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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 Reviewer 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.

**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 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 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 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 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.”.**

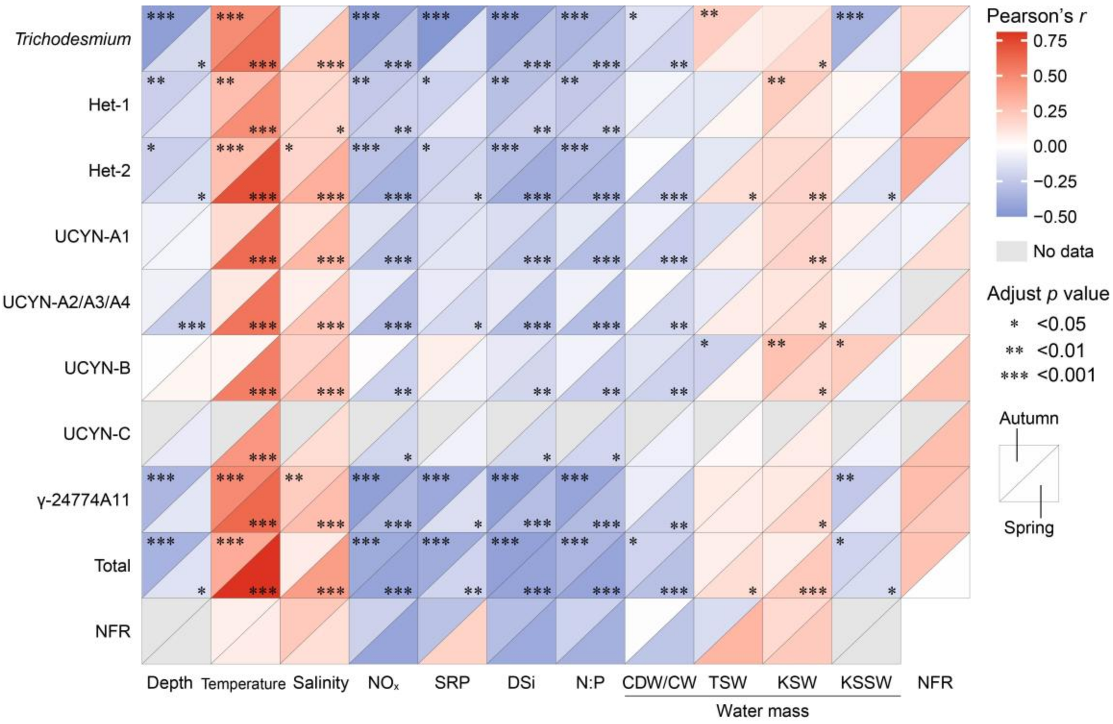


Figure 7. Pearson correlation between diazotroph abundances, $N_2$ fixation rates (NFRs) and environmental variables in distinct water masses on the East China Sea shelf during autumn and spring. Color gradient denotes Pearson correlation coefficients. Total, combined *nifH* gene abundances of the eight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

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 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 for the comment. We have added the Conclusion section to 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 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 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 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 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 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 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 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 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).".**

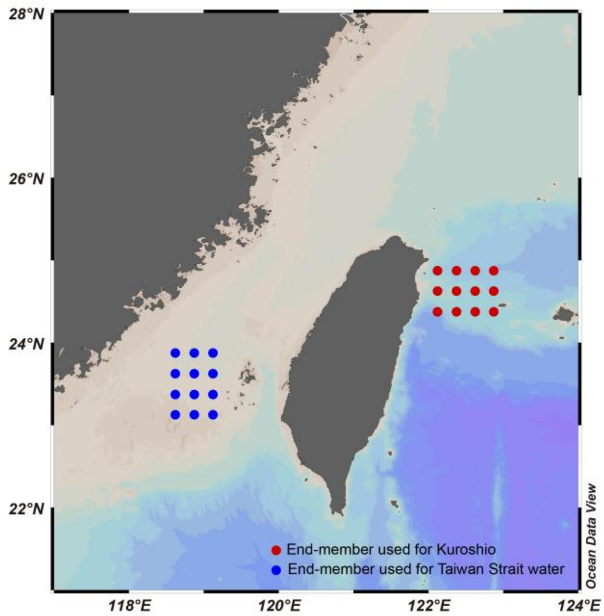


Figure S2. Data points from the World Ocean Atlas 2023 used as end-members for Kuroshio and Taiwan Strait Water. The map was created using Ocean Data View 5.7.2 (Schlitzer, Reiner, Ocean Data View, https://odv.awi.de, last access: 24 January 2025).

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

Response:

We thank the Reviewer 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 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.”.**

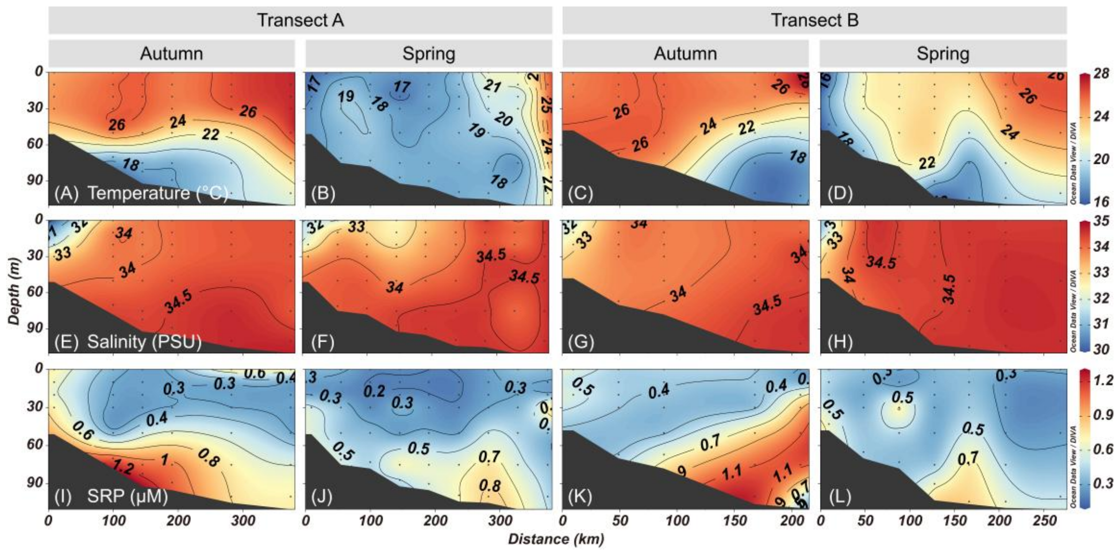


Figure S4. Vertical distributions in temperature (A–D), salinity (E–H) and SRP (I–L) along the transects A (stations 22–30) and B (stations 36–42) on the East China Sea shelf during autumn and spring. The transect plots were created using Ocean Data View 5.7.2 (Schlitzer, Reiner, Ocean Data View, https://odv.awi.de,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

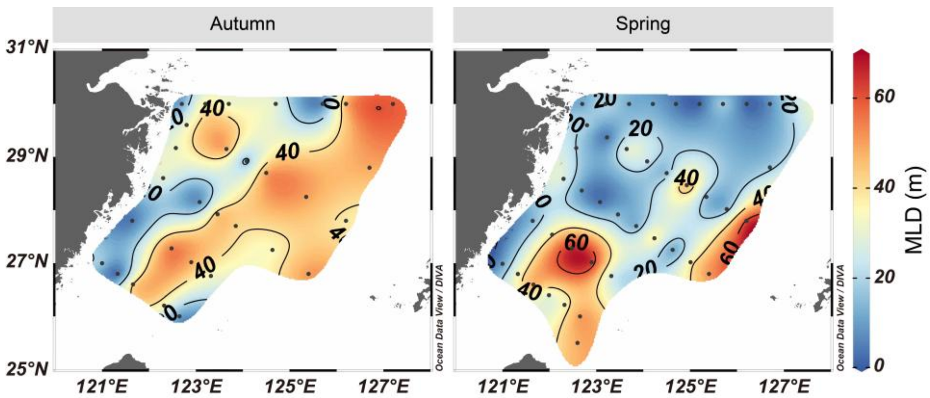


Figure S5. Variations in mixed-layer depth (MLD) on the East China Sea shelf during autumn and spring. The MLD was defined as the depth where the water density exceeded the surface value by 0.125 kg $m^{-3}$ (Thomson and Fine, 2003). The map was created using Ocean Data View 5.7.2 (Schlitzer, Reiner, 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 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 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 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 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 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 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 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 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 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 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.

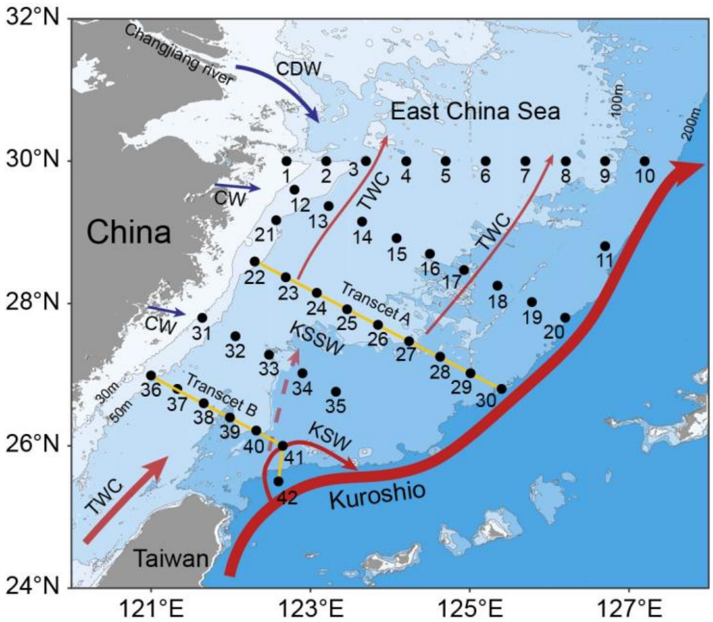


Figure 1. Sampling on the East China Sea (ECS) shelf during the 2023 autumn and 2024 spring cruises. A total of 42 stations along 5 transects were selected for the collection of biological samples and environmental parameters. The station numbers are positioned adjacent to corresponding points. Transects 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 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.".**

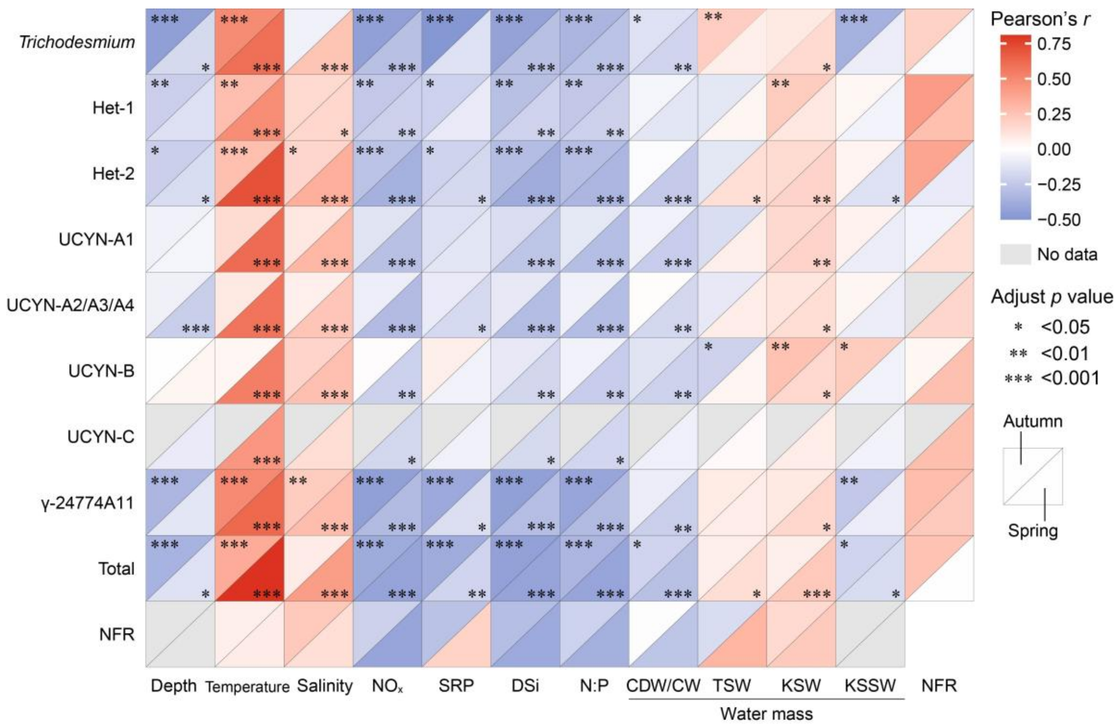


Figure 7. Pearson correlation between diazotroph abundances, $N_2$ fixation rates (NFRs) and environmental variables in distinct water masses on the East China Sea continental shelf during autumn and spring. Color gradient denotes Pearson correlation coefficients. Total, combined *nifH* gene abundances 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 for the comment. We have updated Fig. S3 (previously Fig. S2) in the supplemental material.

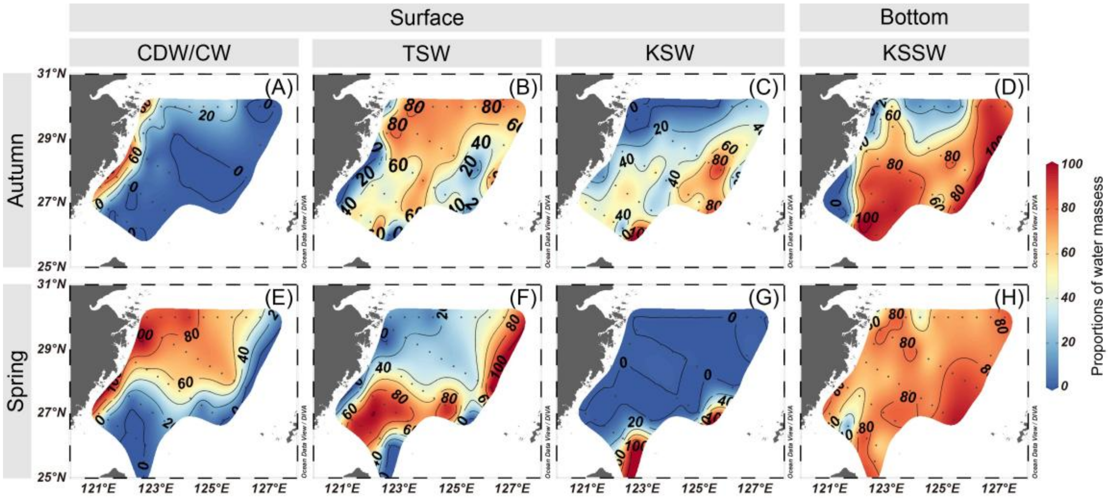


Figure S3. Proportions of water masses in the surface and bottom layers on the East China Sea shelf during autumn (A–D) and spring (E–H) as determined with OMPA. CDW, Changjiang diluted water; CW, Coastal water; TSW, Taiwan Strait water; KSW, Kuroshio surface water; KSSW, Kuroshio subsurface water. The 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 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.".**

**References**

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