Response to RC2: Elemental and isotopic constraints on zooplankton-mediated carbon fluxes in Ryder Bay, Western Antarctic Peninsula

Biogeosciences

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We thank the reviewer for their comments on our manuscript and address them below each point as follows.

The novelty of the study is the unique setting, but more work needs to be done to better contextualize what is found here relative to the large body of work that has been done elsewhere using similar methods and generating similar results.

We thank the reviewer for this comment and would like to clarify what we consider the central novel contribution of this study, as we recognise the reviewer's framing of "unique setting" does not reflect out intended emphasis. While Ryder Bay itself has not been studied using this specific combination of elemental and isotopic faecal pellet analysis, this is not the novelty we intended to convey alone. Similar methodological approaches elsewhere in the wider Western Antarctic Peninsula and Southern Ocean, although not combining all of the analyses presented here together in one study, are cited and discussed extensively throughout the manuscript (e.g. Section 4.1, lines 248 – 266, Section 4.2, lines 286 – 291).

Rather, the central novel finding of this study is that POC:PN ratios observed here exceed those previously reported anywhere in the wider Western Antarctic Peninsula region, despite the region being subject to the same broad range of potential governing processes (Section 4.2, lines 285 – 291). Furthermore, we show that this elevated signature is specifically attributable to the elemental composition of cylinder zooplankton faecal pellets, which contribute a substantial to dominant fraction of the sediment trap material (Section 3.1, Section 4.1), rather than reflecting a system-wide environmental control. This particle-type-specific attribution, evidenced by the contrast between cylinder and round faecal pellets (Section 4.2.2, lines 344 -351), indicates the presence of unresolved processes modifying organic material in this system via the specific vector of zooplankton feeding and egestion, which we consider to be the study's principal contribution. We believe this is stated in the abstract (lines 6-12), though we recognise the framing may benefit from further clarification and will review this throughout the manuscript to ensure it is conveyed clearly.

The two biggest issues with this manuscript are as follows:

1) Very limited sample size

The study only has one sample location and only 4 samples from that location. How can significant findings be justified with such a small sample size. Additionally, if it was estimated that thousands of fecal pellets were captured per sample, why were only 15 used for elemental and isotopic analysis. There is so much extrapolation occurring in this manuscript from very limited actual data. All the conclusions are being formed based on a small time-window as well (only from mid-January to mid-March).

We thank the reviewer for these comments and address each in turn.

Regarding the use of the term "significant" to describe our findings, we would like to clarify two replated points. First, following changes made in response to comments from all reviewers regarding the statistical approach of this manuscript, in the revised manuscript we will be reporting descriptive statistics rather than formal significance tests, addressing the concern that statistical significance testing is not appropriate given the sample sizes involved. Second, we would note that a finding need not depend on formal statistical significance testing to represent a meaningful scientific contribution. The elemental and isotopic composition of faecal pellets and sediment trap material reported here constitute new empirical observations of this coastal Antarctic system that were previously undocumented, regardless of the sample size from which they were obtained.

Regarding the narrow sampling window (14th January – 11th March 2020), we would like to reiterate that this reflects an equipment limitation as described in Section 2.2, lines 115 – 118, rather than a deliberate restriction of scope. We do not consider a shorter observation window to categorically preclude meaningful findings; we note that the discussion and conclusions are consistently framed around interpreting this specific dataset and the system studied here, by considering literature from similar environments, rather than generalising beyond it, and we consider the data obtained during this window to represent a meaningful empirical contribution characterising a specific flux event in a system that remains generally understudied.

Regarding the selection of two replicates of 15 pellets per shape category for elemental and isotopic analysis, we note that this subsample was not treated in isolation from the broader pellet population. As described in Section 2.5, a larger number of pellets (169 – 192 pellets per shape per sample) were measured for size and volume, and this broader dataset was used to scale the elemental and isotopic measurements from the smaller analysed subsample to total faecal pellet carbon and nitrogen flux. Pellets analysed for elemental and isotopic composition were selected at random from each aliquot, minimising the risk of bias towards particular pellet sizes or conditions, and the same subsample size was applied consistently across both pellet shape categories and all sampling periods. We acknowledge, however, that the sample size of 15 pellets per category reflects a practical constraint, rather than a value derived from a formal power calculation or limitation on pellet availability. We recognise that a larger subsample per

category may have more precisely constrained the mean composition reported for each shape and time period, and we will acknowledge the limited sample size as a consideration in the revised manuscript.

We would also like to emphasise that a key strength of our approach is that we directly measured the elemental and isotopic composition of faecal pellets themselves, rather than relying solely on a generic biovolume-to-carbon conversion factor, as is commonly done in sediment trap studies; such factors assume relatively consistent carbon content per unit pellet volume and may not capture variability associated with pellet morphology, producer, feeding history, or sampling period. The composition of the analysed subsample was used to provide a representative estimate of mean pellet composition for scaling to total flux, rather than to characterise the full range of compositional variability across the entire population of pellets collected.

2) Very broad and sweeping statements based on significant literature findings but very little actual sample evidence from this study

There is a good amount of literature review occurring for the study site but very little of it can be traced back to results that were found in this study, let alone unique to this study. Very little, at the present iteration of this article, seems to be new about the results that were generated.

We thank the reviewer for this comment, which relates closely to the earlier comment regarding the novelty of this study's contributions; our response to that comment sets out in detail what we consider to be the central novel findings (elevated POC:PN ratios exceeding previous reports for this region, the particle type-specific contrast between cylinder and round faecal pellets, and the isotopic evidence supporting benthic feeding and trophic reworking as a potential source of this signature).

We recognise that the structure of the discussion could better foreground these points. Several subsections test our results against literature-derived expectations and ranges before returning to our own findings; while this reflects a deliberate approach of using established mechanisms to constrain which processes are consistent with our observations, we can see how the current structuring may read as literature-led rather than results-led. We will revise the discussion to state our own results first in each subsection, using existing literature to contextualise and test candidate mechanisms rather than to introduce them, and will shorten passages where literature serves primarily to rule out mechanisms not supported by our data.

Additionally, the methods section needs a lot more work and whether small particles (single micron particles) are captured/considered needs to at the very least be addressed.

We thank the reviewer for this comment. Regarding filtration, aliquots of both bulk sediment trap material and isolated faecal pellets were filtered onto pre-combusted 25 mm GF/F filters, which have a nominal pore size of 0.7 µm (this will be added to the Methods) and are widely used for the retention of particulate organic carbon and nitrogen in oceanographic sampling. While the very smallest sub-micron particulate material may not be fully retained by filters of this pore size, GF/F filtration is standard practice for POC/PN quantification and is considered appropriate for this purpose.

More broadly, we note that as a passive collection device, the sediment trap itself is designed to capture gravitationally sinking particulate material, and would not be expected to capture very small, slowly sinking or non-aggregated particles (e.g. free-living bacteria or phytoplankton) regardless of subsequent filtration, unless incorporated into larger aggregates or faecal material. This is a recognised characteristic of sediment trap methodology generally, rather than a limitation specific to this study, and reflected the intended scope of the method: quantifying gravitational particle flux rather than total suspended or dissolved organic matter in the water column.

We acknowledge the reviewer's broader view that the methods section requires substantial further work, a concern also reflected in specific comments raised across the three reviewers. We have sought to address these individually through our responses, detailing the corresponding revisions to be made to the methods section as a result. We hope that, taken together, these changes satisfy the reviewer's request for improvement to this section; however, we would welcome further clarification should specific aspects remain unaddressed.

Also, more qualification in the abstract is required. Much of what is presented in the abstract as novel has been well documented in many of the global ocean regions.

We thank the reviewer for this comment. We agree that elevated POC:PN ratios have been documented in various forms across global ocean regions. However, we would like to note that the mechanisms generating elevated POC:PN ratios and their associated isotopic signatures are not necessarily transferable between systems. The specific taxa producing faecal pellets, their feeding ecology, and the environmental and biogeochemical conditions modifying organic matter differ substantially between regions. In the Southern Ocean specifically, faecal pellet-driven carbon flux is often dominated by krill and large calanoid copepods, as found in this study, distinct from the taxa responsible for comparable observations elsewhere. Consequently, an elevated POC:PN ratio in one region cannot be assumed to arise from the same underlying process as a similar observation elsewhere; studies examining the specific mechanisms responsible in a given system remain necessary to interpret such signatures. The elevated POC:PN ratios observed here substantially exceed those previously reported for the Western Antarctic Peninsula region specifically, despite

numerous prior studies using comparable methods. We consider this specifically to indicate the presence of a mechanism not previously identified as significant in this region, which we investigate in detail in this manuscript, and consider to be the central novel contribution of this study. We will revise the abstract to foreground both the regionally unprecedented magnitude observed here and the particle-type-specific mechanism we identify as underlying it.

There was significant discussion of the possibility of isotopic analysis to tease apart processes but no isotope mixing models or higher order analysis was performed (CSIA, Bayesian statistics, etc.).

We thank the reviewer for this comment. We agree that the framing of the isotopic analysis in the discussion, which states that isotopic composition may allow the origin of organic matter to be linked to specific processes and distinguished between pathways, may have created an expectation of formal source-partitioning approaches, such as isotope mixing models or compound-specific isotope analysis, which were not the intended scope of this study. Our approach instead used the direction and magnitude of $\delta^{13}C$ and $\delta^{15}N$ shifts, evaluated against established literature ranges for specific processes (e.g. sea ice algal production, trophic enrichment, benthic reworking), to assess the consistency of candidate mechanisms with our observations, rather than to quantitatively apportion their relative contributions. We consider this qualitative, elimination-based approach appropriate given the absence of well-constrained isotopic endmembers for the multiple co-occurring processes (primary production, trophic processing, remineralisation, and potential non-marine inputs) operating in this coastal system, which would be required for robust quantitative mixing model application. We will revise the introduction (lines 62-65) and stated aims (lines 90-98) to more accurately reflect this analytical approach.

Lastly, the phytoplankton section of the discussion was particularly problematic. There was much too much extrapolation. There are too many processes that could be affecting the isotopic compositions of the bulk material to assume it's only due to changes in primary production. Trophic processing is not discussed in this section at all. Neither is microbial reworking of the fecal pellets (see Wojtal et al. 2023). Additionally, the authors should think about the efficiency of the gut of the zooplankton (see Doherty et al. 2021).

We thank the reviewer for this comment. We would like to clarify the intended scope of this subsection, which is not to attribute the observed elemental and isotopic signatures solely to changes in primary production, but to evaluate phytoplankton production related processes as one of several candidate categories of mechanism, alongside remineralisation related processes (4.2.2) (including trophic processing and gut passage efficiency), and non-marine material inputs (4.2.3). This premise is set out on lines 298-304. The phytoplankton production section itself concludes that

production-stage mechanisms alone are insufficient to account for the full magnitude of the observed deviation. We recognise, however, that the overall structure of Section 4.2 may not have been evident in the text from Section 4.2.1 if read in isolation. In the revised manuscript, we will therefore include some additional signposting to serve as a reminder that factors such as trophic processing and microbial reworking will be examined in subsequent sections.

Regarding the microbial reworking of faecal pellets, we thank the reviewer for drawing our attention to Wojtal et al. (2023). We recognise that the discussion of this topic within section 4.2.2 was unclear, and we will revise the opening of this section to discuss this mechanism explicitly, incorporating this reference alongside Belcher et al. (2016) to address both the general recognition of microbial reworking as a degradation pathway for sinking particulate organic matter and its comparatively minor role relative to zooplankton-mediated processes in productive, actively grazed Southern Ocean systems such as the one sampled here. Please see the revised text for this paragraph below:

Elevated C:N ratios attributed to preferential nitrogen remineralisation have been widely reported in sediment trap studies, reflecting progressive degradation of sinking organic matter through microbial reworking whereby heterotrophic bacterial activity preferentially releases nitrogen-rich compounds relative to carbon (Anadon et al., 2002; Copin-Montegut and Copin-Montegut, 1983; Gordon, 1971; Martin et al., 1987; Treguer et al., 1990). This mechanism has been directly identified in other ocean systems (e.g. Wojtal et al., 2023) and invoked to explain increases in particulate C:N with depth along the WAP, with reported values reaching up to 16.4 in sediment trap material (Anadon et al., 2002). However, the relative influence of microbial reworking appears to vary regionally and seasonally, and has been found to play a comparatively minor role relative to zooplankton-mediated FP degradation during periods of high productivity and active grazing, such as that sampled in this study (Belcher et al., 2016). The magnitude of C:N ratios observed here, exceeding 20 in both total sediment trap material and cylinder FPs, lies beyond the highest values previously reported for this region, indicating that microbial reworking alone is likely insufficient to account for the most extreme values observed, and that additional zooplankton-mediated and particle-specific processes must also be considered.

Regarding the efficiency of zooplankton gut passage, this is already addressed in Section 4.2.2 (lines 251-356), where we discuss selective nitrogen assimilation by zooplankton as a mechanism generating elevated FP C:N ratios relative to their food source, and note that the magnitude of C:N elevation observed in our study exceeds that which gut assimilation efficiency alone would be expected to produce. We do not consider Doherty et al. (2021) directly applicable to this point, as it addresses compound-specific isotopic discrimination of end-member sources rather than the

influence of gut passage processes on elemental ratios, but we would be happy to reconsider this if the reviewer can point to a specific aspect of the paper they feel is relevant.

Major questions

- *No where is diel vertical migration discussed or explored. Why?*

We thank the reviewer for raising this point. Diel vertical migration was not explicitly discussed in the manuscript, and we agree that it is a relevant process in the context of zooplankton-mediated carbon flux more broadly. We will add mention of diel vertical migration to the introduction when discussing the role of zooplankton faecal pellets in the biological carbon pump (lines 32 – 40).

However, we would like to note that distinguishing between active and passive transport mechanisms is not possible using the sediment trap approach employed in this study, which measures material collected at a single fixed depth rather than resolving the horizontal or vertical movement of the zooplankton producing it. We therefore consider a detailed assessment of the role of diel vertical migration specifically to be beyond the scope of this study.

- *The Redfield ratio was conceptualized for phytoplankton physiology. Fecal pellets have presumably undergone significant reworking. Why would one expect that the Redfield ratio of zooplankton fecal pellets would match open ocean phytoplankton cellular elemental ratios?*

We thank the reviewer for raising this important conceptual point. We would like to clarify that our use of the Redfield ratio in this study is not to imply an expectation that the elemental composition of particulate organic matter or zooplankton faecal pellets should match that of open-ocean phytoplankton. Rather, the Redfield ratio is used as a fixed, widely recognised reference baseline against which departures in elemental composition can be interpreted diagnostically. The extent and direction of deviation from this baseline, and how this deviation differs between particles types (cylinder versus round faecal pellets) and from the presumed phytoplankton source material, is used throughout the discussion to constrain which processes, operating between primary production and pellet formation or recovery, are most likely responsible for the observed signatures. This approach, wherein deviation from Redfield stoichiometry is treated as diagnostic rather than anomalous, is well established in studies of particulate organic matter and sediment trap material more broadly (e.g. Schneider and Schlitzer, 2003; Weston et al., 2013; Rembauville et al., 2015), and specifically within zooplankton faecal pellets (e.g. Atkinson, 2012; Tamelander, 2012). We will revise the opening paragraph of section 4.2 to make clear this framing, to avoid the impression that matching the Redfield ratio was an expectation of this study.

- *Why no zooplankton measurements if you talk about carcasses? Why no experiments trying to make zooplankton poop and isolating their fecal pellets to measure for an end member?*

We thank the reviewer for this comment. Zooplankton carcasses are mentioned only in the introduction, in the context of biological pathways shown elsewhere to contribute to Southern Ocean POC flux, and were not treated as a separate component of this study. During sample processing, an attempt was made to distinguish carcasses from swimmers based on physical condition; however, no zooplankton with the characteristics of carcasses (damaged appendages, degraded or broken body, dull colouration) were identified. We will add data of swimmer numbers into the supplementary material to make this clear.

Regarding the suggestion of incubation-based experiments to isolate faecal pellets from known zooplankton populations for elemental endmember characterisation, we consider this outside the scope of this study. Nevertheless, we agree that controlled incubation experiments represent an important next step and would provide a valuable complementary dataset to the observations presented here. We will therefore highlight this as a potential avenue for future work in the revised manuscript.

- *Need to say somewhere that large and small cylinder were grouped together and that oval and spherical were grouped together*

We thank the reviewer for highlighting this omission. We will revise the methods sections 2.3 and 2.5 to state clearly how the four shape categories used for pellet counting and sizing relate to the two categories reported in the results and discussion.

- *What are error bars on the figures? Just the standard deviation of the two replicates?*

We thank the reviewer for this question. In light of this comment and related comments from other reviewers with regards to our statistical approach, we will revise our reporting in the manuscript to use descriptive statistics only. As part of this revision, we will replace the 95 % confidence intervals shown in the figures with the standard deviation of the two replicate measurements per sample, consistent with how variability will be reported in the main text.

Comments

- *Line 6 – why is there a ~ above the 1 in 100%? Also, why is the % sign on the next line?*

We thank the reviewer for catching this. Both instances are typesetting errors (a misplaced tilde character and an incorrectly wrapped % sign) and will be corrected in the revised manuscript.

- *Line 9 – should be "the Redfield Ratio"?*

We thank the reviewer for catching this. This will be corrected to "the Redfield ratio" as suggested.

- *Line 10 and 11 – this has been shown repeatedly. Why is it being stated like a new finding?*

We thank the reviewer for this comment. We would like to clarify that this finding is intended, and presented, as new specifically for this environmental setting, which is the basis for this manuscript. As set out in our response to a related comment above ("the novelty of this study is the unique setting…."), the processes governing POC:PN ratios and their isotopic expression are not necessarily transferable between oceanographic systems: the taxa producing dominant faecal pellets, their feeding ecology, and the biogeochemical conditions modifying organic matter differ substantially between regions, meaning an elevated ratio observed elsewhere does not establish the mechanism responsible for an elevated ratio observed here. The finding reported at this point in the abstract is that POC:PN ratios in Ryder Bay exceed those previously documented anywhere in the Western Antarctic Peninsula specifically, despite the region having been the subject of considerable prior study using comparable methods; this regional context is stated on lines 9-10 ("well beyond the range previous reported for inshore Western Antarctic Peninsula environments"). We consider the magnitude of the ratio, and our subsequent demonstration that it is attributable specifically to the dominance of Southern Ocean-specific zooplankton faecal pellets in the particulate material, to constitute the central novel contribution of this study, rather than the general phenomenon of Redfield deviation of particulate material, which we agree is well documented elsewhere and do not intend to present as new.

- *Sentence starting at Line 28 – all at the same time?*

We thank the reviewer for catching this. We agree that the current phrasing could be misread as implying that all listed particle types dominate POC flux simultaneously. We will revise the sentence to make clear that each particle type has been shown, in different studies and contexts, to form a dominant contribution to POC flux at different times.

- *What about diel vertical migration? (lines 33 to 36)*

We thank the reviewer for this comment and agree that diel vertical migration represents an important pathway of vertical carbon transport that is not currently addressed in this section of the introduction. As discussed in response to the similar comment above, we will revise the text from line 33 to explicitly recognise diel vertical migration as a mechanism of active carbon transport, complementary to the passive

gravitational transport of faecal pellets described here, and will incorporate appropriate supporting literature.

- *Line 40 – what about Doherty et al. (2021), Colleen Durkin's work, or Shea et al. (2023)?*

We thank the reviewer for drawing our attention to this literature. This comment highlighted that the original statement at lines 38–40 did not sufficiently clarify the specific gap we aim to address. We have therefore revised the text from the sentence beginning on line 38, as shown below, to explicitly reference existing work that has made valuable contributions to distinguishing faecal pellets from other particle types and to the use of geochemical approaches for understanding processes affecting faecal pellet contributions to carbon flux. We then identify the specific literature gap this study addresses: understanding the bulk elemental and isotopic signatures of different particle types contributing to flux, in order to improve our ability to use bulk stable isotope signatures of particulate organic material to interpret the drivers of changing particle flux in Southern Ocean environments. The text from line 38 until the end of this paragraph will be revised as the following:

However, while the contribution of FPs to export has been widely recognised (Cavan et al., 2015, 2017; Karaku ş et al., 2021; Perhirin et al., 2025; Turner, 2015), the extent to which the elemental and isotopic composition of faecal pellets varies among morphologically distinct pellet types, and how this reflects differences in the feeding behaviour of producing taxa and subsequent remineralisation processes remains comparatively unexplored. Existing compound-specific isotopic approaches have proven valuable for distinguishing faecal pellets as a source endmember within bulk particulate organic matter (Doherty et al., 2021) and for partitioning particle sources and trophic structure more broadly (Shea et al., 2023; Wojtal et al., 2023). Optical and genetic approaches have also resolved the compositional diversity of sinking particle types and their contribution to flux (Durkin et al., 2021, 2022). However, none of these approaches directly characterise bulk elemental and isotopic composition across distinct faecal pellet morphotypes. In studies that have considered elemental and isotopic composition of distinct FP types, there is considerable inconsistency in the isotopic offset between FPs and their food sources, with no consistent fractionation effect identified for either $\delta^{13}C$ and $\delta^{15}N$ (Altabet and Small, 1990; Schmidt et al., 2003; Tamelander et al., 2006). Consequently, particle-specific isotopic analysis of common sediment trap constituents, such as FPs, phytoplankton detritus, and zooplankton moults, has been identified as necessary to resolve their respective contributions to bulk isotopic signatures to improve understanding of how stable isotope compositions can be used to understand export dynamics (Belcher et al., 2023).

- *The sentence starting at Line 45 is hard to follow*

We thank the reviewer for flagging this. We agree the sentence is difficult to follow as currently written and will revise it, likely by separating the description of CDW modification (via mixing and sediment fluxes) from the statement of its nutrient supply role, into two or more shorter sentences.

- *Sentence starting at Line 48 is confusing*

We thank the reviewer for flagging this. We agree that the sentence is unclear. We will revise to make the connection between nutrient supply and export explicit.

- *Sentence starting at Line 50 – could it not just be recycling?*

We thank the reviewer for this comment, and for prompting us to revisit the framing of this point. On review of Stukel and Ducklow (2017), we recognise that our original phrasing oversimplified their argument. Rather than identifying a deficit between independently measured new production and gravitational particle flux and invoking additional pathways to close it, Stukel and Ducklow derive an independent, first-order estimate of the magnitude of passive transport of particulate and dissolved organic matter via vertical mixing, based on the ratio of vertical gradients of particulate nitrogen and nitrate. We will revise the text as follows below to reflect this more accurately:

Passive transport of organic matter via vertical mixing and diffusion is estimated to account for around one quarter or the biological carbon pump across the Southern Ocean, rising the approximately half in productive coastal regions along the WAP (Stuckel and Ducklow, 2017). Distinct from this diffusive pathway, physically mediated subduction can transport organic matter along isopycnals and diapycnal pathways, redistributing carbon in three dimensions rather than solely through vertical settling (Boyd et al., 2019). In the WAP, such subduction is facilitated by cross-shelf exchange through glacially carved troughs, such as Marguerite Trough, which acts as a conduit for both the on-shelf transport of mCDW (Brearley et al., 2017) and the offshore export of shelf-modified waters via eddies (Brearley et al., 2019), driving carbon export to the ocean interior through the eddy subduction pump (Boyd et al., 2019).

- *Sentence starting at Line 60 – EXPORTS did this*
    - *Look at Siegel et al. (2021) or Siegel et al. (2023) as a place to start (also Ken Buesseler's work and Margaret Estapa's work on the EXPORTS project)*

We thank the reviewer for pointing us towards the EXPORTS project literature, and we have reviewed this work, including Siegel et al. (2021) and Siegel et al. (2023), as well as relevant work by Ken Buesseler (Buesseler et al., 2020a, b) and Margaret Estapa (Estapa et al., 2019, 2021), together with related EXPORTS-affiliated studies using compositional tracers (Doherty et al., 2021; Wojtal et al., 2023). We would like to clarify that the "origins" referred to in this sentence relate specifically to the geographic origin

of particulate organic matter, in particular the distinction between on-shelf and off-shelf source of production, rather than to biological or trophic source (e.g. phytoplankton versus faecal pellet material). On review, we find that the EXPORTS-associated literature we have examined addresses flux magnitude, biological carbon pump efficiency metrics, and the partitioning of gravitational, migrant, and mixing export pathways (Siegel et al., 2021, 2023; Buesseler et al., 2020a, b; Estapa et al., 2019, 2021), or the use of compositional signatures to distinguish biological source endmembers and trophic processing history (Doherty et al., 2021; Wojtal et al., 2023). We did not find literature from the EXPORTS project that addresses the use of particulate organic matter composition to trace its geographic origin, and have therefore retained the original framing of the sentence, but will add the word "geographic" before "origins" to make this explicit. We would welcome further clarification from the reviewer if there are specific studies within this body of work that address this point directly.

- *Line 112 – what was the size of the baffle?*

We thank the reviewer for this comment. The baffle is a grid, where individual cells have a diameter of 2.5 cm. We will add this detail to line 112 of the Methods.

- *Line 124 – were the zooplankton alive during recovery? What about carcasses?*

We thank the reviewer for this comment. By the time of recovery, all material within the trap was preserved and no organisms remained alive. During sample processing, we aimed to visually distinguish between zooplankton presumed to have entered the trap alive (swimmers) from carcasses based on physical condition (colouration, gut contents, damage to appendages, degradation of tissues). However, we found very small swimmer numbers, and did not identify any zooplankton individuals resembling carcasses. We will add further detail of this process to lines 122 – 125 of Section 2.3 and include swimmer data in the supplementary material.

- *Line 125 – how was the material examined under dissecting microscope? Was it on a mesh screen? What was the size of that mesh? What happened to smaller particles*
- *Line 126 – was material removed? What happened to it? How was it determined that material should be removed?*

We thank the reviewer for these related comments. Upon recovery, samples were passed through a 500 µm mesh, and the material retained on the mesh was rinsed into a petri dish for examination under a dissecting microscope. Material retained on the mesh and identified as swimmers (zooplankton individuals with intact bodies, undamaged appendages, and absence of decomposition), was removed and excluded from flux calculations, as noted in our response to the related comment. All other

material retained by the mesh, including large passively sunk particles such as large faecal pellets and aggregates, was returned to the sample. Exuviae were the exception to this process- although retained on the mesh, they were counted, retained, and analysed separately for carbon content, with their contribution considered part of the passive flux. Material smaller than the 500 µm mesh aperture passed through and remained within the bulk sample throughout. We will revise the text to ensure this process is explicit with regards to material included in or excluded from analyses.

- *Line 131 – how were those fecal pellets selected? Why only 15? What happened if there were more?*

We thank the reviewer for this comment, which relates closely to a comment from earlier in this review. Pellets analysed for elemental and isotopic composition were selected at random from each aliquot, minimising the risk of bias toward particular pellet sizes or conditions, and the same subsample size was applied consistently across both pellet shape categories and all sampling periods. We acknowledge, however, that the sample size of 15 pellets per category reflects a practical constraint, rather than a value derived from a formal power calculation or limitation on pellet availability. We recognise that a larger subsample per category may have more precisely constrained the mean composition reported for each shape and time period, and we note this is a limitation of the analysis.

- *Line 133 – why air dried? What method are you following?*
- *Line 134 – reference for method following?*

We thank the reviewer for these comments. Filters were first air dried to evaporate residual Milli-Q water remaining after the rinsing step, which was itself intended to remove any residual preservative from the sample prior to analysis. Filters were subsequently acid fumigated and oven dried following the method originally published in Lorrain et al. (2003), which has since become common practice in studies of particulate organic carbon, often applied without further citation. We will revise the text to state this rationale explicitly and include this citation.

- *References for method section overall?*

We thank the reviewer for this comment. In addition to adding specific citations as requested through specific comments from all reviewers, we will seek to add further references for more general sediment trap processing methodologies throughout the Methods section.

- *Was EA data correction only done with 1 point calibration? How was data processing done?*

We thank the reviewer for this comment. Samples were analysed using a CHN elemental analyser calibrated daily with acetanilide standards, using a three-point calibration curve (0.2 – 2.0 mg C) to verify instrument linearity, rather than a single-point calibration. Blank corrections were performed using pre-combusted GF/F filter blanks, processed identically to samples and 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 Section 2.4 of the Methods.

- *Line 161 – what was the criteria for oval vs. round? Was that only done by one person to keep consistent?*

We thank the reviewer for this comment. Round pellets were defined as having a nearly circular outline, with similar length and width and smooth, round margins, while oval pellets were defined as having an elliptical outline, with length visibly exceeding width while retaining rounded ends and a regular, symmetrical shape. Classification was informed by reference images of faecal pellets collected in similar regions from the citations given in lines 160 – 166. All pellets were classified by the same observer throughout the study to ensure consistency.

- *Line 169 – how was total pellet count determined? Also by scaling?*

We thank the reviewer for this comment. The method for determining total pellet count by scaling is described in Section 2.5 of the methods (lines 158-159): the total number of faecal pellets in each sample was estimated by counting pellets in an aliquot representing a known fraction of the total sample, and then scaling to the full sample. The total pellet count is then used, together with mean pellet volume derived from the larger measured subsample (n = 169–192 pellets per shape per sample), to estimate total faecal pellet volume, as described on lines 165-169. We will revise the text to make this connection explicit, so it is clear without requiring the reader to cross-reference to an earlier passage.

- *Line 171 – I don't get how you get a proportion of the total POC or PN that is fecal pellet from what is described*

We thank the reviewer for this comment. We agree that the final step of this calculation is not clear, and will revise the sentence to improve this. The percentage contribution of each faecal pellet category to the total POC and PN flux was determined as the calculated flux contribution (%), by dividing the relevant faecal pellet flux (FPC or FPN) by the corresponding total POC or PN flux, and multiplying by 100. We will state this explicitly in the methods, and will also revise the terminology used throughout the manuscript to refer to these values as "calculated flux contribution (%)" to make clear that they are derived from the ratio of independently measured fluxes, rather than representing a directly observed proportion of the material, in response to a comment from Reviewer 3.

- *Line 173 – so this was just ignored?*

We thank the reviewer for this comment. Partially degraded faecal pellet material ("faecal fluff") was not excluded from the dataset; it remained within, and is captured by, the bulk sediment trap material measurements, and total POC/PN flux calculations. However, because this material could not be reliably attributed to a specific pellet shape category once degraded, it is not included in the faecal pellet specific (cylinder or round) flux estimates. As a result, the contribution of intact faecal pellets to total flux, as reported in this study, may represent an underestimate of the true contribution of faecal pellet derived material, since some fraction of the bulk attributed to "total sediment trap material" is itself degraded pellet material that could not be assigned back to a pellet category. We will revise the text to make this explanation clearer.

- *Line 178 – how do you test for normality with 4 samples?? How do you do any statistics with 4 samples?*

We thank the reviewer for this comment, and for the related comments raised by this and other reviewers regarding the statistical approach used in this manuscript. We agree that testing for normality and applying formal significance tests (ANOVA, Tukey HSD) to data comprising only four sampling periods, each represented by two replicate aliquots, is not statistically robust, and that this approach was not appropriate given the scale of replication available in this study.

In light of this, we will revise the overall approach to statistical reporting throughout the revised manuscript. Rather than reporting formal significance tests, we will report descriptive statistics only, including means, standard deviations (calculated from the two replicate aliquots per sample), and will provide qualitative descriptions of patterns of variation across sampling periods and material types. Figure error bars will also be revised to show the standard deviation of the two replicates, consistent with in-text reporting, rather than the previously reported 95 % confidence intervals. We will also review reported values throughout the manuscript to ensure appropriate and consistent significant figures, given the level of precision the data can support.

We would like to emphasise that these changes affect that statistical framing and presentation of the data only. The underlying values, patterns of variation between sampling periods and material types, and their interpretation in the discussion remain unchanged. Our conclusions do not rely on the statistical significance testing that will be removed, but on the consistency and magnitude of the patterns observed across the dataset.

Lines 185-186 – how can you say this if you are ignoring so much of the POC (small particles)?

We thank the reviewer for this comment, and would like to clarify that small particles are not excluded from the total POC flux value referenced in this comparison. As described in the Methods, and clarified in response to the comments above about lines 125 and 126, samples were filtered onto GF/F filters with a nominal pore size of 0.7 µm, meaning total POC flux, against which cylinder faecal pellet carbon (FPC) flux is compared in this sentence, includes contributions from small particulate material, excluding only the smallest particles that would pass through a 0.7 µm filter.

- *Line 188 – reference figure 2*

We thank the reviewer for this comment. We will add a reference to Figure 2 at this point in the text.

- *Line 202 – so FPC and PFN don't correlate?*

We thank the reviewer for this comment. We would like to clarify that this sentence reports the result of a test for significant variation in round faecal pellet nitrogen (FPN) flux across the four sampling periods; it is not a statement about, or test of, correlation between faecal pellet carbon (FPC) and FPN flux.

- *Sentence starting at Line 263 – a very bold claim for a very small sample size*

We thank the reviewer for this comment. This statement is intended to describe the mechanism underlying the specific period of elevated POC flux observed within the dataset, rather than to generalise about the drivers of carbon flux in Ryder Bay at a wider timescale, or across the WAP more broadly; as such, we do not consider additional temporal scaling would necessarily provide stronger support for a claim scoped to this specific, discrete flux event. However, we recognise that the wording may currently read as more strongly generalised, or as asserting a greater degree of certainty than is supported by a single sampling period captured in this signal. We will revise the sentence as written below to state this more precisely as an observation grounded in the observed compositional dominance of faecal pellets during this period.

The near-total contribution of FPs during the period of elevated POC flux observed in this study suggests that organic carbon flux during this event was predominantly grazer-driven, with phytodetrital aggregation likely playing a comparatively minor role, although the contribution of degraded or fragmented material not attributable to a specific particle type (Section 2.5) means this cannot be fully excluded.

- *Line 274 – how do you get week-scale changes from sampling periods that were much longer than that?*

We thank the reviewer for this comment. We agree that "week-scale" inaccurately implies a temporal resolution finer than that supported by the sampling design, given that individual sampling periods spanned 12-15 days. We will revise this to reflect the

actual temporal resolution of the dataset, describing the observed changes as occurring between sampling periods of approximately two weeks' duration, rather than implying a shorter, sub-period timescale of variability.

- *Paragraph starting at Line 327 – was there any evidence of TEP present?*

We thank the reviewer for this comment. No direct evidence of TEP was observed or sought in this study; TEP was not a target of the sampling or analytical methods employed here, and its inclusion in the discussion is intended to be explicitly speculative, reflecting its recognised role as a contributor to elevated C:N ratios in other Antarctic marine and sea ice studies, rather than a claim that TEP contributed to the material observed in this study. We believe this is already reflected in the existing text (stating that "TEP was not directly measured in this study, and its potential role remains speculative"), but we will review the wording of this paragraph to ensure this distinction, between a literature-supported candidate mechanism raised for completeness and a mechanism with direct supporting evidence from this study, is unambiguous.

- *Line 340 – what is the range for the region??*

We thank the reviewer for this comment. We agree that "the upper range reported for this region" is imprecise, since the preceding sentence cites a single maximum value (16.4; Anadón et al., 2002) rather than an established range. We will revise the sentence to state clearly that the C:N ratios observed in this study exceed this specific previously reported maximum value, rather than implying a broader defined range.

- *Could this be a constructive interference (synergistic) effect?*

We thank the reviewer for this suggestion. We agree that a synergistic, or constructive interference, effect, between preferential nitrogen remineralisation and zooplankton-mediated processes is plausible, and may in fact be likely given the mechanism proposed in this section, whereby zooplankton feed on already partially degraded material and further process it during gut passage. However, we do not consider that the data presented in this study allow additive and synergistic contributions to be distinguished from one another. We will revise the text to acknowledge both possibilities explicitly, making clear that the observed elevation in C:N ratios is consistent with the combined influence of degradation and zooplankton-mediated processes.

- *Line 381 – what upper and lower traps?*

We thank the reviewer for this comment. We agree that the sentence as written is unclear and, on review, incorrectly attributes the upper/lower sediment trap comparison to this study rather than to Weston et al. (2013), which deployed traps at multiple depths to draw this conclusion. This study deployed a single sediment trap at

one depth and does not include a comparison between multiple trap depths. We will revise the sentence to correctly attribute this comparison.