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"Spatiotemporal heterogeneity in diazotrophic communities reveals novel niche zonation in the East China Sea" by Guangming Mai et al.

We have taken all the comments of the Reviewers into account in the revision. Our point-by-point responses are provided below in blue fonts. Please note that all the line numbers mentioned in the response refer to those in the Marked-up Manuscript.

Response to comments from Reviewer #1

General comments:

Mai et al. investigated diazotroph abundance and activity in the East China Sea using qPCR and in situ incubations, and attempted to elucidate niche zonation with a maximum-entropy ecological model. While the data collection effort is acknowledged, the data analysis and discussion are regrettably inadequate. The modeling framework is introduced without sufficient explanation or validation, and the many results diverge from established patterns (e.g., this study suggests Trichodesmium favors low temperature and high nutrients). Inconsistent results with previous knowledge can be valuable, but the authors do not examine them critically and instead cite mostly only studies that support their observations. Consequently, the discussion remains largely descriptive and does not elucidate or discuss the physio-ecological mechanisms underlying diazotroph niche partitioning. Because reports of diazotrophy around the Kuroshio region and importance of Kuroshio intrusion are no longer novel, the modeling approach could have been the key contribution of this study. However, the absence of careful interpretation and the lack of clear validation leave the novelty and significance of the work unclear.

Response:

We thank the Reviewer #1 for the very professional and constructive feedback. We have thoroughly revised the manuscript to address the issues and concerns, and accommodate them in so far as possible:

1. Enhanced model explanation and validation: Add detailed methodology and validation analyses for the maximum-entropy model (Section 2.5.3 and Table S4).

2. In-depth interpretation and discussion of inconsistent results with previous knowledge: Critically examine unexpected findings with expanded physiological and environmental interpretations (Section 4.3).

3. Balanced literature citation: Cite papers showing both consistent and conflicting results, and

strengthen the mechanistic discussion of niche partitioning (Section 4.3).

4. Emphasis on the importance of modeling approaches: Clarify the ecological insights gained from the models in the context of Kuroshio dynamics (Section 4.3).

We believe these revisions substantially improve the rigor and impact of our work.

Major Comments:

1. Model justification and validation

The rationale for using the maximum-entropy model is not sufficiently explained, nor is its validity demonstrated. The authors should describe the model in more detail and clarify why it is appropriate for their dataset. Also, I suppose that such models are not necessarily valid for all datasets, and thus model significance validation should be done carefully. For example, Brun et al. (2015) evaluated model performance/validity using the AUC of the ROC curve and a student's t-test with randomizations to exclude invalid models. A comparable validation is needed here. Without it, the model results cannot be considered reliable.

Response:

We thank the Reviewer #1 for this important point. We have added the description of maximum entropy model and its rationale in both the Introduction and Materials and Methods (Section 2.5.3). To validate the model, we followed the approach recommended by Brun et al. (2015), assessing the AUC of ROC curves and performing randomization Student's *t*-test. These analyses confirm the model's statistical significance and reliability, as summarized in Table S4.

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
Trichodesmium	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***	0.7***	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***	0.7***	0.74***	0.76***

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

2. Seemingly selective and sometimes inappropriate citations

References often appear cherry-picked to match the authors' results. For instance, in discussing phylotype-specific niche partitioning, they cite only studies whose outcomes align with their data, ignoring meta-analyses and culture experiments that already provide robust estimates of optimal temperature and nutrient ranges (details are mentioned in the specific comments). They should revise the citation thoroughly.

Response:

We thank the Reviewer #1 for this important point. We have revised the manuscript to include a more comprehensive and balanced set of references, such as the meta-analyses (Jiang et al., 2025; Tang and Cassar, 2019) and culture experiments (Knapp et al., 2012; Knapp, 2012), to better contextualize our findings.

3. RDA analysis

The RDA explains little of the variance of datasets (autumn: ~12 %, spring: ~34 %), yet the discussion relies on such weak relationship. RDA compresses relationships among multiple variables, but authors use them to claim direct correlations between specific taxa and single environmental parameters from RDA results. Also, collinearity among variables such as SRP, NOx, and depth is not tested or reported, VIF threshold should be set and reported. The authors should (i) report overall R^2 and significance of their RDA, (ii) test the significance of each variable's significance and contribution in RDA. More simple analysis such as simple correlation and multiple-variable linear regression such as GLM may be better for understanding controlling factors on each diazotroph.

Response:

We thank the Reviewer #1 for the insightful comment regarding the limitations of the RDA. We have removed the RDA entirely from the revised manuscript and replaced it with Pearson correlation analysis and significance test (Figure 7). This method allows us to directly evaluate the relationships between specific diazotrophs and individual environmental factors, providing a clear and statistically robust assessment of pairwise associations, thereby avoiding the multicollinearity issues inherent in the RDA. The updated correlation results are now presented and discussed as the main statistical support for our interpretations.

Line 208-210: "Pearson correlation analysis was adopted to explore relationship among *nifH* gene and transcript abundances (Log10 transformation), NFRs, environmental factors (z-score scaling), and distinct water masses (centered log ratio transformation) obtained from OMPA.".

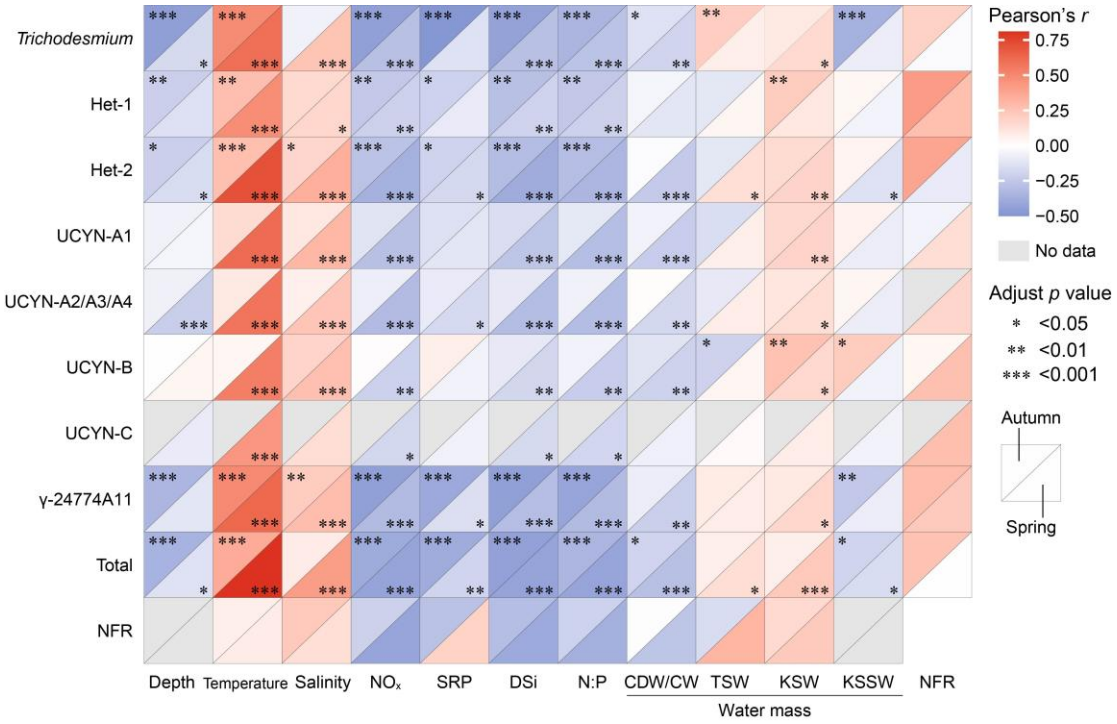


Figure 7. Pearson correlation between diazotroph abundances, N_2 fixation rates (NFRs) and environmental variables in distinct water masses on the East China Sea shelf during autumn and spring. Color gradient denotes Pearson correlation coefficients. Total, combined *nifH* gene abundances of the eight diazotrophs detected; CDW, Changjiang diluted water; CW, Coastal water; TSW, Taiwan Strait water; KSW, Kuroshio surface water; KSSW, Kuroshio subsurface water.

Specific comments:

Introduction

L69–70: Note that Shiozaki et al. (2018) also examined diazotroph abundance using qPCR.

Response:

We thank the Reviewer #1 for the comment. We have cited Shiozaki et al. (2018) in the revised manuscript.

Line 73-77: "Given the dominance of these diazotrophs in Kuroshio (Chen et al., 2014; Cheung et al., 2017, 2019; Shiozaki et al. 2018; Wu et al., 2018) and a currently disproportionate research focus on the filamentous *Trichodesmium* populations in individual seasons (Jiang et al., 2017, 2019,

2023a, b; Yue et al., 2021), systematic investigation into the compositional dynamics of diazotrophs across the ECS shelf is urgently needed.".

L68–73: Because authors study phylotype-specific niches, the introduction should briefly describe the major unicellular diazotrophs rather than grouping them simply as "unicellular."

Response:

We thank the Reviewer #1 for the comment. We have added the description of the unicellular diazotrophs in the revised manuscript.

Line 70-73: "However, the influence of water mass-driven hydrographic processes on other globally distributed diazotrophs in the ECS shelf, such as the unicellular cyanobacterial diazotroph (e.g., UCYN-B) and Haptophyta-associated nitroplasts (early-stage N_2-fixing organelles) (Coale et al., 2024; Cornejo-Castillo et al., 2024), have not been adequately examined.".

L77: The term "ecotype" generally refers to distinct strains within a species.

Response:

We thank the Reviewer #1 for the comment. We have replaced "ecotype" with "phylotype" in the revised manuscript.

Line 78-82: "Furthermore, water mass movements on the ECS shelf are seasonally modulated by the East Asian monsoon (Yin et al., 2018), and the seasonal variability in current patterns is projected to be strengthened in climate models (Vélez-Belchí et al., 2013; Yang et al., 2024), thus necessitating a comprehensive understanding of how increasingly intensified water mass movements may restructure the distribution of distinct diazotrophic phylotypes on the ECS shelf (Tang and Cassar, 2019).".

L81-84. Because most readers should not be familiar with maximum entropy method, authors should explain the method more detail.

Response:

We thank the Reviewer #1 for the comment. We have added the description of maximum entropy method in both the Introduction and Materials and Methods.

Line 86-93: "The Maximum Entropy model has proven particularly effective for

characterizing species distribution in realized niches, even from sparse field observations (Irwin et al., 2012; Phillips et al., 2006). This model is capable of identifying multidimensional niche spaces along key environmental gradients (e.g., temperature and nutrients) through regularization that maximizes environmental dependency while minimizing observational bias (Irwin et al., 2012; Phillips et al., 2006). A major advantage of this method is its ability to operate without absence data or uniform sampling effort in space or time, making it suitable for oceanographic studies that rely on opportunistic sampling (Brun et al., 2015; Irwin et al., 2012).".

Line 215-219: "The MaxEnt is a widely used species distribution model that estimates niches by combining presence-only species records with background-adjusted environmental data (Irwin et al., 2012). The quality predictive performance of MaxEnt allows it to effectively characterize species distributions even with limited data (Brun et al., 2015; Irwin et al., 2012).".

L89-90: Brun et al. studied *Trichodesmium* and *Richelia* realized niche. It should be mentioned here and in the discussion.

Response:

We thank the Reviewer #1 for the comment. We have mentioned the work of Brun et al. (2015) in both the Introduction and Discussion.

Line 97-100: "Despite the progress in modeling phytoplankton ecology in general, our understanding of the realized niches of diazotrophic communities remains fragmented, with only limited documentation on the modeling of realized niches of *Trichodesmium* and *Richelia* in the open ocean (Brun et al., 2015).".

Line 556-558: "However, the realized niches here were slightly cooler in temperature ($\mu_{temperature}$ = 24.2°C, $\sigma_{temperature}$ = 3.2°C) but substantially higher and broader in NO_x (μ_{NOx} = 2.5 µM, σ_{NOx} = 2.54 µM) relative to those of the open ocean (Brun et al., 2015).".

Line 598-600: "Compared with the niche conditions suitable for open-ocean populations (temperature: 24.2°C; NO_x: 1.38 µM) (Brun et al., 2015), Hets inhabited niches of slightly higher temperature but lower NO_x levels (Fig. 8A and C).".

Materials and Methods

L103: “summer” should be “autumn.”

Response:

We thank the Reviewer #1 for the comment. We have replaced “summer” with “autumn”.

Line 112-113: “We conducted a cross-season survey at 42 stations in the ECS shelf aboard the research vessel *Xiang Yang Hong 18* during the 2023 autumn (October 13–30) and 2024 spring (April 9–24) (Fig. 1).”.

L114: Anderson and Sarmiento (1994) did not introduce P*. To my knowledge, Deutsch et al. (2007) is the first.

Response:

We thank the Reviewer #1 for the comment. Given the high collinearity between *P** and soluble reactive phosphorus (SRP), we have deleted the questionable sentences in the revised manuscript.

L120: 200 μm mesh was used for RNA sample as well?

Response:

We thank the Reviewer #1 for the comment. We have clarified this by adding “(prefiltered with 200 μm)” in the sentence.

Line 124-127: “Seawater samples (prefiltered with 200 μm pore size mesh) for RNA analysis were collected at 7 stations in the upper 50 m using acid-rinsed Nalgene polycarbonate bottles during the day (13:00–15:30 local time) or at night (21:40–3:40 local time) to mirror diel variations in nitrogenase reductase gene (*nifH*) expression (Church et al., 2005b; Moisander et al., 2014).”.

L122–123: Fig. 6 here is inappropriate, considering the order of figure.

Response:

We thank the Reviewer #1 for the comment. We have removed “(Fig. 6)” from the sentence.

Line 130-131: “Surface seawater was also obtained from 24 stations of the study area to measure the rates of N_2 fixation, with details described below.”.

L147: Please provide the detection limit of the qPCR.

Response:

We thank the Reviewer #1 for the comment. We have added the description of the detection limit of qPCR in the revised manuscript:

Line 165-167: "The detection limit of the qPCR reactions was 10 *nifH* gene copies per reaction, corresponding to approximately 56–250 copies per liter of seawater.".

L148–150: Recent studies show that UCYN-C (Schvarcz et al. 2022, 2024) and γ-24774A11 (Tschitschko et al. 2024) are also likely diatom symbionts, almost organelle-like. This simplification should be described more carefully.

Response:

We thank the Reviewer #1 for the comment. We have deleted the sentence and provided clarification on the diazotrophic phylotypes in the revised manuscript.

Line 146-152: "Recent studies have demonstrated UCYN-A2, UCYN-C and γ-24774A11 as nitroplasts in the haptophyte *Braarudosphaera bigelowii* (Coale et al., 2024; Cornejo-Castillo et al., 2024) and diatoms (Schvarcz et al., 2022, 2024; Tschitschko et al., 2024). In contrast, other UCYN-A sublineages such as UCYN-A1 have not yet been confirmed to possess the same defining characteristics in its association with haptophytes (Coale et al., 2024; Kantor et al., 2024). Therefore, for clarity and consistency with prior literature, we classified UCYN-A2, UCYN-C, and γ-24774A11 as distinct haptophyte/diatom nitroplasts, while designating UCYN-A1 as other UCYN-A sublineages.".

L167: For transparency as suggested in (White et al. 2020), report the minimum quantifiable N2-fixation rate for each data and the value for each replicate as supplementary material.

Response:

We thank the Reviewer #1 for the comment. We have included the N_2 fixation rates and their respective detection limits for each replicate in Table S2.

Line 184-186: "The NFRs and detection limits were determined according to Montoya et al. (1996). The calculated detection limits for the NFRs ranged from 0.11 to 0.76 nmol N L^{-1} d^{-1} across the stations (Table S2).".

Table S2. N_2 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.

L179–183: Many readers will not be familiar with the MaxEnt model; provide a detailed description. Past studies sometimes exclude absence data, did this study do the same?

Response:

We thank the Reviewer #1 for the comment. We have added the description of MaxEnt and clarified the use of presence-only data in the revised manuscript.

Line 213-219: "Realized niches of diazotrophs in relation to environmental variables on the ECS shelf were determined in a combined framework implementing the Maximum Entropy (MaxEnt) (Phillips et al., 2006) and generalized additive model (GAM), as described by Irwin et al. (2012) and Xiao et al. (2018). The MaxEnt is a widely used species distribution model that estimates

niches by combining presence-only species records with background-adjusted environmental data (Irwin et al., 2012). The quality predictive performance of MaxEnt allows it to effectively characterize species distributions even with limited data (Brun et al., 2015; Irwin et al., 2012).”

Line 223-241: “To address this, an integrated MaxEnt-GAM framework was applied in this study, which estimates species presence probability and abundance before combining them through multiplication. This framework has been used to identify the realized niches of marine phytoplankton such as diatoms, haptophytes and cyanobacteria in the western Pacific marginal seas (Xiao et al., 2018; Zhong et al., 2020).

Given the complicated effects of collinearity among environmental variables on multivariate models in the MaxEnt and GAM, we assessed the response of each diazotrophic phylotype to each individual environmental driver (i.e., the univariate model). The models are formulated as follows:

$$f(x) = P(y = 1|x) \times C(x) \quad (1)$$

$$P(y = 1|x) = P(y = 1)g_1(x)/g(x) \quad (2)$$

$$C(x) = \alpha + s(x) + \varepsilon \quad (3)$$

where $f(x)$ denotes the diazotroph abundance determined by a specific environment, x. The conditional probability of detecting diazotrophic phylotype in the environment, $P(y = 1|x)$, is evaluated using Bayes' theorem of the MaxEnt. $P(y = 1)$ denotes the probability that diazotrophic phylotype would be found in a random sample. The probability distribution functions $g(x)$ and $g_1(x)$ are estimated from the environmental condition of all available background observations and from the conditions where the phylotype is present, respectively. $C(x)$ represents the estimated abundance of the diazotrophic phylotype as derived from GAM, using the same observational data as $g_1(x)$, but with abundance data (Log_{10} transformation) instead of presence-only data. The term $s(x)$ refers to a one-dimensional nonlinear function based on cubic regression splines, α is the grand mean, and ε denotes the error term.”.

L179: Define “breadth (σ).”, what does this mean?

Response:

We thank the Reviewer #1 for the comment. We have added the description of “breadth (σ)” in the revised manuscript:

Line 256-262: "Following Irwin et al. (2012), we characterized the univariate response curves, $f(x)$, with two parameters: niche mean (μ) and breadth (σ), calculated as:

$$\mu = \frac{\int x f(x) dx}{\int f(x) dx} \quad (4)$$

$$\sigma^2 = \frac{\int (x-\mu)^2 f(x) dx}{\int f(x) dx} \quad (5)$$

We used the μ and σ of all environmental variables to define the realized niches of each diazotrophic phylotype. Here, μ represents the central environmental condition, while σ indicates the phylotype's tolerance range for a given variable.".

L180: After integration, how many observations (n) were included?

Response:

We thank the Reviewer #1 for the comment. We have added the description of the observational data used in the MaxEnt-GAM framework in the revised manuscript:

Line 263-266: "Owing to limited data for some diazotrophic phylotypes, we integrated published data (Cheung et al., 2019; Sato et al., 2025; Shiozaki et al., 2018) with our own field observations on the ECS shelf (this study plus unpublished surface data from October–November 2022 and April–May 2023). The total number of data points utilized in the MaxEnt-GAM framework for each diazotrophic phylotype was presented in Table S4.".

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
Trichodesmium	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***	0.7***	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***	0.7***	0.74***	0.76***

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

L181: All parameter settings of MaxEnt should be clearly stated. Is it default setting?

Response:

We thank the Reviewer #1 for the comment. We have added the description of the MaxEnt parameter settings in the revised manuscript:

Line 242-244: "We employed MaxEnt software (version 3.4.3) for model fitting and related statistics. To minimize model overfitting and complexity, threshold features were disabled (Irwin et al., 2012), while linear, quadratic, product and hinge features were enabled. All other parameters remained at default values.".

L182: The GAM parameters is not explained and GAM result does not appear here after.

Response:

We thank the Reviewer #1 for the comment. We have added the description of the GAM parameter settings. The GAM results have been incorporated into the niche mean (μ) and breadth (σ).

Line 227-241: "Given the complicated effects of collinearity among environmental variables on multivariate models in the MaxEnt and GAM, we assessed the response of each diazotrophic phylotype to each individual environmental driver (i.e., the univariate model). The models are formulated as follows:

$$f(x) = P(y = 1|x) \times C(x) \quad (1)$$

$$P(y = 1|x) = P(y = 1)g_1(x)/g(x) \quad (2)$$

$$C(x) = \alpha + s(x) + \varepsilon \quad (3)$$

where $f(x)$ denotes the diazotroph abundance determined by a specific environment, x. The conditional probability of detecting diazotrophic phylotype in the environment, $P(y = 1|x)$, is evaluated using Bayes' theorem of the MaxEnt. $P(y = 1)$ denotes the probability that diazotrophic phylotype would be found in a random sample. The probability distribution functions $g(x)$ and $g_1(x)$ are estimated from the environmental condition of all available background observations and from the conditions where the phylotype is present, respectively. $C(x)$ represents the estimated abundance (Log_{10} transformation) of the diazotrophic phylotype as derived from GAM, using the same observational data as $g_1(x)$, but with abundance data instead of presence-only data. The term $s(x)$ refers to a one-dimensional nonlinear function based on cubic regression splines, α is the grand mean, and ε denotes the error term.".

Line 254-262: "The GAMs with default settings were similarly applied to the same bootstrapped samples using abundance data (Log_{10} transformation).

Following Irwin et al. (2012), we characterized the univariate response curves, $f(x)$, with two parameters: niche mean (μ) and breadth (σ), calculated as:

$$\mu = \frac{\int x f(x) dx}{\int f(x) dx} \quad (4)$$

$$\sigma^2 = \frac{\int (x-\mu)^2 f(x) dx}{\int f(x) dx} \quad (5)$$

We used the μ and σ of all environmental variables to define the realized niches of each diazotrophic phylotype. Here, μ represents the central environmental condition, while σ indicates the phylotype's tolerance range for a given variable."

L191: Z-score-scaling applied to PCA, as well?

Response:

We thank the Reviewer #1 for the comment. We have clarified the use of Z-score scaling in PCA in the revised manuscript.

Line 267-268: "Principal component analysis (PCA) was utilized to discern the relationships between diazotrophic phylotypes and the realized niches (z-score scaling).".

L193–194: Please clarify the VIF threshold used to avoid multicollinearity.

Response:

We thank the Reviewer #1 for the comment. In our study, Pearson correlation analysis coupled with significance test was used to examine the relationship between diazotroph abundances and individual environmental factors. Since this approach does not require assessing the variance inflation factor (VIF) of the environmental variables, VIF analysis was not performed in our study.

Results

L214–216: Provide more specific spatiotemporal deviations from the Redfield ratio (16).

Response:

We thank the Reviewer #1 for the comment. We have extended the description about spatiotemporal deviations from the Redfield ratio (16) in the revised manuscript.

Line 298-301: "The N:P ratio exhibited a lower range in autumn (1.3–19.5) compared to spring (0.4–37.3), but exceeded the Redfield ratio (16) in coastal waters in both seasons (autumn: 18.2 ± 1.18 at stations 1, 22 and 31; spring: 25.7 ± 6.98 at stations 1, 4, 12, 13, 14, 21 and 31), mirroring the spatial distribution pattern of NO_x (Fig. 2E and J).".

L222: Because nifH was not comprehensively amplified, "nifH gene pool of targeted diazotrophs" is more appropriate.

Response:

We thank the Reviewer #1 for the comment. For precision, we have revised manuscript accordingly.

Line 307-309: "*Trichodesmium* accounted for approximately 90% of the *nifH* gene pool of the targeted diazotrophs on the ECS shelf, with both the surface and depth-integrated abundances increasing along the coast-to-offshore transects.".

L223–225: Present quantitative numerical values.

Response:

We thank the Reviewer #1 for the comment. We have added quantitative numerical values in the revised manuscript.

Line 309-312: "Additionally, Hets (Het-1: 11533 ± 11039 copies L^{-1}; Het-2: 5439 ± 7317 copies L^{-1}), UCYN-B (9107 ± 13009 copies L^{-1}) and γ-24774A11 (1961 ± 2316 copies L^{-1}) were found primarily on the southeastern ECS shelf, with 1–2 orders of magnitude lower in abundance than *Trichodesmium*.".

L238: Transects A and B should be mentioned in Methods and figures 1.

Response:

We thank the Reviewer #1 for the comment. We have marked transects A and B in Figure 1 and have also referenced these transects in the Materials and Methods.

Line 131-132: "Additionally, two transects (A and B) spanning from nearshore to offshore waters were chosen to analyze vertical gradients of biological and environmental factors (Fig. 1).".

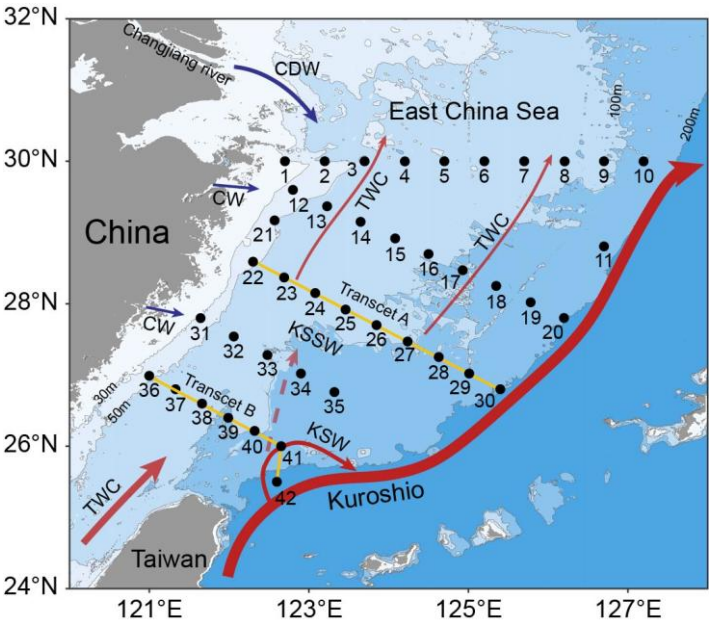


Figure 1. Sampling on the East China Sea (ECS) shelf during the 2023 autumn and 2024 spring cruises. A total of 42 stations along 5 transects were selected for the collection of biological samples and environmental parameters. The station numbers are positioned adjacent to corresponding points. Transects A (yellow line, stations 22–30) and B (yellow line, stations 36–42) were chosen to investigate variations in biological and environmental factors along the vertical gradient extending from inshore to offshore. Major circulations are indicated, including the Changjiang diluted water (CDW), Coastal water (CW), Taiwan warm current (TWC), Kuroshio current, Kuroshio surface water (KSW) and Kuroshio subsurface water (KSSW, dashed arrows) (Yang et al., 2012, 2018). Arrow sizes denote specific discharge rates (Liu et al., 2021). Land topography and ocean bathymetry data were obtained from the General Bathymetric Chart of the Oceans (GEBCO, https://www.gebco.net/, last access: 24 January 2025).

L242–243: The term “broader” is vague; specify how many of how many stations, or give quantitative metrics.

Response:

We thank the Reviewer #1 for the comment. We have added the number of stations detected for Het-1 and Het-2.

Line 329-331: “Among them, Het-2 showed a broader distribution (detected at 47 stations in autumn and 38 stations in spring) than Het-1 (12 stations per season) and peaked in the subsurface (30-m depth) (Fig. 4, S6E–L).”.

L243: UCYN-A, UCYN-C, and γ-24774A11 are now recognized as likely symbionts/organelles; grouping them as "unicellular diazotrophs" is questionable.

Response:

We thank the Reviewer #1 for the comment. We have removed "unicellular diazotrophs" from the sentence.

Line 331-335: "The diatom symbionts/organelles (i.e., UCYN-C and γ-24774A11) were most abundant (>10^3 copies L^{-1}) at depths of 0–50 m (Fig. 4, S6U–Z), demonstrating moderately deeper distributions than Hets. UCYN-A2/A3/A4 was similar to *Trichodesmium* in vertical distribution but with lower abundances (~10^4 copies L^{-1}) in spring, while UCYN-A1 and UCYN-B reached their highest abundance (>10^4 copies L^{-1}) at depths of 0–30 m (Fig. S6M–T).".

L243–245: This description conflicts with Fig. 5 (4). Het-2 peaks in the subsurface, and UCYN-A2/A3/A4 show profiles similar to *Trichodesmium*.

Response:

We thank the Reviewer #1 for the comment. We have revised the sentences accordingly.

Line 329-331: "Among them, Het-2 showed a broader distribution (detected at 47 stations in autumn and 38 stations in spring) than Het-1 (12 stations per season) and peaked in the subsurface (30-m depth) (Fig. 4, S6E–L).".

Line 333-335: "UCYN-A2/A3/A4 was similar to *Trichodesmium* in vertical distribution but with lower abundances (~10^4 copies L^{-1}) in spring, while UCYN-A1 and UCYN-B reached their highest abundance (>10^4 copies L^{-1}) at depths of 0–30 m (Fig. S6M–T).".

L246: Sampling was not performed at 60 m. If values are interpolated, please state that and use the actual sample depth (e.g., 50 m).

Response:

We thank the Reviewer #1 for the comment. We have replaced "60-m" with "50-m" in the revised manuscript.

Line 335-337: "Overall, diazotroph abundances decreased markedly below the 50-m layer, except for UCYN-A1 and UCYN-B which exhibited a moderate decline, possibly due to low temperature caused by KSSW intrusion (Fig. S4 and S6).".

L251: The phrase “were the most abundant among” is unclear; perhaps “were detected as the most abundant.”?

Response:

We thank the Reviewer #1 for the comment. We have replaced “were the most abundant” with “were detected as the most abundant” in the revised manuscript.

Line 341-344: “In autumn, the *nifH* transcripts of *Trichodesmium* were detected as the most abundant among 6 of the 7 stations sampled, ranging from 6.8×10^{3} to 1.7×10^{5} *nifH* transcripts L^{-1} at surface and from 5.1×10^{4} to 2.4×10^{6} *nifH* transcripts m^{-2} in the upper 50 m (Fig. 5A and C).”.

L269, L272: “CDW/CW-affected regions” should be defined quantitatively, e.g., by proportion of water masses.

Response:

We thank the Reviewer #1 for the comment. We have clarified the definition of the CDW/CW-affected regions by including the proportion of water masses in the revised manuscript.

Line 363-365: “In both seasons, the rates were higher at Kuroshio-influenced stations (e.g., 20, 30 and 42), but lower in the CDW/CW-affected regions (>60% of water mass; e.g., stations 1 and 31; Fig. S3A, C, E and G).”.

L270: Figure 6 does not have station 1.

Response:

We thank the Reviewer #1 for the comment. We have updated Figure 6 in the revised manuscript.

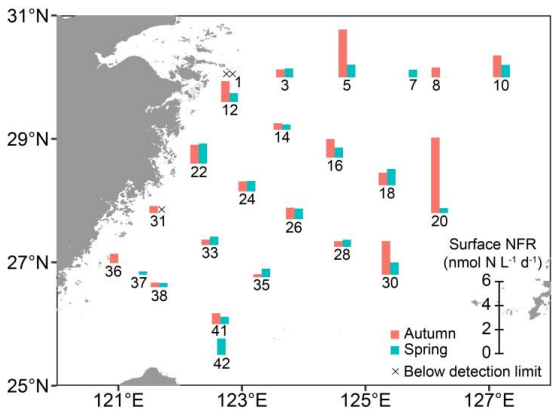


Figure 6. N_2 fixation rates in surface waters at designated stations on the East China Sea shelf during autumn and spring as determined with in situ isotope tracing. The station numbers are positioned adjacent to corresponding bar charts and crossover points. Sampling excluded stations 7, 37 and 42 in autumn, and stations 8 and 36 in spring. Land topography was obtained from the General Bathymetric Chart of the Oceans (GEBCO, https://www.gebco.net/, last access: 24 January 2025).

L271: "About 60%" is more appropriate.

Response:

We thank the Reviewer #1 for the comment. After re-evaluating the NFRs, which yielded a value of 54.8% (0.74/1.35), we feel that describing this proportion as "half" is now appropriate.

Line 360-363: "In autumn, the NFRs ranged from undetectable to 6.28 nmol N L^{-1} d^{-1} (1.35 ± 1.45 nmol N L^{-1} d^{-1} on average). In spring, the NFRs ranged from undetectable to 1.67 nmol N L^{-1} d^{-1} (0.74 ± 0.42 nmol N L^{-1} d^{-1} on average) and were about half of the autumn rates on average.".

L276–277: To support this, I recommend to perform multivariable regression for each phylotype and report the coefficients of determination, so that importance of each variable can be quantified.

Response:

We thank the Reviewer #1 for the suggestion. In our study, we used Pearson correlation analysis to examine the relationships between diazotroph abundances and environmental variables, including centered log ratio-transformed water masses. As a parametric method, Pearson correlation analysis is effective for identifying linear associations, which aligned with our goal of directly linking diazotroph distributions with distinct water masses. The resulting correlation coefficients are analogous to R^2 values in regression, effectively quantifying the strength and direction of each variable's link with phylotypes.

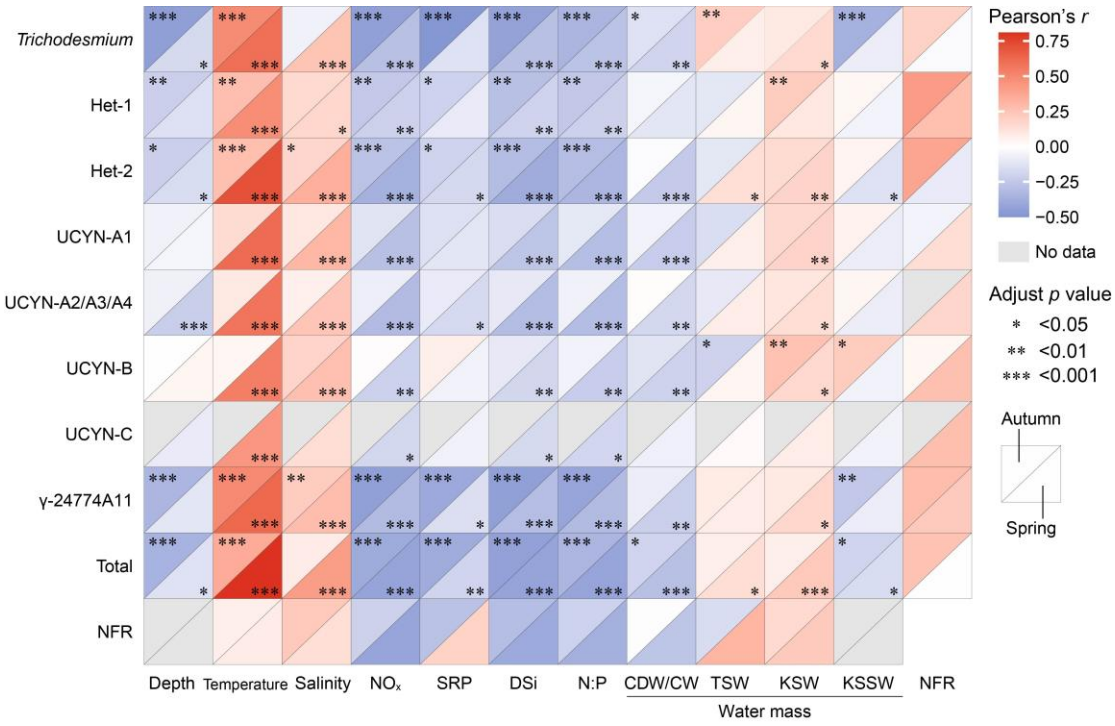


Figure 7. Pearson correlation between diazotroph abundances, N_2 fixation rates (NFRs) and environmental variables in distinct water masses on the East China Sea shelf during autumn and spring. Color gradient denotes Pearson correlation coefficients. Total, combined *nifH* gene abundances of the eight diazotrophs detected; CDW, Changjiang diluted water; CW, Coastal water; TSW, Taiwan Strait water; KSW, Kuroshio surface water; KSSW, Kuroshio subsurface water.

L281: Figure S6 should be moved into the main text; otherwise readers cannot follow the discussion of μ and σ. Also, please evaluate model validity as mentioned in major comments.

Response:

We thank the Reviewer #1 for the suggestion. After re-evaluating the data, we have merged the former Figures 8 and S6 into a new Figure 8 and have added the description of model validity in the revised manuscript.

Line 382-391: "Prior to the MaxEnt-GAM framework, model performance for the realized niches of each diazotrophic phylotype in relation to each individual environmental factor was assessed using AUC scores from MaxEnt. The AUC values ranged from 0.61 to 0.94 across diazotrophic phylotypes and environmental variables, with an average of 0.77 (Table S4), suggesting better predictive accuracy of the univariate models than the random predictions. Notably, UCYN-C achieved the highest model performance with an average AUC of 0.87 across all the variables, likely due to the relatively small dataset (N = 30; Table S4). In contrast, *Trichodesmium* yielded the lowest average AUC (0.66), which may be attributed to its larger sample

size (N = 242).

We characterized the realized niche mean (μ) and breadth (σ) of diazotrophs on the ECS shelf with combined hydrographic, environmental and biological data, identifying three distinct clusters among the diazotrophs (Fig. 8 and S8).".

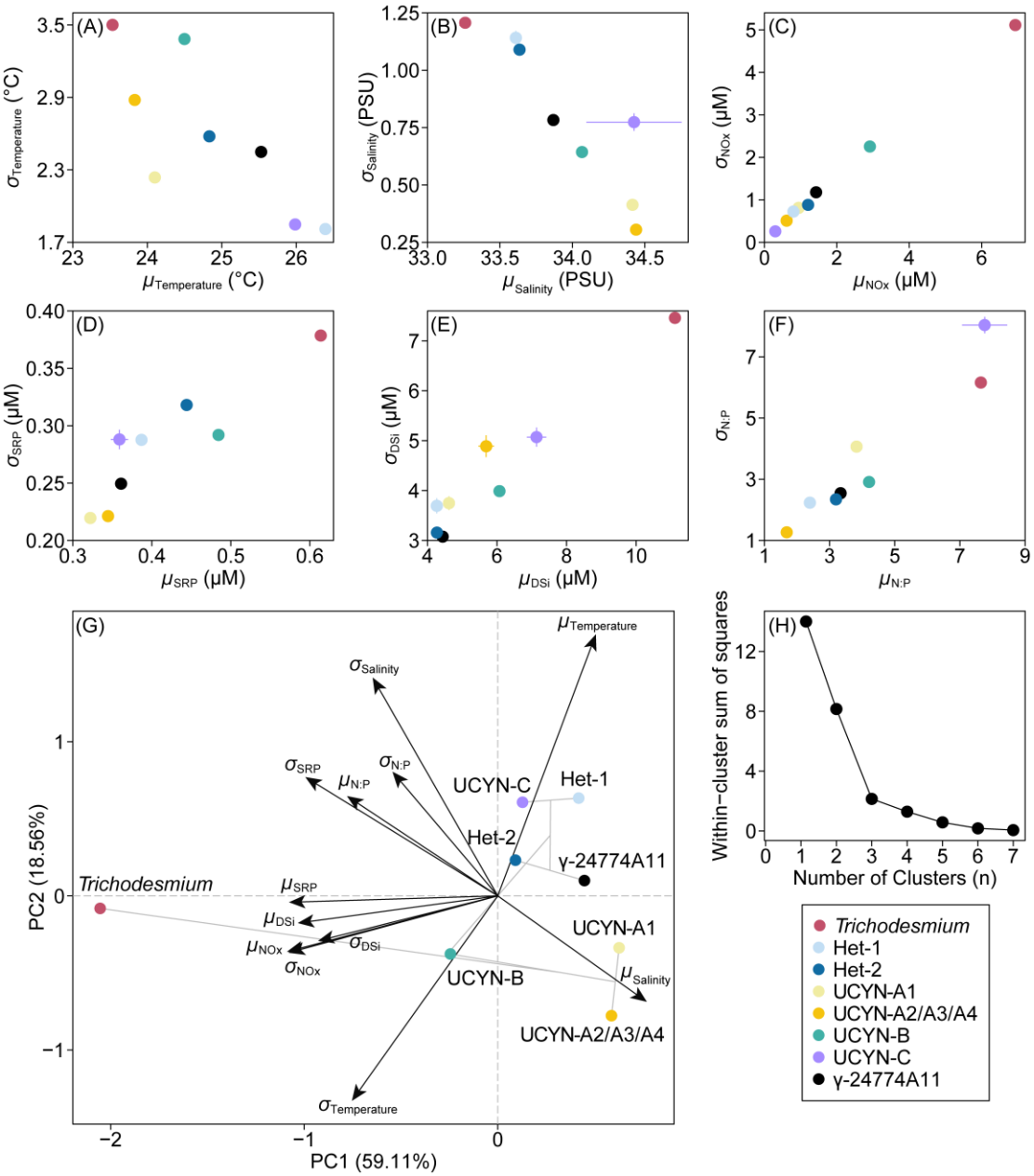


Figure 8. Scatter plots (A–F) and principal component analysis (G) depicting niche mean (μ) and breadth (σ) of each diazotrophic phylotype relative to environmental variables on the East China Sea shelf. Lines (A–F) across the data points indicate 95% confidence intervals for each variable derived from 1000 bootstrap resampling. Clustering (G) among the diazotrophs is shown in grey lines. Elbow plot (H) for determining the optimal clusters based on within-cluster sum of squares.

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
Trichodesmium	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***	0.7***	0.74***	0.76***

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

L281: From Fig. S7, n = 3 seems most appropriate; consider adding a hierarchical cluster tree to illustrate the threshold objectively.

Response:

We thank the Reviewer #1 for the comment. We have set n = 3 and incorporated a hierarchical cluster tree in the supplementary materials (now Figure S8), which provides an objective basis for this choice. We also have replaced "five" with "three" in the revised manuscript.

Line 389-391: "We characterized the realized niche mean (μ) and breadth (σ) of diazotrophs on the ECS shelf with combined hydrographic, environmental and biological data, identifying three distinct clusters among the diazotrophs (Fig. 8 and S8).".

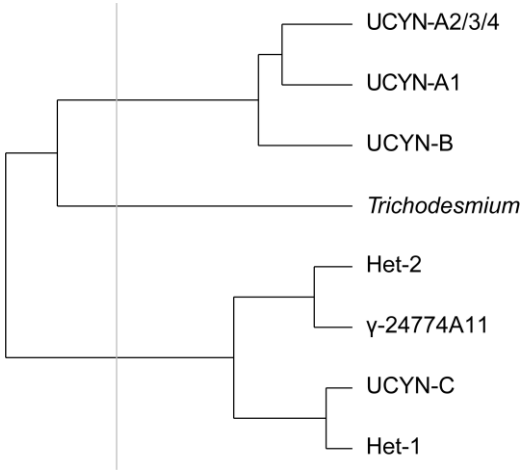


Figure S8. Hierarchical clustering of diazotrophs based on the first two PCA components, with optimal cluster number indicated by a gray line derived from elbow plot (Figure 8H).

L290: “Relatively high” should be described with quantitative data.

Response:

We thank the Reviewer #1 for the comment. We have revised the sentence and incorporated quantitative data to clarify the result accordingly.

Line 396-399: “The diatom-diazotroph symbioses (i.e., Hets, UCYN-C and γ-24774A11) were associated with high-temperature (25.7°C on average), low-NOx (0.98 μM on average) and moderate-SRP conditions (0.39 μM on average), demonstrating substantial ecological overlap among them.”.

Discussion

L295–297: Current ecological model (Fig. S6) shows Trichodesmium preferring low temperature, low salinity, and high nitrate waters. This contradicts both claim here and previous studies.

Response:

We thank the Reviewer #1 for the comment. The pattern in Fig. 8 (previously Fig. S6) reflects a new and careful interpretation of our model result, which stresses statistical correlation between *Trichodesmium* distribution and environmental variables in the study region. The seemingly controversy “preference for lower temperature (Fig. 8A) and higher NO_x (Fig. 8C)” is only used to describe *Trichodesmium*’s presence in the CDW/CW-influenced waters (Fig. 2A, 3A and S3A). But more importantly, the model demonstrates that *Trichodesmium* exhibits a broad range of both temperature and NO_x tolerance (Fig. 8A and C), suggesting its ability to survive in both cool, NO_x-rich and warm, NO_x-poor waters. On the warm, NO_x-depleted outer ECS shelf, the capability of nitrogen fixation is advantageous to *Trichodesmium* distribution, which outcompete non-diazotrophs under severe nitrogen limitation. Thus, the model unfolds the whole picture of *Trichodesmium* habitats. Our observation is overall consistent with previous knowledge on *Trichosdesmium* physiology, in the conclusion that nitrogen limitation is the key factor enabling its preference on the oligotrophic outer ECS shelf. We have also clarified this competitive advantage of *Trichodesmium* under the warm, NO_x-depleted waters in the revised manuscript.

Line 417-420: “Notably, while our modeling result indicated *Trichodesmium* can survive in relatively cool (23.5°C), NO_x-rich environments (6.93 μM; Fig. 8), it was detected the most abundant on the warm, NO_x-depleted outer ECS shelf (Fig. 3), a pattern that is consistent with the

basal physiology of *Trichodesmium* (Brun et al., 2015; Jiang et al., 2025; Tang and Cassar, 2019).".

L298: growth was not measured in this study.

Response:

We thank the Reviewer #1 for the comment. We have removed "growth" from the sentence in the revised manuscript.

Line 406-408: "In contrast, their abundances were considerably lower in the cold, NO_x-rich coastal waters influenced by the CDW/CW (Fig. S3A and E), showing positive correlation with temperatures but negative correlation with nutrient concentrations and N:P ratios (Fig. 7).".

L300–301: Relationships inferred from RDA are weak and not checked stastically.

Response:

We thank the Reviewer #1 for the comment. We have replaced the RDA with Pearson correlation analysis, supplemented with statistical significance test.

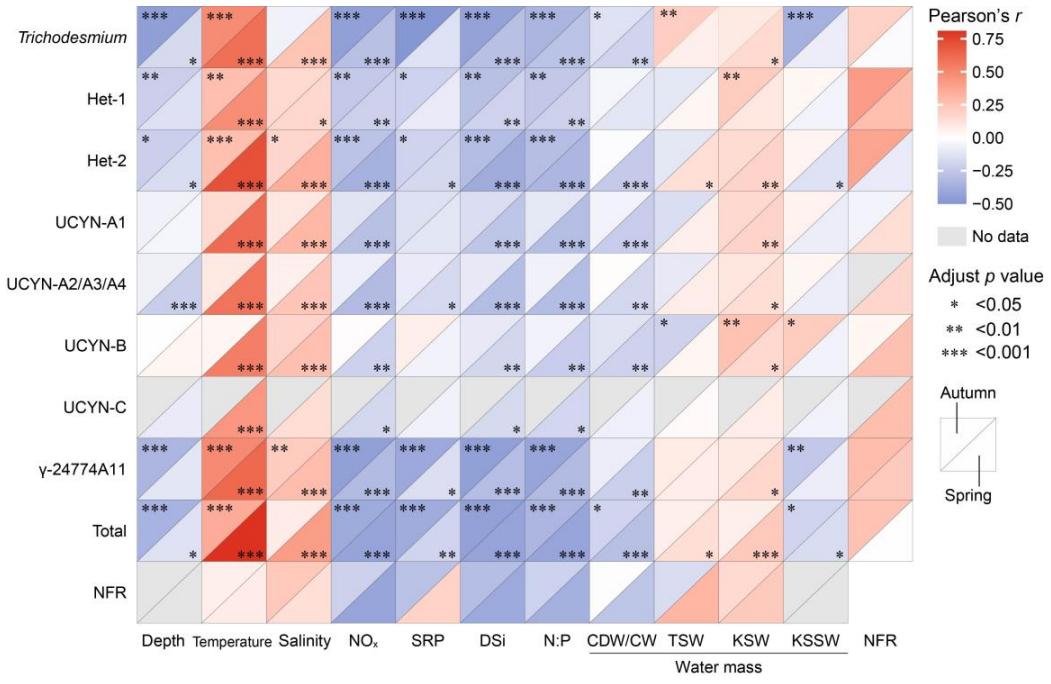


Figure 7. Pearson correlation between diazotroph abundances, N_2 fixation rates (NFRs) and environmental variables in distinct water masses on the East China Sea shelf during autumn and spring. Color gradient denotes Pearson correlation coefficients. Total, combined *nifH* gene abundances of the eight diazotrophs detected; CDW, Changjiang diluted water; CW, Coastal water; TSW, Taiwan Strait water; KSW, Kuroshio surface water; KSSW, Kuroshio subsurface water.

L303: “Below detection limit” does not mean complete absence.

Response:

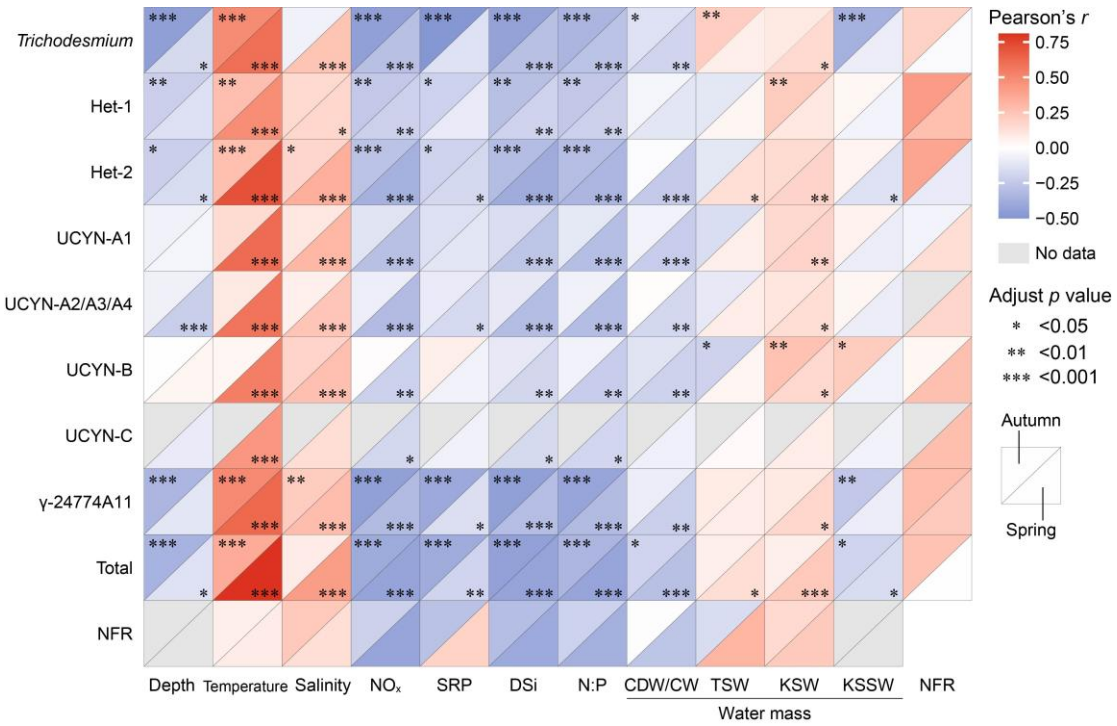
We thank the Reviewer #1 for the comment. We have replaced “but complete absence” with “while these phylotypes were not detected” in the revised manuscript.

Line 421-423: “Additionally, non-filamentous diazotrophs (UCYN-A, UCYN-B, UCYN-C and γ-24774A11) were abundant (>5×10^4 *nifH* gene copies L^{-1}) in Kuroshio-influenced waters, but not detectable in the CDW/CW-dominated regions (Fig. 3 and S3).”.

L307–309: Conclusions based solely on weak RDA correlations are too qualitative.

Response:

We thank the Reviewer #1 for the comment. We have replaced the RDA with Pearson correlation analysis, supplemented with statistical significance test.



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L309–311: To demonstrate major N2 fixers, statistically test relationships between gene (or transcript) abundance and N2-fixation rate. High nifH abundance alone is insufficient.

Response:

We thank the Reviewer #1 for the comment. We have performed Pearson correlation analysis to assess the relationship between diazotroph abundances and N_2 fixation rates in the revised manuscript.

Line 428-433: "Overall, filamentous diazotrophs were the primary N_2 fixers on the ECS shelf (Jiang et al., 2023a, b), as illustrated by the prevalence of their *nifH* gene (~84% of all the *nifH* copies detected; Fig. 3) that was positively correlated with NFRs (Fig. 7) across most of the surveyed areas. On the other hand, UCYN-A1, UCYN-B and γ-24774A11 were only occasionally found in the Kuroshio-influenced regions (Fig. 3 and S3), and the correlation of their *nifH* gene abundances with NFRs (Fig. 7) suggests their potential contribution to regional N_2 fixation (Chen et al., 2014; Wu et al., 2018).".

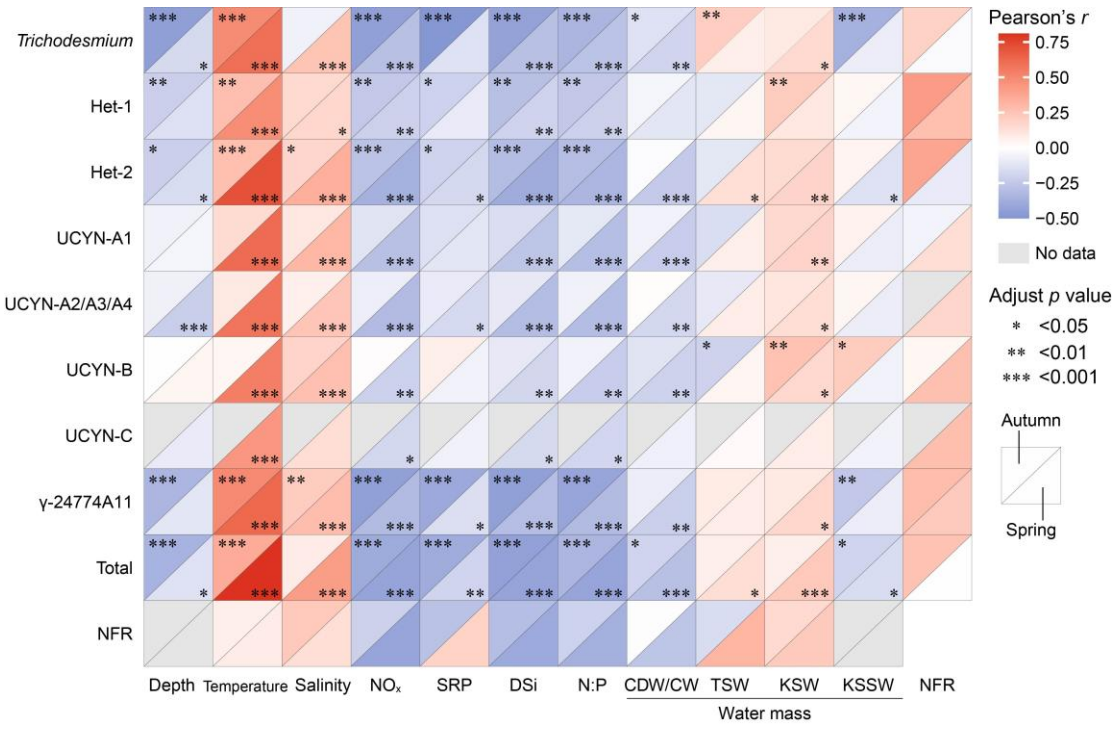

Figure 7. Pearson correlation between diazotroph abundances, N_2 fixation rates (NFRs) and environmental variables in distinct water masses on the East China Sea shelf during autumn and spring. Color gradient denotes Pearson correlation coefficients. Total, combined *nifH* gene abundances of the eight diazotrophs detected; CDW, Changjiang diluted water; CW, Coastal water; TSW, Taiwan Strait water; KSW, Kuroshio surface water; KSSW, Kuroshio subsurface water.

L314–315: Many studies detected Trichodesmium colonies in the ECS (e. g. Marumo and Asaoka 1974, Jiang et al. 2023). Richelia colonies are also expected to removed by this mesh filtering. Was microscopy performed in this study? They should provide more solid evidence of not-underestimating, otherwise it is not convincing.

Response:

We completely agree with the Reviewer #1 that prefiltration using a 200 μm pore-size nylon mesh may potentially remove large colonies of *Trichodesmium* and *Richelia*. As microscopy was not performed, we have revised the Discussion section to explicitly state that the abundance for these colonial diazotrophs may be underestimated.

Line 433-435: "It should be noted that the abundance of colonial diazotrophs like *Trichodesmium* and Hets may be potentially underestimated due to the use of <200-μm size fraction in our study (e.g., Jiang et al., 2023a).".

L319: Jiang et al. (2018, 2019) reported highest densities at the surface and 10–50 m, consistent with your results. Carpenter et al. (2004) also found maxima at ~20 m. Discussion here seems incorrect.

Response:

We thank the Reviewer #1 for the comment. We have revised the sentence accordingly.

Line 437-440: "Specifically, *Trichodesmium* showed maximum abundance in the upper 30 m (Fig. S6A–D), consistent with earlier reports of surface or subsurface maxima (10–30 m) on the ECS shelf (Jiang et al., 2018, 2019) and other regions (Carpenter et al., 2004; Chen et al., 2003; Lu et al, 2019).".

L323: If eddy is important, provide and check the SSH and its anomaly information during study period.

Response:

We thank the Reviewer #1 for the comment. We have included the sea surface height anomaly and geostrophic current vectors during the study period in Figure S9 in the revised manuscript.

Line 442-444: "Additionally, the absence of anticyclonic eddies during our surveys is likely to eliminate the downward entrainment of *Trichodesmium* in deeper water column (e.g., 50–60 m) (Fig. S9) (Jiang et al., 2018).".

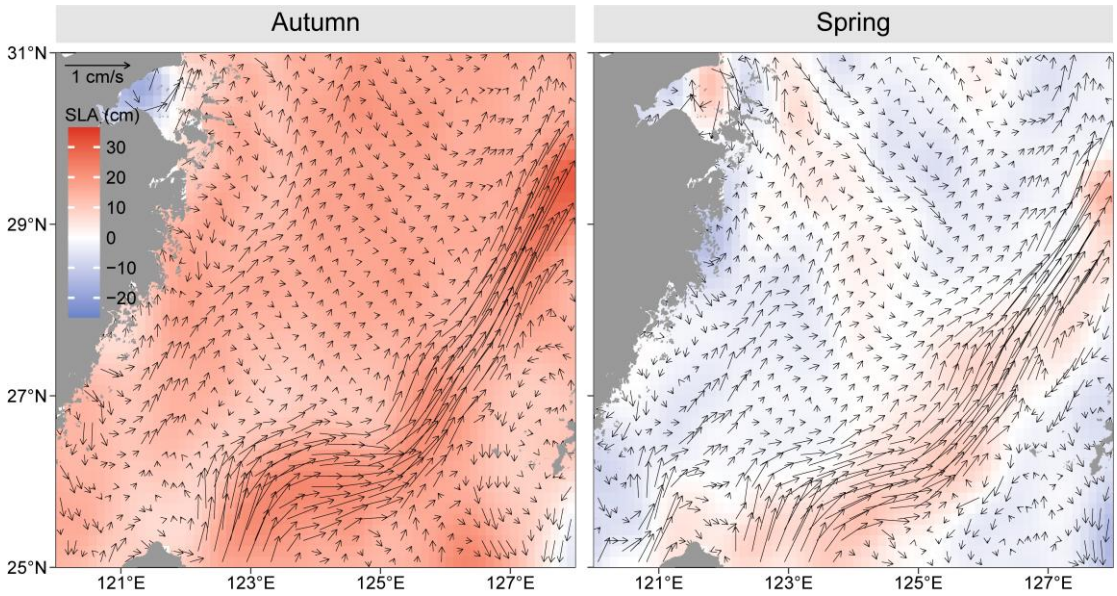

Figure S9. Average sea level anomaly (SLA, cm) and geostrophic current vectors (arrows) on the East China Sea shelf during autumn and spring cruises. The SLA and current data were obtained from Copernicus Marine and Environment Monitoring Service website (http://marine.copernicus.eu/), and land topography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

L324: Only UCYN-C and γ-24774A11 peak at 50 m; others peak above 30 m. Thus filamentous and unicellular diazotrophs are not "in contrast."

Response:

We thank the Reviewer #1 for the comment. We have revised the sentences accordingly.

Line 437-459: "Specifically, *Trichodesmium* showed maximum abundance in the upper 30 m (Fig. S6A–D), consistent with earlier reports of surface or subsurface maxima (10–30 m) on the ECS shelf (Jiang et al., 2018, 2019) and other regions (Carpenter et al., 2004; Chen et al., 2003; Lu et al, 2019). This depth distribution may be attributed to favorable nutrient conditions (e.g., SRP and iron) and light intensity (Turk-Kubo et al., 2018; Wen et al., 2022), which support *Trichodesmium* growth on the site of the ongoing surveys (Fig. 2D and I). Additionally, the absence of anticyclonic eddies during our surveys is likely to eliminate the downward entrainment of *Trichodesmium* in deeper water column (e.g., 50–60 m) (Fig. S9) (Jiang et al., 2018). The filamentous diazotrophs Het-1 and Het-2 showed distinct maximum depths (surface versus 30 m; Fig. 4), contrasting with previously documented subsurface maxima (10–40 m) for both phylotypes on the ECS shelf (Jiang et al., 2019), the tropical North Atlantic (Foster et al., 2007; Goebel et al., 2010) and the western tropical South Pacific (Stenegren et al., 2018). This may be due to different

habitat preferences between the Het hosts, as the Het-1 host was found mainly under NO_x-depleted conditions but not in the deeper, NO_x-repleted water column (Tuo et al., 2014). Alternatively, the differences in thermal sensitivity between Het-1 and Het-2 may explain their vertical distribution, as Het-1 lives within a relatively narrower temperature range (20.4–27.5°C) than Het-2 (17.6–27.6°C; Fig. S4). In contrast, the other two diatom-diazotroph symbioses (UCYN-C and γ-24774A11) showed both surface and subsurface maxima (~50 m; Fig. 4). The deep distributions may result from the low light tolerance of their diatom hosts, as both symbionts lack photosynthetic pigments and rely entirely on carbon fixed by their hosts (Schvarcz et al., 2022, 2024; Tschitschko et al., 2024). Similar subsurface distributions have been observed in UCYN-C at Station ALOHA (Schvarcz et al., 2022) and γ-24774A11 in the northern South China Sea (SCS; Chen et al., 2019; Lu et al., 2019; Shao and Luo, 2022).

Similar to the filamentous diazotrophs, UCYN-A and UCYN-B were mostly confined to the upper 30 m, matching observations from the northern SCS (Lu et al., 2019) and global oceans (Tang and Cassar, 2019).".

L329: Lu et al. 2018 does not seem to provide the information of light adaptation of unicellular cyanobacterial diazotrophs?

Response:

We thank the Reviewer #1 for the comment. We have removed the citation of Lu et al. (2018) and revised the sentence accordingly.

Line 460-462: "However, UCYN-A1 and UCYN-B were moderately abundant (~10^3 copies L^{-1}) in deep layers (Fig. S6M, N, Q–T), potentially due to their low light saturation coefficients (Garcia et al., 2013; Gradoville et al., 2021; Shen et al., 2024).".

L332: ~22°C for optimal temp. of UCYN-B is not common understanding. Also, Webb et al 2009 report 26-30°C for its optimal temperature. Other studies also reported ~25–30 °C (Tang and Cassar 2019; Mauda 2024).

Response:

We thank the Reviewer #1 for the comment. We have revised the sentences and clarified UCYN-B's persistence in cold environments in the revised manuscript.

Line 466-473: "However, such low temperatures may inhibit UCYN-B, which has an optimal growth temperature around 25–30°C (Fu et al., 2014; Jiang et al., 2025; Tang and Cassar, 2019; Webb et al., 2009). Nevertheless, studies have indicated that UCYN-B can persist in cold environments, albeit at low abundances (Jiang et al., 2025; Tang and Cassar, 2019). Low-temperature tolerance of UCYN-B has also been demonstrated in the laboratory (Deng et al. 2022), however, the lowest threshold temperature used for the bottle incubation (25°C) is substantially higher than the in situ temperatures (~10–30°C) in the field (Tang and Cassar, 2019). Therefore, the distribution of UCYN-B under such low-temperature conditions warrants further investigation.".

L334–338: Tschitschko et al. 2024 in Nature strongly (almost conclusively) indicate that γ-24774A11 is a diatom symbiont. Include this information.

Response:

We thank the Reviewer #1 for the comment. We have incorporated this information into the sentence accordingly.

Line 451-453: "In contrast, the other two diatom-diazotroph symbioses (UCYN-C and γ-24774A11) showed both surface and subsurface maxima (~50 m; Fig. 4).".

L346: Koening et al. (2009) only speculated about an r-strategy for Trichodesmium; they did not conclude it. Citing this paper as proof in current manner is inappropriate.

Response:

We thank the Reviewer #1 for the comment. We have removed the citation of Koening et al. (2009) and revised the sentence accordingly.

Line 482-486: "It has been suggested that iron deficiency limits *Trichodesmium* growth (Berman-Frank et al., 2001). Given that SRP (0.2–0.4 μM) did not appear to be a limiting factor (Fig. 2D), the dominance of *Trichodesmium* on the ECS shelf is likely due to the high level of dissolved iron (0.76–30 nM) transported via the Kuroshio and TSW (Shiozaki et al., 2015; Su et al., 2015) or delivered via aerial dust deposition (Guo et al., 2014).".

L351–353: This statement seems contradict the Maxent based discussion.

Response:

We thank the Reviewer #1 for the comment. The MaxEnt-GAM framework predicts the potential distribution based on the species' occurrence, which spans a wide range of temperatures and NO_x. However, the discussion here focuses on its ecological performance, where it becomes dominant. Although it can exist in cool, NO_x-rich waters (as shown by the MaxEnt-GAM; Fig. S6A), its abundance is significantly higher in the warm, NO_x-poor waters (i.e., preferred and dominant habitat). Thus, there is no contradiction between our statement and the MaxEnt-GAM-based discussion.

L360: Please quantitatively compare your data values with those of Jiang et al. 2023 with actual value.

Response:

We thank the Reviewer #1 for the comment. We have included the quantitative comparison of our data with Jiang et al. (2023) in the revised manuscript.

Line 500-502: "The averaged autumn NFR (1.35 nmol N L^{-1} d^{-1}) was similar to what has been reported during summer on the ECS shelf (1.54 nmol N L^{-1} d^{-1}) (Jiang et al., 2023a), despite the differences in NFR measurement between the two studies (i.e., dissolution versus bubble methods).".

L364–367: Only one phylotype of non-cyanobacterial diazotroph (NCD) was quantified. Many other NCDs likely exist; please acknowledge this limitation.

Response:

We thank the Reviewer #1 for the comment. We have incorporated the discussion of this limitation in the revised manuscript.

Line 505-509: "On the other hand, the low temperature (14–20°C) in spring could otherwise promote the prevalence of non-cyanobacterial diazotrophs (NCDs) and their contribution to N_2 fixation, particularly in CDW/CW-affected waters (Jiang et al., 2023b), but this pattern was not comprehensively analyzed in this study since only one phylotype of NCD (γ-24774A11) was quantified with qPCR (Fig. 3 and 6).".

L367–368: A simple correlation analysis between N2-fixation rate and nifH transcript abundance is needed to support this argument.

Response:

We thank the Reviewer #1 for the comment. We have performed Pearson correlation analysis between N_2 fixation rate and *nifH* transcript abundance and have included the results in Table S5 in the revised manuscript.

Line 509-512: "Notably, the seasonal dynamics of NFRs showed negative, but not significant, correlation with transcriptional abundances of diazotrophs (Fig. 5 and 6; Table S5), suggesting that the expression of *nifH* gene in diazotrophs may not be synchronized with actual nitrogenase activity (Turk-Kubo et al., 2012).".

Table S5. Pearson correlation coefficients between surface N_2 fixation rates (NFRs) and diazotroph transcripts on the East China Sea shelf.

Parameter	*Trichodesmium*	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: [a] NFRs 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.

L370: Recent studies report cell-specific rates for UCYN-C (Schvarcz et al. 2022) and γ-24774A11 (Tschitschko et al. 2024). Also, please provide standard deviations.

Response:

We thank the Reviewer #1 for the comment. We have incorporated the cell-specific NFRs of UCYN-C and γ-24774A11 into the calculation of bulk rates and added standard deviations of estimated NFRs in Table S6.

Line 513-514: "To assess the contribution of key diazotrophs to the N budget, we extrapolated water-column NFRs using published, cell-specific rates for the targeted diazotrophs (Table S6).".

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 ± 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})
Trichodesmium	12	53	0.73 ± 0.73	15.49 ± 12.15	0.11 ± 0.43	2.58 ± 8.67
Het-1	2.6	216	0.30 ± 0.84	7.10 ± 17.36	0.09 ± 0.35	3.97 ± 19.10
Het-2	2.6	216	0.18 ± 0.48	10.42 ± 22.46	0.09 ± 0.22	4.45 ± 11.74
UCYN-A1	14	2.7	0.00009	0.007	0.002 ± 0.01	0.11 ± 0.38
UCYN-A2	14	55	—	0.01 ± 0.04	0.01 ± 0.01	0.14 ± 0.38
UCYN-B	3.6	12.4	0.02 ± 0.04	0.86 ± 2.16	0.01 ± 0.02	0.28 ± 0.86
UCYN-C	1	92.4	—	—	0.04 ± 0.16	1.79 ± 8.37
γ-24774A11	1	162.5	0.23 ± 0.39	10.26 ± 16.18	0.26 ± 0.80	16.19 ± 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.

L375: The spring value of 58.5 seems incorrect; re-check Table S3.

Response:

We thank the Reviewer #1 for the comment. After re-calculating the NFR estimates, we have revised the spring value in Table S6 (formerly Table S3) and updated the manuscript accordingly.

Line 523-524: "Given the depth-integrated diazotroph abundances, the averaged NFRs on the ECS shelf ranged from 29.51 μmol N m^{-2} d^{-1} in spring to 44.14 μmol N m^{-2} d^{-1} in autumn (Table S6).".

L376: Because this study directly measured surface N2-fixation rates as well, author should verify consistency with values inferred from nifH copies and per-cell rates and measured rates by incubations; otherwise the subsequent quantitative discussion can be not-reliable.

Response:

We thank the Reviewer #1 for the comment. We have added a comparison between the estimated and measured NFRs in Figure S10 and included the relevant discussion in the revised manuscript.

Line 519-523: "Under these assumptions, the estimated and measured surface NFRs were highly correlated (Slope = 1.04, R^2 = 0.39, p < 0.001; Fig. S10). Despite the uncertainties and low level of explained variance (Fig. S10), the significantly positive correlation between the two approaches validates the use of cell-specific NFRs in assessing regional N budget.".

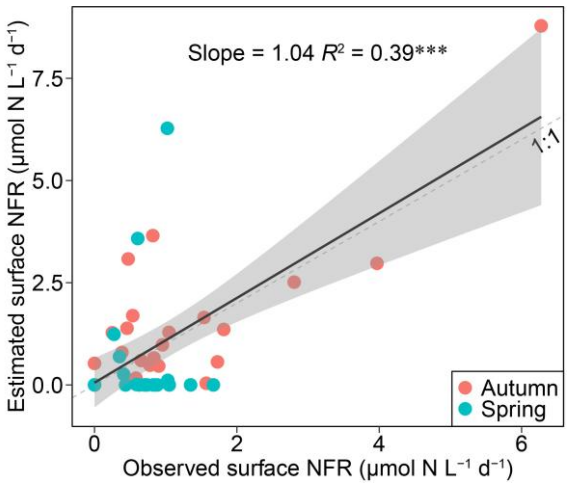

Figure S10. Linear regression of estimated versus measured surface N_2 fixation rates (NFRs). Estimates were derived from cell-specific NFRs, *nifH* gene polyploidy, and phylotype-specific *nifH* gene abundance. The shaded area represents the 95% confidence interval.

L399: Is this broad niche of Trichodesmium consistent with the earlier claim of an r-strategy for Trichodesmium?

Response:

We thank the Reviewer #1 for the comment. We realized that the use of the term "r-strategy" might be inappropriate and could potentially lead to conceptual ambiguity. Therefore, we have removed all the descriptions that characterize *Trichodesmium* as an r-strategist in the revised manuscript.

L399–404: Of course there is intra- and inter-specific metabolic variability; explain how such variability could produce the observed differences.

Response:

We thank the Reviewer #1 for the comment. We have added the discussion on how intra- and inter-specific metabolic variability in *Trichodesmium* could account for the expanded NO_x niche breadth in the revised manuscript.

Line 559-565: "Specifically, intra-specific plasticity may enable endemic *Trichodesmium* populations to modulate N_2 fixation and growth by exploiting ambient SRP under fluctuating NO_x regimes (Knapp et al., 2012; Knapp, 2012), or utilizing NO_x directly (Boatman et al., 2018). Meanwhile, inter-specific distributions of *Trichodesmium* were evident, with *T. erythraeum* dominating coastal waters whereas *T. thiebautii* prevailing on the ECS shelf (Jiang et al., 2018; Zhang et al., 2019), possibly owing to species-specific physiological optima and NO_x tolerance thresholds (Carpenter et al., 1993; Confesor et al., 2022; Rodier and Le Borgne, 2008, 2010).".

L411–412: Zehr et al. (2007) did not study genotype-specific optimal temperatures. Webb et al. (2009) reported an optimum of 26–30 °C, which is not as broad and is inconsistently higher than current result (<24 °C).

Response:

We thank the Reviewer #1 for the comment. We have revised the sentence and incorporated additional evidence from the literature.

Line 582-588: "The latter seems in contrast to the well-known preference of UCYN-B for warm, oligotrophic waters (Jiang et al., 2025; Webb et al., 2009). Nevertheless, global metadata analysis suggests that UCYN-B may survive under low temperatures (20–23°C) and high NO_x (up to 20 μM) and SRP levels (1–2 μM) (Tang and Cassar, 2019). Both field and laboratory studies have shown that UCYN-B remains viable under nitrate-rich conditions (5–20 μM) when SRP is sufficient (Knapp et al., 2012; Knapp, 2012; Turk-Kubo et al., 2018). These results indicate that UCYN-B has the capacity to cope with lower temperatures and elevated nutrient concentrations.".

L414: Brum et al. 2015 analyzed diatom-associated diazotrophs, please discuss it?

Response:

We thank the Reviewer #1 for the comment. We have added the discussion comparing our results on diatom-associated diazotrophs with those reported in Brum et al. (2015) in the revised manuscript.

Line 598-609: "Compared with the niche conditions suitable for open-ocean populations (temperature: 24.2°C; NO_x: 1.38 μM) (Brun et al., 2015), Hets inhabited niches of slightly higher temperature but lower NO_x levels (Fig. 8A and C). This result is different from previous findings based on studies analyzing all the *Richelia* diatom hosts, including *Rhizosolenia clevei* (Het-1 host), *Hemiaulus hauckii* (Het-2 host) and *H. membranaceus* (Brun et al., 2015; Luo et al., 2012), which may have distinct temperature and nutrient optima (Stenegren et al., 2017; Tuo et al., 2014, 2021). In fact, the different niche preferences for temperature and SRP between Het-1 and Het-2 were verified in this study (Fig. 8A and D), as well as in other studies (Foster et al., 2007; Tuo et al., 2021; Stenegren et al., 2017). Furthermore, the modeled realized niche space based on regional environmental gradients (e.g., temperature: 17.6–27.6°C) may be narrower than that derived from global datasets (16.5–31.6°C) (Brun et al., 2015; Jiang et al., 2025; Tang and Cassar, 2019). Thus, the prediction of realized niche of Hets may be affected by a variety of factors, including physiological traits of the diazotrophs, their hosts, and the scale of spatial sampling.".

K420-425: Please refer Tschitschko et al. 2024 which report important ecology and physiology of γ-24774A11.

Response:

We thank the Reviewer #1 for the comment. We have incorporated additional information on the ecology and physiology of γ-24774A11 and its diatom host in the revised manuscript.

Line 609-618: "Finally, our niche modeling result suggests a cosmopolitan distribution of γ-24774A11 in oligotrophic surface waters (Fig. 8) (Cheung et al., 2020; Shiozaki et al., 2018; Tschitschko et al., 2024), despite the fact that it is frequently found in environments with elevated NO_x concentrations (Bird and Wyman, 2013; Shao and Luo, 2022; Shiozaki et al., 2014). This feature may be related to its diatom host, which acquires a substantial fraction of fixed nitrogen from γ-24774A11 (Tschitschko et al., 2024). Nevertheless, in high-NO_x environments, the host might leverage ambient NO_x for growth, thereby partially reducing its metabolic dependency on the

symbiont. To illuminate the mechanisms governing the biogeographic distribution of the γ-24774A11-diatom association (Cornejo-Castillo and Zehr, 2021; Shao and Luo, 2022; Tschitschko et al., 2024), more systematic studies are needed.".

L432: To my knowledge, Cheung et al. 2019 did not mention ecological niche similarity between UCYN-C and Hets.

Response:

We thank the Reviewer #1 for the comment. We have removed the citation of Cheung et al. (2019) in the sentence.

L438–439: Cabello et al. (2016) did not report a distinct "reproductive strategy" for UCYN-A.

Response:

We thank the Reviewer #1 for the comment. We have removed the statement accordingly.

L440: Cabello et al. (2016) speculated on such a strategy, but many opposing studies also exist: UCYN-A2 is considered coastal ecotype, whereas UCYN-A1 is open-ocean ecotype (e.g., Turk-Kubo et al. 2017, 2021; Henke et al. 2018). Cite a more objective literature rather than only studies consistent with your results.

Response:

We thank the Reviewer #1 for the comment. We have revised the manuscript to include a more balanced perspective by including references to studies (Henke et al., 2018; Turk-Kubo et al., 2017, 2021) that describe distinct distribution patterns of UCYN-A.

Line 570-579: "It has been suggested that UCYN-A1 is an open-ocean ecotype and UCYN-A2 a coastal ecotype (Henke et al., 2018; Turk-Kubo et al., 2017, 2021). However, we found a co-occurrence pattern between these two ecotypes, with UCYN-A2/A3/A4 exhibiting lower abundance and narrower vertical distribution range (Fig. 3 and S6M–P) (Cabello et al., 2016; Gérikas Ribeiro et al., 2018; Wen et al., 2022). The distinct UCYN-A2 distributions between this and previous studies may be due to the phenotypical plasticity of the UCYN-A2-haptophyte symbioses, allowing them to inhabit either in the oligotrophic open oceans with small cell appearance (4–5 µm) or large one (7–10 µm) in the eutrophic coastal regions (Cabello et al., 2016; Hagino et al., 2013). Although

genes related to N_2 fixation and energy production are actively transcribed in both UCYN-A ecotypes, the mechanisms underlying their niche overlap and differentiation require further studies (Muñoz-Marín et al., 2023; Nguyen et al., 2025).".

Figures

Figure 4: Clarify whether data represent an average across multiple stations and include error bars if so.

Response:

We thank the Reviewer #1 for the comment. We have updated the Figure 4 caption to clarify that the data represent "average abundances" and have added error lines to the figure.

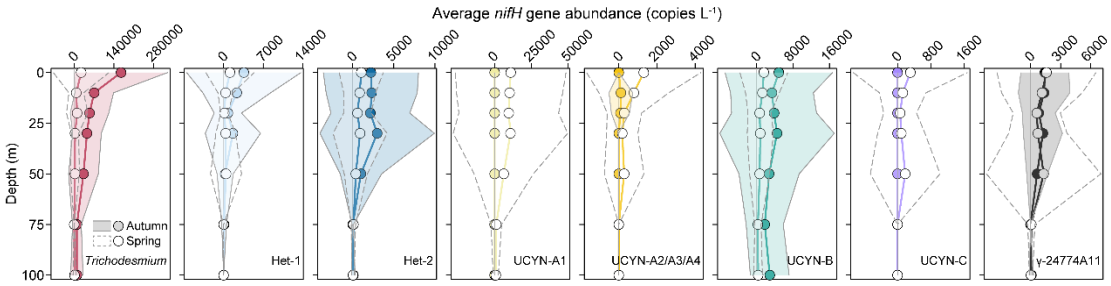

Figure 4. Vertical distributions in the average abundances of the eight major diazotrophic phylotypes on the East China Sea shelf during autumn and spring as determined based on the TaqMan qPCR assay of the *nifH* gene. The grey solid and dashed lines represent ± 1 standard deviation for autumn and spring, respectively.

Figures 5 and 6: The numbers near the pie/bar charts appear to be station numbers—please clarify in the captions.

Response:

We thank the Reviewer #1 for the comment. We have added the description of station numbers to the captions of Figures 5 and 6.

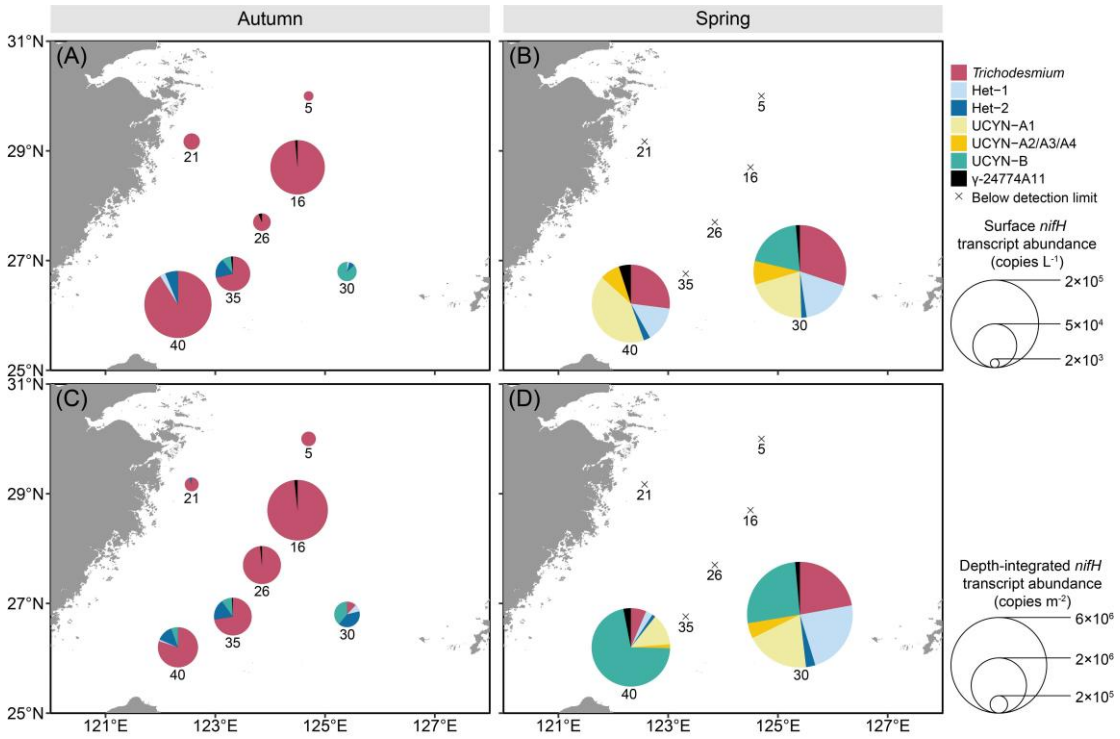

Figure 5. Surface (A, B) and depth-integrated (C, D) abundances of the seven major diazotrophic phylotypes on the East China Sea shelf during autumn and spring as determined based on TaqMan qPCR assay of the *nifH* transcript (i.e., RNA-based). Note that the transcript abundance of UCYN-B was derived from nighttime samples, whereas that of the other groups was from daytime samples. The depth-integrated transcript abundance was calculated based on the integration of the upper 50 m of the water columns. The station numbers are positioned adjacent to corresponding pie charts and crossover points. Land topography was obtained from the General Bathymetric Chart of the Oceans (GEBCO, https://www.gebco.net/, last access: 24 January 2025).

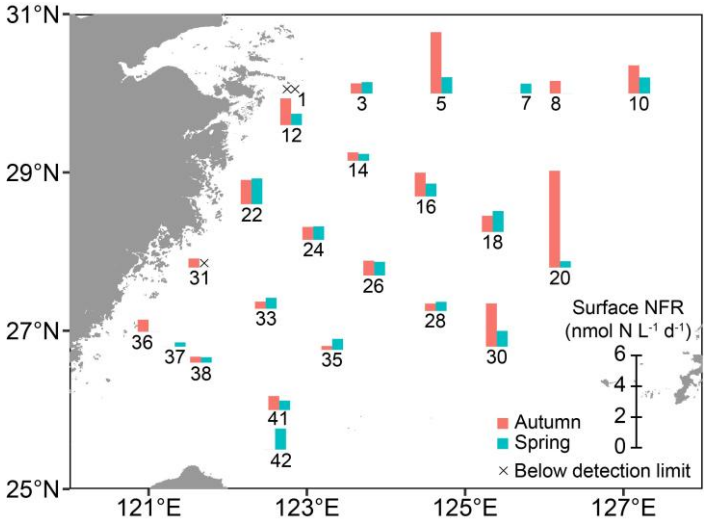

Figure 6. N_2 fixation rates in surface waters at designated stations on the East China Sea shelf during autumn and spring as determined with in situ isotope tracing. The station numbers are positioned adjacent to corresponding bar charts and crossover points. Sampling excluded stations 7, 37 and 42 in autumn, and stations 8 and 36 in spring. Land topography was obtained from the General Bathymetric Chart of the Oceans (GEBCO, https://www.gebco.net/, last access: 24 January 2025).

Figure 3: If station numbers are important here, add them for consistency.

Response:

We thank the Reviewer #1 for the comment. We have added the station numbers to Figure 3 and clarified them in the caption.

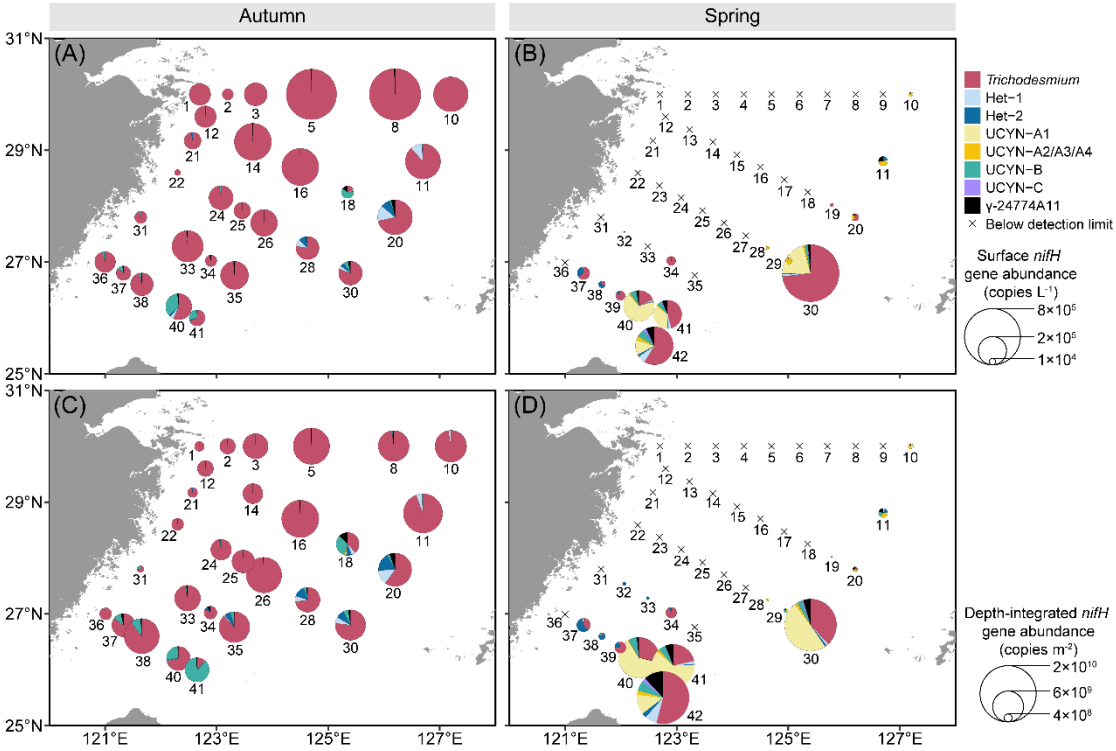

Figure 3. Surface (A, B) and depth-integrated (C, D) abundances of the eight major diazotrophic phylotypes on the East China Sea shelf during autumn and spring as determined based on the quantification of the *nifH* gene (i.e., DNA-based) with TaqMan qPCR. The station numbers are positioned adjacent to corresponding pie charts and crossover points. Land topography was obtained from the General Bathymetric Chart of the Oceans (GEBCO, https://www.gebco.net/, last access: 24 January 2025).

Response to comments from Reviewer #2

General Comments:

This manuscript presents a comprehensive dataset on nifH gene copy numbers, expression levels of major diazotroph groups, and surface nitrogen fixation rates in the East China Sea (ECS). By applying maximum entropy methods and generalized additive models, the study delineates the realized ecological niches of these diazotrophic groups. These findings enhance our understanding of diazotrophic community composition and nitrogen fixation in the ECS. Overall, this study represents a rigorous and valuable contribution. However, certain aspects of the presentation require improvement. I recommend considering this manuscript for publication pending minor revisions.

Response:

We thank the Reviewer #2 for the very professional and constructive feedback, and recommendation of the manuscript for acceptance. We have thoroughly revised the manuscript to address the issues and concerns, and accommodate them in so far as possible.

1. The Kuroshio region is a recognized hotspot for nitrogen fixation, which forms a key premise for this study. However, the survey design did not cover the Kuroshio mainstream but was confined to its intruded waters. Strictly speaking, therefore, the study area is more accurately defined as the East China Sea (ECS) continental shelf. Given the potential for significant variations in nifH gene copy numbers, expression, and nitrogen fixation rates across different water masses, I recommend redefining the study area throughout the manuscript to "ECS continental shelf" to ensure precise interpretation. Correspondingly, the title should be updated to "Spatiotemporal heterogeneity in diazotrophic communities reveals novel niche zonation on the continental shelf of the East China Sea" to accurately reflect the spatial scope of the work.

Response:

We thank the Reviewer #2 for the pertinent and very constructive comment. We have updated the title and redefined the study area to “ECS continental shelf ” throughout the manuscript.

Line 1-3: “Spatiotemporal heterogeneity in diazotrophic communities reveals novel niche zonation on the continental shelf of the East China Sea”.

2. The authors state: "Arguably, given the lack of observed Trichodesmium colonies in our samples, the pre-filtration through a 200-μm pore-size nylon mesh did not underestimate its abundance." This rationale requires clarification. How were Trichodesmium colonies determined – via microscopic examination or visual inspection? Pre-filtration through a 200-μm mesh is intended to remove large zooplankton but may also inadvertently remove Trichodesmium, including both free and colonial trichomes, potentially leading to an underestimation of its abundance. This potential limitation should be acknowledged and discussed in the manuscript.

Response:

We completely agree with the Reviewer #2 that prefiltration using a 200 μm pore-size nylon mesh may potentially remove large colonies of *Trichodesmium*. As microscopy was not performed, we have revised the Discussion section to explicitly state that the abundance for these colonial diazotrophs may be underestimated.

Line 433-435: "It should be noted that the abundance of colonial diazotrophs like *Trichodesmium* and Hets may be potentially underestimated due to the use of <200-μm size fraction in our study (e.g., Jiang et al., 2023a).".

3. The study did not clarify how the surface nitrogen fixation rate is related to diazotrophic biomass and physicochemical conditions.

Response:

We thank the Reviewer #2 for the comment. We have replaced the RDA with Pearson correlation analysis to better illustrate the relationships between surface nitrogen fixation rates, diazotrophic abundance, and physicochemical conditions, as shown in Fig. 7.

Line 208-210: "Pearson correlation analysis was adopted to explore relationship among *nifH* gene and transcript abundances (Log_{10} transformation), NFRs, environmental factors (z-score scaling), and distinct water masses (centered log ratio transformation) obtained from OMPA.".

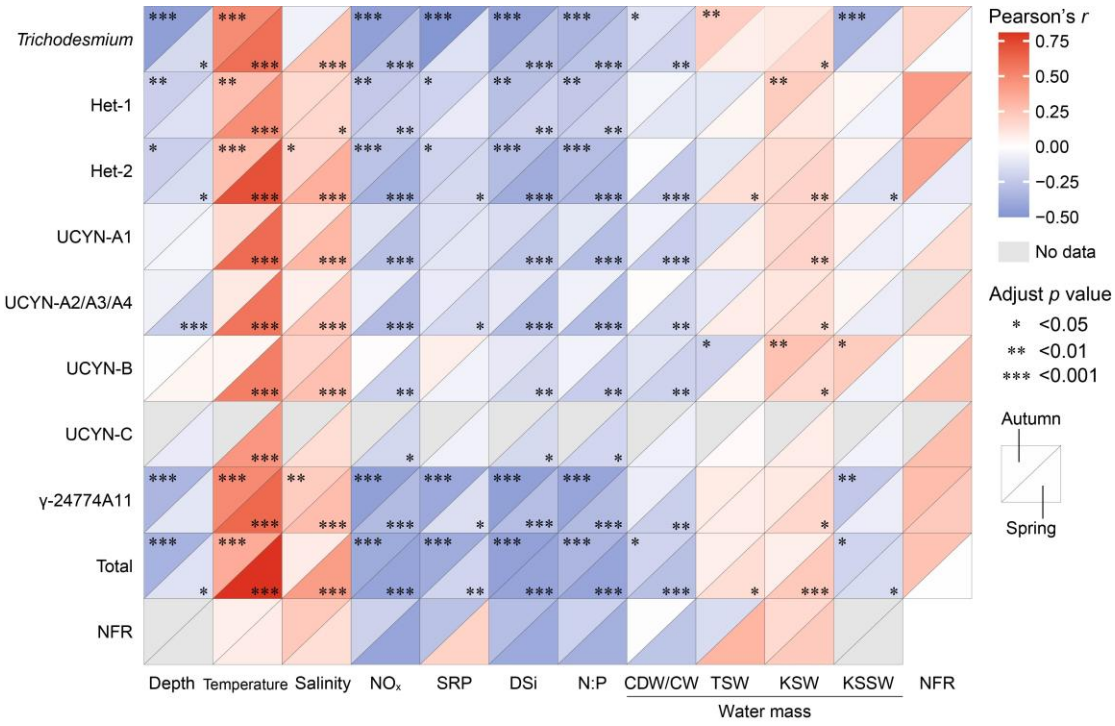

Figure 7. Pearson correlation between diazotroph abundances, N2 fixation rates (NFRs) and environmental variables in distinct water masses on the East China Sea shelf during autumn and spring. Color gradient denotes Pearson correlation coefficients. Total, combined *nifH* gene abundances of the eight diazotrophs detected; CDW, Changjiang diluted water; CW, Coastal water; TSW, Taiwan Strait water; KSW, Kuroshio surface water; KSSW, Kuroshio subsurface water.

4. The analysis of mean and breadth realized niches for diazotrophs is constrained by the lack of seasonal survey data. The ECS, influenced by the Kuroshio and Changjiang Diluted Water, exhibits strong seasonal variability in its physical and chemical environment (e.g., temperature, salinity, nutrient availability). The current niche modeling, which lacks data from summer and winter, may therefore not fully capture the annual ecological dynamics, potentially affecting the robustness of the conclusions. I therefore recommend that the authors include a clarification on this matter in their Discussion or Conclusions.

Response:

We thank the Reviewer #2 for the comment. We have clarified the limitation of lacking full-season survey data in niche modeling in the Conclusion section.

Line 629-632: "However, due to the lack of summer and winter survey data, the current niche modeling may not be able to capture the full annual ecosystem dynamics, potentially affecting the modeling robustness and accurate estimate of realized niches to some extent.".

5. The manuscript would be significantly strengthened by the addition of a dedicated Conclusions section. This section should succinctly summarize the key findings, explicitly state the scientific innovations of the work, and thoughtfully discuss the study's limitations alongside suggestions for future research directions.

Response:

We thank the Reviewer #2 for the comment. We have added a Conclusions section in the revised manuscript.

Line 620-639: "In this study, we conducted a cross-season survey on the distribution and activity of the major diazotrophic phylotypes on the continental shelf of ECS, alongside hydrographic analysis and integrated niche modeling. Our results demonstrate pronounced spatiotemporal heterogeneity in composition, distribution, and activity of the diazotrophs that is closely related to distinct water masses. The patterns of taxon-specific niche zonation underscore how dominant physical forcing (e.g., Kuroshio intrusion) may shape the diversity and biogeography of diazotrophs in this marginal sea with complex land-ocean interactions. Consequently, diazotrophic abundances and activities are relatively higher on the outer ECS shelf frequently intruded by the warm, saline, N-limited Kuroshio and TSW. The comprehensive biological, hydrographic, and modeling datasets provided here are, therefore, of utmost importance in assessing the dynamics of diazotrophic communities on the ECS shelf and its contribution to the regional N budget. However, due to the lack of summer and winter survey data, the current niche modeling may not be able to capture the full annual ecosystem dynamics, potentially affecting the modeling robustness and accurate estimate of realized niches to some extent. Additional uncertainties may arise from the omission of key environmental drivers such as light intensity, iron availability, and biotic interactions (e.g., microzooplankton grazing, competition from non-diazotrophic microorganisms), which strongly influence diazotrophic abundance and activity. Moving forward, to refine the niche modeling and resolve the spatiotemporal variations of marine diazotrophs in dynamic marginal seas such as ECS, a multidisciplinary framework should be prioritized in future research to integrate approaches such as microscopic cell counts, comprehensive ecosystem monitoring and environmental parameter collection, and molecular diagnostics (e.g., qPCR, amplicon sequencing, and multi-omics profiling).".

Specific Comments:

Line 11: Please replace "in the ECS" with "on the ECS shelf".

Response:

We thank the Reviewer #2 for the comment. We have replaced “in the ECS” with “on the ECS shelf” and updated the descriptions of the study area throughout the manuscript.

Lines 17-19: Please describe the key spatial distribution patterns and their primary

Response:

We thank the Reviewer #2 for the comment. We have added the description of the key spatial distribution patterns of N_2 fixation rates and their primary N_2-fixers in the revised manuscript.

Line 18-21: “The nitrogen fixation rates were generally higher in autumn than in spring, particularly in Kuroshio-affected waters dominated by *Trichodesmium*, Het-1 and Het-2, suggesting a seasonal variability primarily regulated by hydrographic conditions (mainly temperature and salinity) associated with water mass movement.”.

Line 56: Please replace "nutrient-rich" with "phosphorus-rich" for accuracy.

Response:

We thank the Reviewer #2 for the comment. We have replaced “nutrient-rich” with “phosphorus-rich” in the revised manuscript.

Line 59-60: “The upwelling of phosphorus-rich KSSW, coupled with terrestrial inputs from CDW and CW, may fuel phytoplankton growth in the coastal regions (e.g., Gao et al., 2025; Sun et al., 2025).”.

Line 70: Please replace "Lee Chen et al." with "Chen et al.".

Response:

We thank the Reviewer #2 for the comment. We have replaced “Lee Chen et al.” with “Chen et al.”. in the revised manuscript.

Line 73-77: “Given the dominance of these diazotrophs in Kuroshio (Chen et al., 2014; Cheung et al., 2017, 2019; Shiozaki et al. 2018; Wu et al., 2018) and a currently disproportionate research

focus on the filamentous *Trichodesmium* populations in individual seasons (Jiang et al., 2017, 2019, 2023a, b; Yue et al., 2021), systematic investigation into the compositional dynamics of diazotrophs across the ECS shelf is urgently needed.".

Line 103: The period October 13–30 should be explicitly referred to as autumn.

Response:

We thank the Reviewer #2 for the comment. We have replaced "summer" with "autumn" in the revised manuscript.

Line 112-113: "We conducted a cross-season survey at 42 stations on the ECS shelf aboard the research vessel *Xiang Yang Hong 18* during the 2023 autumn (October 13–30) and 2024 spring (April 9–24) (Fig. 1).".

Line 164: Please specify whether this refers to particulate organic nitrogen or total particulate nitrogen.

Response:

We thank the Reviewer #2 for the comment. We have added the "PON" in the sentence for clarity.

Line 181-182: "Unamended natural seawater samples for PON were also collected for comparison as a blank control.".

Line 166: Please provide the limits of detection for the nitrogen fixation rates, as per the methodology of Montoya et al. (1996).

Response:

We thank the Reviewer #2 for the comment. We have added the detection limits of N_2 fixation rate in the revised manuscript as per Montoya et al. (1996).

Line 184-186: "The NFRs and detection limits were determined according to Montoya et al. (1996). The calculated detection limits for the NFRs ranged from 0.11 to 0.76 nmol N L^{-1} d^{-1} across the stations (Table S2).".

Lines 174-176: Since the Taiwan Warm Current is a mixture of Kuroshio Subsurface Water and water from the Taiwan Strait, using the Taiwan Strait water as an end-member, rather than the Taiwan Warm Current itself, might provide a better framework for interpreting the influence of Kuroshio intrusion on diazotrophs.

Response:

We thank the Reviewer #2 for the comment. We agree that using the Taiwan Strait water as an end-member provides a clearer interpretation and we have revised the relevant sentences accordingly.

Line 197-200: "Given that the TWC represents a mixture of Kuroshio and Taiwan Strait water (TSW), we used TSW, rather than TWC, as an end-member to better evaluate the influence of Kuroshio intrusion on diazotrophs. Accordingly, the properties of TSW, KSW and KSSW were obtained from the World Ocean Atlas 2023 dataset (Fig. S2; Table S3) (Locarnini et al., 2024; Reagan et al., 2024).".

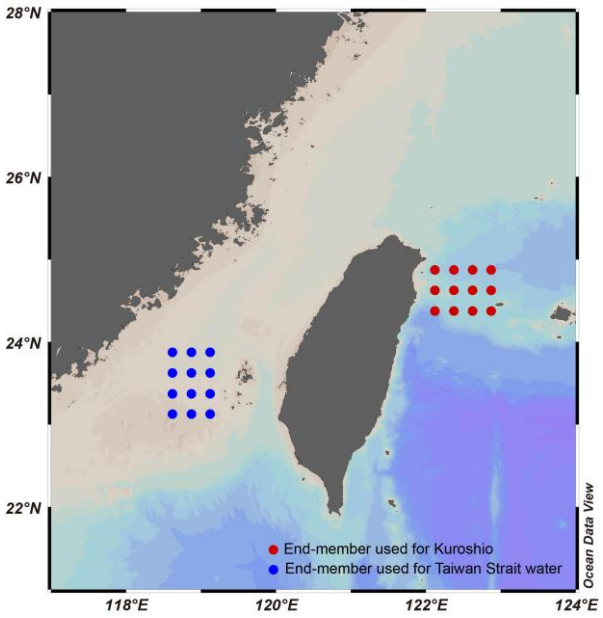

Figure S2. Data points from the World Ocean Atlas 2023 used as end-members for Kuroshio and Taiwan Strait Water. The map was created using Ocean Data View 5.7.2 (Schlitzer, Reiner, Ocean Data View, https://odv.awi.de, last access: 24 January 2025).

Lines 209-211: Please provide clear definitions for "nearshore waters" and "offshore regions" as used in this context.

Response:

We thank the Reviewer #2 for the comment. We have added the station numbers to clarify the nearshore and offshore regions in the sentence.

Line 291-294: "Surface NO_x concentration in autumn (0.44–17.71 μM) was in a narrower range than that in spring (0.16–19.11 μM), but the values were 6–12 times higher in nearshore waters (stations 1, 12, 21, 22, 31 and 36) relative to offshore regions (stations 5–11, 15–20, 25–30, 33–35 and 38–42) in both seasons (p<0.05; Fig. 2C and H).".

Lines 212-214: Please replace "content" with "concentration". Was there any notable climatic anomaly during the survey period? The reported high SRP concentrations associated with Kuroshio Surface Water seem inconsistent with its typical oligotrophic character, as described in Line 277. Furthermore, Figures S3A and S3E suggest a slight upwelling at transect A; including data on the mixed layer depth and SRP profiles for this transect would be informative.

Response:

We thank the Reviewer #2 for the comment. We have replaced "content" with "concentration" in the sentence. Regarding climatic conditions, a late-arriving summer marine heatwave occurred from August 12 to October 13, 2023, accompanied by weakened northerly winds and increased humid southerly winds over the ECS (Oh et al., 2024). Our autumn survey was conducted from October 13 to 30, immediately following this event. However, it remains unclear whether this heatwave contributed to the elevated SRP concentrations observed in offshore surface waters during autumn. We have revised the sentence accordingly. Although Fig. S4A and E (previously Fig. S3A and E) indicate slight upwelling along transect A during autumn, the mixed-layer depth (Fig. S5) and SRP profiles (Fig. S4I) in this transect suggests that this upwelling did not significantly influence the high SRP levels in the surface offshore regions.

Line 289-291: "The mixed-layer depth was high (>40 m) on the eastern and southeastern ECS shelf (Fig. S5), corresponding approximately to Kuroshio and TSW intrusion (Fig. S3B, C, F and G).".

Line 297-298: "A trend of increasing SRP with depth was observed, with the concentrations

ranging from ~0.3 μM at the surface to 0.7–1.2 μM at the bottom (Fig. S3I–L).".

Line 377-379: "Collectively, these findings demonstrate that the distribution and activity of targeted diazotrophs were primarily constrained by KSW, characterized by high temperature and salinity, shallow water depth, and limited N.".

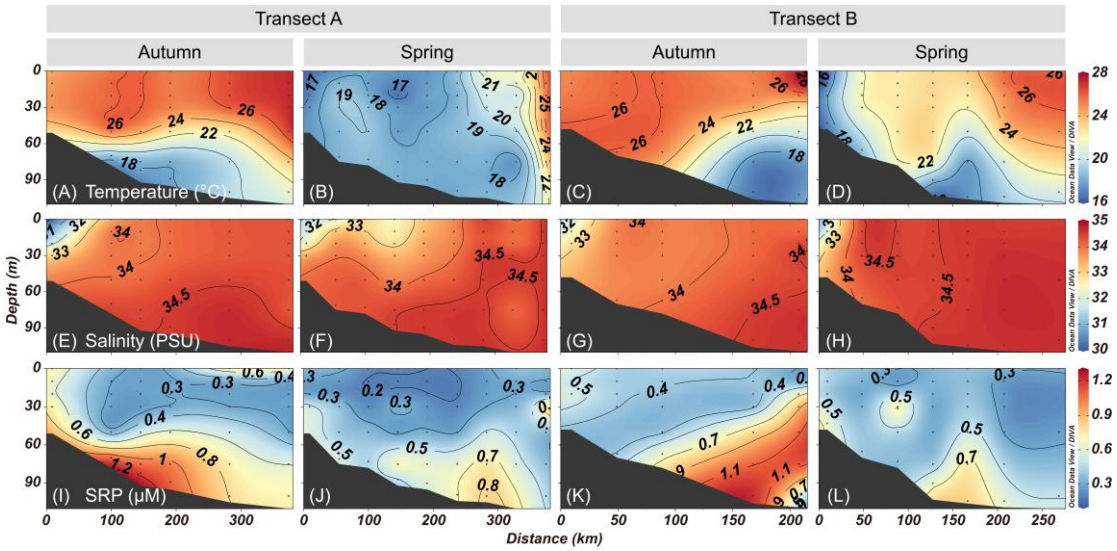

Figure S4. Vertical distributions in temperature (A–D), salinity (E–H) and SRP (I–L) along the transects A (stations 22–30) and B (stations 36–42) on the East China Sea shelf during autumn and spring. The transect plots were created using Ocean Data View 5.7.2 (Schlitzer, Reiner, Ocean Data View, https://odv.awi.de,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

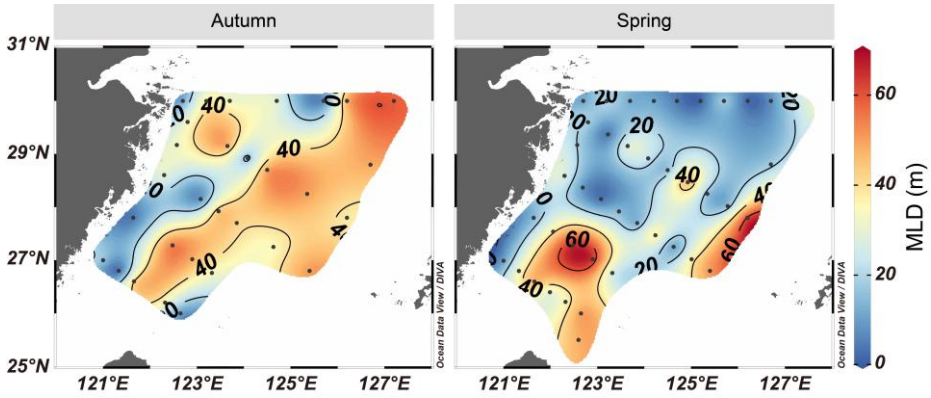

Figure S5. Variations in mixed-layer depth (MLD) on the East China Sea shelf during autumn and spring. The MLD was defined as the depth where the water density exceeded the surface value by 0.125 kg m–3 (Thomson and Fine, 2003). The map was created using Ocean Data View 5.7.2 (Schlitzer, Reiner, Ocean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

Line 223: Please briefly specify which diatoms Het1 and Het2 are associated with and highlight their key ecological differences.

Response:

We thank the Reviewer #2 for the comment. We have updated the relevant sentences accordingly to specify the diatom hosts of Het-1 and Het-2, as well as the key ecological differences between Hets.

Line 152-157: "We then performed TaqMan quantitative PCR (qPCR) to quantify *nifH* gene and transcript abundances of nine diazotrophic phylotypes, including *Trichodesmium*, *Richelia* (Het-1 and Het-2 associated with diatoms in the genera *Rhizosolenia* and *Hemiaulus*, respectively), *Calothrix* (Het-3 associated with *Chaetocerous*), UCYN-B as well as the N_2-fixing haptophytes and diatoms mentioned above, using established primer and probe sets (Table S1) (Church et al., 2005a, b; Foster et al., 2007; Langlois et al., 2008; Moisander et al., 2008, 2010; Thompson et al., 2014).".

Line 327-329: "The Hets were mainly found in the upper 30 m, and most abundant (>10^4 copies L^{-1}) on the southeastern ECS shelf during spring (Fig. S6E–L). Among them, Het-2 showed a broader distribution (detected at 47 stations in autumn and 38 stations in spring) than Het-1 (12 stations per season) and peaked in the subsurface (30-m depth) (Fig. 4, S6E–L).".

Line 275: Please clarify whether "water depth" refers to the sampling depth or the station depth.

Response:

We thank the Reviewer #2 for the comment. We have replaced "water depth" with "sampling depth" in the revised manuscript.

Line 369-371: "In both seasons, diazotroph abundances were positively correlated with temperature and salinity, and negatively with sampling depth, nutrient concentrations (NO_x, SRP and DSi), and N:P ratio, although UCYN-B deviated from these trends in autumn (Fig. 7).".

Lines 313-315: The manuscript does not detail a microscopic examination protocol. Please clarify how Trichodesmium colonies were observed and identified.

Response:

We thank the Reviewer #2 for the comment. As microscopy was not performed, we have revised the Discussion section to explicitly state that the abundance of colonial *Trichodesmium* may be underestimated.

Line 433-435: "It should be noted that the abundance of colonial diazotrophs like *Trichodesmium* and Hets may be potentially underestimated due to the use of <200-μm size fraction in our study (e.g., Jiang et al., 2023a).".

Line 347: Please replace the general term "nutrients" with the specific "SRP and iron".

Response:

We thank the Reviewer #2 for the comment. We have removed the phrase mentioning "nutrients (e.g., SRP and iron)" and revised the sentences accordingly.

Line 478-486: "In autumn, the southwest monsoon intensified the northward and shoreward penetration of Kuroshio and TSW onto the ECS shelf (~80%; Fig. S3B and C) (Oh et al., 2024), establishing warm (>24°C), NO_x-deplete (<1 μM) water mass (Fig. 2 and S3) that favored the proliferation of *Trichodesmium* (Fig. 3A and C), while increasing the coastal shoaling of CDW/CW. It has been suggested that iron deficiency limits *Trichodesmium* growth (Berman-Frank et al., 2001). Given that SRP (0.2–0.4 μM) did not appear to be a limiting factor (Fig. 2D), the dominance of *Trichodesmium* on the ECS shelf is likely due to the high level of dissolved iron (0.76–30 nM) transported via the Kuroshio and TSW (Shiozaki et al., 2015; Su et al., 2015) or delivered via aerial dust deposition (Guo et al., 2014).".

Lines 350-353: Please elaborate on the proposed mechanism by which the northeast monsoon enhances Trichodesmium abundance in the southern ECS during spring.

Response:

We thank the Reviewer #2 for the comment. We have revised the relevant sentences to clarify the cause of the springtime increase in *Trichodesmium* abundance on the southern ECS shelf.

Line 488-493: "In spring, however, the northeast monsoon facilitated the offshore expansion of the CDW/CW while weakening Kuroshio and TSW intrusion into the ECS shelf (Fig. S3A–C, E–G), resulting in decreased *Trichodesmium* abundance on the cold (<20°C), NO_x-replete (>1 μM) northern ECS shelf (Fig. 3B and D). The high *Trichodesmium* abundance on the southern ECS shelf (Fig. 3B and D) may be related to its physiological preferences for warm, NO_x-deplete waters brought by Kuroshio and TSW intrusion (Fig. 2F and H) (Jiang et al., 2018, 2019).".

Line 360: "similar to" or "comparable to" would be more appropriate phrasing.

Response:

We thank the Reviewer #2 for the comment. The phrasing has been changed to "similar to" in the revised manuscript.

Line 500-502: "The averaged autumn NFR (1.35 nmol N L^{-1} d^{-1}) was similar to what has been reported during summer on the ECS shelf (1.54 nmol N L^{-1} d^{-1}) (Jiang et al., 2023a), despite the differences in NFR measurement between the two studies (i.e., dissolution versus bubble methods).".

Lines 377-378: Please rephrase for clarity, e.g., "In spring, the nitrogen fixation efficiency of Trichodesmium was likely suppressed by low temperatures."

Response:

We thank the Reviewer #2 for the comment. We have revised the sentence accordingly.

Line 502-505: "The decreased NFRs in spring may be related to a substantial decrease in abundance and activity of filamentous diazotrophs (Fig. 3A, B and 7), which has been suggested to account for approximately 60% of the bulk NFRs on the outer ECS shelf (Jiang et al., 2023a, b).".

Lines 379-381: Please specify the exact stations used to estimate NFRs in the high-salinity water during autumn and spring, as these cannot be readily identified in Fig. 3 or Table S3.

Response:

We thank the Reviewer #2 for the comment. We have added the station numbers to specify the KSW-affected waters (previously termed "high-salinity water") in the revised manuscript.

Line 526-529: "Notably, the estimated NFRs in KSW-affected waters were considerably higher in spring (averaged 344 µmol N m^{-2} d^{-1} at stations 30, 41 and 42) than in autumn (averaged 110 µmol N m^{-2} d^{-1} at stations 18, 20, 28, 30, 40 and 41), yet both values fell within previously reported ranges on the southeastern ECS shelf (100–428 µmol N m^{-2} d^{-1}) (Jiang et al., 2023a; Sato et al., 2025; Zhang et al., 2012).".

Lines 385-388: If the high nifH gene copy number in Trichodesmium is attributed to cell cycle-dependent polyploidy, please explain why this would lead to an overestimation of its relative contribution specifically in spring and not in other seasons.

Response:

We thank the Reviewer #2 for the comment. We have revised the relevant sentences accordingly.

Line 530-535: "In terms of community composition, filamentous diazotrophs contributed 75% and 37% of the bulk NFRs in autumn and spring, respectively (Table S6), consistent with earlier observations (26–80%) on the ECS shelf (Jiang et al., 2023b), the northern SCS and upstream Kuroshio (Chen et al., 2014), and the western Pacific (Bonnet et al., 2009; Kitajima et al., 2009). However, caution is warranted in interpreting these contributions, as the values (25% and 63%) for non-filamentous diazotrophs may be overestimated due to the low cellular *nifH* gene copy number used for UCYN-C and γ-24774A11.".

Figure and Table Comments:

Figure 1: The path of the Kuroshio Subsurface Water (KSSW) shown appears to be based on its summer pattern (Yang et al., 2012). According to Yang et al. (2018), the branch of KSSW intruding off the Zhejiang coast typically occurs in April and recedes by July. Please ensure the path depicted is accurate for the study period. Additionally, please label the two transects (A and B) on the map.

Response:

We thank the Reviewer #2 for the comment. We have removed the nearshore and offshore Kuroshio branch currents and have marked transects A and B in Fig. 1 in the revised manuscript.

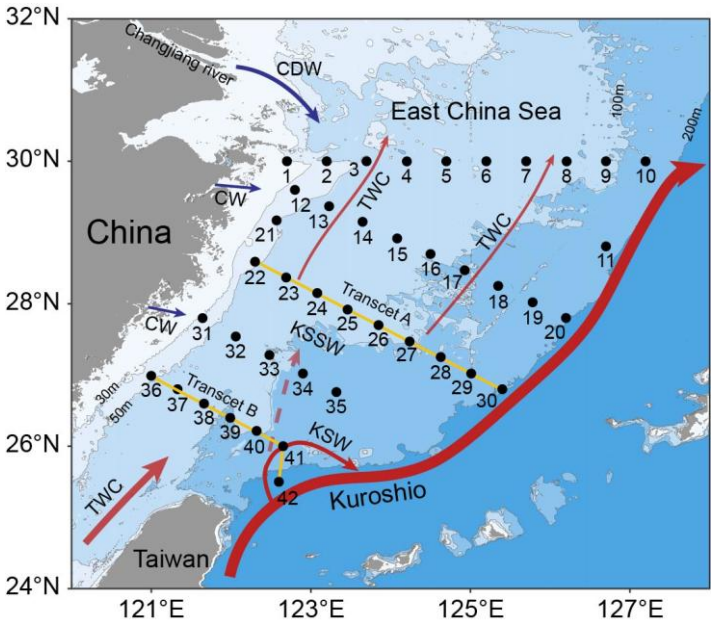

Figure 1. Sampling on the East China Sea (ECS) shelf during the 2023 autumn and 2024 spring cruises. A total of 42 stations along 5 transects were selected for the collection of biological samples and environmental parameters. The station numbers are positioned adjacent to corresponding points. Transects A (yellow line, stations 22–30) and B (yellow line, stations 36–42) were chosen to investigate variations in biological and environmental factors along the vertical gradient extending from inshore to offshore. Major circulations are indicated, including the Changjiang diluted water (CDW), Coastal water (CW), Taiwan warm current (TWC), Kuroshio current, Kuroshio surface water (KSW) and Kuroshio subsurface water (KSSW, dashed arrows) (Yang et al., 2012, 2018). Arrow sizes denote specific discharge rates (Liu et al., 2021). Land topography and ocean bathymetry data were obtained from the General Bathymetric Chart of the Oceans (GEBCO, https://www.gebco.net/, last access: 24 January 2025).

Figure 7: Please describe in the Materials and Methods section how the environmental factors related to KSW, TWC, and CDW/CW were quantified for the RDA analysis.

Response:

We thank the Reviewer #2 for the comment. We have replaced the RDA with Pearson correlation analysis to better illustrate the relationships between surface nitrogen fixation rates, diazotrophic abundance, and physicochemical conditions, as shown in Fig. 7. The corresponding methodological details have been clarified in the Materials and Methods section.

Line 208-210: "Pearson correlation analysis was adopted to explore relationship among *nifH* gene and transcript abundances (Log_{10} transformation), NFRs, environmental factors (z-score scaling), and distinct water masses (centered log ratio transformation) obtained from OMPA.".

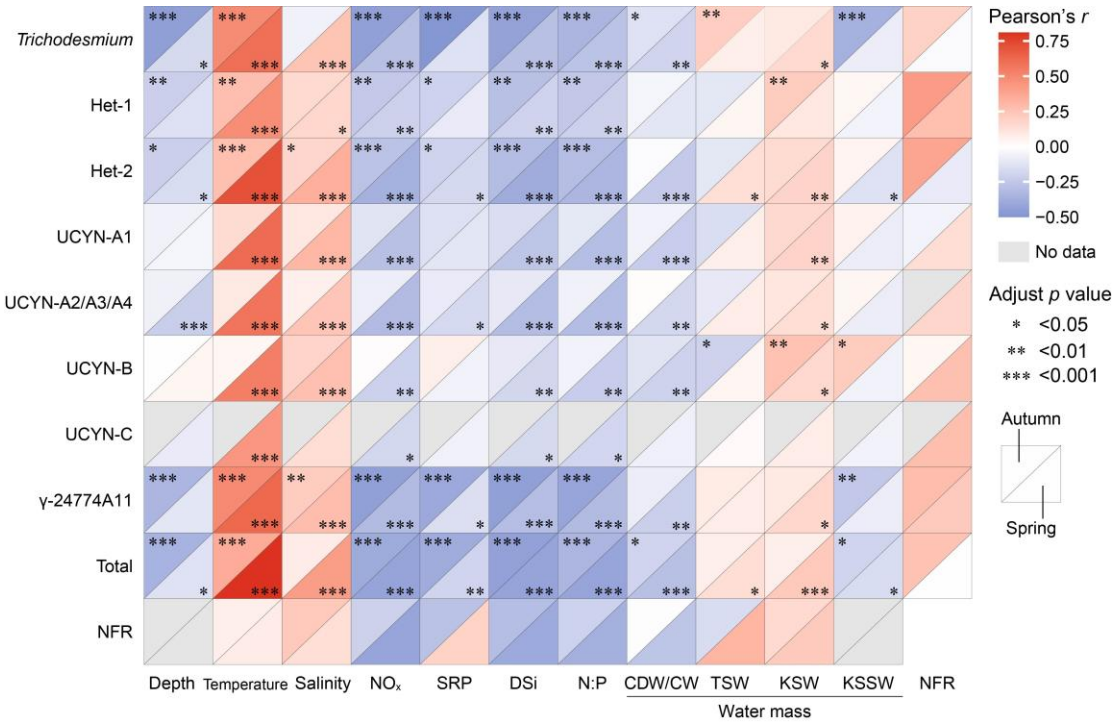

Figure 7. Pearson correlation between diazotroph abundances, N_2 fixation rates (NFRs) and environmental variables in distinct water masses on the East China Sea continental shelf during autumn and spring. Color gradient denotes Pearson correlation coefficients. Total, combined *nifH* gene abundances of the eight diazotrophs detected; CDW, Changjiang diluted water; CW, Coastal water; TSW, Taiwan Strait water; KSW, Kuroshio surface water; KSSW, Kuroshio subsurface water.

Figure S2: The finding of nearly 100% contribution from Taiwan Warm Current Water in the coastal waters of Zhejiang (Panel B) seems unusually high. As the Taiwan Warm Current flows northward, it typically mixes with coastal water, diluted water, and shelf mixed water, making a 100% contribution improbable. Please review this data or provide further justification.

Response:

We thank the Reviewer #2 for the comment. We have updated Fig. S3 (previously Fig. S2) in the supplemental material.

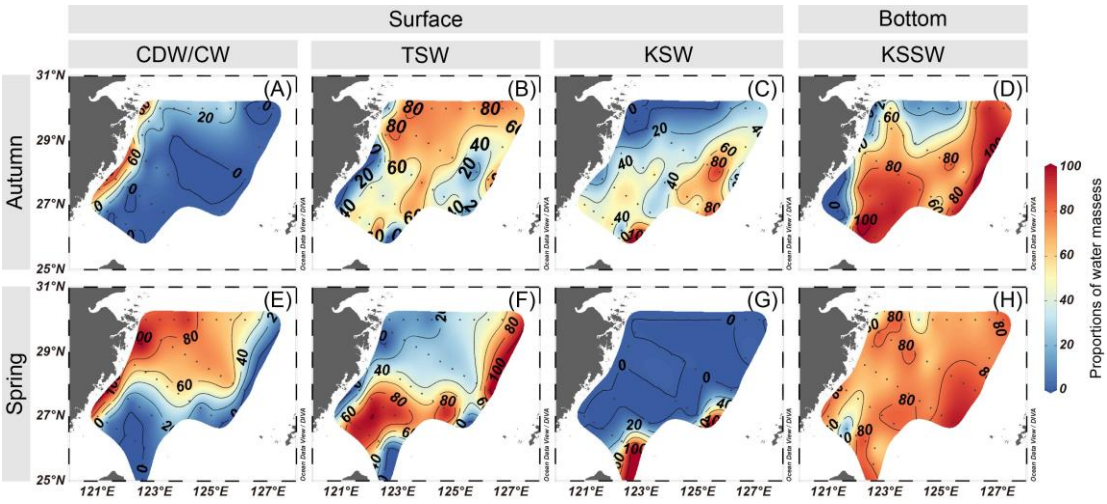

Figure S3. Proportions of water masses in the surface and bottom layers on the East China Sea shelf during autumn (A–D) and spring (E–H) as determined with OMPA. CDW, Changjiang diluted water; CW, Coastal water; TSW, Taiwan Strait water; KSW, Kuroshio surface water; KSSW, Kuroshio subsurface water. The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

Table S3: The estimation of Trichodesmium NFRs based solely on qPCR data might be inflated, as nitrogen fixation does not occur in every single cell. This potential source of overestimation should be acknowledged as a limitation of the methodology.

Response:

We thank the Reviewer #2 for the comment. We have added the description of the methodology's limitation in the revised manuscript.

Line 537-540: "It should also be noted that the calculated NFRs reflect an estimate of the maximum diazotrophic potential, not the actual in situ activity, as the method cannot distinguish between metabolically active (N_2-fixing) and inactive (non-N_2-fixing) cells.".

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