Response to RC3: Elemental and isotopic constraints on zooplankton-mediated carbon fluxes in Ryder Bay, Western Antarctic Peninsula

Biogeosciences

Preprint: https://doi.org/10.5194/egusphere-2026-2459

General comments

The authors present bulk C, N, and isotopic data from bulk sediment trap material and isolated fecal pellets from four time segments during a bottom-moored sediment trap deployment off the Western Antarctic Peninsula. The authors aim to describe the importance of fecal pellets and other sources of particulate organic material to sinking flux and its properties, such as C:N ratio. The overall background of the manuscript is well presented, but the reader currently cannot assess the quality of the data due to several missing methodological details.

We thank the reviewer for this positive assessment of the manuscript's background and framing, and for highlighting the need for greater methodological detail. We address the reviewer's specific comments individually below, and note that further improvements have also been made to the methods section as a result of comments raised by reviewers 1 and 2, which are addressed in the corresponding responses.

Specific comments

Missing methodological or quality-control details:

- *The limits of detection and quantification for C & N analyses are not described, specifically in relation to the amount of C & N obtained from the samples. If, for example, the small number of fecal pellets analyzed resulted in measured quantities of N that were close to the limits of detection or quantification, this could explain a high measured C:N ratio.*

We thank the reviewer for this important comment. We can confirm that the measured quantities of carbon and nitrogen in all samples, including the isolated faecal pellet subsamples, were above the limits of detection and quantification for this instrument. We will add the limits of detection and quantification, alongside the measured C and N quantities for all sample types, to the revised manuscript so that this can be assessed directly by the reader.

- *Blank corrections are also not described; contribution from a C blank, which is common on pre-combusted GF/Fs, could also account for high measured C:N ratios, particularly in samples with low organic loading on the filter.*

We thank the reviewer for this comment. Blank corrections were performed as part of the standard analytical procedure, with pre-combusted GF/F filter blanks, processed identically to samples, run at a frequency of approximately two blanks per 15 samples. Reported carbon and nitrogen values are blank corrected. We will add this information explicitly to the methods (Section 2.4), alongside the calibration details described in response to a comment from Reviewer 2.

- *The methods by which the fecal pellet volumes were estimated are described, but the volumes are not reported. Thus, the reporting of fecal pellet C mg/mm^3 cannot be related back to the amount of C in a single fecal pellet, which would both be useful for the reader and important for understanding how much C & N was actually measured on the 15 isolated pellets (related to limit of detection/quantification above).*

We thank the reviewer for this comment. We agree that reporting faecal pellet volumes directly would allow the carbon and nitrogen content of individual replicates to be assessed, and would support evaluation of these values relative to instrument detection and quantification limits. We will add this data to the revised manuscript.

- *Uncertainty in the reported fecal pellet C & N flux is not adequately described. There appear to be at least four sources of uncertainty, and it is unclear which sources have or have not been included in the "95% confidence intervals" reported. The four sources of uncertainty that I can see are: uncertainty in the geometric method for estimating volume, standard deviation in the mean volume assumed in converting from FP volume to C & N (based on means of volume estimated across a large number of pellets), analytical uncertainty in the instrumental method, and variation across the two replicate samples analyzed for each set of FP. Along with ensuring that adequate uncertainty is expressed, reporting proportional fluxes to two decimal places may be inappropriate once all sources of uncertainty are considered (e.g., Lines 193-194). To highlight this point, this analysis by the authors results in the statement in both the abstract and the text that fecal pellets constituted 100% of the flux in some samples. However, the authors should be able to validate this statement directly, as they performed visual inspection of the samples under magnification; in these samples, did they observe that the samples in fact contained absolutely no material other than identifiable fecal pellets?*

We thank the reviewer for this detailed and important comment. We address the sources of uncertainty, decimal precision, and the specific question regarding faecal pellet flux contribution in turn.

Regarding uncertainty, we agree that the four sources identified by the reviewer (geometric volume estimation, variability in mean pellet volume across the measured

subsample, analytical/instrumental precision, and variation between the two replicate samples analysed per set sample type) all contribute to the overall uncertainty in faecal pellet carbon and nitrogen flux, and that these sources were not independently described in the manuscript. We do not consider formal propagation of these uncertainties through the calculation to be appropriate, as several of these sources are not independent of one another (for example, the same pellets contribute to both the geometric volume estimate and the scaling calculation), which formal error propagation methods generally assume. As described in response to related comments from Reviewers 1 and 2, we will be revising our reporting throughout the manuscript to present descriptive statistics only, reporting the standard deviation of the two replicate analyses per sample as the primary expression of variability, and no longer applying significance testing to these values. We will also revise the methods to describe the scaling calculation from pellet volume to total faecal pellet carbon and nitrogen flux in more explicit detail, so that the contribution of each processing step to the final reported value is clearer to the reader, and will note the additional, unquantified sources of uncertainty as limitations of the approach.

Regarding decimal precision, we agree that the proportional flux values should be reported to a level of precision consistent with the underlying variability, and we will revise decimal precision throughout the results, including the values at lines 193 – 194, accordingly.

Regarding the specific statement that faecal pellets constituted ~ 100 % of the flux in one sample, we agree that reporting this as a single value does not adequately convey the uncertainty inherent in a ratio calculated from two independently measured, and themselves variable, flux estimates. We do not consider formal propagation of uncertainty through this calculation to be appropriate, as several of the underlying sources of variability are not independent of one another. Instead, consistent the revised approach we will be taking to statistical reporting throughout the manuscript, we will report the calculated flux contribution (%) as a range across the two replicate measurements, rather than as a single value, to better reflect the variability underlying this calculation.

- *Figure 3 displays POC and PN as a fraction of total trap material, but the quantification of total trap material was not described. Is this a weight %? How was total trap material weighed?*

We thank the reviewer for this comment. Percent contribution of POC and PN to total sediment trap material, as shown in Figure 3, was calculated on a weight percentage basis. We will add a description of how total sediment trap material was weighed to the methods.

Statistics: Averages and standard deviations across all sampling periods are reported throughout the Results section. In some cases, these time-averages for different measurements are statistically compared against each other (e.g., Lines 197-198). Reporting of these averages across sampling periods for the most part is not useful, since there is a high amount of variability across periods in most measurements; this also means that comparison of two such means against one another is not a reasonable comparison.

We thank the reviewer for this important point, which relates closely to comments raised both in this review and by the other reviewers regarding our statistical approach. We agree that reporting a single mean and standard deviation across sampling periods that themselves show substantial and structured variability, and subsequently comparing such means to one another using formal significance testing, is not an appropriate or informative approach given the degree of heterogeneity between periods and the limited replication underlying each estimate.

As described in response to related comments, we will revise our statistical reporting throughout the manuscript to present descriptive statistics only, describing the pattern of variation between sampling periods directly, rather than through significance testing or period-averaged means. We will review the results section to ensure that comparisons between sampling periods and material types are carried out in such a way that internal variability is not obscured.

Other commenters have provided input about missing literature comparisons; I also noted these but will not comment further, as these issues can be found in the other reviews.

Please see responses to reviewers 1 and 2 to consider how the literature comparisons raised have been addressed.

Technical comments

- *Isotopic data with precision of +/-0.2 per mille or greater should not be reported to two decimal places—please edit to display one decimal place.*

We thank the reviewer for this comment. We agree that reporting isotopic values to two decimal places implies a precision greater than that supported by the analytical reproducibility of ± 0.2 ‰ (as stated in section 2.4). We will revise all reported $\delta^{13}C$ and $\delta^{15}N$ values throughout the manuscript to one decimal place.

- *The journal's style guide may need to clarify, but it is my understanding that percent and per mille signs should not be separated from numbers by a space. This is because these signs are not units, but rather part of the number itself.*

We thank the reviewer for this comment. We have checked the Biogeosciences author submission guide on this point, which states "Spaces must be included between

number and unit (e.g. 1 %, 1 m)". We have therefore retained the spaced format throughout the manuscript.

- *Line 136-137: Were bulk C & N quantities determined in a separate analysis from the isotopic measurement, or the same analysis? The type of detector used for quantification is not mentioned.*

We thank the reviewer for this comment. We can confirm that bulk C and N quantities and isotopic composition were determined from the same analysis, rather than separate analyses: the elemental analyser (CE Instruments NA2500) is coupled in-line with the isotope ratio mass spectrometer (Thermo Finnigan Delta-V Advantage) in a continuous-flow configuration, such that a single combustion of each sample provides both elemental quantification and isotopic composition. We will state this explicitly in the methods rather than relying on "in line with" to convey this.

- *Line 165: What is ImageJ?*

We thank the reviewer for this comment. ImageJ is an open-source image analysis software (developed by the U.S. National Institutes of Health) used here to measure faecal pellet dimensions from dissecting microscope images. We will add a brief description and appropriate citation to the methods.

- *In all cases, how 95% confidence intervals were determined needs to be described.*

We thank the reviewer for this comment. We acknowledge that in the submitted manuscript, this was not adequately described. In light of other comments regarding our statistical approach, in the revised manuscript we will instead present the mean and standard deviation from the two replicate measurements per sample on the plots, consistent with how variability is reported in the main text. Figure captions will also be updated to clearly state that the error bars represent this.