Titles and Legends Supplementary Figures

Supplementary Figure 1: Microbial mats at the intertidal zone at Laguna Ojo de Liebre near Guerrero Negro, Baja California Sur, Mexico.

Supplementary Figure 2: Cross section of the microbial mat from Laguna Ojo de Liebre. The green and purple layers comprise ~2 mm thickness of the mat.

Supplementary Figure 3: Micrograph of a subsample from the green layer of the Laguna Ojo de Liebre microbial mat displaying *Lyngbya* spp. (indicated by arrows) and the diversity of small filamentous *Cyanobacteria*. Scale bar represents 10 μm.

Supplementary Figure 4: Phylogenetic tree based on 16S rRNA sequences depicting the diversity of *Cyanobacteria* in the upper 2 mm of the intertidal mats from Laguna Ojo de Liebre. This maximum likelihood tree was calculated with full lengths sequences identified as closest relatives of the partial sequences from the investigated mats by SILVA (http://www.arb-silva.de/). Strain sequences were included to facilitate orientation for the reader. The OTU_{97} representatives of the partial 16S rRNA sequences of the investigated mats were added with the quick add parsimony function in ARB. The number of sequences per OTU is depicted in parentheses. Scale bar represents 10% estimated sequence divergence.

Supplementary Figure 5: Phylogenetic tree of cyanobacterial dinitrogenase reductase (*NifH*) amino acid sequences from intertidal mats (bold) together with their closest relatives and with representative strains (based on the amino acid sequences). The neighbor-joining tree depicts the OTU_{97} representative sequences of the three major cyanobacterial clusters (see Figure 4). The number of sequences derived from DNA (■), cDNA (◆) and cDNA from molybdate addition experiments (★) within an OTU are indicated. Bootstrap values calculated with the PhyML algorithm that were ≥ 50% are displayed in the tree. Scale bar represents 10% estimated sequence divergence.

Supplementary Figure 6: Representative epifluorescence micrographs of *E. coli* (upper panels) and *B. subtilis* (lower panels) cells after CARD-FISH with the general bacterial probe EUB338 (panel A and C) and the nonsense probe non338 (panel B and D). Images were taken on an epifluorescence laser microdissection microscope (LMD, Leica LMD 7000) using a 40x air objective prior to the NanoSIMS measurements. Scale bars represent 50 μm.

Supplementary Figure 7: Single-cell isotope measurements by NanoSIMS of ^{13}C and ^{15}N co-labeled *E. coli* (circles) and *B. subtilis* (triangles) cells. The effect of the CARD-FISH procedure on the enrichment in ^{13}C and ^{15}N of ~99 at% (large graph) and ~6 at% (small graph) labeled cells was investigated. ^{13}C and ^{15}N isotope fractions are displayed for formaldehyde-fixed *E. coli* (blue circles) and ethanol-fixed *B. subtilis* cells (blue triangles), which served as references in calculations of dilution factors (see Equation 1 in Supplementary Information). Both species show a significant decrease in the tracer contents after CARD-FISH using a probe targeting most *Bacteria* (EUB338, red symbols). Deposition of C- and N-atoms within the cells during the CARD-FISH

procedure independent from a hybridized probe was analyzed using the negative control probe nonEUB338 (green symbols).
Displayed data refer to the arithmetic mean ± the standard deviation (SD) over the analyzed cells per treatment (partly performed in replicate - as indicated by multiple identical symbols of the same color). On average 30 cells were measured in each analysis. Supplementary Figure 8 depicts the same data as in Figure 7, including identifiers for each sample, which enables cross-referencing with the data obtained from individual cells, listed in Supplementary Tables 4 to 6.

Supplementary Figure 8: Single-cell isotope measurements by NanoSIMS of ^{13}C and ^{15}N co-labeled *E. coli* (circles) and *B. subtilis* (triangles) cells. Codes next to symbols depict the wafer numbers and can be cross-referenced with the original data in Supplementary Tables 4 to 6. The effect of the CARD-FISH procedure on the enrichment in ^{13}C and ^{15}N of ~99 at% (large graph) and ~6 at% (small graph) enriched cells was investigated. ^{13}C and ^{15}N at% are indicated for formaldehyde-fixed *E. coli* (blue circles) and ethanol-fixed *B. subtilis* cells (blue triangles), as well as for cells hybridized with the negative control probe nonEUB338 (green symbols) and the probe targeting most bacteria (EUB338, red symbols).
Displayed data refer to the arithmetic mean ± standard deviation (SD) over the analyzed cells per treatment (partly performed in replicate - as indicated by multiple identical symbols of the same color). On average 30 cells were measured in each analysis.