**Supplementary Information**

***I. Isolation and 15N enrichment of nitrogen-fixing bacteria***

1. *Coral diazotroph isolates*

Nitrogen-fixing bacteria were isolated using endophyte, nitrogen-free selective medium (NFb) ([Baldani *et al.* 1997](#_ENREF_4)) supplemented with 3%NaCl ( 4g.L-1 mannitol; 2 g.L-1 MgSO4; 0.2 g.L-1 CaCl2; 4 g.L-1 KOH; 0.2 g.L-1 Na2MoO4; 0.5 g.L-1 K2HPO4; 0.28 g.L-1 H3BO3; 0.024 g.L-1 ZnSO4.7H2O; 0.008 g.L-1 CuSO4.5H2O; 0.235 g.L-1 MnSO4; 0.01 g.L-1 biotin; 0.02 g.L-1 pyridoxin; 4ml FeEDTA 1.6% solution; pH=7.2). Diazotrophs were isolated from coral juveniles that had been settled onto terracotta tiles and reared for six months on the reef in Nelly Bay, Magnetic Island, Australia (S 19°10’ E 146°50’) (a detailed description of coral juvenile rearing, settlement and field deployment can be found in ([Lema *et al.* 2014](#_ENREF_9))). After thoroughly rinsing the terracotta tiles with artificial seawater (ASW), 10 juveniles where scraped off, rinsed again with ASW and crushed with a sterile mortar and pestle. All crushed material was vortexed for 10 min and serially diluted to 10−6 of its original concentration. A 100 µl aliquot of each dilution was spread-plated onto the nitrogen-free media.

A dominant, morphologically-distinct bacterial colony was isolated to purity, and total genomic DNA was extracted from overnight cultures using the wizard genomic DNA extraction kit according to the manufacturers’ instructions (Promega, Madison, WI, USA). This dominant bacterial strain was identified by amplification and sequencing of the 16S rRNA gene ([Bourne *et al.* 2008](#_ENREF_5); [Lane 1991](#_ENREF_8)), checked against the closest match using the online BLASTN function available from Genbank ([Altschul *et al.* 1997](#_ENREF_1)), and affiliated to a *Vibrio* sp. (98%). The 16S rRNA sequence of the isolated nitrogen-fixing *Vibrio* sp. has been deposited in GenBank under nucleotide accession number KF691569.

1. *15N enrichment of diazotroph isolates*

For 15N isotopic enrichment, the *Vibrio* sp*.* (acc. Num. KF691569)isolate was grown in 125 mL serum vials with crimp-seal silicone rubber closures that were filled to the top with liquid NFb media, thus without head space. Incubations were initiated by injecting 1 mL of 99% 15N2 Gas (Cambridge Isotope Laboratories Inc., Cambridge, MA, USA; gas cylinder) directly into the vial’s septa with gas-tight Hamilton syringes and grown for ~36 hours. Experimental controls consisted of cultures grown in the same type of serum vials but without 15N injection or aeration (no crimp-sealed closure, and not filled with NFb media to top). Bacterial cultures were then transferred into sterile 50 mL falcon tubes, pelleted through centrifugation (3,000 r.c.f for 10 min), and washed three times by resuspending the pellet in phosphate saline buffer (PBS) prior to experimental incubations with coral larvae.

***II. Rearing of A.millepora larvae***

Larvae of *Acropora millepora* were reared as described in Lema et al., (2014). Briefly, gametes released from adult coral colonies (11th November 2011) were randomly mixed and the fertilized embryos reared in 500 L tanks containing 1 μm filtered and UV-treated seawater. Five days after spawning, larvae were removed from the rearing tanks and observed microscopically. Larvae at a similar developmental stage (i.e. elongated with a distinct oral pore starting to develop) were selected for the experiment.

***III. Experimental set up and sampling***

Larvae selected from the large rearing tanks were thoroughly rinsed (UV treated and 0.2 μm filtered sea water, FSW) and transferred into 6 well sterile plates (10 larvae per well) (BD Falcon) filled with 0.2 μm FSW (n=10 larvae per well and 4 wells per treatment). Larvae were incubated with one of three bacterial treatments : 1) 15Nlabeled *Vibrio* sp. cells (1 x106 bacteria mL-1 FSW) (Positive treatment (**PT**)); 2) unlabeled *Vibrio* sp. cells (1 x106 bacteria mL-1 FSW) (Control 1 (**C1**)); 3) a supernatant of dead 15N labeled *Vibrio* sp. cells, where cells were sonicated at 80 watts (UltraSonic Processor, Cole Palmer Inc., IL, USA) 6 times for 30 seconds and centrifuged at 13,000 r.c.f for 20 min (Control 2 (**C2**)).

After a 4 hour incubation larvae were sampled and thoroughly rinsed in PBS prior to fixation. Larvae were fixed in ice cold 4% paraformaldehyde in PBS and refrigerated overnight at 4oC, washed 3 times in PBS, and then stored in 1:1 PBS/ethanol for long-term preservation at -20oC. Additional samples containing only bacterial cells were collected and fixed just prior to experimental incubations to enable testing of fluorescence *in situ* hybridization (FISH) techniques and as 15N uptake controls for NanoSIMS (supplementary Figure 1).

***IV. Fluorescence in situ hybridization (FISH) and confocal laser scanning microscopy (CLSM) analyses***

1. *FISH preparation*

FISH was performed on whole larval samples carefully placed on microscopy slides (a ring was drawn around larvae with a liquid wax pen to ensure reagents remained in contact with the sample), which were then dehydrated in a dilution series of 50%, 80% and 100% ethanol at 3 min intervals. Following dehydration, hybridization was conducted in buffer (0.9M NaCl, 0.01% sodium dodecyl sulphate (SDS), 20 mM Tris-HCl , pH 7.4) with 30% formamide for 90 min at 46°C. Samples were probed with either a mix of universal eubacterial EUB338 (5’-GCT GCC TCC CGT AGG AGT -3’) ([Amann *et al.* 1990](#_ENREF_2)) and specific Vibrio-GV (5'- AGG CCA CAA CCT CCA AGT AG -3') ([Eilers *et al.* 2000](#_ENREF_6)) probes, or a negative control nonEUB338 probe (5’-ACT CCT ACG GGA GGC AGC-3’) ([Manz *et al.* 1992](#_ENREF_11)) at a final concentration of 5 ng μl-1 (i.e. to cover a larvae ~50 μl of hybridization buffer was used).Vibrio-GV and nonEUB338 probes were fluorescently-labelled with fluorophore ATTO647 (Thermo Fisher Scientific,ULM, Germany) and the general EUB338 was labelled with fluorophore AlexaFluor 546 (Invitrogen life technologies,Vic, Australia; see supplementary Table 1 for details of the emission spectra of these probes). After hybridization, samples were washed for 15 min in pre-warmed buffer (0.1M NaCl, 0.01% SDS, 20 mM Tris-HCl, 5mM EDTA) at 48°C. Fluorescently-labelled larvae were rinsed and preserved in PBS at 4°C in the dark until analysis using CLSM. Because negative controls hybridized with the nonEUB338 (ATTO647) probe showed unspecific binding (supplementary Figure 2, 3 and 4), additional inhibition assays were carried out as described by ([Heiniger *et al.* 2007](#_ENREF_7)) to further assess probe specificity and non-specific binding of the ATTO 647 fluorophore to other coral cellular structures (i.e nematocysts and gland cells morphologically different to bacteria). Briefly, inhibition assays consisted of larval samples hybridized with a non-fluorescently-tagged sequence probe (i.e sequence Vibrio-GV and negative control nonEUB338 sequence) using the same conditions as previously detailed. Following this hybridization with the unlabeled probe, samples underwent treatment with the fluorescently-labeled ATTO 647 probes. The outer layer of the nematocyst and gland cells stained with the fluorophore (supplementary Figure 5) in both the negative controls in which larvae were probed with nonEUB338-ATTO647 and in the inhibition assays. From this apparent unspecific binding, we conclude that there is a compound in the outer layer of nematocyst and in gland cells which binds to a compound within the fluorophore rather than to the hybridization probe itself. Unspecific binding has been recognized in previous coral studies (e.g Apprill *et al*. 2009; Sharp *et al.* 2010). Further studies assessing the bonding properties of these structures to specific components of the fluorophores will be essential to enable specific labelling within corals in the future. Importantly, the PT showed specific bacterial hybridization only in those samples treated with the Vibrio-GV probe.

1. *CLSM preparation and observation*

Single whole larvae (n=16) were carefully placed in a cover slip chamber with 1 drop of 30% glycerol low-fade mounting media (20% Polyvinyl alcohol (PVA), 1g.L-1Chlorobutanol, 50ml Tris-PO4 Buffer) and observed using a Nikon A1Si CLSM (Nikon Instruments, Japan) for 3 dimensional (3D) imaging (40x oil lens). Eight larvae, 6 PT and 2 C1, were observed with their respective negative controls (supplementary Figures 3 and 4). Images of single larvae were recorded in a XY stack series that covered the whole individual and a Z stack that could penetrate to a depth of 50 μm (3 μm step) into a larvae (~16 to 17 images per sample). All images were set to the same optimized settings for each channel of interest (Supplementary Table 1) and automatically processed through the Nikon NIS element software (version 3.22). Regions of interest were identified and further imaged at higher magnification. For analysis of each larval sample, all Z stacks were combined into a single maximum intensity projection image using the Nikon NIS element software (version 3.22; see Supplementary Table 1for details of emission wavelengths for each probe).

**V. *NanoSIMS sample preparation and analysis***

1. *Sample preparation*

One larval sample containing numerous *Vibrio* sp. detected through FISH and confocal analysis was selected and subsequently prepared for 15N analysis by NanoSIMS, together with its two respective control samples, C1 and C2. All three samples were dehydrated in a graded series of ethanol (50%, 70%, 90%, 100%, anhydrous 100%) and anhydrous acetone (100%). After dehydration, samples were gradually infiltrated in anhydrous acetone: araldite resin mixtures until total resin embedding (100% araldite concentration). Samples were mounted and cured for 36 h at 60°C. Resin blocks with intact larvae were sectioned to 600 nm on a Leica EM UC6 Ultramicrotome (Leica Microsystems, Wetzlar, Germany), with a diamond knife and mounted on silicon wafer disks. Disks were coated with 5 nm gold for subsequent analysis in a CAMECA NanoSIMS 50 ion microprobe (CAMECA, France) at the Centre for Microscopy, Characterisation and Analysis (CMCA), University of Western Australia. Distributions of the secondary ion species 12C15N and 12C14N were determined in the exact same areas where bacteria were observed in FISH analysis. For controls C1 and C2, similar areas (epiderm at the aboral ends of larvae) were scanned.

Vibrio sp. cells incubated under 15N2 gas were isolated on Si wafers and analysed by NanoSIMS to determine the enrichment levels prior to incubation with the larvae. The cells were found to be highly enriched with 15N, with values ranging from ~2 to 8 atom% (Supplementary Table 2). It should be noted that these isolated bacteria were not embedded in resin, and represent the enrichment level of the bacteria at the start of the experiment.

1. *Image analysis*

The NanoSIMS instrument was calibrated using a yeast standard with a known abundance ratio for naturally occurring15N/14N. Images were acquired at a 256x256 pixel resolution across an area of 40 μm x 40 μm (detailed images were acquired across a 15 μm2 area). Images were processed and analysed using the OpenMIMS data analysis software plugin in ImageJ (http://www.nrims.hms.harvard.edu/software.php). Individual images were first processed using a pixel-by-pixel detector dead time correction (44ns), and data were extracted from pixels within manually drawn regions-of-interest (ROI) encircling enriched bacteria for quantification of 12C15N /12C14N ratios (*n*=29) (data shown as 15N Atom %).

Additional regions of interest were also randomly drawn next to enriched areas, on non-enriched coral tissue areas to test possible 15N translocation into tissues in the same positive samples (ROI *n* =29) (supplementary Figure 6). Control samples (C1 and C2) were also measured for isotope enrichment in random tissue areas (ROI *n* =29) in similar ectodermal layers at the aboral end of larvae (see supplementary Table 2 for data, supplementary Figure 6).

Enrichment of the 15N isotope in ratio images is expressed as colour on a Hue Saturation and Intensity (HSI) scale, where the minimum (blue) was set to natural isotopic abundance of nitrogen (0.0037), and the maximum (magenta) was set to an arbitrary ratio value of 0.010. The HSI image uses a ratio scale factor of 10,000, which emphasises very small changes in the 15N/14N by expanding the digital scale between the maximum and minimum – i.e, 0.0037 becomes 37 and 0.010 becomes 100, giving a dynamic range of 63 units. A median filter radius of 1 pixel was also used to smooth out noise. Unenriched areas appear blue, and any increase in the 15N/14N ratio would be seen as a shift along the colour scale to magenta. Any 15N hotspots enriched to a value above the 0.01 maximum therefore appear magenta. The HSI images are only used to identify regions of enrichment; data extracted from the image ROIs are not affected by any image processing, and the actual ratios are derived from the deadtime-correct total counts within a ROI for each isotope. The ratio is converted to atom % using the formula:

Atom% = 15N/(15N + 14N) x 100

The values displayed along the colour scale for all HSI images is in atom %.

1. *Statistical analysis*

For NanoSIMS images, differences in 15N atom % abundances between enriched versus non-enriched and control regions were tested using permutational analysis of variance PERMANOVA ([Anderson 2001](#_ENREF_3)) (since 15N enriched bacteria in larvae resulted in abundances with high variance) in Primer 6 (PRIMER-E Ltd, Plymouth, UK) with the PERMANOVA+ add-on.

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