Dear Editor,

Thank you for sharing the reviewer's feedback on the revised version of our manuscript, "Estimating Particulate Organic Matter Flux from In-Situ Optics: A Framework for Correcting for Suspended Particles and Incorporating Depth-Dependent Degradation" (N. Moradi et al.).

We appreciate the constructive insights provided during this second evaluation, which have been highly beneficial for the improvement of our manuscript. We have carefully updated the manuscript according to all the suggestions.

Please find below our comprehensive response to each of the reviewer's comments.

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Response to Reviewer #1
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Review of " Estimating Particulate Organic Matter Flux from In-Situ Optics: A Framework for Correcting for Suspended Particles and Incorporating Depth-Dependent Degradation" by Moradi and co-authors.

The authors have resubmitted their analysis of field observations of sinking particle fluxes and its modeling from particle size distribution (PSD) observations from in situ imagery from near the Cape Verde Islands. The paper now introduces three distinct methods for modeling sinking particle fluxes from in situ particle imagery. These are 1) removing suspended particles from the in situ imagery PSD using gel trap PSD determinations so that only sinking particles are considered in estimating fluxes, 2) accounting for changes in carbon content in sinking particles as function of depth/time and 3) considering changes in nitrogen to carbon ratio vs. depth due to the preferential remineralization of PON. The method results in assessments of the sinking particle PSD separately from suspended and POC and PON fluxes. The resubmitted paper is much improved, both in its focus and execution. This is greatly appreciated. I also appreciate their inclusion of uncertainty analyses in this work. It was missing before. However, there remain issues that need to be resolved. Detailed presentation of my larger issues are followed by some specific comments.

Response: We are glad to hear that the improvements in focus, execution, and the new uncertainty analysis were appreciated. We have taken all the remaining concerns into consideration and addressed each point carefully. We believe these revisions have further strengthened the manuscript.

General Issues.

As I understand it, Method 1 attempts to correct for the non-sinking particles seen by the UVP before sinking fluxes are calculated by removing the non-sinking fraction from the UVP PSD. Paired gel trap and UVP PSD are used to fit log-log relationships vs diameter (D) for each member of the ensemble of paired observations available. The UVP PSD is then mapped onto the gel trap PSD using by minimizing the square errors of the areas of a trapezoid under each PSD vs. D curve summed over the ensemble. I see no physical rationale of why this is done using the areas of the trapezoid. I think that this would bias the corrections towards focusing on the smaller D values (which is counter to what one thinks about suspended and sinking PSD slopes). The authors must provide some rationale of why this is the right way to do it. Why can't the slopes and offset factors be simply modeled from each other using a linear regression approach? It seems to me that there may be an environmental parameter or two, like the UVP PSD slope or total particle volume, that can be used to better constrain the correction.
Was this attempted? One of my concerns is that the details of the method presented may be specific to the particular characteristics of the data set and environmental characteristics and may be difficult to replicate in other settings.

Response:

We thank the Reviewer for highlighting this point. We wish to clarify that the proposed correction method (Method I) is fundamentally a mathematical and statistical optimization rather than a physics-based derivation. Its primary objective is to empirically align the characteristic power-law distribution of the optical (UVP) PSD flux—which manifests as a linear relationship ($y = ax + b$) when plotted in a log-log space—with that of the

physical gel trap reference. The reasoning for Method I is based on the nature of the in-situ camera (UVP) and gel traps; the UVP takes an image of a known volume of water, which may contain both settling and non-sinking particles (suspended particles and zooplankton), whereas the gel traps only collect particles that physically sink into the trap (i.e., they only detect settling particles). Since the UVP detects both sinking and non-sinking particles, the uncorrected UVP-derived particle flux overestimates the number of particles that effectively contribute to the flux through gravitational sinking. In contrast, suspended particles do not settle in the gel trap, and if any zooplankton are found in the gel, they are removed during image analysis. Therefore, it is assumed that the gel provides a reliable estimate of the size distribution of sinking particles. To obtain a more realistic estimate of the number of particles contributing to the flux estimated from the UVP PSD, we perform a simultaneous statistical adjustment of the slope and intercept of the UVP's characteristic regression line (in log-log space) to ensure that it aligns with the corresponding regression line derived from the directly observed size distribution of settling particle flux in the gel trap.

Rationale for the trapezoidal area and addressing bias. In Method I, we used the area under the regression line in log–log space (representing a trapezoid) to calibrate the UVP-derived size distribution of particle flux. While the area under the regression line characterizing the particle flux size spectrum in log-log space does not represent the total integrated particle flux (which would require integration in linear space), it serves as a robust geometric proxy for the alignment of the two characteristic regression lines. Geometrically, within the fixed overlapping size range (x_1 to x_2), we define a trapezoid bounded by fixed left and right vertical sides, a fixed horizontal bottom side (along the x-axis), and a variable oblique upper side (defined by the regression line). Thus, the area of the trapezoid is determined exclusively by the vertical position and slope of this regression line.
Since the difference between the two characteristic lines in log–log space is not substantial, a slight simultaneous adjustment of the position and slope of the UVP regression line (via α and β) is sufficient to align it to the gel trap reference. Consequently, minimizing the difference in their associated trapezoidal areas serves as a robust metric to optimize this alignment, effectively accounting for the coupled nature of the slope and intercept of the regression line. **We have added this rationale in the main text (lines 210–223).**

Addressing the Bias toward Small Particles. We agree with the Reviewer that the higher frequency of small-particle bins in log-space increases their numerical weight. However, we chose to remain in log–log space for reasons of practicality and robustness. Log-transformation is the established convention for empirical data with power-law distributions—particularly for marine particle data spanning several orders of magnitude (e.g., Jackson, 1997; Guidi et al., 2008; Iversen et al., 2010; Kiko et al., 2022). This approach effectively accounts for both abundant and rare observations while ensuring mathematical consistency in the distribution's shape, regardless of whether particle size is characterized by diameter or by volume.

Why Optimization instead of regression. We avoided independent linear regressions of slope (b) and intercept (a) because these parameters are mathematically coupled. Correcting them independently could lead to significant error propagation, where a minor deviation in the slope induces a substantial error in the predicted intercept. Our optimization method treats the PSD as a holistic distribution; by simultaneously tuning α and β, we identify the optimal parameter combination that best aligns the UVP characteristic regression line with the Gel trap 'ground truth.' We agree that relevant environmental parameters could be used to constrain the correction; however, our goal is to provide a robust and simple method without leading to over-parameterization.

Applicability and future use. We acknowledge that the derived coefficients (α and β) are specific to the environmental conditions of our dataset, as is standard for trap-camera calibrations (see e.g., Iversen et al., 2010; McDonnell & Buesseler, 2012). However, this methodological framework—leveraging the integrated area in log-log space to statistically align high-frequency optical profiles with physical trap data—represents a robust and replicable technique for any region where paired datasets are available. Currently, the combined use of in situ optics and gel traps is becoming a standard tool in biogeochemical studies of the driving mechanisms of the biological carbon pump (e.g., McDonnell & Buesseler, 2012; Fender et al., 2019; Giering et al., 2020; Durkin et al., 2021; Xiang et al., 2022; Svensen et al., 2024; Song et al., 2025). While this approach was pioneered in our lab and the Laboratoire d'Océanographie de Villefranche (LOV) (e.g., Guidi et al., 2008; Iversen et al., 2010; Ramondenc et al., 2016; Seifert et al., 2019; Baker et al., 2020; Markussen et al., 2020; van der Jagt et al., 2020; Fadeev et al., 2021; Pauli et al., 2021), it is now being used by many leading groups performing in situ observations of the processes driving carbon export and attenuation, including MBARI, WHOI, MIO, UAF (Fairbanks), UiT (Tromsø), NOC, AWI, and MARUM. In our laboratory alone, we have datasets combining in situ optics with conventional and gel traps from more than 20 cruises spanning the Central Arctic, the Fram

Strait, the Irish Shelf, the Porcupine Abyssal Plain, the Cape Blanc upwelling region, the Scotia Sea, the Bransfield Strait, and the Weddell Sea. We are currently applying this methodology to those datasets to optimize parameterizations that will help both global biogeochemical models and the utilization of BGC-Argo floats. We hope that by sharing this method of calibrating UVP PSD data, other groups can perform similar optimizations on their in situ optics and gel trap data in order to improve the quality of in situ flux estimations.

Once the corrections (maybe more correctly termed "calibrations") are made, then the UVP data can be used to estimate fields of sinking particle PSD. This would be a very useful thing to know. It is a shame that the authors do not take advantage of this and address what fraction of the total particle pool is sinking particles in section 3.1.2 (lines 429-435). The comparison of the coefficients presented is not very intuitive. This seems like a missed opportunity

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Response:

To address the suggestion regarding the visualization of these fields, we have added a new set of plots (Fig. 4m–o) showing the UVP-derived size distributions of particle flux after calibration, complementing the uncorrected distributions shown in Fig. 4a–c (previously Fig. 4b–d).

Regarding the fraction of the total particle pool that is sinking, we previously provided an estimate in the Discussion (lines 580–590 of the first revision), utilizing the average ratio of the uncalibrated UVP-based flux to the gel trap particle flux—approximately 2.6 (Fig. 3j in the current manuscript; previously Fig. 4a)
From this, we estimated that roughly 70% of small particles are non-sinking (either suspended or sinking extremely slowly). This suggests that approximately 30% of the particles detected by the UVP were part of the effectively gravitationally settling pool. We acknowledge, however, that these estimates rely on the gel trap-based particle flux data as a reference, as well as the application of the in situ measured size-velocity relationship, which are specific to the study area and sampling period. It is worth noting that overestimations of particle flux from uncalibrated UVP data stem not only from the detection of suspended material in smaller size classes but also from zooplankton in the larger size

We have **incorporated this explanation into Section 3.1.2** of the current manuscript revision (lines 415–422).

The second method uses measurements of particle O2 consumption rates to address the loss of sinking particle carbon content vs. depth. They show that they retrieve similar values for POC fluxes whether they model the carbon losses as a zeroth or first order terms. Again, zeroth order kinetics implies that the rate of carbon loss is independent of the mass of carbon present, which simply is not physical. Given the uncertainty in all of the data used to model the POC fluxes, the differences between the two methods are small. That said, I would drop the zeroth order case as it adds unnecessary complexity to the paper (and is fundamentally flawed). Regardless, they adjust constants to make the method "work" using trap measured POC fluxes.

Response:

We acknowledge that the first-order degradation model is an established approach for modeling biological degradation processes. Our initial choice to apply zero-order kinetics was intended to directly leverage our microbial respiration data, under the assumption that the microbial enzymes responsible for degradation are saturated with substrate (resulting in a constant rate; see lines 240–245 in the first revision).
However, we agree with the Reviewer that including both models adds unnecessary complexity, especially given that they produce similar results. Therefore, **we have followed the recommendation to drop the zero-order model entirely** and now present only the first-order model results. **We have removed all tex**t and comparisons regarding the zero-order model from the manuscript.

Accordingly, **Figs. 5 and 6 have been updated** to show these first-order calculations for POC, PON, and C:N ratios. We note that the uncertainty for the POC flux estimates is slightly larger under the first-order method. This is expected, as degradation in a first-order model is proportional to the POC mass; the model is therefore more sensitive to the variations of the degradation rate constant (λ), which leads to higher propagated uncertainty during the analysis.

Finally, to **ensure full reproducibility** of the updated results, we fixed the random seed across the entire computational framework (bootstrapping statistical analysis and initial randomization for model parameter optimization) and reran the calculation pipeline.

The third method models PON losses relative to POC as function of particle age (depth / sinking speed) using a hyperbolic function. This makes sense to me. It is logically calibrated using the trap PON fluxes. No issue here, assuming that the POC loss rates make sense.

Response:

We thank the Reviewer for their feedback and appreciate their positive assessment of Method III. The PON flux estimates shown in the current manuscript **have been calculated using the POC values from the first-order model.**

The uncertainty calculations are appreciated as mentioned before. However, given the large uncertainties in the inputs (sinking speed spectra [fig 3a], particle O2 respiration rates [fig 3d], results of the UVP to gel trap PSD correction [fig 4gh], etc.), the error bars for the flux profiles are tiny (fig 6). Further the mismatches with the flux and C/N ratio profiles (fig 6) lie outside of the 95% confidence intervals more than 5% of the time. Something does not seem right here.

Response:

Flux profile uncertainty. The proposed framework consists of a sequence of methods where uncertainty propagates through each stage. To capture this, we implemented a nested bootstrap procedure, as described in **Section 3.2**. The resulting error bars appear relatively small because the model parameters are directly dependent on the input variables (e.g., sinking velocity) and are re-optimized against the reference trap fluxes during every bootstrap iteration. This **re-optimization** effectively constrains the estimated fluxes and their associated uncertainties.

C:N ratio mismatches. The C:N ratio is not optimized directly; rather, it is a derived quantity obtained by dividing the results of the optimized POC model by those of the optimized PON model. Consequently, the derived C:N estimate incorporates the propagated uncertainties of both models. Therefore, unlike the POC and PON flux profiles, the C:N fit is not directly constrained by the observations. This results in a higher frequency of data points falling outside the 95% confidence intervals compared to the directly optimized POC and PON profiles, where the fit error is actively minimized against the data. If the C:N ratio were the primary variable of interest, reformulating the PON model to optimize directly against measured C:N ratios would reduce this mismatch.

This explanation **has now been added to the current revision** of the manuscript (lines 601–610).

Another issue with the paper is that it seems that the methods presented here were created for a particular experimental setting and the various parameters estimated would not necessarily work for another setting. This means that the many measurements - bulk and gel sediment trap fluxes, in situ particle profiling, analysis of gel trap imagery, particle-specific remineralization rate determinations, etc.- need to made again for another setting and the various coefficients and uncertainty bounds need to be recalculated. This would make it extremely difficult to recreate for another setting, especially by another group of investigators. Clearly, we are a long way from applying these methods on without the detailed experimental work. But it seems that a discussion of the paths forward to achieve this would make some sense. What are the most important things to estimate, how best to automate some of this, etc., etc.? As it stands, the methods introduced would be hard to adopt widely.

Response

We appreciate the Reviewer's perspective on the broader applicability of this framework. We acknowledge that the specific model parameters derived here are specific to the environmental conditions of the study site. However, the methodological framework itself is designed to be transferable, and the necessary observational tools are becoming standard practice on many research cruises. We have already addressed this point above, but for the sake of readability, we include the response again here: "Currently, the combined use of in situ optics

and gel traps is becoming a standard tool in biogeochemical studies of the driving mechanisms of the biological carbon pump (e.g., McDonnell & Buesseler, 2012; Fender et al., 2019; Giering et al., 2020; Durkin et al., 2021; Xiang et al., 2022; Svensen et al., 2024; Song et al., 2025). While this approach was pioneered in our lab and the Laboratoire d'Océanographie de Villefranche (LOV) (e.g., Guidi et al., 2008; Iversen et al., 2010; Ramondenc et al., 2016; Seifert et al., 2019; Baker et al., 2020; Markussen et al., 2020; van der Jagt et al., 2020; Fadeev et al., 2021; Pauli et al., 2021), it is now being used by many leading groups performing in situ observations of the processes driving carbon export and attenuation, including MBARI, WHOI, MIO, UAF (Fairbanks), UiT (Tromsø), NOC, AWI, and MARUM. In our laboratory alone, we have datasets combining in situ optics with conventional and gel traps from more than 20 cruises spanning the Central Arctic, the Fram Strait, the Irish Shelf, the Porcupine Abyssal Plain, the Cape Blanc upwelling region, the Scotia Sea, the Bransfield Strait, and the Weddell Sea. We are currently applying this methodology to those datasets to optimize parameterizations that will help both global biogeochemical models and the utilization of BGC-Argo floats. We hope that by sharing this method of calibrating UVP PSD data, other groups can perform similar optimizations on their in situ optics and gel trap data in order to improve the quality of in situ flux estimations."

Regarding the complexity of experimental work, we agree that, in particular, direct respiration measurements on individual in situ formed aggregates are rare and in fact only performed in a handful of laboratories. However, increasing efforts are being directed toward the development of in situ respiration measurements for both short- and long-term deployments. We have developed long-term in situ instruments for direct measurements of in situ settling velocities and the capture of aggregates in gel traps, which can easily be combined with the commercially available UVP camera systems. However, as noted in our response above, following the Reviewer's recommendation, we have removed the zero-order model primarily to avoid unnecessary complexity. This decision also directly addresses the concern regarding the detailed experimental work. Unlike the zero-order approach, the first-order kinetic model does not require direct respiration measurements as an input, as the degradation rate is optimized directly from the trap data.

Indeed, the increasing availability of paired optical and biogeochemical observations was a primary motivation for developing this framework. We hope that the underlying logic and modeling approach presented here can be adopted or adapted by other research groups to suit their specific needs and improve flux estimations from in situ optical observations.

We have **incorporated a discussion based on these points** into the current revision of the manuscript (lines 650–664).

A few additional comments follow.

The abstract needs to state that the methods introduced improve the estimates of sinking particle fluxes from the experimentally optimized conventional method. You do not want to bury the lead...

Response:

We appreciate the advice to highlight our main contribution. We have updated the abstract to clearly state that the new framework yields improved flux estimates relative to the conventional method.

No link or reference is given for the Gel-PISA gel analysis software that is used to determine the PSD of sinking particles. The authors need to provide a link to a github repository (or similar) for the software so the phyton scripts are available. That seems important enable any open science to happen with this approach.

Response:

The Python scripts for our custom image analysis software, Gel-PISA, **are now publicly available on GitHub** at https://github.com/namoradi/Gel-PISA. The repository includes a detailed step-by-step user guide located in the 'docs' folder. We have added this link to the Data Availability section in the current revision.

Figure 7 compares flux calculations made with two different profiles with differing amounts of large particles in the mesopelagic. It is super hard to see the differences in the particle sizes from the number density spectra profiles shown and some better metric is needed. Even a profile of the abundance of large particles greater than some size would make this argument easier to follow.

Response:

We thank the Reviewer for this suggestion. To better illustrate the differences in particle size composition between the two stations, we have **revised Fig. 7.** The updated figure now displays the total volume of both small (< 0.5 mm) and large (> 0.5 mm) particles within the studied size range.

Sincerely,

We hope and trust that the current manuscript now meets the standards for publication in Biogeosciences (BG).

Corresponding author

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