

Author Responses:

*We deeply appreciate the thorough and helpful comments from the Reviewer. We will carefully revise the manuscript (EGUSPHERE-2026-216) carefully following each comment from the reviewer. The response (**in bold**) was made to each comment, and we also marked changes, which is helpful to find how we changed.*

CC1's comments to the Author:

Estuaries act as critical filters for terrestrial nutrients transporting to the ocean. With the increasing anthropogenic nitrogen loading worldwide, understanding the transformation and bioavailability of organic nitrogen (ON) in estuarine mixing zones is becoming crucial for predicting coastal eutrophication. However, most previous studies focused on inorganic nitrogen, while the dynamics of the organic fraction remains less understood. This manuscript investigates the ON transformation and bioavailability dynamics during estuarine mixing, which addresses an important gap. The experimental design is logical, and the data is solid. It is a meaningful paper.

Response:

We sincerely thank the reviewer for the positive evaluation and encouraging comments on the significance, experimental design, and data quality of our study.

Question 1: In Introduction, add quantitative data or recent estimates regarding the global flux of estuarine ON to highlight the significance of estuarine organic nitrogen.

Response:

Thank you for this helpful suggestion. We have added quantitative global estimates of riverine nitrogen export in the Introduction to better emphasize the importance of estuarine organic nitrogen.

The revised text reads:

[Introduction, L35-40] “Globally, rivers deliver an estimated 36–60 Tg N yr⁻¹ to coastal waters, including approximately 11.8 Tg N yr⁻¹ as dissolved organic nitrogen (DON), highlighting the substantial role of organic nitrogen in land–ocean nitrogen

transfer (Mayorga et al., 2010; Tivig et al., 2021). Recent global modeling further suggests that riverine total nitrogen export to the ocean increased from 27.5 Tg N yr⁻¹ in 1901–1920 to 40.0 Tg N yr⁻¹ in 1995–2014, with DON export increasing by 50.6%, emphasizing the growing importance of estuarine ON transformation under global environmental change (Ma et al., 2025)”.

Question 2: The incubation experiments were conducted for 3 days. While this effectively captures rapid mixing processes, the manuscript discusses "long-term" nitrogen sequestration. It would be better to briefly mention the limitations of this short-term incubation in the Discussion section to ensure the extrapolation to long-term sinks is robust.

Response:

Thank you for this helpful suggestion. We agree that the 3-day incubation mainly captures rapid estuarine mixing processes, including flocculation, adsorption, and initial biological transformation, and cannot directly verify long-term nitrogen sequestration. We have therefore revised the Discussion to clarify this limitation and softened the relevant statements.

The revised text reads:

[Section 4.3, L452] “This transformation process likely enhances the potential for ON burial during transport from river to sea”;

[Section 4.4, L478-480] “Collectively, these findings, along with the results from the current study, suggest that coupled biological and physicochemical interactions during mixing not only promote short-term ON transfer into the particulate phase through a top-down PON pump (Fig. 7b, Pathway 1 & 3), but also alter...”;

[Section 4.5, L510-512] “Second, the 3-day incubation is sufficient to capture rapid flocculation, adsorption, and initial microbial transformation, but may underestimate slower processes such as long-term mineralization, sedimentation, and RDON' stabilization”.

Question 3: Since phytoplankton uptake of DIN involves isotopic fractionation, could

this process also contribute to the elevated $\delta^{15}\text{N}$ values in PON?

Response:

Thank you for this helpful comment. We agree that planktonic assimilation of dissolved inorganic nitrogen (DIN) can contribute to the biological production of PON during the mixing experiments. We have revised the Discussion to explicitly account for this process. However, phytoplankton DIN assimilation generally involves isotope fractionation that preferentially incorporates ^{14}N , thereby producing isotopically lighter PON and tending to decrease $\delta^{15}\text{N-PON}$. This process therefore cannot explain the positive $\delta^{15}\text{N-PON}$ deviation observed in the BAM treatments.

The observed enrichment instead suggests that a ^{15}N -enriched source was added to or retained within the particulate pool and that this process outweighed the opposing isotopically light contribution from newly produced planktonic PON. We therefore clarified that microbial processing can leave residual DON relatively enriched in ^{15}N , and that the selective re-adsorption of this microbially modified refractory DON (RDON') onto particles most likely increased $\delta^{15}\text{N-PON}$. Accordingly, the revised text emphasizes that planktonic DIN assimilation may have contributed to PON production but likely acted as a counteracting process, whereas RDON' re-adsorption dominated the isotopic budget and promoted the accumulation of refractory, isotopically enriched PON.

The revised text reads:

[Section 4.3, L415-437] “The isotopic deviations further constrain this mechanism by indicating that refractory PON accumulation involved the selective re-adsorption of microbially modified, ^{15}N -enriched RDON. In a simple dilution scenario, $\delta^{15}\text{N-PON}$ would be expected to decrease linearly toward the seawater end-member value. Phytoplankton assimilation of DIN may further lower $\delta^{15}\text{N-PON}$ through isotopic fractionation (Chen et al., 2024). However, in the BAM treatments, both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ exceeded the theoretical mixing predictions at intermediate salinities, with enrichments of +0.10‰ and +0.45‰, respectively (Fig. 4c, d). This indicates that a process introducing or retaining ^{15}N -enriched material in the PON

pool must have operated and outweighed the opposing input of isotopically light phytoplankton-derived PON. ON modified by microbial activity, including microbial products and residues, tends to have a higher C/N ratio, more negative $\delta^{13}\text{C}$ values, and elevated $\delta^{15}\text{N}$ values. These isotopic shifts are known to result from fractionation during the biosynthesis of cellular components (Abraham and Hesse, 2003; Ogawa et al., 2001). Because phytoplankton DIN assimilation would tend to lower rather than raise $\delta^{15}\text{N}$ -PON, the observed positive deviations strongly implicate the selective re-adsorption of microbially modified, ^{15}N -enriched and residual refractory DON (RDON') onto particles as the dominant mechanism. Several complementary observations support this interpretation. The increase in C/N ratios with salinity (Fig. 4f) suggests a shift toward microbially processed organic matter rather than fresh phytoplankton biomass, which typically has lower C/N ratios. In addition, free energy calculations have shown that microbial transformation products, such as amino sugars, are thermodynamically favored for adsorption onto particles (Liu et al., 2024). As no external particles were introduced during the mixing experiments, the net positive $\delta^{15}\text{N}$ deviation most likely reflects in-situ RDON' adsorption and particle-phase retention, contributing to the accumulation of refractory, isotopically enriched ON in the particulate pool".

Question 4: Figure 7 presents an "Enhanced nitrogen pump model." What exactly is "enhanced"?

Response:

Thank you for raising this important point. We agree that the meaning of "enhanced" was not sufficiently defined in the original version. In our conceptual model, "enhanced" does not simply refer to a quantitatively larger nitrogen flux. Rather, it refers to the mechanistic expansion of the classical nitrogen pump by incorporating microbial transformation processes into the physicochemical framework of estuarine mixing. Therefore, the term "enhanced" refers to the additional microbial transformation pathway and its coupling with adsorption/flocculation processes. This coupling not only strengthens the transfer

of ON from the dissolved to particulate phase, but also alters DON composition and bioavailability during estuarine mixing. We have revised the figure caption and related text to clarify this definition.

The revised text reads:

[Figure 7] “Figure 7. Conceptual model of nitrogen cycling under estuarine mixing, highlighting the role of organic nitrogen (ON) transformation. (a) Classical nitrogen pump model dominated by physicochemical processes, including dissolved organic nitrogen (DON) adsorption/flocculation, particulate organic nitrogen (PON) deposition and burial, resuspension, and mineralization. (b) Microbially enhanced nitrogen pump model, in which microbial utilization and transformation of terrestrial DON promote the degradation of humic-like DON, the production of NH_4^+ and labile DON (LDON), and the formation of microbially modified refractory DON (RDON’). The subsequent re-adsorption of RDON’ onto particles further promotes refractory PON accumulation and nitrogen retention during estuarine mixing”.

Question 5: The authors mention using parametric tests (e.g., t-tests or ANOVA) to compare treatments. It would be rigorous to briefly clarify whether the normality of the data distribution was checked.

Response:

Thank you for this helpful suggestion. We agree that the assumptions underlying the parametric analyses should be clarified. In the revised manuscript, we have added information to the Statistical analysis section stating that data normality was assessed using the Shapiro–Wilk test and homogeneity of variance was evaluated using Levene’s test prior to one-way ANOVA. Tukey’s post hoc test was applied when the assumptions of normality and equal variance were satisfied.

The revised text reads:

[Section 2.4, L158-161] “Prior to one-way ANOVA, the normality of data distribution was assessed using the Shapiro–Wilk test, and homogeneity of variance was evaluated using Levene’s test. Group differences were assessed by one-way ANOVA followed by Tukey’s post hoc tests when the assumptions of normality and

equal variance were satisfied”.

Question 6: I noticed several grammatical and formatting errors that should be corrected:

Line 127: “p” is not italic.

Line 188: "The non-conservative changes... are govern by..." should be "are governed by".

Line 223: "...particle characteristics , typically..." There is an extra space before the comma.

Line 309: "This study highlight that..." should be "highlights".

Line 424: "Crucially, our isotopic evidence that biologically-modified DON re-adsorbs onto particles..." This sentence appears to be a fragment or grammatically incomplete (missing a main verb for "evidence" or "that" should be removed).

Response:

Thank you for carefully checking the manuscript and pointing out these grammatical and formatting issues. We have revised the manuscript accordingly. Specifically, “p” has been italicized in Line 127; the extra space before the comma in Line 223 has been removed; and “This study highlight that” in Line 309 has been corrected to “This study highlights that.” In addition, the sentence containing “are govern by” in Line 188 and the incomplete sentence in Line 424 have been removed from the revised manuscript in response to related comments from another reviewer, so these issues no longer occur in the current text.
