microbial communities are produced in low quantities relative to those contributed by the endosymbionts or obtained through heterotrophy (Fitzgerald & Szmant, 1997), suggesting that de novo synthesis is likely not the primary source of AA_{ESS} used by corals to maintain protein homeostasis. The fidelity of AA_{ESS} $\delta^{13}C$ fingerprints within producer groups is directly linked to taxon-specific biochemical pathways of amino acid synthesis (Elliott Smith et al., 2018; Larsen et al., 2013), which suggests that the $\delta^{13}C$ fingerprint of AA_{ESS} synthesized by corals de novo would likely be distinct from those of their primary autotrophic or heterotrophic food sources. Corals thus represent a model system to explore the power of AA_{ESS} isotope analysis to disentangle auto- versus heterotrophic nutrition in mixotrophs and to examine the possibility of de novo AA_{ESS} synthesis in corals and other taxa.

Here, we use AA_{ESS} $\delta^{13}C$ analysis to address a fundamental yet poorly understood question in coral reef ecology: How variable is auto- versus heterotrophic nutrition among coral colonies? We collected a widely distributed Indo-Pacific coral species from Palmyra Atoll in the central Pacific. We sampled corals and the dominant coral food sources (e.g. zooplankton and particulate organic matter) from four sites around Palmyra which differ in food supply to determine: (a) the spatial scale at which coral diets are most variable (within or among sites), (b) whether heterotrophic carbon from different habitats (lagoon vs. pelagic) is distinguishable in coral tissue, (c) if AA_{ESS} $\delta^{13}C$ analysis estimates coral nutrition more precisely than bulk tissue $\delta^{13}C$ and $\delta^{15}N$ data, and (d) if there is evidence of de novo AA_{ESS} synthesis in the coral host. Collectively, our study provides new insights into the trophic plasticity of a widely distributed reef-building coral and a quantitative framework that will facilitate future studies seeking to unravel the trophic ecology of mixotrophs using AA_{ESS} $\delta^{13}C$ analysis.

2 | MATERIALS AND METHODS

2.1 | Data collection

Samples were collected from Palmyra Atoll National Wildlife Refuge in the Northern Line Islands (5°53′N, 162°50′W) during September 2015. Notably, a widespread bleaching event occurred on Palmyra in October 2015 (Fox, Carter, et al., 2019). The samples for this study were collected early in September prior to the onset of bleaching, and all colonies sampled were not visibly pale or discoloured. However, physiological responses to thermal stress that did not manifest in pigmentation change could still have influenced the relative degree of heterotrophy in sampled colonies (Grottoli et al., 2006). To maximize variation in isotopic values and coral trophic strategies around Palmyra, we collected samples from the fore reef habitat at 10 m depth at four sites on opposing corners of the atoll (see Figure S1) that experience differences in food supply and environmental conditions (Gove et al., 2015; Williams et al., 2018). At each site, we measured AA $\delta^{13}C$ values in coral host (consumer, n = 4–5) and endosymbiont tissues (autotrophic source, n = 3) as well as the dominant heterotrophic food sources for corals including zooplankton (>163 μm) and particulate organic matter (POM; n = 1 each per site). Light attenuation with depth is consistent across sites on Palmyra's fore reef (Williams et al., 2018), and all coral samples were collected from the top-centre portion of un-shaded colonies. All samples from the four sites were pooled for statistical analyses due to the high spatial variability of particulate resources on reefs and the limited time window of our sampling. Zooplankton from the lagoon on Palmyra can be transported onto the fore reef habitat where they provide an additional food resource for corals that promotes increased heterotrophy at the NW site (Figure S1; Williams et al., 2018). To account for the possibility of lagoon-derived resources being consumed by corals, we collected zooplankton and POM samples for AA $\delta^{13}C$ analysis at four locations (n = 4 per food source) across the lagoon.

To compare estimates of coral heterotrophy using AA_{ESS} $\delta^{13}C$ fingerprints to those from bulk tissue $\delta^{13}C$ and $\delta^{15}N$ analysis, we sampled corals, endosymbionts, and food resources from 17 sites (including the four corner sites sampled for AA $\delta^{13}C$ analysis) that encircled the atoll perimeter (Figure S1, Table S1). This larger sample size helped constrain the natural variation in bulk tissue measurements. At each site, coral host and endosymbiont fractions (n = 5), along with the heterotrophic resources (zooplankton and POM, n = 1 each), were analysed for bulk tissue $\delta^{13}C$ and $\delta^{15}N$ (Table S1). Collections for food resources within the lagoon were also made for bulk tissue $\delta^{13}C$ and $\delta^{15}N$ analysis at 8 locations (n = 8).

Reef-associated zooplankton (>163 μm) were collected with net tows 3–4 m above the benthos along the fore reef slope. All tows in reef and lagoon habitats were conducted at idle speed for 500 m, and the contents were filtered onto pre-combusted GF/F filters. Diving and boating regulations on Palmyra prohibit nighttime sampling of zooplankton on the fore reef. However, the mean bulk $\delta^{13}C$ values of our zooplankton samples are consistent with values determined from zooplankton communities sampled over several diurnal cycles during the same month of the year at or near the study sites (Williams et al., 2018) and thus accurately reflect what corals may consume on the reef during the day or at night. POM samples were concentrated from 4 L of water collected just above the benthos onto 25-mm pre-combusted GF/F filters (>0.7 μm, Whatman). All samples were frozen at −20°C until analysis. In the laboratory, coral host and endosymbiont fractions were separated and loaded onto pre-combusted GF/F filters following established methods (Fox et al., 2018). All samples were briefly acidified using drop-wise addition of 1N HCl to remove $CaCO_3$ and dried at 60°C for 48 hr.

2.2 | Laboratory analysis

Bulk tissue samples for all sources were analysed for $\delta^{13}C$ and $\delta^{15}N$ with a Costech 4010 Elemental Analyzer interfaced with a Thermo Finnigan Delta Plus XP stable isotope mass spectrometer at Scripps Institution of Oceanography. Isotope values are expressed as delta

(δ) values (δ^3C or $\delta^{15}N$), where $\delta = 1{,}000 \times [(R_{sample}/R_{standard}) - 1]$ and R_{sample} or $R_{standard}$ are the ratio of the heavy to light isotope in parts per thousand, or per mil (‰). The international reference standards are Vienna-Pee Dee Belemnite (V-PDB) for $\delta^{13}C$ and atmospheric N_2 for $\delta^{15}N$. Repeated measurements of internal reference materials calibrated against NBS-18 and IAEA-1 produced a precision (*SD*) of <0.1‰ for $\delta^{13}C$ and <0.2‰ for $\delta^{15}N$.

For AA $\delta^{13}C$ analysis, samples were first hydrolysed with 1.5 ml of 6N HCl at 110°C for 20 hr. Hydrolysates were passed through a cation exchange resin column (Dowex 50WX8 100–200 mesh) to isolate AAs from other metabolites (Amelung & Zhang, 2001). After Dowex purification, amino acids were derivatized to N-trifluoroacetic acid isopropyl esters (Newsome, Fogel, Kelly, & Martínez del Rio, 2011; O'Brien, Fogel, & Boggs, 2002). Samples were derivatized along with an in-house reference material containing all AAs measured for $\delta^{13}C$. Derivatized samples were injected into a 60 m BPX5 gas chromatograph column for AA separation (0.32 ID, 1.0 μm film thickness, SGE Analytical Science) in a Thermo Scientific Trace 1300, then combusted into CO_2 with a GC Isolink II interfaced to a Thermo Scientific Delta V Plus isotope ratio mass spectrometer at the University of New Mexico Center for Stable Isotopes. Samples were run in duplicate and bracketed with in-house AA reference material; within-run standard deviations of $\delta^{13}C$ values in this reference material ranged from 0.3‰ (isoleucine) to 0.5‰ (tyrosine). We reliably measured $\delta^{13}C$ values of thirteen AAs including seven considered non-essential: alanine (Ala), aspartic acid (Asp), glutamic acid (Glu), glycine (Gly), proline (Pro), serine (Ser) and tyrosine (Tyr); and six considered essential: isoleucine (Ile), leucine (Leu), lysine (Lys), phenylalanine (Phe), threonine (Thr) and valine (Val). The reagents used during derivatization add carbon to AA side chains, and hence, measured $\delta^{13}C$ values reflect a combination of the intrinsic carbon in each AA and reagent carbon (Silfer, Engel, Macko, & Jumeau, 1991); see the supplementary material for the equations used to correct each AA $\delta^{13}C$ value.

2.3 | Data analysis

Our approach for studying coral nutrition depends on clear separation between the AA_{ESS} $\delta^{13}C$ fingerprints of different nutritional sources. We used MANOVA to determine separation between the AA_{ESS} $\delta^{13}C$ values of the putative autotrophic (endosymbionts) and heterotrophic sources (zooplankton and POM) of coral nutrition and between resources from different reef habitats (i.e. lagoon vs. fore reef). Individual ANOVAs were used to compare differences in $\delta^{13}C$ values for each AA between the coral host, endosymbionts and heterotrophic sources. Pairwise contrasts between groups were conducted using Tukey's HSD. Assumptions of normality and equal variances within and among groups were determined using the Shapiro–Wilk test with quantile–quantile plots and Levene's test, respectively. Because zooplankton obtain AA_{ESS} directly from their diet (i.e. free-living phytoplankton), we hypothesized that POM and zooplankton AA $\delta^{13}C$ values would be statistically similar. Indeed, zooplankton and POM AA $\delta^{13}C$ values were indistinguishable from each other and were consistent across habitats for both AA_{ESS} (Pillai trace = 0.27, $F_{(6,8)} = 0.50$, $p = .79$) and AA_{NESS} (Pillai trace = 0.71, $F_{(7,4)} = 0.70$, $p = .70$). Therefore, in all multivariate analyses and for the classification of coral host tissue with one group or the other, zooplankton and POM were pooled a posteriori into a single heterotrophic resource category ($n = 17$) to more accurately represent the natural variation the heterotrophic sources available to corals. Little is known about the carbon isotope fractionation associated with de novo synthesis of AA_{NESS} in symbiotic cnidarians, so we focused our analyses on AA_{ESS}. Lastly, statistical comparisons (ANOVA) of the bulk tissue $\delta^{13}C$ and $\delta^{15}N$ data are described in the electronic Supplementary Material.

To quantify the contribution of autotrophic versus heterotrophic resources to coral diets, we used linear discriminant analysis (LDA; R package MASS; Venables & Ripley, 2002). LDA provides a powerful approach for maximizing separation among source groups (autotrophic vs. heterotrophic) and then classifying consumers (corals) as belonging more to one group than another based on the amino acid $\delta^{13}C$ 'fingerprints' of potential sources (Larsen et al., 2009). Most importantly, LDA offers a less rigid framework than Bayesian mixing models for examining diet contributions within undetermined mixing spaces caused by unquantified diet sources; see the Electronic Supplementary Material for a detailed comparison of the two methods (Supplemental methods and Figure S2). For example, if the corals in our study were obtaining nutrition from a third, unquantified source such as coral-associated microbes or de novo AA_{ESS} synthesis by the coral animal, we would expect to find poor or variable classification rates of the coral host within the prescribed autotrophic or heterotrophic groups. We examined the classification error rate for the autotrophic (endosymbionts) and heterotrophic (zooplankton and POM) sources using leave-one-out cross-validation to establish whether our two sources are statistically distinct. A high reclassification rate of samples within their own group (>80%) is critical for establishing reliable separation between sources. We then used this training dataset to predict group membership for each coral host sample (i.e. autotrophic or heterotrophic). We examined classification rates of the coral consumers using the measured AA_{ESS} $\delta^{13}C$ data and values normalized to the sample mean (Larsen et al., 2013) to account for possible temporal variation in coral food sources, growth rates or environmental conditions prior to the time of sampling. Normalization had no effect on the results of our analysis; therefore, we used the measured (non-normalized) AA_{ESS} $\delta^{13}C$ values (Figure S3). We investigated the strength of individual AA_{ESS} in separating groups using biplots and 95% confidence ellipses and report the means and standard deviation of each AA_{ESS} (Table S5).

The binary classification of group membership (heterotrophic *or* autotrophic) within LDA is not necessarily accurate for mixotrophic organisms that rely on a continuum of both sources. To more accurately calculate the proportional contribution of both nutritional sources to coral diets, we modified the traditional LDA with a two-part bootstrap resampling approach. First, we ran 10,000 permutations of the training dataset used to define the source groups and then classify the coral host, using random draws (with replacement)

from the distributions of AA_{ESS} $\delta^{13}C$ within each group. Group classification for individual corals was determined for each permutation of the training dataset, which provided a distribution of possible classifications, based on subtle variations in the source group error distributions. From this distribution, we calculated the global classification percentage with 95% CI to describe what percentage of the *P. meandrina* population on Palmyra relies more heavily on heterotrophic nutrition. Second, to more precisely quantify the contributions of both sources to individual corals, we created a continuum of autotrophic to heterotrophic nutrition using the first linear discriminant (LD_1), which explained 98% of the variation between the source groups. For each permutation of the LDA (n = 10,000), we determined the centroid (LD_1 mean) of the source groups and calculated the distance between the actual LD_1 value of a given coral and the modelled autotrophic centroid (d_a). We then standardized this value to the total distance between the centroids of the autotrophic and heterotrophic sources (d_{h-a}) to obtain a scale of heterotrophic contribution that ranged from 0 to 1. Thus, the proportional contribution of heterotrophy can be calculated as:

$$\%\text{heterotrophy} = \frac{LD1_{coral} - d_a}{d_{h-a}}$$

We calculated this value for every coral in each of the 10,000 random permutations of the source data and calculated 95% CI of the mean per cent contribution of heterotrophy to each individual (Figure 4). Importantly, this analysis is designed to identify unquantified diet contributions (e.g. microbially or coral-synthesized AA_{ESS}, as large deviations from 0 or 1 are indicative of the first linear discriminant poorly constraining possible diet sources. For any coral with a CI that slightly overlapped 0 or 1, we are unable to distinguish that individual from being 100% of the respective source. Notably, we developed this approach using only LD_1 because of how well it explained the variation in our data. For future studies on other coral taxa or for sources that separate along more than one linear discriminant, a similar approach should be developed using Euclidian distance between the source centroids.

3 | RESULTS

We observed significant differences in $\delta^{13}C$ of both AA_{ESS} and AA_{NESS} between the coral host and the autotrophic (endosymbionts) and heterotrophic (zooplankton and POM) sources ($\delta^{13}C_{ESS}$: Pillai trace = 1.11, $F_{(12,80)}$ = 8.40, p < .001; $\delta^{13}C_{NESS}$: Pillai trace = 1.34, $F_{(14,68)}$ = 9.87, p < .001). There was clear separation between the AA_{ESS} $\delta^{13}C$ values of the heterotrophic source and endosymbiont with isoleucine, lysine and threonine having the greatest differences (Figure 1, Tables S2 and S5). There was greater variation among AA_{NESS} $\delta^{13}C$ values. Coral and endosymbiont AA_{NESS} $\delta^{13}C$ values were generally similar and more enriched than heterotrophic sources with the exceptions of glycine that was most enriched in the coral host tissue and serine, which had similar $\delta^{13}C$ values across all groups (Figure 1, Table S2).

We obtained 100% successful reclassification within each of our source groups indicating highly differentiated autotrophic and heterotrophic $\delta^{13}C$ fingerprints for AA_{ESS}. Based on the first linear discriminant (LD_1), which explained 98% of the overall variation between groups, the most important AA_{ESS} for separating the autotrophic and heterotrophic sources were isoleucine, leucine and threonine (Table S4). LDA classified 6 of 19 (32%) coral host samples as heterotrophic (Figure 2a). The bootstrapped reclassification indicated 34.4% (95% CI: 21%–53%) of the coral host samples obtain significantly more of their AA_{ESS} from heterotrophic sources, while 65.6% (95% CI: 47%–79%) relied primarily on AA_{ESS} from

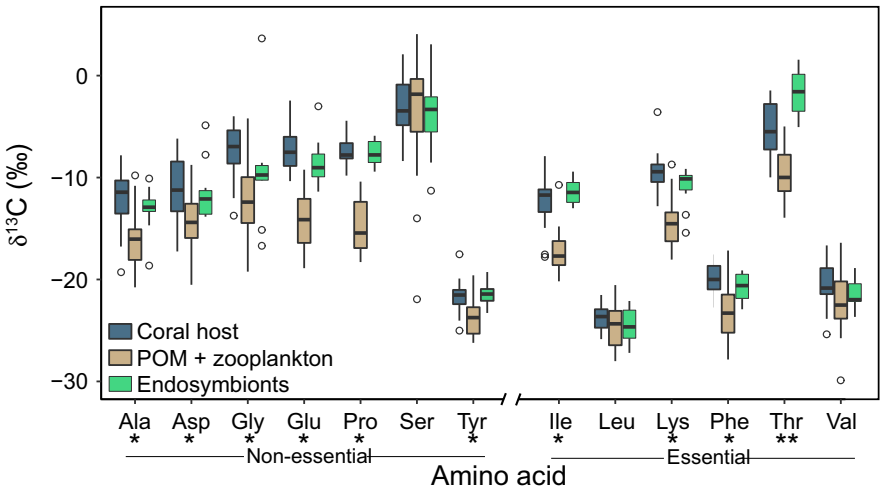

FIGURE 1 Variation in $\delta^{13}C$ values of seven non-essential (AA_{NESS}) and six essential (AA_{ESS}) amino acids in *Pocillopora meandrina* relative to endosymbiont tissue (autotrophic resource) and POM+ zooplankton (heterotrophic resource). The median for each group is indicated with a horizontal line. Boxes encompass the first and third quartiles of the data, and the whiskers are calculated as 1.58*IQR. Points beyond this range are plotted individually. Significant differences between the mean value of each amino acid across groups at the p < .05 level are indicated with (*). With the exception of glycine (Gly), this difference is always between the endosymbiont and POM+ zooplankton tissues. Differences among all three groups are denoted with (**). Please see Table S3 for pairwise contrasts

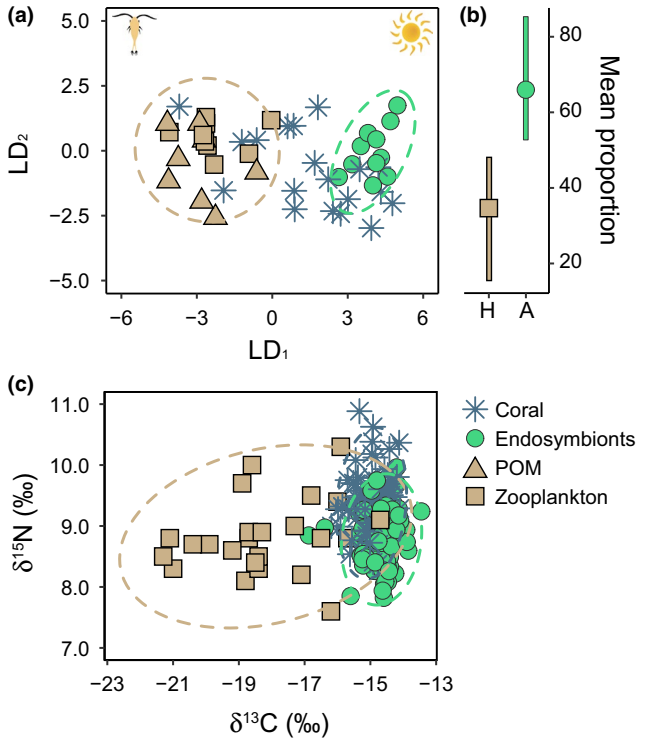

FIGURE 2 A comparison between $\delta^{13}C$ fingerprints based on six essential amino acids (AA_{ESS}) and bulk tissue $\delta^{13}C$ and $\delta^{15}N$ analysis of a mixotrophic coral and the common autotrophic (endosymbiont) and heterotrophic (zooplankton and POM) resources on Palmyra Atoll. (a) Linear discriminant analysis of six AA_{ESS} for coral tissue (n = 19), endosymbionts (n = 11), zooplankton (n = 9) and POM (n = 8). Dashed lines represent 95% confidence ellipses around each source group. (b) The mean proportion and 95% confidence intervals of colonies that were classified as belonging to the autotrophic (A) versus heterotrophic (H) groups based on 10,000 random permutations of the source data. (c) Biplot of bulk tissue $\delta^{13}C$ and $\delta^{15}N$ values for all coral and endosymbiont samples collected around Palmyra (n = 87) and zooplankton samples from the lagoon and fore reef (n = 24); data for POM are not shown for visual clarity due to high variation among samples. For reference, the POM means (±95% CI) are −19.9‰ (−26.6 to −13.3) for $\delta^{13}C$ and 5.2‰ (1.9 to 8.4) for $\delta^{15}N$

their endosymbionts (Figure 2b). Our results suggest that the more individual AA_{ESS} included in a fingerprinting framework, the more robust group separation is likely to be. Indeed, group separation was strongest using $\delta^{13}C$ fingerprints derived from all six AA_{ESS}. In contrast, using only the AA_{ESS} (Ile, Lys, Thr) with mean $\delta^{13}C$ values that differed among the potential sources (Figure 1) provided less statistical support for separation between the heterotrophic resources and endosymbionts (Figure 3a–c). When the most informative linear discriminant coefficients were considered (Ile, Thr, Leu; Table S4), stronger separation between groups and a more informative spread of the individual coral colonies was achieved (Figure 3d–f). Notably, Leu $\delta^{13}C$ values do not differ among the three groups but Leu is more powerful in separating them than Lys, which does have distinct values between autotrophic and heterotrophic sources (Figure 1). This illustrates that the relative spacing between $\delta^{13}C$ values of individual AA_{ESS} within a sample (fingerprints) help maximize the separation of AA_{ESS} $\delta^{13}C$ values that truly differ among groups (Figure 3f).

Site location had no consistent influence on the diets of individual corals; colonies separated by metres were just as likely to have different diet compositions than those on opposite sides of the atoll (Figure 4a,b). On average, the SW corner of the atoll near the main channel into the lagoon had the highest proportion of corals classified as heterotrophic (56.7%, 95% CI [7.4%–99.9%]) and the SE corner had the lowest (13.7%, 95% CI [0%–60.6%]). The large confidence intervals of heterotrophic diet proportions for each location were driven by high inter-colony variation in coral AA_{ESS} $\delta^{13}C$ values at the site level. Across 19 colonies, the relative contribution of heterotrophic and autotrophic sources ranged from 0% to 100%, with an average of 41.34%, 95% CI [28.93%–53.93%], indicating exceptional variation in trophic plasticity (Figure 4a).

In contrast, coral and endosymbiont bulk tissue $\delta^{13}C$ and $\delta^{15}N$ values showed minimal variation on an atoll scale and provided no additional insight to patterns of coral nutrition around Palmyra. Mean (±*SD*) coral host $\delta^{13}C$ values (−15.0 ± 0.4‰) were slightly but not significantly lower than those of the endosymbionts (−14.7 ± 0.5‰: Figure 1c, Table S3). Mean (±*SD*) $\delta^{15}N$ values for corals (9.4 ± 0.5‰) and endosymbionts (8.8 ± 0.4‰) were variable but on average differed by <1‰ (Figure 2c, Table S2). There was substantial overlap in the 95% confidence ellipses around all groups (Figure 2c), and the statistical separation between sources was inconsistent (Table S3). POM samples from the lagoon and fore reef habitats were not distinguishable and showed a high degree of variation in both mean (±*SD*) $\delta^{13}C$ (−19.9 ± 3.4‰) and $\delta^{15}N$ (5.2 ± 1.6‰) values. Finally, the bulk tissue isotope proxy for estimating coral heterotrophy ($\Delta^{13}C = \delta^{13}C_{host} - \delta^{13}C_{endosymbiont}$) was correlated with our estimates of coral nutrition using AA_{ESS} $\delta^{13}C$. Corals that consumed >40% of heterotrophic carbon tended to have lower mean (±*SD*) $\Delta^{13}C$ (−0.6 ± 0.2‰) relative to those that relied on <40% heterotrophic nutrition (−0.3 ± 2‰), but there was high variation in $\Delta^{13}C$ among colonies (r = −.54, p = .03, Figure 4c). This suggests that despite high variability among colonies, $\Delta^{13}C$ values of *Pocillopora* tend to track coarse changes in heterotrophic nutrition.

4 | DISCUSSION

The physiological benefits of heterotrophic nutrition to mixotrophic corals are diverse and may be critical for survival and recovery following disturbance events, yet methodological limitations have historically impeded our ability to quantify coral nutrition in situ. Here, we demonstrate that AA_{ESS} $\delta^{13}C$ analysis is a promising technique for partitioning heterotrophic and autotrophic nutrition in reef-building corals. Our study reveals that the trophic ecology of a widely distributed Indo-Pacific coral can be highly variable among conspecific colonies within a small area (~10 m^2). These results provide a

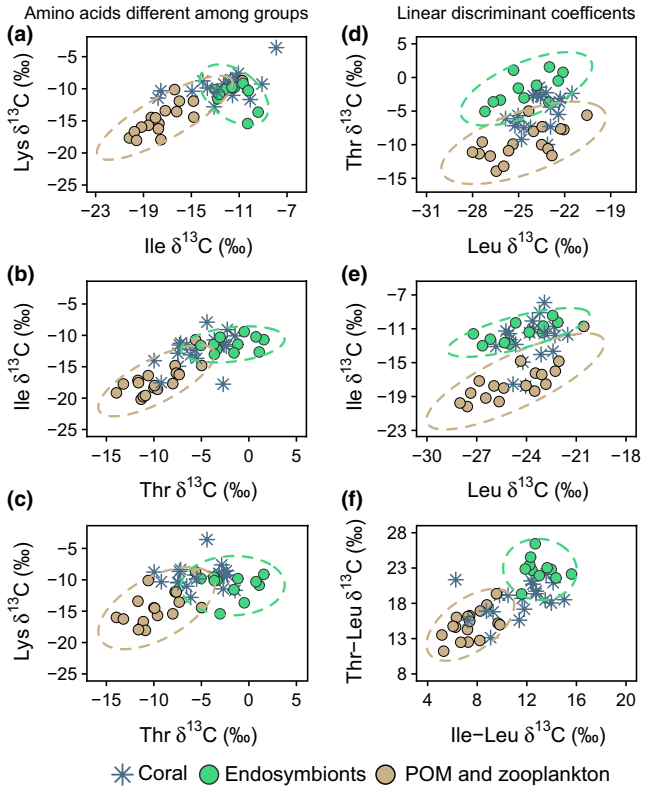

FIGURE 3 Biplots of measured AA_{ESS} $\delta^{13}C$ values displaying the autotrophic (endosymbionts) and heterotrophic (POM and zooplankton) resources in relation to the individual coral hosts. The left column (a–c) shows separation patterns based on the three AA_{ESS} (Ile, Lys, Thr) that differ significantly in raw values between at least two groups (Figure 1). The right column (d–f) presents group separation as a function of the three most informative linear discriminant coefficients, which includes Leu. Note Leu $\delta^{13}C$ values do not differ among groups (Figure 1), but separation among autotrophic and heterotrophic resources is greatest when all groups are normalized to Leu (f). Dashed lines indicate 95% CI

much-needed framework for rigorously investigating coral trophic plasticity across multiple species and spatiotemporal scales.

Recent research has shown that heterotrophic nutrition is more important to coral ecology than previously considered (Ferrier-Pagès et al., 2011) but is highly variable and difficult to quantify. For example, bulk tissue $\delta^{13}C$ and $\delta^{15}N$ data have shown that coral trophic ecology varies spatially and can be directly influenced by resource supply (Fox et al., 2018; Radice, Hoegh-Guldberg, Fry, Fox, & Dove, 2019; Williams et al., 2018). This approach, however, has been hindered by small (~1‰) differences in $\delta^{13}C$ values between the coral host and endosymbionts ($\Delta^{13}C_{host-endosymbiont}$), coupled with a high degree of isotopic variation in bulk tissues among coral species (Hoogenboom et al., 2015; Nahon et al., 2013). While we did observe correlation between our modelled AA_{ESS} $\delta^{13}C$-based estimates of coral heterotrophy and the commonly used bulk tissue $\Delta^{13}C_{host-symbiont}$ metric (Figure 4c), the AA_{ESS} $\delta^{13}C$-based estimates of heterotrophic nutrition provided a more quantitative assessment of coral nutrition among individual colonies. Coral bulk tissue $\delta^{13}C$ values can vary as a function of the relative amounts of proteins, lipids and carbohydrates contained in the host (Wall, Ritson-Williams, Popp, & Gates, 2019). Thus, by isolating a single component of coral tissue (i.e. protein) AA_{ESS} $\delta^{13}C$ analysis can provide estimates of coral nutrition that are less confounded by other aspects of host physiology. Lipid synthesis and catabolism are critical components of coral physiology that cannot be assessed with this technique (Baumann et al., 2014; Grottoli & Rodrigues, 2011) and future work that combines fatty acid $\delta^{13}C$ and $\delta^{13}C$ AA_{ESS} analysis will likely develop a more holistic understanding of coral nutrition. Nevertheless, AAs are major conduits of carbon flow through food webs that provide reliable estimates of source contributions to consumer diets across multiple biomes (Elliott Smith et al., 2018; Larsen et al., 2013; Liew et al., 2019; McMahon et al., 2016) and our results suggest this holds true within mixotrophic corals.

We achieved 100% separation and reclassification of the autotrophic (endosymbionts) and common heterotrophic resources for corals (zooplankton and POM) based on LDA of AA_{ESS} $\delta^{13}C$ values. Notably, patterns of AA_{ESS} $\delta^{13}C$ values from 19 colonies of *P. meandrina* separated cleanly (98% of variation explained) along a one-dimensional continuum of autotrophic and heterotrophic nutrition, allowing for quantitative estimates of the proportion of heterotrophic carbon incorporated by individual corals (Figures 2a and 4a). Our results support previous observations that multivariate AA_{ESS} $\delta^{13}C$ fingerprints provided more robust separation among groups than $\delta^{13}C$ values of individual AA_{ESS} (Figures 2a,b and 3) and support the quantitative power of developing source-specific fingerprints with LDA based on multiple AA_{ESS} (Arthur, Kelez, Larsen, Choy, & Popp, 2014; Elliott Smith et al., 2018; Larsen et al., 2009, 2013, 2016; Liew et al., 2019; McMahon et al., 2016). The definitive separation of source groups revealed by our analysis does not strongly support a missing or unquantified source of AA_{ESS} in our model of coral nutrition for *P. meandrina* on Palmyra. We also used a permutational LDA to explore the sensitivity of our analysis to variation in resource group AA_{ESS} $\delta^{13}C$ values. We found that 5 of 19 modelled coral diets had 95% confidence intervals overlapping 0% (n = 4) or 100% heterotrophy (n = 1). For these individuals, we cannot statistically distinguish their diets from being purely autotrophic versus purely heterotrophic. However, in both cases the mean percentages for each individual were calculated beyond 0% or 100% for 5.5% and 3.7% of 10,000 permutations, respectively. The infrequent occurrence of this statistical limitation provides confidence in our approach for assessing nutritional contributions in corals that consume a mixture of these two resource types.

The reliability of AA_{ESS} $\delta^{13}C$ fingerprinting in organisms that can synthesize their own AA_{ESS} or have complex assemblages of microbial symbionts (e.g. the coral microbiome) has not been extensively tested. This may create challenges for interpreting AA_{ESS} $\delta^{13}C$ data for some coral species as they may be capable of synthesizing certain AA_{ESS} (Fitzgerald & Szmant, 1997; Ying et al., 2018). Presently, the best evidence for AA_{ESS} de novo synthesis in scleractinian

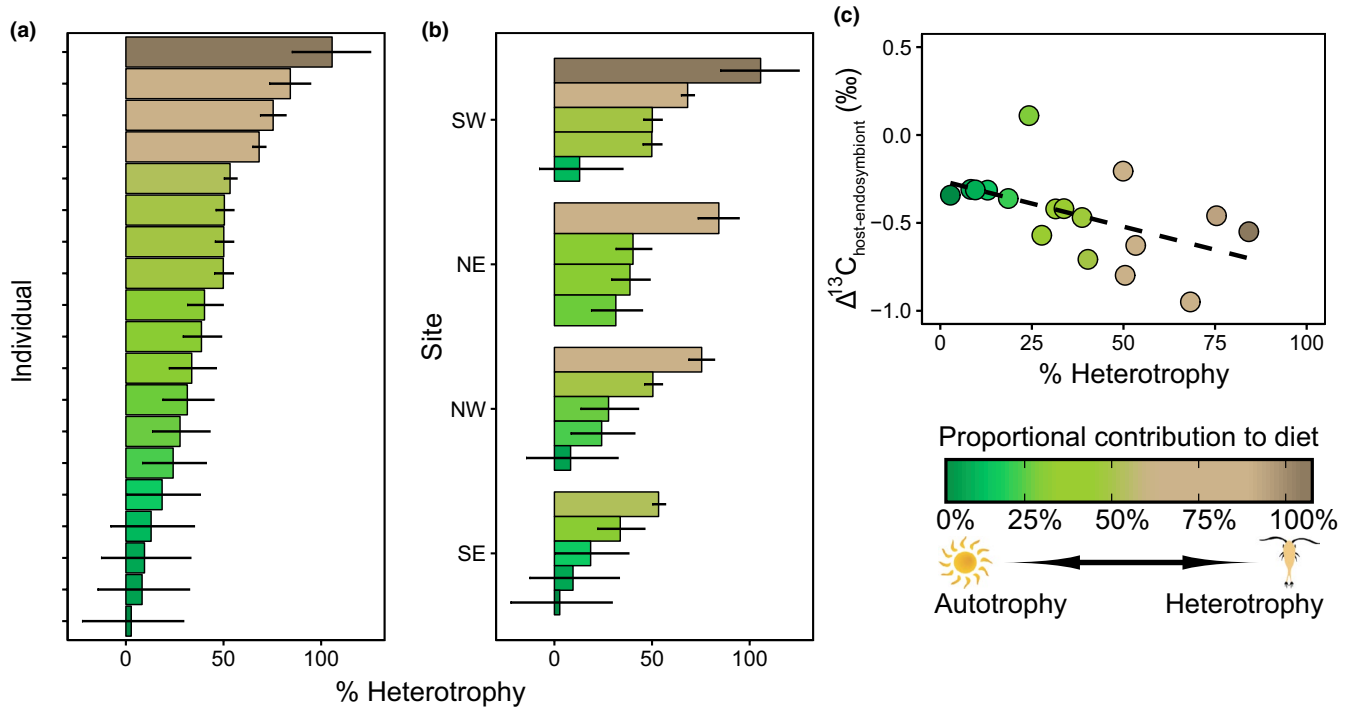

FIGURE 4 Individual variation in coral nutrition across 19 colonies from four locations around Palmyra Atoll. (a) The estimated mean per cent contribution of heterotrophy to coral nutrition. Error bars denote 95% CI and for any individual with an error bar that overlaps 0 (100% autotrophy) or 1 (100% heterotrophy) is statistically indistinguishable from the respective source. (b) Individual coral colonies from (a) arranged by site. Error bars denote 95% CI. (c) $\Delta^{13}C_{host-endosymbiont}$ as a function of the average per cent contribution of heterotrophic resources to coral nutrition. See the supporting information for details on calculating relative proportions of autotrophy and heterotrophy to coral nutrition using AA_{ESS} $\delta^{13}C$ data

corals is for histidine and it appears only to be present in corals from the *Robusta* clade (Ying et al., 2018). We were unable to reliably measure histidine in this study; although considered an essential amino acid, it is not a common constituent of AA_{ESS} $\delta^{13}C$ fingerprints (Larsen et al., 2009, 2013) because of its low concentration in many tissues (Beach, Munks, & Robinson, 1943). Furthermore, some corals (e.g. *Acropora digitifera*) are unable to synthesize tyrosine, which is considered an AA_{NESS} for most animals (Lin et al., 2015). As such, the classical definitions of AA_{ESS} versus AA_{NESS} may not be strictly applicable to corals, and we anticipate that emerging techniques in functional genomics will provide valuable insight to which AA_{ESS} corals can synthesize and how widespread such pathways are across taxa (Ying et al., 2018). Such studies will also help resolve the role of coral microbial communities as a possible third source of AA_{ESS} to the coral host. To date, microbial contribution cannot be ruled out (Fitzgerald & Szmant, 1997), in large part due to the difficulty of physically isolating coral host tissue from embedded cell-associated microbial aggregates (David, Kathleen, & Nicole, 2016; Work & Aeby, 2014). Our permutational analysis was designed to allow for evidence of such an unquantified source, which would have resulted in reduced model performance and reduced confidence around estimated mean autotrophic and heterotrophic contributions. However, the strength of separation we obtained between our two sampled resource groups indicate that for *P. meandrina* on Palmyra, we did not find compelling evidence of de novo synthesis of AA_{ESS} or contributions from another unknown (e.g. microbial) source.

We do not interpret our results to suggest that *Pocillopora* colonies are persistently in a state of trophic extremes (complete autotrophy or heterotrophy). Instead, our data provide compelling evidence that *P. meandrina* has the capacity to alter reliance on different nutritional modes and that this trophic plasticity can vary widely among individuals that live only metres apart. Our samples were collected during the onset of thermal stress and several weeks prior to a widespread coral bleaching on Palmyra Atoll (Fox, Carter, et al., 2019). While none of the sampled colonies were visibly bleached or discoloured, it is possible that the magnitude of trophic plasticity we measured was driven by differential responses to thermal stress among colonies. Both *P. meandrina* and its congener, *P. verrucosa*, are efficient predators that are responsive to patterns of resource availability (Fox et al., 2018; Radice et al., 2019; Roder et al., 2010; Williams et al., 2018; Ziegler, Roder, Büchel, & Voolstra, 2014). Our expectation was that *P. meandrina* would exhibit more homogenous reliance on heterotrophy within sites and that site-specific patterns of resource supply would be the dominant driver in trophic response (Williams et al., 2018). The overwhelming variability at the colony level may indicate that some colonies increased heterotrophy in response to thermal stress (Grottoli et al., 2006), or that variation in boundary layer dynamics and flow associated with reef topography or colony morphology can exert strong

control on the trophic ecology of individual colonies (Helmuth & Sebens, 1993; Sebens, Witting, & Helmuth, 1997). Analysis of samples collected during non-stressful environmental conditions will be valuable for evaluating the baseline reliance on heterotrophy in *P. meandrina* and determining if temperature stress can influence coral AA_{ESS} $\delta^{13}C$ values by destabilizing the coral-algal symbiosis.

The tight recycling of carbon and nitrogen between coral hosts and algal endosymbionts (Tremblay, Maguer, Grover, & Ferrier-Pagès, 2015) and the ability of corals to translocate AAs from ingested prey to their endosymbionts (Piniak, Lipschultz, & McClelland, 2003) may also drive variation in coral AA_{ESS} $\delta^{13}C$ values. However, resource sharing did not appear to influence the AA_{ESS} $\delta^{13}C$ values of *P. meandrina* endosymbionts on Palmyra as the endosymbiont fingerprints and raw AA_{ESS} $\delta^{13}C$ values were highly consistent across all samples and did not vary as a function of heterotrophic nutrition across colonies. Therefore, it is possible that carbon sharing does not strongly affect $\delta^{13}C$ values of AA_{ESS} synthesized by the endosymbionts in *P. meandrina*. The same may not be true for AA_{NESS} as these are typically subjected to larger isotopic fractionations during de novo synthesis by basal sources or consumers themselves (Whiteman et al., 2019), but this has yet to be investigated within a mutualistic symbiosis. We also do not know the exact time period reflected in our samples, as turnover rates for AA_{ESS} have not been established for mixotrophic corals. Based on $\delta^{13}C$ analysis of several coral species, bulk tissue turnover typically occurs over a period of 6–8 weeks (Rodrigues & Grottoli, 2006; Tanaka, Suzuki, & Sakai, 2018; Treignier, Tolosa, Grover, Reynaud, & sa, 2009). Thus, our estimates of *Pocillopora* diets on Palmyra likely reflect nutritional inputs over at least the preceding 2 months. Future studies should place particular emphasis on experimental validation of the AA_{ESS} fingerprinting approach across coral species. Feeding studies will be critical for validating patterns of carbon and nitrogen isotopic discrimination and turnover for both AA_{ESS} and AA_{NESS} as these processes can vary among AAs (Downs, Popp, & Holl, 2014), which could impact the temporal window reflected in our analyses. Comprehensive sampling of local autotrophic and heterotrophic resources for corals should also be prioritized to assess the spatial generality of the AA_{ESS} $\delta^{13}C$ fingerprints for different endosymbionts groups within the *Symbiodiniaceae* and planktonic communities on coral reefs. We found that AA_{ESS} $\delta^{13}C$ fingerprints did not differ between POM and zooplankton communities from the lagoon and fore reef habitats, which suggests that the planktonic communities in these two habitats may be taxonomically similar or have similar amino acid synthesis pathways. The similarity between our lagoon and fore reef samples is consistent with the observation that pelagic and lagoon zooplankton on Palmyra have similar bulk tissue $\delta^{13}C$ and $\delta^{15}N$ values and can only be distinguished isotopically by the more deplete $\delta^{34}S$ values of lagoon plankton (McCauley et al., 2014). Therefore, the combination of AA_{ESS} $\delta^{13}C$ analysis with additional isotopic tracers may be a powerful approach for quantifying spatial variation in heterotrophic resource supply to corals (McCauley et al., 2014; Vander Zanden, Soto, Bowen, & Hobson, 2016).

The variation in heterotrophic nutrition we observed among individual colonies of *P. meandrina* reveals the potential for an unexpected degree of trophic plasticity among coral colonies at small spatial scales. Reliance on heterotrophy can vary widely among species (Hoogenboom et al., 2015), and the degree of trophic flexibility may be important for determining population trajectories following disturbances such as bleaching events (Grottoli et al., 2006). Our findings support previous estimates of high inter-colony variation in heterotrophic nutrition among three coral species in the Florida Keys (Teece, Estes, Gelsleichter, & Lirman, 2011). Individual- and species-level variation in nutritional strategy has largely been ignored in studies of coral population dynamics and community structure but is likely a critical driver in these processes. This is particularly relevant as allochthonous subsidies to coral reef ecosystems are increasingly recognized as important drivers in nutrient cycling (Graham et al., 2018; Radice et al., 2019; Reid et al., 2019), coral nutrition (Fox et al., 2018; Williams et al., 2018) and ecosystem functioning (Gove et al., 2016; Morais & Bellwood, 2019). Collectively, this evidence suggests that energy flow in coral reef ecosystems is likely more complex and spatially variable than previously considered.

5 | CONCLUSIONS

AA_{ESS} $\delta^{13}C$ analysis of a ubiquitous Indo-Pacific coral species as well as dominant autotrophic (endosymbiont) and heterotrophic (zooplankton and POM) resources revealed that nutrition in *P. meandrina* can be most variable at the level of individual colonies within a single reef system. Our results demonstrate that AA_{ESS} $\delta^{13}C$ analysis is capable of resolving the relative contribution of auto- versus heterotrophic sources to the diet of mixotrophic corals. Overall, the AA_{ESS} $\delta^{13}C$ fingerprinting approach applied here offers a novel framework for quantifying trophic plasticity within scleractinian corals and refining the drivers of variation in coral trophic ecology. Such studies have the potential to advance our understanding of spatial and taxonomic variation in coral survival and recovery following disturbances and reveal fundamental patterns in coral trophic ecology across environmental gradients.

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CONFLICT OF INTEREST

The authors declare no competing interests.

AUTHORS' CONTRIBUTIONS

All authors conceived the study; M.D.F. collected the samples; M.D.F., E.A.E.S. and S.D.N. conducted laboratory work and analysed the data; M.D.F. led the writing of the manuscript. All authors contributed to revisions and gave final approval for publication.

DATA AVAILABILITY STATEMENT

Data and code are deposited in the Dryad Digital Repository: https://doi.org/10.5061/dryad.tv8v6k3 (Fox, Elliott Smith, Smith, & Newsome, 2019).

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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