Alphaproteobacteria facilitate *Trichodesmium* community trimethylamine utilization

Asa E. Conover\textsuperscript{a,b}, Michael Morando\textsuperscript{a}, Yiming Zhao\textsuperscript{a}, Jacob Semones\textsuperscript{a}, David A. Hutchins\textsuperscript{a}, Eric A. Webb\textsuperscript{a}

\textsuperscript{a} Department of Marine and Environmental Biology, University of Southern California, California, U.S.A

\textsuperscript{b} Department of Ecology and Evolutionary Biology, University of California Santa Cruz, California, U.S.A. (current affiliation)

**Correspondence**

Eric A. Webb
Marine and Environmental Biology
Dept. of Biological Sciences at University of Southern California, AHF 137
3616 Trousdale Parkway
Los Angeles, CA 90089-0371
Phone: 213-740-7954
Email: eawebb@usc.edu

**Competing interests:** The authors declare no competing interests.

**Running Title:** Metabolism of TMA in the *Trichodesmium* consortium
Originality-Significance Statement

Colonies and filaments of the globally-significant nitrogen-fixing cyanobacterium *Trichodesmium* host a diverse consortium of heterotrophic bacteria. It has been predicted that these bacteria support the growth of *Trichodesmium* by performing useful metabolic functions. Herein, we provide evidence that associated microbiota can metabolize the dissolved organic nitrogen compound trimethylamine, and in doing so, provide *Trichodesmium* with a viable nitrogen source alternative to the costly process of N2 fixation. The results highlight the significance of associated microbiota in *Trichodesmium* physiology, and further implicate the microbial loop and organic nitrogen supply in modulating global N, fixation patterns.

Summary

In the surface waters of the warm oligotrophic ocean, filaments and aggregated colonies of the nitrogen (N)-fixing cyanobacterium *Trichodesmium* create microscale nutrient-rich oases. These hotspots fuel primary productivity and harbor a diverse consortium of heterotrophs. Interactions with associated microbiota can affect the physiology of *Trichodesmium*, often in ways that have been predicted to support its growth. Recently, it was found that trimethylamine (TMA), a globally-abundant organic N compound, inhibits N2 fixation in cultures of *Trichodesmium* without impairing growth rate, suggesting that *Trichodesmium* receives nitrogen from TMA. In this study, 15N-TMA DNA stable isotope probing (SIP) of a *Trichodesmium* enrichment was employed to further investigate TMA metabolism and determine if TMA-N is incorporated directly or secondarily via cross-feeding facilitated by microbial associates. Herein we identify two members of the marine *Roseobacter* clade (MRC) of Alphaproteobacteria as the likely metabolizers of TMA and provide
genomic evidence that they converted TMA into a more readily available form of N, e.g., NH$_4^+$, which was subsequently used by *Trichodesmium* and the rest of the community. The results implicate microbiome-mediated carbon (C) and N transformations in modulating N$_2$ fixation, and thus highlight the involvement of host-associated heterotrophs in global biogeochemical cycling.

**Introduction**

The N-fixing cyanobacterium *Trichodesmium* contributes a substantial portion of the new nitrogen and carbon in the warm oligotrophic ocean (Capone *et al.*, 1997; Karl *et al.*, 1997; Capone *et al.*, 2005; Zehr and Capone, 2020). *Trichodesmium* grows in multicell filaments called trichomes, which can aggregate to form macroscopic colonies. Trichomes and colonies of *Trichodesmium* create micro-scale nutrient hotspots, referred to as the phycosphere or trichosphere, that attract and support the growth of many heterotrophic organisms (e.g., Hmelo *et al.*, 2012; Frischkorn *et al.*, 2017; Klawonn *et al.*, 2020; Zehr and Capone, 2020). Evidence suggests that such heterotrophs may confer physiological benefits to *Trichodesmium*, such as through drawdown of oxygen (an inhibitor of N$_2$ fixation), production of CO$_2$ (required for photosynthesis), detoxification of radical oxygen species, and production of siderophores and alkaline phosphatases for acquisition of Fe and P, respectively (Paerl *et al.*, 1989; Webb *et al.*, 2007; Hynes *et al.*, 2009; Roe *et al.*, 2012; Lee *et al.*, 2017; Basu *et al.*, 2019). Attempts to culture *Trichodesmium* axenically have been challenging, though a single successful effort in the early 2000s by Waterbury found that axenic cultures of *Trichodesmium erythraeum* IMS101 grow at a reduced rate (personal communication), further suggesting that associated microbiota play an important role in *Trichodesmium* physiology.
The tight association between *Trichodesmium* and its heterotrophic consortium has made it historically difficult to discern which metabolic functions specific taxa perform and what chemical compounds mediate these interactions. Recent studies have suggested that methylated amines (MAs) may play important functions in *Trichodesmium* consortium dynamics. Pade et al. (2016) found that *Trichodesmium erythraeum* IMS101 produces the quaternary ammonium compound N,N,N-trimethyl homoserine (or homoserine betaine), a member of the methylamine family, as its main compatible solute to maintain osmotic balance. Walworth et al. (2018) showed that TMA, a globally-abundant organic N compound, can support growth of *T. erythraeum* IMS101 in non-axenic culture while suppressing N$_2$ fixation, suggesting that *Trichodesmium* can use TMA as a N source. The latter study further found that long-term growth with elevated CO$_2$ and limiting nutrients led *Trichodesmium* to downregulate transcription of the N$_2$-fixing nitrogenase enzyme and upregulate production of a gene predicted to encode trimethylamine monooxygenase (Tmm) — an enzyme that catalyzes the oxidation of TMA to trimethylamine N-oxide (TMAO), the first step in aerobic TMA catabolism (Chen et al., 2011; Lidbury et al., 2014; Sun et al., 2019). But while *T. erythraeum* IMS101 possesses a likely Tmm homologue, it lacks other known genes required for TMA utilization (Walworth et al., 2015). It thus remains unclear if *Trichodesmium* is capable of consuming TMA directly, or if TMA is first metabolized by associated microbiota and then transferred to *Trichodesmium* as an alternate form of nitrogen. *Trichodesmium* colonies have been found to host likely metabolizers of MAs. *Roseibium* sp. TrichSKD4, a member of the marine *Roseobacter* clade (MRC) of Alphaproteobacteria, was isolated from *Trichodesmium* colonies in defined media and subsequently found to possess the full set of genes required for TMAO
catabolism (Lidbury, 2015). However, it lacks the \textit{tmm} gene for conversion of TMA to TMAO (Lidbury, 2015), suggesting potential to benefit from \textit{Trichodesmium} derived Tmm activity.

Chen \textit{et al.} (2012) demonstrate that several members of the MRC can use MAs, including TMA, as a sole N source, and that many have the genetic potential to do so. Lidbury \textit{et al.} (2015) show that TMA catabolism in MRC member \textit{Ruegeria pomeroyi} DSS-3 generates cellular energy and remineralizes ammonium, which supports the growth of co-cultured bacteria. The production of ammonium, a known N source for \textit{Trichodesmium} (Mulholland and Capone, 1999), highlights a potential mechanism whereby \textit{Trichodesmium} may receive N through exogenous TMA metabolism. However, the occurrence of such secondary utilization, and more broadly, the potential influence that recycling of N compounds by heterotrophs has on rates of N$_2$ fixation, has yet to be resolved.

In this study, we examine whether \textit{Trichodesmium} receives nitrogen from TMA directly (Figure 1, Option A) or secondarily via conversion to an alternate nitrogen species by a co-cultured heterotroph (Figure 1, Option B). $^{15}$N-TMA DNA SIP was used to investigate TMA metabolism in non-axenic cultures of \textit{Trichodesmium erythraeum} GBRTRLIN201 (hereafter LIN). Our results suggest that TMA catabolism by co-cultured MRC taxa has the ability to provide \textit{Trichodesmium} with a N source, and thereby modulate N$_2$ fixation.

\textbf{Results}

As seen in Walworth \textit{et al.} (2018) with \textit{T. erythraeum} IMS101, the addition of 80 \textmu M TMA suppressed N$_2$ fixation by day 1 in both heavy ($^{15}$N) and light ($^{14}$N) treatments of \textit{T. erythraeum} LIN culture, reducing fixation rates to 10 - 30\% that of the no-addition control cultures (Figure 2, bottom panel). On day 2, N$_2$ fixation
remained at about 10% that of the no-addition control in both TMA-addition groups. Fixation rates climbed on day 3 in two of three light TMA-addition cultures but remained suppressed in all others. On day 4, N₂ fixation increased in all TMA-addition cultures, though still at 50 - 75% that of the no-TMA control cultures. Over the four days, despite the reduced levels of N₂ fixation, heavy and light TMA-addition cultures grew at similar rates to the no-addition controls, as measured by in vivo chlorophyll A fluorescence measurements (Figure 2, top panel).

¹⁵N-SIP was used to track utilization of the isotopically-labeled TMA. Density fractionation yielded 18-19 fractions per sample with sufficient DNA for PCR amplification. Metabarcode sequencing of 16S/18S genes generated a total of 1,171,632 reads following denoising and sparsity filtering, with a mean of 15,832 reads per fraction (maximum = 33,064, minimum = 8,280). Twenty bacterial ASVs were common to both treatments and present in all three timepoints. No 18S ASVs passed the filtering threshold. Atom fraction excess was calculated for each ASV using the qSIP function of the HTSSIP R package (Youngblut et al., 2018). On day 1, 3 ASVs showed label incorporation—two belonging to Alphaproteobacteria of the Rhodobacteraceae family (Seq13, Seq14), and one to a Gammaproteobacterium of unknown family (Seq05). By day 3, all of the community, including Trichodesmium (Seq04), showed atom fraction excess values indicative of ¹⁵N incorporation. On day 4, ¹⁵N enrichment remained high, with atom fraction excess values dropping below the enrichment threshold in just two of the 20 ASVs—Seq18 and Seq19, both belonging to the Bacillales order of Firmicutes.

The two day 1 Rhodobacteraceae incorporators share 100% identity with V4-V5 region 16S sequences from published genomes available on IMG/M. Specifically, Seq13 is 100% identical to Mameliella spp. (10 genomes), while Seq14 is 100%
identical to Phaeobacter gallaeciensis (7 genomes). Both Mameliella and Phaeobacter are members of the marine Roseobacter clade (MRC) of Alphaproteobacteria.

To determine whether Mameliella spp. or P. gallaeciensis are indeed present in Trichodesmium cultures, genomes of each were obtained from IMG/M and used as read-mapping references for metagenomic reads from cultures of T. erythraeum LIN and other Trichodesmium strains. Anvi’o visualization of the Bowtie2 read-mapping showed that Mameliella spp. genomes were detectable at low concentration in the non-TMA-treated LIN metagenome, but P. gallaeciensis genomes were not (Supplemental Figure 2). Mameliella spp. was also detected in metagenomes of other strains of T. erythraeum, as well as in T. thiebautii VI-I (data not shown). It is possible that a relative of P. gallaeciensis is present in the enrichments but was not detected in this analysis either because its genome is different from the ones obtained from IMG/M or its abundance was low in the non-TMA-enriched cultures used for sequencing.

To determine whether Seq13 and Seq14 associate with Trichodesmium in the field, a BLAST database was constructed using 16S sequences of the Trichodesmium consortium collected by Rouco et al. (2016) across the Atlantic and Pacific Ocean. Multiple 100% matches to both ASVs were found in colonies of “puff” morphology from the South Pacific, but no identical hits appeared in colonies of other oceanic regions or of the “raft” morphology (Supplemental File 1).

BLASTP was used to assess genomic potential for TMA catabolism in Mameliella spp., T. erythraeum, and environmental collections of the Trichodesmium consortium (Supplemental File 2, Figure 4). All 10 publicly-available Mameliella genomes were found to possess likely homologs for the full suite of genes required
for the aerobic TMA catabolism pathway described by Lidbury et al. (2015) (Figure 4a). In contrast, the three queried strains of *T. erythraeum*, including LIN, were found to possess possible homologs of Tmm and GmaS, but none of the other 12 genes in the pathway. Possible homologs for all 14 genes appeared in at least one of the three metagenomes of *Trichodesmium* colonies collected near Station ALOHA (Figure 4, Supplemental File 2).

**Discussion**

Associated microbiota dramatically expand the metabolic potential and functional significance of *Trichodesmium* (Frischkorn et al., 2017; Lee et al., 2017; Lee et al., 2018). As such, the role of the *Trichodesmium* consortium in N cycling stretches beyond N$_2$ fixation. High rates of nitrate and nitrite assimilation and reduction are detected in *Trichodesmium* colonies, with negligible detected loss of N to denitrification (Klawonn et al., 2020). This network of N transformation processes demonstrates high potential for recycling N, a limiting nutrient for many sympatric, oligotrophic organisms. Metagenomic and transcriptomic surveys of *Trichodesmium* colonies implicate associated heterotrophs in many of these recycling processes (Frischkorn et al., 2017; Gradoville et al., 2017; Lee et al., 2018). Although it has been shown that *Trichodesmium* derives benefit from its microbiota, it is unknown whether it receives N through these recycling processes.

TMA is a useful compound for investigating this question. While cultures of *T. erythraeum* appear to use TMA as a N source in lieu of N$_2$ fixation (Figure 2, Walworth et al., 2018), the genome of *T. erythraeum* lacks most of the known genes for TMA catabolism (Figure 4, Walworth et al., 2015). Therefore, unless *T.
*erythraeum* metabolizes TMA through a novel biochemical pathway, it must receive TMA-N in an alternate form, i.e., following TMA catabolism by associated microbiota.

In this study, SIP was employed to determine if TMA-N was incorporated directly or secondarily via cross-feeding facilitated by the *Trichodesmium* consortium. Our results revealed that two Alphaproteobacteria of the MRC were $^{15}$N-enriched before other taxa (Figure 3) and may have converted this N source into a more bioavailable form that was subsequently used by the rest of the community.

Additionally, we found genomic evidence that these MRC taxa have the metabolic potential to catabolize TMA, thereby transforming TMA nitrogen to ammonium, further demonstrating the potential for cross-feeding over time (Figure 3 and 4).

The MRC are globally-distributed and abundant in a wide variety of environments—from the coast to the open ocean, and from surface waters to deep seafloor sediments (Buchan *et al.*, 2005; Luo and Moran, 2014). Members of the MRC are commonly found in association with phytoplankton (Geng and Belas, 2010) and have been shown to benefit their phytoplankton hosts in some of these relationships, such as through production of growth-stimulating auxins (Seyedsayamdost *et al.*, 2011) and protective antibiotics (Rao *et al.*, 2007). MRC members typically possess comparatively large bacterial genomes and demonstrate high metabolic versatility (Newton *et al.*, 2010). Surveys of the *Trichodesmium* consortium in culture and in the field have regularly found MRC taxa present (Hmelo *et al.*, 2012; Rao *et al.*, 2015; Rouco *et al.*, 2016; Lee *et al.*, 2017). Thus, members of the MRC may play an important role in *Trichodesmium* ecology and physiology.

Genes for TMA and TMAO metabolism are common but not universal among the MRC (Chen, 2012; Lidbury *et al.*, 2014) and enable some taxa to use TMA as a sole N source (Chen, 2012). Landa *et al.* (2017) suggest that the patchy distribution
of TMA utilization genes among the MRC may implicate TMA as a currency of specific relationships between individual roseobacters and phytoplankton species, many of which (including *Trichodesmium*) produce MAs as compatible solutes to maintain osmotic balance in saline environments (Mackay *et al.*, 1984; Oren, 1990; Pade *et al.*, 2016). These compounds accumulate in high concentrations and represent substantial pools of organic N, which are liberated during cellular lysis (Welsh, 2000).

While the metabolic fate of the major compatible solute in *Trichodesmium*, trimethylated homoserine, remains unknown, other quaternary amines, such as glycine betaine and choline, are known to degrade to TMA (King, 1984; Oren, 1990; Bain *et al.*, 2004). Thus, trimethylated homoserine constitutes a likely source of TMA *in situ*, potentially providing an ecological incentive to microbiota that can metabolize TMA and other MAs. Not surprisingly, a member of the MRC isolated from *Trichodesmium* colonies, *Roseibium sp*. TrichSDK4, was found to possess the genes for TMAO metabolism (Lidbury *et al.*, 2014).

Lidbury *et al.* (2015) propose a model for TMA catabolism via aerobic oxidation in the MRC member *R. pomeroyi* DSS-3. They demonstrate that this pathway generates ATP and leads to remineralization of ammonium—some of which is kept as an N source while the rest is exported. Our results are consistent with this model: high levels of $^{15}$N incorporation by the two MRC ASVs on day 1 were followed by incorporation in nearly all of the community, including *Trichodesmium*, on day 3, and further incorporation on day 4. Such widespread incorporation implies conversion of TMA to a more accessible nitrogen species, such as ammonium, as is predicted by the aerobic oxidation pathway (Lidbury *et al.*, 2015). This trophic cascade suggests that TMA metabolism in *T. erythraeum* LIN cultures proceeds as
outlined in Figure 1, Option B—in which MRC taxa Seq13 and Seq14 perform TMA
catabolism and release ammonium, which then serves as an N source for

*Trichodesmium* and the rest of the community. In this scenario, the rapid inhibition of

$N_2$ fixation in *Trichodesmium* apparent on day 1 (Figure 2) is explained by MRC
ammonium production.

While the results support this model, they do not rule out the possibility that

*Trichodesmium* is also capable of TMA metabolism. It is possible that both MRC and

*Trichodesmium* utilize TMA (i.e., Figure 1, Option C) and that we saw MRC
incorporation first because they had faster generation times, leading to more rapid
incorporation of $^{15}$N. Indeed, genomic-based estimates collected in the EGGO
database predict a maximal doubling time of ~2.4 hours for *Mameliella alba* CGMCC
[GCF_900101505.1] (Weissman *et al.*, 2020), while the doubling time of *T.
erythraeum* LIN observed here was ~48 hours. However, if *Trichodesmium* were to
utilize TMA directly, it would need to do so by a yet unknown pathway, since *T.
erythraeum* LIN (along with IMS101 and all other genome-sequenced strains) lack
most of the known genes required for TMA metabolism (Figure 4, Walworth *et al.*, 2018). In contrast, ammonium is a well-documented N source for *Trichodesmium*
(Mulholland and Capone, 2000), and as described above, was likely made available
via MRC TMA catabolism. Therefore, it appears likely that suppression of $N_2$ fixation
in cultures of *T. erythraeum* LIN (and by proxy IMS101 as well) following TMA
addition was the result of TMA catabolism by co-cultured MRC taxa.

It is not difficult to envision how such an association could prove mutually
beneficial. Through TMA catabolism MRC could gain access to the energy and N
source offered by methylated amines released by *Trichodesmium*, such as its
primary compatible solute, trimethylated homoserine. In turn, *Trichodesmium* could
recover remineralized N released by the MRC associates, thereby saving some of the expenses required for N₂ fixation. Such an association would promote the consortium’s ability to recycle N and conserve resources. The association may also improve the consortium’s ability to acquire new N. Presumably, the MRC routinely benefits by obtaining N released by *Trichodesmium* (both as MAs, but also as ammonium and other forms of dissolved organic nitrogen), as evidenced by their decades-long perseverance in laboratory cultures in N-free media. But when TMA becomes available to the consortium, the N flux goes the other way—from MRC to *Trichodesmium*. Thus, the direction of N transfer within the *Trichodesmium/MRC* association depends on whether the N source is N₂ or TMA. This flexibility could benefit both partners as it broadens the entire consortium’s nitrogen source niche.

The environmental data assembled here suggests that this relationship may exist beyond the laboratory. The appearance of Seq13 and Seq14 in *Trichodesmium* colonies from the South Pacific sequenced by Rouco *et al.* (2016) indicates that the same or highly similar MRC taxa associate with *Trichodesmium* in natural environments. And though these same MRC taxa do not appear in colonies globally, the presence of likely TMA catabolism genes in metagenomes collected circa Station ALOHA (near Hawaii) suggests that the aerobic TMA oxidation pathway may be functionally conserved within the consortium. Testing whether TMA addition regularly inhibits N₂ fixation in the field will be important for assessing the implications of these relationships.

The findings presented here are novel, but not without precedent. Frischkorn *et al.* (2018) demonstrate that microbiota can influence *Trichodesmium* N₂ fixation rates, but the means by which this happens remain uncertain. This study provides the first evidence we know of that microbiome-mediated N recycling can modulate N₂
fixation rates. Such evidence elevates the significance of the *Trichodesmium*-associated heterotrophic consortium, which likely plays a significant role in global $N_2$ fixation patterns.

### Experimental Procedures

#### Culture conditions

Cultures of *T. erythraeum* strain GBRTRL1N201 (Bell *et al.*, 2005) were grown from a single inoculum and divided into three TMA treatment groups: final concentration 80 µM ‘heavy TMA’ ($^{15}$N), 80 µM ‘light’ TMA ($^{14}$N), and no TMA addition. Triplicates of each treatment were grown in sterile acid-washed 2 L polypropylene baffled Erlenmeyer flasks with 1.6 L of YBC-II artificial seawater medium (Chen *et al.*, 1996). Cultures were housed in Percival (I-66 and I-30) incubators at a temperature of 26.0 °C and light intensity of 200 µEinsteins m$^{-2}$s$^{-1}$ with warm and cool white fluorescent lights operating on a 12 hour light : 12 hour dark cycle. They were kept on an orbital shaker operating at 50 rpm to ensure homogeneity of trichomes. Over the next four days, subsamples were withdrawn from each culture daily to monitor $N_2$ fixation and growth rate, as well as to collect biomass for DNA extraction.

#### Nitrogen fixation

Nitrogenase activity was quantified by the acetylene reduction assay described by Capone and Montoya (2001) as modified by Chappell *et al.* (2010). This assay leverages the nitrogenase enzyme’s ability to reduce triple-bonded acetylene gas ($C_2H_2$) to double-bonded ethylene gas ($C_2H_4$), both of which can be quantified by gas chromatography. In short, a culture sample in a closed container is...
injected with acetylene gas, after which ethylene production is measured over time and used as a proxy for calculating the sample’s ability to reduce nitrogen gas (N₂) to ammonium (NH₄⁺).

In this study, acetylene gas was generated by mixing 15.0 g of calcium carbide with 50 ml of ddH₂O in a glass sidearm flask. The evolved gas was collected and stored in a rubber bladder. At the midpoint of the incubator’s daylight cycle 20 ml subsamples of each culture were transferred to clear 30 ml polycarbonate centrifuge tubes with silicone-PTFE septa caps (I-Chem, Thermo Fisher Scientific). Caps were tightly screwed and 3 ml of acetylene gas was injected into each. Tubes were inverted gently to mix. 0.5 ml samples of headspace were withdrawn from each sample using a 1.0 ml disposable syringe. Samples were taken hourly over a two-hour period for a total of three time points. Filled syringes were stuck into a rubber stopper prior to analysis to prevent leaking. 0.2 ml from each syringe was injected in a Shimadzu (GC-8a) gas chromatograph equipped with a flame ionization detector and Porapak R column. Peak height readings were integrated using PeakSimple software (SRI Instruments). A 9.0 ppm ethylene standard was used to calibrate peak heights. Ethylene formed was calculated according to Capone and Montoya (2001) with a Bunsen coefficient of 0.084, as calculated according to Breitbarth et al. (2004) for samples at 26°C with salinity of ~35 ppt. Estimates of nitrogen-fixing capacity were calculated assuming an equivalence of 4 moles ethylene to 1 mole nitrogen gas and values were normalized to biomass using relative fluorescence of chlorophyll A (see below).

**Growth data**
Culture growth was monitored via relative fluorescence of chlorophyll A, measured *in vivo* using a Trilogy Laboratory Fluorometer (Turner Designs). Following the conclusion of the daily acetylene reduction assay, *in vitro* chlorophyll concentrations were measured for each sample (Welschmeyer, 1994; Wasmund, 2006).

**DNA extraction**

Subsamples were collected from each culture daily for DNA extraction — starting with 250 ml on day 1, 200 ml on day 2, 150 ml on day 3, and 100 ml on day 4. The volume was reduced each day to account for the increasing density of biomass in each culture. Cells were collected on 0.2 µm Supor membrane disc filters (Pall Life Sciences) by gentle vacuum filtration. DNA extraction was performed using a DNeasy PowerSoil Pro kit (Qiagen) following the manufacturer's protocol.

**Density-gradient stable isotope probing**

Density-gradient formation, fractionation, and clean-up was performed similarly to Morando and Capone (2018). Briefly, DNA extracts were mixed with a gradient buffer (0.1 M Tris-HCl, 0.1 M KCl, 1 mM EDTA) and cesium chloride (7.163 M) in 3.3 ml polyallomer centrifuge tubes (Beckman Coulter) to a final density of 1.700 g ml⁻¹. Tubes were loaded into a TLN-100 near-vertical rotor (Beckman Coulter) and spun for 72 hours at 136,000 × $g_{av}$ and 20°C. Tubes were then fractionated in ~100 µl increments via displacement with mineral oil. Fraction densities were calculated using an AR200 Digital Refractometer (Reichert Technologies). DNA was precipitated from each fraction with PEG-NaCl (30% PEG, 1.6 M NaCl) and linear polyacrylamide (Invitrogen, Thermo Fisher Scientific),
washed with 70% ethanol, dried, and resuspended in TE buffer (10 mM Tris-HCl and 1 mM EDTA, pH 8.0). Recovered DNA was quantified using a Qubit fluorometer with a dsDNA BR Assay Kit (Invitrogen, Thermo Fisher Scientific).

**PCR and sequencing**

Fractions from Light TMA day 1 and Heavy TMA days 1, 3, and 4 with quantifiable DNA were sent to a commercial vender (Molecular Research LP, MR DNA) for amplification and MiSeq paired-end (2 × 300 bp) sequencing of the 16S V4-V5 hypervariable regions and partial 18S sequences using the 515F-Y / 926R primer pair (515F-Y: 5' - GTGYCAGCMGCCGCGGTAA, 926R: 5' - CCGYCAATTTYMTTTRAGTTT) (Parada *et al.*, 2016). Library preparation and sequencing was carried out by MR DNA following Illumina library preparation protocols using MiSeq Reagent Kit v3 (Illumina).

Raw 16S/18S sequence data is available in the NCBI Sequence Read Archive with BioProject ID PRJNA703412 and BioSample IDs SAMN18016931:18017004.

**Inferring sequence variants**

Exact sequence variants were inferred from raw reads following the Deblur variant of the Fuhrman Lab eASV Pipeline (dx.doi.org/10.17504/protocols.io.vi9e4h6). Primers were removed using cutadapt (Martin, 2011), and reads were sorted into 16S and 18S pools using BBSplit (Bushnell, 2014). Forward and reverse 16S reads were merged using VSEARCH (Rognes *et al.*, 2016) and then trimmed to 365 bases. Forward and reverse 18S reads generated with the 515F-Y / 926R primer pair are non-overlapping, and,
therefore, could not be merged. Instead, 18S forward reads were trimmed to 220 bases, reverse reads were trimmed to 179 bases, and the two were concatenated. 16S and 18S reads were denoised separately using Deblur (Amir et al., 2017) within QIIME 2 (Bolyen et al., 2019). Taxonomy was assigned to ASVs by QIIME 2’s q2-feature-classifier plugin (Bokulich et al., 2018) using the SILVA 132 database (Quast et al., 2013).

**Identifying $^{15}$N incorporators**

Enrichment with $^{15}$N was determined using the quantitative SIP (qSIP) function of the HTSSIP software package (v1.4.1; Youngblut et al., 2018) in R (v3.6.1; R Core Team 2020). qSIP utilizes ASV-specific density curves from labeled and unlabeled treatments to calculate the shift in density in response to isotope labeling for each ASV (Hungate et al., 2015). This shift divided by the calculated maximum shift that would occur with 100% enrichment is termed atom fraction excess and was calculated following Hungate et al. (2015) with appropriate parameters for $^{15}$N taken from Morrissey et al. (2018). Data were pre-filtered to remove ASVs that constituted less than 0.01% of the total reads for each treatment, since such low read counts preclude the ability to accurately compare density distributions between treatments.

Without sequencing replicates from each day of the experiment, we were unable to calculate confidence intervals for atom fraction excess values. Instead, we assessed the range of potential error in these values by examining the variation in ASV enrichment on day 1 (Figure 3). Since $^{15}$N incorporation cannot be less than 0, negative atom fraction excess values obtained on day 1 must be generated by variance in the data. To determine a threshold for likely incorporation of $^{15}$N, we
averaged the negative values seen on day 1 (mean = -0.0447), multiplied by 3, and
took the absolute value as the sample deviation, similar to Morando and Capone
(2016). We concluded that atom fraction excess values above 0.134 g ml\(^{-1}\) are
greater than what we might obtain from measurement error and are likely generated
by true \(^{15}\)N enrichment. Regardless of this threshold, it is clear that significant
incorporation occurred in the majority of the ASVs analyzed during the course of the
experiment (Figure 3).

Generating \textit{T. erythraeum} LIN metagenome

DNA extraction was carried out as described above. DNA was sent to
Novogene (Sacramento, CA) for library preparation using NEBNext DNA Library
Prep Kit (300 bp inserts) and Illumina PE150 sequencing. Reads were processed as
described in the “Genome Extraction from Shotgun Metagenome Sequence Data”
narrative (https://kbase.us/n/33233/351/) constructed at KBase (Arkin \textit{et al.}, 2018) as
follows: Reads were quality-checked with FastQC (v0.11.5; Andrew, 2010) and
linkers were trimmed using Trimmomatic (v0.36; Bolger \textit{et al.}, 2014). Contigs were
assembled with metaSPAdes (v3.12.0; Nurk \textit{et al.}, 2017) and binned with MaxBin2
(v2.2.4; Wu \textit{et al.}, 2016) using kmer frequencies and read depth. PROKKA (v1.12;
Seemann, 2014) was used to annotate the assembled bins.

Raw metagenomic sequence data is available in the NCBI Sequence Read
Archive with BioSample ID SAMN18208363 within BioProject ID PRJNA703412. The
\textit{T. erythraeum} LIN metagenome-assembled genome is available in Supplemental
File S4.
Ten genomes of *Mameliella* spp. and seven of *P. gallaeciensis* with 16S sequences 100% identical to Seq13 and Seq14, respectively, were identified using BLASTN searching of the JGI IMG/M database (Chen et al., 2020) (accessed March 05, 2021). Genomes were downloaded and checked for similarity using FastANI (Jain et al., 2018), and highly similar genomes (most with >99% average nucleotide identity) were dereplicated. To avoid search redundancy and random read mapping, the two most divergent genomes of each taxon were selected as representative strains—these were *Mameliella alba* F15 (ID 2788500142), *Mameliella sp.* LZ28 (ID 2891139733), *P. gallaeciensis* P128 (ID 2814123083), and *P. gallaeciensis* DSM26640 (ID 2558309061). Bowtie2 (Langmead and Salzberg, 2012) was used to map metagenomic reads from *T. erythraeum* LIN to these representative genomes. Samtools (Li et al., 2009) was used to screen out poor scoring reads in the SAM file and prepare the Bowtie2 output for visualization with Anvi’o (v6.2; Eren et al., 2015).

To determine if TMA utilizing MRC strains (Seq13 and 14 from LIN) co-occur with natural *Trichodesmium* samples, we made a custom BLAST database of all 16S SSU V4 amplicon reads from a global *Trichodesmium* colony microbial diversity study (Rouco et al., 2016). Briefly, sequences were obtained in FASTA format from the NCBI Sequence Read Archive using fastq-dump (https://ncbi.github.io/sra-tools/) and then used to generate a custom BLASTN database in Geneious Prime (www.geneious.com). MegaBLASTN default settings were used and only hits greater than 99.6% across the whole V4 region were reported.
Surveying genomic TMA catabolism potential

We searched for TMA catabolism proteins in the genomes of *Mameliella* spp. (there are 10 publicly available genomes on IMG/M) and *T. erythraeum* (strains LIN, IMS101, and 2175), as well as in environmental metagenomes generated from *Trichodesmium* colonies (using 3 published from samples collected near Station ALOHA). BLASTP was used to search each genome for the 14 TMA catabolism proteins using reference sequences from *R. pomeroyi* DSS-3, in which most of the proteins were identified (Chen, 2012; Lidbury *et al.*, 2014; Lidbury *et al.*, 2017). With the exception of *T. erythraeum* LIN, the genome for which was generated in this study, BLASTP searches were conducted using JGI’s IMG/M database. To query the genome of *T. erythraeum* LIN, a custom BLAST+ 2.10.0 database was constructed (Camacho *et al.*, 2009).

Acknowledgements

We would like to thank Doug Capone, John Heidelberg, Jed Fuhrman, and the members of their labs for sharing equipment and space with us. Additionally, we thank Jesse McNichol for guidance with sequence processing and to Mike Lee and Maggi Mars Brisbin for invaluable guidance and conversations. This research was supported by funding from the National Science Foundation (For DAH and EAW: OCE 1657757 and OCE 1851222).

References
Amir, A., McDonald, D., Navas-Molina, J.A., Kopylova, E., Morton, J.T., Xu, Z.Z., et al. (2017) Deblur rapidly resolves single-nucleotide community sequence patterns. *mSystems* **2**: e00191-16.

Andrews, S. (2010). FastQC: a quality control tool for high throughput sequence data. [WWW document]. URL https://www.bioinformatics.babraham.ac.uk/projects/fastqc/.

Arkin, A.P., Cottingham, R.W., Henry, C.S., Harris, N.L., Stevens, R.L., Maslov, S., et al. (2018) KBase: The United States Department of Energy Systems Biology Knowledgebase. *Nat Biotechnol* **36**: 566–569.

Bain, M.A., Faull, R., Fornasini, G., Milne, R.W., Schumann, R., and Evans, A.M. (2004) Quantifying trimethylamine and trimethylamine-N-oxide in human plasma: interference from endogenous quaternary ammonium compounds. *Anal Biochem* **334**: 403–405.

Basu, S., Gledhill, M., de Beer, D., Prabhu Matondkar, S.G., and Shaked, Y. (2019) Colonies of marine cyanobacteria *Trichodesmium* interact with associated bacteria to acquire iron from dust. *Commun Biol* **2**: 1–8.

Bell, P.R.F., Uwins, P.J.R., Elmetri, I., Phillips, J.A., Fu, F.-X., and Yago, A.J.E. (2005) Laboratory culture studies of *Trichodesmium* isolated from the Great Barrier Reef Lagoon, Australia. *Hydrobiologia* **532**: 9–21.

Bokulich, N.A., Kaehtler, B.D., Rideout, J.R., Dillon, M., Bolyen, E., Knight, R., et al. (2018) Optimizing taxonomic classification of marker-gene amplicon sequences with QIIME 2’s q2-feature-classifier plugin. *Microbiome* **6**: 90.

Bolyen, E., Rideout, J.R., Dillon, M.R., Bokulich, N.A., Abnet, C.C., Al-Ghalith, G.A., et al. (2019) Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nat Biotechnol* **37**: 852–857.
Bolger, A.M., Lohse, M., and Usadel, B. (2014) Trimmomatic: a flexible trimer for Illumina sequence data. Bioinformatics 30: 2114–2120.

Breitbarth, E., Mills, M.M., Friedrichs, G., and LaRoche, J. (2004) The Bunsen gas solubility coefficient of ethylene as a function of temperature and salinity and its importance for nitrogen fixation assays. Limnol Oceanogr Methods 2: 282–288.

Buchan, A., González, J.M., and Moran, M.A. (2005) Overview of the Marine Roseobacter Lineage. Appl Environ Microbiol 71: 5665–5677.

Bushnell, B. (2014) BBMap: A Fast, Accurate, Splice-Aware Aligner, Lawrence Berkeley National Lab. (LBNL), Berkeley, CA (United States).

Camacho, C., Coulouris, G., Avagyan, V., Ma, N., Papadopoulos, J., Bealer, K., and Madden, T.L. (2009) BLAST+: architecture and applications. BMC Bioinform 10: 421.

Capone, D.G., Burns, J.A., Montoya, J.P., Subramaniam, A., Mahaffey, C., Gunderson, T., et al. (2005) Nitrogen fixation by Trichodesmium spp.: An important source of new nitrogen to the tropical and subtropical North Atlantic Ocean. Global Biogeochem Cycles 19:

Capone, D.G. and Montoya, J.P. (2001) Nitrogen fixation and denitrification. In Methods in Microbiology. Marine Microbiology. Academic Press, pp. 501–515.

Capone, D.G., Zehr, J.P., Paerl, H.W., Bergman, B., and Carpenter, E.J. (1997) Trichodesmium, a Globally Significant Marine Cyanobacterium. Science 276: 1221–1229.

Chappell, P.D. and Webb, E.A. (2010) A molecular assessment of the iron stress response in the two phylogenetic clades of Trichodesmium. Environ Microbiol 12: 13–27.
Chen, I.-M.A., Chu, K., Palaniappan, K., Ratner, A., Huang, J., Huntemann, M., et al. (2021) The IMG/M data management and analysis system v.6.0: new tools and advanced capabilities. *Nucleic Acids Res* **49**: D751–D763.

Chen, Y. (2012) Comparative genomics of methylated amine utilization by marine Roseobacter clade bacteria and development of functional gene markers (*trmm, gmaS*). *Environ Microbiol* **14**: 2308–2322.

Chen, Y., Patel, N.A., Crombie, A., Scrivens, J.H., and Murrell, J.C. (2011) Bacterial flavin-containing monooxygenase is trimethylamine monooxygenase. *Proc Natl Acad Sci USA* **108**: 17791–17796.

Chen, Y.-B., Zehr, J.P., and Mellon, M. (1996) Growth and nitrogen fixation of the diazotrophic filamentous nonheterocystous cyanobacterium *Trichodesmium* sp. IMS101 in defined media: evidence for a circadian rhythm. *J Phycol* **32**: 916–923.

Eren, A.M., Esen, Ö.C., Quince, C., Vineis, J.H., Morrison, H.G., Sogin, M.L., and Delmont, T.O. (2015) Anvi'o: an advanced analysis and visualization platform for 'omics data. *PeerJ* **3**: e1319.

Frischkorn, K.R., Rouco, M., Mooy, B.A.S.V., and Dyhrman, S.T. (2018) The *Trichodesmium* microbiome can modulate host N2 fixation. *Limnol Oceanogr Lett* **3**: 401–408.

Frischkorn, K.R., Rouco, M., Van Mooy, B.A.S., and Dyhrman, S.T. (2017) Epibionts dominate metabolic functional potential of *Trichodesmium* colonies from the oligotrophic ocean. *ISME J* **11**: 2090–2101.

Geng, H. and Belas, R. (2010) Molecular mechanisms underlying roseobacter–phytoplankton symbioses. *Curr Opin Biotechnol* **21**: 332–338.
Gradoville, M.R., Crump, B.C., Letelier, R.M., Church, M.J., and White, A.E. (2017) Microbiome of *Trichodesmium* Colonies from the North Pacific Subtropical Gyre. *Front Microbiol* **8**: 1-16.

Hmelo, L., Van Mooy, B., and Mincer, T. (2012) Characterization of bacterial epibionts on the cyanobacterium *Trichodesmium*. *Aquat Microb Ecol* **67**: 1–14.

Hungate, B.A., Mau, R.L., Schwartz, E., Caporaso, J.G., Dijkstra, P., van Gestel, N., *et al.* (2015) Quantitative microbial ecology through stable isotope probing. *Appl Environ Microbiol* **81**: 7570–7581.

Hynes, A.M., Chappell, P.D., Dyhrman, S.T., Doney, S.C., and Webb, E.A. (2009) Cross-basin comparison of phosphorus stress and nitrogen fixation in *Trichodesmium*. *Limnol Oceanogr* **54**: 1438–1448.

Jain, C., Rodriguez-R, L.M., Phillippy, A.M., Konstantinidis, K.T., and Aluru, S. (2018) High throughput ANI analysis of 90K prokaryotic genomes reveals clear species boundaries. *Nat Commun* **9**: 5114.

Karl, D., Letelier, R., Tupas, L., Dore, J., Christian, J., and Hebel, D. (1997) The role of nitrogen fixation in biogeochemical cycling in the subtropical North Pacific Ocean. *Nature* **388**: 533–538.

King, G.M. Metabolism of trimethylamine, choline, and glycine betaine by sulfate-reducing and methanogenic bacteria in marine sediments. *Appl Environ Microbiol*. **48**: 719-725.

Klawonn, I., Eichner, M.J., Wilson, S.T., Moradi, N., Thamdrup, B., Kümmel, S., *et al.* (2020) Distinct nitrogen cycling and steep chemical gradients in *Trichodesmium* colonies. *ISME J* **14**: 399–412.
Landa, M., Burns, A.S., Roth, S.J., and Moran, M.A. (2017) Bacterial transcriptome remodeling during sequential co-culture with a marine dinoflagellate and diatom. *ISME J* 11: 2677–2690.

Langmead, B. and Salzberg, S.L. (2012) Fast gapped-read alignment with Bowtie 2. *Nat Methods* 9: 357–359.

Lee, M.D., Walworth, N.G., McParland, E.L., Fu, F.-X., Mincer, T.J., Levine, N.M., et al. (2017) The *Trichodesmium* consortium: conserved heterotrophic co-occurrence and genomic signatures of potential interactions. *ISME J* 11: 1813–1824.

Lee, M.D., Webb, E.A., Walworth, N.G., Fu, F.-X., Held, N.A., Saito, M.A., and Hutchins, D.A. (2018) Transcriptional activities of the microbial consortium living with the marine nitrogen-fixing cyanobacterium *Trichodesmium* reveal potential roles in community-level nitrogen cycling. *Appl Environ Microbiol* 84: e02026-17.

Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., et al. (2009) The Sequence Alignment/Map format and SAMtools. *Bioinformatics* 25: 2078–2079.

Lidbury, I. (2015) Microbial methylated amine metabolism in marine surface waters. PhD thesis, University of Warwick.

Lidbury, I., Mausz, M.A., Scanlan, D.J., and Chen, Y. (2017) Identification of dimethylamine monooxygenase in marine bacteria reveals a metabolic bottleneck in the methylated amine degradation pathway. *ISME J* 11: 1592–1601.

Lidbury, I., Murrell, J.C., and Chen, Y. (2014) Trimethylamine N-oxide metabolism by abundant marine heterotrophic bacteria. *Proc Natl Acad Sci USA* 111: 2710–2715.
Lidbury, I.D., Murrell, J.C., and Chen, Y. (2015) Trimethylamine and trimethylamine N-oxide are supplementary energy sources for a marine heterotrophic bacterium: implications for marine carbon and nitrogen cycling. *ISME J* 9: 760–769.

Luo, H. and Moran, M.A. (2014) Evolutionary Ecology of the Marine Roseobacter Clade. *Microbiol Mol Biol Rev* 78: 573–587.

Mackay, M.A., Norton, R.S., and Borowitzka, L.J. (1984) Organic osmoregulatory solutes in cyanobacteria. *Microbiology*, 130: 2177–2191.

Martin, M. (2011) Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet.journal* 17: 10–12.

McNichol, J. (2019) Exact Amplicon Sequence Variant (eASV) Pipeline for 16S/18S Sequences Derived From 515Y/926R Primers (Deblur). [WWW document]. URL https://www.protocols.io/private/6C81EC4BC2074A76D7ACF80E2F0603B7

Morando, M. and Capone, D.G. (2018) Direct Utilization of Organic Nitrogen by Phytoplankton and Its Role in Nitrogen Cycling Within the Southern California Bight. *Front Microbiol* 9: 2118.

Morando, M. and Capone, D.G. (2016) Intraclade Heterogeneity in Nitrogen Utilization by Marine Prokaryotes Revealed Using Stable Isotope Probing Coupled with Tag Sequencing (Tag-SIP). *Front Microbiol* 7: 1932.

Morrissey, E.M., Mau, R.L., Schwartz, E., Koch, B.J., Hayer, M., and Hungate, B.A. (2018) Taxonomic patterns in the nitrogen assimilation of soil prokaryotes: Nitrogen assimilation of soil prokaryotes. *Environ Microbiol* 20: 1112–1119.

Mulholland, M.R. and Capone, D.G. (1999) Nitrogen fixation, uptake and metabolism in natural and cultured populations of *Trichodesmium* spp. *Mar Ecol Prog Ser* 188: 33–49.
Mulholland, M.R. and Capone, D.G. (2000) The nitrogen physiology of the marine N2-fixing cyanobacteria *Trichodesmium* spp. *Trends Plant Sci* 5: 148–153.

Newton, R.J., Griffin, L.E., Bowles, K.M., Meile, C., Gifford, S., Givens, C.E., *et al.* (2010) Genome characteristics of a generalist marine bacterial lineage. *ISME J* 4: 784–798.

Nurk, S., Meleshko, D., Korobeynikov, A., and Pevzner, P.A. (2017) *metaSPAdes*: a new versatile metagenomic assembler. *Genome Res* 27: 824–834.

Oren, A. (1990) Formation and breakdown of glycine betaine and trimethylamine in hypersaline environments. *Antonie van Leeuwenhoek* 58: 291–298.

Pade, N., Michalik, D., Ruth, W., Belkin, N., Hess, W.R., Berman-Frank, I., and Hagemann, M. (2016) Trimethylated homoserine functions as the major compatible solute in the globally significant oceanic cyanobacterium *Trichodesmium*. *Proc Natl Acad Sci USA* 113: 13191–13196.

Paerl, H.W., Priscu, J.C., and Brawner, D.L. (1989) Immunochemical Localization of Nitrogenase in Marine *Trichodesmium* Aggregates: Relationship to N2 Fixation Potential. *Appl Environ Microbiol* 55: 2965–2975.

Parada, A.E., Needham, D.M., and Fuhrman, J.A. (2016) Every base matters: assessing small subunit rRNA primers for marine microbiomes with mock communities, time series and global field samples. *Environ Microbiol* 18: 1403–1414.

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., *et al.* (2013) The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Res* 41: D590–D596.
Rao, D., Webb, J.S., Holmström, C., Case, R., Low, A., Steinberg, P., and Kjelleberg, S. (2007) Low densities of epiphytic bacteria from the marine alga *Ulva australis* inhibit settlement of fouling organisms. *Appl Environ Microbiol* 73: 7844–7852.

Rao, M., Smith, B.C., and Marletta, M.A. (2015) Nitric oxide mediates biofilm formation and symbiosis in *Silicibacter sp.* strain TrichCH4B. *mBio* 6: e00206-15.

Roe, K.L., Barbeau, K., Mann, E.L., and Haygood, M.G. (2012) Acquisition of iron by *Trichodesmium* and associated bacteria in culture. *Environ Microbiol* 14: 1681–1695.

Rognes, T., Flouri, T., Nichols, B., Quince, C., and Mahé, F. (2016) VSEARCH: a versatile open source tool for metagenomics. *PeerJ* 4: e2584.

Rouco, M., Haley, S.T., and Dyhrman, S.T. (2016) Microbial diversity within the *Trichodesmium* holobiont. *Environ Microbiol* 18: 5151–5160.

Seemann, T. (2014) Prokka: rapid prokaryotic genome annotation. *Bioinformatics* 30: 2068–2069.

Seyedsayamdost, M.R., Case, R.J., Kolter, R., and Clardy, J. (2011) The Jekyll-and-Hyde chemistry of *Phaeobacter gallaeciensis*. *Nat Chem* 3: 331–335.

Sun, J., Mausz, M.A., Chen, Y., and Giovannoni, S.J. (2019) Microbial trimethylamine metabolism in marine environments: Microbial TMA metabolism. *Environ Microbiol* 21: 513–520.

Walworth, N., Pfreundt, U., Nelson, W.C., Mincer, T., Heidelberg, J.F., Fu, F., et al. (2015) *Trichodesmium* genome maintains abundant, widespread noncoding DNA in situ, despite oligotrophic lifestyle. *Proc Natl Acad Sci USA* 112: 4251–4256.
Walworth, N.G., Fu, F.-X., Lee, M.D., Cai, X., Saito, M.A., Webb, E.A., and Hutchins, D.A. (2018) Nutrient-colimited Trichodesmium as a nitrogen source or sink in a future ocean. *Appl Environ Microbiol* **84**: e02137-17.

Wasmund, N., Topp, I., and Schories, D. (2006). Optimising the storage and extraction of chlorophyll samples. *Oceanologia* **48**: 125-144.

Webb, E.A., Jakuba, R.W., Moffett, J.W., and Dyhrman, S.T. (2007) Molecular assessment of phosphorus and iron physiology in *Trichodesmium* populations from the western Central and western South Atlantic. *Limnol Oceanogr* **52**: 2221–2232.

Weissman, J.L., Hou, S., and Fuhrman, J.A. (2020) Estimating maximal microbial growth rates from cultures, metagenomes, and single cells via codon usage patterns. *bioRxiv* 2020.07.25.221176.

Welschmeyer, N.A. (1994) Fluorometric analysis of chlorophyll a in the presence of chlorophyll b and pheopigments. *Limnol Oceanogr* **39**: 1985–1992.

Welsh, D.T. (2000) Ecological significance of compatible solute accumulation by micro-organisms: from single cells to global climate. *FEMS Microbiol Rev** **24**: 263–290.

Wu, Y.-W., Simmons, B.A., and Singer, S.W. (2016) MaxBin 2.0: an automated binning algorithm to recover genomes from multiple metagenomic datasets. *Bioinformatics* **32**: 605–607.

Youngblut, N.D., Barnett, S.E., and Buckley, D.H. (2018) HTSSIP: An R package for analysis of high throughput sequencing data from nucleic acid stable isotope probing (SIP) experiments. *PLoS ONE* **13**: e0189616.

Zehr, J.P. and Capone, D.G. (2020) Changing perspectives in marine nitrogen fixation. *Science* **368**: eaay9514.
Figure Legends

**Figure 1. Potential paths of TMA incorporation in the *Trichodesmium* consortium.** Option A - *Trichodesmium* metabolizes TMA directly, possibly then releasing a nitrogen compound that is incorporated by associated taxa. Option B - *Trichodesmium* receives TMA-N secondarily following conversion of TMA to an alternate N compound by one or more of the associated taxa. Option C - both *Trichodesmium* and one or more of the associated taxa metabolizes TMA.

**Figure 2. Growth and N₂ fixation rates for ¹⁴N TMA, ¹⁵N TMA, and no TMA control cultures over the four days following TMA addition.** A = *Trichodesmium* growth, represented by ln-transformed chlorophyll A fluorescence, measured in vivo in relative fluorescence units (RFU). B = rate of N₂ fixation divided by each culture’s chlorophyll A fluorescence and normalized to the value of the no-addition control treatment. N₂ fixation rates were measured by gas chromatography using the acetylene reduction proxy method.

**Figure 3. Atom fraction excess of ¹⁵N for each ASV on days 1, 3, and 4.** The dotted vertical line at 0.1340 represents our empirical threshold for incorporation of ¹⁵N. Each ASV is listed on the X-axis along with the finest-level classification assigned by QIIME2’s q2-feature-classifier plugin at the default confidence threshold of 0.7 using the SILVA132 database.

**Figure 4. Proposed model for methylated amine catabolism and presence/absence of corresponding proteins in the genomes of**
**Trichodesmium** and **Mameliella spp.** (a) Proposed MA catabolism pathway as described by Lidbury et al. (2015). (b) Presence/absence of gene homologs in *T. erythraeum*, *Mameliella spp.*, and environmental *Trichodesmium* metagenomes based on evidence from BLASTP search for TMA utilization proteins from *R. pomeroyii* DSS-3 (Chen et al., 2011; Lidbury et al., 2014; Lidbury et al., 2015; Lidbury et al., 2017). Black rectangles denote BLASTP hits with ≥ 90% coverage and ≥ 50% identity. Gray rectangles denote BLASTP hits with ≥ 90% coverage and ≥ 25% identity or ≥ 50% coverage and ≥ 50% identity. For *Mameliella spp.* (10 strains), rectangles indicate hits in genomes from all 10 strains. For *Trichodesmium* metagenomes (collected near Station ALOHA), rectangles indicate hits in one or more of the three metagenomes. Tmm, TMA monooxygenase (Chen et al., 2011); Tdm, TMAO demethylease (Lidbury et al., 2014); Dmm, DMA monooxygenase (Lidbury et al., 2017); GmaS, gamma-glutamylmethylamide synthetase (Lidbury et al., 2015); MgsABC, N-methylglutamate synthase (Lidbury et al., 2014); MgdABCD, N-methylglutamate dehydrogenase (Lidbury et al., 2014). TMA, trimethylamine; TMAO, trimethylamine N-oxide; DMA, dimethylamine; MMA, monomethylamine; GMA, gamma-glutamylmethylamide; NMG, N-methylglutamate.
