Taxonomic distribution of metabolic functions underpins nutrient cycling in *Trichodesmium* consortia

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Abstract

The photosynthetic and diazotrophic cyanobacterium *Trichodesmium* is a key contributor to marine biogeochemical cycles in the subtropical-oligotrophic oceans. *Trichodesmium* forms colonies that harbor a distinct microbial community, which expands their functional potential and is predicted to influence the cycling of carbon, nitrogen, phosphorus and iron (C, N, P, and Fe). To link key traits to taxa and elucidate how community structure influences nutrient cycling, we assessed Red Sea *Trichodesmium* colonies using metagenomics and metaproteomics. This diverse consortium comprises bacteria that typically associate with algae and particles, such as the ubiquitous *Alteromonas macleodii,* but also lineages specific to *Trichodesmium*, such as members from the order Balneolales. These bacteria carry functional traits that would influence resource cycling in the consortium, including siderophore biosynthesis, reduced phosphorus metabolism, vitamins, denitrification, and dissimilatory-nitrate-reduction-to-ammonium (DNRA) pathways. Denitrification and DNRA appeared to be modular as bacteria collectively completed the steps for these pathways. The vast majority of associated bacteria were auxotrophic for vitamins, indicating the interdependency of consortium members. *Trichodesmium* in turn may rely on associated bacteria to meet its high Fe demand as several lineages can synthesize the photolabile siderophores vibrioferrin, rhizoferrin, and petrobactin, enhancing the bioavailability of particulate-Fe to the entire consortium. Our results highlight that *Trichodesmium* is a hotspot for C, N, P, Fe, and vitamin exchange. The functional redundancy of nutrient cycling in the consortium likely underpins its resilience within an ever-changing global environment.

Importance (150 words)

Colonies of the cyanobacteria *Trichodesmium* act as a biological hotspot for the usage and recycling of key resources such as C, N, P and Fe within an otherwise oligotrophic environment. While *Trichodesmium* colonies are known to interact with a unique community of algae and particle-associated microbes, our understanding of the taxa that populate these colonies and the gene functions they encode is still limited. Characterizing the taxa and adaptive strategies that influence consortium physiology and its concomitant biogeochemistry is critical in a future ocean predicted to have increasing particulate fluxes and resource-depleted regions.

Introduction

Trichodesmium spp. are a globally relevant group of cyanobacteria that can form surface blooms visible from space (1). Owing to their high abundance and capability to fix both carbon and nitrogen, they are considered key players in the biogeochemical cycling of C and N within the oligotrophic tropical and subtropical oceans (2–5). A key trait underpinning the functionality of *Trichodesmium* is that its filaments can cluster together to form large colonies of 1-2 mm in size. The formation of colonies allows *Trichodesmium* to capture dust that is deposited on the ocean surface and *Trichodesmium* is reported to actively select for Fe and P-rich particles and shuffle them to their colony core (6–8). Colony formation hereby adds an intriguing spatial component where *Trichodesmium* spp. is predicted to act as a biological hotspot not only for C and N, but also for the uptake and recycling of key limiting nutrients such as Fe and P.

Trichodesmium colonies harbor a unique microbial consortium of epibiotic bacteria. Amplicon sequencing has shown these associated bacteria are distinct from populations in the surrounding oligotrophic surface waters (9–11). At a genetic level, the associated microbial community was found to enrich the total functional potential of the collective colony beyond the traits of N_2 and C fixation (12–14). By enriching the genetic repertoire of metabolic functions, or traits, related to the nutrients C, N, Fe, and P, consortia members were predicted to influence the internal cycling of these nutrients (14–17). To understand the biogeochemical contributions of *Trichodesmium* within the global ocean it is therefore critical to look beyond the metabolic functions present in *Trichodesmium* and towards the processes taking place within the larger microbial community. While metagenomic studies of *Trichodesmium* colonies in the Atlantic (13) and the Pacific Oceans (12, 17, 18) highlighted key functional traits within the consortium, only one study linked pathways to the associated bacteria from 8

56 metagenome-assembled bins (13). Expanding these studies, and characterizing how functional
57 traits are distributed across different taxa is critical to understanding the role of *Trichodesmium*
58 associated bacteria in consortium physiology and its concomitant influence over the cycling of
59 key resources such as C and N.
60
61 Recent evidence from the Red Sea highlights the unique ability of *Trichodesmium* consortia
62 members to acquire particulate Fe from dust through the production of Fe-chelating
63 siderophore molecules (7, 8, 16, 19, 20). Siderophores dissolve particulate Fe from dust and
64 are predicted to increase its bioavailability for both *Trichodesmium* and its associated bacteria
65 (16, 21, 22). In this manner, *Trichodesmium* spp. interacts with their associated bacteria in
66 order to meet the high Fe-requirements for the processes of photosynthesis and N_2 fixation (23).
67 This finding is particularly relevant in light of climate model simulations, which predict an
68 increase in regional dust emissions in areas such as the Red Sea where *Trichodesmium* is known
69 to occur (24, 25). While *Trichodesmium* blooms are a re-occurring phenomenon in this area
70 (26), it represents an under sampled region where the taxonomic composition of its consortium
71 has not been explored genetically. Furthermore, the presence and taxonomic distribution of
72 siderophore biosynthesis pathways within *Trichodesmium* colonies has not yet been
73 interrogated in depth. Understanding which consortium members positively enhance the
74 bioavailability of Fe within the *Trichodesmium* consortium will help elucidate key interactions
75 and community dynamics taking place within the colony that influence the ability of
76 *Trichodesmium* to fix C and N. This information is critical to evaluating *Trichodesmium*
77 contributions to C and N cycling in the future ocean.
78
79 In this study, we conducted an in-depth metagenomic and proteomic study of *Trichodesmium*
80 colonies collected from the Red Sea. To elucidate how the community structure influences the

81 functional dynamics of the *Trichodesmium* consortium we identified the different taxa making
82 up the *Trichodesmium* consortium and examined the presence of functional traits involved in
83 nutrient cycling. We probed our dataset for genes, proteins and pathways involved in the uptake
84 and metabolism of N, P, Fe, and vitamins. Finally, we contextualize these findings by
85 comparing them to previous metagenomic studies of *Trichodesmium* colonies obtained from
86 the Atlantic and the Pacific oceans.
87

88 ## Results and Discussion

89 A total of 52 metagenomic-assembled genomes (MAGs) were obtained from *Trichodesmium*
90 colonies, of which 42 MAGs were of high quality (90% complete and <5% redundancy
91 (Supplementary Table 1) (27). The relative abundance of the different MAGs was consistent
92 across the three samples with *T. thiebautii* MAG 52 making up 71% ± 6 of the total relative
93 abundance (Supplementary Table 1). In parallel to the metagenomic analysis, a proteomic
94 dataset consisting of 14161 peptide sequences matching to protein sequences of 52 different
95 MAGs further probed the activity within the Red Sea *Trichodesmium* consortia
96 (Supplementary Table 2; Supplementary Table 3).

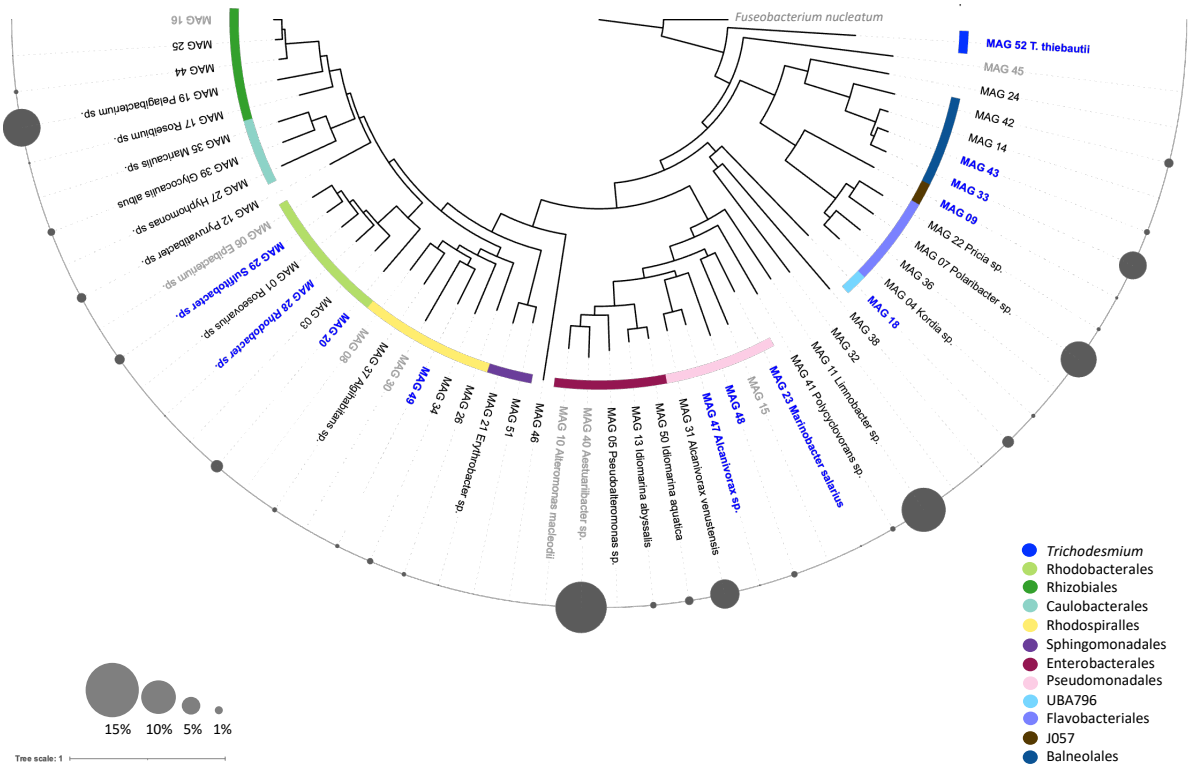


Figure 1. Phylogenetic tree of the 52 MAGs assembled from *Trichodesmium* colonies collected in the Red Sea. The tree is rooted by the outgroup *Fuseobacterium nucleatum*. Known bacterial orders are marked in different colours and several MAGs could taxonomically be identified at the genus level. The relative abundance of each MAG within the *Trichodesmium* consortium, excluding the abundance of Trichodesmium MAG 52 (±70% of samples), is presented as a bubble chart. The 11 MAGs that matched to MAGs assembled from a previous metagenomic dataset of Red Sea *Trichodesmium* colonies (28) are highlighted in blue and tentatively represent the core-consortium (see Supplementary Figure 2; Supplementary Table 4). MAGs that did not meet the requirements of a high-quality genome (>90% completeness; <5% redundancy) are indicated in grey. Note that re-occurring MAGs are not necessarily the most abundant MAGs present within the Trichodesmium colony. (a full taxonomic description of each MAG in this dataset can be found in Supplementary Table 1).

Trichodesmium colonies host diverse and flexible associated bacteria

Trichodesmium colonies consisted of a single *Trichodesmium* species (*T. thiebautii* MAG 52) and a diverse consortium of 51 associated bacteria spanning at least 10 different taxonomic orders (Figure 1). Unlike a previous study (28), our samples did not include any non-diazotrophic *Trichodesmium* populations and the 51 MAGs hereby represent the associated bacteria of *T. thiebautii* MAG 52. The majority of the associated bacterial MAGs belonged to taxonomic orders known to be present in *Trichodesmium* colonies and included the orders Rhodobacterales, Pseudomonadales, Balneolales, Rhodospirillales, Flavobacterales, Enterobacterales, Rhizobiales, and Sphingomonadales (9, 11–13).

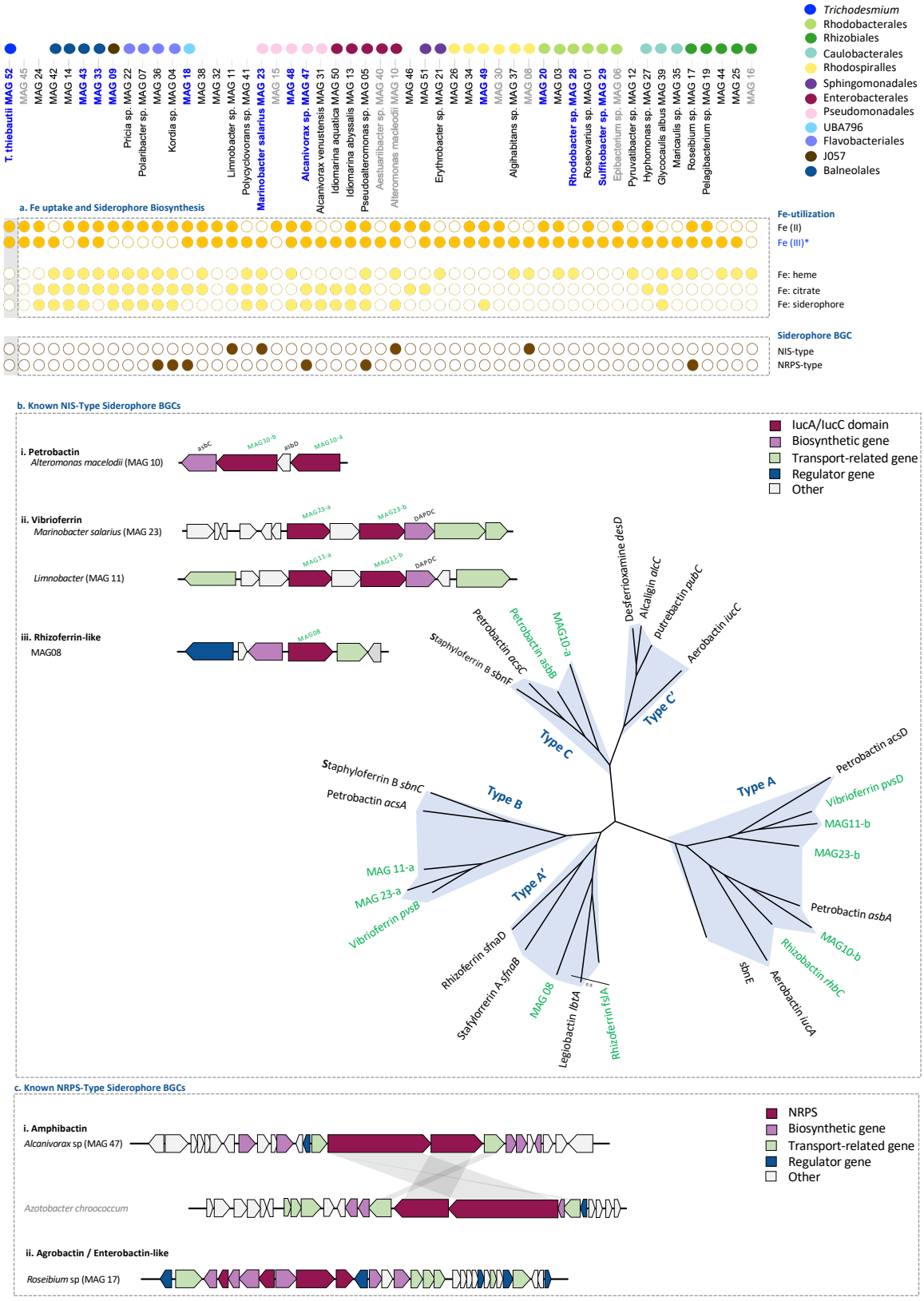
119 An intercomparison between the MAGs observed here and a previous metagenomic dataset
120 from the Red Sea (28) , identified a total of 11 MAGs present in both datasets (Supplementary
121 Figure 2; Supplementary Table 4). These 11 MAGs included the particle- and algae-associated
122 lineages (29–31) of *Marinobacter* sp. (MAG23) and *Alcanivorax* sp. (MAG 47) from the order
123 Pseudomonadales; *Rhodobacter* sp. (MAG 28) and *Sulfitobacter* sp. (MAG29) from the order
124 Rhodobacterales, and *Alteromonas macleodii* (MAG 10), a ubiquitous bacterium often found
125 in association with phototrophs (32) and previously isolated from *T. erythraeum* IMS101
126 cultures (33). If these observations are consistent across a larger time-period of observations,
127 these MAGs may represent a core component of the *Trichodesmium* consortium. Further
128 comparison of our metagenomic dataset with those obtained from the Atlantic, North or South
129 Pacific oceans, and the Red Sea (12, 13, 17, 18, 28), showed taxonomic similarities at the order
130 level rather than at the species level. While most taxonomic orders re-occurred in all datasets,
131 only one Balneolales genome (MAG 33) was present amongst all *Trichodesmium* metagenomic
132 datasets. As none of the re-occurring MAGs represented highly abundant genomes within our
133 samples (Figure 1), this may partially be the result of a higher sequencing depth (500 million
134 reads) compared to past datasets. Nonetheless, the wide diversity coupled with the recurrence
135 of only a few MAGs indicates the presence of a flexible assemblage of associated bacteria,
136 which may have important implications for the community structure and functioning of the
137 *Trichodesmium* consortium.
138
139 ***Trichodesmium associated bacteria synthesize diverse photolabile siderophores, which may***
140 ***increase iron availability from dust***
141 We explored the different Fe-uptake mechanisms present within the *Trichodesmium*
142 consortium, as N_2 and C fixation in *Trichodesmium* is often constrained by Fe availability (23,
143 34). Despite the high Fe-requirements of *Trichodesmium* (23), *T. thiebautii* (MAG 52) only

144 contained genes for the uptake of free Fe^{+2} and Fe^{+3}, but not organic Fe, suggesting it is limited
145 to a narrow range of forms it can directly take up (Figure 2). In contrast, MAGs of the
146 associated bacteria contained a larger diversity of Fe-uptake genes which included the ability
147 to take up organically bound Fe, such as heme, citrate, and siderophores (Figure 2). The
148 proteome of the *Trichodesmium* consortium indicated that, at the time of sampling,
149 *Trichodesmium* was actively taking up Fe through the presence of a ferric Fe-uptake
150 transporters (FbpC, FbpA) and Fe-storage proteins (Dps, Bfr) (Supplementary Table 2).
151
152 The genomes of 10 *Trichodesmium* associated bacteria spread across 6 different taxonomic
153 orders encoded different siderophore biosynthesis gene clusters (BGCs) (Figure 2). The
154 presence of a siderophore biosynthesis pathway was strain-specific and not present in an entire
155 clade or lineage. Similar to previous studies, *T. thiebautii* MAG 52 did not contain any known
156 siderophore biosynthesis pathways (12, 35). Siderophore BGCs could further be separated into
157 4 NIS-type and 6 NRPS-type siderophores (36, 37). Based on the presence of one or more
158 *iucA/iucC* domains, the 4 NIS-types BGCs matched closely to petrobactin (*Alteromonas*
159 *macleodii* MAG 10), vibrioferrin (*Marinobacter salarius* MAG 23; *Limnobacter sp.* MAG 11),
160 and rhizoferrin (Rhodospirillales MAG 08) biosynthesis pathways (Figure 2). From the 6
161 different NRPS-type siderophores, only 2 matched a known BGC, namely that of the
162 membrane-bound amphibactin (100%) (*Alcanivorax venustensis* MAG 47) and one resembling
163 the BGC of the siderophores agrobactin (93%) or enterobactin (±83%) (*Roseibium* sp MAG
164 18) (Figure 2). Our results additionally indicated the presence of several novel NRPS-type
165 siderophore biosynthesis pathways (Supplementary Figure 3). NRPS-type-siderophore BGCs
166 are not as well characterized as NIS-type-siderophores BGCs and can be difficult to distinguish
167 from other secondary metabolites such as toxins and antibiotics (38). While the 4 NRPS-type-
168 siderophore BGCs could not be linked to known siderophore BGCs, several unknown

169 metallophores have previously been identified and associated with *Trichodesmium* colonies
170 and we speculate that some may be linked to these uncharacterized NRPS BGCs (19). The
171 presence of multiple NRPS pathways containing a putative siderophore TonB-dependent
172 receptor (SMCOG1082) likely involved in Fe uptake, highlights the potential for novel
173 siderophore pathways within the *Trichodesmium* consortium and indicates the importance of
174 additional culture-based research linking such pathways to a siderophore molecule.
175
176 Our results indicate that the *Trichodesmium* consortium in the Red Sea may interact with
177 photolabile siderophore-producing bacteria to mine Fe from dust. The re-occurring consortium
178 members *Marinobacter salarius* (MAG 23), *Alcanivorax venustensis* (MAG 47), and
179 *Alteromonas macleodii* (MAG 10) are all able to synthesize the NIS-type siderophores
180 petrobactin, vibrioferrin, and rhizoferrin (Figure 2) whose citrate-moiety results in their
181 photolability (39). In the natural illumination of surface waters, where *Trichodesmium* resides,
182 sunlight can reductively disassociate Fe from these siderophore complexes and provide a flux
183 of highly available dissolved inorganic Fe (40, 41). While *Trichodesmium* does not contain a
184 TonB-receptor, the presence of photolabile siderophores implies that Fe is also bioavailable to
185 consortium members that lack a specific TonB-receptor for that particular siderophore. These
186 photolabile siderophores act as a "common good" within the *Trichodesmium* consortia, where
187 only a few members carrying the trait are needed to benefit the entire consortium (42). This
188 contrasts with the uptake mode of photostable siderophores, such as desferrioxamine-B
189 (DFOB), which require a specialized TonB-siderophore transport system (43). Similarly,
190 amphibactin is a membrane-bound siderophore (44), which prevents its diffusion in the
191 environment and subsequently its ability to act as a public good to the *Trichodesmium*
192 consortium. A recent gene-knockout study in *Alteromonas macleodii* verified the ability of
193 petrobactin to enhance the bioavailability of particulate Fe (22). This further supports the idea

194 that *Trichodesmium* can interact with consortium members that produce photolabile
195 siderophores to acquire Fe from dust.
196
197 Most of the siderophore BGCs detected here were identified in ubiquitous and copiotrophic
198 bacteria typically found in association with particles and algae (11, 22) , including
199 *Marinobacter salarius* (MAG 23), *Alcanivorax venustensis* (MAG 47), and *Alteromonas*
200 *macleodii* (MAG 10) (Figure 2). Siderophore-production within the *Trichodesmium*
201 consortium appears to be linked to a lifestyle associated with a rapidly changing environment
202 rich in N and C, where regulating Fe-bioavailability can provide a competitive advantage. In
203 line with particle and algae associations, siderophore production has also been associated with
204 other ecological functions, including heavy metal detoxification and intra-specific
205 communication (45) (Supplementary Figure 4; Supplementary Table 5). Whether siderophore
206 production can benefit the *Trichodesmium* consortium in functions other than Fe-dissolution
207 from dust requires further investigation. Nonetheless, the presence of a few, yet reoccurring,
208 siderophore producing MAGs augments previous measurements of siderophore production
209 within the consortium (16, 19, 20) and reveals additional layers of *Trichodesmium* colonies'
210 intriguing ability to sequester Fe from dust.

211 **Figure 2 (below).** MAGs were probed for Fe-uptake and siderophore biosynthesis genes which are noted in
212 Supplementary Table 5. **(a)** *Trichodesmium* MAG 52 does not contain a wide diversity of Fe-uptake genes, and
213 the majority of MAGs of associated bacteria contain an uptake receptor for organic Fe (heme, citrate,
214 siderophores). Several MAGs of associated bacteria contained an NRPS or NIS-type siderophore biosynthesis
215 pathways (BGCs). **(b)** Siderophore biosynthetic gene clusters (BGCs) of known NIS-type siderophore BGCs can
216 be clustered according to the presence of one or multiple *iuc*A/*iuc*C domains which enables one to target the
217 closest siderophore BGC match (52). Using this method, we were able to link these pathways to the synthesis of
218 the photolabile **(i)** petrobactin, **(ii)** vibrioferrin and **(iii)** rhizoferrin. **(c)** Siderophore BGCs of known NRPS
219 siderophores. Using antiSMASH 7.0, which aligns BGCs to known siderophores within the MIBiG database, we
220 were able to link 2 of the 6 putative siderophore NRPS-type pathways to **(i)** the membrane-bound amphibactin
221 and **(ii)** agrobactin/enterobactin. (See Supplementary Figure 3 for more details on the siderophore biosynthesis
222 pathways).


Trichodesmium
Rhodobacterales
Rhizobiales
Caulobacterales
Rhodospiralles
Sphingomonadales
Enterobacterales
Pseudomonadales
UBA796
Flavobacteriales
J057
Balneolales
T. thiebautii MAG 52
MAG 45
MAG 24
MAG 42
MAG 14
MAG 43
MAG 33
MAG 09
Pricia sp. MAG 22
Polaribacter sp. MAG 07
MAG 36
Kordia sp. MAG 04
MAG 18
MAG 38
MAG 32
Limnobacter sp. MAG 11
Polycyclovorans sp. MAG 41
Marinobacter salarius MAG 23
MAG 15
MAG 48
Alcanivorax sp. MAG 47
Alcanivorax venustensis MAG 31
Idiomarina aquatica MAG 50
Idiomarina abyssalis MAG 13
Pseudoalteromonas sp. MAG 05
Aestuarilibacter sp. MAG 40
Alteromonas macleodii MAG 10
MAG 46
MAG 51
Erythrobacter sp. MAG 21
MAG 26
MAG 34
MAG 49
MAG 30
Algihabitans sp. MAG 37
MAG 08
MAG 20
MAG 03
Rhodobacter sp. MAG 28
Roseovarius sp. MAG 01
Sulfitobacter sp. MAG 29
Epibacterium sp. MAG 06
Pyruvatibacter sp. MAG 12
Hyphomonas sp. MAG 27
Glycocaulis albus MAG 39
Maricaulis sp. MAG 35
Roseibium sp. MAG 17
Pelagibacterium sp. MAG 19
MAG 44
MAG 25
MAG 16
a. Fe uptake and Siderophore Biosynthesis
Fe-utilization
Fe (II)
Fe (III)*
Fe: heme
Fe: citrate
Fe: siderophore
Siderophore BGC
NIS-type
NRPS-type
b. Known NIS-Type Siderophore BGCs
IucA/IucC domain
Biosynthetic gene
Transport-related gene
Regulator gene
Other
i. Petrobactin
Alteromonas macelodii (MAG 10)
asbC
MAG10-b
asbD
MAG10-a
ii. Vibrioferrin
Marinobacter salarius (MAG 23)
MAG23-a
MAG23-b
DAPDC
Limnobacter (MAG 11)
MAG11-a
MAG11-b
DAPDC
iii. Rhizoferrin-like
MAG08
Desferrioxamine desD
Alcaligin alcC
putrebactin pubC
Aerobactin iucC
Type C'
MAG10-a
Petrobactin asbB
Petrobactin acsC
Staphyloferrin B sbnF
Type C
Staphyloferrin B sbnC
Petrobactin acsA
Type B
MAG 11-a
MAG 23-a
Vibrioferrin pvsB
Type A'
Rhizoferrin sfnaD
Staflyoferrin A sfnaB
MAG08
Legiobactin lbtA
Rhizoferrin fslA
Type A
Petrobactin acsD
Vibrioferrin pvsD
MAG11-b
MAG23-b
Petrobactin asbA
MAG10-b
Rhizobactin rhbC
sbnE
Aerobactin iucA
c. Known NRPS-Type Siderophore BGCs
NRPS
Biosynthetic gene
Transport-related gene
Regulator gene
Other
i. Amphibactin
Alcanivorax sp (MAG 47)
Azotobacter chroococcum
ii. Agrobactin / Enterobactin-like
Roseibium sp (MAG 17)

The *Trichodesmium* consortium is heterogeneous in genes related to the uptake and use of inorganic and organic phosphorous.

We predicted that the majority of bacteria associated with *Trichodesmium* have the ability to metabolize phosphite or phosphonate. This can offer a key competitive advantage amongst consortium members in a P-limited environment (18, 46) including the Gulf of Aqaba in the Red Sea (47). Within a low-P environment, dissolved inorganic phosphorus is scarce, and bacteria must scavenge phosphate from dissolved organic phosphorus to meet their P-requirements (48). *Trichodesmium* is highly efficient at scavenging and utilizing organic-P through the production of alkaline phosphatase (AP), and the uptake of phosphonate and phosphite (49). Multiple studies have shown that *Trichodesmium* colonies in the Atlantic and Pacific Ocean are hotspots for the reduced P-cycling of phosphite or phosphonate (46, 50) suggesting that the majority of consortium members can process reduced-P.

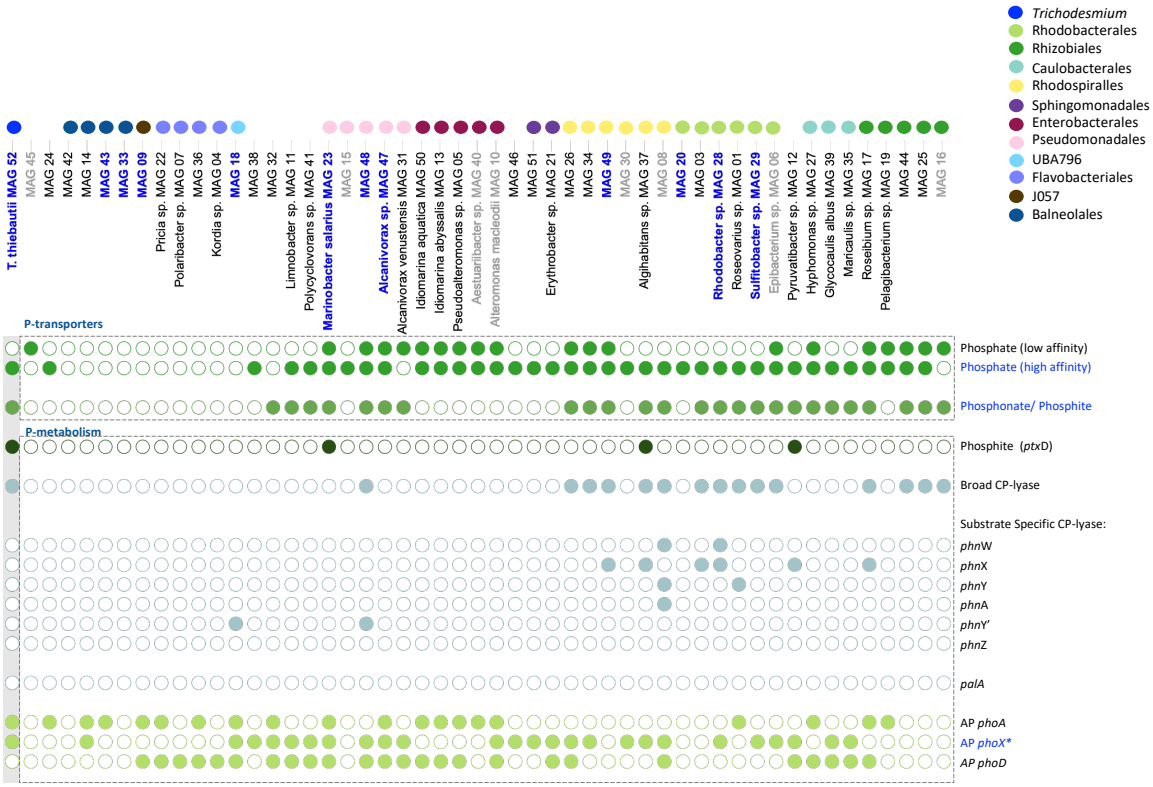


Figure 3. MAGs were probed for several different P-metabolism genes which are noted in Supplementary Table 5. *Trichodesmium* is known to be highly competitive for P, as exemplified by the multitude of **(a)** P-uptake systems present in its genome for phosphate (*pst*S, *pst*B), phosphonate (*phn*CDE), and phosphonate/phosphite (*phn*CDE/*ptx*ABC). **(b)** Within the *Trichodesmium* consortium, the ability to metabolize a broad range of phosphonate using a broad C-P lyase (*phn*GHIJKLM) is more prominent than the substrate specific hydrolysis of

2-aminoethylphosphonate (2-AEP; *phnWXYZA*) or phosphonopyruvate (*pal*A) and the phosphite metabolism (*ptx*D).

Confirming past studies (18, 51), *T. thiebautii* MAG 52 encodes a set of functions to transport and metabolize diverse forms of P (Figure 3). This includes the presence of a high-affinity phosphate transporter (*pst*SCAB), and a phosphonate/phosphite transporter (*phn*CDE/*pxt*ABC). In addition to these transport systems, genes related to utilization and metabolism of organic-P included alkaline phosphatase (AP) (*pho*A, *pho*X), and a carbon-phosphorus (C-P) lyase (*phn*GHIJKLM) which hydrolyses a broad range of phosphonate compounds. *T. thiebautii* MAG 52 also encodes phosphite dehydrogenase (*ptxD)* which catalyzes the oxidation of phosphite to phosphate. The presence of PstB, PhoX, and PhnD within the proteome (Supplementary Table 2), suggests *Trichodesmium* was taking up phosphate, phosphonate, and hydrolyzing phosphoester compounds to meet its P demand. Several of these proteins are regulated by P (51), and their detection in the proteome is consistent with intense competition for P in the low P Red Sea.

As KEGG annotation cannot accurately distinguish between a phosphonate (*phn*CDE) and a phosphite (*pxt*ABC) transporter, the presence of adjacent P-metabolism genes can further indicate the P-source linked to it. Specifically, the presence of phosphite dehydrogenase (*ptxD)* points to phosphite metabolism while a broad-specificity C-P lyase (*phnGHIJKLM*) or the substrate-specific hydrolysis of phosphonopyruvate (*pal*A) or 2-aminoethylphosphonate (2-AEP) to acetaldehyde (*phn*WX), acetate (*phn*WYA), or glycine (*phn*Y'Z), can point to the metabolism of phosphonate (52, 53). Our results showed that the presence of a *phn*CDE/*ptx*ABC uptake system was specific to certain taxonomic orders including Pseudomonadales, Rhodospirillales, Rhodobacterales, Caulobacterales, and Rhizobiales (Figure 3). Within the *Trichodesmium* consortium, these taxonomic orders may have a competitive advantage over members that lack them, particularly in low P conditions. Of these

270 25 MAGs, 16 could be linked to phosphonate metabolism through the presence of a broad-
271 specificity C-P lyase. In contrast, the presence of phosphite dehydrogenase was species-
272 specific where only 4 MAGs appeared to be able to process phosphite spread across different
273 taxonomic orders (Figure 3). Within the *Trichodesmium* consortium, phosphonate metabolism
274 appears to be the more common reduced P-currency of the two. 7 MAGs contained genes
275 associated with the substrate-specific hydrolysis of 2-AEP but pathways were not complete,
276 making it difficult to draw conclusions (Figure 3). Nonetheless, the presence of a broad-
277 specificity C-P lyase appeared to be the most prevalent strategy for phosphonate utilization
278 within the *Trichodesmium* consortium. This finding is in contrast to a meta-analysis of marine
279 metagenomes where the most common phosphonate degradation strategy within the water
280 column was the substrate-specific hydrolysis of 2-AEP (54). 2-AEP is the most abundant
281 phosphonate source in the marine environment (55), and the differences in enzyme distribution
282 within the Trichodesmium consortium may reflect heterogeneity in the phosphonate
283 compounds present in the colony, relative to the water column. Differences in enzyme
284 distribution may also be a function of the taxonomic composition of *Trichodesmium* associated
285 bacteria relative to those in in the water column. Regardless, these observations suggest that a
286 diversity of P compounds are being cycled by members of the consortia in the low P Red Sea
287 and offer a new reference dataset for future comparisons with *Trichodesmium* consortia from
288 other regions.
289

290 **Denitrification and dissimilatory nitrate reduction to ammonia (DNRA) pathways are**
291 **modular in the *Trichodesmium* consortium.**

292 We explored the presence of multiple N-metabolic pathways as they are predicted to influence
293 the biogeochemical contribution of *Trichodesmium* regarding N_2 fixation. Our results showed
294 that, in the *Trichodesmium* colonies sampled from the Red Sea, N_2-fixation appears to be

295 uniquely attributed to *T. thiebautii* MAG 52 (Figure 4). This contrasts with a previous study
296 that has observed *nifH* genes in both *Trichodesmium* and their associated bacteria (12). In our
297 study, however, only *T. thiebautii* (MAG 52) contained the full N_2 fixation pathway
298 (*nifDKHEB*: M00175) and therefore the ability to fix N_2 within the colony. Similar to other
299 studies (12, 17, 56), genes of the two additional N-transformation pathways of dissimilatory
300 nitrate reduction to ammonium (DNRA) and denitrification were present within the
301 *Trichodesmium* consortium (Figure 4). Respectively, denitrification and DNRA are N-loss and
302 N-recycling processes and are predicted to enable the residing community to conserve and fully
303 utilize the N_2 fixed by *Trichodesmium* (12) but also alter the net contribution of *Trichodesmium*
304 colonies to local N-cycling dynamics (15, 57). Our results indicated a relatively high number
305 of 24 MAGs encoding genes involved in DNRA and 10 MAGs encoding genes involved in
306 denitrification (Figure 4). Intriguingly, none of the MAGs analyzed contained the complete
307 pathways for denitrification and DNRA, with the exception of Rhizobiales for the latter (Figure
308 4). Collectively, MAGs were able to complete all steps within either pathway, indicating that
309 these pathways appear to be modular within the *Trichodesmium* consortium, where a bacteria
310 can perform one step within the pathway but not necessarily all steps. While Rhizobiales (MAG
311 16, 17 and 44) contained the full DNRA pathway, which includes the reduction of nitrate to
312 nitrite (*napAB, narGH*), and nitrite to ammonium (*nrfAH, nirBD*), the remaining MAGs
313 contained only one of two steps (Figure 4). Similar to DNRA, several MAGs from the phyla
314 Alphaproteobacteria contained a part of the denitrification steps, including the reduction of
315 nitrite to nitric-oxide (*nir*KS) and nitric-oxide to nitrous oxide (*nor*BC), while members of the
316 phyla Bacteroidetes could perform the final step within the denitrification pathway (*nosZ)*
317 which converts nitrous oxide to nitrogen (Figure 4).

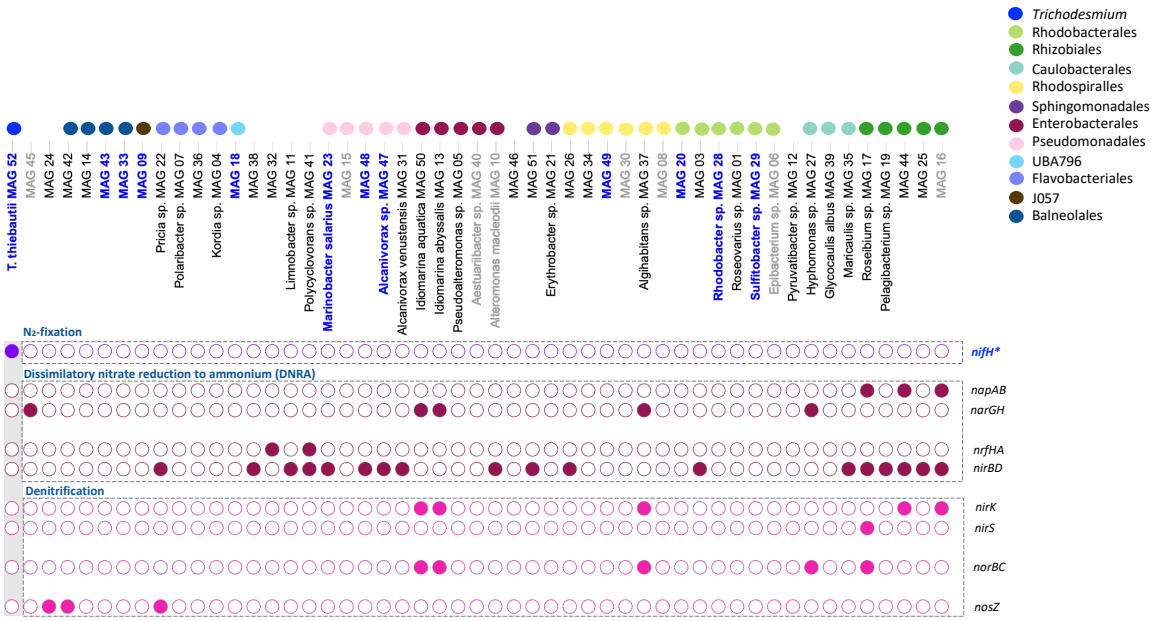


Figure 4. MAGs were probed for N-metabolic pathways and the genes for each step within the pathway are noted in Supplementary Table 5. *Trichodesmium* MAG 52 is the only MAG with a N_2 fixation pathway. Several MAGs contained one or two steps within the pathways of denitrification or dissimilatory nitrate to reduction to ammonia (DNRA) indicating their modularity. Collectively, MAGs completed the denitrification and DNRA pathways, but none contained the genes for the entire pathway.

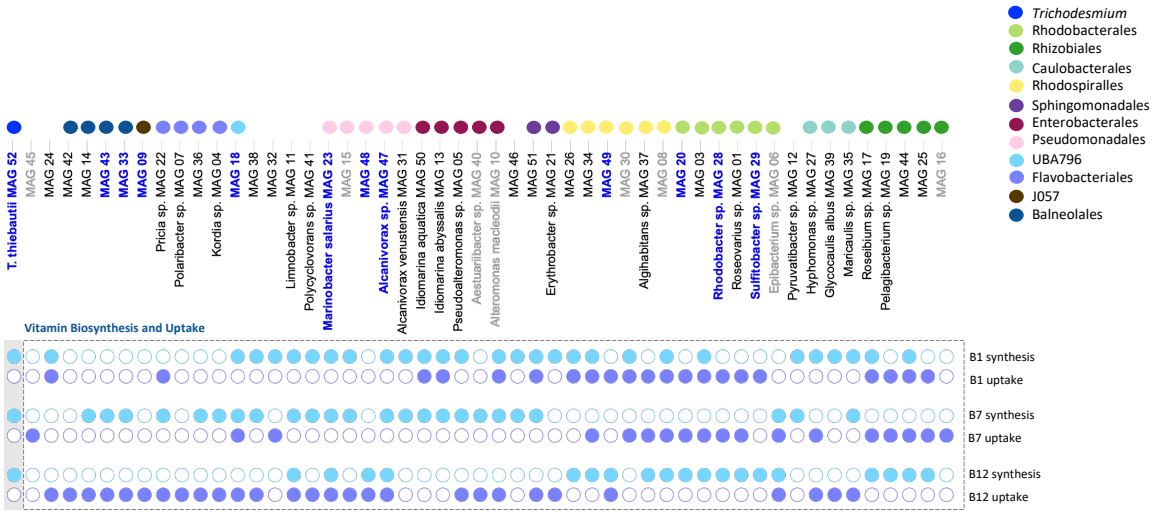
While the presence of modularity in denitrification and DNRA pathways itself is not uncommon (58–60) finding it present within the *Trichodesmium* consortium highlights the spatial role *Trichodesmium* plays in providing a platform where each step can be performed by a distinct subset of the microbial consortium. While the re-occurring consortium members *Alteromonas macleodii* (MAG 10), *Marinobacter salarius* MAG 23), and *Alcanivorax sp* (MAG 47, MAG 48) encoded genes (*nir*BD) within the DNRA pathway, it did not appear to be a key characteristic within the *Trichodesmium* associated bacteria of the Red Sea. The presence of DNRA and denitrification pathways is rare in the oxic surface ocean, as these processes require anoxic and suboxic conditions (61). In the surface waters, suboxic as well as anoxic micro-environments were detected in marine aggregates, such as marine snow and fecal pellets, where enhanced microbial respiration results in the consumption of O_2 (62, 63). *Trichodesmium* colonies may represent an additional environment where such processes may be taking place within the surface waters of the ocean. While it is not entirely clear when and where these N-cycling pathways are actively taking place within the colony studies have

339 measured DNRA and denitrification processes within natural *Trichodesmium* colonies (15, 57).
340 While the obligatory suboxic conditions for denitrification and DNRA may occur in the
341 presence of dust and the rapid O_2 consumption by bacteria (64), the formation of these
342 microenvironments have thus far not been observed in *Trichodesmium* colonies (57).
343 Altogether, these findings pinpoint the intricate community structure needed for such modular
344 processes to take place within the consortium. The exact tradeoffs between *Trichodesmium* and
345 denitrifying or DNRA bacteria, particularly in light of a changing ocean with higher dust
346 deposition requires further investigation.
347

348 **_Trichodesmium_ consortium members are interdependent for vitamins B1, B7 and B12.**

349 We explored the presence of vitamin auxotrophy within the consortium as an indicator for
350 community interdependence. Previous studies have shown that *Trichodesmium* can synthesize
351 and secrete the vitamin cobalamin (B12) to the environment (17). Our results show that while
352 *Trichodesmium* MAG 52 encodes the biosynthesis pathways for B12, biotin (B7) and thiamin
353 (B1), the majority of associated bacteria (49 of the 52 MAGs) lack a biosynthesis pathway for
354 at least one of these vitamins (Figure 5). Bacteroidetes, Sphingomonadales, and
355 Enterobacterales species, including *Alteromonas macleodii* (MAG 10), were all auxotrophic
356 for vitamin B12. MAGs from the order Rhodospirillales lacked the ability to synthesize vitamin
357 B7, whereas Rhizobiales and Rhodobacterales appeared to be auxotrophic for both vitamin B1
358 and B7. Only *Pseudoalteromonas* encoded all three biosynthesis pathways, including the re-
359 occurring taxa *Alcanivorax* sp. (MAG 47) and *Marinobacter salarius* (MAG 23). These
360 consortium members may also contribute to the synthesis and provision of vitamins B1, B7,
361 and B12 to their auxotrophic counterparts (Figure 5) but, based on overall relative abundance,
362 *Trichodesmium* represents the most likely source of B vitamins. In sum, the majority of
363 associated bacteria (49 of 52 MAGs) were predicted to be auxotrophic for B12 and likely

364 dependent on *Trichodesmium* to meet their intracellular requirements. Our results indicate that
365 the production and secretion of different vitamins by *Trichodesmium* is a key determinant of
366 community composition, stimulating vitamin-based interactions among auxotrophic
367 consortium members.



368

369 **Figure 5.** MAGs were probed for vitamin B1, B7, and B12 biosynthesis and uptake pathways. The genes used to
370 indicate the biosynthesis or uptake of these vitamins are noted in Supplementary Table 5. *Trichodesmium* contains
371 the biosynthesis pathways for all three vitamins, but the vast majority of associated bacteria were auxotrophic for
372 one or more vitamins. These results suggest that the associated bacteria rely on *Trichodesmium* to obtain their
373 vitamin requirements.
374

375 **Interactions between copiotrophic microbes underpin nutrient cycling in *Trichodesmium***
376 **consortia**

377 A literature study of the bacterial lineages present within the *Trichodesmium* consortium
378 describes these taxonomic orders as typical primary colonizers of marine surfaces such as algae
379 or sinking particles (29–31). Strains of *Alteromonas*, *Marinobacter,* and *Pseudomonas* often
380 co-occur with phototrophs such as cyanobacteria as well as oil spills, likely as a result of their
381 ability to degrade hydrocarbons and aromatic compounds (32, 65). While the prevalence of
382 these copiotrophic lifestyles within the *Trichodesmium* consortium has been observed in
383 previous studies (11, 13) it is still unclear whether their association with *Trichodesmium* is
384 specific or reflective of a more general interaction between algae or particle associations.
385 Copiotrophy is a widely encompassing term, but it generally concerns opportunistic bacteria

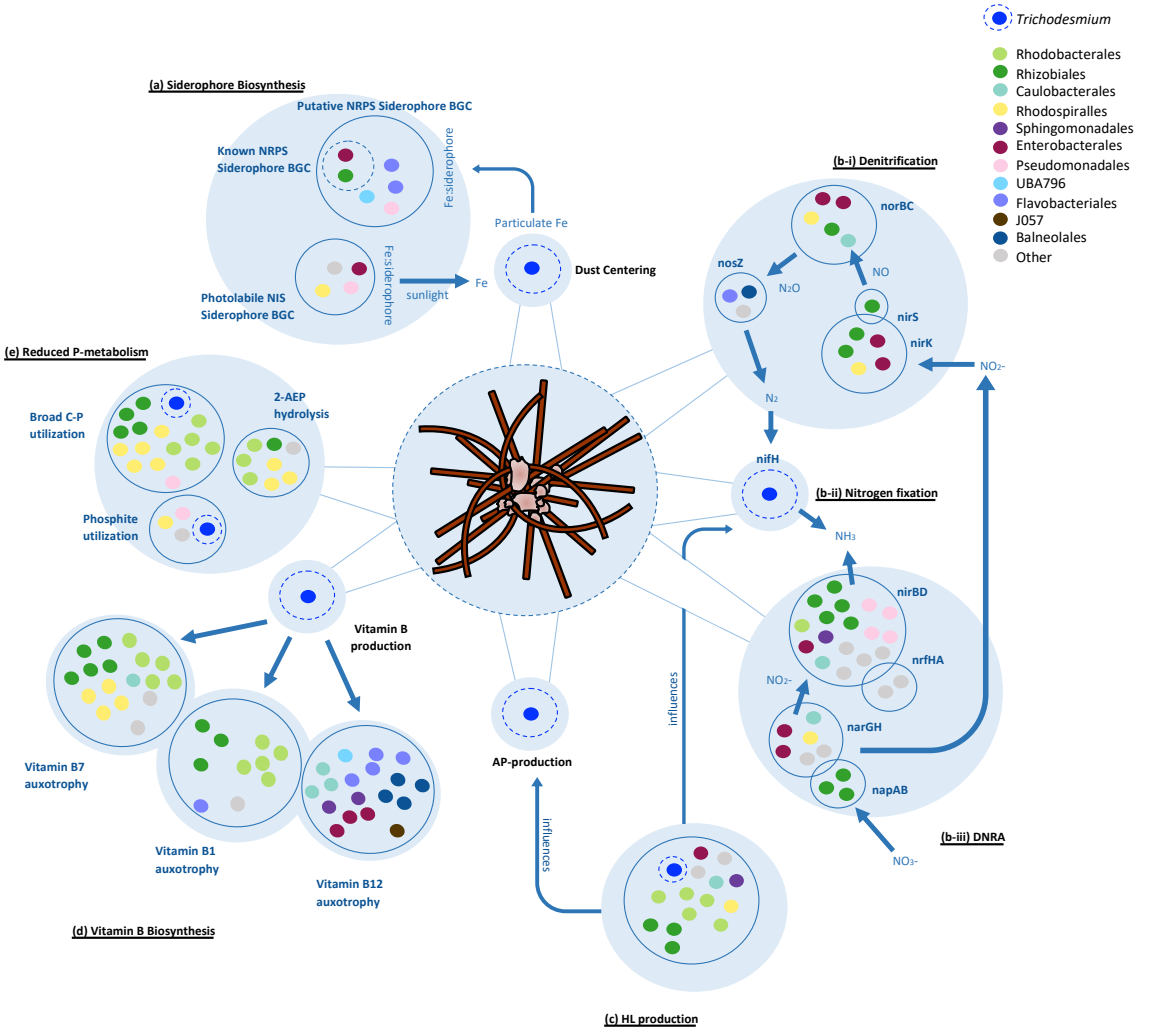
386 with a versatile metabolic capacity to use a wide range of substrates, acting as important
387 remineralizers by rapidly responding towards areas enriched in organic matter (66, 67). It is
388 therefore possible to consider the majority of the consortium as active remineralizers of the C,
389 N, P, Fe, and vitamins *Trichodesmium* is providing. Viewing *Trichodesmium* as a hotspot for
390 nutrients is supported by our proteomic samples where the majority of protein hits attributed
391 to *T. thiebautii* MAG 52 were related to photosynthetic (K05376; K05377; K02284) and
392 diazotrophic machinery (K03839, K02588, K02591) indicating that the processes of N and C
393 fixation were actively taking place at the time of collection (Supplementary Table 2). In turn,
394 the majority of protein hits associated to consortia members were involved in the uptake of
395 amino-acids (K01999; K09969), sugars (K02055; K02027; K10552), and peptides (K02035;
396 K02032) which is reflective of typical copiotrophic activity (Supplementary Table 3).
397
398 Copiotrophic bacteria are also characterised by their strong ability to interact within their
399 associations (68). This is supported by the prominence of several bacterial-interactive and
400 particle-associated traits in the MAGs studied here (68, 69) (Supplementary Figure 4;
401 Supplementary Table 5). The presence of bacterial-associated interactions taking place
402 between *Trichodesmium,* and their consortium members could further be implied through the
403 presence of several proteins involved in pilus formation (K02658, K02662, K02669) and
404 secretion systems (K11003; K03072; K03070, K03110, K03073; K03217) within *T. thiebautii*
405 MAG 52 (Supplementary Table 2). Collectively, these findings are indicative of an intricate
406 network of interaction between *Trichodesmium* and its associated bacteria. For example, *T.*
407 *thiebautii* MAG 52 and several MAGs, including re-occurring members from the order
408 Rhodobacterales, contained a homoserine lactone biosynthesis pathway (Supplementary
409 Figure 4a). Previous studies have predicted that consortium members can directly interact with
410 *Trichodesmium* through the production of homoserine lactones (HLs), where the presence of

411 HLs was shown to modulate N_2 fixation rates (14) and increase alkaline phosphatase (AP)
412 activity in *Trichodesmium* (70). The functional association of poorly studied bacterial clades,
413 in particular Balneolales (Supplementary Table 1), however, remained more elusive and
414 indicated that we still lack a clear understanding of these bacteria and their role within the
415 *Trichodesmium* consortium. Further investigations will be required to untangle the different
416 dynamics of both general and specific interactions taking place between members of the
417 *Trichodesmium* consortium.
418

419 ## *Conclusions and Future Perspectives*

420 The complex *Trichodesmium* consortium represents an ideal system to study the intricate
421 interactions taking place within a particle-rich system (Figure 6), asking how these processes
422 may impact the C, N, P and Fe-cycles in a rapidly changing ocean where both microbial
423 community composition and changes in microbial abundance are expected to occur (25, 71).
424 The *Trichodesmium* consortium is redundant for multiple traits related to nutrient cycling
425 including siderophore production, denitrification, DNRA, and reduced phosphate uptake. The
426 processes of denitrification and DNRA were modular, indicating an interactive and spatial
427 coupling between different consortium members. The associated bacteria were largely
428 auxotrophic for one or more vitamins that *Trichodesmium* can synthesize, and this is likely a
429 major trait influencing the community structure (Figure 6). The vast majority of the associated
430 bacteria could further be characterized according to a copiotrophic lifestyle where
431 *Trichodesmium* represents a hotspot for C, N, P, Fe, and vitamins to the residing community.
432 On the other hand, Fe-uptake through the production of siderophores appeared to be a key
433 beneficial function *Trichodesmium* can obtain from its residing community. *Trichodesmium*
434 harbored several bacteria that can secrete photolabile siderophores, supporting the idea that
435 *Trichodesmium* can meet their high Fe-demand by interacting with siderophore-producing

436 bacteria to mine Fe from dust particles collected within the colony. Other, as of yet
437 uncharacterized, traits will likely further elucidate the factors that drive the community
438 structure of *Trichodesmium* colonies. Our results highlight the presence of a dynamic and
439 flexible consortium where functional traits are well conserved within the *Trichodesmium*
440 consortium, underpinning the resilience of the colony within an ever-changing ocean.



441
442 **Figure 6. Schematic summary of the potential interactions taking place within the *Trichodesmium* consortia**
443 **as discussed in this study.** Each blue bubble indicates a different function, and each blue circle pools together
444 the different MAGs containing a particular trait for that function. Each MAG is represented by a coloured dot
445 reflective of the taxonomic order it is associated with (grey dots represent MAGs that were not attributed to a
446 specific taxonomic order). *Trichodesmium* is highlighted in cobalt-blue, and the important functions
447 *Trichodesmium* is known to display are highlighted with a dotted circle. (See Supplementary Table 5 for the full
448 list of functional traits and their presence/absence). **(a)** Several siderophore biosynthesis genes are present in a
449 diverse set of MAGs. Siderophores are predicted to mine Fe from dust that has been collected and centered within
450 *Trichodesmium* colonies. 6 of the 4 were identified as known NIS-type siderophores of which petrobactin,
451 vibrioferrin and rhizoferrin are photolabile. In the presence of sunlight, the Fe complexed to these siderophores
452 disassociates and acts as a public good to the larger consortium. The genes and pathways for **(b-i)** denitrification,
453 **(b-ii)** N_2 fixation, and **(b-iii)** dissimilatory- nitrate- reduction- to- nitrate (DNRA) are present in several MAGs of
454 the consortium. These processes appear to be modular within the *Trichodesmium* consortium as different
455 taxonomic groups complete the steps for these pathways. **(c)** Several MAGs contain a homoserine lactone

synthesis pathway (HL), including *Trichodesmium*. HL acts as an intercommunicating quorum sensing molecule and has been shown to influence the production of alkaline phosphatase (AP) and N_2 fixation in *Trichodesmium*. **(d)** *Trichodesmium* is capable of producing the vitamins cobalamin (B12), thiamin (B1) and biotin (B7). Different subsets of MAGs lack the biosynthesis pathways for one or more of these vitamins with very few capable of synthesizing all three (associated bacteria can also produce vitamins but is not depicted here - see Figure 2). Consortia members and *Trichodesmium* likely exhibit an interdependent relationship for these vitamins. **(e)** *Trichodesmium* colonies have been shown to take up and utilize phosphonate and phosphite in a low-P environment and MAGs containing these pathways may have a competitive advantage over members that lack them.

Material and Methods

Trichodesmium metagenomic sampling and extraction

Trichodesmium puff colonies were hand-picked during the spring bloom (6th May, 2019) and separated into three samples for metagenomics (~100-200 colonies each) using a 100 µm phytoplankton net at 20 m depth in the Gulf of Aqaba (Eilat, Israel) (29.56ºN, 34.95ºE)**.** Colonies were washed 3 times, by gently picking colonies with a pipette and placing them in new petri dishes with fresh 0.2 µm filtered seawater, before being filtered on a 0.2 µm, 45 mm polycarbonate (PC) filter using a vacuum pump. Filters were flash frozen in liquid N_2 and kept at -80ºC. DNA was extracted from the three *Trichodesmium* samples using the DNeasy Plant Pro Kit (Qiagen), with a minor modification to the lysis procedure. The kit-provided tissue disruption tubes were not used. Rather, ~250 µL zirconia/silica beads (0.5 mm) were added to each sample tube before the addition of Solution CD1, and then samples were vortexed for 5 min. The resulting lysate was processed as per the remainder of the manufacturer instructions. DNA was quantified fluorometrically using the dsDNA HS kit (ThermoFisher Scientific) on a Qubit. From the three samples, metagenomic libraries were prepared and sequenced with 0.7 billion reads 2 × 151 bp reads on Illumina NovaSeq S4 at the DOE Joint Genome Institute (California, USA). This study can be found under JGI Gold Study ID number Gs0149370.

Metagenomic raw read assembly and binning

Sequences were analysed from this study and compared to previously published metagenomic studies from the Red Sea (PRJNA804487) (28) and metagenomes of *Trichodesmium* colonies

487 isolated from the Pacific (PRJNA435427) (17) (PRJNA358796) (12) and the Atlantic
488 (PRJNA330990) (13) Oceans. All raw sequences were analyzed using the same protocol
489 described below.
490
491 Metagenomes were assembled, binned, and quantified using the ATLAS (v2) pipeline (72).
492 Briefly, raw sequences underwent quality control through the BBTools suite (73, 74) and were
493 assembled using metaSPAdes (75) (k-mer lengths: 21, 33, 55, 99, 121 bp). MAGs were binned
494 from each sample using MetaBAT 2 (76), MaxBin 2.0 (77) and VAMB (78). The completeness
495 and redundancy of each bin were subsequently assessed using CheckM2 v0.1.2 (79). MAGs
496 were considered of high-quality if they reached 90% completeness and 10% redundancy and
497 were highlighted throughout our results and figures (27). A non-redundant set of bins was
498 produced using DAS Tool (80) and dRep (81) based on an average nucleotide identity (ANI)
499 cutoff of 97.5%. Genes were predicted using Prodigal (82). The coverage of each MAG was
500 quantified across each sample by mapping reads back to each MAG. We refer to the MAGs
501 from this study as MAG XX, bacterial MAGs from a previous Red Sea study as MAG R-XX
502 (28) and bacterial MAGs from other metagenomic studies from the Pacific and Atlantic Oceans
503 (12, 13, 17) as MAG T-XX.
504
505 *Phylogenetic Diversity*
506 Metagenomically assembled genomes (MAGs) were taxonomically characterized using the
507 genome taxonomy database tool kit GTDB-tk v2.1.1 (83). MAGs were classified according to
508 GTDB taxonomy and NCBI taxonomy (Supplementary Table 1; Supplementary Table 4).
509 GTDB taxonomy is used throughout the analysis, but to simplify comparisons with the
510 literature, we used the larger NCBI taxonomic order Rhodospirillales which encompasses the
511 smaller GTDB taxonomic groups of Kiloniellales and Thalassobaculaceae.

512

513 Phylogeny was inferred using GTo-Tree (v.1.16.12; default settings) (84) from a concatenation
514 of 74 conserved single-copy HMM markers for bacteria using the best-fit model Q.pfam+R7
515 model in IQ-Tree (v2.1.3), using the Bayesian information criterion (BIC) (85, 86).
516 Shimodaira–Hasegawa approximate likelihood-ratio test (SH-aLRT) and ultrafast bootstrap
517 approximation (UFBoot) branch support values were estimated from 1000 bootstraps. The tree
518 was visualized using FigTree (v1.4.4) and rooted using the genome of *Fuseobacterium*
519 *nucleatum* (PRJNA1419) as an outgroup according to (87). MAG were considered to re-occur
520 in another metagenomic dataset if its genome sequence identity was >97.5% similar
521 (Supplementary Figure 1).

522

523 *Functional Diversity*

524 The amino-acid sequence for each MAG was annotated using hmmscan and GhostKoala under
525 default parameters (50% identity cutoff), utilizing the PFAM and KEGG databases,
526 respectively (88, 89). Secondary metabolites (e.g. biosynthesis of siderophores and homoserine
527 lactones) were screened for each MAG using AntiSMASH 7.0. In certain cases, the Rapid
528 Annotation using Subsystem Technology (RAST) server was used to annotate amino-acid
529 sequences using the collection of protein families, FIGfams (90, 91). In addition, each MAG
530 was assessed using METABOLIC (v4.0) which lists the presence of interactive KEGG
531 modules for each MAG using a default completeness cutoff of 0.75 (92). Based on these
532 different annotation formats, we inspected each MAG for the presence of functional traits. For
533 each functional pathway or gene, the annotation method and coinciding KEGG and PFAM ID
534 number can be found in Supplementary Table 5. The presence and absence of different traits
535 for each MAG was displayed using the Interactive Tree Of Life (iTOL) visualization tool (93).

536

537 *Iron metabolism*

538 To explore the relationship between *Trichodesmium* and its associated bacteria for the
539 bioavailability of Fe from dust, we searched MAGs for the KEGG IDs of multiple Fe-uptake
540 pathways. Fe-uptake receptors were separated according to abiotic Fe^{+2} (*feoB*:K04759), Fe^{+3}
541 (*afuA*:K02012), and organic Fe uptake which is further separated into Fe-heme
542 (*hemR*:K16087), Fe-citrate (*fecA*:K16091) and Fe-siderophores (*fevS*:K02016). The presence
543 of an Fe:siderophore receptor K02016 should be taken cautiously as its KEGG annotation does
544 not clearly distinguish between siderophores or cobalamin as a substrate.

545

546 Siderophore biosynthesis pathways within MAGs were identified using a conservative
547 approach that required a combination of both KEGG and HMM annotation for each MAG and
548 the use of AntiSMASH 7.0 and FeGenie tools (94) (Supplementary Figure 4). Pathways were
549 separately analyzed for both Non-Ribosomal Peptide Synthetase (NRPS) Type and NRPS
550 Independent Synthetase (NIS) Type biosynthesis pathways. NIS-type siderophore biosynthesis
551 pathways are more easily identifiable of the two, and we searched for the presence of the Fe-
552 uptake chelate (*iuc)*A/*iuc*C domain (PF04183). NIS genes were clustered into different types
553 using known NIS biosynthesis genes as defined and described by Carroll and Moore (36).
554 Briefly, genes were aligned using the online Multiple Alignment Fast Fourier Transformation
555 (MAFFT v7.490; L-INS-i) software (95). A phylogenetic tree was constructed from this
556 alignment using IQtree2 (v2.1.3). The resulting best-fit substitution model Q.pfam+F+R4 was
557 selected using Bayesian information criteria (BIC). Branches were assigned using Shimodira–
558 Hasegawa approximate likelihood-ratio test (SH-aLRT) and ultrafast bootstrap approximation
559 (UFBoot) branch support values were estimated from 1,000 bootstraps. The resulting
560 consensus tree was visualized using FigTree (v1.4.4).

561

562 The identification of NRPS-type siderophore biosynthesis pathways required FeGenie, which
563 utilizes biosynthetic pHMMs, and was used to highlight putative NRPS-type siderophores that
564 were further inspected using AntiSMASH 7.0. MAGs containing an NRPS pathway coupled
565 with a siderophore transport-receptor (SMCOG1082) within the gene-cluster were marked as
566 putative NRPS-siderophore producers. These pathways were further compared to known
567 NRPS siderophore pathways using the MiBIG database (96) and the KEGG pathway map for
568 NRPS-type siderophores biosynthesis gene cluster (BGC) map01053.
569

570 *Vitamin B1, B7, and B12 biosynthesis and uptake*

571 We assume that a bacterial strain is auxotrophic for thiamin (B1), biotin (B7) or cobalamin
572 (B12) if their coinciding genome lacks a cobalamin biosynthesis pathway and contains a
573 transporter for its uptake. We searched MAGs for the KEGG IDs and determined the presence
574 of a vitamin biosynthesis pathway using a KEGG map for each vitamin. The presence of a
575 thiamin biosynthesis pathway (M00127) was determined by the presence of *thi*DCEGL
576 (K00946; K00788; K03149; K00941; K03147). A thiamin uptake system was present if a
577 coinciding transporter system (M00192) was observed. The presence of a biotin biosynthesis
578 pathway (M00581) was determined by the presence of *bio*ABDFH (K01012; K01935;
579 K00833; K00652; K02170). A biotin uptake system was present if a coinciding transporter
580 system (M00581) was observed. The presence of a cobalamin biosynthesis pathway was
581 determined by the presence of *cob*ACDUQS (K19221; K02226; K02227; K02231:K02233).
582 A putative vitamin B12 uptake system was considered present in a MAG if the specific
583 transporter *btu*B (K16092) was observed.

584

585 *Nitrogen metabolism*

586 N-metabolism pathways om MAGs were compiled from a selected set of KEGG ID numbers
587 using METABOLIC-C (v4.0) and the KEGG pathway map ko00910. MAGs were queried for
588 the presence of genes related to N_2 fixation, dissimilatory nitrate reduction to ammonium
589 (DNRA), and denitrification. The ability to fix N_2 was determined through the presence of
590 *nif*HKD (K02588, K02591, K02586). DNRA can be separated into two steps and includes the
591 reduction of nitrate to nitrite (*nap*AB: K02567, K02568*; nar*GH: K00370-K00374) and nitrite
592 to ammonia (*nrf*AH: K15876, K03385*, nir*BD: K00362, K00363). Denitrification can further
593 be separated into the reduction of nitrite to nitric oxide (*nir*S:K15864*; nir*K: K00368), nitric
594 oxide to nitrous oxide (*nor*BC: K04561, K02305) and nitrous oxide to N_2 (*nos*Z: K00376).

595

596 *Phosphorus metabolism*

597 The presence of multiple P-metabolic pathways within the *Trichodesmium* consortium were
598 explored. We explored several transporters for high-affinity phosphate (*pst*SCAB: K02036-
599 K02038, K02040), low-affinity phosphate (*pitA*:K03306), and a
600 phosphite/phosphonate transporter (*phn*CDE: K02041; K02042; K02044). We searched
601 MAGs for KEGG ID numbers for the presence of phosphite dehydrogenase (*ptx*D:K18916),
602 which oxidizes phosphite to phosphate, and the broad specificity C-P lyase (*phn*GHIJKLM:
603 K06162-6, K05780-1), which hydrolyses phosphonate bonds in organic P. Additionally,
604 MAGs were queried for the presence of the substrate-specific phosphonate metabolism
605 enzymes including *phn*A (K19670), *phn*X (K05306), and *phn*W (K03430) and collectively
606 these genes were used to indicate the functional potential for reduced P-metabolism. Lastly,
607 MAGs were examined for the presence of alkaline phosphatases (AP) *pho*A (K01077), *pho*X

608 (K07093), and *pho*D (K01113). AP hydrolyses phosphoesters enabling the metabolism of
609 organic-P.
610

611 ### *Bacterial and Particle Interaction traits*

612 Known traits that are indicative of putative bacterial interactions were previously summarized
613 and identified by Zoccarato *et al* for marine bacterial genomes (68). In addition to siderophore
614 biosynthesis and vitamin B1, B7 and B12 biosynthesis (see previously), MAGs were probed
615 for the presence of homoserine lactone (HLs) biosynthesis genes using AntiSMASH 7.0. The
616 presence of auxin efflux genes was determined according to RAST annotation. MAGs were
617 screened for KEGG ID numbers for the presence of the quorum sensing regulation gene *lux*R
618 (K07782) and *bja*R1 (K18098), secretion systems (KEGG map03070), and motility and
619 adhesion genes (KEGG map02020) (Supplementary Table 5). Traits involved in particle-
620 interaction were previously described and summarized by Debeljak *et al* (69). In addition to
621 Fe-uptake and siderophore biosynthesis pathways, MAGs were screened for KEGG ID
622 numbers involved in metal-response, metal-efflux and metal-storage pathways (Supplementary
623 Table 5).
624

625 ### *Trichodesmium proteomic sampling and extraction*

626 In parallel to the metagenomic sampling, 20 puff colonies were picked, in triplicate, for
627 proteomic analysis at the Environmental Molecular Sciences Laboratory (EMSL) at Pacific
628 Northwest National Laboratory according to standardized protocols. Colonies were washed 3
629 times with filtered seawater, as described above. Samples were centrifuged, and the resulting
630 pellet was diluted in 200 µl 8 M urea and transferred to 2 mL pre-filled Micro-Organism Lysing
631 Mix glass bead tubes and bead beat in a Bead Ruptor Elite bead mill homogenizer (OMNI
632 International, Kennesaw Georgia) at speed 5.5 for 45 sec. After bead beating, the lysate was

633 transferred to a new 4 mL tube and immediately placed in an ice block and spun at 1,000 x g
634 for 10 mins at 4°C. 200 µl of the lysed samples were transferred into 2 mL centrifuge tubes. A
635 bicinchoninic acid (BCA) assay (Thermo Scientific, MA USA) was performed to determine
636 protein concentration. Following the assay, 10 mM dithiothreitol (DTT) was added to the
637 samples and incubated at 60°C for 30 mins with constant shaking at 800 rpm. Samples were
638 diluted 8-fold for preparation for digestion with 100 mM NH_4HCO_3, 1 mM $CaCl_2$ and
639 sequencing grade trypsin (Promega, WI) was added to all protein samples at a 1:50 (w/w)
640 trypsin-to-protein ratio for 3 hrs at 37˚C, 450 rpm. Digested samples were desalted using a 4-
641 probe positive pressure Gilson GX-274 ASPEC™ system (Gilson Inc., WI) with Discovery
642 C18 50 mg/1 mL solid phase extraction tubes (Supelco, MO), using the following protocol: 3
643 mL of methanol was added for conditioning followed by 3 mL of 0.1% trifluoroacetic acid
644 (TFA) in H_2O. The samples were then loaded onto each column followed by 4 mL of 95:5:
645 H_2O:ACN, 0.1% TFA. Samples were eluted with 1mL 80:20 ACN:H_2O, 0.1% TFA. The
646 samples were concentrated using a speed vac and a final BCA was performed to determine the
647 peptide concentration and samples were fractionated into 12 fractions for LC-MS/MS analysis.
648
649 MS analysis was performed using a Q-Exactive Plus mass spectrometer (Thermo Scientific)
650 outfitted with a homemade nano-electrospray ionization interface. Electrospray emitters used
651 150 µm o.d. × 20 µm i.d. chemically etched fused silica (126). The ion transfer tube
652 temperature and spray voltage were 300°C and 2.2 kV, respectively. Data was collected for
653 120 min following a 10 min delay after completion of sample trapping and start of gradient.
654 FT-MS spectra were acquired from 300 to 1800 m/z at a resolution of 70 k (AGC target 3e6)
655 and the top 12 FT-HCD-MS/MS spectra were acquired in data-dependent mode with an
656 isolation window of 1.5 m/z at a resolution of 17.5 k (AGC target 1e5) using a normalized
657 collision energy of 30, dynamic exclusion time of 30 s, and detected charge state of an ion 2 or

658 higher. The resulting proteomic samples from this study was deposited in MassIVE
659 (MSV000091416).
660

661 ### *Proteomic Analysis*

662 Peptide matching was performed using MSGF+ software (97) against the amino-acid
663 sequences obtained from the matching metagenome dataset through PROKKA (98) using the
664 ATLAS pipeline (v2) (72) resulting in 201374 protein sequences. MSGF+ was used in
665 target/decoy mode with 20 ppm parent ion tolerance, partial tryptic rule, and M-oxidation
666 (+15.9949) as a dynamic modification. Best matches from the MSGF+ searches were filtered
667 at 1% FDR based on the target/decoy model; this set of peptides was used in consequent
668 quantitation analysis. Spectral counts were performed without consideration of peptide
669 specificity for proteins and MAGs. 92% of peptides mapped to unique proteins while 93% of
670 peptides were found in only one MAG. Spectral count normalization was performed using the
671 normalized spectral abundance factor (NSAF) approach (99). Briefly, counts were divided by
672 the matching peptide length and their relative abundance was calculated from the total number
673 of spectral hits per sample.
674

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Competing Interests

The authors declare no conflicting interests.

Data Availability Statement

Raw sequences are available under the Gold Study ID number Gs0149370. The 52 metagenomic assembled genomes analysed in this study are available at NCBI under the BioProject number PRJNA944101. Proteomic samples are available at MassIVE under the project accession number MSV000091416.

Author Contributions

YS, RB, MG, SB, conceived of this study. SB, FZ, SW collected the DNA and protein samples. SB performed the experiments together with FZ, SW and YS. SD, SH, TG extracted and sequenced the DNA. CDN extracted and processed protein samples that were analyzed by NT and RB. MRB and CK performed the metagenomic analysis. CK wrote the manuscript under guidance of YS, MRB, RB, MG, SD, with further input from SW, SB, FZ, NT.

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FIGURES

Figure 1. Phylogenetic tree of the 52 MAGs assembled from *Trichodesmium* colonies collected in the Red Sea. The tree is rooted by the outgroup *Fuseobacterium nucleatum*. Known bacterial orders are marked in different colors and several MAGs could taxonomically be identified at the genus level. The relative abundance of each MAG within the *Trichodesmium* consortium, excluding the abundance of *Trichodesmium* MAG 52 (±70% of samples), is presented as a bubble chart. The 11 MAGs that matched to MAGs assembled from a previous metagenomic dataset of Red Sea *Trichodesmium* colonies (28) are highlighted in blue and tentatively represent the core-consortium (see Supplementary Figure 2; Supplementary Table 4). MAGs that did not meet the requirements of a high-quality genome (>90% completeness; <5% redundancy) are indicated in grey. Note that re-occurring MAGs are not necessarily the most abundant MAGs present within the *Trichodesmium* colony. (a full taxonomic description of each MAG in this dataset can be found in Supplementary Table 1).

Figure 2. MAGs were probed for Fe-uptake and siderophore biosynthesis genes which are noted in Supplementary Table 5. **(a)** *T. thiebautii* MAG 52 does not contain a wide diversity of Fe-uptake genes, and the majority of MAGs of associated bacteria contain an uptake receptor for organic Fe (heme, citrate, siderophores). Several MAGs of associated bacteria contained an NRPS or NIS-type siderophore biosynthesis pathways (BGCs). **(b)** Siderophore biosynthetic gene clusters (BGCs) of known NIS-type siderophore BGCs can be clustered according to the presence of one or multiple iucA/iucC domains which enables one to target the closest siderophore BGC match (36). Using this method, we were able to link these pathways to the synthesis of the photolabile **(i)** petrobactin, **(ii)** vibrioferrin and **(iii)** rhizoferrin.

1016 (c) Siderophore BGCs of known NRPS siderophores. Using antiSMASH 7.0, which aligns
1017 BGCs to known siderophores within the MIBiG database, we were able to link 2 of the 6
1018 putative siderophore NRPS-type pathways to **(i)** the membrane-bound amphibactin and **(ii)**
1019 agrobactin/enterobactin. (See Supplementary Figure 3 for more details on the siderophore
1020 biosynthesis pathways).
1021
1022 **Figure 3.** MAGs were probed for several different P-metabolism genes which are noted in
1023 Supplementary Table 5. *Trichodesmium* is known to be highly competitive for P, as
1024 exemplified by the multitude of **(a)** P-uptake systems for phosphate (*pst*S, *pst*B), phosphonate
1025 (*phn*CDE), and phosphonate/phosphite (*phn*CDE/*ptxABC*). **(b)** Within the *Trichodesmium*
1026 consortium, the ability to metabolize a range of phosphonate compounds using a broad
1027 specificity C-P lyase (*phn*GHIJKLM), is more prominent than the substrate-specific hydrolysis
1028 of 2-aminoethylphosphonate (2-AEP; *phn*WXYZA) or phosphonopyruvate (*pal*A) and
1029 phosphite metabolism (*ptx*D).
1030
1031 **Figure 4.** MAGs were probed for N-metabolic pathways and the genes for each step within the
1032 pathway are noted in Supplementary Table 5. *T. thiebautii* MAG 52 is the only MAG with a
1033 N_2 fixation pathway. Several MAGs contained one or two steps within the pathways of
1034 denitrification or dissimilatory nitrate to reduction to ammonia (DNRA) indicating their
1035 modularity. Collectively, MAGs completed the denitrification and DNRA pathways, but none
1036 contained the genes for the entire pathway.
1037
1038 **Figure 5.** MAGs were probed for vitamin B1, B7, and B12 biosynthesis and uptake pathways.
1039 The genes used to indicate the biosynthesis or uptake of these vitamins are noted in
1040 Supplementary Table 5. *Trichodesmium* contains the biosynthesis pathways for all three
1041 vitamins but the vast majority of associated bacteria were auxotrophic for one or more
1042 vitamins. These results suggest that the associated bacteria rely on *Trichodesmium* to obtain
1043 their vitamin requirements.
1044
1045 **Figure 6. Schematic summary of the potential interactions taking place within the**
1046 **Trichodesmium consortia as discussed in this study.** Genes for each trait are noted in
1047 Supplementary Table 5**.** Each blue bubble indicates a different function, and each blue circle
1048 pools together the different MAGs containing a particular trait for that function. Each MAG is
1049 represented by a colored dot reflective of the taxonomic order it is associated with (grey dots
1050 represent MAGs that were not attributed to a specific taxonomic order). *Trichodesmium* is
1051 highlighted in cobalt-blue, and the important functions *Trichodesmium* is known to display are
1052 highlighted with a dotted circle. (See Supplementary Table 5 for the full list of functional traits
1053 and their presence/absence). **(a)** Several siderophore biosynthesis genes are present in a diverse
1054 set of MAGs. Siderophores are predicted to mine Fe from dust that has been collected and
1055 centered within *Trichodesmium* colonies. 6 of the 4 were identified as known NIS-type
1056 siderophores of which petrobactin, vibrioferrin and rhizoferrin are photolabile. In the presence
1057 of sunlight, the Fe complexed to these siderophores disassociates and acts as a public good to
1058 the larger consortium. The genes and pathways for **(b-i)** denitrification, **(b-ii)** N_2 fixation, and
1059 **(b-iii)** dissimilatory- nitrate- reduction- to- nitrate (DNRA) are present in several MAGs of the
1060 consortium. These processes appear to be modular within the *Trichodesmium* consortium as
1061 different taxonomic groups complete the steps for these pathways. **(c)** Several MAGs contain
1062 a homoserine lactone synthesis pathway (HL), including *Trichodesmium*. HL acts as an
1063 intercommunicating quorum sensing molecule and has been shown to influence the production
1064 of alkaline phosphatase (AP) and N_2 fixation in *Trichodesmium*. (associated bacteria can also
1065 produce AP but is not depicted here - see Figure 3). **(d)** *Trichodesmium* is capable of producing

1066 the vitamins cobalamin (B12), thiamin (B1) and biotin (B7). Different subsets of MAGs lack
1067 the biosynthesis pathways for one or more of these vitamins with very few capable of
1068 synthesizing all three (associated bacteria can also produce vitamins but is not depicted here -
1069 see Figure 5). Consortia members and *Trichodesmium* likely exhibit an interdependent
1070 relationship for these vitamins. **(e)** *Trichodesmium* colonies have been shown to take up and
1071 utilize phosphonate and phosphite in a low-P environment and MAGs containing these
1072 pathways may have a competitive advantage over members that lack them.
1073

1074 ## SUPPLEMENTARY FIGURES

1075 **Supplementary Figure 1. Relative abundance of bacterial MAGs from sampled**
1076 ***Trichodesmium* colonies indicate the associated bacterial distribution is consistent.** The 51
1077 MAGs are stacked, and colors reflect the different bacterial orders each MAG belongs to and
1078 'other' in grey. The exact relative abundance value (%) for each MAG and its standard
1079 deviation can be viewed in Supplementary Table 1.
1080

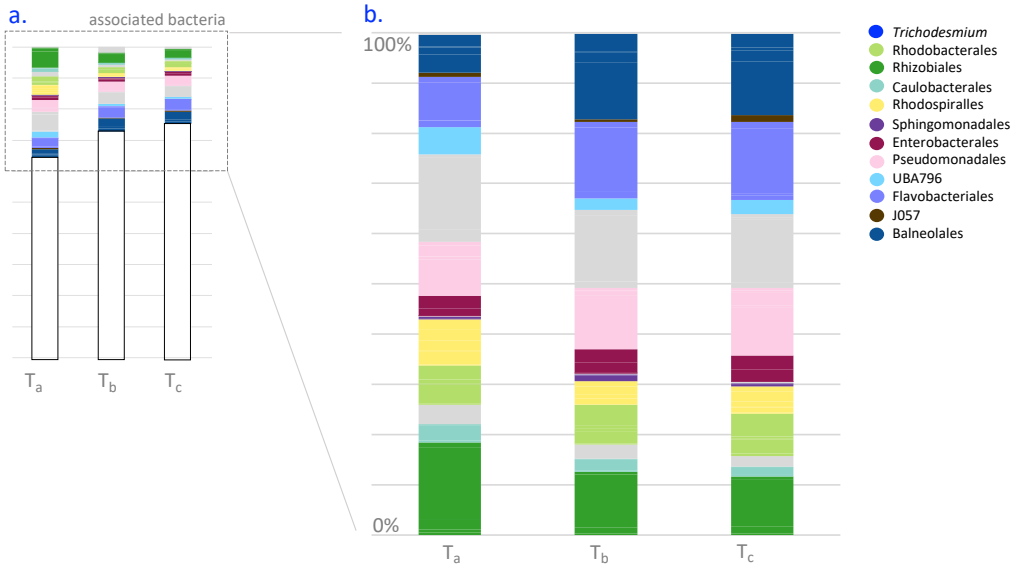
1081 **Supplementary Figure 2. Phylogenetic tree of the 52 MAGs together with MAGs**
1082 **assembled from 3 other Trichodesmium metagenomic datasets from colonies collected in**
1083 **the Red Sea (28), the Pacific (12, 17) and the Atlantic (13) Oceans.** MAGs from the 3 other
1084 datasets are highlighted in grey. MAGs deriving from samples of the Atlantic or Pacific Ocean
1085 are listed as MAG T-XX and those from the Red Sea are listed as MAG R-XX. The 11 MAGs
1086 that matched to those assembled from previous metagenomic datasets, using a 97.5 % identity
1087 cutoff, are highlighted in blue. Known bacterial orders are marked using different colored
1088 squares. The tree is rooted by the outgroup *Fuseobacterium nucleatum*. The full taxonomic
1089 description of each MAG in this dataset and others can be found in Supplementary Table 4.
1090

1091 **Supplementary Figure 3. (a)** NIS and NRPS-type siderophore biosynthesis pathways
1092 identified in MAGs of associated bacteria. (b) The siderophore BGC analysis tools used to
1093 probe and identify the different siderophore BGCs. Most tools are limited in their ability to
1094 discover siderophore BGCs while antiSMASH 7.0 was the most conclusive but also time-
1095 consuming (53). **(c)** Putative NRPS-type siderophore BGCs that did not match to a known
1096 siderophore within the MIBiG database but did containing a TonB-siderophore receptor
1097 (SMCOG 1208). This made us speculate that these are putative siderophores whose identity
1098 remains to be resolved.
1099

1100 **Supplementary Figure 4. (a) MAGs were probed for bacterial-interactive traits** (68)**.**
1101 MAGs from the phyla Proteobacter, contain the presence several interactive traits, including
1102 quorum sensing, gliding motility, adhesion, cell-to-cell signaling, and antibiotic resistance
1103 marker genes. Intriguingly, both the Trichodesmium MAG and several Proteobacter MAGs
1104 (e.g. Rhodobacter) contain genes for homoserine lactones (HLs), which are putative quorum
1105 sensing molecules. **(b) MAGs were probed for particle-interactive traits** (69)**.** In
1106 comparison to *T. thiebautii* MAG 52, many MAGs from associated bacteria contained a wide
1107 variety of heavy metal efflux systems and metal-transcription factors. While *Trichodesmium*,
1108 like most Cyanobacteria, is known to be sensitive to heavy metals, our results indicate that the
1109 consortium is attuned towards an environment rich in metals, including particulate dust. Genes
1110 for each trait are noted in Supplementary Table 5
1111

1112 ## SUPPLEMENTARY TABLES

1113 **Supplementary Table 1.** General information about the MAGs making up the Red Sea
1114 *Trichodesmium* consortium. The relative abundance of associated bacteria (%) and standard
1115 deviation of the triplicates (SD) excludes the abundance of *T. thiebautii* (MAG 52). The relative
1116 abundance of MAGs was consistent across our samples. MAGs that did not meet the high-
1117 quality standard of 90% completeness and <5% redundancy are marked in green (10 of 52) and
1118 the functional analysis from these MAGs needs to be taken with more caution. The % of the
1119 genomes annotated by RAST is also indicated, showcasing how MAGs from the phyla
1120 Bacteroidetes are marked with a low annotation percentage. The closest taxonomic identity is
1121 given using both NCBI and GTDB-taxonomy. Throughout this paper we use GTDB-taxonomy
1122 except for the order Rhodospirillales.
1123
1124 **Supplementary Table 2:** Top proteomic hits matching to *T. thiebautii* MAG52. Spectrum
1125 counts that mapped to KEGG ID numbers mentioned in Supplementary Table 5 are highlighted
1126 in blue. Most proteins are geared towards the processes of photosynthesis, nitrogen fixation,
1127 carbon metabolism, amino acid uptake, protein synthesis, and phosphate uptake. Total spectral
1128 counts (T-total) and their associated KEGG or PFAM ID number and their description are
1129 presented together with the average relative contribution (T0%) of each protein hit to all
1130 Trichodesmium-related proteins and for all three samples (%T1-3). Collectively, the top 50
1131 proteins represented >50% of the total spectral counts.
1132
1133 **Supplementary Table 3:** Top proteomic hits matching to MAGs of *Trichodesmium* associated
1134 bacteria. Many proteins are geared towards a typical heterotrophic and copiotrophic lifestyle
1135 including proteins related to amino-acid uptake and carbon metabolism are highlighted in bold.
1136 Total spectral counts (%) and their associated KEGG or PFAM ID numbers and their
1137 description are presented together with the average relative contribution (Avg%) of each
1138 protein hit to all Trichodesmium-related proteins and for all three samples (%T1-3).
1139
1140 **Supplementary Table 4. Taxonomic identity of MAGs used in this study are compared to**
1141 **MAGs assembled from other metagenomic studies of *Trichodesmium* colonies** (Red Sea
1142 samples: MAG R-XX (28), the Pacific (12, 17) and Atlantic samples (13): MAG T-XX. Their
1143 closest taxonomic identity is given using both NCBI and GTDB-taxonomy. Throughout this
1144 paper we use GTDB-taxonomy except for the order Rhodospirillales. Highlighted in blue are
1145 the 11 MAGs from this dataset that matched to those assembled from these other datasets (in
1146 bold), using a 97.5 % identity cutoff. The phylogenetic tree of all MAGs from all datasets
1147 combined can be seen in Supplementary Figure 2.
1148
1149 **Supplementary Table 5.** The presence and absence of functional traits separated into larger
1150 groups including Fe-uptake, P-metabolism, N-metabolism, Vitamin B synthesis and uptake,
1151 metal regulators, metal efflux, metal storage, signaling, secretion systems. motility and
1152 adhesion genes. The associated analysis tool, KEGG id number or module is given for each
1153 trait. The total number of MAGs containing a specific trait and (MAGs %) is calculated and
1154 show below. Traits that were detected within our proteomic samples are also highlighted in
1155 blue.
1156



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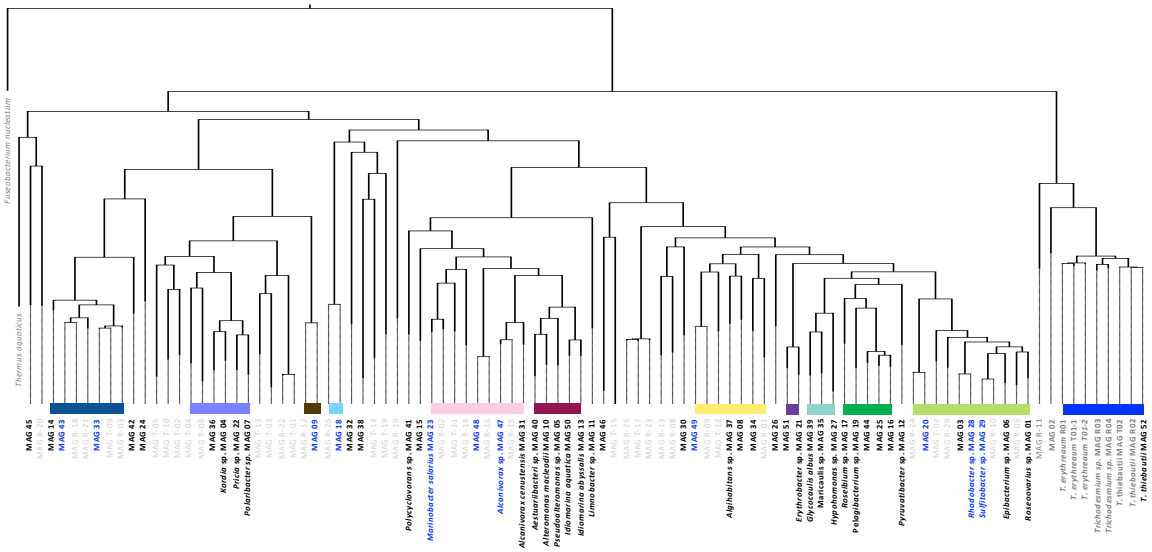
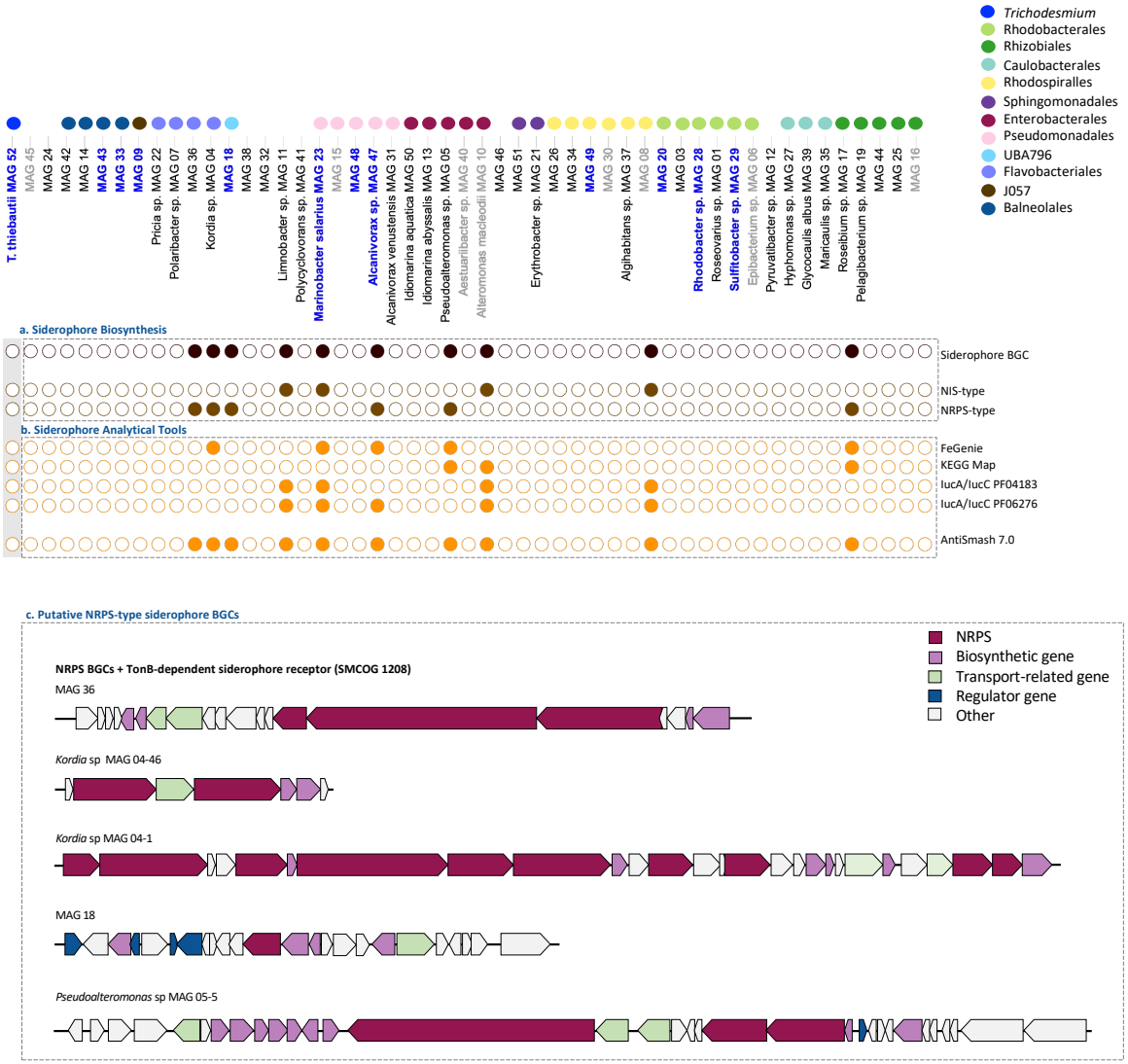


Phylogenetic tree of the 52 MAGs together with MAGs assembled from 3 other *Trichodesmium* metagenomic datasets from colonies collected in the Red Sea (28)**, the Pacific and the Atlantic Ocean.** MAGs from the 3 other datasets are highlighted in grey. MAGs deriving from samples of the Atlantic or Pacific Ocean are listed as MAG T-XX and those from the Red Sea are listed as MAG R-XX. The 11 MAGs that matched to those assembled from previous metagenomic datasets, using a 97.5 % identity cutoff, are highlighted in blue. Known bacterial orders are marked using different colored squares. The tree is rooted by the outgroup *Fuseobacterium nucleatum*. The full taxonomic description of each MAG in this dataset and others can be found in Supplementary Table 4.



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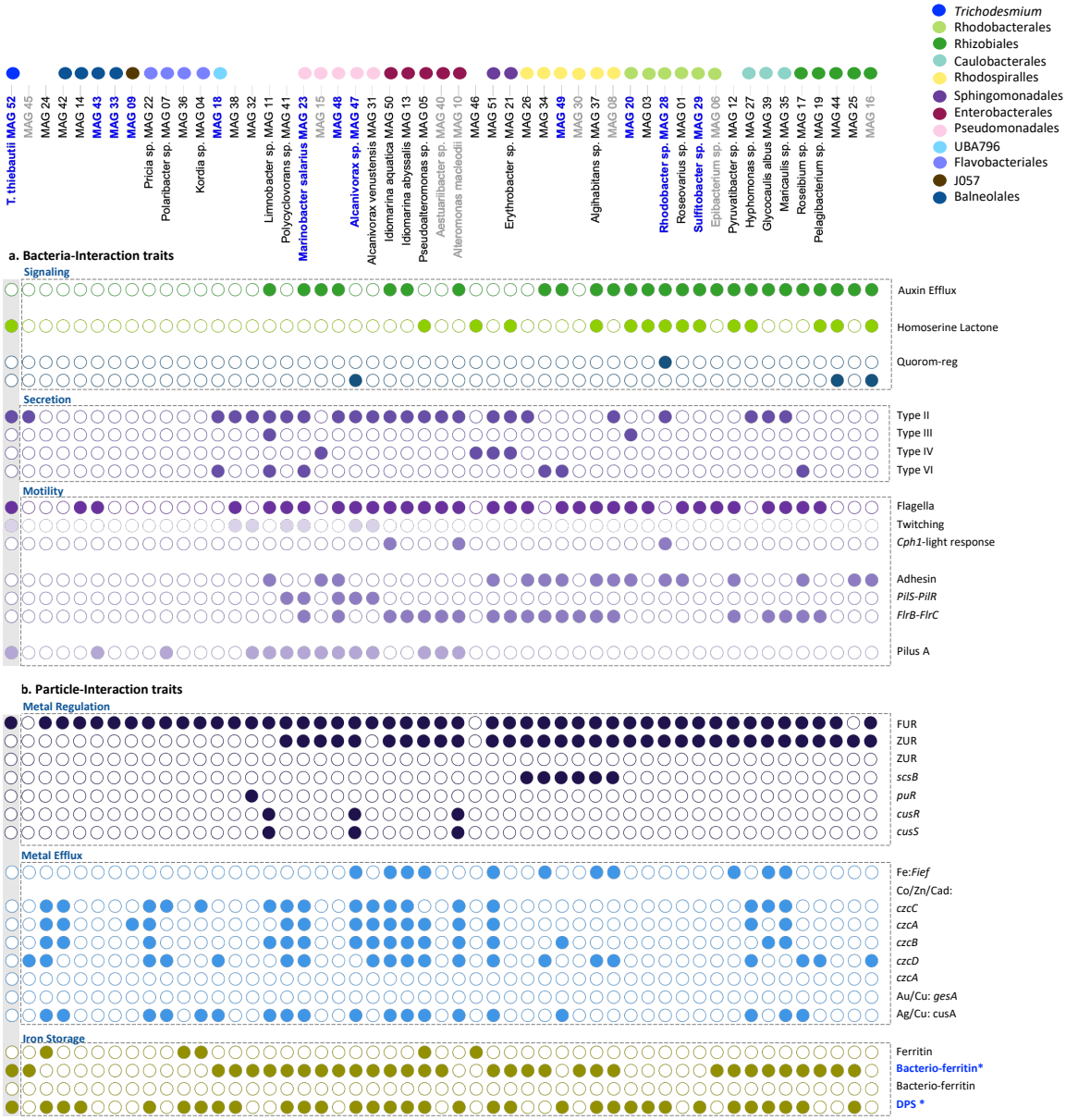

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