# Amino acid $\delta^{13}C$ and $\delta^{15}N$ analyses reveal distinct species-specific patterns of trophic plasticity in a marine symbiosis

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*Supplemental Information*

## Bulk tissue isotope analysis

Sample analytical accuracy and precision ($\delta^{13}C$ and $\delta^{15}N$) was <0.2 ‰ based on repeated analysis of laboratory reference material (glycine and a homogenate of tuna white muscle tissue) run before and after every 10 samples. Quantification and isotopic corrections were calculated using the glycine laboratory reference material run alongside samples, and the isotopic composition of the glycine was characterized using international reference materials including USGS 32, USGS 34, USGS 35, IAEA N3, USGS 40, USGS 41, NBS 18 and NBS 19. The known values for glycine are +11.36 ‰ vs. AIR for $\delta^{15}N$, and -36.49 ‰ vs. V-PDB for $\delta^{13}C$.

## Individual amino acid isotope analyses

Isotopic analysis of amino acids in coral host, symbiont algae, and a pooled plankton sample were performed by subjecting tissue samples to acid hydrolysis, carboxyl terminus esterification, and amine group trifluoroacetylation (Hannides et al. 2013; Shih et al. 2020). Acid hydrolysis was performed by heating (150 °C) tissues (approx. 15 mg) in 6N HCl for 70 min in a culture tube fitted with a Teflon-lined cap and filled with $N_2$ gas. HCl was evaporated to dryness, hydrolysate redissolved in 0.01N HCl, and filtered through a 0.2 µm polyethersulfone filter with a 0.01N HCl wash. The hydrolysate solubilized in 0.01N HCl was purified by cation exchange

(Dowex 50WX8-400), and amino acids were eluted with ammonium hydroxide. Hydrolyzed tissues were esterified by heating (110 °C) for 60 min in 1:4 acetyl chloride : isopropanol and the amine group was trifluoroacetylated by adding 3:1 methylene : trifluoroacetic anhydride (TFAA) solution to vials and heating (100 °C) for 15 min. Finally, solvent extraction in P-buffer ($KH_2PO_4 + Na_2HPO_4$ in milli-Q water, pH 7) was used to further purify samples (Ueda et al. 1989). Chloroform was used to partition acylated amino acids, and following solvent evaporation, sample trifluoroacetylation was repeated to maximize derivitization. Samples were stored frozen in 3:1 methylene chloride : TFAA at -20 °C until analyzed.

## Trophic position calculations

While values for β and Δ have been widely used in the study of a marine invertebrates and fishes to calculate trophic positions (Chikaraishi et al. 2009; Hannides et al. 2013; Shih et al. 2020) and more recently in reef corals (Fujii et al. 2020), we tested the applicability of the canonical β and Δ values for $TP_{\text{Glx-Phe}}$. The β values calculated for isolated Symbiodiniaceae (3.7 ± 0.8 ‰, propagated error, $n = 6$) were similar to that found by (Fujii et al. 2020) and not statistically different from 3.4 ± 1.0 ‰ ($p$=0.407). In addition, the average trophic position for Symbiodiniaceae calculated using equation 1 is not different from 1 (1.04 ± 0.22), which correctly identifies Symbiodiniaceae as a primary producer and indicates that the $\Delta_{\text{Glx-Phe}}$ value of 7.6 ‰ is applicable for this alga. In other works, trophic positions in Symbiodiniaceae and hermatypic corals have been reported to range from 0.7-1.5 and 0.8-1.8, respectively (Fuji et al. 2020; Martinez et al. 2020), with trophic positions in temperate heterotrophic cnidarians (sea jellies, octocorals) ranging from 2.2-3.7 (Grossowicz et al. 2020).

## *Supplemental Tables*

**Table S1**. Bulk isotope samples in plankton and suspended particles in seawater adjacent to six reefs spanning Kāne‘ohe Bay in August and December 2016. Isotopes values in the pooled plankton sample used in the present study are presented for comparison (collected January 2018).

| Size fraction (μm) | $\delta^{13}C$ | $\delta^{15}N$ |
|---|---|---|
| <10 | -21.04 ± 0.79 | 5.33 ± 0.14 |
| 10-100 | -18.06 ± 0.44 | 7.41 ± 0.12 |
| 100-243 | -21.06 ± 0.19 | 6.50 ± 0.11 |
| <243 | -20.44 ± 0.50 | 5.92 ± 0.17 |
| >243[a] | -20.75 ± 0.28 | 6.77 ± 0.22 |
| 63-243[b] | -21.8 | 7.1 |

Values are mean±SE (*n*=12), except [b] = pooled plankton sample (*n*=1, this study). All samples were filtered from seawater collected at 3 m depth, except [a,b] = collected from vertical and horizontal plankton tows (63 μm mesh) (data from Wall et al., 2020).

**Table S2.** Statistical analyses of carbon and nitrogen isotope values ($\delta^{13}C$, $\delta^{15}N$) in bulk tissues of coral and Symbiodiniaceae exposed to Light-by-Feeding nutrition treatments*.

| *Bulk tissue isotope values* | *Effect* | *df* | *SS* | *MS* | *F* | *p* |
|---|---|---|---|---|---|---|
| $\delta^{13}C$ | Fraction | 1 | 1.401 | 1.401 | 1.532 | 0.251 |
| | Treatment | 2 | 3.772 | 1.886 | 2.062 | 0.190 |
| | Residual | 8 | 7.317 | 0.915 | | |
| $\delta^{15}N$ | Fraction | 1 | 4.083 | 4.083 | 14.770 | **0.005** |
| | Treatment | 2 | 0.105 | 0.053 | 0.190 | 0.831 |
| | Residual | 8 | 2.212 | 0.277 | | |
| C:N | Fraction | 1 | 0.132 | 0.132 | 0.723 | 0.420 |
| | Treatment | 2 | 2.218 | 1.109 | 6.086 | **0.025** |
| | Residual | 8 | 1.457 | 0.182 | | |
| $\delta^{13}C_{H\text{-}S}$ | Treatment | 1 | 0.763 | 0.382 | 7.897 | 0.064 |
| | Residual | 2 | 0.145 | 0.048 | | |
| $\delta^{15}N_{H\text{-}S}$ | Treatment | 1 | 0.063 | 0.032 | 0.288 | 0.769 |
| | Residual | 2 | 0.330 | 0.110 | 0.000 | |

*'Fraction' is host coral (H) or symbiont Symbiodiniaceae tissue (S). 'Treatment' represents combination of Light-by-Feeding nutrition treatments: Light–Not Fed, Light–Fed, Dark–Fed. Significant p-values ($p$<0.05) are in bold.

**Table S3.** Carbon and nitrogen isotope values ($\delta^{13}C$, $\delta^{15}N$) of amino acids measured in pooled samples of coral host (*Montipora capitata*) and endosymbiont algae (Symbiodiniaceae) along with a size-fractioned plankton sample*.

| *Amino acid $\delta^{13}C$ (‰)* | *Coral* | *Symbiodiniaceae* | *Pooled plankton* |
|---|---|---|---|
| Alanine (Ala) | -15.35 ± 0.29 | -16.98 ± 0.66 | -20.83 |
| Aspartic acid (Asp) | -10.35 ± 0.39 | -9.55 ± 0.69 | -15.38 |
| Glutamic acid (Glx) | -8.11 ± 0.54 | -10.38 ± 0.47 | -16.12 |
| Glycine (Gly) | -13.44 ± 0.66 | -17.25 ± 1.26 | -19.48 |
| Isoleucine (Ile) | -14.21 ± 0.59 | -15.09 ± 0.93 | -21.81 |
| Leucine (Leu) | -24.69 ± 0.43 | -24.94 ± 0.45 | -27.84 |
| Lysine (Lys) | -11.59 ± 0.33 | -12.08 ± 0.56 | -19.34 |
| Phenylalanine (Phe) | -19.94 ± 0.50 | -20.34 ± 0.42 | -24.88 |
| Proline (Pro) | -10.32 ± 0.26 | -12.16 ± 0.73 | -17.28 |
| Serine (Ser) | -9.13 ± 0.70 | -8.72 ± 0.81 | -11.03 |
| Threonine (Thr) | -10.53 ± 1.01 | -9.67 ± 0.70 | -17.90 |
| Tyrosine (Tyr) | -21.08 ± 0.61 | -20.70 ± 0.53 | -25.22 |
| Valine (Val) | -24.26 ± 0.55 | -25.27 ± 0.69 | -27.20 |
| *Amino acid $\delta^{15}N$ (‰)* | | | |
| Alanine (Ala) | 7.02 ± 0.47 | 5.37 ± 0.69 | 12.53 |
| Aspartic acid (Asp) | 6.44 ± 0.36 | 5.38 ± 0.25 | 8.94 |
| Glutamic acid (Glx) | 6.38 ± 0.23 | 5.77 ± 0.33 | 11.55 |
| Glycine (Gly) | 2.84 ± 0.58 | 4.03 ± 0.63 | 5.60 |
| Isoleucine (Ile) | 6.26 ± 0.79 | 5.93 ± 0.70 | 7.25 |
| Leucine (Leu) | 4.65 ± 0.41 | 2.82 ± 0.30 | 6.72 |
| Lysine (Lys) | 3.08 ± 0.28 | 2.89 ± 0.53 | 3.84 |
| Phenylalanine (Phe) | 1.35 ± 0.38 | 2.03 ± 0.76 | 0.57 |
| Proline (Pro) | 6.81 ± 0.43 | 4.05 ± 0.41 | 11.99 |
| Serine (Ser) | 3.73 ± 0.58 | 3.72 ± 0.48 | 6.05 |
| Threonine (Thr) | -1.06 ± 0.77 | 1.04 ± 0.93 | -1.98 |
| Tyrosine (Tyr) | 2.11 ± 0.35 | 4.43± 0.60 | 4.62 |
| Valine (Val) | 6.24 ± 0.42 | 5.97 ± 0.32 | 9.79 |

*Coral and Symbiodiniaceae values are pooled across three nutrition treatments, which had limited effects on carbon and nitrogen isotope values. Values are mean ± SE (*n* = 6), except for the pooled plankton sample (63 – 250 µm) (*n* = 1).

**Table S4.** Statistical analyses of carbon isotope values ($\delta^{13}C$) in coral and Symbiodiniaceae amino acids exposed to Light-by-Feeding nutrition treatments*.

| *Amino acid $\delta^{13}C$* | *Effect* | *df* | *SS* | *MS* | *F* | *p* |
|---|---|---|---|---|---|---|
| *Nonessential amino acids* | | | | | | |
| Alanine (Ala) | Fraction | 1 | 7.928 | 7.928 | 4.849 | 0.059 |
| | Treatment | 2 | 2.386 | 1.193 | 0.730 | 0.512 |
| | Residual | 8 | 13.080 | 1.635 | | |
| Aspartic acid (Asp) | Fraction | 1 | 1.937 | 1.937 | 0.870 | 0.378 |
| | Treatment | 2 | 1.110 | 0.555 | 0.249 | 0.785 |
| | Residual | 8 | 17.798 | 2.225 | | |
| Glycine (Gly) | Fraction | 1 | 43.514 | 43.514 | 16.281 | **0.004** |
| | Treatment | 2 | 38.910 | 19.455 | 7.279 | **0.016** |
| | Residual | 8 | 21.382 | 2.673 | | |
| Glutamic acid (Glx) | Fraction | 1 | 15.418 | 15.418 | 15.500 | **0.004** |
| | Treatment | 2 | 7.177 | 3.589 | 3.608 | 0.076 |
| | Residual | 8 | 7.958 | 0.995 | | |
| Proline (Pro) | Fraction | 1 | 10.096 | 10.096 | 5.023 | 0.055 |
| | Treatment | 2 | 1.963 | 0.981 | 0.488 | 0.631 |
| | Residual | 8 | 16.081 | 2.010 | | |
| Serine (Ser) | Fraction | 1 | 0.510 | 0.510 | 0.191 | 0.674 |
| | Treatment | 2 | 13.333 | 6.667 | 2.498 | 0.144 |
| | Residual | 8 | 21.353 | 2.669 | | |
| Tyrosine (Tyr) | Fraction | 1 | 0.427 | 0.427 | 0.220 | 0.652 |
| | Treatment | 2 | 3.794 | 1.897 | 0.974 | 0.418 |
| | Residual | 8 | 15.577 | 1.947 | | |
| *Essential amino acids* | | | | | | |
| Isoleucine (Ile) | Fraction | 1 | 2.301 | 2.301 | 0.509 | 0.496 |
| | Treatment | 2 | 0.536 | 0.268 | 0.059 | 0.943 |
| | Residual | 8 | 36.157 | 4.520 | | |
| Leucine (Leu) | Fraction | 1 | 0.181 | 0.181 | 0.146 | 0.713 |
| | Treatment | 2 | 1.819 | 0.910 | 0.734 | 0.510 |
| | Residual | 8 | 9.913 | 1.239 | | |
| Lysine (Lys) | Fraction | 1 | 0.715 | 0.715 | 0.458 | 0.518 |
| | Treatment | 2 | 0.083 | 0.041 | 0.027 | 0.974 |
| | Residual | 8 | 12.498 | 1.562 | | |
| Phenylalanine (Phe) | Fraction | 1 | 0.472 | 0.472 | 0.340 | 0.576 |
| | Treatment | 2 | 1.717 | 0.858 | 0.619 | 0.562 |
| | Residual | 8 | 11.093 | 1.387 | | |
| Threonine (Thr) | Fraction | 1 | 2.197 | 2.197 | 0.446 | 0.523 |
| | Treatment | 2 | 5.526 | 2.763 | 0.561 | 0.591 |
| | Residual | 8 | 39.372 | 4.922 | | |
| Valine (Val) | Fraction | 1 | 3.055 | 3.055 | 1.273 | 0.292 |
| | Treatment | 2 | 4.477 | 2.239 | 0.933 | 0.432 |
| | Residual | 8 | 19.199 | 2.400 | | |

*'Fraction' is host coral or symbiont Symbiodiniaceae tissue. 'Treatment' represents combination of Light-by-Feeding nutrition treatments: Light–Not Fed, Light–Fed, Dark–Fed. Significant p-values ($p<0.05$) are in bold.

**Table S5.** Statistical analyses of nitrogen isotope values ($\delta^{15}N$) in coral and Symbiodiniaceae amino acids exposed to Light-by-Feeding nutrition treatments*.

| *Amino acid $\delta^{15}N$* | *Effect* | *df* | *SS* | *MS* | *F* | *p* |
|---|---|---|---|---|---|---|
| *Trophic amino acids* | | | | | | |
| Alanine (Ala) | Fraction | 1 | 8.168 | 8.168 | 3.392 | 0.103 |
| | Treatment | 2 | 1.858 | 0.929 | 0.386 | 0.692 |
| | Residual | 8 | 19.262 | 2.408 | | |
| Aspartic acid (Asp) | Fraction | 1 | 3.333 | 3.333 | 6.317 | **0.036** |
| | Treatment | 2 | 1.420 | 0.710 | 1.346 | 0.314 |
| | Residual | 8 | 4.221 | 0.528 | | |
| Glutamic acid (Glx) | Fraction | 1 | 1.115 | 1.115 | 2.361 | 0.163 |
| | Treatment | 2 | 0.935 | 0.467 | 0.990 | 0.413 |
| | Residual | 8 | 3.777 | 0.472 | | |
| Isoleucine (Ile) | Fraction | 1 | 0.338 | 0.338 | 0.091 | 0.771 |
| | Treatment | 2 | 3.858 | 1.929 | 0.518 | 0.615 |
| | Residual | 8 | 29.800 | 3.725 | | |
| Leucine (Leu) | Fraction | 1 | 9.995 | 9.995 | 19.904 | **0.002** |
| | Treatment | 2 | 3.809 | 1.904 | 3.792 | 0.069 |
| | Residual | 8 | 4.017 | 0.502 | | |
| Proline (Pro) | Fraction | 1 | 22.935 | 22.935 | 28.936 | **0.001** |
| | Treatment | 2 | 4.117 | 2.059 | 2.597 | 0.135 |
| | Residual | 8 | 6.341 | 0.793 | | |
| Valine (Val) | Fraction | 1 | 0.225 | 0.225 | 0.220 | 0.652 |
| | Treatment | 2 | 0.042 | 0.021 | 0.020 | 0.980 |
| | Residual | 8 | 8.170 | 1.021 | | |
| *Source amino acids* | | | | | | |
| Glycine (Gly) | Fraction | 1 | 4.226 | 4.226 | 1.650 | 0.235 |
| | Treatment | 2 | 1.569 | 0.785 | 0.306 | 0.744 |
| | Residual | 8 | 20.487 | 2.561 | | |
| Lysine (Lys) | Fraction | 1 | 0.105 | 0.105 | 0.080 | 0.785 |
| | Treatment | 2 | 0.245 | 0.123 | 0.093 | 0.912 |
| | Residual | 8 | 10.530 | 1.316 | | |
| Serine (Ser) | Fraction | 1 | 0.001 | 0.001 | 0.000 | 0.986 |
| | Treatment | 2 | 1.661 | 0.831 | 0.440 | 0.659 |
| | Residual | 8 | 15.113 | 1.889 | | |
| Phenylalanine (Phe) | Fraction | 1 | 1.400 | 1.400 | 0.536 | 0.485 |
| | Treatment | 2 | 0.801 | 0.400 | 0.153 | 0.861 |
| | Residual | 8 | 20.916 | 2.615 | | |
| Threonine (Thr) | Fraction | 1 | 13.327 | 13.327 | 2.658 | 0.142 |
| | Treatment | 2 | 3.612 | 1.806 | 0.360 | 0.708 |
| | Residual | 8 | 40.117 | 5.015 | | |
| Tyrosine (Tyr) | Fraction | 1 | 16.163 | 16.163 | 8.950 | **0.017** |
| | Treatment | 2 | 0.128 | 0.064 | 0.035 | 0.965 |
| | Residual | 8 | 14.448 | 1.806 | | |

*'Fraction' is host coral or symbiont Symbiodiniaceae tissue. 'Treatment' represents combination of Light-by-Feeding nutrition treatments: Light–Not Fed, Light–Fed, Dark–Fed. Significant p-values ($p<0.05$) are in bold.

**Table S6.** Fractional contribution of heterotrophy (%) to coral nutrition calculated using a simple two component linear mixing model and $\delta^{15}$N amino acid trophic position (TP$_{\text{Glx-Phe}}$). End-member TP$_{\text{Glx-Phe}}$ used to calculate % heterotrophy are (*i*) *Montipora capitata* coral hosts (*ii*) symbionts isolated from *M. capitata,* (*iii*) a pooled Kāne‘ohe Bay plankton sample, and (*iv*) modeled values for marine consumers feeding on zooplankton prey. All uncertainties represent standard deviation of propagated errors.

| ***Treatment*** | ***TP$_{\text{Glx-Phe}}$*** | | | | ***% Primary consumers [b] vs. Translocation [a]*** | ***% Zooplanktivory [c] vs. Translocation*** |
|---|---|---|---|---|---|---|
| | *Coral host* | *Symbiont algae* | *Primary consumers* | *Zooplankton consumers* | | |
| Light-Not Fed | 1.35 ± 0.17 | 0.90 ± 0.17 | 2.00 ± 0.22 | 3.00 ± 0.20 | 41 ± 15% | 21 ± 7% |
| Light-Fed | 1.13 ± 0.17 | 1.12 ± 0.16 | 2.00 ± 0.22 | 3.00 ± 0.20 | 1 ± 19% | 1 ± 9% |
| Dark-Fed | 1.16 ± 0.16 | 1.11 ± 0.18 | 2.00 ± 0.22 | 3.00 ± 0.20 | 6 ± 19% | 3 ± 9% |

[a] *Translocation* TP$_{\text{Glx-Phe}}$ of symbionts = autotrophy, a diet consisting symbiont products

[b] *Primary production* TP$_{\text{Glx-Phe}}$ based on digested symbiont cells, free-living primary producers, and/or allochthonous particulates preyed upon by zooplankton

[c] *Zooplanktivory* TP$_{\text{Glx-Phe}}$ of zooplankton consumers, with trophic enrichment (+1 TP$_{\text{Glx-Phe}}$) of predators *vs.* prey

## *Supplemental Figures*

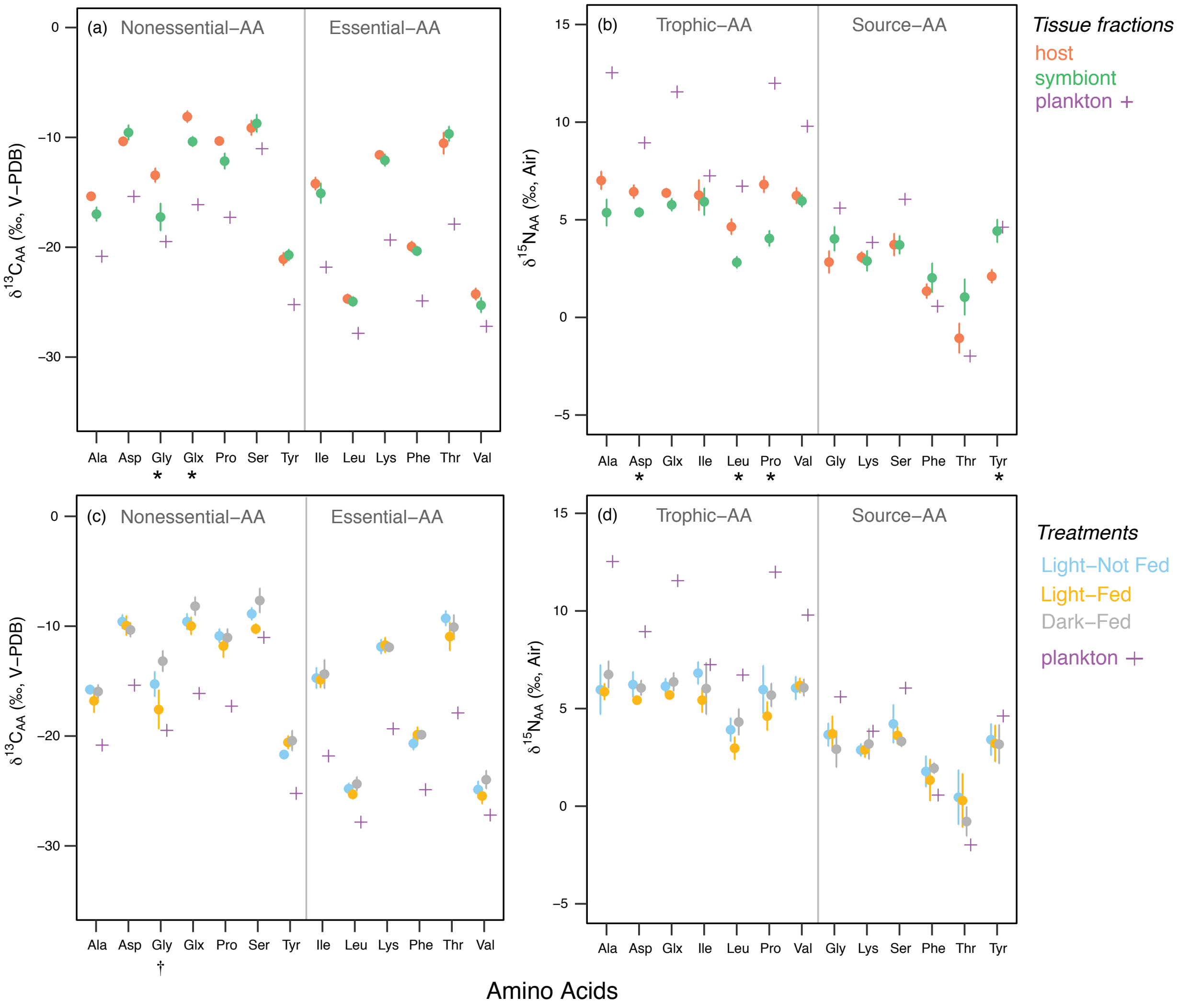

**Figure S1**. (*a*,*c*) $\delta^{13}$C and (*b*,*d*) $\delta^{15}$N values of individual amino acids in coral hosts and Symbiodiniaceae symbionts according to tissue fraction (*top panel*) and nutrition treatments (*bottom panel*) in relationship to a pooled plankton sample. Values are mean ± SD ($n = 2$) [*top*] and mean ± SE ($n = 6$) [*bottom*], except for the plankton sample ($n = 1$). X-axis *symbols* indicate significant differences ($p<0.05$) between fractions (host and symbiont, *) and treatments (†).

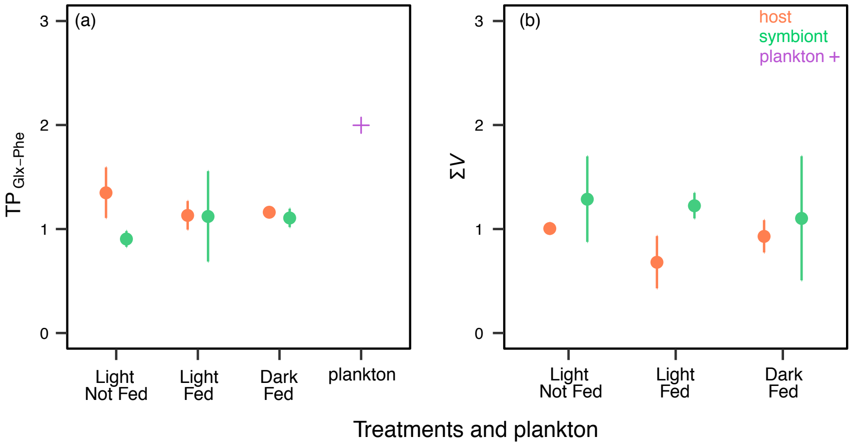

**Figure S2**. (*a*) Trophic position and (*b*) summed variance ($\Sigma V$) index calculations for individual amino acid $\delta^{15}N$ values in coral hosts and Symbiodiniaceae symbionts across nutrition treatments in relation to a pooled plankton sample. Values are mean ± SD ($n = 2$), except for the plankton sample ($n = 1$), where $\Sigma V$ cannot be calculated.

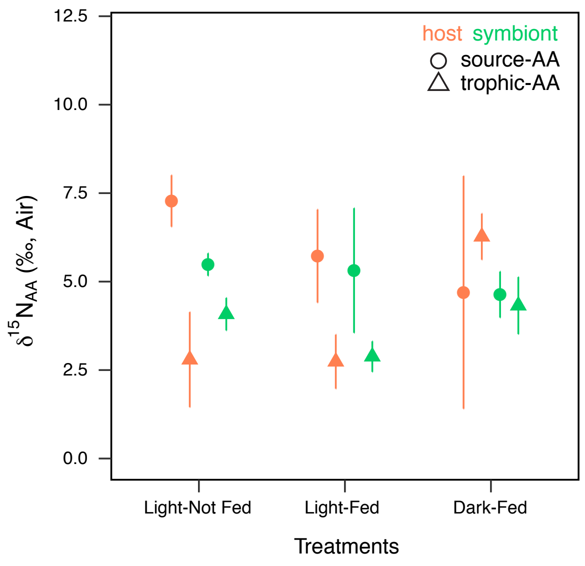

**Figure S3**. $\delta^{15}$N weighted means for trophic and source amino acids according to coral tissue fractions (host, symbiont) and experimental nutrition treatments. Values are mean ± SD (*n* = 2).

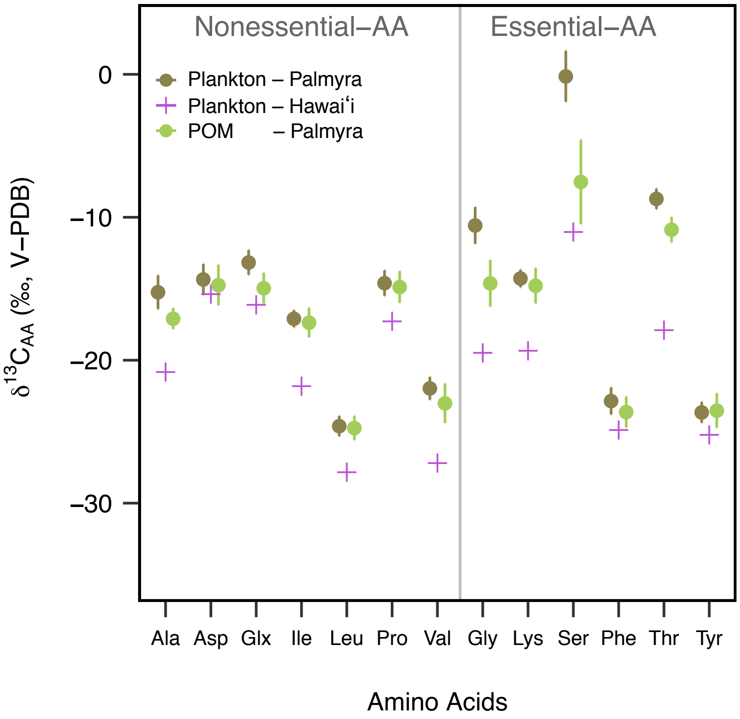

**Figure S4**. Comparison of $\delta^{13}$C values for nonessential and essential amino acids in a plankton sample from Kāneʻohe Bay, Oʻahu, Hawaiʻi and plankton and particulate organic material (POM) from Palmyra (reported in Fox et al., 2019). Values are mean ± SE (*n* = 6 – 9), except for the Hawaiʻi plankton (*n* = 1).

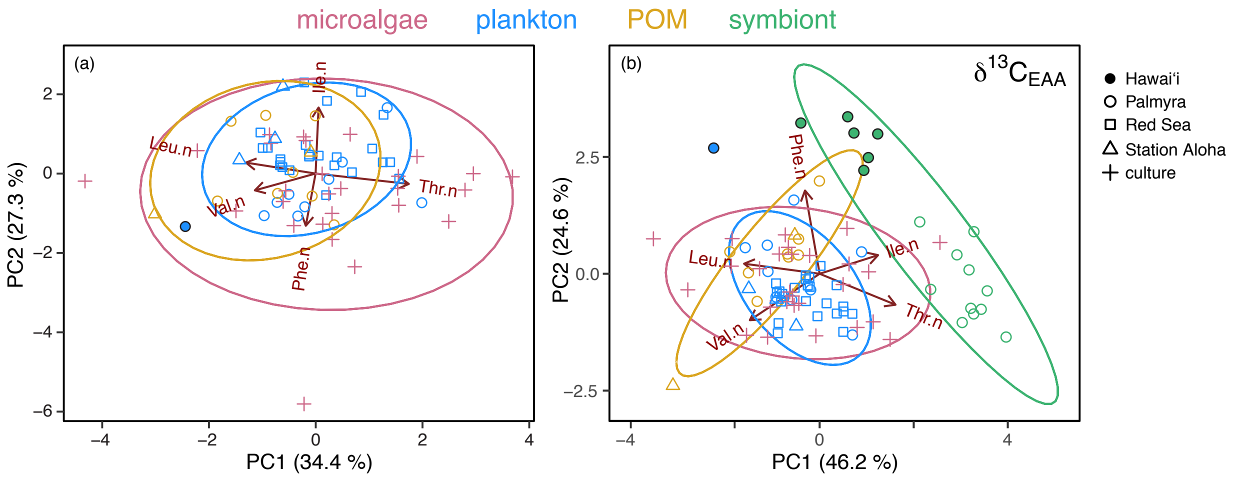

**Figure S5**. Principal component (PC) analyses of mean-normalized essential amino acid $\delta^{13}C$ ($\delta^{13}C_{EAA}$) values in cultured microalgae, coastal and oceanic plankton and particulate organic matter (POM), and Symbiodiniaceae symbionts isolated from two coral species. Data matrixes in (*a*) and (*b*) are identical, except for the inclusion of symbiont samples in (*b*). Ellipses represent 90 % standard deviation with arrows for individual mean-normalized amino acids being significant ($p<0.05$) correlation vectors. Data from Hawai‘i (this study), Palmyra (Fox et al. 2019), the Red Sea (McMahon et al. 2015), Station Aloha (Hannides et al. 2008), and cultures (Larsen et al. 2013).

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