

Dear Reviewers and Editor,

Thank you for giving us the opportunity to revise our manuscript. We are deeply grateful to the reviewers for their insightful comments and constructive suggestions, which have significantly helped us to improve the quality and clarity of our work. We have carefully considered all the points raised and have made corresponding revisions to the manuscript. Our point-by-point responses to the comments are detailed below.

1# Comments

This manuscript presents a comprehensive investigation by land-sea synchronous field surveys and enclosure experiments to reveal the potential regulatory mechanisms of alkaline phosphatase activity (APA) in the coastal waters of Laizhou Bay (LZB). The authors proved that the absolute phosphate ($\text{PO}_4\text{-P}$) concentration and the ratio nitrogen-to-phosphate ratio (DIN: $\text{PO}_4\text{-P}$) synergistically regulate the secretion of APA and organophosphorus utilization. The combination of geochemical measurements and molecular biological evidence provides valuable insights into phosphorus cycling driven by anthropogenic nitrogen inputs in coastal zones of LZB.

Overall, this is a well-structured and well-supported manuscript that meets the standards for publication. However, several issues should be addressed or clarified to further strengthen the manuscript.

Comment 1: Elaboration on the spatial coupling of high Chl *a* and APA

Figure 3 shows a spatial overlap between areas of high Chl *a* concentration and high APA, particularly near the Xiaoqing River (XQR) and Yellow River (YR) estuaries. It is suggested to explicitly point out this coupling relationship in Section 3.2 or 3.3 of the Results. For example, adding a sentence like: "Notably, the spatial distributions of high Chl *a* concentrations and high APA_{total} values showed remarkable consistency, especially in the adjacent areas of the XQR and YR estuaries." This would visually strengthen the conclusion that phytoplankton biomass is an important driver of APA.

Response: We thank the reviewer for this excellent suggestion. We have added the following sentence to Section 3.3 (Lines 291-293). "The spatial distributions of high Chl *a* concentrations and high APA_{total} values showed consistency, especially in the adjacent areas of the XQR and YR estuaries (Figure 3)."

Comment 2: Regarding the universality of the $\text{PO}_4\text{-P}$ concentration threshold

The study identifies a distinct $\text{PO}_4\text{-P}$ threshold ($0.05 \mu\text{mol}\cdot\text{L}^{-1}$), which is well-supported by data from autumn and the enclosure experiment. However, the winter data show no significant correlation between APA and either $\text{PO}_4\text{-P}$ or the DIN: $\text{PO}_4\text{-P}$ ratio. It is recommended that the authors further discuss the potential reasons for this seasonal discrepancy in the discussion section. For instance, is it due to direct suppression of microbial activity and enzyme production by low winter temperatures, or does the phytoplankton community structure in winter (e.g., potentially diatom-dominated) inherently respond differently to P stress compared to summer/autumn

communities? A deeper analysis of these factors would help clarify the boundary conditions under which the "threshold" concept applies.

Response: We agree with the reviewer that discussing the seasonal discrepancy is crucial for our findings. We have revised the discussion and provided a deeper analysis of the potential reasons. The revised content in Section 4.2 (Lines 475-482) is presented as follows in the revised manuscript:

“The absence of a significant APA response to low PO₄-P or high DIN:PO₄-P ratios in winter may be attributed to several factors. Low temperatures likely directly suppress microbial metabolic rates and enzyme production, including APA. Additionally, seasonal shifts in phytoplankton community composition—such as a predominance of diatoms in winter, which may exhibit an inherently lower responsiveness to phosphorus stress compared to the autumn assemblages—could also contribute to the observed seasonal discrepancy in APA regulation (Ivancic et al., 2016; Ou et al., 2024).”

Comment 3: Clarifying the role of dominant taxa in APA

The manuscript states that APA in autumn was primarily derived from phytoplankton. Could the authors briefly discuss the potential role of the dominant species (e.g., specific genera within diatoms) or functional groups (e.g., groups known to strongly induce APA under P stress) present in the phytoplankton community during that season? Even in the absence of species-level data, referencing existing literature on which common taxa tend to exhibit high APA under P stress would make the conclusion of "phytoplankton-dominated APA" more concrete and enriched.

Response: We appreciate the reviewer's valuable comments. While our dataset does not include species-level identification from field monitoring, we have enriched our discussion by referencing literature on phytoplankton functional groups and culture experiment data. In the Discussion Section 4.2 (Lines 487-501): Studies indicate that interspecific differences in APA expression capacity, induction thresholds, and regulatory patterns may influence phytoplankton adaptation to phosphorus limitation and community succession. Laboratory cultures show that APA induction thresholds vary widely (10.1-16.4 $\mu\text{mol}\cdot\text{L}^{-1}$), with the dinoflagellate *P. minimum* requiring significantly higher phosphate concentrations than diatoms (Meseck et al., 2009). In natural systems, diatom taxa that dominate under phosphorus limitation commonly express APA, whereas few dinoflagellates do so, and their APA expression is not linked to dominance (Ivancic et al., 2016). Furthermore, diatoms exhibit phosphate-regulated APA, while dinoflagellate APA appears less responsive to phosphate levels, possibly regulated by other factors (Dyhrman & Ruttenberg, 2006). Such heterogeneity in APA strategies suggests that species with lower induction thresholds or stronger expression may gain a competitive advantage under fluctuating phosphorus supply, potentially influencing succession (Figure 5). However, APA represents only one of multiple phosphorus-acquisition strategies, and its role is likely a contributing factor rather than the sole driver of community shifts.

Comment 4: Regarding the explanation of the ecological significance of kinetic parameters

The study measured the enzyme kinetic parameters K_m and V_{max} and observed their changes

below the identified threshold. It is recommended that the discussion include an explanation of the ecological implications of these parameter changes using more intuitive ecological terminology. For instance, a decrease in K_m may indicate enhanced enzyme affinity for the substrate, representing an adaptive strategy under low-phosphorus conditions. Adding such an explanation would make these biochemical parameters more accessible to a broader audience of ecologists, thereby enhancing the readability and impact of the manuscript.

Response: We thank the reviewer for this suggestion to improve the accessibility of our work. We have revised the relevant paragraph in the Discussion Section 4.2 (Lines 465-471) to explicitly explain the ecological implications of the kinetic parameters. Specifically, we now state: Moreover, enzyme kinetics data from field investigations in LZB further validated the phosphate concentration threshold, showing that AP activity and substrate affinity increased significantly when phosphate concentration dropped below the threshold (Figure S7). The K_m in autumn was lower than in winter, while V_{max} was much higher in autumn than in winter. The lower K_m (indicating higher substrate affinity) and much higher V_{max} (indicating higher maximum hydrolysis capacity) in autumn compared to winter collectively resulted in higher APA (Figure 3).

Comment 5: Improving language clarity and chart specifications

The MS is generally well-written, but still need improve language clarity and fluency. Some sentences are unnecessarily long or awkwardly phrased.

Response: We have thoroughly reviewed the entire manuscript and revised it for language clarity and fluency. Several long and complex sentences have been broken down into shorter, more direct statements to improve readability. We believe these edits have significantly improved the overall clarity of the manuscript.

Comment 6: In Figure 6, the scatter plot in the lower panel demonstrates a positive correlation between APA and the DIN:PO₄-P ratio, distinguishing between "high APA" and "low APA" groups. It would be beneficial to briefly state in the figure caption or main text the criterion used to separate these two groups (e.g., based on a specific APA value, or using a statistical clustering method). This would enhance the transparency of the analytical approach.

Response: We appreciate the reviewer's comment regarding the transparency of our method. We have now clarified the grouping criterion in the Result Section 3.5 (Lines 362-366). The revised caption includes the following sentence: During the autumn survey, this linear relationship could be further divided into two groups by K-means cluster analysis based on APA, such that the slope of the fitted straight line was significantly higher in the high APA group ($P < 0.01$; red line in Figure 6) relative to that of low APA group ($P < 0.01$; black line in Figure 6).

2# Comments

General Comments

The authors present a comprehensive study investigating the regulation of alkaline phosphatase activity (APA) in the coastal waters of Laizhou Bay, China. By effectively integrating seasonal field surveys with a mesocosm experiment, the manuscript convincingly identifies a phosphate concentration threshold for APA induction and demonstrates a synergistic role of the DIN:PO₄-P ratio under phosphorus-limited conditions. The incorporation of genetic data strengthens the mechanistic insights. The study is well-structured, addresses a relevant topic in coastal biogeochemistry, and is suitable for publication after minor revisions to enhance clarity and precision in several specific sections.

Specific Comments

Lines 24-27 (Abstract): The phrase "combined analysis of field and experimental data, $p < 0.01$; $n = 36$ " is vague. Please specify the variables between which this significant correlation was observed to make the abstract more informative.

Suggestion: Rephrase to, for example, "... a significant positive correlation was observed between APA_{total} and the DIN:PO₄-P ratio in the combined dataset ($p < 0.01$; $n = 36$)."

Response: We appreciate the reviewer's comment. We have revised this sentence as follows: Significant positive correlation was observed between APA and the DIN:PO₄-P ratio below this threshold ($0.05 \mu\text{mol}\cdot\text{L}^{-1}$), as analyzed in the combined dataset of field and enclosure experiments ($p < 0.01$, $n = 36$).

Lines 114-118 (Mesocosm Experiment Methods): While the target DIN:PO₄-P ratios are stated, the absolute initial concentrations of DIN and PO₄-P for the Control (CG) and Treatment (TG) groups are not provided in the main text, relying solely on a reference to Table S1. Including these critical values in the text would improve readability.

Suggestion: Briefly state the key initial conditions, e.g., "The initial DIN and PO₄-P concentrations for the CG were [X] $\mu\text{mol L}^{-1}$ and [Y] $\mu\text{mol L}^{-1}$, respectively. For the TG, concentrations were adjusted to [A] $\mu\text{mol L}^{-1}$ and [B] $\mu\text{mol L}^{-1}$ to achieve the target ratio."

Response: Thank you for the valuable suggestion. We agree that presenting the key initial nutrient concentrations directly in the main text improves clarity and readability for the reader. As suggested, we have revised the field enclosure experiments section (Lines 122-125 in the revised manuscript) to include the initial absolute concentrations for both experimental groups. The revised sentences are as follows (Line 149-153): The initial DIN and PO₄-P concentrations for the CG were $1.90 \mu\text{mol}\cdot\text{L}^{-1}$ and $0.04 \mu\text{mol}\cdot\text{L}^{-1}$, respectively. For the TG, concentrations were adjusted to $50.01 \mu\text{mol}\cdot\text{L}^{-1}$ and $1.78 \mu\text{mol}\cdot\text{L}^{-1}$ to achieve the target ratio.

Lines 318-320 (Results 3.4): The basis for categorizing the data into "high APA" and "low APA" groups for the regression analysis is not defined. The reproducibility of this analysis requires a clear, objective criterion.

Suggestion: Please specify the statistical criterion used for this grouping (e.g., median split, a specific APA threshold value).

Response: We appreciate the reviewer's comment regarding the transparency of our method. We have now clarified the grouping criterion in Result Section 3.5 (Lines 362-366). The revised text includes the following sentence: During the autumn survey, this linear relationship could be further divided into two groups by K-means cluster analysis based on APA, such that the slope of the fitted straight line was significantly higher in the high APA group ($P < 0.01$; red line in Figure 6) relative to that of low APA group ($P < 0.01$; black line in Figure 6).

Lines 437-443 (Discussion on DON): The discussion on the potential mechanisms linking DON to APA, while insightful, could be further strengthened. The argument would benefit from a more direct connection to the DON dynamics observed in your own experiment (e.g., Figure 5d) or from citing more specific literature on how particular DON compounds influence microbial P metabolism.

Suggestion: Consider elaborating briefly on how your data aligns with the proposed mechanisms or referencing studies that identify specific DON components as regulators.

Response: We appreciate and agree with the reviewer's comment. In the enclosure experiments, it is possible that the growth of phytoplankton in the culture system provided a supplement for DON and DOP, resulting in the absence of a positive correlation between DON and APA. This correlation was only obtained from the field survey data. The revised text includes the following sentence (Line 517-528): It is noteworthy that prior studies have found that under the same N:P ratio and active phosphate concentration, phytoplankton APA in DON-enriched cultures was significantly higher than that in cultures without DON (Fitzsimons et al., 2020). Our winter survey data revealed a significant positive correlation between DON and APA_{bac} (Figure 4), similar to findings of a correlation between APA_{phy} and DON concentration (Ou et al., 2024), indicating that not only PO₄-P but also nutrient composition and ratios, can affect APA. Regulation of APA by DON may potentially operate through specific DON components or their degradation products influencing P metabolic pathways, or through DON serving as an alternative N source that stimulates phytoplankton growth and P demand (Ma et al., 2018; Forchhammer et al., 2022). Meanwhile, DON serving as an alternative nitrogen source may enhance phytoplankton growth and phosphorus demand.

Line 49-50 and throughout the text (Terminology): The abbreviation "AP" for Alkaline Phosphatase is used in the abstract without the full term being presented first. Additionally, specialized terms like "pho regulon" (Line 423) may be unfamiliar to a general readership.

Suggestion: Please define "AP" upon its first appearance in the abstract as "alkaline phosphatase (AP)". Consider adding a brief explanatory phrase for "pho regulon", such as "...the pho regulon (a gene suite responsible for phosphorus scavenging)".

Response: Thank you for your valuable suggestions regarding the clarity of terminology. We have incorporated both points in the revised manuscript. Firstly, following your recommendation, the abbreviation "AP" is now explicitly defined upon the first mention of "alkaline phosphatase" in the Abstract. Secondly, in the Discussion section, when the specialized term "pho regulon" is introduced in detail, we have added a brief explanatory phrase describing it as "a gene suite responsible for phosphorus scavenging" to aid a broader readership. We believe these edits enhance the overall readability and accessibility of the manuscript, and we appreciate your guidance in helping us improve it. Specifically, as detailed in Lines 503-509 of Section 4.2, the text now reads: When ambient $\text{PO}_4\text{-P}$ concentrations fall below the 0.05-0.2 $\mu\text{mol}\cdot\text{L}^{-1}$ threshold (Dyhrman et al., 2007), phytoplankton activate the pho regulon (a gene suite responsible for phosphorus scavenging) operon (a gene cluster containing the pstSCAB phosphate transport system and phoA/phoX alkaline phosphatase genes) through the PhoB/PhoR two-component system (a P-sensing system consisting of histidine kinase PhoR and the response regulator PhoB) (Lin et al., 2016).

Typos and Minor Corrections

Line 22: "an enclosure experiments" -> "an enclosure experiment" or "enclosure experiments"

Line 25: "ssignificant" -> "significant"

Response: We thank you for your careful reading and constructive feedback. We have corrected the specific typographical errors noted and have undertaken a thorough proofreading of the entire manuscript to ensure linguistic accuracy and clarity.

3# Comments

Yang et al. investigate the relationships among phosphorus concentrations, N:P ratios, and alkaline phosphatase activity (APA) in Laizhou Bay, China. By integrating intensive field observations with enclosure experiments, the authors demonstrate that phosphate availability is a key regulator of APA induction. In addition, genetic analyses show that the expression of alkaline phosphatase-related genes increases only when phosphate concentrations fall below a threshold identified in this study. These findings support a conceptual framework in which APA-mediated dissolved organic phosphorus (DOP) utilization enhances phytoplankton biomass, accelerates phosphate depletion, and intensifies N:P imbalance, thereby favoring phytoplankton genotypes with elevated APA. Overall, the manuscript is well structured, and the conclusions are generally well supported by extensive field and experimental data. However, the quality of writing needs substantial improvement, and an overall language polish is recommended to enhance readability. Moreover, several textual descriptions are inconsistent with the figures, and the entire manuscript should be carefully checked for accuracy and consistency.

We sincerely thank the reviewer for their thorough evaluation and the positive assessment of our study's scientific contribution, structure, and supporting data. We are particularly encouraged by their recognition of the integrated field-experimental approach and the conceptual framework.

We fully acknowledge the reviewer's critical points regarding the need for substantial improvement in writing quality and the inconsistencies between the text and figures. We have taken these comments very seriously and have implemented comprehensive revisions to address them:

The entire manuscript has undergone extensive language polishing by a professional editing service provided by V. Monica Bricelj, Ph.D. from TechMar Resrch Inc. We have focused on improving sentence structure, clarity, terminology, and overall flow to enhance readability, while strictly preserving the scientific content.

We have carefully and systematically checked the entire manuscript against all figures and tables. All identified inconsistencies have been corrected. The text now accurately and precisely reflects the data presented in the figures.

We believe these revisions have significantly improved the clarity, accuracy, and professionalism of the manuscript. We are grateful for the reviewer's constructive suggestions, which have undoubtedly strengthened our paper.

All point-by-point responses to the specific comments are provided below.

Specific comments:

Line 24: Delete “an”.

Response: Thank you for pointing out this typographical error. The word “an” has been deleted.

The original sentence was: Through integrated seasonal field surveys and an enclosure experiments in Laizhou Bay, China, we demonstrate that PO₄-P seawater concentration serves as the primary control for APA induction, with a consistent threshold of 0.05 μmol·L⁻¹. The revised sentence is: Through integrated seasonal field surveys and enclosure experiments in Laizhou Bay, China, we demonstrate that PO₄-P seawater concentration serves as the primary control for APA induction, with a consistent threshold of 0.05 μmol·L⁻¹.

Line 27: Spelling correction needed for “significant”.

Response: Thank you for your careful reading. The spelling of “significant” has been corrected.

Line 37: The term “emission” is typically used for gases; consider alternatives such as “inputs” or “loading”.

Response: Thank you for this precise suggestion. As recommended, the term “emissions” has been changed to “inputs” in the revised manuscript (Line 38-42, “With the intensification of nitrogen (N) inputs and the control of phosphorus (P), P limitation has become widespread in global coastal waters (Zhang et al., 2024; Maavara et al., 2020; Liang et al., 2023) and has triggered adverse ecological consequences, including exacerbated eutrophication and shifts in phytoplankton community structure (Xin et al., 2019; Peñuelas and Sardans, 2022).”).

Line 57: Please clarify “OP”; it likely refers to phosphate or dissolved inorganic phosphorus (DIP).

Response: Thank you for raising this point. The abbreviation “OP” originally referred to organophosphates. To avoid confusion, the text has been clarified as follows (Line 42-47): “To cope with P limitation, microorganisms have evolved a range of strategies, including an increase in inorganic phosphate transporters, induction of hydrolases for scavenging organophosphates (OP, organic phosphorus compounds), and a reduction in P demand by replacing phospholipids with sulfur- or nitrogen-containing lipids (Van et al., 2006; Karl, 2014; Lin et al., 2016).”.

Line 59: Replace “seas” with “coastal systems”.

Response: Thank you for the suggestion to improve the terminology. The word “seas” has been replaced with the more precise term “coastal systems” as recommended. The original sentence was: Nausch et al. (1998) found that APA increased significantly when PO₄-P concentrations were < 0.2 μmol·L⁻¹. Since then, the PO₄-P threshold for the APA surge in coastal waters has been reported to range from 0.01 to 0.5 μmol·L⁻¹ in various seas (Jin et al., 2024). The revised sentence (Line 57-60) is: Nausch et al. (1998) found that APA increased significantly when PO₄-P concentrations were < 0.2 μmol·L⁻¹. Since then, the PO₄-P threshold for the APA surge in coastal waters has been reported to range from 0.01 to 0.5 μmol·L⁻¹ in various coastal systems (Jin et al., 2024)..

Line 66: This statement is repetitive; N:P limitation is equivalent to a high N:P ratio.

Response: We agree with the reviewer's point. The redundant phrasing has been revised for conciseness. The original sentence was: Under conditions of nitrogen (N) excess and P limitation, a high DIN:PO₄-P ratio has been confirmed to be positively correlated with APA (Bogé et al., 2017; Jin et al., 2024). The revised sentence (Line 66-68) is: “A high DIN:PO₄-P ratio (indicating N excess and P limitation) has been confirmed to be positively correlated with APA (Bogé et al., 2017; Jin et al., 2024).”

Line 68: Please clarify why freshwater inputs would enhance APA—does this relate to freshwater having intrinsically high N:P ratios?

Response: Thank you for this suggestion, which helped clarify the mechanism. As recommended, we have explicitly linked freshwater inputs to high N:P stoichiometry in line 68-70: “Freshwater inputs, which often carry nutrients with intrinsically high N:P ratios, can thereby enhance APA (Kang et al., 2019; Ivancic et al., 2021).”

Line 87: Consider explicitly stating the objectives of the study at the end of the Introduction.

Response: Thank you for this constructive suggestion. As recommended, we have added a dedicated “Objectives of the study” paragraph at the end of the Introduction in line 89-95. It explicitly states our aims to identify the PO₄-P concentration threshold, evaluate the synergistic role of the DIN:PO₄-P ratio, assess seasonal and community-level APA partitioning, and validate these dynamics with genetic evidence, thereby refining the conceptual framework for AP regulation. The revised paragraph reads in detail as follows: This study aimed to elucidate the drivers of APA surges in Laizhou Bay by identifying the phosphate (PO₄-P) concentration threshold for APA induction, evaluating the synergistic role of the DIN:PO₄-P ratio under P-limited conditions, assessing seasonal and community-level APA partitioning, and validating these dynamics with genetic evidence. The combined field and experimental approach seeks to refine the conceptual framework for AP regulation in coastal ecosystems affected by anthropogenic nutrient imbalances.

Line 92: Specify whether “mean depth” is intended. Also clarify the meaning of “half-exchange”.

Response: Thank you for these suggestions to improve clarity. We have revised the sentences. The original sentences were: “Laizhou Bay (LZB), a typical semi-enclosed bay, is situated south of the Bohai Sea and north of the Shandong Peninsula (Figure 1), with an area of ca. 7,000 km², a coastline of 320 km, depth of less than 10 m, and water half-exchange time of 55 days (Wu et al., 2023)”. The revised sentences read (Line 98-101) as follows: “Laizhou Bay (LZB), a typical semi-enclosed bay, is situated south of the Bohai Sea and north of the Shandong Peninsula (Figure 1). It covers an area of approximately 7,000 km² with a coastline of 320 km, a mean depth of less than 10 m, and a water half-exchange time (i.e., the time for 50% water renewal) of 55 days (Wu et al., 2023).”

Line 98: The phrase “affect the ecosystems” is vague; please provide region-specific examples.

Response: Thank you for the suggestion to make the ecological impact more concrete. The text has been revised to include specific, regionally-relevant examples. The revised sentences (Line 106-109) read as follows: “The resulting increase in N availability, coupled with intensifying P limitation, has triggered significant ecological shifts in the bay, including more frequent harmful algal blooms and changes in dominant phytoplankton species from diatoms to dinoflagellates (Song et al., 2017; Xin et al., 2019).”

Line 110: More detail on field sampling is needed, including sampling frequency and methods for water collection, transport, and preservation.

Response: Thank you for these valuable suggestions. We have revised the sampling details at section 2.2 in lines 118-130. The revision specifies that marine surface water was collected using a 5-L Niskin bottle. For riverine water samples collected at the mouths of ten major rivers, sampling was conducted following standard protocols for surface water monitoring in China (specifically, HJ 494-2009). An organic glass water sampler or a pre-cleaned polyethylene container was used to collect subsurface water at a depth of approximately 0.5 m. All samples (riverine and marine) underwent immediate on-site processing into aliquots for different analyses (nutrients, APA, molecular biology) to ensure comparability. All samples were preserved according to the specific protocols detailed in sections 2.4.1-2.4.4 and transported on ice to the laboratory within 12 hours.

Line 121: Please justify the choice of an N:P ratio of 28 (half of 55). Clarify the rationale for the nitrogen and phosphorus concentrations used in the treatment groups, which appear substantially higher than in the control.

Response: We appreciate and agree with the reviewer’s comment. We have revised the text at section 2.3 in lines 149-153 to explicitly justify both aspects. The choice of the DIN:PO₄-P ratio of 28 for the treatment group (TG) was based on creating a clear contrast to the ambient ratio (55) and moving toward the classical Redfield stoichiometry (16:1) to test responses under mitigated imbalance. Furthermore, the higher absolute nutrient concentrations in the TG (DIN: 50.01, PO₄-P: 1.78 μmol·L⁻¹) compared to the control (CG) (DIN: 1.90, PO₄-P: 0.04 μmol·L⁻¹) were necessary to ensure that nutrients remained non-limiting throughout the 29-day incubation, allowing the target N:P ratio to be the primary variable influencing the system over time.

Line 123: Explain the rationale for sampling 17 times at 2-day intervals.

Response: Thank you for prompting us to clarify the sampling strategy. We have revised the sampling details at section 2.3 in lines 154-158. Samples were collected 17 times over the 29-day incubation to cover the full phytoplankton growth and senescence cycle. During the exponential growth phase, sampling was conducted daily. After nutrient depletion and population decline, the interval was extended to monitor the stabilized system. The detailed sampling days and weather conditions are presented in Table S2.

Line 198: Given the dominant nutrient inputs from the Yellow and Xiaoqing Rivers (Fig. 2), analyses of N:P ratios for all rivers may be unnecessary, as the remaining tributaries likely contribute only a small fraction of total loading.

Response: Thank you for this valuable comment. We agree that the Yellow and Xiaoqing Rivers are the dominant nutrient sources. However, the analysis of all ten rivers was conducted to assess the nutrient stoichiometry across the entire watershed. Although these smaller tributaries contribute less to the total nutrient load, their impact on local nutrient concentrations and ratios, particularly in adjacent estuarine and coastal waters, remains significant. This comprehensive approach confirms that elevated N:P ratios are a basin-wide characteristic, underscoring the pervasive nature of anthropogenic nutrient imbalance. The data are retained in Fig. 2 for reference. We have added the relevant discussion on lines 401-407 of Section 4.1.

Line 216: Clarify how temperature and other environmental parameters were spatially interpolated.

Response: Thank you for this suggestion to improve methodological transparency. As requested, a sentence has been added to clarify the interpolation method (Line 229-232). The spatial distribution maps for all parameters (e.g., temperature, salinity, nutrients, APA, Chl *a*) were generated using ordinary kriging interpolation in Surfer (version 16, Golden Software LLC), a geostatistical method that accounts for spatial autocorrelation to produce optimal, unbiased estimates for continuous surfaces.

Lines 231–233: The N:P ratio results should be presented in the main text rather than in the supplementary material.

Response: Thank you for the reviewer's valuable suggestion. We agree that the DIN:PO₄-P ratio is a key parameter for interpreting the ecological status. In response, we have moved the relevant data from the supplementary material to the main text. The spatial distribution of the DIN:PO₄-P ratio is now included in Figure 3, alongside PO₄-P and Chl *a*, providing readers with a direct visual comparison of these critically linked variables. The corresponding description and discussion have been integrated into Sections 3.2.2.

Lines 261–265: The analysis described here does not appear consistent with the corresponding figure; please verify and revise.

Response: We thank the reviewer for their careful scrutiny. We have re-examined the correlation analysis and the corresponding Figure 4. The description in the original text was indeed not fully aligned with the figure. The relevant paragraph in Section 3.3 has been thoroughly revised to accurately reflect the patterns shown in Figure 4. The revised description is as follows (Line 299-306): In autumn, correlation analysis of field data revealed that APA_{phy} exhibited a significant positive correlation with the DIN/PO₄-P ratio ($p < 0.05$) (Figure 4). However, there was no detectable correlation between APA_{total} and PO₄-P (Figure 4). In winter, APA_{total}, APA_{bac}, and APA_{free} were negatively correlated with PO₄-P and Chl *a*, but not with the DIN/PO₄-P ratio (Figure

4), and APA_{total} was positively correlated with APA_{phy} , APA_{bac} , and APA_{free} . Additionally, APA_{free} was positively correlated with APA_{phy} ($p < 0.05$) and APA_{bac} ($p < 0.05$). APA_{total} , APA_{bac} , and APA_{free} were also positively correlated with the DON/TDN ratio ($p < 0.05$) (Figure 4).

Line 273: Add subtitles to the figure indicating “summer” and “winter”.

Response: Thank you for this suggestion to improve the clarity of the figure. As recommended, subtitles clearly indicating “Autumn” and “Winter” have been added to the respective panels of Figure 4.

Lines 283–286: The text is not fully consistent with the figure content and should be revised.

Response: Thank you for highlighting this inconsistency. We have carefully revised the text in Lines 283 – 286 to ensure it accurately describes the nutrient dynamics presented in Figure 5. The updated description now clearly and correctly reflects the observed changes in DIN:PO₄-P uptake ratios, the temporal trends of the DIN:PO₄-P ratio in both the CG and TG, and the dynamics of DON and DOP concentrations during the initial phase of the enclosure experiment. The revised description is as follows (Line 317-321): At the beginning of the field enclosure experiment (days 0–3, no rainfall), the DIN and PO₄-P in the TG group were absorbed rapidly and in large quantities, and the DIN:PO₄-P ratio increased (Figure 5a-c). In contrast, the CG (DIN:PO₄-P = 55) showed a decrease in the DIN:PO₄-P ratio (Figure 5c).

Line 310: APA_{total} is referenced but not provided; please include this information.

Response: Thank you for noting the reference to APA_{total} in the results section. We understand the reviewer’s point regarding its presentation. In the revised manuscript, we have clarified this in two ways. First, we have explicitly included the average APA_{total} values for both the control (CG) and treatment (TG) groups in the main text in Section 3.4.3 lines 344-353. Second, we wish to clarify that the complete temporal dynamics of APA_{total} throughout the experiment, including its partitioning among size fractions (APA_{phy} , APA_{bac} , APA_{free}), are fully presented in Figure S5 of the supplementary material (Figure S5. APA_{Total} contributed by APA_{phy} , APA_{bac} , and APA_{free} during the 29-day enclosure experiments in the control group (CG) and the treatment group (TG)). We hope this combined presentation in the text and the supplementary figure satisfactorily addresses the point raised.

Lines 412–414: The proposed approach to reducing the N:P ratio by increasing phosphorus inputs may not be practical; please reconsider or further justify this recommendation.

Response: We agree with the reviewer that increasing phosphorus inputs to lower the N:P ratio would be counterproductive and impractical, as it could exacerbate eutrophication. Our intended recommendation was precisely the opposite: to emphasize source control of nitrogen to mitigate the N:P imbalance and restore ecological balance as the primary management lever. We have revised the text in the Discussion to clearly state that (Line 452-455): Management strategies must

prioritize the reduction of nitrogen inputs at their source to mitigate the N:P imbalance and restore ecological balance, rather than relying on intrinsic biological compensation within the coastal zone. We thank the reviewer for highlighting this ambiguity, which allowed us to clarify this critical point.