Tissue- and cellular-level allocation of autotrophic and heterotrophic nutrients in the coral symbiosis – A NanoSIMS study

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Supplementary Information

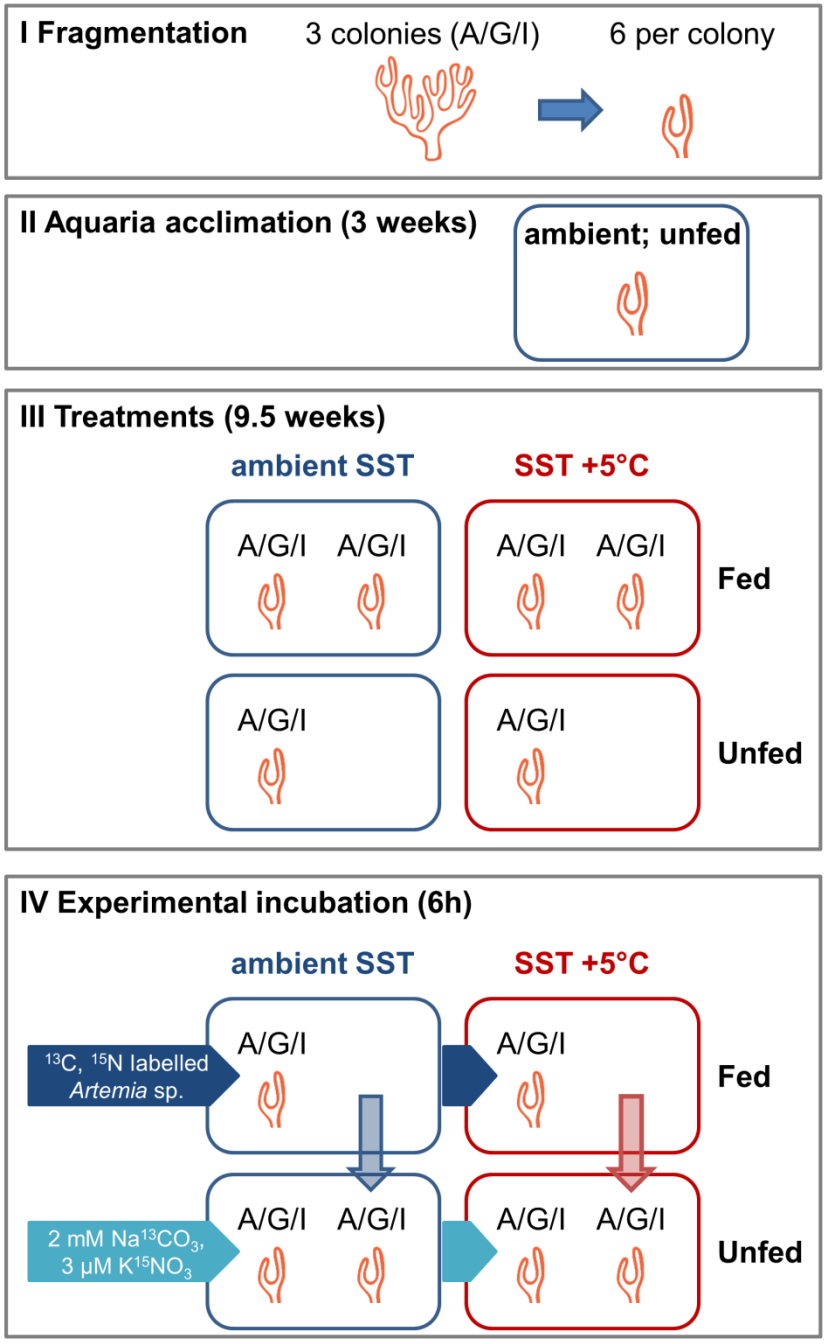

Figure S1. Schematic overview of experimental setup and coral acclimation. After collection from the sea, three coral colonies (A/G/I) were fragmented (I) and acclimated to the aquarium setup (II). Corals were subsequently maintained under a 2x2 factorial treatment design with 2 levels for temperature (ambient SST/SST+5°C) and food supply (Fed/Unfed) (III). For the experimental incubation (IV), the isotopic label was either provided in the form of inorganic nutrients to assess autotrophic assimilation or as organic prey (*Artemia salina*) for heterotrophic assimilation. To contrast autotrophic performance in corals that are acclimated to a regular food input vs. corals that are dependent on autotrophy, fed coral fragments from each temperature treatment were moved into the aquaria that received inorganic isotopic labels.

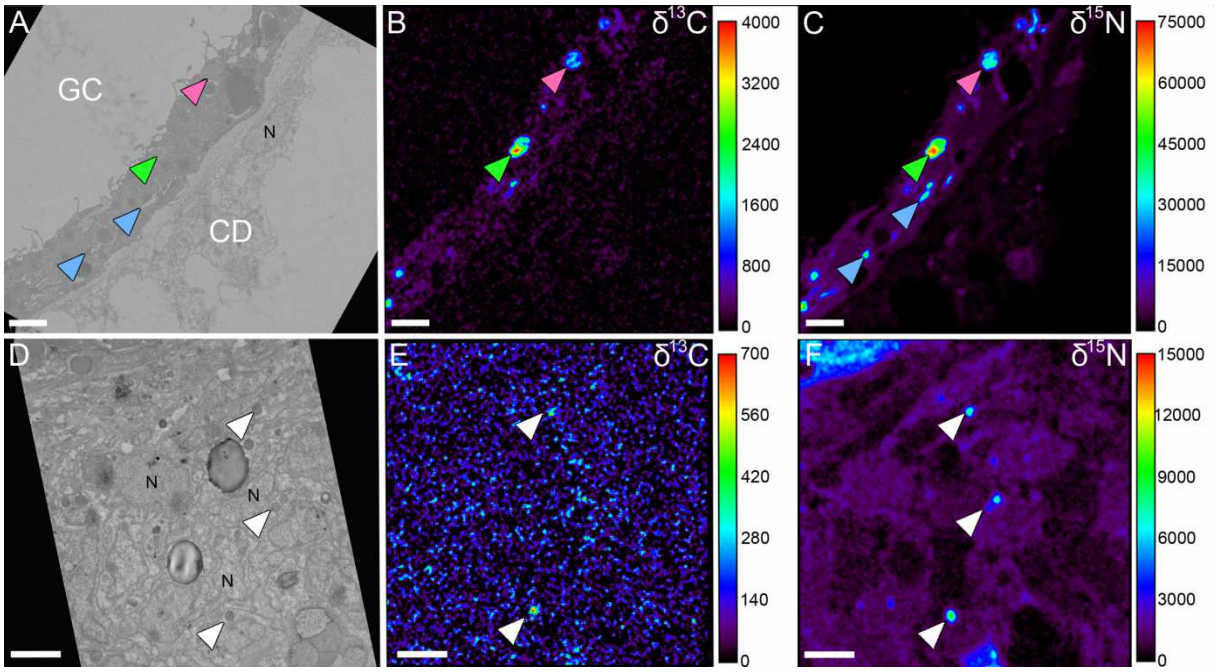

Figure S2. Heterotrophic carbon and nitrogen allocation in the basal body wall gastrodermis and surface body wall epidermis. Representative images for the distribution of prey ^{13}C and ^{15}N (c) in the coral basal body wall gastrodermis (A-C) and the surface body wall epidermis (D-F), originating from ingestion of labelled brine shrimps after 6h. Colour codes of arrows in A-C correspond to the three described types of gastrodermal hotspots (cf. main text, Fig. 4). White arrows in D-F indicate multivesicular bodies. Colours in NanoSIMS maps display enrichment relative to an unlabelled tissue in δ-notation (black to red). GC: gastrovascular canal, CD: calicodermis, N: nucleus. Scale bars are 2 µm.

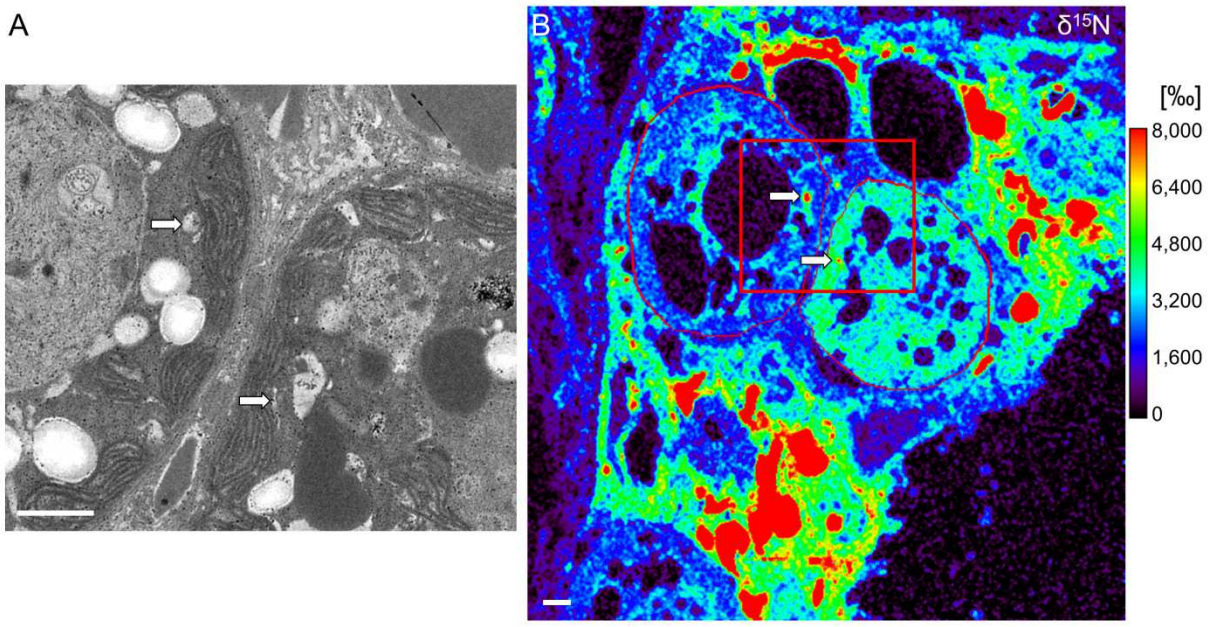

Figure S3. Heterotrophic nitrogen hotspots in *Symbiodinium*. TEM (a) and corresponding ^{15}N NanoSIMS image (b) reveals homogenous heterotrophic nitrogen labelling in the symbiont cells (thin red outline) with occasional vesicle-like hotspots (black arrows). Note that (b) shows the same image as Fig. 2f with a δ-value cut-off at 8000. Scale bars are 1 µm.

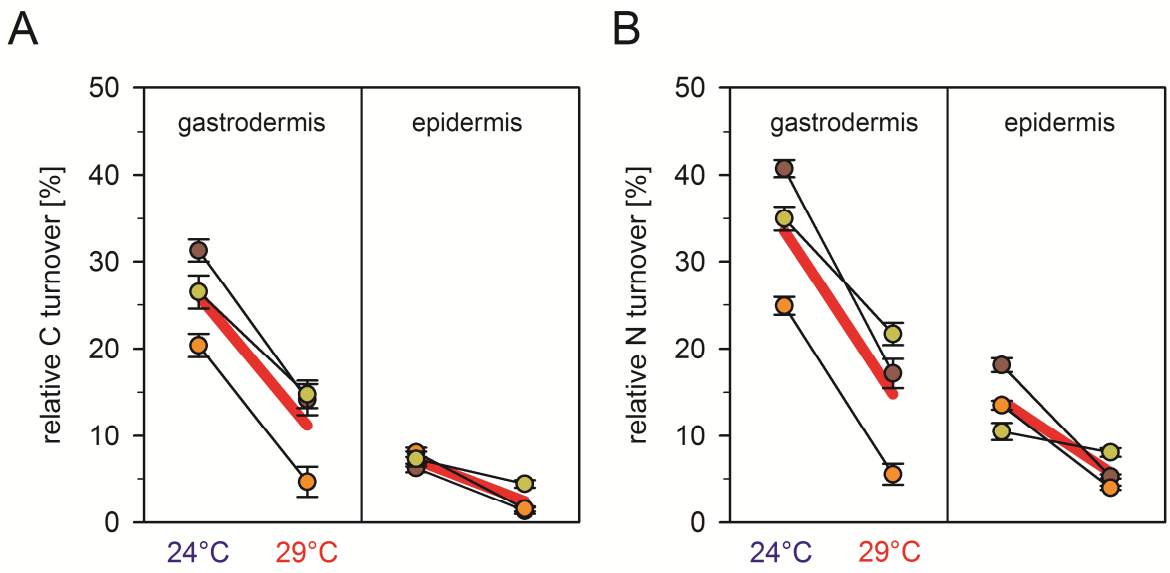

Figure S4. Heterotrophic hotspot enrichment. Relative carbon (a) and nitrogen (b) turnover of gastrodermal and epidermal heterotrophy hotspots, irrespective of type. Individual colony responses (dots; green, orange, red) and overall species response (thick red line) are shown for the surface body wall of the coral coenenchyme (Table S4). Due to the strong imbalance in N between thermal treatments, all data points within each replicate were reduced to a mean value and the thermal response tested as one-sided paired t-test with N=3. Gastrodermal hotspots: ^{13}C: t(2)=-9.173, p=0.0058*; ^{15}N: t(2)=-6.297, p=0.0122*. Epidermal hotspots: ^{13}C: t(2)=-4.213, p=0.0232*; ^{15}N: t(2)=-2.689, p=0.0575.

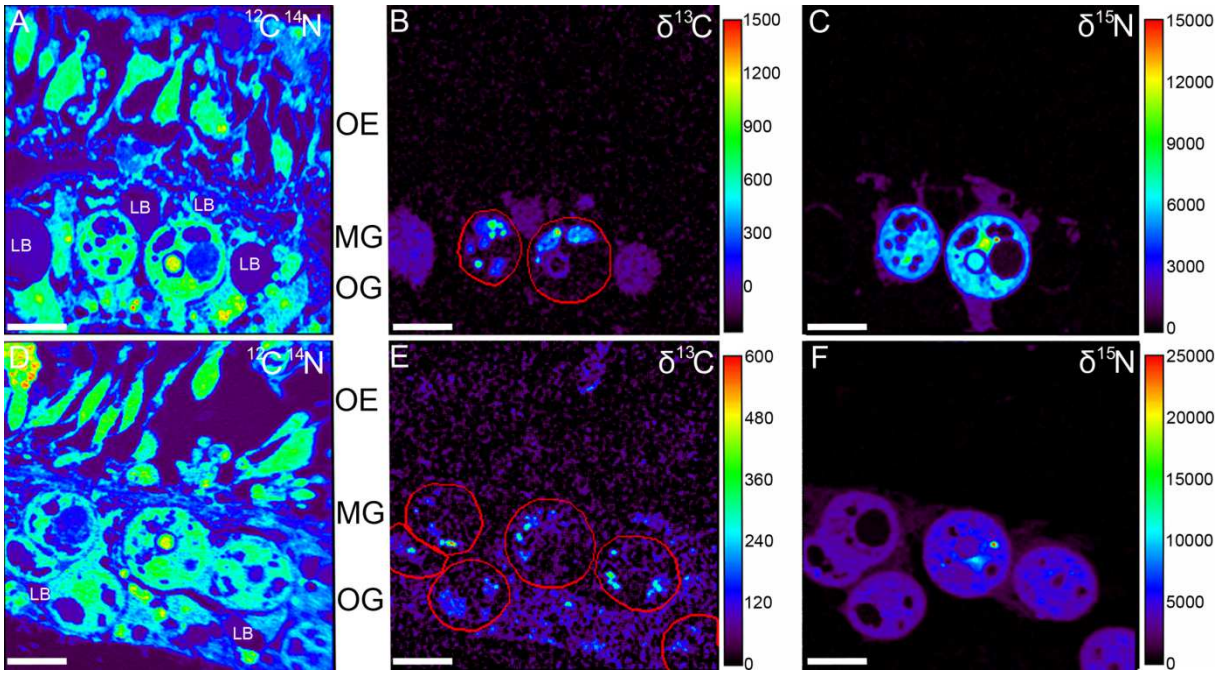

Figure S5. The fate of pyruvate carbon. Enrichment patterns of coenenchyme tissue of *Stylophora pistillata* after incubation with 1 mM [1-^{13}C]-pyruvate + 3 µM $K^{15}NO_3$ (A-C) and [3-^{13}C]-pyruvate + 3 µM $K^{15}NO_3$ (D-E) after 3h in the light. General tissue structure (A, B) showing oral epidermis (OE), mesoglea (MG), and oral gastrodermis containing symbionts and host lipid bodies (LB). Scale bars are 5 µm. Average symbiont $\delta^{13}C$ of shown symbiont cells is 159‰ *vs*. 37‰ (B *vs*. E) and 4944 *vs*. 3565 (C *vs*. F)

Table S1. Statistical results of the Three-Way ANOVA on the effects of feeding, temperature, and replicate on the autotrophic fixation of bicarbonate and nitrate for each region of interest (cf. Fig. 5 Aut [U] *vs*. Aut [F]). Note that ^{15}N ROI data was not considered for the extra-algal lipid bodies due to their close proximity to the highly enriched symbiont cell and the labelled symbiosome membrane (cf. LB* in Fig. 1C). In contrast ^{13}C enrichment was clearly assessable (Fig 1B). Asterisks indicate statistical significance.

element	compartment	*Symbiodinium*	gastrodermis	epidermis	host lipid bodies	extra-algal lipid bodies
^{13}C	feeding acclimation (F)	$F_{1,607}$ = 9.893, p=0.0017*	$F_{1,158}$ = 0.187, p=0.6663	$F_{1,158}$ = 6.751, p=0.0103*	$F_{1,522}$ = 2.532, p=0.1122	$F_{1,129}$ = 0.203, p=0.6534
	temperature acclimation (T)	$F_{1,607}$ = 54.047, p<0.0001*	$F_{1,158}$ = 69.107, p<0.0001*	$F_{1,158}$ = 27.203, p<0.0001*	$F_{1,522}$ = 24.527, p<0.0001*	$F_{1,129}$ = 2.677, p=0.1044
	replicate (R)	$F_{2,606}$ = 7.307, p=0.0007*	$F_{2,157}$ = 0.431, p=0.6508	$F_{2,157}$ = 46.836, p<0.0001*	$F_{2,521}$ = 19.674, p<0.0001*	$F_{2,128}$ = 1.787, p=0.1718
	F x T	N/A	$F_{1,156}$ = 5.877, p=0.0165*	$F_{1,156}$ = 22.731, p<0.0001*	$F_{1,520}$ = 2.956, p=0.0862	N/A
	T x R	$F_{2,603}$ = 8.552, p=0.0002*	$F_{2,154}$ = 18.585, p<0.0001*	$F_{2,154}$ = 27.344, p<0.0001*	$F_{2,518}$ = 8.519, p=0.0002*	$F_{2,125}$ = 3.560, p=0.0314*
	F x R	N/A	$F_{2,154}$ = 30.607, p<0.0001*	$F_{2,154}$ = 14.204, p<0.0001*	$F_{2,518}$ = 0.717, p=0.4889	$F_{2,125}$ = 3.448, p=0.0349*
	F x T x R	N/A	N/A	N/A	$F_{2,512}$ = 10.200, p<0.0001*	N/A
^{15}N	feeding acclimation (F)	$F_{1,607}$ = 1.890, p=0.1697	$F_{1,158}$ = 0.548, p=0.4602	$F_{1,158}$ = 0.158, p=0.6913	$F_{1,522}$ = 0.003, p=0.9558	-
	temperature acclimation (T)	$F_{1,607}$ = 22.872, p<0.0001*	$F_{1,158}$ = 45.346, p<0.0001*	$F_{1,158}$ = 58.990, p<0.0001*	$F_{1,522}$ = 4.764, p=0.0295*	-
	replicate (R)	$F_{2,606}$ = 151.771, p<0.0001*	$F_{2,157}$ = 6.219, p=0.0025*	$F_{2,157}$ = 5.934, p=0.0033*	$F_{2,521}$ = 14.396, p<0.0001*	-
	F x T	$F_{1,605}$ = 0.054, p=0.8159	$F_{1,156}$ = 4.541, p=0.0347*	$F_{1,156}$ = 0.789, p=0.3760	N/A	-
	T x R	$F_{2,603}$ = 60.557, p<0.0001*	$F_{2,154}$ = 18.570, p<0.0001*	$F_{2,154}$ = 0.8326, p=0.4370	N/A	-
	F x R	$F_{2,603}$ = 34.306, p<0.0001*	$F_{2,154}$ = 9.4011, p<0.0001*	$F_{2,154}$ = 6.149, p=0.0027*	$F_{2,518}$ = 6.182, p=0.0022*	-
	F x T x R	$F_{2,597}$ = 6.777, p=0.0012*	$F_{2,148}$ = 12.173, p<0.0001*	$F_{2,148}$ = 9.474, p=0.0001*	N/A	-

Table S2. Statistical results of the Two-Way ANOVA on the effects of temperature and replicate on the heterotrophic fixation of carbon and nitrogen from a brine shrimp for each region of interest (cf. Fig. 6: Het [F]). Asterisks indicate statistical significance.

element	compartment	Symbiont	Gastrodermis	Epidermis	Host lipid bodies
^{13}C	replicate (R)	$F_{2,271}$ = 17.158, p<0.0001*	$F_{2,65}$ = 4.183, p=0.0198*	$F_{2,65}$ = 63.356, p<0.0001*	$F_{2,371}$ = 11.267, p<0.0001*
	temperature acclimation (T)	$F_{1,272}$ = 298.391, p<0.0001*	$F_{1,66}$ = 113.063, p<0.0001*	$F_{1,66}$ = 244.079, p<0.0001*	$F_{1,372}$ = 69.298 p<0.0001*
	R x T	$F_{2,268}$ = 109.604, p<0.0001*	$F_{2,62}$ = 4.895, p=0.0106*	$F_{2,62}$ = 3.745, p=0.0291*	$F_{2,368}$ = 14.018, p<0.0001*
^{15}N	replicate (R)	$F_{2,271}$ = 167.730, p<0.0001*	$F_{2,65}$ = 21.822, p<0.0001*	$F_{2,65}$ = 37.411, p<0.0001*	$F_{2,371}$ = 10.174, p<0.0001*
	temperature acclimation (T)	$F_{1,272}$ = 715.600, p<0.0001*	$F_{1,66}$ = 155.285, p<0.0001*	$F_{1,66}$ = 365.760, p<0.0001*	$F_{1,372}$ = 84.389 p<0.0001*
	R x T	$F_{2,268}$ = 150.906, p<0.0001*	$F_{2,62}$ = 12.587, p<0.0001*	$F_{2,62}$ = 5.742, p=0.0052*	$F_{2,368}$ = 5.387, p=0.0049*

Table S3. Statistical results of the Three-Way ANOVA on the effects of feeding mode (autotrophic vs. heterotrophic), temperature, and replicate on the carbon and nitrogen turnover for each region of interest (cf. Fig. 6: Aut [F] *vs*. Het [F]). Asterisks indicate statistical significance.

element	compartment	*Symbiodinium*	gastrodermis	epidermis	host lipid bodies
^{13}C	feeding mode (F)	$F_{1,565}$ = 1031.004, p<0.0001*	$F_{1,140}$ = 108.896, p<0.0001*	$F_{1,140}$ = 1576.139, p<0.0001*	$F_{1,618}$ = 1257.353, p<0.0001*
	temperature acclimation (T)	$F_{1,565}$ = 100.112, p<0.0001*	$F_{1,140}$ = 164.544, p<0.0001*	$F_{1,140}$ = 286.319, p<0.0001*	$F_{1,618}$ = 72.467, p<0.0001*
	replicate (R)	$F_{2,564}$ = 7.305, p=0.0007*	$F_{2,139}$ = 4.487, p=0.0130*	$F_{2,139}$ = 82.625, p<0.0001*	$F_{2,617}$ = 2.838, p=0.0593
	F x T	$F_{1,563}$ = 0.457, p=0.4995	$F_{1,138}$ = 43.660, p<0.0001*	$F_{1,138}$ = 229.191, p<0.0001*	N/A
	T x R	$F_{2,561}$ = 11.542, p<0.0001*	$F_{2,136}$ = 8.538, p=0.0003*	$F_{2,136}$ = 3.231, p=0.0427*	$F_{2,614}$ = 13.504, p<0.0001*
	F x R	$F_{2,561}$ = 0.696, p=0.4993	$F_{2,136}$ = 7.723, p=0.0007*	$F_{2,136}$ = 58.039, p<0.0001*	$F_{2,614}$ = 13.814, p<0.0001*
	F x T x R	$F_{2,555}$ = 7.762, p=0.0005*	$F_{2,130}$ = 3.871, p=0.0233*	$F_{2,130}$ = 5.359, p=0.0058*	N/A
^{15}N	feeding mode (F)	$F_{1,565}$ = 495.822, p<0.0001*	$F_{1,140}$ = 649.590, p<0.0001*	$F_{1,140}$ = 2422.978, p<0.0001*	$F_{1,618}$ = 503.026, p<0.0001*
	temperature acclimation (T)	$F_{1,565}$ = 657.368, p<0.0001*	$F_{1,140}$ = 171.770, p<0.0001*	$F_{1,140}$ = 394.396, p<0.0001*	$F_{1,618}$ = 72.560, p<0.0001*
	replicate (R)	$F_{2,564}$ = 293.411, p<0.0001*	$F_{2,139}$ = 23.576, p<0.0001*	$F_{2,139}$ = 40.764, p<0.0001*	$F_{2,617}$ = 18.860, p<0.0001*
	F x T	$F_{1,520}$ = 507.797, p<0.0001*	$F_{1,138}$ = 166.140, p<0.0001*	$F_{1,138}$ = 388.653, p<0.0001*	$F_{1,616}$ = 43.136, p<0.0001*
	T x R	$F_{2,561}$ = 182.553, p<0.0001*	$F_{2,136}$ = 14.416, p<0.0001*	$F_{2,136}$ = 6.100, p=0.0029*	$F_{2,614}$ = 12.670, p<0.0001*
	F x R	$F_{2,561}$ = 41.064, p<0.0001*	$F_{2,136}$ = 22.471, p<0.0001*	$F_{2,136}$ = 40.183, p<0.0001*	$F_{2,614}$ = 21.014, p<0.0001*
	F x T x R	$F_{2,555}$ = 75.621, p<0.0001*	$F_{2,130}$ = 12.959, p<0.0001*	$F_{2,130}$ = 6.375, p=0.0023*	$F_{2,608}$ = 5.741, p=0.0034*

Table S4. Statistical effects of feeding and temperature on selected physiological variables of the *Stylophora pistillata* holobiont in colonies A, G, I, based on mixed model analysis of a paired factorial design. Asterisks indicate statistical significance with arrows indicating the direction of the effect.

variable	feeding	temperature	feeding x temperature	REML variance component estimate
host protein [mg cm^{-2}]	$F_{1,6}$ = 4.002 p=0.0924	$F_{1,6}$ = 0.699 p=0.4350	$F_{1,6}$ = 0.0355 p=0.8568	10%
host carbohydrates [µmol cm^{-2}]	$F_{1,6}$ = 0.458 p=0.5240	$F_{1,6}$ = 0.327 p=0.5882	$F_{1,6}$ = 0.231 p=0.6480	9%
symbiont protein [pg $cell^{-1}$]	$F_{1,6}$ = 4.056 p=0.0906	$F_{1,6}$ = 1.973 p=0.2097	$F_{1,6}$ = 0.386 p=0.5575	75%
symbiont carbohydrate [pg $cell^{-1}$]	$F_{1,6}$ = 0.017 p=0.8995	$F_{1,6}$ = 2.195 p=0.1890	$F_{1,6}$ = 0.279 p=0.6165	0%
symbiont density [cells cm^{-2}]	$F_{1,6}$ = 5.797 p=0.0527	$F_{1,6}$ = 1.331 p=0.2926	$F_{1,6}$ = 0.4645 p=0.5209	82%
symbiont density [cells mg host $protein^{-1}$]	$F_{1,6}$ = 0.008 p=0.9304	$F_{1,6}$ = 3.133 p=0.1271	$F_{1,6}$ = 0.396 p=0.5523	59%
total Chl content [pg $cell^{-1}$]	$F_{1,6}$ = 6.268 p=0.0463* ↑	$F_{1,6}$ = 20.487 p=0.0040* ↑	$F_{1,6}$ = 4.166 p=0.0873	0%
O_2 per Chl [mg min^{-1} µg Chl^{-1}]	$F_{1,6}$ = 8.491 p=0.0268* ↓	$F_{1,6}$ = 0.490 p=0.5102	$F_{1,6}$ = 3.857 p=0.0972	22%
O_2 per surface [mg min^{-1} cm^{-2}]	$F_{1,6}$ = 0.244 p=0.6392	$F_{1,6}$ = 9.374 p=0.0222* ↑	$F_{1,6}$ = 0.570 p=0.4788	6%
respiration [mg O_2 min^{-1} mg $protein^{-1}$]	$F_{1,6}$ = 0.590 p=0.4715	$F_{1,6}$ = 0.057 p=0.8190	$F_{1,6}$ = 0.083 p=0.7832	9%
respiration [mg O_2 min^{-1} cm^{-2}]	$F_{1,6}$ = 0.188 p=0.6795	$F_{1,6}$ = 0.069 p=0.8019	$F_{1,6}$ = 0.057 p=0.8191	0%
P_{gross}:R	$F_{1,6}$ = 2.154 p=0.1926	$F_{1,6}$ = 21.079 p=0.0037* ↑	$F_{1,6}$ = 0.562 p=0.4819	50%

Table S5. (see excel file)