1 **Evidence for polyploidy in the globally important diazotroph *Trichodesmium***

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20

21 **Running Title:** Evidence for polyploidy in *Trichodesmium* (40 characters)

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23 **Abstract**

24 Polyploidy is a well-described trait in some prokaryotic organisms; however, it is
25 unusual in marine microbes from oligotrophic environments, which typically display a
26 tendency towards genome streamlining. The biogeochemically significant diazotrophic
27 cyanobacterium *Trichodesmium* is a potential exception. With a relatively large genome and
28 a comparatively high proportion of non-protein-coding DNA *Trichodesmium* appears to
29 allocate relatively more resources to genetic material than closely related organisms and
30 microbes within the same environment. Through simultaneous analysis of gene abundance
31 and direct cell counts we show for the first time that *Trichodesmium spp*. can also be highly
32 polyploid, containing as many as 100 genome copies per cell in field-collected samples and
33 >600 copies per cell in laboratory cultures. These findings have implications for the
34 widespread use of the abundance of the *nifH* gene (encoding a subunit of the N_2-fixing
35 enzyme nitrogenase) as an approach for quantifying the abundance and distribution of
36 marine diazotrophs. Moreover, polyploidy may combine with the unusual genomic
37 characteristics of this genus both in reflecting evolutionary dynamics and influencing
38 phenotypic plasticity and ecological resilience.

39

40 **173 words**

41 **Introduction**

42 Smaller genomes and reduced gene diversity are characteristics of many microbes
43 adapted to life in the oligotrophic oceans (Swan *et al*., 2013). Such a strategy can be
44 advantageous under nutrient-limited conditions as fewer resources are required to maintain
45 and duplicate a genome, but comes at the cost of reduced physiological flexibility (Yooseph
46 *et al.*, 2010). In stark contrast is the cyanobacterium *Trichodesmium*, a genus of colony-
47 forming marine diazotrophs prolific in N_2-fixation in oligotrophic tropical and sub-tropical
48 oceans (Capone *et al*., 2005). *Trichodesmium* has a large (7.75 Mbp) genome with low protein
49 coding capacity (~40% non-protein-coding DNA) and a high level of gene duplication (10%
50 of all genes) (Bergman *et al*. 2013; Walworth *et al*. 2015). The abundant non-protein-coding
51 sequences, over 80% of which are transcribed, consist of a combination of non-coding RNAs
52 (ncRNAs), selfish DNA elements, transposases and introns, which possibly contribute to the
53 genome expansion and metabolic flexibility observed in this group (Pfreundt *et al*. 2014;
54 2015; Walworth *et al*., 2015). These features may also contribute to the versatility and
55 ecological success of *Trichodesmium* (Bergman *et al*. 2013; Pfreundt *et al*. 2014; Walworth *et*
56 *al*., 2015).

57 In contrast to the small-celled picocyanobacterial lineages *Prochlorococcus* and
58 *Synechococcus*, which co-inhabit and numerically dominate low latitude oligotrophic marine
59 environments, cell sizes are also much greater for species of *Trichodesmium*, being >3 orders
60 of magnitude larger by volume (Carpenter *et al*. 2004). Moreover, *Trichodesmium* are often
61 found in association with a diverse community of other microbes (Pearl et al. 1989; Hewson
62 et al. 2009), factors indicating that *Trichodesmium* is an unusual and unique inhabitant in
63 oligotrophic tropical environments (Walworth *et al.*, 2015).

64 Polyploidy, the presence of multiple genome copies per cell, has received little
65 assessment in marine microbes. Maintaining additional genome copies represents a
66 significant nutrient investment of both nitrogen and phosphorus, which is a scarce resource
67 in the oligotrophic ocean (Elser *et al.*, 2003; Karl, 2014), with the latter nutrient potentially
68 particularly important in constraining the growth of diazotrophs such as *Trichodesmium*
69 (Sanudo-Wilhelmy *et al*. 2001). Despite this, polyploidy in cyanobacteria has been widely
70 documented with multiple genome copies per cell commonly reported for a variety of model
71 and ecologically important species (Griese *et al*. 2011, Zerulla *et al*. 2016). In this study we
72 investigated polyploidy in *Trichodesmium* and the implications this may have for both the
73 success of this genera and the use of gene copy numbers in assessing the biogeography and
74 abundance of marine diazotrophic species.
75
76 **Materials and methods**
77 **Sample collection**
78 *Culture: Trichodesmium* IMS101 was grown in YBC-II medium under a 12/12-h
79 light/dark cycle at 25°C (Richier *et al.*, 2012). For DNA analysis 10 ml samples from triplicate
80 exponential phase cultures were filtered onto 0.22-μm Durapore (Millipore) filters under
81 low (2 mbar) vacuum pressure. After filtration, filters were flash frozen in liquid nitrogen
82 and stored at -80˚C until DNA extraction. For cell counts, 10 mL of culture was collected in
83 parallel from triplicate exponential phase cultures and preserved in 2% acidic Lugol's iodine
84 (Throndsen, 1978).
85 *Field:* Samples were collected during the AMT17 (Oct-Nov 2005) and D361 (Feb-Mar
86 2011) research cruises in the tropical and subtropical Atlantic (see Snow *et al*. 2015 for

87 cruise tracks). During these cruises, *Trichodesmium* cell count samples were collected
88 through filtration of a 20 L surface CTD bottle drained through a 10 μm polycarbonate filter.
89 The resulting retentate was rinsed into a 50 mL amber bottle using 0.2 μm filtered seawater,
90 and was preserved with 2% acidic Lugol's iodine (Throndsen, 1978). For DNA analysis, 2 L
91 of seawater was collected from a replicate surface CTD bottle and was filtered in duplicate
92 onto 0.22 μm Durapore (Millipore) filters under low (2 mbar) vacuum pressure. After
93 filtration, filters were flash frozen in liquid nitrogen and stored at -80°C until DNA extraction.
94

95 **DNA extraction, gene abundance and genome copy**

96 Frozen filters were crushed using a nucleic acid free, sterile plastic pestle and DNA
97 was extracted using the Qiagen DNeasy mini plant kit according to the manufacturer's
98 protocol. Following extraction, DNA concentrations were determined using the RediPlate 96
99 dsDNA Quantitation Kit (Molecular Probes) and read on a Fluoroscan Ascent microplate
100 reader.

101 Quantitative (q)PCR was performed using primers and a probe chosen for
102 amplification of the filamentous *nifH* phylotypes (Langlois *et al.*, 2008), or primers designed
103 to specifically amplify predicted double or triple copy genes from the *Trichodesmium*
104 *erythraeum* IMS101 genome (Table S1). For the multi-copy number genes the primers were
105 chosen such that they would amplify the same product from each of the individual copies.
106 For *nifH* analysis, qPCRs were run on an ABI Prism 7000 (Applied Biosystems) using cycling
107 conditions and reaction parameters as described previously (Langlois *et al*. 2008). For
108 analysing the ratio of *nifH* to other genes, qPCR was performed using a Mx3005P qPCR
109 System using Brilliant III Ulta-FAST SYBR Green QPCR Master Mix (Agilent Technologies,

110 Santa Clara CA, USA) with a thermal profile of an initial denaturation at 95°C for 3 min
111 followed by 40 cycles of denaturation for 15 s at 95°C and combined annealing/elongation
112 for 20 s at 60°C. Dissociation curves were collected between 55-95°C and revealed a single
113 melting point, which was confirmed to be due to amplification of single products by agarose
114 gel electrophoresis. The gene copy number ratio was estimated using the mean (n=3)
115 difference in Ct-value from a gDNA standard curve (2-fold serial dilution from 1 to 1:64) for
116 each primer pair and the primer efficiency calculated over the same dilution series. Standard
117 curves all had R^2 values for linearity > 0.99 and primer efficiencies between 102-106%. The
118 formula for calculating the ratio between two given genes A and B was:

119 (Primer efficiency gene A^CT gene A)/(Primer efficiency gene B^CT gene B).

120

121 **Cell counts, chlorophyll concentration and DNA staining**

122 Cell abundances for *Trichodesmium* were directly measured from free trichomes via
123 light microscopy at 200x magnification. Chlorophyll concentrations were obtained from
124 culture studies using 10 ml of cell cultures filtered onto Whatman GF/F filters, which were
125 flash frozen in liquid nitrogen and stored at -20°C until extraction and fluorometric
126 chlorophyll determination (Welschmeyer, 1994). For field sampling, *in situ* community
127 chlorophyll concentrations were measured through collecting 200 mL of seawater from a
128 surface CTD bottle, which was filtered onto a Whatman GF/F filter and then extracted
129 immediately. Extraction was performed in the dark in 8 mL 90% acetone overnight at 4˚C,
130 with subsequent concentrations in the extract determined using a TD-700 fluorometer
131 calibrated using an RS Aqua red solid standard.

132 *Trichodesmium erythraeum* IMS101 was examined under confocal microscopy
133 following 4',6-diamidino-2-phenylindole (DAPI) staining to assess intracellular DNA
134 distribution. Following gravitational settling and aspiration of 5 mL culture, *Trichodesmium*
135 biomass was fixed with 1% glutaraldehyde, 3% formaldehyde, 14% sucrose in piperazine-
136 N,N′-bis(2-ethanesulfonic acid) (PIPES) buffer. After 20 minutes at 4°C, samples were
137 filtered onto 0.8 µm polycarbonate black filters, transferred to microscope slides, and
138 mounted with VECTASHIELD HardSet Mounting Medium with DAPI (Vector Laboratories) at
139 1.5 µg mL^{-1}. Slides were allowed to set overnight at 4°C. Samples were imaged on a Leica
140 SP5 confocal microscope under UV excitation, which excites DAPI associated with DNA, and
141 488 nm excitation, which excites chlorophyll *a* and phycoerythrin. DAPI associated with RNA
142 is also excited at this wavelength, but the associated emission spectrum is weak (Suzuki *et*
143 *al.* 1997).

144

145 **Results and discussion**

146 ***Comparison of gene and cellular abundance***

147 Direct measurement of *nif*H gene copies (*nif*H L^{-1}; as described in Langlois *et al*., 2008)
148 and cell counts (cells L^{-1}) of *in situ Trichodesmium* populations, sampled from two research
149 cruises in the Atlantic Ocean and laboratory-grown cultures, revealed that *nifH* abundance
150 exceeds cell abundance by 1-2 orders of magnitude (Table 1 and Figure 1). Thus, despite a
151 strong correlation between cell count based abundances and *nif*H abundances (R^2 = 0.89)
152 (Figure 1) the latter considerably exceed the former, which is consistent with unexplained
153 discrepancies previously reported in the literature (Luo *et al.*, 2012; Rouco *et al*. 2014).

154 As *nifH* is a single copy gene in the *Trichodesmium* genome (Zehr *et al*., 2008;
155 Walworth *et al*. 2015), we estimated the degree of ploidy (genome copies cell^{-1}) using an
156 established approach (genome copies cell^{-1} = *nifH* L^{-1} / cells L^{-1}; Pecoraro *et al*. 2011). The
157 estimated degree of ploidy ranged from 1-120 genome copies per cell for *in situ* samples
158 (n=31) excluding those samples where <1 copy number per cell was estimated. Errors in
159 both cell counts and copy number estimates may have been more significant for such
160 samples, which were all at low overall biomass (Figure 1). For example, the low total copy
161 numbers may have been too low for the quantitative PCR (qPCR) technique to work reliably.
162 Additionally, dead cells with partially degraded DNA may also have contributed
163 disproportionately within the low biomass field sampled locations. Estimated copies per cell
164 exceeded 600 in laboratory-cultured *Trichodesmium erythraeum* IMS 101 (n=2) (Table 1).
165 Thus, both field and cultured *Trichodesmium* displayed substantial, albeit variable, degrees
166 of polyploidy, with our limited dataset further suggesting that *nifH* copy number per cell was
167 higher in culture as opposed to field collected samples.
168 To confirm that the observed mismatch between measured gene abundances and cell
169 counts was not specific to the *nifH* amplicon, genes present in the *Trichodesmium* IMS101
170 genome in double (16S rRNA) or triple (*psb*A) copies were also analyzed by qPCR from
171 cultured samples. Our measured ratios (Table 2) compared well with the predicted copy
172 numbers from the genome, arguing against cryptic amplification of *nifH* copies and
173 suggesting robust estimates of the degree of ploidy.
174

175 ***Localisation of DNA in Trichodesmium cells***

176 To visualise the localisation of DNA in *Trichodesmium* cells, confocal microscopy
177 following DAPI staining of *T. erythraeum* IMS101 DNA was performed. The markedly
178 segregated DAPI staining throughout the cell implies intracellular DNA distribution in *T.*
179 *erythraeum* IMS101 is scattered and extensive (Figures 1B and 1C); an observation that is
180 consistent with *Trichodesmium* possessing multiple copies of the genome per cell, as have
181 been reported in other cyanobacteia (Schneider *et al.*, 2007; Lane and Martin 2010; Sukenik
182 *et al.* 2012; Zerulla et al. 2016).
183
184 ***Implications of polyploidy for abundance estimates***
185 Quantitative (q)PCR is commonly used to quantify the environmental abundance of the
186 highly conserved *nif*H gene that encodes the iron-binding component of the nitrogenase
187 enzyme present in all diazotrophs (Zehr *et al.* 1998; Luo *et al*. 2012). Such approaches can
188 be used to assess the biogeography of diazotrophic phylotypes and have been crucial in
189 increasing our understanding of the intricacies of the marine nitrogen cycle (Fernández *et*
190 *al.*, 2010; Goebel *et al.*, 2010; Zehr, 2011; Thompson and Zehr, 2013). In the Atlantic Ocean,
191 *Trichodesmium nif*H constitutes as much as 50% of the total detectable *nif*H (Langlois *et al.*,
192 2008) and can exceed contributions by unicellular diazotrophic phylotypes by up to an order
193 of magnitude (Rijkenberg *et al.*, 2011). However, in the case of *Trichodesmium*, failure to
194 account for any degree of polyploidy (Table 1) would result in an overestimate of cell
195 abundance if extrapolations were made from gene abundance to cell abundance assuming a
196 1:1 cell:genome ratio.
197 To further highlight this potential issue and provide additional circumstantial evidence
198 for significant polyploidy within field communities, we extrapolated estimates of

199 *Trichodesmium* abundances to corresponding *in situ* chlorophyll concentrations (Table 3).
200 For example, using a typical cellular chlorophyll content of 1.1 pg Chl per cell (LaRoche and
201 Breitbarth, 2005) we can estimate the contribution of *Trichodesimum* to total measured *in*
202 *situ* whole community chlorophyll from a range of published data alongside that collected
203 within the current study (Table 3). Consistent with previous estimates (Carpenter *et al*.
204 2004), *Trichodesmium* accounted for as much as 30% of measured *in situ* whole community
205 chlorophyll when estimated from cell counts. In contrast, performing a similar calculation
206 based on measured *nifH* gene copies and assuming only one *nifH* copy per cell (i.e. no
207 polyploidy) would result in a ten-fold increase in the average *Trichodesmium* contribution to
208 total chlorophyll (Table 3). Moreover, under an assumption of monoploidy, *Trichodesmium*
209 alone might be estimated to contribute up to an order of magnitude higher chlorophyll than
210 the actual measured community chlorophyll concentration, highlighting that the number of
211 *nifH* copies per cell must be >1.
212
213 ***Ecophysiological implications of polyploidy in Trichodesmium spp.***
214 There may be several ecological advantages to polyploidy in *Trichodesmium* that would
215 overcome the obvious disadvantages of maintaining multiple genome copies per cell. For
216 instance, multiple genome copies distributed throughout each cell (suggested in Figure 1B
217 and C) would allow efficient transcription in localised areas of the cell. This is an
218 advantageous feature, as *Trichodesmium* needs to simultaneously reconcile oxygen-evolving
219 photosynthesis and anaerobic nitrogen fixation processes within a large cellular volume
220 (Bergman *et al.*, 2013). Being polyploid is thought to enable quick instigation of metabolic
221 activity following dormancy and also long-term preservation of genome integrity in

222 *Aphanizomenon ovalisporum* (Sukenik *et al.*, 2012). The ability to quickly instigate
223 alterations in metabolism through polyploidy may be a considerable advantage for
224 *Trichodesmium,* enabling exploitation of ephemeral nutrient pulses, such as through aeolian
225 iron deposition which is a key factor in the biogeography of this group (Moore *et al*., 2009).

226 While there are considerable advantages to polyploidy, there are also costs associated
227 with the higher resource requirement to maintain and duplicate multiple genomes. The
228 considerable range of polyploidy observed in *Trichodesmium*, may reflect variability with
229 growth phase, diel cycle, nutrient availability or intercellular variability within a colony (e.g.
230 the presence of diazocytes). The increased nitrogen requirement for multiple genome copies
231 is presumably not as disadvantageous for *Trichodesmium* as it would be for non-diazotrophic
232 organisms such as *Prochlorococcus* and *Synechococcus*. However, considering the potential
233 range in genome copies per cell (Table 1), using an estimated genomic phosphorus content
234 of 8×10^{-4} pg genome^{-1} (Walworth *et al.*, 2015) and a typical range of intracellular
235 phosphorus of 0.5-1 pg cell^{-1}, (Nuester *et al*. 2012; Tovar-Sanchez & Sañudo-Wilhelmy 2011),
236 100 genome copies per cell (maximum estimated in marine environment, Table 1), would
237 account for 8-16% of the total cellular phosphorus content. The degree of polyploidy may
238 therefore significantly impact the phosphorus resource allocation in *Trichodesmium*
239 (Sanudo-Wilhelmy *et al*. 2001; Elser *et al.*, 2003; Nuester *et al.*, 2012). Indeed recent results
240 on the single-celled cyanobacteria *Synechocystis* sp. suggest that phosphorus availability has
241 an impact on the degree of ploidy observed in this cyanobacterial species (Zerulla *et al.*
242 2016). Our data reveal higher polyploidy within phosphorous-replete cultures compared to
243 field samples collected within low phosphorous environments (Sanudo-Wilhelmy *et al*.

244 2001; Moore *et al*. 2009), which is consistent with such a response (Figure 1), although
245 further work would clearly be required to substantiate such suggestions.

246 In summary, we provide direct evidence linking gene abundance to cell number that
247 indicates that *Trichodesmium* is highly polyploid in culture and exhibits a highly diverse
248 degree of ploidy in the field. This observation highlights that caution should be applied in
249 extrapolating *nifH* gene abundance data to estimate diazotrophic cell abundances and
250 suggests that a wider assessment of the extent of polyploidy in ecologically significant
251 marine (cyano-)bacterial taxa would be desirable. A high degree of polyploidy in
252 *Trichdesmium* adds to the unique genomic characteristics of this organism when compared
253 to the majority of cyanobacteria in oligotrophic oceanic environments (Walworth *et al.*,
254 2015). *Trichodesmium* appears to have evolved to capitalise on some of the benefits of
255 maintaining multiple copies of a large genome; features that may enable *Trichodesmium* to
256 continue to be a prolific and robust player in a changing future ocean. Further work is
257 required to determine how environmental conditions, growth phase and cell differentiation
258 may impact the degree and function of ploidy observed in this keystone oceanic microbe.

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273

274 **Conflict of interest statement**

275 The authors declare no conflict of interest.

276

277 **References**

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374

375 **Table and figure legends**

376

377 **Table 1. Estimated genome copies in *Trichodesmium* spp.** Genome copies per cell are
378 calculated by division of gene copies L^{-1} by cells L^{-1}, as described by Pecoraro *et al*. (2011).
379 The table shows the ranges of cell abundance and *nifH* gene copy numbers measured from
380 cultures of *Trichodesmium* or surface seawater samples collected from two research cruises
381 to the Atlantic (AMT17 and D361). The range of derived genome copies per cell (i.e. level of
382 ploidy) is also shown. Sampling procedures for cultures and *in situ* samples, as well as
383 methods for measurement of cell count by microscopy and gene abundance by qPCR, are
384 described in material and methods.

385

386 **Table 2. Predicted and measured ratio of multi-copy number genes in *Trichodesmium***
387 ***erythraeum* ISM101 genome.** The *Trichodesmium erythraeum* ISM101 genome predicts
388 that the *nifH*, 16S rRNA and *psbAII* genes are present in the *T. erythraeum* ISM101genome
389 (Walworth *et al.* 2015) in single (Tery_4136), double (Tery_R0014, Tery_R0029) and triple
390 (Tery_0182, Tery_0183, Tery_4763) copies respectively. The abundances of these genes in
391 cultures of *T. erythraeum* ISM101 were measured using qPCR from DNA extracted from the
392 same culture. The corresponding measured ratio of these genes is shown as an average and
393 standard deviation of n=3 samples.

394

395 **Table 3. Calculated potential contributions to total *in situ* chlorophyll by**
396 ***Trichodesmium spp*.** The calculated contribution that *Trichodesmium* makes to measured *in*
397 *situ* whole community chlorophyll derived from cells counts and gene copies, with the latter

398 assuming monoploidy (i.e. assuming 1 copy per cell), from published data and this study.
399 Conservative estimates are made using the lowest reported chlorophyll content per cell (1.1.
400 pg Chl per cell, La Roche and Breitbarth 2005). Note that the large variation in gene copy-
401 based contributions to total chlorophyll under the assumption of monoploidy are likely due
402 to variability in the actual level of ploidy in natural populations of *Trichodesmium*.
403
404 **Figure 1.** Relationship between *Trichodesmium* cell counts and *nifH* gene copies (Table 1).
405 (**A**) A combined assessment of *in situ* data from surface samples along the AMT17 transect
406 (blue) and D361 transect (black) and *Trichodesmium* erythraeum IMS101 culture samples
407 (red). A significant correlation is observed (R^2 = 0.89, y = 0.5423x + 0.9624) Gene copies
408 consistently exceed cell counts by 1-2 orders of magnitude and thus deviated from a 1:1
409 correlation (dotted line). (**B**) Confocal microscopy of DAPI stained *T. erythraeum* IMS101
410 cultures observed under UV and 488 nm excitation. DNA (blue) and chlorophyll
411 autofluorescence (orange) in a single *T. erythraeum* IMS101 cell demonstrating marked
412 scattered intracellular DNA distribution. (**C**) Scattered DNA distribution was observed in
413 cells along a trichome. Scale bars represent 2 μm, variation in intensity is due to changes in
414 cellular morphology along the z-plane. See materials and methods for detailed microscopy
415 methods.
416

417 **Tables**

418

419 **Table 1**

***Trichodesmium* Sample**	***nifH* copies L^{-1}**	**cells L^{-1}**	**Genome copies per cell [range (avg ± SD)]**
Cruise AMT17 (n=15)	ND – 2.7E+5	56 – 1.8E+4	1-120 (12 ± 13)
Cruise D361 (n=16)	ND – 6.7E+5	0 – 1.9E+4	1-50 (31 ± 30)
Trichodesmium erythraeum IMS 101 (n=2)	2.5E+7 – 1.4E+8	3.6E+4 – 2.1E+5	639-697 (668 ± 41)

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Table 2

Gene targets	**Predicted ratio**	**Measured ratio**
nifH:16S rRNA	1:2	1:1.53 (±0.06)
nifH:*psbAII*	1:3	1:3.32 (±0.13)

424

425 **Table 3**

Trichodesmium contribution to total chlorophyll (%) estimated from either cell counts or gene copies (assuming monoploidy)					
Cell Counts		**Gene Copies**			
Range	**Avg ± SD**	**Range**	**Avg ± SD**	**n**	**Source**
0-8.0	4.1 ± 1.7	NA	NA	16	This study, AMT 21
0-11.6	4.4 ± 7.9	NA	NA	33	Fernández et al. 2010**
0.2-13.7	7.2 ± 4.5	NA	NA	22	Letelier & Karl 1996**
0-175.5	18.8 ± 30.3	NA	NA	336	Capone et al. 2004**
0-243.9	22.9 ± 34.0	NA	NA	335	Borstad 1978**
0-6	2.1 ± 2.5	0-98.4	10 ± 24.9	31	This study, AMT 17
0-17.8	4.1 ± 4.3	0-138.7	44.3 ± 63.9	15	This study, D361
NA	NA	1-440	136.0 ± 126.1	13	Goebel et al. 2010*
NA	NA	0-1163.8	99.6 ± 275.6	23	Kong et al. 2011**
NA	NA	0-2065.5	120.3 ± 319.1	125	Moisander et al. 2010**

*Data estimated from figures, **Data from Luo *et al.* (2012) dataset.

426
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428 **Figure 1**
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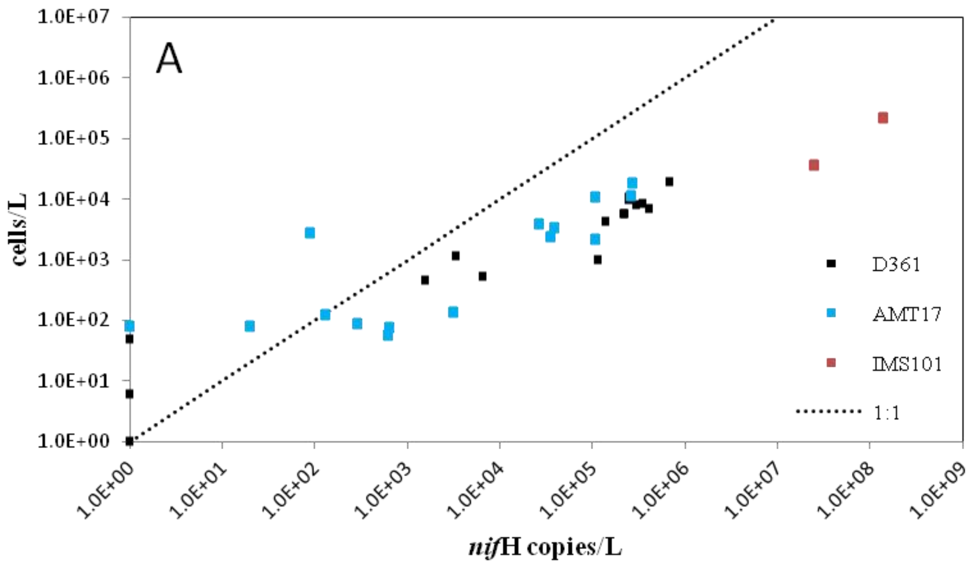

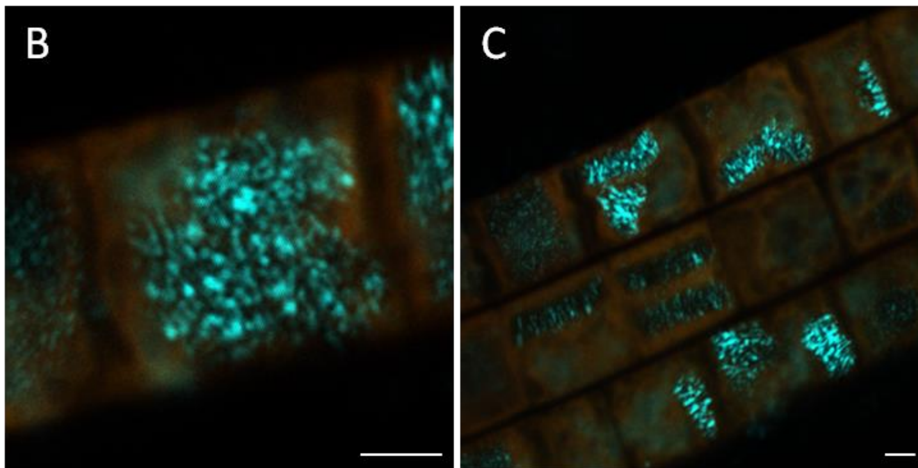

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Table S1. Genes analysed by quantitative PCR in this study and corresponding *Trichodesmium*-specific qPCR oligonucleotide primers. Primer specificity determined by BLAST analysis (Altschul *et al*. 1997).

Gene	Homologues in *T. erythraeum* IMS 101[a]	Primer	Sequence (5′-3′)	Amplicon size (bp)
*nif*H	*Tery_4136*	Forward	TGGCCGTGGTATTATTACTGCTATC	111
		Reverse	GCAAATCCACCGCAAACAAC	
		Probe	AAGGAGCTTATACAGATCTA	-
16S rRNA	*Tery_R0014, Tery_R0029*	Forward	CCACACTGGGACTGAGACAC	117
		Reverse	AACCCTAGAGCCTTCCTCCC	
*psb*A	*Tery_0182, Tery_0183, Tery_4763*	Forward	AATGCACCCATTCCACATGC	192
		Reverse	CGACCGAAGTAGCCATGAG	

[a] annotated in the *Trichodesmium erythraeum* IMS 101 genome (Genbank accession NC_008312).

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