Supplement of

Spatiotemporal heterogeneity in diazotrophic communities reveals novel niche zonation in 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
Trichodesmium	GACGAAGTATTGAAGCCAGGTTTC	CATTAAGTGTGTTGAATCTGGTGGTCCTGAGC	CGGCCAGCGCAACCTA	L00689	Church et al., 2005a
Het-1	CGGTTTCCGTGGTGTACGTT	TCCGGTGGTCCTGAGCCTGGTGT	AATACCACGACCCGCACAAC	DQ118191	Church et al., 2005b
Het-2	TGGTTACCGTGATGTACGTT	TCTGGTGGTCCTGAGCCTGGTGT	AATGCCGCGACCAGCACAAC	DQ225754	Foster et al., 2007
Het-3	CGGTTTCCGTGGCGTACGTT	TCTGGTGGTCCAGAACCTGGTGT	AATACCACGACCAGCACAAC	DQ225762	Foster et al., 2007
UCYN-A1	AGCTATAACAACGTTTATGCGTTGA	TCTGGTGGTCCTGAGCCTGGA	ACCACGACCAGCACATCCA	AF059627	Church et al., 2005a
UCYN-A2/A3/A4	GGTTACAACAACGTTTATGTGTTGA	TCTGGTGGTCCTGAGCCCGGA	ACCACGACCAGCACATCCA	KF806604	Thompson et al., 2014
UCYN-B	CGTAATGCTCGAAGGGTTTGA	CAAGTGTGTAGAATCTGGTGGTCCTGAGCC	CACGACCAGCACAACCAACT	AF299418	Moisander et al., 2010
UCYN-C	GGTATCCTTCAAGTAGTACTTCGTCTAGCT	AAACTACCATTCTTCACTTAGCAG	TCTACCCGTTTGATGCTACACACTAA	HQ455916	Langlois et al., 2008
γ-24774A11	CGGTAGAGGATCTTGAGCTTGAA	AAGTGCTTAAGGTTGGCTTTGGCGACA	CACCTGACTCCACGCACTTG	EU052318	Moisander et al., 2008, 2010

Table S2. Characteristics and parameter weights of each seawater type incorporated in the OMP analysis.

	CDW/CW	TWC	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; TWC, Taiwan warm current; KSW, Kuroshio surface water; KSSW, Kuroshio subsurface water.

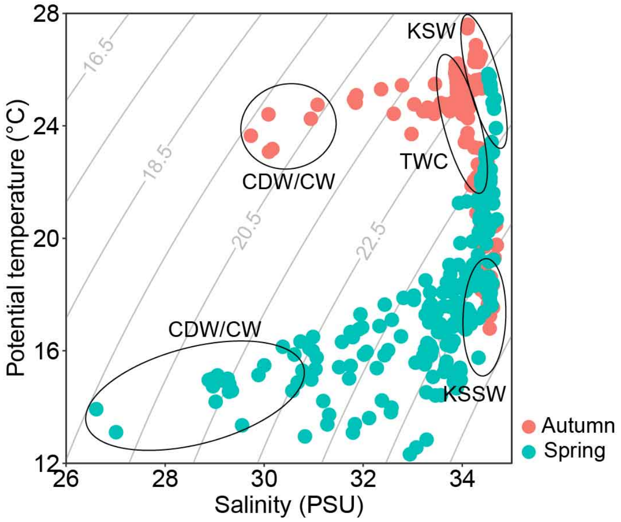

Figure S1. Plot of potential temperature and salinity at sampling depths influenced by mixing of distinct water massess during autumn and spring, with isopycnal density contours (grey lines) superimposed. CDW, Changjiang diluted water; CW, Coastal water; TWC, Taiwan warm current; KSW, Kuroshio surface water; KSSW, Kuroshio subsurface water.

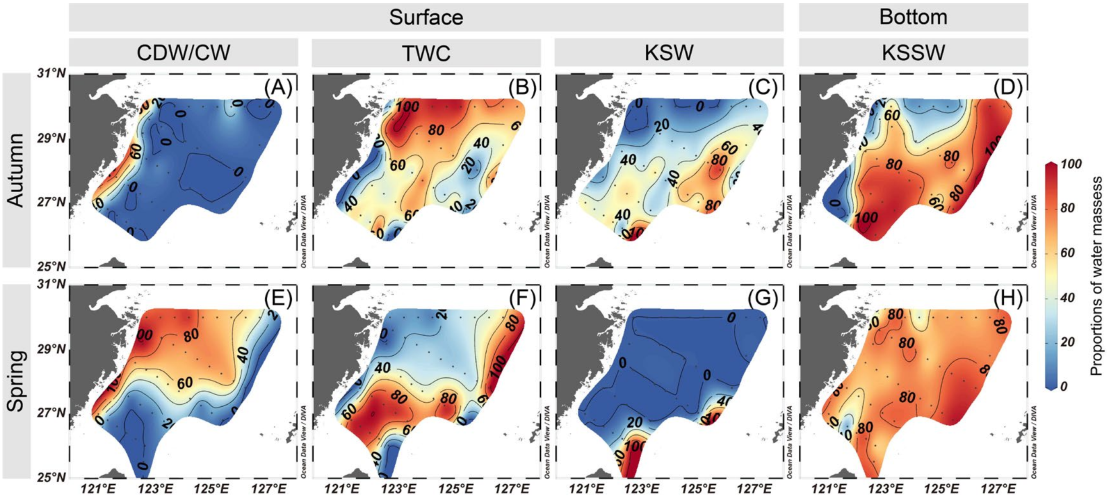

Figure S2. Proportions of water masses in the surface and bottom layers in the ECS during autumn (A–D) and spring (E–H) as determined with OMP analysis. CDW, Changjiang diluted water; CW, Coastal water; TWC, Taiwan warm current; KSW, Kuroshio surface water; KSSW, Kuroshio subsurface water.

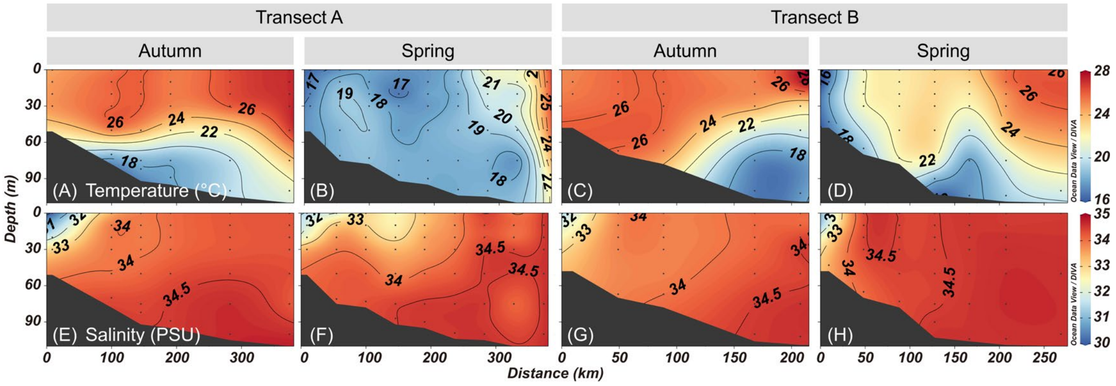

Figure S3. Vertical distributions in temperature (A–D) and salinity (E–H) along the transects A (stations 22–30) and B (stations 36–42) in the ECS during autumn and spring. The transect plots were created using Ocean Data View 5.7.2 (Schlitzer, Reiner, Ocean Data View, https://odv.awi.de, last access: 24 January 2025).

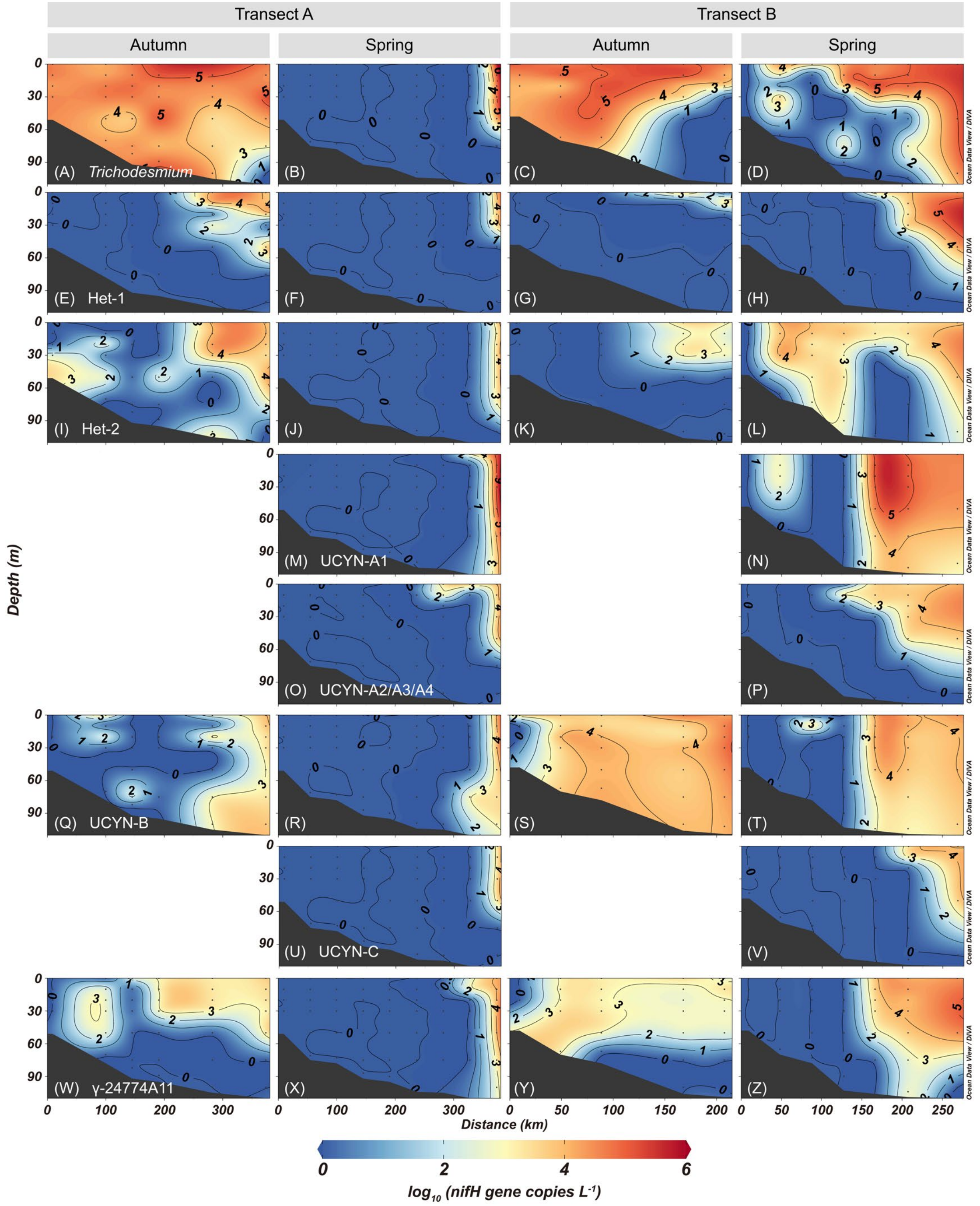

Figure S4. Vertical distributions in the abundances (Log$_{10}$ transformation) of the eight major diazotrophic phylotypes along the transects A (stations 22–30) and B (stations 36–42) in the ECS during autumn and spring as determined with TaqMan qPCR assay of the *nifH* gene. Note that UYCN-A1, UCYN-A2/A3/A4 and UCYN-C were undetectable along the transects A and B during autumn.

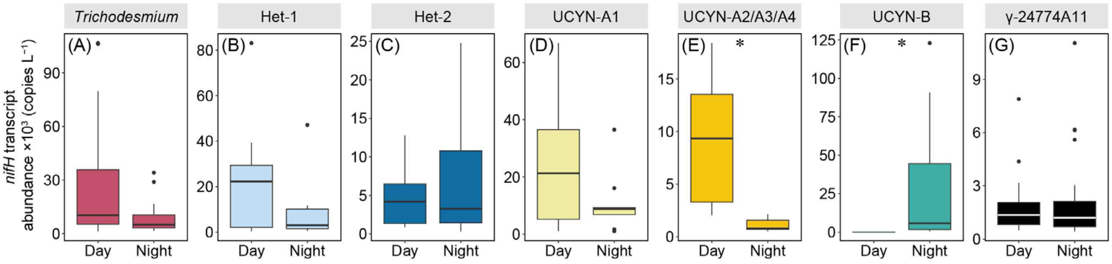

Figure S5. Differences in *nifH* transcript abundances of the seven major diazotrophic phylotypes in the ECS between day and night as determined by TaqMan qPCR assay. Note: *p<0.05.

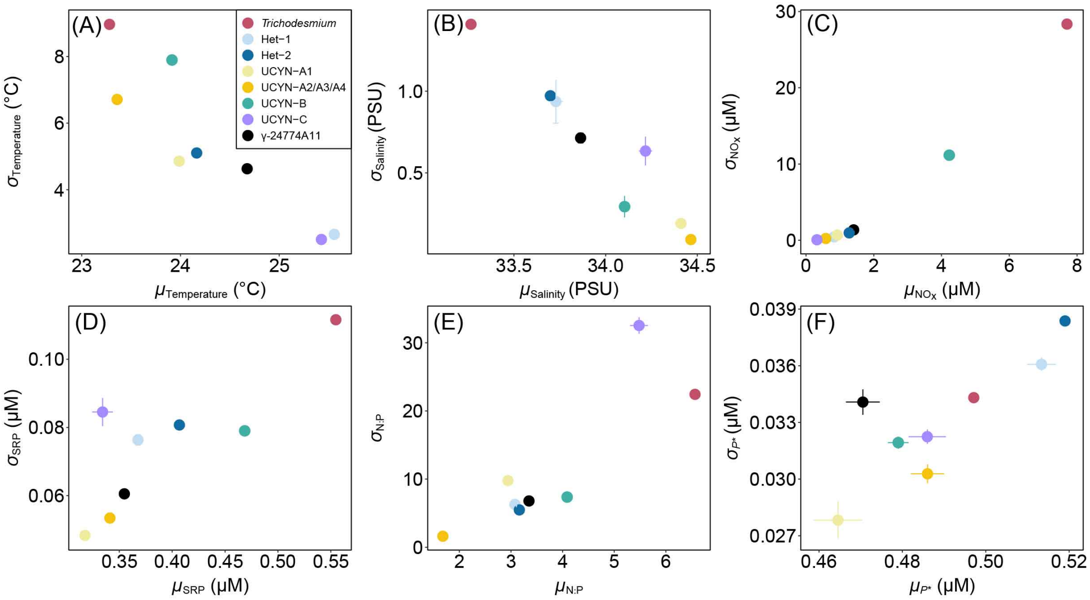

Figure S6. Niche mean (μ) and breadth (σ) corresponding to each individual environmental variable relative to each diazotrophic phylotype in the ECS. Colored lines associated with points indicate 95% confidence intervals for each variable derived from 100 bootstrap resampling.

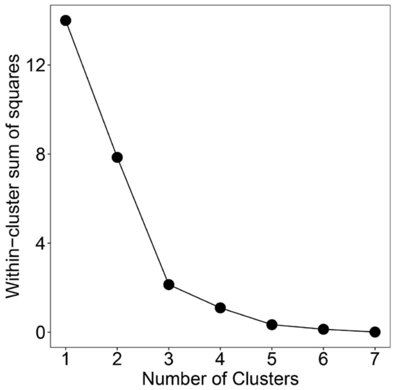

Figure S7. Elbow plot for determining the optimal clusters based on within-cluster sum of squares.

Table S3. The estimated N_2 fixation rate (NFR) in the ECS during autumn and spring derived from published, cell-specific NFR and depth-integrated abundances of the targeted diazotrophic phylotypes.

Phylotype	Cell-specific NFR (fmol N $cell^{-1}$ d^{-1})	Autumn NFR (μmol N m^{-2} d^{-1})	Spring NFR (μmol N m^{-2} d^{-1})
Trichodesmium	53	185.8	40.0
Het-1	216	18.5	10.3
Het-2	216	27.1	11.5
UCYN-A1	2.7	0.003	1.6
UCYN-A2	55	0.1	1.9
UCYN-B	26	6.5	2.1

Note: Cell-specific NFRs of diazotrophic phylotypes were calculated as mean values from published data (Shao et al., 2024).

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