1 *Supplement of*

2 **Change in diazotrophic community structure associated with**
3 **Kuroshio succession in the northern South China Sea**

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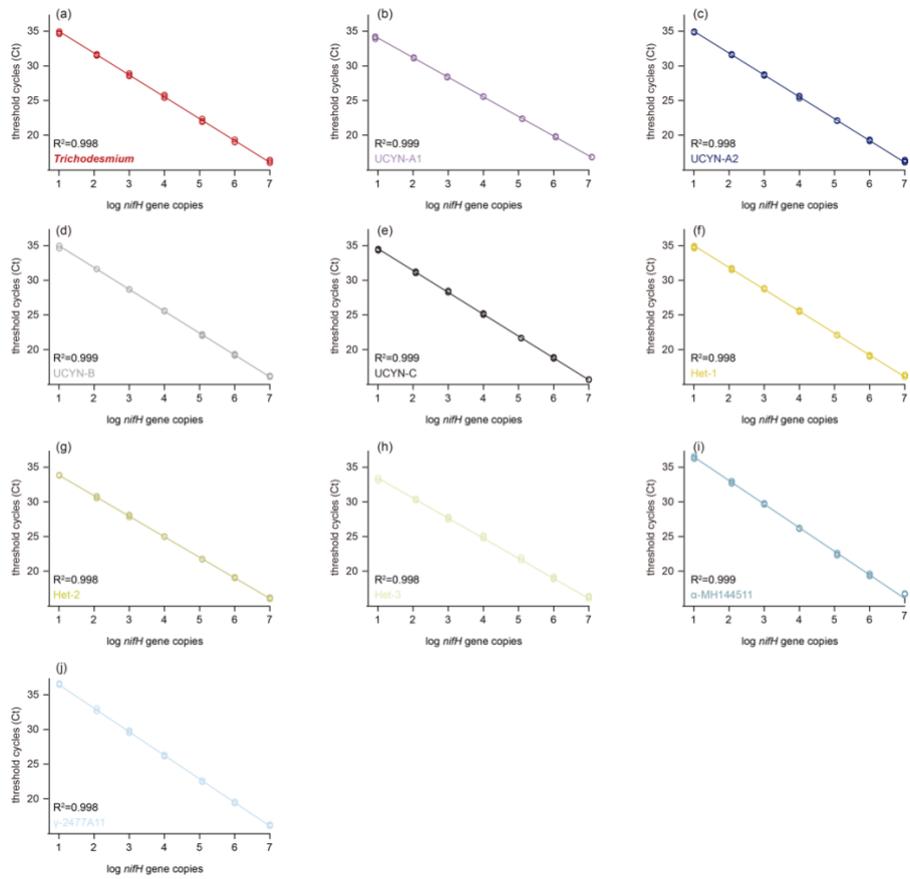
7 This file includes:

8   Figure S1

9   Tables S1 to S8

10   References for supplement

12

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14   Figure S1. Representative standard curves for qPCR. (a) *Trichodesmium*; (b) UCYN-A1; (c) UCYN-A2; (d) UCYN-B;

15   (e) UCYN-C; (f) Het-1; (g) Het-2; (h) Het-3; (i) α-MH144511; (j) γ-24774A11.

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17   Table S1. Sampling information.

Station [a]	Location	Sampling time (yyyy/mm/dd)	Sampling depth (m) [b]
stn1	123.023°E, 22.016°N	2017/07/14	5; 25; 50; 75; 100; 115
stn4*	119.500°E, 21.000°N	2017/07/19	5; 25; 50; 75; 100; 115
stn9*	116.000°E, 18.000°N	2017/07/27	5; 25; 50; 85; 100; 150
stn11	115.432°E, 16.550°N	2017/08/01	5; 25; 55; 75; 100; 150
stn12	114.789°E, 15.154°N	2017/08/04	5; 25; 50; 60; 100; 150
stn2	122.500°E, 22.000°N	2018/06/27	5; 50; 75; 100; 150
stn3	122.500°E, 21.500°N	2018/06/25	5; 25; 75; 100; 150
stn4*	119.500°E, 21.000°N	2018/06/22	5; 25; 75; 100; 150
stn5	119.000°E, 20.500°N	2018/06/21	5; 25; 75; 100; 150
stn6	118.400°E, 20.000°N	2018/06/22	5; 25; 75; 100; 150
stn7	117.980°E, 19.500°N	2018/06/30	5; 25; 75; 100; 150
stn8	117.000°E, 19.000°N	2018/07/03	5; 25; 75; 100; 150
stn9*	116.000°E, 18.000°N	2018/07/05	5; 25; 75; 100; 150
stn10	115.000°E, 17.000°N	2018/07/07	5; 25; 75; 100; 150

18   Note: a) The same stations sampled in both 2017 and 2018 are marked with an asterisk (*). b) Samples collected at the 5
19   m water depth were used for qPCR and NGS.
20
21   Table S2. GenBank accession numbers of sequence standards used to target specific N_2-fixing taxa.

Species	Accession	Reference
UCYN-A1	AF059627	Zehr et al., 1998
UCYN-A2	KF806604	Thompson et al., 2014
UCYN-B	AF299418	Zehr et al., 2001
Het-1	DQ118191	Church et al., 2005b
Het-2	DQ225754	Foster and Zehr, 2006
Het-3	DQ225762	Foster and Zehr, 2006
α-MH144511	MH144511	Chen et al., 2019
γ-24774A11	EU052318	Moisander et al., 2008
UCYN-C	HQ455916	This study
Trichodesmium	L00689	This study

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23   Table S3. Oligonucleotide primer and probe sequences utilized for TaqMan qPCR.

Species	Forward (5′→3′)	Probe (5′→3′)	Reverse (5′→3′)	Reference
Trichodesmium	GACGAAGTATTGAAGCCAGGTTTC	CATTAAGTGTGTTGAATCTGGTGGTCCTGAGC	CGGCCAGCGCAACCTA	Church et al., 2005b
UCYN-A1	TAGCTGCAGAAAGAGGAACTGTAGAAG	TAATTCCTGGCTATAACAAC	TCAGGACCACCGGACTCAAC	Langlois et al., 2008
UCYN-A2	GGTTACAACAACGTTTTATGTGTTGA	TCTGGTGGTCCTGAGCCCGGA	ACCACGACCAGCACATCCA	Thompson et al., 2014
UCYN-B	CGTAATGCTCGAAGGGTTTGA	CAAGTGTGTAGAATCTGGTGGTCCTGAGCC	CACGACCAGCACAACCAACT	Moisander et al., 2010
UCYN-C	TCTACCCGTTTGATGCTACACACTAA	AAACTACCATTCTTCACTTAGCAG	GGTATCCTTCAAGTAGTACTTCGTCTAGCT	Langlois et al., 2008
Het-1	CGGTTTCCGTGGTGTACGTT	TCCGGTGGTCCTGAGCCTGGTGT	AATACCACGACCCGCACAAC	Church et al., 2005b
Het-2	TGGTTACCGTGATGTACGTT	TCTGGTGGTCCTGAGCCTGGTGT	AATGCCGCGACCAGCACAAC	Moisander et al., 2010
Het-3	CGGTTTCCGTGGCGTACGTT	TCCGGTGGTCCAGAACCTGGTGT	AATACCACGACCAGCACAAC	Foster et al., 2007
α-MH144511	ACGGCGCCTACGAGGATATCGATT	ACGTGCTGGGCGACGTTGTCTGC	CTGCGCCTTGTTCTCGCGGAT	Chen et al., 2019
γ-24774A11	CGGTAGAGGATCTTGAGCTTGAA	AAGTGCTTAAGGTTGGCTTTGGCGACA	CACCTGACTCCACGCACTTG	Moisander et al., 2008

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25   Table S4. Summary of environmental factors detected in this study.

Year	Station [a]	SST (°C)	SSS	MLD (m)	DCM (m)	Nit (m)	I_{DIN} (mmol m^{-2})	I_{DIP} (mmol m^{-2})	I_{NFR} (µmol N m^{-2} d^{-1})	S_{NFR} (nmol N L^{-1} d^{-1})	I_{PP} (mmol C m^{-2} d^{-1})	S_{PP} (µmol C L^{-1} d^{-1})	R_{K_100} (%)
2017	stn1	30.81	34.59	40	137	ND	ND	ND	271.88	0.90	4.67	0.06	100.00
2017	stn4*	29.95	33.85	37	98	ND	ND	ND	86.40	0.71	21.11	0.24	82.01
2017	stn9*	29.71	33.60	34	69	ND	ND	ND	129.81	0.11	18.09	0.29	11.81
2017	stn11	27.96	33.64	32	63	ND	ND	ND	11.73	0.25	20.87	0.23	28.34
2017	stn12	29.20	33.43	22	63	ND	ND	ND	27.27	0.39	27.7	0.26	5.89
2018	stn2	29.12	34.45	44	102	114	10.50	8.40	52.00	0.32	18.84	0.20	100.00
2018	stn3	29.17	34.51	15	117	131	1.00	8.00	64.38	0.48	27.76	0.32	92.57
2018	stn4*	28.87	33.66	21	44	ND	ND	16.52	77.95	0.96	90.00	0.61	11.01
2018	stn5	28.59	33.30	36	52	ND	ND	19.41	86.82	1.57	121.16	0.43	5.83
2018	stn6	29.35	33.46	22	64	26	25.00	179.50	52.19	1.17	27.11	0.52	6.23
2018	stn7	29.50	33.78	40	83	50	28.00	230.11	56.49	1.75	19.39	0.20	3.75
2018	stn8	29.20	33.84	27	78	50	272.23	23.38	105.90	1.38	26.89	0.31	0.44
2018	stn9*	29.55	33.81	31	71	44	177.42	17.21	94.03	1.41	21.09	0.30	0.00
2018	stn10	30.24	33.45	27	82	56	95.80	12.18	93.61	2.86	31.55	0.51	0.00

26   Note: a) The same stations sampled in both 2017 and 2018 are marked with an asterisk (*). Abbreviations: SST, sea surface temperature; SSS, sea surface salinity; MLD, mixed
27   layer depth; DCM, depth of chlorophyll maximum; Nit, depth of nitracline; I_{DIN}, depth-integrated dissolved inorganic nitrogen; I_{DIP}, depth-integrated dissolved inorganic
28   phosphorus; S_{NFR}, surface N$_2$ fixation rate; I_{PP}, depth-integrated primary production; S_{PP}, surface primary production; R_{K_100}, averaged Kuroshio fractions in the upper 100 m;
29   ND, not determined.

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32 Table S5. The abundance of the ten major diazotrophic groups determined based on TaqMan qPCR assay of the *nifH* gene (copies L^{-1}).

Year	Station [a)]	Time (hh: mm)	*Trichodesmium*	UCYN-A1	UCYN-A2	UCYN-B	UCYN-C	Het-1	Het-2	Het-3	α-MH144511	γ-2477A11
2017	stn1	10: 00	5.79×10^5	4.82×10^2	3.00×10^3	8.31×10^4	3.70×10^2	1.32×10^4	BDL	9.37×10^2	8.84×10^4	1.44×10^4
2017	stn1	19: 00	4.00×10^4	2.07×10^4	1.44×10^3	2.31×10^6	3.19×10^3	4.31×10^2	BDL	BDL	4.67×10^4	2.44×10^4
2017	stn4*	13: 00	1.28×10^6	6.32×10^4	1.83×10^3	3.31×10^6	6.34×10^3	2.00×10^4	BDL	BDL	2.46×10^5	2.97×10^4
2017	stn4*	19: 00	1.63×10^5	1.81×10^4	2.35×10^3	3.61×10^4	1.39×10^4	2.37×10^2	2.95×10^2	BDL	3.85×10^5	1.94×10^4
2017	stn9*	10: 00	5.47×10^5	5.69×10^4	5.72×10^2	1.59×10^6	8.53×10^3	1.06×10^3	BDL	3.85×10^4	7.47×10^4	5.18×10^4
2017	stn9*	19: 30	6.19×10^4	4.50×10^4	1.96×10^3	4.18×10^4	3.78×10^3	1.05×10^2	BDL	9.04×10^3	1.17×10^4	7.78×10^3
2017	stn11	15: 00	3.78×10^6	4.80×10^4	2.47×10^4	3.10×10^6	9.20×10^3	9.38×10^2	4.54×10^2	6.71×10^3	5.49×10^5	1.09×10^4
2017	stn11	20: 30	6.29×10^4	9.61×10^5	1.71×10^4	2.39×10^5	2.21×10^4	9.58×10^2	BDL	4.93×10^3	7.80×10^4	3.58×10^3
2017	stn12	13: 00	5.47×10^5	1.28×10^3	1.49×10^3	3.17×10^3	4.80×10^3	3.98×10^2	BDL	4.47×10^2	1.77×10^4	2.15×10^4
2017	stn12	20: 00	7.62×10^4	2.30×10^3	3.34×10^3	4.15×10^4	5.22×10^3	1.16×10^4	BDL	1.67×10^3	1.58×10^4	8.40×10^3
2018	stn2	13: 30	1.04×10^3	3.40×10^3	5.62×10^2	1.08×10^4	2.18×10^2	4.87×10^2	1.01×10^2	1.53×10^2	1.04×10^2	2.29×10^2
2018	stn2	03: 00	BDL	2.59×10^3	5.63×10^2	1.11×10^3	8.79×10^2	BDL	1.25×10^2	3.40×10^2	1.26×10^2	2.36×10^2
2018	stn3	15: 30	1.35×10^5	1.74×10^2	1.87×10^2	8.88×10^3	5.31×10^2	2.56×10^3	1.01×10^2	1.92×10^2	BDL	BDL
2018	stn3	19: 00	1.66×10^2	8.99×10^2	3.34×10^3	6.22×10^5	2.25×10^3	2.39×10^2	2.41×10^2	1.25×10^2	2.94×10^2	7.06×10^2
2018	stn4*	15: 00	5.24×10^3	1.60×10^2	1.44×10^2	2.55×10^4	6.27×10^2	9.20×10^1	BDL	BDL	BDL	BDL
2018	stn4*	20: 00	BDL	7.58×10^2	5.60×10^3	5.11×10^5	BDL	BDL	1.25×10^2	3.40×10^2	1.26×10^2	4.36×10^2
2018	stn7	10: 40	5.81×10^4	2.76×10^3	2.58×10^3	1.45×10^5	1.89×10^3	2.38×10^2	1.48×10^2	BDL	BDL	BDL
2018	stn7	03: 00	7.47×10^2	2.46×10^3	5.61×10^3	1.10×10^7	3.31×10^3	7.65×10^3	4.43×10^3	2.81×10^3	1.23×10^3	1.28×10^3
2018	stn9*	14: 30	5.24×10^3	5.80×10^2	5.44×10^2	1.22×10^5	1.05×10^3	3.19×10^2	BDL	5.39×10^2	1.62×10^2	3.85×10^2
2018	stn9*	20: 00	2.81×10^3	1.55×10^3	5.08×10^3	5.55×10^5	8.70×10^2	2.03×10^3	5.06×10^2	1.55×10^3	1.23×10^3	1.79×10^3

33 Note: a) The same stations sampled in both 2017 and 2018 are marked with an asterisk (*). BDL, below detection limit.

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36   Table S6. The number of OTUs corresponding to various diazotrophic lineages at designated stations as detected via NGS.

Station [a]	2017					2018								
Taxonomy	stn1	stn4*	stn9*	stn11	stn12	stn2	stn3	stn4*	stn5	stn6	stn7	stn8	stn9*	stn10
Trichodesmium	26910	16480	10983	5567	6050	19142	13541	12310	4542	6816	5644	13222	5683	5139
UCYN-A	0	26	0	7	4	336	252	5	566	259	123	772	1	1
UCYN-B	4345	797	3398	28	7473	5214	7267	18765	9276	10029	13139	10282	13459	17818
UCYN-C	234	1	0	24599	0	0	7	3	3	27	17	6	3	5
Other Cyanobacteria	42	391	21	64	45	570	414	12	33	8	1942	223	575	261
α-proteobacteria	0	0	0	0	0	64	37	10	2	0	0	48	46	27
β-proteobacteria	0	0	0	0	0	99	195	1	2	0	377	32	455	105
γ-proteobacteria	4539	9339	18059	4254	22889	7451	9479	1051	13529	10161	7521	6199	7356	6157
δ-proteobacteria	0	0	0	0	0	5	2	4	0	8	1	0	0	8
Other Proteobacteria	31	1618	55	1414	46	0	0	0	0	0	0	0	0	0
Actinobacteria	0	0	0	258	0	0	0	0	0	0	0	0	0	0
Archaea	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Bacteroidetes	0	247	1	0	0	0	0	0	0	0	0	0	0	0
Firmicutes	9	168	0	194	6	0	12	0	0	0	16	6	0	0
Planctomycetes	7	0	0	43	0	0	0	0	0	0	0	0	0	0
Spirochaetes	0	26	0	0	0	0	0	0	0	0	0	0	0	0
Verrucomicrobia	0	0	0	49	4	170	20	84	52	3	2812	206	447	130
Others	402	7426	4002	41	2	526	2351	1332	5572	6266	1985	2581	5552	3926

37   Note: The same stations sampled in both 2017 and 2018 are marked with an asterisk (*).

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Table S7. The number and proportion (in brackets) of OTUs corresponding to specific diazotrophic subcommunities as detected via NGS with a 97% sequence similarity cutoff.

OTU number Diazotrophic subcommunities	2017	2018
All OTUs	360	114
Abundant taxa	0	1 (0.9%)
Conditionally abundant taxa	5 (1.4%)	3 (2.6%)
Moderate taxa	0	0
Rare taxa	32 (8.9%)	11 (9.6%)
Conditionally rare taxa	290 (80.6%)	77 (67.5%)
Conditionally rare or abundant taxa	33 (9.2%)	22 (19.3%)

Table S8. Mantel's tests of correlations of geographical distance, environmental distance, and individual environmental factors with the diazotrophic subcommunities.

Sampling time	Factors	All		AT+CAT+CRAT		RT		CRT	
		r	P	r	P	r	P	r	P
2017	Geo	0.058	0.255	0.251	0.002	-0.039	0.651	-0.032	0.65
	Env	0.378	0.001	0.428	0.001	0.228	0.015	0.392	0.001
	Stat_depth	0.348	0.001	0.348	0.001	0.161	0.007	0.256	0.012
	Samp_depth	0.135	0.065	0.132	0.064	0.123	0.113	0.272	0.004
	Temp	0.356	0.001	0.351	0.001	0.135	0.081	0.309	0.001
	DO	0.040	0.310	0.044	0.290	0.012	0.441	0.039	0.299
	Sal	0.428	0.002	0.425	0.001	0.178	0.036	0.376	0.001
	MLD	0.426	0.001	0.421	0.001	0.210	0.022	0.422	0.001
	Chl *a*	0.405	0.001	0.398	0.002	0.201	0.023	0.417	0.001
	SSW	0.012	0.347	0.008	0.405	0.102	0.158	0.175	0.014
2018	Geo	0.054	0.376	0.050	0.386	NA	NA	0.211	0.159
	Env	-0.060	0.648	-0.060	0.638	NA	NA	-0.166	0.851
	Stat_depth	-0.036	0.539	-0.038	0.586	NA	NA	-0.096	0.727
	Temp	0.050	0.384	0.050	0.379	NA	NA	0.069	0.397
	DO	0.008	-0.450	0.009	0.444	NA	NA	-0.078	0.695
	Sal	0.025	0.395	0.024	0.405	NA	NA	0.273	0.059
	MLD	-0.133	0.828	-0.135	0.85	NA	NA	0.136	0.178
	Chl *a*	0.461	0.003	0.460	0.004	NA	NA	0.136	0.174
	SSW	-0.061	0.650	-0.064	0.641	NA	NA	-0.121	0.782

Abbreviations: Geo, geographical distance; Env, environmental distance; Stat_depth, station depth; Samp_depth, sampling depth; Temp, temperature; Sal, salinity; MLD, mixed layer depth; Chl *a*, Chlorophyll *a*; DO, dissolved oxygen; SSW, sea surface wind. All, all taxa; AT, abundant taxa; CAT, conditionally abundant taxa; CRAT, conditionally rare and abundant taxa; CRT, conditionally rare taxa; RT, rare taxa. NA, not applicable.

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