Supplementary Material for

Discovery and quantification of anaerobic nitrogen metabolisms among oxygenated tropical Cuban stony corals

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Supplementary Figure 3. Phenotypic nitrate and nitrite reduction assays results
| Reef ID | Site # | Coral species         | Surface area (cm$^2$) | Polyp count | Estimated dry weight (g) | Volume (cm$^3$) | Polyp density (polyps cm$^{-3}$) |
|--------|--------|-----------------------|------------------------|-------------|--------------------------|----------------|----------------------------------|
| 5B     | 1      | *Porites astreoides*  | 7.1                    | 191         | 0.19                     | 3.9            | 2700                             |
|        |        |                       | 8.3                    | 212         | 0.22                     | 3.4            | 2600                             |
|        |        |                       | 6.3                    | 182         | 0.17                     | 2.6            | 2900                             |
|        |        |                       | 5.2                    | 142         | 0.13                     | 1.6            | 2700                             |
|        |        |                       | 14.3                   | 331         | 0.22                     | 2.8            | 2300                             |
|        |        |                       | 11.2                   | 301         | 0.16                     | 4.1            | 2700                             |
|        |        |                       | 9.3                    | 247         | 0.21                     | 2.9            | 2700                             |
|        |        |                       | 7.8                    | 191         | 0.20                     | 2.7            | 2400                             |
|        |        |                       | 4.5                    | 123         | 0.11                     | 3.2            | 2700                             |
|        |        |                       | 6.8                    | 162         | 0.11                     | 1.1            | 2400                             |
|        |        |                       | 7.1                    | 187         | 0.19                     | 1.7            | 2600                             |
|        |        |                       | 8.8                    | 236         | 0.19                     | 2.0            | 2700                             |
|        |        |                       | **mean ± sd**          |             |                          |                | **2610 ± 160**                   |
| 6B     | 2      | *Porites porites*     | 25.3                   | 703         | 0.26                     | 5.9            | 2800                             |
|        |        |                       | 12.6                   | 351         | 0.19                     | 2.5            | 2800                             |
|        |        |                       | 8.1                    | 285         | 1.35                     | 1.4            | 3500                             |
|        |        |                       | 18.4                   | 567         | 0.67                     | 3.0            | 3100                             |
|        |        |                       | 21.0                   | 563         | 0.24                     | 3.6            | 2700                             |
|        |        |                       | 18.8                   | 524         | 0.20                     | 4.3            | 2800                             |
|        |        |                       | 13.9                   | 376         | 0.22                     | 3.8            | 2700                             |
|        |        |                       | 8.6                    | 248         | 0.15                     | 2.1            | 2900                             |
|        |        |                       | 7.9                    | 218         | 0.21                     | 1.5            | 2800                             |
|        |        |                       | 19.3                   | 589         | 0.10                     | 4.2            | 3100                             |
|        |        |                       | 24.2                   | 611         | 0.21                     | 4.5            | 2500                             |
|        |        |                       | 21.6                   | 595         | 0.27                     | 7.8            | 2800                             |
|        |        |                       | **mean ± sd**          |             |                          |                | **2800 ± 250**                   |
| 10B    | 3      | *Porites porites*     | 12.6                   | 362         | 0.22                     | 2.0            | 2900                             |
|        |        |                       | 14.6                   | 374         | 0.35                     | 1.6            | 2600                             |
|        |        |                       | 11.8                   | 321         | 0.12                     | 2.1            | 2700                             |
|        |        |                       | 7.4                    | 211         | 1.06                     | 1.2            | 2900                             |
|        |        |                       | 18.3                   | 533         | 1.04                     | 3.5            | 2900                             |
|        |        |                       | 10.4                   | 289         | 0.21                     | 2.3            | 2800                             |
|        |        |                       | 6.7                    | 187         | 0.50                     | 1.1            | 2800                             |
|        |        |                       | 9.7                    | 264         | 0.38                     | 1.5            | 2700                             |
|        |        |                       | 10.5                   | 312         | 0.20                     | 1.9            | 3000                             |
|        |        |                       | 19.1                   | 554         | 0.10                     | 4.8            | 2900                             |
|        |        |                       | 15.3                   | 402         | 0.21                     | 3.8            | 2600                             |
|        |        |                       | 14.3                   | 397         | 0.27                     | 4.9            | 2800                             |
|        |        |                       | **mean ± sd**          |             |                          |                | **2800 ± 120**                   |
| 16C    | 4      | *Orcibella faveolata* | 4.9                    | 22          | 0.20                     | 3.1            | 450                              |
|        |        |                       | 7.5                    | 38          | 0.17                     | 4.3            | 510                              |
|        |        |                       | 3.6                    | 21          | 0.14                     | 7.2            | 590                              |
|        |        |                       | 5.5                    | 31          | 0.18                     | 7.4            | 560                              |
|        |        |                       | 5.6                    | 36          | 0.18                     | 4.7            | 640                              |
|        |        |                       | 4.9                    | 23          | 0.47                     | 9.9            | 470                              |
|        |        |                       | 5.0                    | 24          | 0.19                     | 4.2            | 480                              |
|        |        |                       | 3.5                    | 14          | 0.07                     | 0.9            | 400                              |
|        |        |                       | 5.1                    | 18          | 0.11                     | 7.8            | 360                              |
|        |        |                       | 5.3                    | 27          | 0.13                     | 3.9            | 510                              |
|        |        |                       | 6.4                    | 38          | 0.15                     | 6.1            | 590                              |
|        |        |                       | 5.5                    | 29          | 0.18                     | 5.3            | 550                              |
|        |        |                       | **mean ± sd**          |             |                          |                | **510 ± 80**                     |

**Supplementary Table 1. Characteristics of incubated coral fragments (continued on next page).** The surface area was determined via wrapping the tissue-exposed portion of the fragment with aluminum foil and weighing the mold; polyps were directly counted; dry weight was estimated by weighing a portion of the sloughed tissue and mucus; skeletal volume was measured via water displacement. Polyps were not counted for the *D. labyrinthiformis* brain coral. The dry weight estimates were the least precise method due to the difficulty in homogenizing the slurry.
| Reef ID | Site # | Species                  | Surface area (cm²) | Polyp count | Estimated dry weight (g) | Volume (cm³) | Polyp density (polyps cm⁻²) |
|--------|-------|--------------------------|--------------------|-------------|--------------------------|--------------|-----------------------------|
| 18A    | 5     | Diploria labyrinthiformis| 3.4                | ND          | 0.17                     | 3.2          | NA                          |
|        |       |                          | 5.0                | ND          | 0.15                     | 4.9          | NA                          |
|        |       |                          | 5.8                | ND          | 0.21                     | 4.7          | NA                          |
|        |       |                          | 4.2                | ND          | 0.12                     | 9.8          | NA                          |
|        |       |                          | 5.1                | ND          | 0.19                     | 2.3          | NA                          |
|        |       |                          | 4.0                | ND          | 0.04                     | 3.1          | NA                          |
|        |       |                          | 3.3                | ND          | 0.14                     | 5.5          | NA                          |
|        |       |                          | 3.6                | ND          | 0.06                     | 3.8          | NA                          |
|        |       |                          | 2.9                | ND          | 0.05                     | 1.6          | NA                          |
|        |       |                          | 2.2                | ND          | 0.07                     | 1.9          | NA                          |
|        |       |                          | 8.0                | ND          | 0.22                     | 10.1         | NA                          |
|        |       |                          | 3.9                | ND          | 0.17                     | 2.5          | NA                          |

| mean ± sd | 4.3 ± 1.5 | 0.13 ± 0.06 | 4.5 ± 2.8 | — |  
|-----------|-----------|-------------|-----------|---|   |
| 15 6      | Acropora palmata  | 33.4        | 287       | 0.25 | 7.2 | 860 |
|           |            | 26.3        | 217       | 0.15 | 5.9 | 820 |
|           |            | 35.8        | 317       | 0.21 | 8.3 | 890 |
|           |            | 28.2        | 231       | 0.12 | 4.3 | 820 |
|           |            | 21.9        | 212       | 0.11 | 3.9 | 970 |
|           |            | 34.3        | 298       | 0.20 | 6.2 | 870 |
|           |            | 37.0        | 306       | 0.15 | 5.1 | 830 |
|           |            | 23.0        | 224       | 0.12 | 4.0 | 970 |
|           |            | 22.9        | 226       | 0.09 | 3.6 | 990 |
|           |            | 12.2        | 102       | 0.04 | 1.1 | 840 |
|           |            | 17.4        | 147       | 0.10 | 5.7 | 840 |
|           |            | 17.4        | 142       | 0.08 | 2.1 | 820 |

| mean ± sd | 25.8 ± 8.1 | 226 ± 69 | 0.13 ± 0.06 | 4.8 ± 2.0 | 880 ± 60 |  
|-----------|------------|----------|-------------|-----------|---------|   |
| 2B 7      | Orbicella faveolata  | 5.1       | 33         | 0.12 | 1.5 | 650 |
|           |            | 6.0       | 40         | 0.19 | 2.0 | 660 |
|           |            | 11.7      | 81         | 0.22 | 5.7 | 690 |
|           |            | 5.5       | 36         | 0.12 | 2.9 | 660 |
|           |            | 4.8       | 33         | 0.06 | 2.2 | 690 |
|           |            | 4.5       | 31         | 0.06 | 1.7 | 680 |
|           |            | 9.3       | 58         | 0.07 | 5.9 | 620 |
|           |            | 7.7       | 47         | 0.13 | 1.8 | 610 |
|           |            | 6.4       | 41         | 1.26 | 1.2 | 640 |
|           |            | 5.2       | 38         | 0.06 | 2.9 | 720 |
|           |            | 2.9       | 21         | 0.03 | 1.4 | 730 |
|           |            | 5.2       | 36         | 0.06 | 0.9 | 690 |

| mean ± sd | 6.2 ± 2.4 | 41 ± 15 | 0.20 ± 0.34 | 2.5 ± 1.7 | 670 ± 40 |  
|-----------|-----------|---------|-------------|-----------|---------|   |

**Supplementary Table 1 (continued). Physical characteristics of incubated coral fragments.**
The surface area was determined via wrapping the tissue-exposed portion of the fragment with aluminum foil and weighing the mold; polyps were directly counted; dry weight was estimated by weighing a portion of the sloughed tissue and mucus; volume was measured via water displacement. Polyps were not counted for the *D. labyrinthiformis* brain coral. The dry weight estimates were the least precise method due to the difficulty in homogenizing the slurry.
|                     | NAR light | NAR dark | AMO light | AMO dark |
|---------------------|-----------|----------|-----------|----------|
|                     | Rep. 1    | Rep. 2   | Rep. 1    | Rep. 2   |
| *P. astreoides*     | 0.71      | 0.15     | 3.28      | 1.70     |
| *P. porites* (Site 2)| 2.83      | 1.05     | 9.57      | 1.73     |
| *P. porites* (Site 3)| 2.35      | 1.73     | 3.74      | 1.36     |
| *O. faveolata* (Site 4)| 4.38      | 0.32     | 0.72      | 0.30     |
| *O. faveolata* (Site 7)| 1.12      | n.m.     | 1.32      | n.m.     |
| *D. labyrinth*.     | 1.72      | 1.14     | 2.42      | 2.32     |
| *A. palmata*         | 0.05      | 0.05     | 0.05      | 0.05     |

Supplementary Table 2. Fixed nitrogen transformation rate data. Rates (nmol-N cm\(^{-2}\) d\(^{-1}\) of transformation for nitrate reduction (NAR) and ammonium oxidation (AMO) are shown for two replicates, for both diurnally varying (light) and 100% dark (dark) conditions. n.d., not detected; n.m., not measured.
Supplementary Table 3. Nitrous oxide production data. Rates (nmol-N cm$^{-2}$ d$^{-1}$) of N$_2$O production from each nitrogen pool (NO$_3^-$, NO$_2^-$, and NH$_4^+$) are shown for two replicates, for both diurnally varying (light) and 100% dark (dark) conditions. n.d., not detected; n.m., not measured.
Supplementary Figure 1. Coral reefs co-locate where surface nitrate levels are low. Shown are the locations of modern coral reefs (circles) from reefdata.org overlain on a surface map of nitrate concentrations from World Ocean Atlas 2018.
Supplementary Figure 2. Customized coral incubation vessels for $^{15}$N tracer amendments. The incubation vessels were constructed from water-tight polycarbonate chambers that were customized with a through-wall bulkhead fitting. Gas-tightness of this connection was ensured with a Viton rubber gasket secured with a polycarbonate washer. The bulkhead fitting contained a chlorobuyl rubber septum (Labco, UK). The chambers were filled completed with reef water and a piece of coral placed in each chamber. Afterward, a mixture of nutrient amendments were injected through the septum port. 18 replicate chambers were used for an experiment, consisting of 6 for each $^{15}$N tracer: 2 light, 2 dark, and 2 controls without corals.
Supplementary Figure 3. Phenotypic reduction assays results. Deep 96-well plates were amended with 50 µmol L\(^{-1}\) nitrate or nitrite and allowed to incubate anaerobically. Each culture was amended into triplicate wells. Nitrite production (from nitrate) or consumption (from nitrite) were observed using the colorimetric Griess assay. This photograph is of the nitrite assay at 144 hours, although the same results are observed for all time points. The negative control was unamended with bacteria whereas the positive control was amended with *Pseudomonas aureofaciens*, a denitrifier able to reduce nitrite slowly. The annotations are of the following: 423-T1A (*Pseudoalteromonas* sp. from *P. astreoides*), 424-T2C and D (*Pseudoalteromonas* sp. from *M. cavernosa*), 425-T1C1 (*Pseudoalteromonas* sp. from *D. labyrinthiformis*), 425-T1C2 and T2A (*Psychrobacter* sp. from *D. labyrinthiformis*). The lack of color observed in H2 and F8 is attributed to cross-contamination in the inocula of those individual wells with a nitrite-reducing organism.