



Supplement of

Spatiotemporal heterogeneity in diazotrophic communities reveals novel niche zonation on the continental shelf of the East China Sea

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Table S1. Primer/probe sequences and GeneBank accession numbers for *nifH* standards employed in TaqMan qPCR assays.

Phylotype	Forward primer (5'→3')	Probe (5'→3')	Reverse primer (5'→3')	Accession	Reference
<i>Trichodesmium</i>	GACGAAGTATTGAAGCCAGG TTTC	CATTAAGTGTGTTGAATCTGG TGGTCCTGAGC	CGGCCAGCGCAACCTA	L00689	Church et al., 2005a
Het-1	CGGTTTCCGTGGTGTACGTT	TCCGGTGGTCCTGAGCCTGGT GT	AATACCACGACCCGCACAAC	DQ118191	Church et al., 2005b
Het-2	TGGTTACCGTGATGTACGTT	TCTGGTGGTCCTGAGCCTGGT GT	AATGCCGCGACCAGCACAAC	DQ225754	Foster et al., 2007
Het-3	CGGTTTCCGTGGCGTACGTT	TCTGGTGGTCCAGAACCTGGT GT	AATACCACGACCAGCACAAC	DQ225762	Foster et al., 2007
UCYN-A1	AGCTATAACAACGTTTTATGC GTTGA	TCTGGTGGTCCTGAGCCTGGA	ACCACGACCAGCACATCCA	AF059627	Church et al., 2005a
UCYN-A2/A3/A4	GGTTACAACAACGTTTTATGT GTTGA	TCTGGTGGTCCTGAGCCCGG A	ACCACGACCAGCACATCCA	KF806604	Thompson et al., 2014
UCYN-B	CGTAATGCTCGAAGGGTTTGA	CAAGTGTGTAGAATCTGGTGG TCCTGAGCC	CACGACCAGCACAACCAACT	AF299418	Moisander et al., 2010
UCYN-C	GGTATCCTTCAAGTAGTACTT CGTCTAGCT	AAACTACCATTCTTCACTTAG CAG	TCTACCCGTTTGATGCTACAC ACTAA	HQ455916	Langlois et al., 2008
γ -24774A11	CGGTAGAGGATCTTGAGCTTG AA	AAGTGCTTAAGGTTGGCTTTG GCGACA	CACCTGACTCCACGCACTTG	EU052318	Moisander et al., 2008, 2010

Table S2. N₂ fixation rates (NFRs) with detection limits in parentheses on the East China Sea shelf during autumn and spring.

Station	Autumn NFR (nmol N L ⁻¹ d ⁻¹)		Spring NFR (nmol N L ⁻¹ d ⁻¹)	
	Replicate1	Replicate2	Replicate1	Replicate2
1	0.39 (0.7) ^a	0.58 (0.69) ^a	0.07 (0.56) ^a	0.16 (0.41) ^a
3	0.66 (0.12)	0.65 (0.13)	0.84 (0.39)	0.63 (0.4)
5	3.04 (0.76)	4.9 (0.72)	1.08 (0.45)	1.02 (0.48)
7	—	—	0.7 (0.37)	0.56 (0.34)
8	0.75 (0.19)	0.89 (0.13)	—	—
10	2.44 (0.18)	1.19 (0.18)	1.02 (0.28)	1.04 (0.29)
12	1.72 (0.35)	1.73 (0.34)	0.5 (0.5)	0.96 (0.47)
14	0.47 (0.17)	0.6 (0.17)	0.08 (0.36) ^a	0.44 (0.37)
16	1.67 (0.2)	1.41 (0.2)	0.68 (0.38)	0.97 (0.38)
18	0.56 (0.12)	1.53 (0.11)	1.31 (0.21)	1.39 (0.26)
20	7.47 (0.17)	5.08 (0.15)	0.53 (0.23)	0.29 (0.2)
22	1.46 (0.35)	1.68 (0.36)	1.75 (0.45)	1.59 (0.44)
24	0.89 (0.2)	0.78 (0.18)	1.25 (0.44)	0.48 (0.44)
26	0.99 (0.12)	0.91 (0.13)	1.1 (0.42)	0.66 (0.48)
28	0.5 (0.16)	0.45 (0.17)	0.78 (0.25)	0.4 (0.23)
30	3.2 (0.35)	2.41 (0.36)	0.41 (0.24)	1.64 (0.24)
31	0.58 (0.37)	0.2 (0.34) ^a	0.15 (0.48) ^a	0.36 (0.5) ^a
33	0.48 (0.27)	0.43 (0.26)	0.7 (0.25)	0.04 (0.2) ^a
35	0.26 (0.16)	0.24 (0.14)	0.7 (0.31)	0.71 (0.38)
36	0.41 (0.51) ^a	0.78 (0.47)	—	—
37	—	—	0.27 (0.22)	0.14 (0.24) ^a
38	0.16 (0.2) ^a	0.38 (0.17)	0.39 (0.24)	0.31 (0.24)
41	0.58 (0.37)	1.22 (0.31)	0.89 (0.14)	0.32 (0.25)
42	—	—	1.12 (0.16)	1.61 (0.17)

Note: ^a NFR below the detection limit. — data not available.

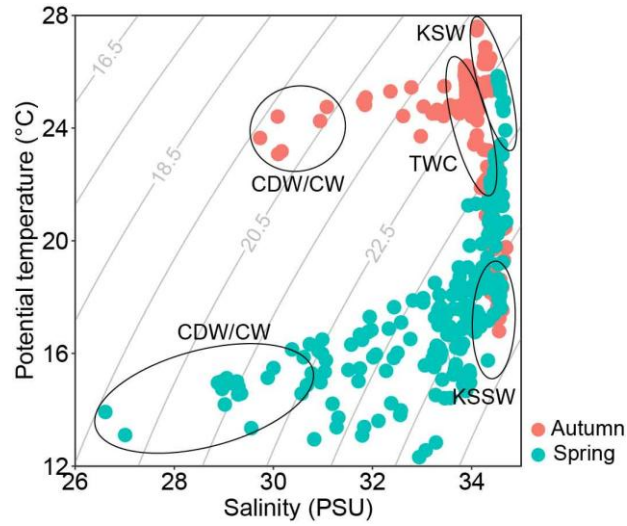


Figure S1. Plot of potential temperature and salinity at sampling depths influenced by mixing of distinct water masses on the East China Sea shelf during autumn and spring, with isopycnal density contours (grey lines) superimposed. CDW, Changjiang diluted water; CW, Coastal water; TSW, Taiwan Strait water; KSW, Kuroshio surface water; KSSW, Kuroshio subsurface water.

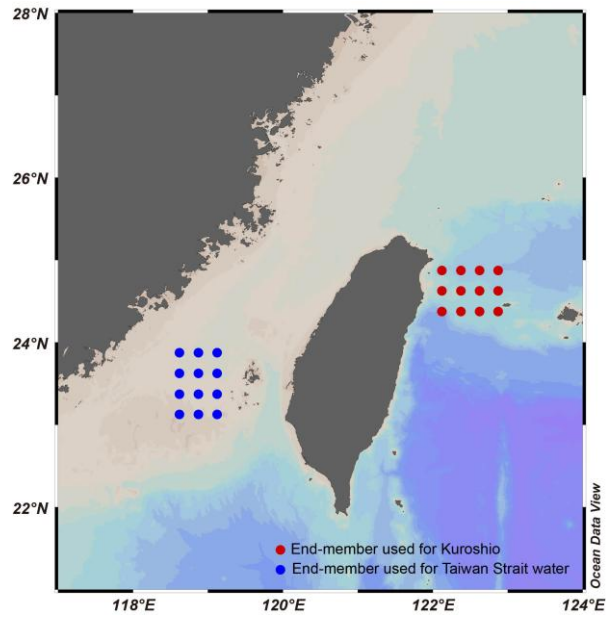


Figure S2. Data points from the World Ocean Atlas 2023 used as end-members for Kuroshio and Taiwan Strait Water. The map was created using Ocean Data View 5.7.2 (Schlitzer, Reiner, Ocean Data View, <https://odv.awi.de>, last access: 24 January 2025).

Table S3. Characteristics and parameter weights of each seawater type incorporated in the OMPA.

	CDW/CW	TSW	KSW	KSSW	Weight
Autumn					
Temperature (°C)	22.8	25.0	27.2	17.0	35.1
Salinity (PSU)	28.4	33.6	34.4	34.7	16.3
Mass	1	1	1	1	35.1
Spring					
Temperature (°C)	14.2	22.9	25.0	16.9	16.6
Salinity (PSU)	26.1	34.3	34.6	34.7	7.5
Mass	1	1	1	1	16.6

Note: CDW, Changjiang diluted water; CW, Coastal water; TSW, Taiwan Strait water; KSW, Kuroshio surface water; KSSW, Kuroshio subsurface water.

Table S4. Summary of dataset size and univariate model performance for the MaxEnt-GAM framework.

Phylotype	N ^a	Temperature	Salinity	NO _x	SRP	DSi	N:P
<i>Trichodesmium</i>	242	0.74***	0.65***	0.67***	0.61***	0.63***	0.66***
Het-1	60	0.88***	0.72***	0.83***	0.75***	0.82***	0.82***
Het-2	134	0.77***	0.69***	0.77***	0.72***	0.77***	0.76***
UCYN-A1	67	0.77***	0.83***	0.85***	0.71***	0.82***	0.84***
UCYN-A2/A3/A4	38	0.75***	0.85***	0.88***	0.70***	0.87***	0.89***
UCYN-B	106	0.79***	0.73***	0.67***	0.65***	0.68***	0.71***
UCYN-C	30	0.88***	0.88***	0.94***	0.84***	0.81***	0.86***
γ -24774A11	153	0.82***	0.68***	0.75***	0.70***	0.74***	0.76***

Note: ^a Number of data points used for the MaxEnt-GAM framework. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

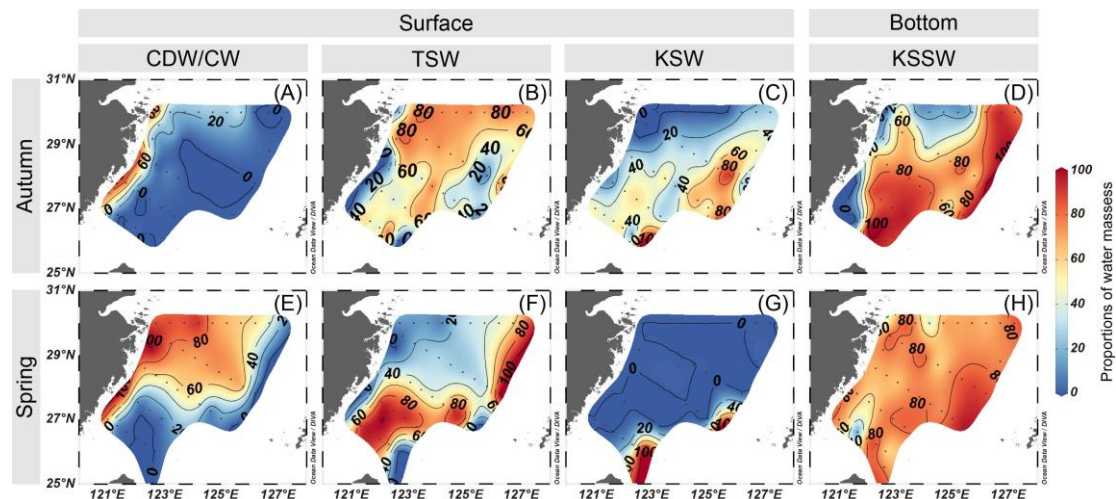


Figure S3. Proportions of water masses in the surface and bottom layers on the East China Sea shelf during autumn (A–D) and spring (E–H) as determined with OMPA. CDW, Changjiang diluted water; CW, Coastal water; TSW, Taiwan Strait water; KSW, Kuroshio surface water; KSSW, Kuroshio subsurface water. The map was created using Ocean Data View 5.7.2 (Schlitzer, Reiner, Ocean Data View, <https://odv.awi.de>, last access: 24 January 2025).

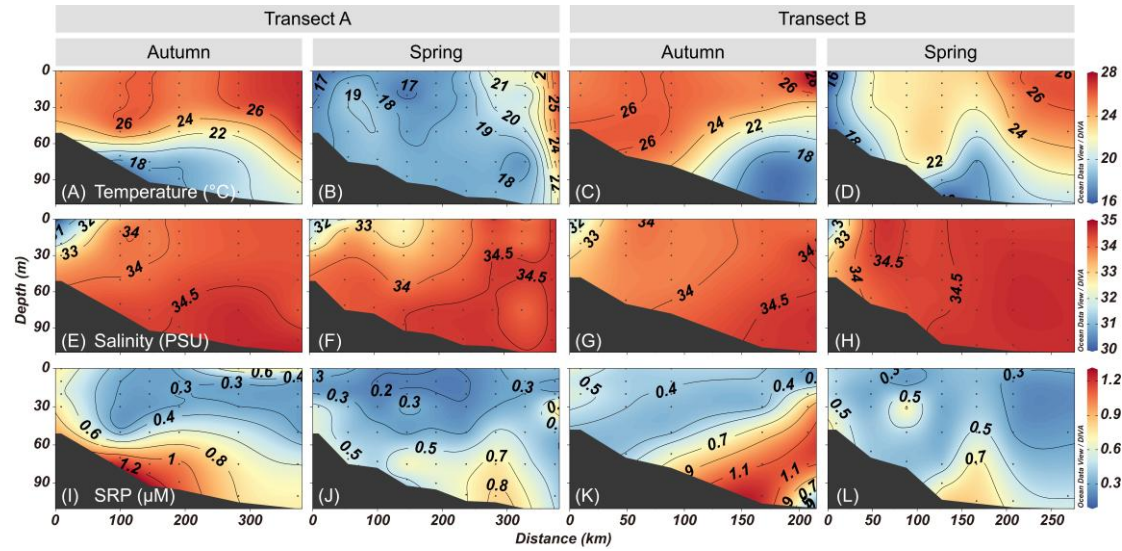


Figure S4. Vertical distributions in temperature (A–D), salinity (E–H) and SRP (I–L) along the transects A (stations 22–30) and B (stations 36–42) on the East China Sea shelf during autumn and spring. The transect plots were created using Ocean Data View 5.7.2 (Schlitzer, Reiner, Ocean Data View, <https://odv.awi.de>, last access: 24 January 2025).

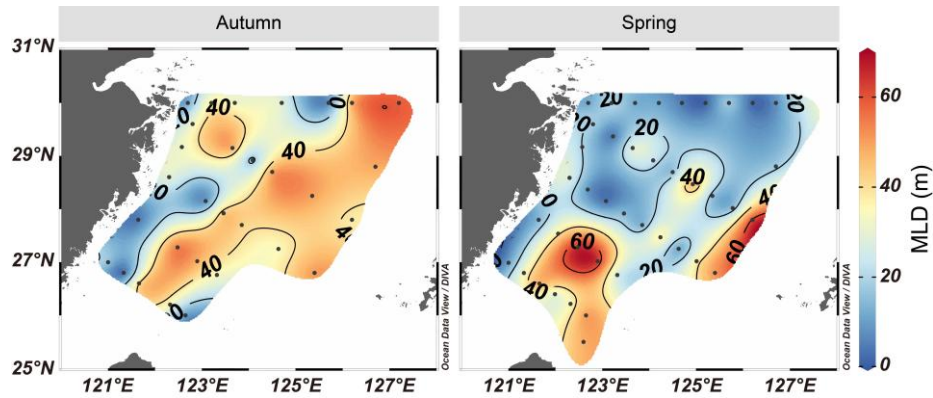


Figure S5. Variations in mixed-layer depth (MLD) on the East China Sea shelf during autumn and spring. The MLD was defined as the depth where water density was higher 0.125 kg m^{-3} than that on the surface (Thomson and Fine, 2003). The map was created using Ocean Data View 5.7.2 (Schlitzer, Reiner, Ocean Data View, <https://odv.awi.de>, last access: 24 January 2025).

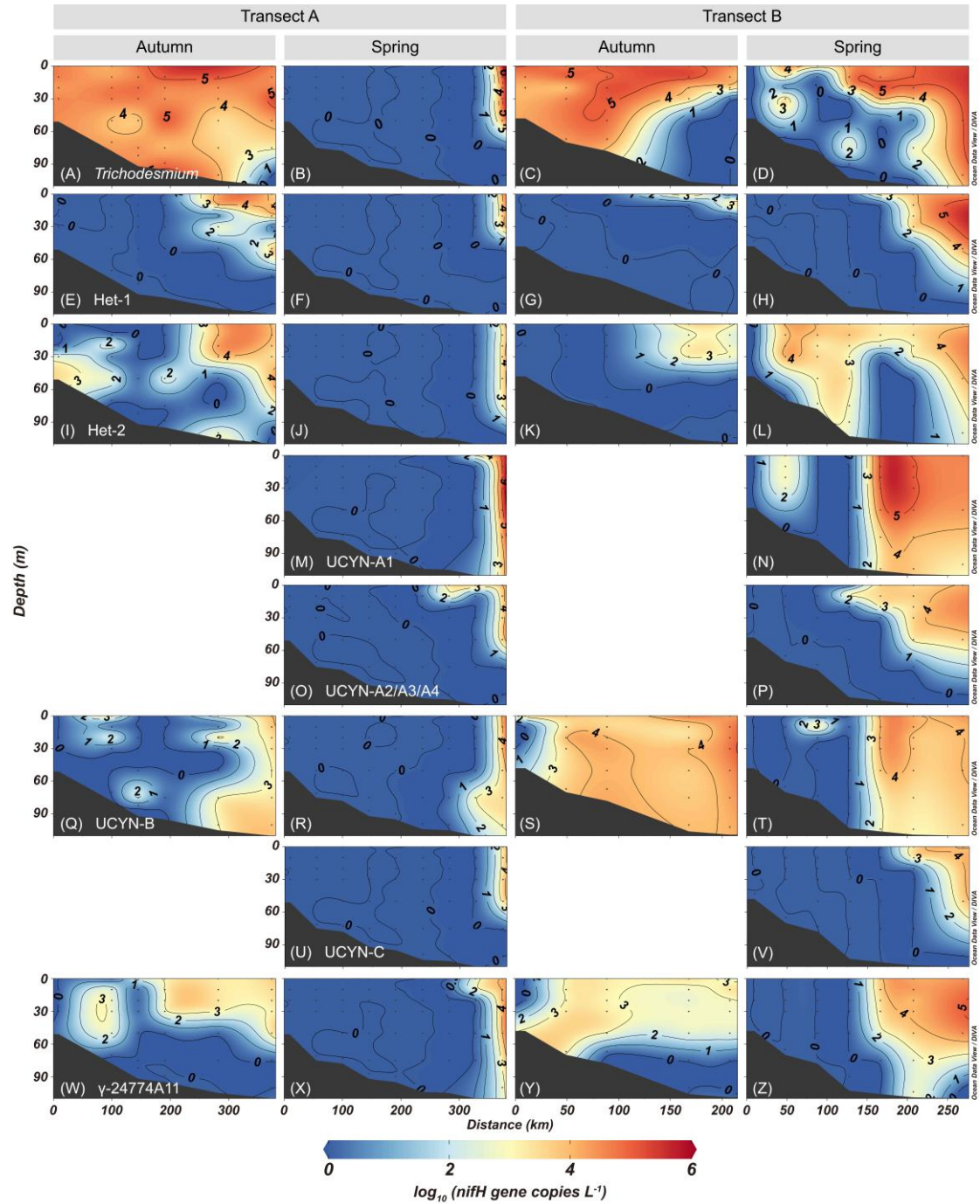


Figure S6. Vertical distributions in the abundances (Log₁₀ transformation) of the eight major diazotrophic phylotypes along the transects A (stations 22–30) and B (stations 36–42) on the East China Sea shelf during autumn and spring as determined with TaqMan qPCR assay of the *nifH* gene. Note that UCYN-A1, UCYN-A2/A3/A4 and UCYN-C were undetectable along the transects A and B during autumn. The transect plots were created using Ocean Data View 5.7.2 (Schlitzer, Reiner, Ocean Data View, <https://odv.awi.de>, last access: 24 January 2025).

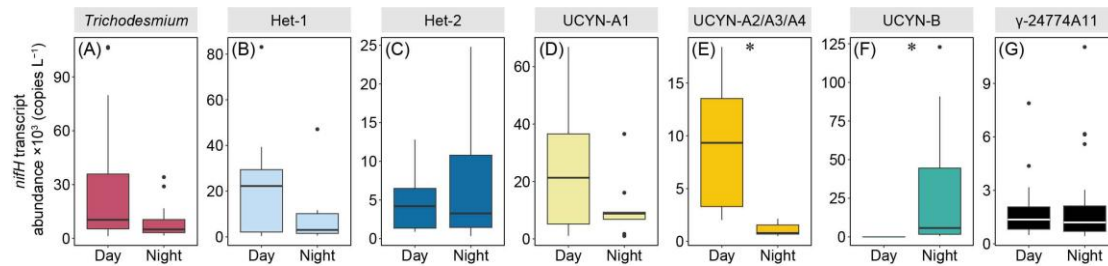


Figure S7. Differences in *nifH* transcript abundances of the seven major diazotrophic phylotypes on the East China Sea shelf between day and night as determined by TaqMan qPCR assay. Note: * $p < 0.05$.

Table S5. Pearson correlation coefficients between surface N₂ fixation rates (NFRs) and diazotroph transcripts on the East China Sea shelf.

Parameter	<i>Trichodesmium</i>	Het-1	Het-2	UCYN-A1	UCYN-A2/A3/A4	UCYN-B	γ-24774A11	Total
NFR ^a	-0.54	-0.05	-0.40	-0.26	-0.26	-0.29	-0.88	-0.66

Note: ^aNFRs and diazotroph transcript abundances from autumn and spring were pooled for Pearson correlation analysis due to scarce data in spring. Total, total *nifH* transcripts of seven diazotrophs detected.

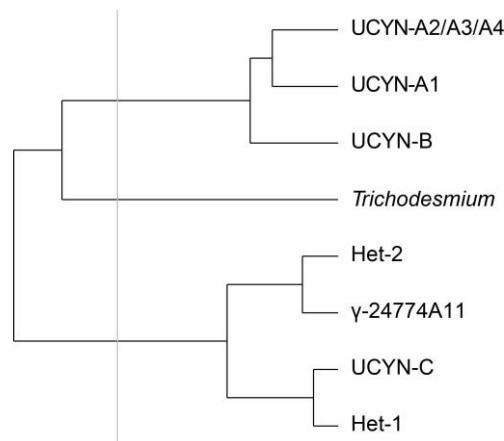


Figure S8. Hierarchical clustering of diazotrophs based on the first two PCA components, with optimal cluster number indicated by a grey line derived from elbow plot (Figure 8H).

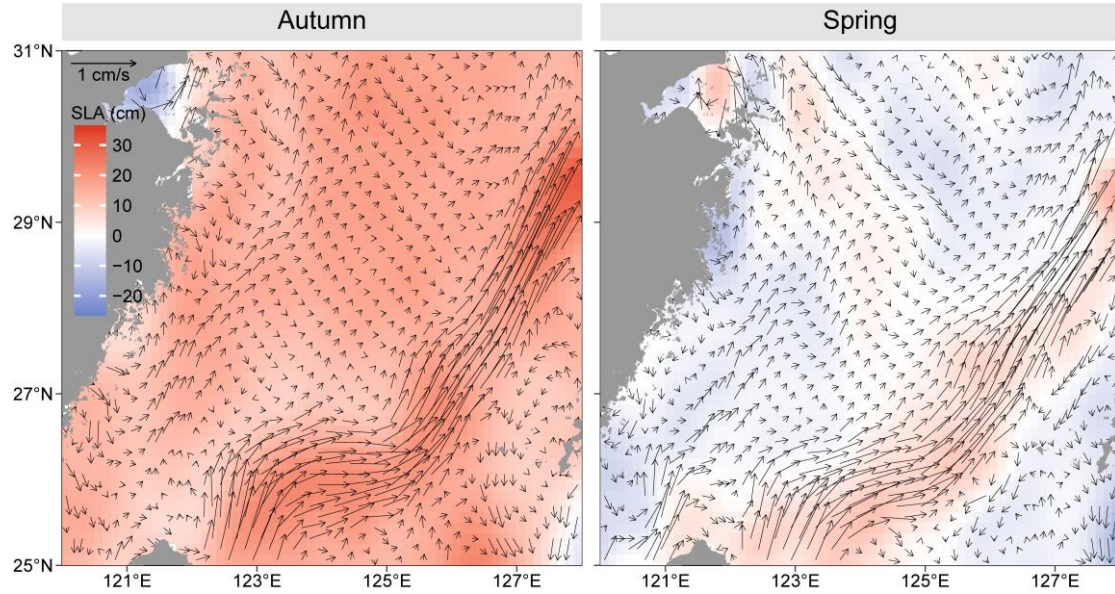


Figure S9. Average sea level anomaly (SLA, cm) and geostrophic current vectors (arrows) on the East China Sea shelf during autumn and spring cruises. The SLA and current data were obtained from Copernicus Marine and Environment Monitoring Service website (<http://marine.copernicus.eu/>), and land topography was obtained from the General Bathymetric Chart of the Oceans (GEBCO, <https://www.gebco.net/>, last access: 24 January 2025).

Table S6. The estimated N_2 fixation rate (NFR) on the East China Sea shelf during autumn and spring derived from polyploidy factor, cell-specific NFR and the targeted diazotroph abundances. The NFRs are shown as mean $\pm$ standard deviation.

Phylotype	Polyploidy factor	Cell-specific NFR (fmol N cell ⁻¹ d ⁻¹)	Autumn NFR		Spring NFR	
			Surface (nmol N L ⁻¹ d ⁻¹)	Depth integrated (μmol N m ⁻² d ⁻¹)	Surface (nmol N L ⁻¹ d ⁻¹)	Depth integrated (μmol N m ⁻² d ⁻¹)
<i>Trichodesmium</i>	12	53	0.73 $\pm$ 0.73	15.49 $\pm$ 12.15	0.11 $\pm$ 0.43	2.58 $\pm$ 8.67
Het-1	2.6	216	0.30 $\pm$ 0.84	7.10 $\pm$ 17.36	0.09 $\pm$ 0.35	3.97 $\pm$ 19.10
Het-2	2.6	216	0.18 $\pm$ 0.48	10.42 $\pm$ 22.46	0.09 $\pm$ 0.22	4.45 $\pm$ 11.74
UCYN-A1	14	2.7	0.00009	0.007	0.002 $\pm$ 0.01	0.11 $\pm$ 0.38
UCYN-A2	14	55	—	0.01 $\pm$ 0.04	0.01 $\pm$ 0.01	0.14 $\pm$ 0.38
UCYN-B	3.6	12.4	0.02 $\pm$ 0.04	0.86 $\pm$ 2.16	0.01 $\pm$ 0.02	0.28 $\pm$ 0.86
UCYN-C	1	92.4	—	—	0.04 $\pm$ 0.16	1.79 $\pm$ 8.37
γ -24774A11	1	162.5	0.23 $\pm$ 0.39	10.26 $\pm$ 16.18	0.26 $\pm$ 0.80	16.19 $\pm$ 63.48

Note: Average polyploidy factors for each diazotrophic phylotype were obtained from Shao et al. (2023) to convert cell-specific NFRs to *nifH*-specific rates. To avoid underestimating the contributions of *Trichodesmium* and Hets to NFRs, which exhibit high variability in cellular *nifH* copy numbers, moderate polyploidy factors were applied. For UCYN-C and γ -24774A11, a polyploidy factor of 1 was assumed due to limited data. Cell-specific NFRs for most phylotypes were calculated as means from published datasets (Shao et al., 2023). Cell-specific NFR for UCYN-C was derived from the average $^{15}N_2$ assimilation rates reported for its host *Epithemia pelagica*, normalized to a 15-hour daily fixation period (Schvarcz et al., 2022). For γ -24774A11, the cell-specific NFR was estimated based on the average NFR of the symbiosis with its diatom host, assuming one host diatom per four γ -24774A11 cells (Tschitschko et al., 2024). — no data.

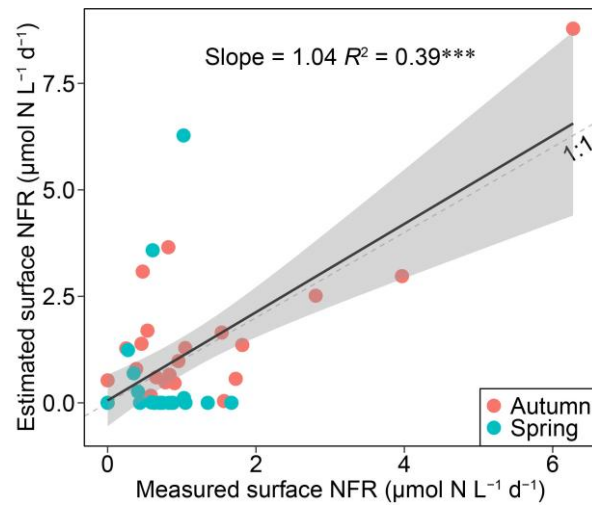


Figure S10. Linear regression of estimated versus measured surface N_2 fixation rates (NFRs). Estimates were derived from cell-specific NFRs, *nifH* gene polyploidy, and phylotype-specific *nifH* gene abundance. The shaded area represents the 95% confidence interval.

References

- Church, M., Jenkins, B., Karl, D., and Zehr, J.: Vertical distributions of nitrogen-fixing phylotypes at Stn ALOHA in the oligotrophic North Pacific Ocean, *Aquat. Microb. Ecol.*, 38, 3–14, <https://doi.org/10.3354/ame038003>, 2005a.
- Church, M. J., Short, C. M., Jenkins, B. D., Karl, D. M., and Zehr, J. P.: Temporal patterns of nitrogenase gene (*nifH*) expression in the oligotrophic North Pacific Ocean, *Appl. Environ. Microbiol.*, 71, 5362–5370, <https://doi.org/10.1128/AEM.71.9.5362-5370.2005>, 2005b.
- Foster, R. A., Subramaniam, A., Mahaffey, C., Carpenter, E. J., Capone, D. G., and Zehr, J. P.: Influence of the Amazon River plume on distributions of free-living and symbiotic cyanobacteria in the western tropical north Atlantic Ocean, *Limnol. Oceanogr.*, 52, 517–532, <https://doi.org/10.4319/lo.2007.52.2.0517>, 2007.
- Langlois, R. J., Hümmer, D., and LaRoche, J.: Abundances and distributions of the dominant *nifH* phylotypes in the northern Atlantic Ocean, *Appl. Environ. Microbiol.*, 74, 1922–1931, <https://doi.org/10.1128/AEM.01720-07>, 2008.
- Moisander, P. H., Beinart, R. A., Voss, M., and Zehr, J. P.: Diversity and abundance of diazotrophic microorganisms in the South China Sea during intermonsoon, *ISME J.*, 2, 954–967, <https://doi.org/10.1038/ismej.2008.51>, 2008.
- Moisander, P. H., Beinart, R. A., Hewson, I., White, A. E., Johnson, K. S., Carlson, C. A., Montoya, J. P., and Zehr, J. P.: Unicellular cyanobacterial distributions broaden the oceanic N₂ fixation domain, *Science*, 327, 1512–1514, <https://doi.org/10.1126/science.1185468>, 2010.
- Schvarcz, C. R., Wilson, S. T., Caffin, M., Stancheva, R., Li, Q., Turk-Kubo, K. A., White, A. E., Karl, D. M., Zehr, J. P., and Steward, G. F.: Overlooked and widespread pennate diatom-diazotroph symbioses in the sea, *Nat. Commun.*, 13, 799, <https://doi.org/10.1038/s41467-022-28065-6>, 2022.
- Shao, Z., Xu, Y., Wang, H., Luo, W., Wang, L., Huang, Y., Agawin, N. S. R., Ahmed, A., Benavides, M., Bentzon-Tilia, M., Berman-Frank, I., Berthelot, H., Biegala, I. C., Bif, M. B., Bode, A., Bonnet, S., Bronk, D. A., Brown, M. V., Campbell, L., Capone, D. G., Carpenter, E. J., Cassar, N., Chang, B. X., Chappell, D., Chen, Y. L., Church, M. J., Cornejo-Castillo, F. M., Detoni, A. M. S., Doney, S. C., Dupouy, C., Estrada, M., Fernandez, C., Fernández-Castro, B., Fonseca-Batista, D., Foster, R. A., Furuya, K., Garcia, N., Goto, K., Gago, J., Gradoville, M. R., Hamersley, M. R., Henke, B. A., Hörstmann, C., Jayakumar, A., Jiang, Z., Kao, S.-J., Karl, D. M., Kittu, L. R., Knapp, A. N., Kumar, S., LaRoche, J., Liu, H., Liu, J., Lory, C., Löscher, C. R., Maraño, E., Messer, L. F., Mills, M. M., Mohr, W., Moisander, P. H., Mahaffey, C., Moore, R., Mouriño-Carballido, B., Mulholland, M. R., Nakaoka, S., Needoba, J. A., Raes, E. J., Rahav, E., Ramírez-Cárdenas, T., Reeder, C. F., Riemann, L., Riou, V., Robidart, J. C., Sarma, V. V. S. S., Sato, T., Saxena, H., Selden, C., Seymour, J. R., Shi, D., Shiozaki, T., Singh, A., Sipler, R. E., Sun, J., Suzuki, K., Takahashi, K., Tan, Y., Tang, W., Tremblay, J.-É., Turk-Kubo, K., Wen, Z., White, A. E., Wilson, S. T., Yoshida, T., Zehr, J. P., Zhang, R., Zhang, Y., and Luo, Y.-W.: Global oceanic diazotroph database version 2 and elevated estimate of global oceanic N₂ fixation, *Earth Syst. Sci. Data*, 15, 3673–3709, <https://doi.org/10.5194/essd-15-3673-2023>, 2023.
- Thompson, A., Carter, B. J., Turk-Kubo, K., Malfatti, F., Azam, F., and Zehr, J. P.: Genetic diversity of the unicellular nitrogen-fixing cyanobacteria UCYN-A and its prymnesiophyte host: UCYN-A genetic diversity, *Environ. Microbiol.*, 16, 3238–3249, <https://doi.org/10.1111/1462-2920.12490>, 2014.

- Thomson, R. E. and Fine, I. V.: Estimating mixed layer depth from oceanic profile data, *J. Atmospheric Ocean. Technol.*, 20, 319–329, [https://doi.org/10.1175/1520-0426\(2003\)020%253C0319:EMLDFO%253E2.0.CO;2](https://doi.org/10.1175/1520-0426(2003)020%253C0319:EMLDFO%253E2.0.CO;2), 2003.
- Tschitschko, B., Esti, M., Philippi, M., Kidane, A. T., Littmann, S., Kitzinger, K., Speth, D. R., Li, S., Kraberg, A., Tienken, D., Marchant, H. K., Kartal, B., Milucka, J., Mohr, W., and Kuypers, M. M. M.: Rhizobia–diatom symbiosis fixes missing nitrogen in the ocean, *Nature*, 630, 899–904, <https://doi.org/10.1038/s41586-024-07495-w>, 2024.