

Response to essd-2024-539 RC2:

We first thank the Reviewer 2 for the thorough review of this manuscript. The feedback provided constructive comments and suggestions and incorporating the feedback to this draft will improve the quality of this work greatly.

Reviewer's comments for "Global database of actual nitrogen loss rates in coastal and marine sediments".

This study compiles global denitrification and anammox data from both open ocean and estuarine environments, providing a valuable dataset for the scientific community, particularly for researchers studying the nitrogen cycle. The database offers insights into nitrogen loss processes and their environmental controls, which can support future studies and biogeochemical modelling. While the study is well-organized, some aspects require clarification. Below are my comments and suggestions

Thanks for your positive comments.

Line 55 The full name of anammox (Anaerobic Ammonium Oxidation) should be provided here.

Response: Thank you for this suggestion. We have added it.

Line 69 Please provide a brief introduction to slurry incubation and intact core incubation methods to clarify their differences and applications.

Response: Thank you for this suggestion. We have improved the expression and provided a brief introduction to slurry incubation and intact core incubation in line 72-78. "Slurry incubations have been used to estimate the potential rates, and have advantages in discovering nitrogen loss processes in the environment (Thamdrup & Dalsgaard, 2002) as well as studying the environmental controls of nitrogen loss pathways, however, the natural gradients of substrates and redox in sediments were disrupted during incubations (Trimmer et al., 2006). The intact core incubations can quantify nitrogen removal processes in intact sediments and reflect the genuine benthic nitrogen transformation rates. The application of intact core incubations will enable us..."

Line 109 Please provide a brief introduction to continuous flow experiments to clarify their methodology.

Response: Thank you for this suggestion. We have amended a brief introduction in line 115-119. "For continuous flow experiments, incubations were carried out in a flow-through system where bottom water was pumped over intact cores using a multi-channel peristaltic pump, and inflow and outflow samples were collected to quantify the nitrogen process rates after the addition of ^{15}N tracer (Gardner & McCarthy, 2009)."

Gardner, W. S. and McCarthy, M. J.: Nitrogen dynamics at the sediment–water interface in shallow, sub-tropical Florida Bay: why denitrification efficiency may decrease with increased eutrophication, *Biogeochemistry*, 95, 185–198, <https://doi.org/10.1007/s10533-009-9329-5>, 2009.

Line 113 Slurry incubation provides valuable data in certain aspects, and completely excluding these measurements may not be appropriate.

Response: Thank you for this comment. We know that slurry incubation provides valuable data and has advantage in certain aspects. We once considered integrating slurry incubation data and whole core incubation data, however, considering the different meanings of rates by slurry incubation and whole core incubation experiments and the differences in potential and actual rates calculation, we did not include slurry incubation data. Firstly, the rates obtained from slurry incubation represent the potential rate, and the whole core incubation can obtain the actual rates. Secondly, some potential rate calculations include both ^{14}N -based and ^{15}N -based rates (Na et al., 2018; Thamdrup and Dalsgaard, 2002), while others only include ^{15}N -based rates (Deng et al., 2015), making it difficult to make comparisons.

Deng, F., Hou, L., Liu, M., Zheng, Y., Yin, G., Li, X., Lin, X., Chen, F., Gao, J., Jiang, X. 2015. Dissimilatory nitrate reduction processes and associated contribution to nitrogen removal in sediments of the Yangtze Estuary. *Journal of Geophysical Research: Biogeosciences*, 120(8), 1521-1531.

Na, T., Thamdrup, B., Kim, B., Kim, S.-H., Vandieken, V., Kang, D.-J. and Hyun, J.-H. 2018. N_2 production through denitrification and anammox across the continental margin (shelf–slope–rise) of the Ulleung Basin, East Sea. *Limnology and Oceanography*, 63, S410-S424.

Thamdrup, B., Dalsgaard, T. 2002. Production of N_2 through anaerobic ammonium oxidation coupled to nitrate reduction in marine sediments. *Applied and Environmental Microbiology*, 68, 1312–1318.

Furthermore, a recent study has summarized the spatial distribution and drivers of nitrogen loss rates by slurry incubations in aquatic systems. To compare the differences in nitrogen removal rates determined by the same method, here we focus on rates measured by intact core incubation and exclude slurry incubation data. We have added this sentence in line 121-126. “Given a recent study has already summarized the data on nitrogen loss rates by slurry incubations in aquatic systems (He et al., 2025), this work only selected data in which denitrification and/or anammox rates were measured using intact core incubations with ^{15}N isotope pairing techniques, excluding measurements derived from slurry incubations.”

He, G., Deng, D., Delgado-Baquerizo, M., Liu, W., and Zhang, Q.: Global Relative Importance of Denitrification and Anammox in Microbial Nitrogen Loss Across Terrestrial and Aquatic Ecosystems, *Advanced Science*, 12, 2406857, <https://doi.org/10.1002/advs.202406857>, 2025.

Line 119 Please clarify why measurements under light incubation were excluded.

Response: Thank you for this suggestion. We have clarified the excluding reason in line 127-130. “Photosynthetic O_2 production can influence O_2 penetration depth and thereby nitrate availability in sediments, interfering with denitrification rates in the nitrate reduction zone (Chen et al., 2021; Bartoli et al., 2021). In cases where nitrogen loss rates were measured under both light and dark conditions, only those measured in the dark were

included to avoid photosynthesis and facilitate comparison with other studies.”

Line 170 Consider adding a figure to summarize the calculation methods for better clarity

Response: Thank you for this suggestion. For the calculation methods mentioned in this part, as Salk et al. (2017) have already summarized the different calculation methods on nitrogen loss rates and presented relevant pictures clearly, here we don't draw pictures. We have added this reference in this part for better clarity and made the following description in line 243-247. “Regarding the aforementioned calculation methods, Salk et al. (2017) have systematically reviewed different methods for quantifying nitrogen loss rates and illustrated their differences with diagrams distinguishing different processes, providing valuable guidance for researchers interested in this field. Therefore, interested researchers can refer to their article.”

Salk, K. R., Erler, D. V., Eyre, B. D., Carlson-Perret, N., and Ostrom, N. E.: Unexpectedly high degree of anammox and DNRA in seagrass sediments: Description and application of a revised isotope pairing technique, *Geochim. Cosmochim. Acta*, 211, 64-78, <https://doi.org/10.1016/j.gca.2017.05.012>, 2017.

Line 286 Other factors, such as iron and sulfide, can also influence denitrification and anammox. Why were these not considered? While some studies may not have measured these parameters, it would be valuable to discuss their potential role.

Response: Thank you for this suggestion. As the reviewer pointed out, due to the limited data on iron and sulfide, we did not include these data in the database. We have discussed their potential influence on denitrification and anammox in line 394-417.

“Other factors, such as iron, manganese, and sulfide, although not included in the database, can also influence denitrification and anammox rates. For example, Fe oxides were observed to be positively correlated with denitrification rates in the Jinpu Bay, China (Yin et al., 2015). The mechanism may be that ferrous iron can supply an electron donor for nitrate, thereby promoting denitrification. Anschutz et al. (2000) found manganese dioxides could also serve as electron donors for denitrification. Deng et al. (2015) showed a positive relationship between denitrification rates and sulfide concentrations in the Changjiang Estuary sediments, revealing that sulfide can act as energy sources for denitrification. In contrast, evidence has shown that sulfide exerts inhibitory effects on nitrogen removal in coastal sediments by inhibiting the metabolism of denitrifying microorganisms (Aelion and Warttinger, 2010). Thus, the impact of sulfide on denitrification remains controversial. For anammox, a study found that sulfide could affect anammox activity. Yin et al. (2015) found that anammox rates were positively correlated with sulfide concentrations. This phenomenon is likely attributed to sulfide-induced nitrite accumulation during incomplete denitrification processes, where sulfide inhibits the activity of nitric oxide reductase and nitrous oxide reductase, thereby enhancing anammox activity. Under anaerobic conditions, ammonium oxidation can be coupled with the reduction of ferric iron, sulfate, and Mn(IV)-oxides. For example, Rios-Del Toro et al. (2018) confirmed that ammonium oxidation was associated with ferric iron and sulfate reduction under anaerobic conditions, thereby stimulating nitrogen loss in marine sediments. Evidence shows ammonium loss is coupled with Fe(III) and Mn(IV) reduction

in coastal environments (Samperio-Ramos et al., 2024), demonstrating the crucial roles of metal oxides in removing reactive nitrogen.”

Aelion, C. M. and Warttinger, U.: Sulfide Inhibition of Nitrate Removal in Coastal Sediments, *Estuaries Coasts*, 33, 798-803, <https://doi.org/10.1007/s12237-010-9275-4>, 2010.

Anschutz, P., Sundby, B., Lefrançois, L., Luther, G. W., and Mucci, A.: Interactions between metal oxides and species of nitrogen and iodine in bioturbated marine sediments, *Geochim. Cosmochim. Acta*, 64, 2751-2763, [https://doi.org/10.1016/S0016-7037\(00\)00400-2](https://doi.org/10.1016/S0016-7037(00)00400-2), 2000.

Deng, F., Hou, L., Liu, M., Zheng, Y., Yin, G., Li, X., Lin, X., Chen, F., Gao, J., and Jiang, X.: Dissimilatory nitrate reduction processes and associated contribution to nitrogen removal in sediments of the Yangtze Estuary, *J. Geophys. Res.:Biogeosci.*, 120, 1521-1531, <https://doi.org/10.1002/2015JG003007>, 2015.

Rios-Del Toro, E. E., Valenzuela, E. I., López-Lozano, N. E., Cortés-Martínez, M. G., Sánchez-Rodríguez, M. A., Calvario-Martínez, O., Sánchez-Carrillo, S., and Cervantes, F. J.: Anaerobic ammonium oxidation linked to sulfate and ferric iron reduction fuels nitrogen loss in marine sediments, *Biodegradation*, 29, 429-442, <https://doi.org/10.1007/s10532-018-9839-8>, 2018.

Samperio-Ramos, G., Hernández-Sánchez, O., Camacho-Ibar, V. F., Pajares, S., Gutiérrez, A., Sandoval-Gil, J. M., Reyes, M., De Gyves, S., Balint, S., Oczkowski, A., Ponce-Jahen, S. J., and Cervantes, F. J.: Ammonium loss microbiologically mediated by Fe(III) and Mn(IV) reduction along a coastal lagoon system, *Chemosphere*, 349, 140933, <https://doi.org/10.1016/j.chemosphere.2023.140933>, 2024.

In addition, this section applies multiple regression analyses to explore the relationships between various controlling factors and denitrification/anammox. I am curious whether the authors were able to determine a threshold value for these factors—beyond which denitrification exceeds anammox. Additionally, based on the compiled data, which parameter is identified as the most significant controlling factor

Response: Thank you for this suggestion. Here we use simple correlation analyses to explore the relationships instead of multiple regression analyses. In most cases denitrification exceeds anammox as we can see from Figure 4 (line 941). From the perspective of nitrogen loss percentage, denitrification generally dominates nitrogen loss processes. In contrast, anammox exceeds denitrification mainly in stations with water depths between 100 and 2342 m, including a continental shelf to slope transect in the North Atlantic (Trimmer and Nicholls, 2009), the deep Norwegian Trench in the Skagerrak (Trimmer et al., 2013), and the continental margin (shelf – slope – rise) of the Ulleung Basin, East Sea (Na et al., 2018). Thus, by far we have been unable to determine a threshold value for these factors—beyond which denitrification exceeds anammox.

Na, T., Thamdrup, B., Kim, B., Kim, S.-H., Vandieken, V., Kang, D.-J., and Hyun, J.-H.: N₂ production through denitrification and anammox across the continental margin (shelf – slope – rise) of the Ulleung Basin, East Sea, *Limnol. Oceanogr.*, 63, S410-S424, <https://doi.org/10.1002/lno.10750>, 2018.

Trimmer, M. and Nicholls, J. C.: Production of nitrogen gas via anammox and

denitrification in intact sediment cores along a continental shelf to slope transect in the North Atlantic, *Limnol. Oceanogr.*, 54, 577-589, <https://doi.org/10.4319/lo.2009.54.2.0577>, 2009.

Trimmer, M., Engström, P., and Thamdrup, B.: Stark Contrast in Denitrification and Anammox across the Deep Norwegian Trench in the Skagerrak, *Appl. Environ. Microbiol.*, 79, 7381-7389, <https://doi.org/10.1128/AEM.01970-13>, 2013.

Given the fact that we use simple correlation analyses to explore the relationships between nitrogen loss rates and environmental factors, we can't identify which parameter is the most significant controlling factor. However, based on correlation analysis, we identified some key factors influencing denitrification and anammox. We have added these sentences in line 388-393. "Through the correlation analysis of global-scale compiled data, we identified that sediment C/N ratios, oxygen penetration depth, water depth, temperature, salinity, dissolved oxygen, and nitrate concentrations were the main factors regulating denitrification rates, whereas sediment organic carbon, C/N ratios, temperature, salinity, and nitrate concentrations primarily controlled anammox rates (Fig. 5 and Fig. 6)."

In line 453-456, "Based on the simple correlation analysis of global-scale compiled data, we identified that sediment C/N ratios, oxygen penetration depth, water depth and temperature were the primary factors governing the relative contribution of anammox to total nitrogen loss (Fig. 8)."

Line 357 I am wondering about the sediment characteristics at these study sites. Do they include vegetated areas? These factors can significantly influence denitrification and anammox rates.

Response: Thank you for this suggestion. We have checked the references and deleted studies by Gao et al. (2017) and Shan et al. (2016) as their study sites were in vegetated areas. We have restated the expression in line 419-422. "Liu et al. (2020) have examined the spatio-temporal changes of *in situ* nitrogen loss processes in intertidal wetlands of the Yangtze Estuary and found that denitrification was linked to anammox, implying the coupling of denitrification and anammox on a local scale."

Some data in the table represent open ocean environments, while others are from riverine systems. Please consider adding water depth to Table 1 to provide clearer context for the different study sites.

Response: Thank you for this suggestion. We have amended water depth to Table 1.