

Text S2: Detailed protocol of the chemical fixation, sample preparation for transmission electron microscopy and NanoSIMS imaging and analysis

At each sampling time point, selected *O. universa* specimens were individually transferred into 0.5 mL microcentrifuge tubes containing filtered seawater with 4 % glutaraldehyde and 2 % paraformaldehyde (pH = 8.1), and fixed for 24 h at room temperature. After three successive seawater rinses, specimens were post-fixed with a solution of 2 % OsO₄ prepared in distilled water for 1 h, rinsed with deionized water and embedded in 3% Agar (Sigma-Aldrich, type VII-A, low gelling temperature) to preserve the location of the symbiotic dinoflagellates outside the foraminiferal shell. After decalcification in two successive 0.1 M EDTA baths (1 h and 48 h), samples were transferred through an ethanol dehydration series from 50 to 100 % EtOH. Finally, samples were embedded in LR white resin and polymerized in an oven at 70°C for 12h. Ultra-thin (70 nm) sections were collected from each specimen using an ultramicrotome (Reichert Ultracut S) equipped with a diamond knife (Diatome Ultra, 45). Ultra-thin sections were placed on formvar-carbon coated copper grids (Electron Microscopy Science, reference FCF200F1-Cu), stained with 2 % uranyl acetate for 10 min and observed with a transmission electron microscope (TEM, Philips 301 CM100, 80 kV). All preparation and imaging were conducted at the Electron Microscopy Facility of the University of Lausanne (Switzerland). Areas of interest for subsequent NanoSIMS imaging were selected from TEM images.

The sections used for TEM imaging were first coated with ca. 10 nm of gold, before analysis with a Cameca NanoSIMS 50L ion microprobe. Sections of *O. universa* were imaged by bombarding them with a Cs⁻ ion beam focused to a spot size of ca. 100 – 150 nm. The secondary molecular ions ¹²C₂⁻, ¹³C¹²C⁻, ¹²C¹⁴N⁻ and ¹²C¹⁵N⁻ were collected simultaneously in electron multiplier detectors at a mass-resolution superior to 8000 (Cameca definition), enough to resolve potential interferences in the mass spectrum. Isotopic images were taken with a 256x256 pixel resolution and a size ranging from 10x10 to 45x45 μm. Quantified ¹³C/¹²C and ¹⁵N/¹⁴N ratios were obtained as follows:

$$\delta^{13}\text{C} (\text{‰}) = \left(\left(\frac{{}^{13}\text{C}/{}^{12}\text{C}_{\text{meas}}}{{}^{13}\text{C}/{}^{12}\text{C}_{\text{control}}} \right) - 1 \right) \times 10^3$$

$$\delta^{15}\text{N} (\text{‰}) = \left(\left(\frac{{}^{15}\text{N}/{}^{14}\text{N}_{\text{meas}}}{{}^{15}\text{N}/{}^{14}\text{N}_{\text{control}}} \right) - 1 \right) \times 10^3$$

where ¹³/₁₂C_{meas} and ¹⁵/₁₄N_{meas} are the ¹²C¹³C⁻/¹²C₂⁻ and ¹²C¹⁵N/¹²C¹⁴N ratios measured in the isotopically labeled samples, and C_{control} and N_{control} are the ¹²C¹³C⁻/¹²C₂⁻ and ¹²C¹⁵N/¹²C¹⁴N ratios measured in control samples from unspiked filtered seawater (see above), prepared and handled in an identical manner. The average ratios of the foraminifer endoplasm of three control specimens were used as standard ratios against which the experimental ¹³C and ¹⁵N-enrichments were quantified as shown in the equations above (i.e. not relative to the VPDB or atmospheric N₂ isotope references, respectively). It is noteworthy that chemical fixation and embedding processes are known to affect the isotopic content of cells, especially the ¹³C-content, due to loss of soluble compounds and ¹²C-dominated resin infiltration (Musat et al., 2014; Nomaki et al., 2018). However, as all the samples were prepared for TEM and NanoSIMS imaging following the same procedure, relative comparisons can be made.

NanoSIMS image processing was carried out using the software Look@NanoSIMS (Polerecky et al., 2012). ROIs were drawn on TEM images previously aligned with NanoSIMS images. Isotopic enrichments were calculated for the foraminifer cytoplasm, dinoflagellate, and cellular compartments. For the average isotopic enrichment of the foraminiferal cytoplasm, three circles of ca. 2 μm in diameter were drawn per image, avoiding highly enriched ^{13}C - and ^{15}N - organelles. Note that in contrast to what was done by LeKieffre et al., 2018b, the dinoflagellate ROIs were drawn around the entire dinoflagellate cell rather than around the starch grains in the symbiont cytosol. Thus, the values expressed here for the dinoflagellate symbionts are averages of the entire exposed surface of the microalgal cell. For each condition, time point, and type of cellular compartments, the average ^{13}C - and ^{15}N -enrichments were calculated based on 3 replicates (unless otherwise specified).

References:

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