



*Supplement of*

## **Changes in diazotrophic community structure associated with Kuroshio succession in the northern South China Sea**

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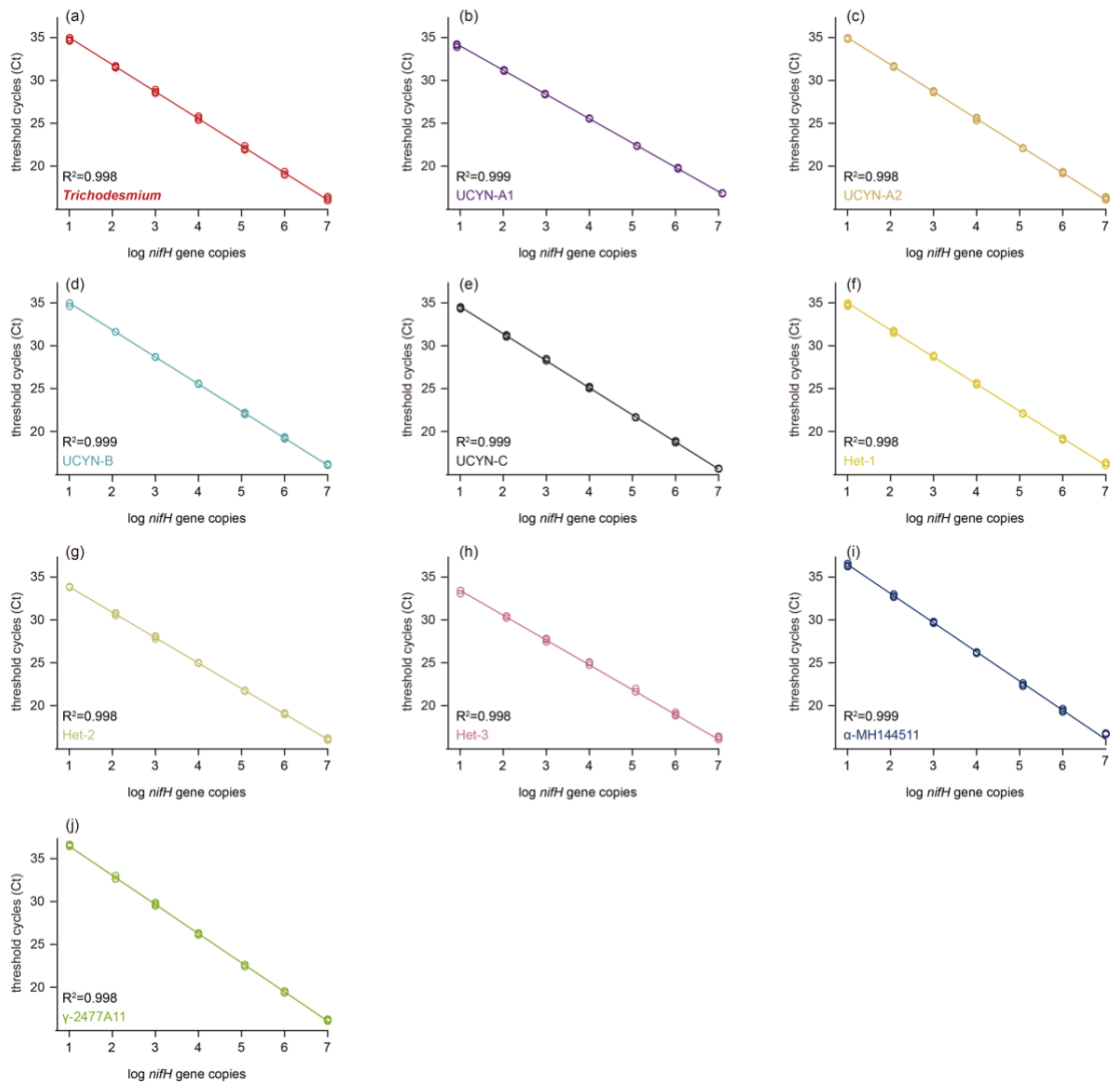


Figure S1. Representative standard curves for qPCR. (a) *Trichodesmium*; (b) UCYN-A1; (c) UCYN-A2; (d) UCYN-B; (e) UCYN-C; (f) Het-1; (g) Het-2; (h) Het-3; (i)  $\alpha$ -MH144511; (j)  $\gamma$ -2477A11.

Table S1. Sampling information.

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

Note: a) The same stations sampled in both 2017 and 2018 are marked with an asterisk (\*). b) Samples collected at the 5 m water depth were used for qPCR and NGS.

Table S2. GenBank accession numbers of sequence standards used to target specific N<sub>2</sub>-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
$\alpha$ -MH144511	MH144511	Chen et al., 2019
$\gamma$ -24774A11	EU052318	Moisander et al., 2008
UCYN-C	HQ455916	This study
<i>Trichodesmium</i>	L00689	This study

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

Species	Forward (5'→3')	Probe (5'→3')	Reverse (5'→3')	Reference
<i>Trichodesmium</i>	GACGAAGTATTGAAGCCAGGTTT C	CATTAAGTGTGTTGAATCTGGTG GTCCTGAGC	CGGCCAGCGCAACCTA	Church et al., 2005a
UCYN-A1	TAGCTGCAGAAAGAGGAACTGT AGAAG	TAATTCCTGGCTATAACAAC	TCAGGACCACCGGACTCAAC	Langlois et al., 2008
UCYN-A2	GGTTACAACAACGTTTTATGTGT TGA	TCTGGTGGTCCTGAGCCCGGA	ACCACGACCAGCACATCCA	Thompson et al., 2014
UCYN-B	CGTAATGCTCGAAGGGTTTGA	CAAGTGTGTAGAATCTGGTGGTC CTGAGCC	CACGACCAGCACAACTCAACT	Moisander et al., 2010
UCYN-C	TCTACCCGTTTGATGCTACACAC TAA	AAACTACCATTCTTCACTTAGCA G	GGTATCCTTCAAGTAGTACTTCG TCTAGCT	Langlois et al., 2008
Het-1	CGGTTTCCGTGGTGTACGTT	TCCGGTGGTCCTGAGCCTGGTGT	AATACCACGACCCGCACAAC	Church et al., 2005b
Het-2	TGGTTACCGTGATGTACGTT	TCTGGTGGTCCTGAGCCTGGTGT	AATGCCGCGACCAGCACACAAC	Moisander et al., 2010
Het-3	CGGTTTCCGTGGCGTACGTT	TCCGGTGGTCCAGAACCTGGTGT	AATACCACGACCAGCACACAAC	Foster et al., 2007
$\alpha$ -MH144511	ACGGCGCCTACGAGGATATCGAT T	ACGTGCTGGGCGACGTTGTCTGC	CTGCGCCTTGTTCTCGCGGAT	Chen et al., 2019
$\gamma$ -24774A11	CGGTAGAGGATCTTGAGCTTGAA	AAGTGCTTAAGGTTGGCTTTGGC GACA	CACCTGACTCCACGCACTTG	Moisander et al., 2008

Table S4. Abundance of the ten major diazotrophic groups determined based on TaqMan qPCR assay of the *nifH* gene (copies L<sup>-1</sup>).

Year	Station	Time (hh: mm)	<i>Trichodesmium</i>	UCYN-A1	UCYN-A2	UCYN-B	UCYN-C	Het-1	Het-2	Het-3	$\alpha$ -MH144511	$\gamma$ -2477A11
2017	stn1	10: 00	$5.79 \times 10^5$	$4.82 \times 10^2$	$3.00 \times 10^3$	$8.31 \times 10^4$	$3.70 \times 10^2$	$1.32 \times 10^4$	BDL	$9.37 \times 10^2$	$8.84 \times 10^4$	$1.44 \times 10^4$
2017	stn4*	13: 00	$1.28 \times 10^6$	$6.32 \times 10^4$	$1.83 \times 10^3$	$3.31 \times 10^6$	$6.34 \times 10^2$	$2.00 \times 10^4$	BDL	BDL	$2.46 \times 10^5$	$2.97 \times 10^4$
2017	stn9*	10: 00	$5.47 \times 10^5$	$5.69 \times 10^4$	$5.72 \times 10^2$	$1.59 \times 10^6$	$8.53 \times 10^2$	$1.06 \times 10^3$	BDL	$3.85 \times 10^4$	$7.47 \times 10^4$	$5.18 \times 10^4$
2017	stn11	15: 00	$3.78 \times 10^6$	$4.80 \times 10^4$	$2.47 \times 10^4$	$3.10 \times 10^6$	$9.20 \times 10^2$	$9.38 \times 10^2$	$4.54 \times 10^2$	$6.71 \times 10^3$	$5.49 \times 10^5$	$1.09 \times 10^4$
2017	stn12	13: 00	$5.47 \times 10^5$	$1.28 \times 10^3$	$1.49 \times 10^3$	$3.17 \times 10^3$	$4.80 \times 10^2$	$3.98 \times 10^2$	BDL	$4.47 \times 10^2$	$1.77 \times 10^4$	$2.15 \times 10^4$
2018	stn3	15: 30	$1.35 \times 10^5$	$1.74 \times 10^2$	$1.87 \times 10^2$	$8.88 \times 10^3$	$5.31 \times 10^3$	$2.56 \times 10^3$	$1.01 \times 10^2$	$1.92 \times 10^2$	$5.68 \times 10^1$	$5.30 \times 10^1$
2018	stn4*	15: 00	$5.24 \times 10^3$	$1.60 \times 10^2$	$1.44 \times 10^2$	$2.55 \times 10^4$	$6.27 \times 10^2$	$9.20 \times 10^1$	$8.24 \times 10^1$	BDL	$6.11 \times 10^1$	$4.56 \times 10^1$
2018	stn7	10: 40	$5.81 \times 10^4$	$2.76 \times 10^3$	$2.58 \times 10^3$	$1.45 \times 10^5$	$1.89 \times 10^3$	$2.38 \times 10^2$	$1.48 \times 10^2$	$3.88 \times 10^1$	BDL	$2.52 \times 10^1$
2018	stn9*	14: 30	$5.24 \times 10^3$	$5.80 \times 10^2$	$5.44 \times 10^2$	$1.22 \times 10^5$	$1.05 \times 10^3$	$3.19 \times 10^2$	BDL	$5.39 \times 10^2$	$1.62 \times 10^2$	$3.85 \times 10^2$
2018	stn10	05: 00	$1.24 \times 10^4$	$2.31 \times 10^3$	$9.15 \times 10^2$	$3.37 \times 10^5$	$1.70 \times 10^3$	$8.99 \times 10^2$	$2.93 \times 10^2$	$7.22 \times 10^2$	$6.45 \times 10^2$	$1.15 \times 10^2$

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

Table S5. Relative abundance of the ten major diazotrophic groups determined based on TaqMan qPCR assay of the *nifH* gene (copies L<sup>-1</sup>).

Year	Station	Time (hh: mm)	<i>Trichodesmium</i>	UCYN-A1	UCYN-A2	UCYN-B	UCYN-C	Het-1	Het-2	Het-3	$\alpha$ -MH144511	$\gamma$ -2477A11
2017	stn1	10: 00	0.74	0.00	0.00	0.11	0.00	0.02	0.00	0.00	0.11	0.02
2017	stn4*	13: 00	0.26	0.01	0.00	0.67	0.00	0.00	0.00	0.00	0.05	0.01
2017	stn9*	10: 00	0.23	0.02	0.00	0.67	0.00	0.00	0.00	0.02	0.03	0.02
2017	stn11	15: 00	0.50	0.00	0.00	0.41	0.00	0.00	0.00	0.00	0.07	0.02
2017	stn12	13: 00	0.92	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.03	0.04
2018	stn3	15: 30	0.89	0.00	0.00	0.06	0.03	0.02	0.00	0.00	0.00	0.00
2018	stn4*	15: 00	0.17	0.01	0.01	0.79	0.02	0.00	0.00	0.00	0.00	0.00
2018	stn7	10: 40	0.28	0.01	0.01	0.69	0.01	0.00	0.00	0.00	0.00	0.00
2018	stn9*	14: 30	0.04	0.01	0.01	0.93	0.01	0.00	0.00	0.01	0.00	0.00
2018	stn10	05: 00	0.03	0.01	0.03	0.92	0.01	0.00	0.00	0.00	0.00	0.00

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

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

Taxonomy	2017					2018									
	stn1	stn4*	stn9*	stn11	stn12	stn2	stn3	stn4*	stn5	stn6	stn7	stn8	stn9*	stn10	
<i>Trichodesmium</i>	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	
$\alpha$ -proteobacteria	0	0	0	0	0	64	37	10	2	0	0	48	46	27	
$\beta$ -proteobacteria	0	0	0	0	0	99	195	1	2	0	377	32	455	105	
$\gamma$ -proteobacteria	4539	9339	18059	4254	22889	7451	9479	1051	13529	10161	7521	6199	7356	6157	
$\delta$ -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	

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

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

<b>Diazotrophic subcommunities</b>	<b>2017</b>	<b>2018</b>
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 test of correlations of geographical distance, environmental distance, and individual environmental factors with the diazotrophic subcommunities.

<b>Sampling time</b>	<b>Factors</b>	<b>ALL</b>		<b>AT+CAT+CRAT</b>		<b>RT</b>		<b>CRT</b>	
		<b>r</b>	<b>P</b>	<b>r</b>	<b>P</b>	<b>r</b>	<b>P</b>	<b>r</b>	<b>P</b>
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 <i>a</i>	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 <i>a</i>	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.



Table S9. The importance components, eigenvalue proportion explained and cumulative proportion in redundancy analysis (RDA)

Components	Eigenvalue	Proportion Explained	Cumulative Proportion
RDA1	0.1640	0.5770	0.5770
RDA2	0.0615	0.2164	0.7935
RDA3	0.0309	0.1086	0.9021
RDA4	0.0121	0.0426	0.9447
RDA5	0.0065	0.0228	0.9675
RDA6	0.0025	0.0087	0.9761
RDA7	0.0011	0.0040	0.9802
RDA8	0.0009	0.0031	0.9833
RDA9	0.0003	0.0012	0.9845
RDA10	0.0001	0.0003	0.9847
RDA11	0.0000	0.0001	0.9848
RDA12	0.0000	0.0000	0.9849
RDA13	0.0043	0.0151	1

Table S10. Contribution of the environments factors and major diazotrophic groups to the variations explained by the RDA. The environmental factors used in this study are shown in Table S4.

		RDA1	RDA2
Environments factors	SST	-0.0952	0.5214
	SSS	-0.1040	0.7065
	MLD	0.1829	0.4228
	DCM	0.0851	0.8747
	I <sub>DIN</sub>	-0.8404	-0.2569
	I <sub>DIP</sub>	-0.9077	-0.1455
	Nit	-0.8394	0.1058
	I <sub>NFR</sub>	-0.3828	0.4466
	I <sub>PP</sub>	-0.3233	-0.5956
	S <sub>NFR</sub>	-0.6608	0.0791
	S <sub>PP</sub>	-0.4627	-0.5505
<i>R<sub>K_100</sub></i>	0.1800	0.6767	
Major diazotrophic groups	<i>Trichodesmium</i>	0.0110	0.5879
	UCYN-B	-0.7612	-0.1394
	UCYN-C	0.5982	-0.1114
	$\gamma$ -proteobacteria	0.2126	-0.1731

Abbreviations: I<sub>NFR</sub>, depth-integrated N<sub>2</sub> fixation rate; SST, surface seawater temperature; SSS, sea surface salinity; MLD, mixed layer depth; I<sub>DIP</sub>, depth-integrated dissolved inorganic phosphorus; I<sub>DIN</sub>, depth-integrated dissolved inorganic nitrogen; Nit, nitracline; S<sub>PP</sub>: surface primary productivity; I<sub>PP</sub>, depth-integrated primary productivity; *R<sub>K\_100</sub>*, averaged Kuroshio fractions in the upper 100 m (*R<sub>K\_100</sub>*, %);

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