We would like to thank the reviewer for their thoughtful and constructive feedback. We have carefully considered all comments and made the necessary revisions. Their comments have substantially improved the quality and rigour of the work. Below, we provide a point-by-point response to each comment. Reviewer comments are shown in *black,* and our responses follow in blue text. Where applicable, revised text from the manuscript is shown in **bold**.

Reviewer 2

The manuscript addresses a genuinely important and understudied topic – root exudation in mangroves – and presents a creative adaptation of the cuvette method for waterlogged, tidal conditions. The dataset, while limited in sample size and spatial scope, provides some of the first in situ exudation rates for Avicennia and Rhizophora. The upscaling attempt is useful for highlighting the potential significance of this flux. However, several methodological weaknesses, statistical limitations, and overstatements in the interpretation require major revision before the work meets the standards for a high-quality journal like Biogeosciences.

We thank the reviewer for the thorough and perceptive evaluation. We address each specific comment below, and we believe the revised manuscript has substantially improved in methodological transparency and interpretive caution in response to these recommendations.

- The sample sizes are highly uneven across species and seasons (e.g., Avicennia dry season: n=5 vs. wet season: n=13). How do the authors account for potential statistical bias from unequal replication, and could they justify the minimum sample size for robust comparisons?

We thank the reviewer for this important point. The unequal sample sizes reflect field constraints. In particular, dry-season sampling at the Can Giờ site was limited by tidal access windows and logistical restrictions, which reduced replication for some species–season combinations (e.g., *Avicennia* dry season: n = 5 vs. wet season: n = 13). We agree that this imbalance must be acknowledged in both the statistical analysis and interpretation of results.

To address potential bias arising from this imbalance, we used a Gamma GLM with a log link rather than an ANOVA-based framework. Unlike classical ANOVA, GLMs do not require balanced replication and are appropriate for handling non-normal, heteroscedastic ecological data. In addition, we report estimated marginal means with associated confidence intervals, which explicitly incorporate the increased uncertainty associated with smaller sample sizes, particularly for the low-replication groups.

Regarding minimum replication, we consider a threshold of at least three independent observations to be the absolute minimum required for statistical tests, but we acknowledge that higher replication is preferable for robust inference. In our case, most treatment groups exceed this minimum (≥5 replicates), which is consistent with, and in several cases comparable to, similar field-based studies in root exudation (Meier et al., 2020; Zhang et al., 2024). Nevertheless, we recognise that without detailed prior knowledge of variability in

exudation rates across the inundation profile, any minimum-sample justification remains rather theoretical. We have now clarified these points in the revised manuscript to better acknowledge the limitations associated with unequal sampling effort.

In the method section, we have now added:

> "To compare root exudation rates between seasons and species, we used Gamma generalised linear models (GLMs) with a log link function, which are well-suited to continuous positive, right-skewed data. **Sample sizes were unequal across species–season combinations (*Avicennia*: wet n = 13, dry n = 5; *Rhizophora*: wet n = 21, dry n = 8), reflecting tidal access constraints at the field site; unlike ANOVA, GLMs do not require balanced replication and are appropriate for heteroscedastic ecological data, with estimated marginal means explicitly propagating the greater uncertainty in smaller groups.** Species or season was included as a covariate in each respective model to control for confounding effects, and we also tested for a species × season interaction. Model fit was assessed using Nagelkerke's R^2 and dispersion ratios via DHARMa residual diagnostics. Estimated marginal means (EMMs) and pairwise contrasts were reported on the response scale as ratios with 95% confidence intervals."

- Section2.5.1 Exudation rates are calculated using the dry mass of roots after they have been incubated for 48 h and then oven-dried. This assumes that root mass did not change during incubation. However, roots may lose mass through respiration, exudation itself, or tissue death. Conversely, they might gain mass if any microbial biofilm develops. This is a critical source of error. Did you measure root fresh mass or estimate initial dry mass from a parallel set of roots?

We thank the reviewer for highlighting this methodological consideration. While, to our knowledge, this has not been addressed in previous root exudation studies, mass change was likely limited given our 48h incubation window and the use of pre-rinsed roots to reduce external microbial and soil organic matter contamination. Unfortunately, the experimental approach does not allow the collection of fresh roots before and after incubation, given that roots must remain attached to the parent tree throughout the incubation. We have now explicitly stated this potential caveat in our method:

> **"This approach assumes limited change of root biomass during the incubation period. A change in root biomass might occur, e.g. through respiration (Lovelock et al., 2006), but any such eventuality was likely limited given the short incubation time, the flushing event performed after 24 h, and by using pre-rinsed roots to reduce external microbial and soil organic matter contamination."**

- Section3.2 The incubation duration test was based on only three roots. No statistical pairwise differences were significant, yet the authors use this to justify the 48 h protocol. This is insufficient. Please either provide a larger time-series

experiment or present the existing data more cautiously, acknowledging that the choice of 48 h remains provisional.

We thank the reviewer for this comment, and we believe we were not clear in the description of our design to test the difference between 24 and 48 h. We tested the incubation time for 49 roots between both times, and the pilot n=3 time series at 0/24/48/72h was a separate, earlier validation experiment. The n=49 paired comparison provides a substantially larger evidential basis for the 48 h choice than the original manuscript made clear. We have now restructured Sect. 2.4.2 and the relevant results and discussion passages to make both experiments and their respective roles explicit.

We have now made it clearer in the manuscript as follows:

> "To determine a suitable recovery and incubation period, we conducted **two** *in situ* time-series **experiments. The first** involved the sealed-cuvette protocol and sampled solutions from the same three roots used in the field waterproof test at 0, 24, 48, and 72 **h (n = 3). The second time-series experiment used more roots (n = 49) and compared only the 24 and 48 h intervals.** These intervals span a full diurnal cycle of exudation, which has been shown to fluctuate significantly over 24 h, and suggests a mechanistic link between the supply of newly assimilated photosynthates and root exudates (Brauner et al., 2018; Tixier et al., 2023).
>
> "**For the second time-series analysis, the paired** 24 h and 48 h intervals were then compared using a paired Wilcoxon signed-rank test, as well as a Lin's concordance correlation coefficient (CCC; Lin, 1989) **to assess measurement agreement, precision, and potential interchangeability between two time points at individual root leve**l."
>
> "Paired measurements (n = 49) showed **no significant difference (V = 506, *p* = 0.40) between** median exudation of 32.7 µg C g^{-1} h^{-1} at 24 h (IQR: 96.8; range: 0.70–2206.34 µg C g^{-1} h^{-1}) and 40.8 µg C g^{-1} h^{-1} at 48 h (IQR: 59.6; range: 9.5–513.2 µg C g^{-1} h^{-1}). **Lin's concordance correlation coefficient, used to assess potential interchangeability between 24 h and 48 h measurements, indicated poor agreement (CCC = 0.45, 95% CI: 0.31–0.57; Fig. 5) – suggesting that exudation measurements at 24 h and 48 h at root level are different and not interchangeable."**
>
> **"The primary justification for the 48 h incubation comes from Lin's CCC of 0.45 indicating a poor agreement at individual root level, meaning that 24 h and 48 h measurements are not interchangeable for individual roots. This suggests that roots had not fully stabilised at 24 h. It is consistent with the idea that some roots require a short recovery period after disturbance (Döll et al., 2024; Oburger & Jones, 2018), and that 48 h measurements are**

therefore more reliable for flux calculations. Similarly, prolonged incubation risks increasing microbial consumption and root re-uptake. This progressively decouples measured DOC from true gross exudation rates, which, when coupled with the need to fit tidal access windows while capturing a complete diurnal cycle in exudation, suggests the use of a 48 h total protocol as an optimal compromise (i.e., 24 h of recovery + 24 h of effective incubation). Nevertheless, the optimal incubation duration remains provisional, and future work should validate this choice with a larger time-series experiment across a greater number of roots."

- Section 4.3 The Spearman correlation matrix shows essentially no significant correlations between exudation rate and any measured variable (temperature, rainfall, humidity, soil EC, pH, bulk density, root traits). The authors suggest this is because the sampled conditions were not sufficiently contrasted. If the study cannot identify any driver, what is the mechanistic contribution of the work? Please discuss more honestly whether the method may be too noisy to detect environmental signals, or whether sample size is simply too small.

We thank the reviewer for this fair point. We have added clarification to Section 4.3 to address this more explicitly: that the method may be too variable to detect moderate environmental signals at low sample sizes.

"…sampled across species and seasons may not have been sufficiently contrasted in order to reveal driver–response relationships. **We also acknowledge that small sample size (n = 47) and high individual-level variability (SD > mean for most groups) limit statistical power to detect moderate environmental effects, independent of whether the relevant drivers were measured."**

- Section 4.4 The manuscript compares exudation fluxes to a "global mean mangrove GPP of 16–20 Mg C ha^{-1} yr^{-1}" from Adame et al. (2024). However, your site-specific GPP may differ. If available, please report local GPP or at least discuss the uncertainty in applying a global average to a specific Vietnamese mangrove.

We thank the reviewer for this valid point. Site-specific GPP data for Cần Giờ were not available to us, and we have now made this limitation explicit in the manuscript, noting that the GPP comparison should be interpreted with caution. We have additionally revised the comparison to use a fixed global mean of 19 Mg C ha^{-1} yr^{-1} (mid-range of Adame et al., 2024) and now report both mean- and median-based estimates to better represent the distribution of values:

"Mean annual inputs were approximately 2140 kg C ha^{-1} yr^{-1} **(SD = 4175; range = 16.6–15270 kg C ha^{-1} yr^{-1}).** in *Avicennia*-dominant plots and 1093 kg C ha^{-1} yr^{-1} **(SD = 1581 kg C ha^{-1} yr^{-1}; range = 23.5–6610 kg C ha^{-1} yr^{-1})** in *Rhizophora*-

dominant plots. **However, given that SD exceeds the mean in both cases, median values are more representative of typical fluxes (*Avicennia*: 519 kg C ha^{-1} yr^{-1}; Rhizophora: 526 kg C ha^{-1} yr^{-1}).** Relative to a global mean mangrove GPP of 16–20 Mg C ha^{-1} yr^{-1} (Adame et al., 2024), **median-based estimates correspond to approximately 2.7% of GPP in *Rhizophora*-dominant and 2.8% of GPP in *Avicennia*-dominant plots, respectively, while mean-based estimates reach 5.7 and 11.3% respectively. Site-specific GPP measurements for Cần Giờ were unavailable, which would have allowed a more precise comparison and should be prioritised in future studies, alongside expansion of measurements across sites with contrasting edaphic conditions, to improve this first back-of-the-envelope assessment."**

- Dry-season exudation was higher but non-significant. Could the authors link this to seasonal changes in salinity, temperature, or plant water stress, and provide mechanistic explanations?

We appreciate this suggestion and have expanded the discussion of potential mechanisms in Section 4.3. We note that soil EC (a proxy for salinity) was indeed higher in the dry season, which could increase osmotic stress and stimulate exudation of compatible solutes. Mean temperature was similar between seasons (dry: 29.6°C; wet: 29.9°C), so thermal effects are unlikely to explain the trend. We have added the following:

" Though SRL did not differ significantly between species (Table 2), SRL and RTD did respectively increase and decrease during the wet season, indicating a shift toward finer, less dense roots under wetter conditions – a pattern shared across both species. Yet this morphological change was not accompanied by **a statistically significant difference in exudation rates**, suggesting that SRL alone is a poor predictor of exudation in these mangroves, contrary to patterns reported in terrestrial systems (Meier et al., 2020; Tückmantel et al., 2017). **The directional trend toward higher exudation in the dry season, when roots were denser and less elongated, may reflect stress-induced changes in carbon allocation**. Along the seasonal and environmental axis, environmental measurements likewise showed that the underlying ecological contrasts across zones and seasons were small, limiting the potential for divergence of exudation rate between species and seasons. Soils beneath *Avicennia* showed higher pH and electrical conductivity but lower bulk density than beneath *Rhizophora*, and EC and bulk density were both elevated in the dry season. These differences were modest in magnitude**, yet the higher dry-season EC is consistent with elevated porewater salinity, which is known to alter root exudation in plants (Vives-Peris et al., 2020) and is thus a plausible contributor to the non-significant trend toward higher exudation in the dry season. However, in the absence of porewater solute data and larger sample sizes, these mechanistic links remain speculative."**

- Porewater salinity, redox potential (ORP), and nutrient concentrations (NH_4^+, NO_3^-) were not measured. These variables strongly regulate root exudation—could the authors supplement these data or discuss how their omission limits mechanistic interpretation?

Yes, thank you. We have added a further discussion on the limitations of omitting these measurements in section 4.5:

> "Finally, while this study provides the first exudation measurements for *Avicennia* and *Rhizophora*, caution is warranted when extrapolating these results. Measurements were limited to two species in a single reserve under relatively homogeneous edaphic conditions. *Avicennia*, for instance, spans a wide range of inundation classes across its global distribution, exudation rates may vary considerably with edaphic context**, and the absence of concurrent porewater chemistry data, such as redox potential and nutrient concentrations, may have limited our ability to detect clear driver–response relationships.**
>
> These limitations point to clear priorities for future research. Expanding measurements to more contrasting sites, encompassing different inundation classes, geomorphological settings, salinity gradients, and mangrove species, **and inclusion of more detailed porewater and soil characterisation** is needed to identify the environmental drivers of exudation and establish whether the patterns observed here are representative of tropical mangroves more broadly."

- The study focuses on one mangrove site. Could the authors contextualize results with data from other tropical/subtropical mangroves to assess generalizability?

We appreciate the comment. Contextualisation with other mangrove sites is limited by the near-absence of published *in situ* root exudation measurements in mangrove ecosystems. To our knowledge, only one comparable study currently exists (Kato et al., 2026), conducted on *Bruguiera gymnorhiza* in a subtropical system. Direct comparison with that study is complicated by methodological and biological differences, and by the fact that both studies sampled only a fraction of the full rooting depth. We have therefore expanded our contextualisation to include terrestrial forest studies. We also note that our study was conducted in stands dominated by *Avicennia* and *Rhizophora*, the two most widely distributed mangrove genera globally, which enhances the potential representativeness of our findings. We have furthermore added explicit caveats around our ecosystem-scale flux estimates, which should be treated as first-order approximations given the high individual-level variability, low replication, and single-season biomass data. Expanding measurements across sites with contrasting edaphic conditions remains a clear priority for future work. We have now been more

careful in presenting our upscaling and have been clearer on the availability of data for contextualisation, and future steps:

:

"Given the low replication, high individual-level variability, and single-season biomass data, the ecosystem-scale flux estimates presented here should be treated as a first, back-of-envelope approximation rather than as precise flux measurements. … Relative to a global mean mangrove GPP of 16–20 Mg C ha^{-1} yr^{-1} (Adame et al., 2024), **median-based estimates correspond to approximately 2.7% of GPP in *Rhizophora*-dominant and 2.8% of GPP in *Avicennia*-dominant plots, respectively, while mean-based estimates reach 5.3–11.3% respectively. Yet, expanding measurements across sites with contrasting edaphic conditions is a critical next step to improve this first assessment.**

Comparison with other mangroves is limited because, to our knowledge, only one other study has until now measured root exudation *in situ* in mangroves. Our measured fluxes are lower than those reported for *Bruguiera gymnorhiza (*Kato et al. 2026). Both estimates are therefore conservative, and the true ecosystem-scale flux is likely higher than reported here. **Compared to terrestrial forests, mass-specific exudation rates at Cần Giờ (mean 90.5 µg C g^{-1} h^{-1}; median 41.1 µg C g^{-1} h^{-1}) fall within the interquartile range reported across upland ecosystems (25–140 µg C g^{-1} h^{-1}; Chari et al., 2024), consistent with the finding that mass-specific exudation rates do not differ significantly among biomes (Chari et al., 2024). At ecosystem scale, mean-based GPP estimates (6.1–11.9%) are broadly compatible with the global terrestrial average of 7–14% of GPP (Chari et al., 2024), while the more conservative median-based estimates (2.6–3.3%) fall at the lower end of this range, likely reflecting the skewed distribution of our data rather than a fundamentally lower exudation capacity in mangroves."**

- The sealed cuvette contains microbes that may re-uptake DOC. Could the authors discuss how incubation time and sterile controls mitigate this bias?

We thank the reviewer for raising this point. The sealed cuvette is not completely sterile, and microbial re-uptake of DOC is a recognised limitation of all cuvette-based approaches. We address this in two ways described in the manuscript: (1) the 48 h incubation duration was chosen in part to limit this effect — the pilot time series showed DOC declining by 72 h, consistent with an increasing likelihood of microbial consumption at longer incubation times (Section 4.1); and (2) root-free control cuvettes, which contain the same microbial background, are subtracted from all samples, partially correcting for microbial DOC turnover in the bulk solution. In Section 4.1, we have expanded the discussion of the 72 h decline to explicitly attribute it to

microbial consumption and root re-uptake progressively decoupling measured DOC from gross exudation at longer incubation times and have clarified that the choice of 48 h was made in part to limit this effect.

> "The pilot time series revealed a biphasic pattern, with DOC rising over the first 48 hours and declining by 72 hours (Fig. 4**). The initial increase is consistent with the accumulation of exudates described for cuvette-based methods, where exudation rates are highest immediately after equilibration (Phillips et al., 2008; Zhang et al., 2024), whereas the subsequent decline may result from microbial degradation and root re-uptake of compounds offsetting gross exudation at longer incubation times (Oburger & Jones, 2018; Phillips et al., 2008; Zhang et al., 2024)"**

References

Adame, M. F., Cormier, N., Taillardat, P., Iram, N., Rovai, A., Sloey, T. M., Yando, E. S., Blanco-Libreros, J. F., Arnaud, M., Jennerjahn, T., Lovelock, C. E., Friess, D., Reithmaier, G. M. S., Buelow, C. A., Muhammad-Nor, S. M., Twilley, R. R., & Ribeiro, R. A. (2024). Deconstructing the mangrove carbon cycle: Gains, transformation, and losses. *Ecosphere*, *15*(3), e4806. https://doi.org/10.1002/ecs2.4806

Brauner, K., Birami, B., Brauner, H. A., & Heyer, A. G. (2018). Diurnal periodicity of assimilate transport shapes resource allocation and whole-plant carbon balance. *The Plant Journal*, *94*(5), 776–789. https://doi.org/10.1111/tpj.13898

Brunn, M., Mueller, C. W., Chari, N. R., Meier, I. C., Obersteiner, S., Phillips, R. P., Taylor, B., Tumber-Dávila, S. J., Ullah, S., & Klein, T. (2025). Tree carbon allocation to root exudates: Implications for carbon budgets, soil sequestration, and drought response. *Tree Physiology*, https://doi.org/10.1093/treephys/tpaf026

Canarini, A., Kaiser, C., Merchant, A., Richter, A., & Wanek, W. (2019). Corrigendum: Root Exudation of Primary Metabolites: Mechanisms and Their Roles in Plant Responses to Environmental Stimuli. *Frontiers in Plant Science*, *10:157*. https://doi.org/10.3389/fpls.2019.00157

Chari, N. R., Tumber-Dávila, S. J., Phillips, R. P., Bauerle, T. L., Brunn, M., Hafner, B. D., Klein, T., Obersteiner, S., Reay, M. K., Ullah, S., & Taylor, B. N. (2024). Estimating the global root exudate carbon flux. *Biogeochemistry*, *167*(7), 895–908. https://doi.org/10.1007/s10533-024-01161-z

Chow, A. T., Ulus, Y., Huang, G., Kline, M. A., & Cheah, W. (2022). Challenges in quantifying and characterizing dissolved organic carbon: Sampling, isolation, storage, and analysis. *Journal of Environmental Quality*, *51*(5), 837–871. https://doi.org/10.1002/jeq2.20392

Cook, S., Peacock, M., Evans, C., Page, S., Whelan, M., Gauci, V., & Kho, L. K. (2016). Cold storage as a method for the long-term preservation of tropical dissolved organic carbon (DOC). *Mires and Peat*, *18*, 1–8. https://doi.org/10.19189/MaP.2016.OMB.249

Döll, S., Koller, H., & Van Dam, N. M. (2024). A simple, cost-effective and optimized protocol for collecting root exudates from soil grown plants. *Rhizosphere*, *30*, 100899. https://doi.org/10.1016/j.rhisph.2024.100899

Kato, N., Osaka, K., Yimatsa, N., Ohtsuka, T., & Iimura, Y. (2026). In Situ Quantification of Root Exudates in a Subtropical Mangrove (Bruguiera gymnorhiza) Forest. *Forests*, *17*(2), 156. https://doi.org/10.3390/f17020156

Kuzyakov, Y., Raskatov, A., & Kaupenjohann, M. (2003). Turnover and distribution of root exudates of Zea mays. *Plant and Soil*, *254*(2), 317–327. https://doi.org/10.1023/A:1025515708093

Lovelock, C. E., Ruess, R. W., & Feller, I. C. (2006). Fine root respiration in the mangrove Rhizophora mangle over variation in forest stature and nutrient availability. *Tree Physiology*, *26*(12), 1601–1606. https://doi.org/10.1093/treephys/26.12.1601

Meier, I. C., Tückmantel, T., Heitkötter, J., Müller, K., Preusser, S., Wrobel, T. J., Kandeler, E., Marschner, B., & Leuschner, C. (2020). Root exudation of mature beech forests across a nutrient availability gradient: The role of root morphology and fungal activity. *New Phytologist*, *226*(2), 583–594. https://doi.org/10.1111/nph.16389

Mencuccini, M., & Hölttä, T. (2010). The significance of phloem transport for the speed with which canopy photosynthesis and belowground respiration are linked. *New Phytologist*, *185*(1), 189–203. https://doi.org/10.1111/j.1469-8137.2009.03050.x

Nimptsch, J., Woelfl, S., Kronvang, B., Giesecke, R., González, H. E., Caputo, L., Gelbrecht, J., Von Tuempling, W., & Graeber, D. (2014). Does filter type and pore size influence spectroscopic analysis of freshwater chromophoric DOM composition? *Limnologica*, *48*, 57–64. https://doi.org/10.1016/j.limno.2014.06.003

Oburger, E., & Jones, D. L. (2018). Sampling root exudates – Mission impossible? *Rhizosphere*, *6*, 116–133. https://doi.org/10.1016/j.rhisph.2018.06.004

Phillips, R. P., Erlitz, Y., Bier, R., & Bernhardt, E. S. (2008). New approach for capturing soluble root exudates in forest soils. *Functional Ecology*, *22*(6), 990–999. https://doi.org/10.1111/j.1365-2435.2008.01495.x

Tixier, A., Forest, M., Prudent, M., Durey, V., Zwieniecki, M., & Barnard, R. L. (2023). Root exudation of carbon and nitrogen compounds varies over the day-night cycle in pea: The role of diurnal changes in internal pools. *Plant, Cell & Environment*, *46*(3), 962–974. https://doi.org/10.1111/pce.14523

Tückmantel, T., Leuschner, C., Preusser, S., Kandeler, E., Angst, G., Mueller, C. W., & Meier, I. C. (2017). Root exudation patterns in a beech forest: Dependence on soil depth, root morphology, and environment. *Soil Biology and Biochemistry*, *107*, 188–197. https://doi.org/10.1016/j.soilbio.2017.01.006

Vives-Peris, V., De Ollas, C., Gómez-Cadenas, A., & Pérez-Clemente, R. M. (2020). Root exudates: From plant to rhizosphere and beyond. *Plant Cell Reports*, *39*(1), 3–17. https://doi.org/10.1007/s00299-019-02447-5

Zhang, C., Cai, Y., Zhao, Q., He, T., Mao, T., Zhang, T., Zhang, L., & Su, W. (2024). The quantification of root exudation by an in-situ method based on root morphology over three incubation periods. *Frontiers in Plant Science*, *15*, 1423703. https://doi.org/10.3389/fpls.2024.1423703