Responses to Reviewer#1's Comments

We sincerely appreciate **Reviewer #1** 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 #1** comments are described below in a point-to-point manner. We hope that the revision addresses your concerns.

The paper «A potential explanation for the anomalously low nitrate to phosphate ratio in the well-oxygenated East/Japan Sea » by Kim et al. attempt to explore the contribution of bacterially mediated N loss for explaining the low N:P ratio encountered in the East/Japan Sea. The study is based on the hypothesis that N reducing pathways, i.e. nitrate reduction and denitrification, may occur at relevant magnitude in their study area (despite the well oxygenated conditions encountered), enough to explain low $NO_3^-:PO_4^{3-}$ ratios. To test their hypothesis, the authors use sequencing data from samples collected between the surface and 1000 m depth during 5 cruises in the EJS, between February and October 2021. 16S sequencing was used to build up a taxonomic classification of bacterial communities at the order level. Furthermore, they applied a computational tool to infer functional properties of the obtained bacterial communities in an attempt to analyse the relative abundance of N-reducing genes.

1) I am not a specialist in genomics approaches but (or maybe because of that) I find that the Methods section lack literature references (with doi) for applied protocols, bioinformatics tools/pipelines and used databases. Hopefully, other reviewers more proficient in the field may assess whether the used methodology is sound.

→ Thank you for pointing out the need for clearer methodological references. In the revised manuscript, we have added literature references (including DOIs) throughout the Methods section to document the sequencing protocol, bioinformatic workflow, and reference databases used in this study. Specifically, references were added for the DNA quality verification step using a NanoDrop spectrophotometer (Desjardins and Conklin, 2010), the Illumina 16S Metagenomic Sequencing Library Preparation protocol (Klindworth et al., 2013), and the Illumina MiSeq sequencing platform and workflow (Kozich et al., 2013). We also added references for the taxonomic annotation and clustering procedures, including the SILVA database and NCBI reference resources (Quast et al., 2013; Sayers et al., 2021) and the UCLUST clustering algorithm (Edgar, 2010). These references are now provided for the key steps of the workflow, including sequence processing, taxonomic assignment, and functional inference. This revision clarifies the methodological framework and allows readers to more easily trace the analytical procedures used in this study.

2) This said, the main and critical caveat of this work is, in my opinion, that the conclusions are not supported by the data and the interpretation of the results is sometimes way too speculative.

→ Thank you for this important comment. In the revised manuscript, we have addressed this by restructuring the Results and Discussion to strengthen the data–interpretation linkage. Specifically, we added depth-resolved vertical profiles of NO_3^-, PO_4^{3-}, and $NO_3^-:PO_4^{3-}$ ratios from the 2021 observations at the beginning of the Results section (revised Section 3.1), using the same vertical resolution as the genomic data. These profiles allow us to explicitly characterize the seasonal and vertical structure of nutrients during the study period and to evaluate, based on the cruise-resolved observations, whether the low $NO_3^-:PO_4^{3-}$ ratios are consistently maintained throughout the water column. By presenting both NO_3^- and PO_4^{3-} distributions together with their stoichiometric ratios, we establish a data-driven nutrient background prior to the genomic results. This addition strengthens the manuscript by shifting the interpretation toward an observation-based framework, in which conclusions are more directly grounded in the measured nutrient structure rather than inferred from aggregated values. This allows the nutrient structure to be presented as an observational constraint prior to the genomic results, thereby providing a clearer basis for subsequent interpretation. This reorganization shifts the manuscript from a literature-style exposition to a nutrient–genomic linkage framework, in which genomic patterns are interpreted within the observed nutrient context.

More importantly, we revised the interpretation to avoid overstating the implications of the genomic data. In the previous version, some expressions could be interpreted as linking the observed gene proportions to quantitative N loss. These have now been carefully moderated to ensure that the results are not presented as direct evidence of N loss rates. We also clarify that this study is based on 16S rRNA gene amplicon sequencing and therefore reflects relative variations in N-reducing genes and taxa across depth and season, rather than direct quantification of N loss. This limitation is now explicitly stated in both the Results and Discussion sections.

Accordingly, the conclusions are reframed to emphasize that our results represent depth- and time-resolved patterns of nitrate-reduction–associated functional potential, which are consistent with—and help constrain—the mechanisms underlying the low $NO_3^-:PO_4^{3-}$ ratios, rather than directly quantifying or explaining N loss. This distinction is now consistently reflected throughout the revised manuscript.

3)The authors base their conclusions in a series of numbers (percentages of N-reducing genes and associated bacterial communities) which do not give sufficient quantitative information.

→ Thank you for this comment. We agree that the definition and interpretation of the reported percentages were not sufficiently clear in the previous version. In the revised manuscript, we explicitly define how these values were calculated. The percentage of N-reducing genes represents the proportion of nitrate-reduction–associated genes (*nar*, *nap*, *nir*, *nor*, *nos*) relative to the total set of detected N-cycling genes (including ammonification, nitrification, and nitrite oxidation, nitrate reduction, denitrification pathways) for each depth and sampling period. Similarly, the associated bacterial community proportion represents the fraction of order-level OTUs linked to nitrate-reduction–associated taxa relative to the total detected bacterial OTUs at each depth and time point. These definitions are now clearly described in the Results and Discussion, and detailed in the revised figure legends (Figures 2 and 3), to ensure that the reported values are interpreted as relative contributions within the N cycling network rather than as standalone quantitative measures. This clarification ensures that the reported values are interpreted within a defined analytical framework and not as isolated quantitative metrics.

4) Throughout the manuscript, the authors state that they found « high » abundances of N-reducing genes; but high compared to what?

→ Thank you for this comment. We agree that the use of qualitative expressions such as "high" was not sufficiently defined in the previous version. In this study, because our analysis is based on relative abundances derived from 16S rRNA gene amplicon data, and because comparable datasets from well-oxygenated, depth-resolved water columns remain limited, the term "high" was originally intended to describe relative differences across depth and season within our dataset rather than absolute or cross-system comparisons. To avoid this ambiguity, we have replaced such expressions throughout the manuscript with more explicit, context-dependent descriptions (e.g., detectable, depth-dependent, or substantial contributions), clearly indicating their vertical and seasonal variability. We now consistently frame these values within an internal, depth- and time-resolved context, rather than as absolute magnitudes or inter-regional comparisons.

We further clarify that these relative abundances represent variations in nitrate-reduction–associated functional potential, not direct or rate-based measurements of N loss. Accordingly, we revised the Discussion to emphasize pattern-based interpretation within the dataset and to avoid direct quantitative comparison with other regions, while providing context through studies linking gene abundance and process rates without implying equivalence. Finally, we highlight that our dataset provides a depth-resolved, time-integrated baseline for nitrate-reduction–associated functional potential in an oxic basin, which can serve as a reference for future studies combining genomic and rate-based measurements.

5) And how can we know that the obtained percentages are « enough » to explain the observed N:P ratios?

→ Thank you for this important question. We agree that the reported percentages cannot, by themselves, determine whether the observed N loss is quantitatively sufficient to explain the low $NO_3^-:PO_4^{3-}$ ratios. In this study, our analysis is based on relative abundances derived from 16S rRNA gene amplicon sequencing data and therefore does not provide direct measurements of N loss rates. Accordingly, we do not interpret these percentages as quantitative estimates of N removal or as sufficient evidence to fully explain the observed nutrient ratios. Instead, we interpret these values as indicators of the presence and distribution of nitrate-reduction–associated functional potential across depth and season. The consistent detection of these genes and taxa throughout the water column suggests that nitrate-reduction processes may be persistently active at a background level, which is consistent with a mechanism contributing to N loss. We have revised the manuscript to clarify this point and to frame our conclusions accordingly, emphasizing that the genomic evidence constrains plausible mechanisms rather than directly quantifying their magnitude. We additionally discuss previous studies reporting relationships between gene abundance and N-loss rates to provide context for interpretation, while avoiding direct rate equivalence.

6) Moreover, seasonal and vertical variability is presented without really assessing the reasons and consequences of this variability.

→ Thank you for this comment. We agree that the previous version did not sufficiently address the drivers and implications of the observed seasonal and vertical variability. Our primary objective in this study is to demonstrate the persistent, depth- and time-integrated presence of nitrate-reduction–associated genes and taxa across the entire water column (0–1000 m) as a potential contributor to the low NO_3^-:PO_4^{3-} ratios in the East/Japan Sea. In this context, the seasonal and vertical variability is presented as part of the overall pattern rather than as the main focus of the study. Nevertheless, we acknowledge that these patterns should be interpreted within an environmental and biogeochemical context. In the revised manuscript, we have therefore expanded the Discussion to include potential drivers of the observed variability (e.g., stratification, oxygen gradients, and particle-associated microenvironments), and we clarify how these factors may influence the distribution of nitrate-reduction–associated functional potential. This addition provides a more complete framework linking the observed variability to its underlying controls and biogeochemical implications, while maintaining the primary focus of the study.

7) Nutrient data collected during the cruises are exploited only superficially although they should be at the center of their study. In the current version, all nutrient data have been merged to produce a unique value of NO_3:PO_4 ratio which is not presented in the results section but in the introduction. I strongly suggest to present the nutrient profiles for each cruise to explore seasonal and vertical variability in the same way as the genomics data. This may allow to compare the variability between nutrients and genomics data which may help assessing the role of N-reducing pathways in N loss in the water column. The Results and Discussion section reads more like a literature

review of the factors that may drive N:P ratios in the study area and I found that the results are not discussed enough.

→ We agree that nutrient data were not sufficiently integrated into the Results section in the previous version and that this limited the connection between nutrient observations and genomic results. In the revised manuscript, we have substantially reorganized the Results and Discussion to place nutrient data at the center of the analysis. Specifically, we now present cruise-resolved vertical profiles of NO_3^-, PO_4^{3-}, and NO_3^-:PO_4^{3-} at the beginning of the Results section (Section 3.1), using the same depth resolution as the genomic dataset. This allows direct comparison of seasonal and vertical variability between nutrient distributions and genomic patterns. We further include an explicit description of nutrient variability during the study period, providing the environmental context for the persistently low NO_3^-:PO_4^{3-} ratios observed in the East/Japan Sea. The genomic results are then presented within this framework, enabling a clearer linkage between nutrient structure and nitrate-reduction–associated functional potential. This reorganization shifts the manuscript from a literature-based exposition to a data-driven nutrient–genomic linkage framework. In addition, we have revised the Discussion to focus more directly on interpreting the results, reducing descriptive literature review content and strengthening the connection between observations and their biogeochemical implications. This change ensures that nutrient observations are directly linked to the genomic analysis throughout the manuscript.

8) Moreover, there are a number of inconsistencies in the arguments given. For instance, the first part of the section claims that external phosphorus inputs are insufficient to explain low N:P ratios but later on, the authors state that N-enriched atmospheric deposition is responsible for high N:P ratios in surface waters. Again, presenting nutrient profiles will be very useful to understand whether N:P ratios are indeed higher in the surface that in deeper waters.

→ Thank you for this important comment. The authors agree that the previous version did not sufficiently distinguish the different depth- and time-scales of the processes discussed, which may have created the impression of inconsistency. In the revised manuscript, we clarify that the proposed dual time-scale feedback refers to two distinct processes operating in different parts of the water column. Atmospheric N deposition is discussed as a short-term surface forcing that may elevate NO_3^-:PO_4^{3-} ratios in the upper ocean, whereas the long-term low N:P signature at depth is interpreted in relation to reduced deep ventilation and progressive deoxygenation, which may enhance the relative importance of particle-associated nitrate-reduction pathways over time. These processes are therefore not presented as contradictory explanations, but as scale-dependent forcings acting in opposite directions in surface and deep waters. To make this distinction explicit, we have added cruise-resolved vertical profiles of NO_3^-, PO_4^{3-}, and NO_3^-:PO_4^{3-} to the Results section and revised the Discussion to separate short-term surface enrichment from long-term deep-water controls more clearly.

9) On specific comments

Lines 57-59. If low N:P ratios are a known feature of the EJA, I suggest the authors refer to literature data in the introduction and present their own data in the results section in a more detailed way.

→ The authors agree with the referee's suggestion. In the revised manuscript, we will strengthen the Introduction by explicitly citing previous studies that have reported persistently low NO_3^-:PO_4^{3-} ratios in the East/Japan Sea, thereby clarifying that this feature has been recognized in earlier work. In addition, we will present our nutrient observations from the 2021 cruises (vertical profile of NO_3^-, PO_4^{3-}, and NO_3^-:PO_4^{3-} ratio, same resolution of genomic data) in the Results section in a more detailed way by expanding the presentation of the NO_3^-:PO_4^{3-} ratio shown in Fig. 1c, rather than relying on a single representative value. This will allow readers to directly assess the observed nutrient distributions before proceeding to interpretation.

Lines 64-66. Since genomics-based techniques are experiences rapid advances, the authors may want to cite more recent references.

→ In the revised manuscript, we have added recent references that (i) establish genomics and metagenomics as key tools for linking marine microbial communities to biogeochemical cycling, (ii) illustrate current approaches to functional interpretation of N cycling microorganisms, and (iii) explicitly discuss the strengths and limitations of inferring functional potential from 16S rRNA gene data. These additions are intended to better situate our approach within the current state of the field.

Line 81. I guess it was filters and not filtered samples that were frozen and used for DNA extraction. Line 83. Same comment as above.

→ Thank you for the referee for pointing out this wording issue. We have revised the text at Lines 81 and 83 to clarify that the membrane filters, rather than the filtered seawater samples, were frozen and used for DNA extraction.

As said before, the results and discussion section is awkward. Indeed, only 3.2. sub-section is properly results and a bit of light discussion. Sub-section 3,1 reads more like a literature review.

→ The authors fully acknowledge the referee's concern that the previous Results and Discussion section did not clearly separate observational results from interpretative discussion and that Section 3.1 read more like a literature review. In response, we have substantially reorganized this part of the manuscript. First, we added depth-resolved nutrient observations from the 2021 cruises (vertical profiles of NO_3^-, PO_4^{3-}, and NO_3^-:PO_4^{3-} ratio) presented at the same resolution as the genomic figures. These data now form the opening subsection (3.1), providing the observational nutrient context for the anomalously low N:P ratios. The subsequent sections have been reorganized to improve the logical flow: Section 3.2 now presents the genomic observations, Section 3.3 discusses potential mechanistic interpretations linking microbial nitrate-reduction potential with the observed nutrient structure, Section 3.4 quantitatively reassesses the P balance (including additional data and clearer exclusion of external sources), and Section 3.5 discusses future implications while moderating the tone of the dual-scale feedback hypothesis. Through this restructuring, the Results and Discussion section is now organized to more clearly emphasize the linkage between nutrient observations and genomic evidence in explaining the low N:P ratios in the East/Japan Sea.

Lines 139-140. Please, add references for the selected biomarker genes.

→ We have added appropriate references supporting the use of the selected biomarker genes for the corresponding N transformation pathways.

Figure 2. Is there a possibility of adding error bars?

→ Figure 2 shows the relative proportion of nitrate-reduction–associated genes (*nar, nap, nir, nor, nos*) within the total N-cycling gene pool for each individual cruise-depth sample. These values are not averages across replicates but represent the observed gene composition at specific depths and months. Because biological replicates are not available and the purpose of the figure is to illustrate the vertical structure and consistency of NO_3^- reduction–associated gene proportions across the water column, rather than to quantify statistical uncertainty, adding conventional error bars would be misleading. We have clarified this point in the figure caption and text.

Line 169. Aren't some of these orders (e.g. *Alteromonadales*, *Pseudomonadales*) ubiquitous in the ocean?

→ We agree with the referee that several of the identified orders, including *Alteromonadales* and *Pseudomonadales*, are widely distributed and commonly observed in marine environments. Our interpretation does not rely on these taxa being region-specific to the East/Japan Sea, but rather on their documented functional potential related to nitrate reduction and their consistent co-occurrence with nitrate reduction–associated genes across the water column. In the revised manuscript, we clarify this point to avoid any implication that these taxa are unique indicators of the region. At the same time, we expand the discussion to place these taxa in an environmental context, noting that many of these broadly distributed heterotrophic groups are known to associate with particles or organic-matter-rich microenvironments where localized oxygen depletion can occur. We therefore discuss how such ecological traits may facilitate nitrate-reduction processes within micro-scale niches even in otherwise well-oxygenated waters. This revision helps link the observed microbial community composition with plausible environmental mechanisms operating in the East/Japan Sea.

Line 178-179. The statement that proportions of 37.7% of N-reducing genes and 20.0% of taxa indicate the potential for bacterially mediated N loss is not very convincing. How these numbers compare with other regions?

→ We appreciate the referee's suggestion to place our findings in a broader quantitative context. We agree that cross-regional comparison and linkage to process rates would strengthen interpretation. However, direct numerical comparison between our reported gene proportions and published rate measurements is inherently limited by differences in methodological focus and spatial scale.

Most previous studies examining nitrate-reduction and denitrification genes have focused on oxygen-deficient zones or sediments, where direct rate measurements and gene abundances are often reported together. In contrast, well-oxygenated pelagic systems have primarily been investigated with respect to oxic pathways such as nitrification, ammonification, or N_2 fixation. Consequently, comparable depth-resolved, time-integrated datasets of nitrate-reduction–associated gene proportions in fully oxygenated basins remain scarce. We also emphasize that our study does not attempt to infer N removal rates from gene proportions. Rather, it characterizes the depth- and time-integrated distribution of nitrate-reduction–associated functional potential across the entire water column (0–1000 m) over five sampling periods. This integrative framework differs fundamentally from most rate-based or site-specific studies and therefore does not permit direct rate equivalence.

In the revised manuscript, we will (i) explicitly clarify the conceptual distinction between functional potential and measured process rates, (ii) discuss the limitations of cross-study numerical benchmarking, and (iii) frame our results as establishing a depth-integrated reference baseline for nitrate-reduction–associated functional potential in an oxic basin, which may facilitate future comparative studies conducted under similar methodological and spatial frameworks. 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.

References

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