Supplemental text

Materials and Methods

a) *Experimental design*

Unless otherwise mentioned, experiments were performed in closed-system glass aquaria, in 20 L seawater (Instant Ocean salts, after equilibration in large, permanent aquarium exhibit tanks), representing a high volume compared to the average ~5 cm size of *Pocillopora damicornis* coral nubbins, ensuring no growth limitation or stress. Corals were not fed with plankton during the acclimatization period and the subsequent experiments. They relied on the photosynthetic activity of their dinoflagellate symbionts for autotrophic nutrition. Illuminance was provided by 8 fluorescent T5 tubes of 39 W (6×10 000 K, 2×20 000 K) on a light/dark cycle of 14 hours/10 hours, respectively. Labeling pulses with ^{15}N-tracers were carried out under light and chase periods under light/dark cycling, except for one experiment carried out in constant darkness to compare ammonium assimilation and the fate of nitrogenous compounds after dark-inhibition of dinoflagellate photosynthesis

1) A short-term experiment was conducted to investigate the dynamics of ^{15}N-ammonium incorporation by the symbiotic association during a pulse of labeling. Eight nubbins of *P. damicornis* were incubated in light for 90 minutes in 0.22 μm filtered artificial seawater enriched with $^{15}NH_4Cl$ (^{15}N isotopic abundance of 98 %, SIGMA) to an estimated ammonium concentration of 20 μM, starting 2 hours after the beginning of the light period. The apex of colony branches (small pieces of corals < 5 mm length) was sampled from each nubbin during the experiment at 0, 5, 15, 30, 45, 60 and 90 minutes.

2) Three nubbins of *P. damicornis* were incubated in light for 4 hours in 0.22 μm filtered artificial seawater enriched with $^{15}NH_4Cl$ to an estimated ammonium concentration of 2 μM, starting 8 hours after the beginning of the light period. The apex of colony branches was sampled from each nubbin during the experiment at 0, 2, and 4 hours.

3) Twenty-four nubbins of *P. damicornis* were labeled in light for 1 hour in 0.22 μm filtered artificial seawater enriched with $^{15}NH_4Cl$ to an estimated ammonium concentration of 20 μM. The labeling pulse started 2 hours after the beginning of the light period. Nubbins were then transferred to seawater with natural isotopic abundances of N and low ammonium concentration (< 1 μM) for a chase period lasting 95 hours under standard light/dark cycle (14 h/10 h).

4) In parallel to experiment 3, twenty-four nubbins of *P. damicornis*, pre-incubated 24 hours in the dark, were pulse-labeled 1 hour in the dark with 20 μM ^{15}N-ammonium, and then transferred to unlabeled seawater for a chase period lasting 95 hours under constant darkness.

5) Another pulse-chase experiment investigated ^{15}N-nitrate assimilation and ^{15}N exchanges between the dinoflagellates and the coral cells. Seven nubbins of *P. damicornis* were pulse-labeled in light for 12 hours in 0.22 μm filtered artificial seawater enriched with $K^{15}NO_3$ (^{15}N isotopic abundance of 98 %, SIGMA) to an estimated nitrate concentration of 30 μM. This pulse started 2 hours after the beginning of the light period. Nubbins were then transferred to seawater with natural isotopic abundances of nitrogen and high nitrate concentration (~30–40 μM) for a chase period under light /dark cycle (14 h/10 h) lasting 84 hours.

For these three pulse-chase experiments, the apex of colony branches were sampled from each nubbin at times 0, 1, 2, 6, 12, 24, 48, and 96 hours.

6) An experiment was performed with ^{15}N-aspartic acid in order to preliminarily investigate assimilation of organic N in the form of dissolved free amino acids (DFAA). Three

nubbins of *P. damicornis* were incubated 6 hours under light in 0.22 μm filtered artificial seawater enriched to an estimated ^{15}N-aspartic acid final concentration of 20 μM (^{15}N isotopic abundance of 98 %, SIGMA), starting 2 hours after the beginning of the light period. The apex of colony branches was sampled from each nubbin at times 0, 2 and 6 hours.

b) *TEM ultrastructural observations*

Coral samples were chemically fixed in either filtered artificial seawater containing both 1.25 % glutaraldehyde and 0.5 % formaldehyde, or in Sörensen-sucrose phosphate buffer (0.1 M phosphate at pH 7.5, 0.65 M sucrose, 2.5 mM CaCl2) containing both 2.5 % glutaraldehyde and 1 % formaldehyde. They were decalcified at 4°C and pH 7.5 in seawater or Sörensen 0.1 M buffer containing 0.5 M EDTA, which was daily renewed until completely demineralization. Rinsed tissue samples were dissected under the stereomicroscope into small pieces containing one or two individual polyps, post-fixed 1 hour at RT in 1% OsO_4 in filtered artificial seawater or Sörensen phosphate buffer (0.1 M), dehydrated in ethanol and embedded in Spürr resin. Tissue was oriented in order to obtain longitudinal sections parallel to the vertical growth direction of the polyps. Sections were cut with a Diatome 35° diamond (Ultracut microtome). Semi-thin sections (~0.5 μm) were stained with Toluidine blue-Borax or with Methylene Blue-Azur II and observed with a light microscope LEICA DMRB equipped with a LEICA DC300F camera (Leica, France). Ultra-thin sections (~70 nm) were mounted on formvar coated alphanumeric grids counterstained with uranyl acetate 2% in 50% ethanol, and were observed at 75 kV with a Hitachi H7100 TEM at the Plateforme d'Imagerie et de Microscopie Electronique of the MNHN (Paris, France) or at 80 kV with a ZEISS 912 Omega TEM at the Institut de Biologie Integrative (IFR 83) (Paris, France).

c) *NanoSIMS isotopic imaging*

To image and quantify the distribution of ^{15}N-enrichment within *Pocillopora damicornis* at the sub-cellular level, the exact same areas of interest selected in ultra-thin sections by TEM observations were analyzed with a NanoSIMS ion microprobe to enable direct comparison of ultrastructural (TEM) and isotopic (NanoSIMS) images.

TEM Grids were mounted on 10 mm aluminum stubs and coated with 7 nm gold. They were bombarded with a 16 keV primary ion beam of (1-3 pA) Cs^+ focused to a spot size of about 100-150 nm on the sample surface. Secondary molecular cyanide ions $^{12}C^{14}N^-$ and $^{12}C^{15}N^-$ were simultaneously collected in electron multipliers at a mass resolution (M/ΔM) of about 9000, enough to resolve the $^{12}C^{15}N^-$ ions from potentially problematic interferences. Charge compensation was not necessary. Typical images of 40×40 μm with 256×256 pixels or 15×15 μm with 128×128 pixels were obtained for $^{12}C^{14}N^-$ and $^{12}C^{15}N^-$ by rastering the primary beam across the sample surface with a dwell-time of 5 milliseconds. The $^{15}N/^{14}N$ ratio distribution was obtained by taking the ratio between the drift-corrected $^{12}C^{15}N^-$ and $^{12}C^{14}N^-$ images, respectively. ^{15}N enrichments were expressed in the delta notation: (1)

$$\delta^{15}N(‰) = \left(\frac{N_{mes}}{N_{nat}} - 1 \right) \times 1000 \text{, where}$$

N_{mes} is the measured $^{15}N/^{14}N$ ratio and N_{nat} is the natural $^{15}N/^{14}N$ ratio measured in non-labeled coral samples. In order to correct for potential drift in the instrument, N_{nat} was obtained on each new day of NanoSIMS analyses. Measured ^{15}N-enrichment from NanoSIMS isotopic images were considered significantly labeled when above the natural fluctuations of the $^{15}N/^{14}N$ ratio measured in similar areas of unlabeled control corals, defined as the average $\delta^{15}N$ values ± 3σ.

d) *NanoSIMS data processing and ROIs definition*

Data were processed using the L'IMAGE® software. Regions Of Interest (ROIs) were defined from the $^{15}N/^{14}N$ images to quantify the bulk ^{15}N enrichment of the dinoflagellates and of the four epithelia composing the coral tissue (1) by drawing the contours around dinoflagellates, and (2) as circles of about 2 μm diameter, covering host tissue and avoiding inter-cellular spaces and mesoglea (as illustrated in Fig. S1). The overall bulk enrichment of each symbiotic partner was then obtained by averaging corresponding ROIs values.

e) *Bulk $^{15}N/^{14}N$ isotopic measurements*

NanoSIMS data of dinoflagellate enrichment were compared with bulk measurements obtained with a classical method, i.e. after tissue dissociation and density centrifugation separation of the dinoflagellate fraction from the coral fraction. For each time point of both pulse-chase experiments with ^{15}N-ammonium (experiments 3 and 4) three nubbins of *P. damicornis* were sampled, frozen in liquid N_2 and stored at -80 °C. Coral tissue was blasted away from skeleton (water-pick method) in phosphate buffer (0.06 M solution KH_2PO_4, pH 6.65), centrifuged (200 g for 10 min at 4 °C), and the pellet containing dinoflagellates was resuspended in phosphate buffer solution and lyophilized. An aliquot was used to count zooxanthellae density on Malassez hemocytometer and to evaluate mitotic index. ^{15}N-enrichment values were determined at the UC Davis Stable Isotope Facility (Department of Plant Sciences, University of California, Davis, California) using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer. Values were expressed using the delta notation as defined

above in the equation (1). The value of N_{nat} was determined from unlabeled coral samples (0.00368 ± 0.00002, n = 48).

f) *Electron diffraction, EFTEM spectrum imaging and EELS*

Selected area electron diffraction (SAED), EFTEM spectrum imaging, and EELS of N-hotspots were performed on coral ultra-thin section (~70 nm) using a JEOL 2200FS with in-column Ω filter and operated at 200 kV acceleration voltage. SAED patterns were made by choosing regions of interest with a selected area aperture of 275 nm diameter when back-projected to the object plane, and were energy filtered using a 21 eV slit to improve clarity by removing diffuse intensity from inelastic scattering. EELS spectra were taken in image mode, with the same selected-area aperture and a broadly focused electron beam to choose the region of interest. For N mapping, EFTEM spectrum images across a 350–450 eV energy loss range were taken using a 10 eV slit and 5 eV step size. Images in the data cubes were aligned using *Automated Spatial Drift Correction for EFTEM Image Series* (1). After alignment, noise was reduced by using principle component analysis to reconstruct the data cubes with only significant data components (2). The N maps were then made by integrating the post-edge N K-edge intensity over a 40 eV wide energy window in EELS spectra extracted from the data cubes, and subtracting a background based on a standard power-law extrapolation of the pre-edge intensity. In the map shown in Fig. 1H, negative counts in the low-signal, noisy regions are discounted from the display. Both EELS and EFTEM used an objective aperture to limit the electron scattering collection semi-angle to ~11 mrad.

g) *GC-MS and GC-C-IRMS analyses of uric acid*

Coral tissue of nubbins incubated 90 min in 20 µM ^{15}N-ammonium was removed from the skeleton by using the water-pick method and Millipore water (PBS was not used to avoid phosphate salts contaminants in the pellet). Zooxanthellae were separated from their coral host tissue by centrifugation (200 g for 10 min at 4 °C) and were ultrasonicated to release the crystals from the cells. Derivatization of uric acid in algal cell material and commercial uric acid standard (Sigma–Aldrich, U0881) for GC analyses was achieved by a modified two-step procedure (3, 4). Methoxymation of carbonyl groups was done with methoxyamine hydrochloride solution in pyridine, followed by formation of trimethylsilyl (TMS) esters using *N*-trimethylsilyl-*N*- methyl trifluoroacetamide (MSTFA).

GC-MS analyses of TMS derivative of uric acid were performed with an Agilent (Palo Alto, USA) model 6890 gas chromatograph attached to an Agilent 5973N quadrupole mass selective detector and equipped with a 30 m Agilent HP-5MS fused–silica capillary column. GC-C-IRMS analyses of the nitrogen isotopic composition of the TMS-derivative of uric acid were determined by the use of an Agilent 6890 GC coupled to a Thermo Fisher Scientific (Bremen, Germany) Delta V isotope ratio mass spectrometer (IRMS) by a combustion (C) interface III (GC-C-IRMS). The GC was operated with the same type of column used for GC-MS analyses. Values are reported using the delta notation as described above, by using the standard air-N_2 as reference. Reproducibility ranged between 0.05 and 0.3‰ for the uric acid standard with natural ^{15}N abundance. For ^{15}N-labeled dinoflagellate uric acid, the reproducibility ranged between 50 and 100‰.

References

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