

Supplementary Information for “Co-occurrence of Fe and P stress in natural populations of the marine diazotroph *Trichodesmium*.”

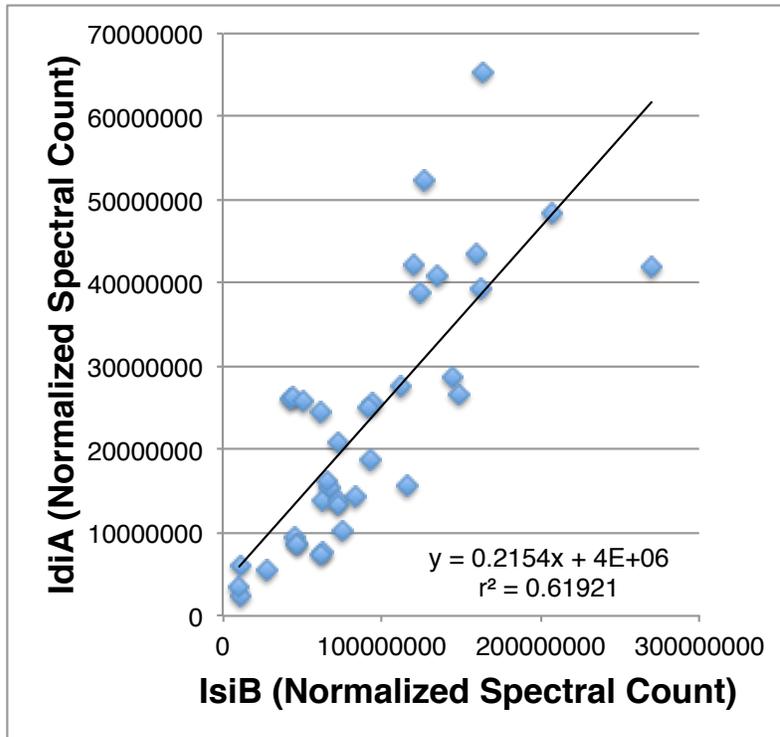


Figure S1. Relative abundance of IdiA and IsiB. Both are biomarkers of iron stress in *Trichodesmium*, and behave similarly in this dataset.

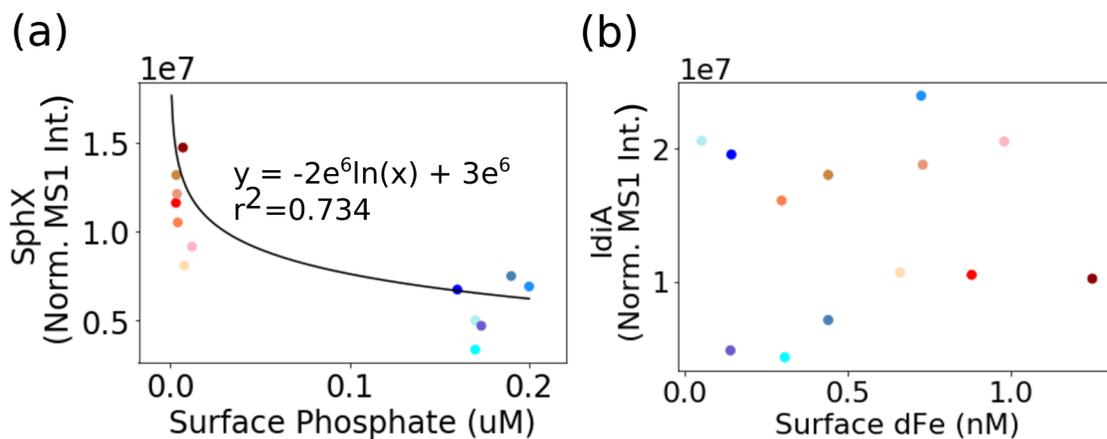


Figure S2. Scatter plot of A) SphX versus surface phosphate and B) IdiA versus surface dFe values. Note that analytical differences between JC150 (red dots) and Tricolim (blue dots) may be forcing the relationship with the surface phosphate values, though all values are above the reported limit of detection.

*Note: Tables S3, S4, S6 are provided as excel files due to their large size

Table S1. Important Fe and P stress biomarkers in *Trichodesmium*

Name	Nutrient sensed	<i>T. erythraeum</i> sp. IMS101 gene name	Gene name in metagenome used in this study (IMG ID 2821474806)
IdiA	Fe	Tery_3377	TCCM_0877.00000020
PstS	Phosphate	Tery_3537	TCCM_0018.00000050
SphX	Phosphate	Tery_3534	TCCM_0018.00000040

Table S2. Sample provenance information

Name	Replication	Cruise	Cruise station #	Time sampled	Date sampled	Net size (uM)	Filter size	Latitude	Longitude
JC150_03	triplicate	JC150	3	pre-dawn	7/10/17	200	0.2uM	22	-50
JC150_04	singlicate	JC150	4	pre-dawn	7/14/17	200	0.4uM	23.22	-44.47
JC150_05	triplicate	JC150	5	pre-dawn	7/19/17	200	0.4uM	23	-39.6
JC150_06	singlicate	JC150	6	pre-dawn	7/24/17	200	0.4uM	22.19	-35.53
JC150_07	triplicate	JC150	7	pre-dawn	7/29/17	200	0.4uM	22	-31
JC150_01	triplicate	JC150	1	pre-dawn	7/3/17	200	0.2uM	22	-58
JC150_02	duplicate	JC150	2	pre-dawn	7/4/17	200	0.2uM	21.26	-54
Tricolim_18	triplicate	Tricolim	18	pre-dawn	3/9/18	130	0.2uM	13.38	-55.5
Tricolim_15	triplicate	Tricolim	15	pre-dawn	3/5/18	130	0.2uM	5.0239	-44.1836
Tricolim_19	triplicate	Tricolim	19	pre-dawn	3/11/18	130	0.2uM	16.5	-57.5
Tricolim_14	duplicate	Tricolim	14	afternoon	3/1/18	130	0.2uM	2.36061	-39.591
Tricolim_15	singlicate	Tricolim	15	9	3/3/18	130	0.2uM	5.0239	-44.1836
Tricolim_03	duplicate	Tricolim	3	11:30	2/15/18	130	0.2uM	12.13	-21.59
Tricolim_16	duplicate	Tricolim	16	14:45	3/4/18	130	0.2uM	7.3	-48.29
Tricolim_13	singlicate	Tricolim	13	15:00	2/27/18	130	0.2uM	0.17976	-30.3984
HOT_01	duplicate	HOT 117	n/a	13:30	7/27/00	130	5uM	22.45	-158
BATS_01	singlicate	BATS	n/a	morning	2/4/15	200	0.2uM	31.4	-64.1

Table S5. Absolute abundance of the PstA protein and calculation of surface area occupied. See Table S6 for list of peptide transition ions used for quantification.

Assuming 30% w/w cyanobacteria protein content (Gonzalez Lopez et al., 2010) - lower bound

Assume a cylindrical cell of height 15um, width 11um (Bergmann et al., 2013)

cell volume (um³) 1424.8

cell surface area (um²) 708.07

Assume average protein contains 0.53 g C / g protein (Rouwenhorst et al)

Assume ATP transporter has cross sectional area of 1.66e-5 um² (Hudson and Morel 1992)

pg C per cell	pg C in protein	pg protein/cell	ug protein/cell
86.65	26.00	49.05	0.00005

from cell volume per Strathman 1967

Station	Avg [Pst] total protein fmol/ug	St Dev	fmol protein per cell	Pst molecules per cell	S.A. occupied per cell (um ²)	% surface area occupied
Tricolim_18	13.00	1.80	0.0006	383994.82	25.34	3.58
Tricolim_15	11.22	3.45	0.0006	331540.40	21.88	3.09
Tricolim_16	89.06	123.06	0.0044	2630552.93	173.62	24.52
JC150_3	38.73	63.34	0.0019	1143789.85	75.49	10.66
JC150_4	89.58	14.74	0.0044	2645858.35	174.63	24.66
JC150_5	74.24	36.42	0.0036	2192888.10	144.73	20.44
JC150_6	61.64	40.07	0.0030	1820590.66	120.16	16.97
JC150_7	165.72		0.0081	4894655.41	323.05	45.62
JC150_1	106.08		0.0052	3133303.68	206.80	29.21
				average		19.86

Assuming 55% w/w cyanobacteria protein content (Gonzalez Lopez et al., 2010) - upper bound

Assume a cylindrical cell of height 15um, width 11um

pg C per cell	pg C in protein	pg protein/cell	ug protein/cell
86.65	47.66	89.92	0.00009

Station	Avg [Pst] total protein fmol/ug	St Dev	fmol protein per cell	Pst molecules per cell	S.A. occupied per cell (um ²)	% surface area occupied
Tricolim_18	13.00	1.80	0.0012	703990.50	46.46	6.56
Tricolim_15	11.22	3.45	0.0010	607824.06	40.12	5.67
Tricolim_16	89.06	123.06	0.0080	4822680.37	318.30	44.95
JC150_3	38.73	63.34	0.0035	2096948.06	138.40	19.55
JC150_4	89.58	14.74	0.0081	4850740.31	320.15	45.21
JC150_5	74.24	36.42	0.0067	4020294.85	265.34	37.47
JC150_6	61.64	40.07	0.0055	3337749.54	220.29	31.11
JC150_7	165.72		0.0149	8973534.91	592.25	83.64
JC150_1	106.08		0.0095	5744390.09	379.13	53.54
				average		36.41

Table S6. Transition ions used for PstA protein quantification (peptide ATDEALQIVPR)

Peptide ATDEALQIVPR	y8 - 925.5465+
	y7 - 796.5039+
	b3 - 288.1190+
	b4 - 417.1616+
	b5 - 488.1987+
	b6 - 601.2828+

Table S7. All peptides targeted in PRM mode (note only PstA data reported here)

Description	Tery #	Metagenome ID	Peptide
IdiA periplasmic binding	Tery_3 377	TCCM_0877.000 00020	IFSEGNNEYPPVVAGIPIAT VLK HYDTDQALYDSFTQK FLEHLVSPEAQK ILYHDQNIYDPDIDPVEIR
phosphate ATP binding protein PstB	Tery_3 540	TCCM_0018.000 00090	LGQSGFALSGGQQQR NIDQQNSAAALSAEK IADVTAFFNAK ATDEALQIVPR GPLSPTLPSLAYLVYEFSR
phosphate permease PstA	Tery_3 539	TCCM_0018.000 00080	ATDEALQIVPR GPLSPTLPSLAYLVYEFSR
phosphate permease PstC	Tery_3 538	TCCM_0018.000 00070	VLIPAAFSGIVGGVMLGL GR AMGETMAVTMLIGNANS IK
SphX periplasmic binding	Tery_3 534	TCCM_0018.000 00040	GAIGYIEFGFAK INLVGAGASFPAPLYQR NSGFVQVDYQSVGSGA GIER
PstS periplasmic binding less phosphate responsive	Tery_3 537	TCCM_0018.000 00050	EVYVDILLGNIK NDGVTAQITQTEGAIGYV EYGYAK VSPELGYIPLPDNVR YIEPTFESAEATLGAVAL PENLR
flavodoxin		TCCM_0640.000 00010	IGLFLGTTTGK FVGLALDDDNQAELTDE R

Table S8. Literature values for IdiA as a biomarker of Fe stress

Study	Fe addition replete (nM)	Fe addition deplete (nM)	IdiA fold change
Webb et al., 2001	50	0	1.54
Walworth et al., 2016	250	10	1.07 at 380 pCO ₂ , 1.31 at 780 pCO ₂
Snow et al., 2015	120	0	2.38

*Note that while the cells in Walworth, et al., 2016 were clearly Fe-limited according to growth rate measurements, they had access to 10 nM total iron as opposed to 0 nM total iron for the other experiments. This may explain the discrepancy in the IdiA fold change values.

Table S9. Literature values for SphX as a biomarker of P stress

Study	P addition replete (μM)	P addition deplete (μM)	SphX fold change
Walworth et al., 2016	0.25	10	2.64 at 380 pCO ₂ , 3.10 at 780 pCO ₂
Frischkorn et al., 2019	0	50	2.62