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.*

RC3's comments to the Author:

Manuscript egusphere-2026-216 employs laboratory incubation experiments to investigate the transformation of dissolved organic nitrogen (DON) and particulate organic nitrogen (PON), as well as nitrogen bioavailability. The study offers valuable insights with clear relevance to future research on nitrogen biogeochemical cycling in coastal waters. Overall, the experimental design is robust and the results are interesting and potentially impactful. However, several aspects require further clarification.

Response:

We sincerely thank the reviewer for the positive evaluation of our experimental design and the significance of this study. We also appreciate the constructive comments, which helped us improve the clarity and rigor of the manuscript. In response, we have added missing references, clarified methodological details, strengthened the explanation of conservative versus non-conservative mixing, added theoretical mixing values and deviations, revised the interpretation of PON isotope trends with statistical support, and defined FI, BIX, and $SUVA_{254}$ in the Methods section. The relevant revised passages are copied below where necessary.

Question 1: Between line 50 and 55: is there a citation for "Humic-like (terrestrial and microbial sources) and protein-like components are considered as the dominant terrestrial DON components entering the sea"?

Response:

We thank the reviewer for noting the missing citation. In the revised manuscript, we have added supporting references for this statement.

The revised text reads:

[Introduction, L59-61] "Humic-like (terrestrial and microbial sources) and protein-like components are considered as the dominant terrestrial DON components entering the sea (Stedmon and Markager, 2005; Yan et al., 2024)".

Stedmon, C. A. and Markager, S.: Resolving the variability in dissolved organic matter fluorescence in a temperate estuary and its catchment using PARAFAC analysis, Limnol. Oceanogr., 50, 686–697, https://doi.org/10.4319/lo.2005.50.2.0686, 2005.

Yan, Z., Xin, Y., Zhong, X., Yi, Y., Li, P., Wang, Y., Zhou, Y., He, Y., He, C., Shi, Q., Xu, W., and He, D.: Evolution of dissolved organic nitrogen chemistry during transportation to the marginal sea: Insights from nitrogen isotope and molecular composition analyses, Water Res., 249, 120942, https://doi.org/10.1016/j.watres.2023.120942, 2024.

Question 2: Between line 110 and 115: authors mentioned the PN was considered equivalent to PON because PIN is less than 5%. Wonder how much would it affect the isotopic ratios? Especially if the nitrogen isotopic values of PIN vary along the salinity gradient, would it affect the observed trend?

Response:

Thank you for this helpful comment. We agree that variations in δ^{15}N-PIN along the salinity gradient could, in principle, affect the measured bulk PN isotopic composition. To address this issue, we added a sensitivity analysis based on isotope mass balance in the Supplementary Information (Text 3). Because PIN accounted for less than 5% of total PN in all samples, even a large isotopic difference of 20‰ between PIN and PON would shift bulk δ^{15}N-PN by only approximately 1‰, and a conservative difference of 40‰ would result in an offset of approximately 2‰. This potential offset is much smaller than the observed δ^{15}N-PON variation in the BAM treatments, which ranged from 1.1‰ to 8.8‰. Therefore, although salinity-dependent variation in δ^{15}N-PIN cannot be completely excluded, its low contribution to total PN indicates that it would only slightly affect absolute δ^{15}N values and would not change the main isotopic trend observed in this study. Moreover, treating PN as equivalent to PON when PIN is negligible (<5%) is a

well-established practice in estuarine and coastal biogeochemistry (Yan et al., 2022), and our approach is consistent with these precedents.

We have added a brief sentence to the revised manuscript to explicitly acknowledge the potential isotopic effect and justify why it is negligible.

The revised text reads:

[Section 2.3, L123-127] "Particulate inorganic nitrogen was determined via HCl extraction (Zuo et al., 2016) and accounted for less than 5% of total PN in all samples; therefore, PN was used as a proxy for PON. The potential influence of PIN on δ^{15}N-PON was further evaluated using an isotope mass-balance sensitivity analysis, which indicated that the low PIN contribution would have only a minor effect on bulk δ^{15}N values and would not alter the observed isotopic trends (Text S3)";

[Text S3] "Sensitivity analysis of the potential influence of particulate inorganic nitrogen on δ^{15}N-PON

Particulate inorganic nitrogen (PIN) accounted for less than 5% of total particulate nitrogen (PN) in all samples. Therefore, PN was used as a proxy for particulate organic nitrogen (PON) in this study. To evaluate whether the small PIN fraction could affect the measured nitrogen isotopic composition, we performed a simple isotope mass-balance assessment.

The measured isotopic composition of bulk PN can be expressed as:

$$\delta^{15}N\_PN = (1 - f\_PIN) \times \delta^{15}N\_PON + f\_PIN \times \delta^{15}N\_PIN$$

where f _PIN is the fraction of PIN in total PN. Therefore, the potential offset caused by PIN can be approximated as:

$$\delta^{15}N \approx f\_PIN \times (\delta^{15}N\_PIN - \delta^{15}N\_PON)$$

Because f _PIN was consistently lower than 0.05, the influence of PIN on bulk δ^{15}N-PN would be limited even if δ^{15}N-PIN differed substantially from δ^{15}N-PON. For example, if δ^{15}N-PIN differed from δ^{15}N-PON by 20‰, the resulting offset in bulk δ^{15}N-PN would be approximately 1‰. Even under a more conservative scenario with a 40‰ isotopic difference, the offset would still be approximately 2‰.

In comparison, the observed δ^{15}N-PON values in the BAM treatments ranged from 1.1‰ to 8.8‰, corresponding to a total variation of 7.7‰. To generate such a

variation solely through a PIN contribution of 5%, δ^{15}N-PIN would need to differ from δ^{15}N-PON by approximately 154‰, which is unrealistic for this system. Therefore, the observed δ^{15}N variation in the BAM treatments cannot be explained by the minor PIN contribution. In the BIM treatments, δ^{15}N-PON varied within a narrower range, from 6.2‰ to 7.2‰. Thus, the possible contribution of PIN may introduce minor uncertainty to small within-treatment differences. However, because PIN remained consistently below 5% of total PN, this effect would mainly influence absolute δ^{15}N values slightly and would not alter the major treatment-level pattern or the interpretation that PN was dominated by PON.

Overall, although salinity-dependent variation in δ^{15}N-PIN cannot be completely excluded, the low contribution of PIN to total PN indicates that its effect on measured δ^{15}N-PN was minor and would not change the main isotopic trends observed in this study".

Yan, X., Yang, J., Xu, M., Wang, H., Dai, M., and Kao, S.: Nitrogen isotope constraint on the zonation of multiple transformations between dissolved and particulate organic nitrogen in the Changjiang plume, Sci. Total Environ., 818, 151678, https://doi.org/10.1016/j.scitotenv.2021.151678, 2022.

Question 3: Between line 115 and 120: does the S in "Additional water quality parameters including S, pH, SPM, and chlorophyll-a (Chl-a) were measured" stand for sulfur? Why is sulfur collected and how would affect nitrogen? I don't think I have seen the relevant discussion in the manuscript.

Response:

We thank the reviewer for pointing out this ambiguity. The "S" in the original text refers to salinity, not sulfur. We apologize for the confusion caused by the undefined abbreviation. In the revised manuscript, we have replaced "S" with "salinity" to eliminate any ambiguity.

The revised text reads:

[Section 2.3, L135] "Additional water quality parameters including salinity, pH, …"

Question 4: In results, the manuscript mentioned the DON indicated a non-conservative mixing and PON indicated a conservative mixing. Do you have data to support it? All I can see from the paragraph and figures is that DON decreases with increasing salinity, which looks like conservative for DON.

Response:

Thank you for this helpful comment. In estuarine mixing studies, conservative behavior is commonly evaluated by comparing observed concentrations with the theoretical two-endmember conservative mixing curve calculated from river-water and seawater endmembers. A decrease in concentration with increasing salinity does not necessarily indicate conservative mixing; it is considered conservative only when the observed values follow the theoretical dilution curve. Recent estuarine studies have applied this approach to DOC, CDOM, nutrients, trace metals, and DON, using deviations from conservative mixing curves to identify net addition or removal (Cheong et al. 2024; Yan et al. 2024).

However, to our knowledge, there is no universal fixed threshold, such as 5% or 10% deviation from the conservative line, that is generally accepted across estuarine mixing studies. The threshold depends on analytical uncertainty, endmember variability, and replicate variability. Therefore, in the revised manuscript, we define non-conservative behavior operationally as a statistically meaningful deviation between the observed concentration and the predicted conservative mixing value, considering the propagated uncertainty from triplicate measurements. We added the calculated theoretical values of nitrogen concentrations and their deviations from conservative mixing to Table S3. To avoid misunderstanding, we have revised the Introduction and Methods section to explicitly define conservative and non-conservative mixing using the two-end-member model, and revised the Results section to clarify the different behaviors of DON and PON in BAM and BIM treatments.

The revised text reads:

[Introduction, L48-51] "Contrary to early models of conservative dilution mixing, in which solute concentrations vary linearly with salinity between the

freshwater and seawater end-members (i.e., following the theoretical dilution line; Liss, 1976; Officer, 1979), ON behavior is often non-conservative, with measured concentrations deviating above (net addition) or below (net removal) the theoretical dilution line";

[Section 2.4, L148-158] "Conservative mixing was evaluated using a two-end-member mixing model based on the river-water and seawater endmembers to distinguish simple physical dilution from internal sources or sinks (Liss, 1976; Langmuir et al., 1978). The theoretical conservative concentration at each salinity was calculated as:

$$X_{mix} = f_r \times X_r + f_s \times X_s$$
$$f_r = S_s - S_i / (S_s - S_r),\ f_s = S_i - S_r / (S_s - S_r)$$

where X_{mix} was the predicted value under conservative mixing, X_r and X_s were the corresponding values in the river-water and seawater endmembers, respectively, f_r and f_s were the corresponding mixing fractions estimated from salinity, S_i, S_r, and S_s represent the salinity of the mixture, river-water endmember, and seawater endmember, respectively, and X represented nitrogen concentrations and PARAFAC component fluorescence intensities. Deviations between the observed concentration and the conservative mixing value (X_{mix}) were used to identify non-conservative behavior. The calculated theoretical values and deviations from conservative mixing for nitrogen concentrations and PARAFAC component fluorescence intensities are provided in Tables S3 and S4, respectively. Positive deviations were interpreted as net addition, whereas negative deviations were interpreted as net removal. Thus, a monotonic decrease in concentration with increasing salinity was not necessarily considered conservative unless the observed values followed the theoretical conservative mixing curve";

[Section 3.1, L174-175] "Conservative mixing was evaluated by comparing the observed concentrations with the theoretical two-end-member dilution curves shown as solid lines in Fig. 1. The observed tDON and CON concentrations deviated from the conservative mixing curve (Fig. 1; Table S3), indicating non-conservative behavior*";*

[Section 3.2, L215-218] "PON showed contrasting behavior between the two treatments. In the BIM treatments, PON concentrations were comparatively closer to the conservative mixing curve, with only slight positive deviation. In contrast, PON in the BAM treatments showed substantial positive deviation from the conservative mixing curve (Fig. 1e, f). Therefore, PON was not described as conservative under both treatments; rather, it behaved more conservatively in BIM than in BAM.";

[Figure 1] "Figure 1. Variations in the concentrations of truly dissolved organic nitrogen (tDON) (a, b), colloidal organic nitrogen (CON) (c, d) and particulate organic nitrogen (PON) (e, f) across different river-seawater mixing ratios in the biologically active mixing (BAM) and biologically inhibited mixing (i.e., physicochemical mixing only, BIM) treatments. Conservative mixing curves (solid lines) were calculated using a two-end-member model based on the river-water and seawater endmembers (Langmuir et al., 1978), while dashed lines represent smoothed fits to the observed data. Deviations of the observed data from the conservative mixing curves indicate non-conservative behavior, with positive deviations representing net addition and negative deviations representing net removal";

[Table S3] "Theoretical conservative mixing concentrations and deviations of observed PON, CON, and tDON concentrations from conservative mixing at each salinity. Observed concentrations used to calculate deviations are provided in Table S2. Theoretical conservative concentrations were calculated using the salinity-based two-end-member mixing model. Positive values of deviations indicate net addition relative to conservative mixing, whereas negative values indicate net removal".

Salinity	*Theoretical concentration*			*Deviation from conservative mixing*		
	PON (mg L $^{-1}$)	*CON (mg L $^{-1}$)*	*tDON (mg L $^{-1}$)*	*PON (mg L $^{-1}$)*	*CON (mg L $^{-1}$)*	*tDON (mg L $^{-1}$)*
Biologically active mixing (i.e., biological and physicochemical processes occurring concurrently, BAM)						
1.52	*0.391*	*0.829*	*1.270*	*0.000*	*0.000*	*0.000*
8.34	*0.338*	*0.701*	*1.028*	*0.049*	*-0.079*	*0.183*
14.67	*0.289*	*0.582*	*0.803*	*0.069*	*-0.143*	*0.298*
17.24	*0.269*	*0.534*	*0.711*	*0.049*	*-0.148*	*0.202*
20.83	*0.241*	*0.466*	*0.584*	*0.041*	*-0.107*	*0.125*
26.06	*0.200*	*0.368*	*0.398*	*0.044*	*-0.072*	*0.106*

30.31	*0.167*	*0.288*	*0.247*	*0.000*	*0.000*	*0.000*
Biologically inhibited mixing (i.e., physicochemical processes mixing only, BIM)						
1.28	*0.381*	*0.756*	*1.309*	*0.000*	*0.000*	*0.000*
8.98	*0.323*	*0.612*	*1.026*	*0.028*	*-0.108*	*-0.103*
15.18	*0.276*	*0.497*	*0.798*	*0.017*	*-0.114*	*-0.168*
18.13	*0.254*	*0.443*	*0.691*	*0.022*	*-0.102*	*-0.249*
20.27	*0.237*	*0.402*	*0.610*	*0.006*	*-0.108*	*-0.192*
27.85	*0.181*	*0.262*	*0.335*	*0.014*	*-0.082*	*-0.037*
31.5	*0.153*	*0.193*	*0.199*	*0.000*	*0.000*	*0.000*

Cheong, A. Y. L., Annammala, K. V., Yong, E. L., Zhou, Y., Nichols, R. S., and Martin, P.: Distribution of nutrients and dissolved organic matter in a eutrophic equatorial estuary: the Johor River and the East Johor Strait, Biogeosciences, 21, 2955–2971, https://doi.org/10.5194/bg-21-2955-2024, 2024.

Yan, Z., Xin, Y., Zhong, X., Yi, Y., Li, P., Wang, Y., Zhou, Y., He, Y., He, C., Shi, Q., Xu, W., and He, D.: Evolution of dissolved organic nitrogen chemistry during transportation to the marginal sea: Insights from nitrogen isotope and molecular composition analyses, Water Res., 249, 120942, https://doi.org/10.1016/j.watres.2023.120942, 2024.

Question 5: Between line 155 and 160: what are the theoretical fluorescence values?

Response:

Thank you for this comment. The theoretical fluorescence values were calculated using the same two-end-member conservative mixing model as that used for nitrogen concentrations. Specifically, the fluorescence intensities of each PARAFAC component expected under conservative mixing were calculated from the river-water and seawater endmembers, and the deviations were determined by comparing the observed fluorescence intensities with these theoretical values. The calculated theoretical fluorescence values and corresponding deviations for C1, C2, and C3 are now provided in Table S4.

The revised text reads:

[Section 3.1 L208-212] "Compared with the theoretical fluorescence values calculated using the same two-end-member conservative mixing model as for nitrogen concentrations, all three components showed negative deviations in the BIM treatments (Fig. 2; Table S4). In contrast, in the BAM treatments, C1 and C3 showed negative deviations, whereas C2 showed positive deviation (Fig. 2; Table S4)";

[Table S4] "Table S4. Theoretical fluorescence values and deviations from conservative mixing for PARAFAC components in the truly dissolved and colloidal phases. Observed fluorescence values used to calculate deviations are provided in Table S2. Theoretical conservative fluorescence values were calculated using the salinity-based two-end-member mixing model. Positive values of deviations indicate net addition relative to conservative mixing, whereas negative values indicate net removal".

Salinity	Theoretical fluorescence values						Deviation from conservative mixing					
	Truly dissolved phase			Colloidal phase			Truly dissolved phase			Colloidal phase		
	C1	C2	C3	C1	C2	C3	C1	C2	C3	C1	C2	C3
Biologically active mixing (i.e., biological and physicochemical processes occurring concurrently, BAM)												
1.52	2.098	2.140	0.396	2.614	2.004	0.398	0.000	0.000	0.000	0.000	0.000	0.000
8.34	1.752	1.769	0.327	2.070	1.645	0.318	-0.314	0.336	-0.143	-0.301	0.059	-0.112
14.67	1.431	1.425	0.263	1.566	1.311	0.243	-0.218	0.108	-0.210	-0.344	0.085	-0.135
17.24	1.301	1.285	0.238	1.361	1.176	0.213	-0.241	0.083	-0.181	-0.155	0.077	-0.135
20.83	1.119	1.090	0.201	1.074	0.987	0.170	-0.136	0.120	-0.176	-0.092	0.107	-0.165
26.06	0.854	0.806	0.148	0.657	0.711	0.109	-0.148	0.083	-0.075	-0.002	0.073	-0.045
30.31	0.638	0.575	0.105	0.318	0.487	0.059	0.000	0.000	0.000	0.000	0.000	0.000
Biologically inhibited mixing (i.e., physicochemical processes mixing only, BIM)												
1.28	1.851	2.582	0.565	2.212	1.856	0.450	0.000	0.000	0.000	0.000	0.000	0.000
8.98	1.455	2.059	0.450	1.821	1.587	0.362	-0.236	-0.021	-0.103	-0.203	-0.008	-0.039
15.18	1.135	1.638	0.358	1.506	1.370	0.292	-0.270	-0.067	-0.081	-0.297	-0.023	-0.040
18.13	0.983	1.437	0.315	1.356	1.266	0.258	-0.161	-0.058	-0.059	-0.125	-0.009	-0.041
20.27	0.873	1.292	0.283	1.247	1.192	0.234	-0.141	-0.053	-0.062	-0.057	0.012	-0.017
27.85	0.483	0.776	0.171	0.861	0.926	0.148	-0.084	-0.037	-0.057	-0.004	0.009	-0.041
31.5	0.295	0.528	0.116	0.676	0.798	0.106	0.000	0.000	0.000	0.000	0.000	0.000

Question 6: Between line 170 and 175: numbers from −28.3‰ to −26.5‰, 1.1‰ to 8.8‰, −26.1‰ to −25.9‰, and 6.2‰ to 7.2‰, are them statistically different? If not, it would be a stretch to say they decline along the salinity gradient, especially Figure 3 didn't look like these numbers decreased when the salinity increased.

Response:

Thank you for the helpful comment. We agree that the previous statement overgeneralized the trends in PON isotopes along the salinity gradient. To clarify, we analyzed the data using both one-way ANOVA and linear regression.

- ***One-way ANOVA: In both treatments, δ^{13}C-PON and δ^{15}N-PON differed***

significantly among salinity levels (BAM: δ^{13}C-PON $F(6,14) = 13.10$, $p < 0.001$; δ^{15}N-PON $F(6,14) = 322.43$, $p < 0.001$; BIM: δ^{13}C-PON $F(6,14) = 19.00$, $p < 0.001$; δ^{15}N-PON $F(6,14) = 7.43$, $p = 0.001$). This indicates that isotope values vary across salinity levels in both treatments.

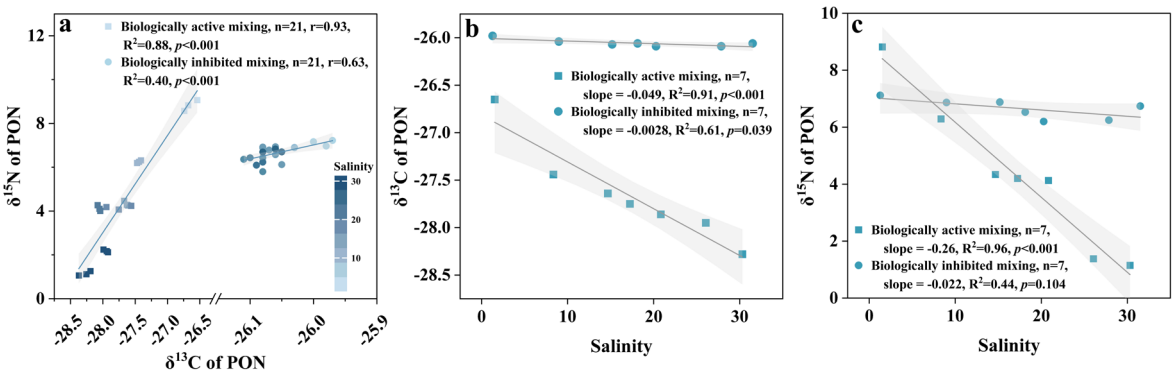
- ***Linear regression: The decrease along the salinity gradient was mainly evident in the BAM treatments (δ^{13}C-PON slope = −0.049‰ per salinity unit, $R^2 = 0.91$, $p = 0.0009$; δ^{15}N-PON slope = −0.26‰ per salinity unit, $R^2 = 0.96$, $p = 0.0001$). In the BIM treatments, δ^{13}C-PON showed only a weak linear decrease (slope = −0.0028‰ per salinity unit, $R^2 = 0.61$, $p = 0.039$), and δ^{15}N-PON showed no significant monotonic trend (slope = −0.022‰ per salinity unit, $R^2 = 0.44$, $p = 0.104$).***

Together, these analyses indicate that the significant decrease in PON isotope values along the salinity gradient is mainly observed in BAM treatments, whereas BIM treatments show only minor or non-monotonic variations. Accordingly, we have revised the text to avoid implying that both isotope values declined along the salinity gradient in both treatments.

The revised text reads:

[Section 3.2, L245-252] "The salinity-related decrease in PON isotope values was mainly evident in the BAM treatments, whereas isotope variations in the BIM treatments were relatively small or non-monotonic. Specifically, both δ^{13}C and δ^{15}N values varied significantly among salinity levels as shown by one-way ANOVA ($p < 0.001$). Linear regression analysis based on the mean values at each salinity level further showed significant decreasing trends with increasing salinity for both δ^{13}C-PON and δ^{15}N-PON in the BAM treatments ($p < 0.001$, Fig. S7). In the BIM treatments, the regression analysis indicated only a weak decrease in δ^{13}C-PON with salinity ($p = 0.039$, Fig. S7), while δ^{15}N-PON showed no significant linear trend ($p = 0.104$, Fig. S7)";

[Figure S7]



"Figure S7. Relationships between PON isotopic compositions and salinity under biologically active and inhibited mixing treatments. (a) Relationship between δ¹³C-PON and δ¹⁵N-PON under biologically active mixing and biologically inhibited mixing treatments, with symbol color indicating salinity. (b) Changes in δ¹³C-PON along the salinity gradient. (c) Changes in δ¹⁵N-PON along the salinity gradient. Regression lines are based on the mean values at each salinity level, and shaded areas indicate 95% confidence intervals. δ¹³C-PON and δ¹⁵N-PON showed significant decreasing trends with increasing salinity in the biologically active mixing treatment, whereas isotope variations in the biologically inhibited mixing treatment were relatively small or non-significant".

Question 7: Between line 225 and 230: How does the Fig S5a indicate the decrease in the intensity of C1? I didn't found a decrease trend in the figure and also decrease with what? With time, treatment numbers, or salinity?

Response:

Thank you for pointing this out. We agree that the original wording was unclear and could cause misunderstanding. Fig. S5a does not indicate a decreasing trend with time, treatment number, or salinity. Instead, Fig. S5 shows the deviation between the measured fluorescence intensity and the theoretical conservative mixing/dilution value. The deviation from conservative mixing is illustrated in Fig. 2, where the measured fluorescence intensity of each component is compared with the theoretical conservative mixing curve. Fig. S5 further summarizes these deviations and statistically compares them between BAM and BIM treatments in the

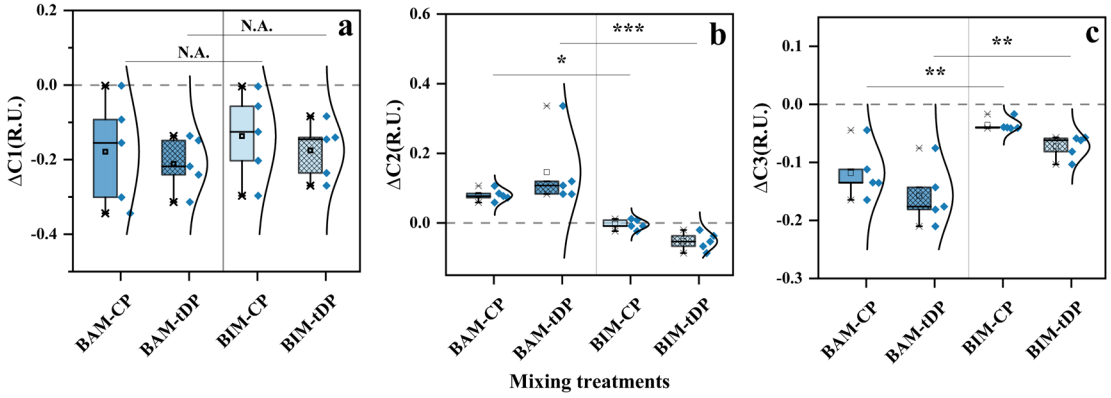
truly dissolved phase and colloidal phase.

For C1, the negative ΔC1 values indicate that the measured fluorescence intensity was lower than the theoretical conservative mixing value. The similar ΔC1 values between BAM and BIM treatments, with no significant difference (p > 0.05; Fig. S5a), suggest that the attenuation of C1 relative to conservative mixing was mainly driven by physicochemical processes, with limited biological influence. We have revised the text to clarify that the "decrease" refers to a negative deviation from conservative mixing rather than a temporal, treatment-number, or salinity-dependent decrease.

The revised text reads:

[Section 4.2, L319-323] "Fig. S6 presents the statistical comparisons of deviations from conservative mixing. For the terrestrial humic-like component C1, ΔC1 values were negative in both treatments, indicating that measured C1 fluorescence intensities were lower than the theoretical conservative mixing values. The absence of a significant difference in ΔC1 between BAM and BIM treatments (p > 0.05; Fig. S6a) suggests that C1 attenuation relative to conservative mixing was mainly controlled by physicochemical processes, with limited additional biological influence (Shutova et al., 2014)";

[Figure S6]



"Figure S6. Deviations of fluorescence component intensities from theoretical conservative mixing values under biologically active and inhibited mixing treatments. Deviations were calculated as ΔC = measured fluorescence intensity − theoretical conservative mixing/dilution value for C1 (a), C2 (b), and C3 (c) in the colloidal

*phase (CP) and truly dissolved phase (tDP) under biologically active mixing (BAM) and biologically inhibited mixing (BIM) treatments. The dashed horizontal line represents conservative mixing (ΔC = 0). Blue diamonds represent individual samples (n = 5), and squares indicate mean values. Statistical significance indicates differences among the corresponding treatments; NS indicates no significant difference, * indicates p < 0.05, ** indicates p < 0.01, and *** indicates p < 0.001".*

Question 8: Between line 230 and 235: what are the fluorescence index and biological index and how are them measured? You would want to address them in the methods section first.

Response:

Thank you for this helpful comment. We agree that the fluorescence index (FI), biological index (BIX), and $SUVA_{254}$ should be defined before they are used in the Results and Discussion section. FI and BIX are not independently measured parameters, but optical indices calculated from corrected EEM fluorescence spectra, whereas $SUVA_{254}$ is calculated from UV absorbance at 254 nm normalized to DOC concentration. We have added a brief description of these indices in the Methods section and provided their detailed calculation procedures in the Supplementary Information as Text S5. We have also revised the corresponding text to clarify that FI, BIX, and SUVA254 were used as optical proxies for DOM source, recent biological contribution, and aromaticity, respectively.

The revised text reads:

[Section 2.3, L140-145] "Optical indices, including the fluorescence index (FI), biological index (BIX), and specific UV absorbance at 254 nm ($SUVA_{254}$), were calculated from the corrected EEM fluorescence and UV–Vis absorbance spectra. FI and BIX were derived from fluorescence intensity ratios at specific excitation/emission wavelengths, whereas $SUVA_{254}$ was calculated by normalizing UV absorbance at 254 nm to the DOC concentration of the corresponding sample or fraction. Detailed equations and interpretations are provided in Text S4";

[Section 4.2, L329-337] "In the BAM treatments, accumulation of tDON was

accompanied by increases in FI and BIX and a concurrent decrease in $SUVA_{254}$ (Fig. 3a–c). Because FI and BIX indicate microbial/autochthonous DOM contribution and recent biological production, respectively, whereas SUVA254 reflects DOM aromaticity, these optical changes suggest an increased contribution of freshly produced, less aromatic DOM and thus potentially enhanced DON bioavailability during estuarine mixing";

[Text S4] "Text S4. Calculation of optical indices

Optical indices, including the fluorescence index (FI), biological index (BIX), and specific UV absorbance at 254 nm ($SUVA_{254}$), were calculated to characterize DOM source, recent biological contribution, and aromaticity. Prior to FI and BIX calculation, EEM spectra were blank-subtracted, corrected for inner-filter effects, and Raman-normalized.

Let $F_{Ex,\ Em}$ represent the corrected fluorescence intensity at a given excitation and emission wavelength.

The fluorescence index was calculated as:

$$FI = F_{370,\ 470} / F_{370,\ 520}$$

where $F_{370,\ 470}$ and $F_{370,\ 520}$ are the fluorescence intensities at emission wavelengths of 470 and 520 nm, respectively, under excitation at 370 nm. FI is commonly used to distinguish terrestrial and microbial/autochthonous DOM sources, with higher FI values generally indicating a greater microbial contribution.

The biological index was calculated as:

$$BIX = F_{310,\ 380} / F_{310,\ 430}$$

where $F_{310,\ 380}$ and $F_{310,\ 430}$ are the fluorescence intensities at emission wavelengths of 380 and 430 nm, respectively, under excitation at 310 nm. BIX reflects the contribution of recently produced or autochthonous DOM, with higher values indicating stronger biological production.

$SUVA_{254}$ was calculated as:

$$SUVA_{254} = A_{254} \times 100 / DOC$$

where A_{254} is the UV absorbance at 254 nm measured with a 1-cm quartz cuvette, and DOC is the dissolved organic carbon concentration of the corresponding sample or

fraction in mg C L^{-1}. $SUVA_{254}$ is reported in L mg C $^{-1}$ m^{-1} and is commonly used as a proxy for DOM aromaticity. Higher $SUVA_{254}$ values indicate higher aromaticity, whereas lower values indicate a lower contribution of aromatic DOM".