

Supplementary Material for

Analysis of *nifH* DNA and RNA reveals a disproportionate contribution to nitrogenase activities by rare plankton-associated diazotrophs

Qingsong Yang^{1,3}, Junde Dong^{1,2}, Manzoor Ahmad^{1,3}, Juan Ling¹, Weiguo Zhou^{1,3}, Yehui Tan¹, Yuanzhou Zhang⁴, Dandan Shen^{5*}, Yanying Zhang^{1,2*}

¹CAS Key Laboratory of Tropical Marine Bio-resources and Ecology, Guangdong Provincial Key Laboratory of Applied Marine Biology, South China Sea Institute of Oceanology, Chinese Academy of Sciences, 510301 Guangzhou, China

²Tropical Marine Biological Research Station in Hainan, South China Sea Institute of Oceanology, Chinese Academy of Sciences, 572000 Sanya, China

³University of Chinese Academy of Sciences, 100049 Beijing, China

⁴State Oceanic Administration Sansha Marine Environmental Monitoring Center Station, 570311, Haikou, China

⁵Section of Biological Oceanography, Leibniz Institute for Baltic Sea Research, 18119, Warnemünde, Germany

***Correspondence:**

Dandan Shen, dand.shen@gmail.com

Yanying Zhang, zyy@scsio.ac.cn

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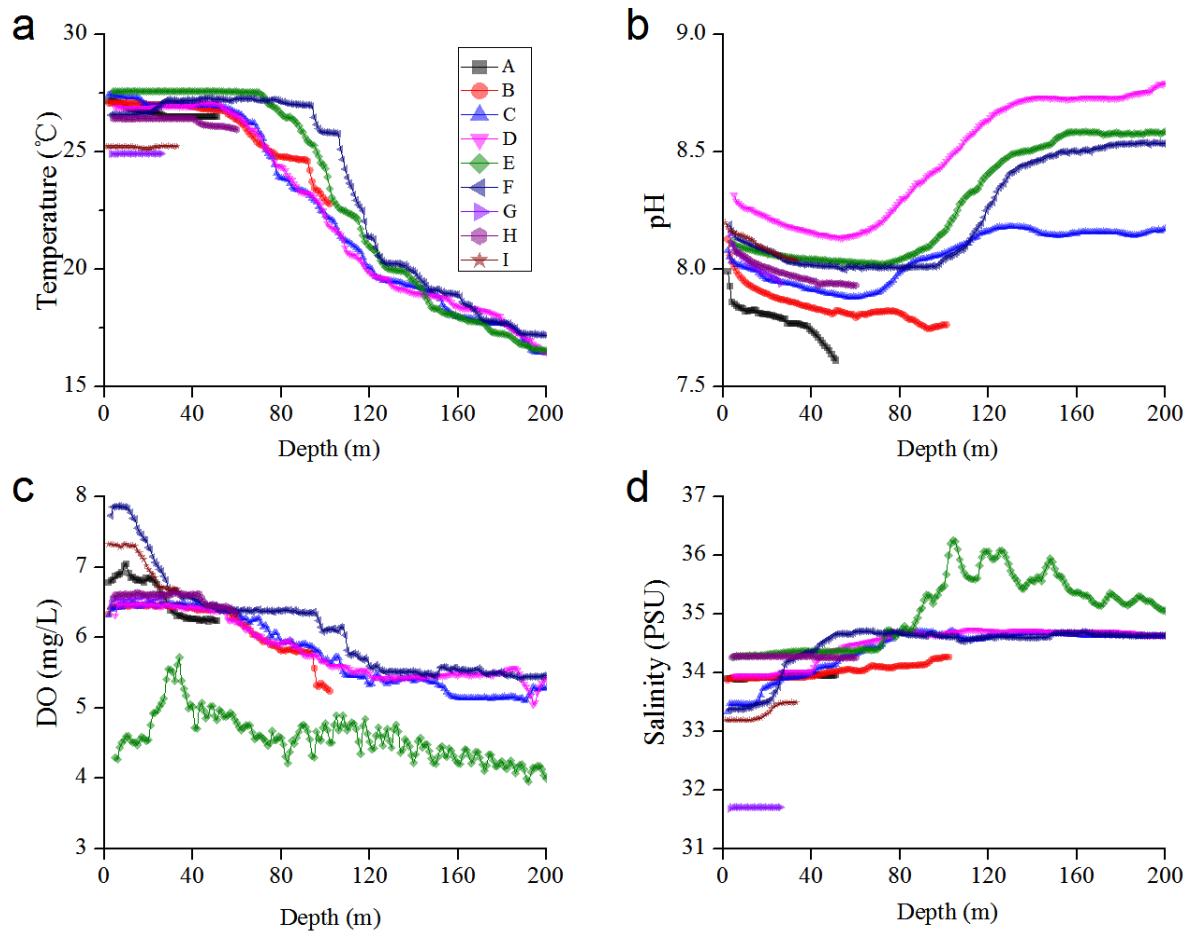


Fig. S1 Water quality profiles at each station as determined by the CTD sampler.

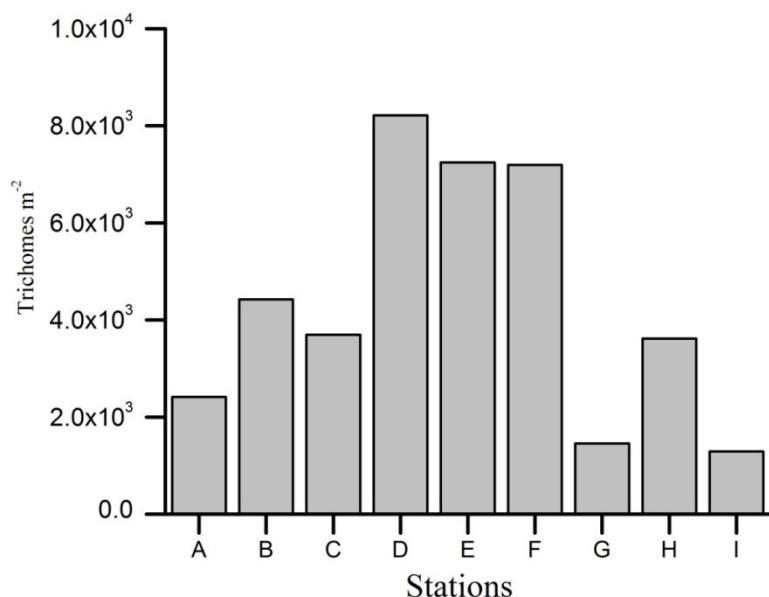


Fig. S2 Abundance of *Trichodesmium* in at the nine stations. Cell numbers were determined by microscopic counting.

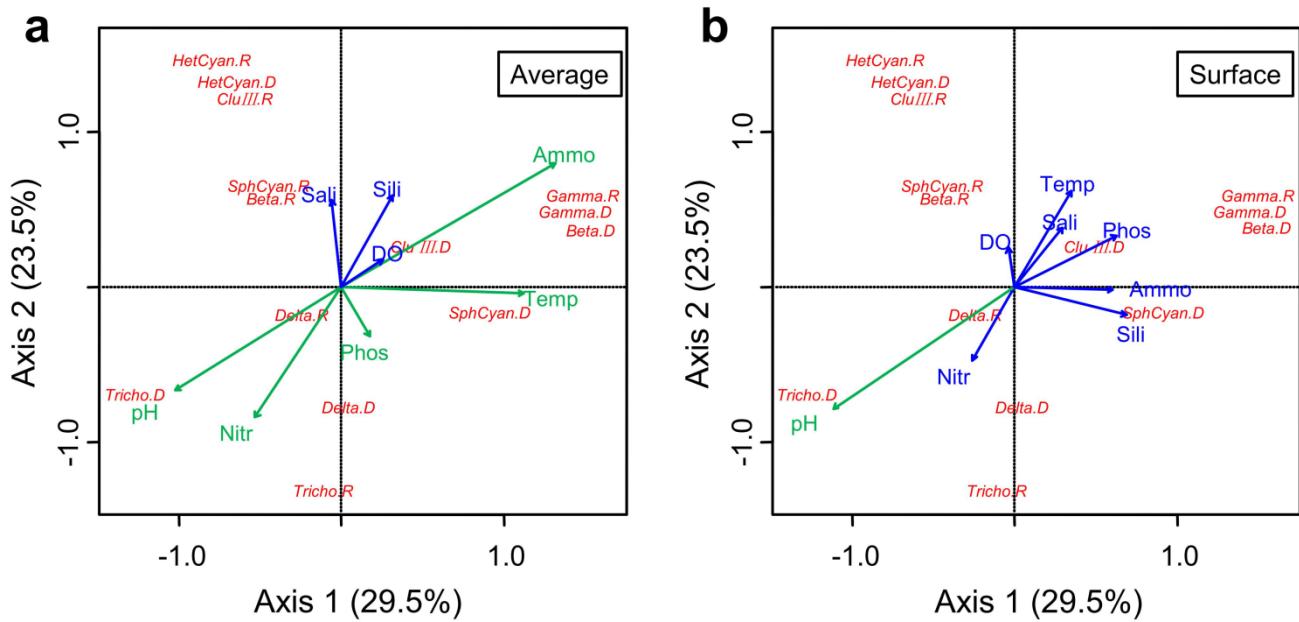


Fig. S3 Redundancy analysis (RDA) ordination plot showing the relationships between environmental variables and taxa in the DNA and RNA libraries: (a) average values from the water column and (b) the values from the sea surface. Taxa are shown in red (R: taxa derived from the RNA library; D: taxa derived from the DNA library). Tricho, *Trichodesmium*; HetCyan, heterocystous cyanobacteria; SphCyan, spherical cyanobacteria; Gamma, Gammaproteobacteria; Delta, Deltaproteobacteria; Beta, Betaproteobacteria; Clu III, cluster III. Environmental variables that correlated significantly with the taxa are indicated in green, otherwise in blue. Temp, temperature; DO, dissolved oxygen; Sali, salinity; Nitr, nitrate+nitrite; Phos, phosphate; Ammo, ammonium; Sili, silicate.

Table S1 Geolocation of the stations, sampling depth, and sample volume

Station	Geolocation	Water depth (m)	Sampling depth (m)	Sample volume (L)
A	21°59.789', 114°59.924'	57.8	55	12304.9
B	21°29.443', 115°29.513'	108.7	105	22549.1
C	20°30.026', 116°30.022'	435.5	200	18840.0
D	21°19.931', 118°0.070'	1475.8	200	41860.1
E	21°30.040', 119°29.665'	2999.0	200	36914.6
F	22°30.142', 119°30.078'	227.0	200	36679.1
G	23°18.133', 117°42.110'	33.9	31	7418.3
H	22°29.836', 118°30.106'	64.3	60	18427.9
I	22°46.199', 116°44.916'	33.3	31	6594.0

Table S2 Alpha-diversity indexes of the total and active communities in each sample

	Station	Shannon index	Simpson index	Pielou evenness	Chao1
Total communities	A	1.21	1.45	0.23	223.02
	B	0.97	1.32	0.19	200.25
	C	1.00	1.33	0.20	200.55
	D	0.86	1.27	0.17	183.77
	E	0.89	1.28	0.18	189.52
	F	0.89	1.28	0.18	187.39
	G	0.91	1.30	0.18	217.62
	H	0.86	1.27	0.17	196.36
	I	1.03	1.33	0.20	200.64
Active communities	A	1.84	2.31	0.35	247.45
	B	0.99	1.33	0.20	192.10
	C	1.55	2.20	0.30	211.91
	D	1.09	1.38	0.21	227.56
	E	1.00	1.32	0.19	214.92
	F	1.56	2.39	0.29	268.32
	G	0.92	1.29	0.18	205.18
	H	1.80	2.21	0.34	264.03
	I	1.07	1.38	0.21	200.90

Table S3 Nutrient concentrations at the sampling stations.

Station	Column average($\mu\text{mol L}^{-1}$)				Surface water($\mu\text{mol L}^{-1}$)			
	Nitrite+ Nitrate	Ammonium	Phosphate	Silicate	Nitrite+ Nitrate	Ammonium	Phosphate	Silicate
A	0.39	0.57	0.27	5.00	0.19	0.44	0.27	3.95
B	0.85	0.14	0.20	4.00	0.30	0.48	0.07	1.94
C	1.79	0.18	0.32	6.32	0.32	0.43	0.12	1.65
D	2.37	0.13	0.42	5.22	0.22	0.00	0.03	2.13
E	3.22	0.04	0.47	4.69	0.08	0.62	0.07	1.56
F	1.45	0.08	0.21	3.69	0.75	0.08	0.12	2.23
G	4.93	0.00	0.38	4.92	5.38	0.00	0.37	5.78
H	0.37	0.28	0.12	1.97	0.63	0.00	0.17	1.56
I	0.84	0.48	0.09	0.93	0.81	0.5.	0.07	0.98

Table S4 Monte Carlo permutation test of the effects of environmental variables on the *nifH*-derived communities

	Water column (average)	Sea surface
Nitrate+Nitrite	0.100*	0.503
Ammonium	0.064*	0.402
Phosphate	0.094*	0.608
Silicate	0.405	0.969
Temperature	0.040**	0.732
Salinity	0.910	0.608
pH	0.021**	0.019**
Dissolved oxygen	0.990	0.290

Asterisks indicate the level of significance: * $P < 0.1$; ** $P < 0.05$

Table S5 The C_T values obtained from the technical triplicate of quantitative PCR.

Sample name	qPCR C _T value				
	Replicate 1	Replicate 2	Replicate 3	Mean	Standard Error
nifH_DNA_A	26.45	26.21	26.29	26.32	0.12
nifH_DNA_B	27.45	27.60	27.04	27.36	0.29
nifH_DNA_C	27.04	27.06	27.16	27.09	0.06
nifH_DNA_D	22.19	22.67	22.22	22.36	0.27
nifH_DNA_E	22.34	22.23	22.11	22.23	0.11
nifH_DNA_F	24.44	24.05	24.02	24.17	0.23
nifH_DNA_G	24.66	25.08	24.54	24.76	0.28
nifH_DNA_H	23.46	23.63	23.44	23.51	0.10
nifH_DNA_I	25.53	25.64	25.67	25.61	0.074
nifH_RNA_A	33.20	33.65	33.67	33.51	0.26
nifH_RNA_B	31.21	31.22	31.52	31.32	0.18
nifH_RNA_C	30.46	30.56	30.01	30.34	0.29
nifH_RNA_D	29.75	29.75	29.91	29.80	0.09
nifH_RNA_E	28.10	28.3	28.2	28.20	0.10
nifH_RNA_F	30.32	30.24	30.25	30.27	0.04
nifH_RNA_G	28.51	28.49	28.64	28.55	0.08
nifH_RNA_H	33.35	33.68	34.18	33.74	0.42
nifH_RNA_I	31.60	31.12	31.10	31.27	0.28