<u>Responses to Reviewer#2's Comments</u>

We sincerely appreciate **Reviewer #2** for taking the time and effort necessary to review our manuscript. We would like to thank all valuable comments and suggestions, which helped us to improve the quality of our manuscript. Our responses to the **Reviewer #2** comments are described below in a point-to-point manner. Below we outline how we will address the major points raised. We hope that the revision addresses your concerns.

In this manuscript, Kim et al. explore the possible causes for the relatively low NO_3:PO_4 ratio in a well-oxygenated water column in the East/Japan Sea. They collected water samples from different depths over five months to understand microbial N cycling. They measured the abundance of N-reducing genes and the distribution of functionally grouped N-reducing bacterial communities. Based on these (non-quantitative) sampling results, they conclude that there is dual feedback driving the low NO_3:PO_4, i.e., enrichment in NO_3 due to atmospheric N deposition and reduction of NO_3 in low-oxygen microenvironments. They conclude that in the future, this process will prevail, as subsurface N removal associated with climate-driven deoxygenation, and the EJS can potentially serve as a sentinel for biogeochemical response in other regions.

1) Overall, the paper is well written and clear. However, I think that the evidence given in the manuscript does not justify the conclusion. First, the water column in the EJS is well oxygenated (Fig. 1B). So, even a proposed reduction in 20 M O_2 will not make the deep ocean anoxic. Therefore, the proposed dual-scale feedback seems to be irrelevant to the EJS.

→ We fully agree that the water column of the East/Japan Sea remains well oxygenated and that a reduction of ~20 μM O_2 would not lead to the development of basin-scale anoxia. Our intention was not to imply the emergence of anoxic conditions. Rather, we aimed to highlight long-term deoxygenation trends associated with reduced deep ventilation that may gradually alter the balance of the reactive N inventory. In particular, our hypothesis is that 'progressive deoxygenation'—even within an overall oxic water column—may expand the prevalence and spatial extent of nitrate-reduction pathways occurring in localized low-oxygen microenvironments (e.g., particle-associated niches). Over decadal timescales, such changes could enhance internal N loss relative to present conditions.

To avoid misunderstanding, we will revise the manuscript to replace references implying "anoxic" conditions with more precise descriptions of long-term deoxygenation and reduced ventilation. In particular, we will clarify that the reported ~20 μM decrease in oxygen concentration reflects a deoxygenation trend rather than the development of anoxic conditions in the water column. In addition, we will revise the description of the subsurface feedback by replacing the term "N removal" with "reactive N inventory" to more accurately convey that the proposed mechanism refers to changes in the balance of the N pool rather than the formation of basin-scale anoxia.

These revisions aim to clarify that the proposed feedback concerns the potential for increased relative importance of nitrate-reduction pathways under gradually declining oxygen conditions, rather than the onset of anoxic environments. The revised text will therefore frame the proposed mechanism as a shift in the balance of the N inventory under gradual deoxygenation, rather than as evidence for anoxic N loss.

2) The author also mentions in the abstract that low NO_3:PO_4 in the well-oxygenated EJS is a "long-standing biogeochemical enigma". This is the first time I have heard of this interesting enigma, and I would like to understand more about how past studies have approached it. The authors only provide a general statement ("Much of our current understanding of marine N cycling ..." LN 50). It is missing to understand what has been done until today.

→ We thank the reviewer for pointing out that the background explaining why the low NO_3^-:PO_4^{3-} ratio of the EJS has been considered an unresolved problem was not sufficiently described in the original manuscript. In the revised version, we have expanded the Introduction to more clearly summarize how previous studies have approached this issue.

Previous studies have consistently reported that the N:P ratio in the East/Japan Sea is lower than the Redfield ratio throughout much of the water column, indicating persistent N limitation. Proposed explanations have included physical circulation and remineralization processes (Kim and Kim, 2013), the inflow of low N:P waters from the East China Sea (Lee et al., 2009), and the possibility of denitrification (Yanagi, 2002). However, the occurrence of substantial denitrification remains difficult to reconcile with the well-oxygenated conditions of the basin, and the mechanism responsible for maintaining the low N:P ratio has therefore remained unresolved.

We have therefore revised the Introduction to briefly synthesize these previous perspectives and clarify why the persistence of low NO_3^-:PO_4^{3-} ratios in an oxic water column remains an open biogeochemical question. This added context better motivates the present study, which examines microbial nitrate-reduction pathways as a potential mechanism contributing to the observed stoichiometry.

3) P-balance: The riverine P balance has not been sufficiently analyzed. I would like to get more quantitative information about the riverine input – when was the dam constructed? What is the NO_3:PO_4 in the CDW? What do you mean by "confined to surface layers"? It is possible that the low NO_3:PO_4 is due to past influx? I would like more quantitative information on the long-term NO_3:PO_4 balance for the EJS.

→ We agree that the discussion of external phosphorus inputs in the current version lacks sufficient quantitative context. In particular, the basis for excluding sustained external P supply as a driver of the depth-integrated low NO_3^-:PO_4^{3-} signal requires clearer numerical support.

First, we now specify that the Nakdong River estuarine barrage was constructed in 1990, which regulates freshwater discharge to the coastal ocean. We also provide the reported mean discharge magnitude (~0.0004 Sv), emphasizing that riverine input represents only a very small fraction of the basin volume. Second, we have added quantitative information on the nutrient characteristics of CDW. Previous studies report very high DIN:DIP ratios (50–100) near the Changjiang mouth and inner shelf, reflecting strong N enrichment, with ratios decreasing offshore due to mixing and biogeochemical processing (Lee et al., 2009). These values are now explicitly described in the revised manuscript. Third, we clarified the meaning of the previous phrase "confined to surface layers." Specifically, we now explain that Changjiang-derived freshwater forms a buoyant plume due to its lower density relative to surrounding seawater, which restricts its influence largely to the upper mixed layer rather than penetrating into deeper waters (Tseng et al., 2014).

Together, these revisions provide clearer physical and biogeochemical context showing that riverine inputs and CDW intrusion are unlikely to exert a strong control on basin-scale nutrient stoichiometry. Specifically, previous studies indicate that CDW is typically N-rich relative to phosphate, its influence is largely restricted to buoyant surface layers due to strong stratification, and its transport exhibits pronounced seasonal variability. These characteristics make it unlikely that CDW can sustain the basin-scale and vertically persistent low NO_3^-:PO_4^{3-} ratios observed in the East/Japan Sea. We have verified these points using the relevant literature, incorporated the corresponding citations, and clarified this interpretation in the revised manuscript.

4) Furthermore, what about the release of P from anoxic sediment?

→ We thank the reviewer for raising the possibility that the low NO_3^-:PO_4^{3-} ratios observed in the East/Japan Sea (EJS) could reflect historical phosphorus enrichment or sedimentary P release rather than N loss processes.

To address this concern, we expanded the manuscript to better constrain the long-term nutrient balance of the basin. Previous studies indicate that deep-water NO_3^-:PO_4^{3-} ratios in the EJS have remained consistently below the Redfield ratio (≈13) over multiple decades (Lee and Rho, 2015), and these references have now been added. In addition, following the reviewer's suggestion, we included vertical NO_3^-:PO_4^{3-} profiles using our dataset to illustrate the depth structure of the ratio.

We also clarified the role of sedimentary phosphorus cycling. Studies of EJS sediments show that phosphorus is largely retained through burial and adsorption processes; dissolved phosphate released during early diagenesis is efficiently scavenged by iron oxides in oxidized sediments (Cha et al., 2005). This suggests that large-scale phosphate release from sediments is unlikely to represent a major phosphorus source to the water column. These clarifications have been incorporated into the revised manuscript to better explain the long-term NO_3^-:PO_4^{3-} balance of the EJS.

5) I am not so familiar with the sequencing protocol, so I am unable to evaluate it. However, to gain more quantitative intuition into the significance of the N-reducing genes, it would be beneficial to compare them with other locations. Perhaps other studies showed the relationship between N-reducing genes and rates?

→ We appreciate the referee's suggestion to place the reported proportions of N-reducing genes in a broader quantitative context and to consider studies linking gene abundance with measured N-loss rates. Our analysis is based on 16S rRNA gene amplicon sequencing, which provides relative taxonomic abundance rather than absolute gene copy numbers; therefore, the dataset is most suitable for examining vertical and seasonal patterns in the distribution of nitrate-reducing taxa rather than deriving quantitative estimates of N loss. In addition, most previous studies investigating nitrate-reduction or denitrification genes have focused on oxygen-deficient zones or sediments, and comparable datasets examining their vertical and seasonal distribution across fully oxygenated water columns remain limited.

To strengthen this contextualization, we will incorporate representative studies reporting N-reducing taxa and related nitrate-reduction genes (i.e., *nir*, *nos,* etc) in oxic open-ocean environments, thereby situating our findings within the broader distribution of these pathways beyond oxygen-deficient systems. In addition, we will cite literature addressing the potential decoupling between gene abundance and measured biogeochemical rates, in order to explicitly clarify that functional gene proportions do not imply proportional N removal fluxes. These additions will ensure that the revised discussion carefully avoids overextending gene-based observations into rate-based interpretations. In addition, we will incorporate discussion of studies reporting positive relationships between denitrification-related gene abundance and measured N-loss rates to provide broader context, while clarifying that such relationships have primarily been established in anoxic environments. Accordingly, we will frame our results as describing the persistent functional potential of nitrate-reducing taxa in an oxic basin rather than implying direct rate equivalence, and as providing a depth-resolved reference baseline for future comparisons in oxic pelagic systems.

6) Is it possible to plot NO_3:PO_4 vs. depth?

→ We agree with the suggestion and will present depth-resolved NO_3^- and PO_4^{3-} profiles, along with explicit NO_3^-:PO_4^{3-} vs. depth representations for each sampling period. In the revised manuscript, we have included depth-resolved NO_3^-:PO_4^{3-} profiles for each cruise, presented alongside NO_3^- and PO_4^{3-} vertical distributions at the beginning of the Results section (Section 3.1). These profiles are shown at the same vertical resolution as the genomic data, allowing direct comparison between nutrient structure and microbial patterns. The NO_3^-:PO_4^{3-} vs. depth plots clearly illustrate the vertical variability in nutrient stoichiometry during the study period, including relatively elevated and variable ratios in surface waters and more persistent lower ratios at depth. This addition helps resolve the scale-dependent interpretation emphasized throughout the manuscript and provides a clearer observational basis for linking nutrient distributions with nitrate-reduction–associated functional potential.

7) Minor comments

line 35 - > cite Tyrell 'The relative influences of nitrogen and phosphorus on oceanic primary production' (1999)

→ We have now cited Tyrrell (1999), which describes how the opposing effects of biological N_2 fixation and denitrification contribute to the regulation of the oceanic N inventory and the long-term stability of marine N:P ratios.

Line 50 -> cite Martiny et al., 'Strong latitudinal patterns in the elemental ratios of marine plankton and organic matter', 2013.

→ We have now cited Martiny et al. (2013) to support the statement that, although the global NO_3^-:PO_4^{3-} ratio approximates the Redfield ratio, substantial regional variability in marine N:P stoichiometry has been widely documented.

Line 50: What do you mean by “circulation terminates”?

→ In the revised manuscript, we replaced this expression with a more precise description indicating that the North Pacific contains relatively old deep waters within the global overturning circulation. The sentence has been revised accordingly to clarify that the lower oxygen concentrations in this region reflect the accumulation of respiratory oxygen consumption along the circulation pathway.

Line 60: “Much of our current understanding of marine N cycling...” is it a general statement or is it specific to the EJS?

→ Our intention was to refer to the broader development of research on microbial nitrate-reduction processes rather than N cycling in general. In the revised manuscript, we have clarified this point by specifying that studies directly examining microbial nitrate-reduction pathways in the East/Japan Sea remain limited, particularly those integrating genomic approaches.

In summary, we appreciate the referee’s constructive critique. We will undertake a substantial revision to strengthen the quantitative framing of external inputs, clarify the scope and limitations of the genomic evidence, and refine the dual-scale feedback interpretation to ensure that conclusions remain consistent with the well-oxygenated nature of the EJS.

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