Point-to-point response to reviews

Response to Reviewer 1

I have read the manuscript (egusphere-2024-3265) entitled “Sea ice-associated algae and zooplankton fecal pellets fuel organic particle export in the seasonally ice-covered northwest Labrador Sea”. This manuscript reports the results of the δ13C and δ15N analyses of amino acids in sinking particles collected by sediment traps in the seasonally ice-covered arctic sea. Applying several statistical tools, the authors found that 1. sinking particles is mainly originated from fecal pellets; 2. sea ice algae is the main ultimate carbon source of sinking particles; 3. sinking particles experienced only minor microbial reworking. In general, I think this research is well-conducted. Although some similar conclusions have been demonstrated in previous studies in some other polar regions, this study reveals the mechanism and dynamics of organic carbon sink in this specific area, and provides a good reference for future researches. Thus, I recommend the publication of this manuscript in BG after revision. Here are some comments to the authors:
RESPONSE: We thank Reviewer 1 for taking the time to review the manuscript and their insightful comments. Please find below our detailed point-to-point responses. All line numbers referenced here refer to the track-changed document.

Line 176: Please clearly indicate which method you applied, and add a few descriptions of it. At least list the name of derivatization method here.
RESPONSE: We used the trifluoroacyl-isopropyl ester method with esterficiation with isopropanol to form the isopropyl esters of AAs and acylation with trifluoroacetic acid anhydride (TFAA). We added the detail of derivatization in the following sentence (LN 178).

Line 205, 208: I think the terms “TPmet” and “TPpro” may be misleading, because Met and Pro are the abbreviations of two amino acids, and someone may think that the TP is calculated from the isotope ratio of these two amino acids. I recommend you to use some other abbreviations, for example, “TPmeta” and “TPproto”.
RESPONSE: Thank you for pointing this out. We made this change throughout.

Table 2: AA-related indices for many samples are not determined. What is the difficulty in obtaining these data? Not enough amount? Some chromatographic problems? Or just didn’t have enough time to analyze all of them?
RESPONSE: Indeed, not all sediment trap cups contained the requisite 10 mg of organic carbon for CSIA-AA. This was mentioned on LN 173-176 of the manuscript. We have added text on LN 238-241 to clarify this.

Figure 5: I think Lys should be EAA, not NEAA. In my understanding, Lys cannot be synthesized by marine consumers.
RESPONSE: Thank you for this catch. Lys is indeed an EAA. Fig. 5a has been redrafted with Lys plotted as a member of the EAA group.

Figure 6. About the PCA analysis, I recommended you to try adding Lys in the PCA model (if you agree that it is an EAA). It may help the classification of different end members, because it is known that Lys has different synthetic pathways in plants and bacteria.
RESPONSE: Thank you for this suggestion. However, due to coelution with tyrosine, the measured $\delta^{13}C$ of Lys may not reflect its true values and thus may not be informative about primary producer end-members. We have added text on LN 198 and in the Fig. 5 caption to clarify this point.

Also, I feel that you can discuss a bit more about the PCA results in the text. It seems that Thr and Leu are two informative amino acids in terms of distinguishing sea ice algae and pelagic algae. Do you think we can propose a new indicator using these two AAs to distinguish the contribution from sea ice algae and pelagic algae?
RESPONSE: This is an interesting suggestion, but the separation of data along PC1 in PCA space is also driven by Phe (in addition to Thr and Leu) in the original figure. In the new Fig. 6a, we included heterotrophic bacteria data in the PCA, where the separation driven by Ile, Thr, and Phe is more obvious. For consistency with existing literature (e.g., Larsen et al. 2013; McMahon et al. 2015; Chen et al. 2022) and clarity of presentation we prefer using the existing "5-EAA" PCA and LDA approaches.

About the LDA results, your sea ice algae and pelagic algae data look like in the middle of microalgae and heterotrophic bacteria, instead of showing "pure algae-like" signal. It makes your sinking particles look even "more like" algae than your algae samples. I think it will be interesting if you use your sea ice algae and pelagic algae data as the training data to construct a new LDA model, and put your sediment trap data into it. It may provide us a better semi-quantitative estimation of the relative contribution from sea ice algae and pelagic algae.
RESPONSE: Based on this suggestion, we have redrafted Fig 6 to show the PCA and LDA to be consistent with each other. The new PCA plot includes heterotrophic bacteria as a potential C source, and the new LDA model is trained by heterotrophic bacteria, sea ice algae, and pelagic algae to predict classes of sinking particles. Both plots are mutually consistent in that they indicate sea ice algae as the dominant C source to the sediment traps. The text on LN 350-357 has been revised accordingly.

Figure 7, 8: I recommend you to add the word "Microbially" before "Degraded OM".
RESPONSE: We made this change.

Line 465: Most lipids and carbohydrates don't contain N, so it sounds strange to me to say that they are responsible for the bulk δ15N values. I prefer to list some other N-containing compounds here, such as heterocyclic molecules (including nucleotides and pigments), and amino sugars.
RESPONSE: Thank you for pointing this out. We detailed other N-containing compounds, including inorganic clays in the revised section (LN 484-487).

Line 472: I don't think "Phe does not undergo deamination reactions during heterotrophic metabolism". A more accurate expression should be like "deamination/transamination reaction is not the first and 'rate-limiting' step in the 'dominant' metabolic pathway of Phe in animals".
RESPONSE: We agree with the reviewer. In response to Reviewer 2, section 4.2 was rewritten. The original statement was removed.

Line 489-491: Could you explain a little about the discrepancy between ΣV values and the Bayesian mixing model using Phe-normalized δ15N of Ala and Thr? Because there are several high ΣV values for sediment trap samples which are comparable to degraded OM, but we don't see the same results in the output of the Bayesian mixing model.
RESPONSE: This is a good point. The ΣV parameter is actually not very sensitive in distinguishing among potential endmember sources. We added text as part of a new section 4.3 on "preservation of AA-specific isotope signals" to clarify this point.

Line 523: While I understand it is necessary to exclude the zooplankton end-member in the mixing model because zooplanktons were removed from the samples before analysis, I wonder what the relative contribution from the zooplankton biomass in the N fraction of sinking particles will be.
RESPONSE: We ran a mixing model including zooplankton and the fraction of zooplankton ranged 7-19% (Table S5). This estimation reflects post-zooplankton-removal samples and is not representative of their original fraction. Nevertheless, swimmers are not considered "sinking" due to their nature of diel vertical migration, and thus the removal of swimmers is a common practice in most sediment trap studies.

Line 531-532: Because copepods are the only dominant type of zooplankton in the area, do you think that using the end-member containing a much larger variety of species will cause a larger uncertainty/error in the estimation of relative N contributions?
RESPONSE: The reviewer raised a good point about the uncertainties from including multiple species in an end-member. We agree that fecal pellet data from various species may lead to a large uncertainty. It would be ideal if only copepod fecal pellet data is used in the mixing model but unfortunately, data of zooplankton fecal pellet

are scarce in general and lacking for copepods. The fecal pellet end-member here was derived from a small number of samples from krill, salp, amphipods, and mixed zooplankton communities (Doherty et al., 2021). To ensure an adequate sample size for the end member in the mixing model, we had to include all the available data. New text on LN 250-256 of the Methods was added to clarify this.

Response to Reviewer 2

In this paper, the effects of sea ice algae and fecal pellets on carbon and nitrogen cycling processes in the Labrador Sea are investigated using an integrated isotope approach, including amino acid stable isotope methods. Overall this paper did a good job of analyzing the bulk and amino acid stable isotope data and obtaining very meaningful results of the analysis. I still have some questions to discuss with the authors at the level of analytical methods.
RESPONSE: We thank Reviewer 2 for taking the time to review the manuscript and their insightful comments. Please find below our detailed point-to-point responses. All line numbers referenced here refer to the track-changed document.

1. The authors used different TDF and beta values cited in the literature as the reference for trophic level calculations for different animal groups, and the system of calculations included not only Glx-Phe but also Ala-Phe. In the same ecosystem, the use of different criteria to calculate trophic levels may lead to biased conclusions, and would it not be better to use a unified method for calculating trophic levels in the same region and ecosystem? In addition, the food chain structure in this study is not complex.
RESPONSE: The reviewer raised concerns about not using a unified method for calculating trophic levels in our study. In fact, the reason why we reported both Glx-Phe and Ala-Phe derived trophic positions for samples from the same ecosystem is to compare the results using different criteria and to verify if the protozoan component is more pronounced in our samples. The criteria based on Ala-Phe has only been recently proposed and less reported compared to the Glu-Phe calculations in CSIA-AA studies. We believe reporting both results from a rarely investigated region (using CSIA-AA) provides useful information and reference for future studies. A reworded section 4.2, particularly new text on LN 492-496 was added.

2. Why estimate the proportional contributions of three end-members by using Phe-normalized δ15N-Ala and δ15N-Thr? I don't particularly understand the actual meaning of Phe-normalized δ15N-Ala and δ15N-Thr. Generally Thr is not a trophic amino acid nor a source amino acid, what is the significance of using Thr here?
RESPONSE: Thank you for pointing out that the description of the Bayesian mixing model was insufficient in the original manuscript. Our use of Phe-normalized δ15N-Ala and δ15N-Thr follows the approach developed in Doherty et al. (2021) and further demonstrated in Wojtal et al. (2023) and Golombek et al, (2024). We added

text on LN 253-256 of the Methods and LN 497-510 of the Discussion to explain and elaborate on the approach.

3. I don't really agree with the estimation of nitrogen sources in Figure 8, which is not in line with the general understanding. And unlike carbon, nitrogen usually undergoes a very complex mineralization and denitrification process that leads to severe isotopic fractionation. Also, using End-menber data from the literature instead of actually measuring it yourself may lead to calculation errors.
RESPONSE: Respectfully, we disagree with the assertions in this comment.
First, our finding that the N in sinking particles is sourced mainly from fecal pellets is consistent with literature on the composition of sediment trap POM. We discuss and cite the evidence extensively on LN 550-572.
Second, while it is true that degradation and remineralization can significantly alter $\delta^{15}N$ in organic matter, the effect is relatively small (< 2-3 per mil) in sinking POM because of more rapid sinking velocities and shorter residence time in the water column (e.g., Altabet, 1988 Deep Sea Res 35:535-554; Altabet, 1992 Nature 354:136-139;). This forms the basis for the long-standing use of $\delta^{15}N$ in sinking POM, as well as sediments, as a quantitative tracer for the $\delta^{15}N$ of euphotic zone NO_3 and NO_3 utilization efficiency (e.g., Altabet et al., 1999 Deep-Sea Res II 46:655-679; Altabet & Francois, 2001 Deep Sea Res II 48:4247-4273; Karl et al., PNAS 109: 1842-1849; Galbraith et al., 2013 Nature Geoscience 6: 579-584, to cite but a few). Moreover, our use of AA-specific $\delta^{15}N$ of sinking particles explicitly accounts for degradation (i.e., via the ΣV parameter) based on known patterns of microbially-mediated isotope fractionation (e.g., McCarthy et al., 2007; Chikaraishi et al., 2009, McMahon and McCarthy, 2016, Ohkouchi et al., 2017; Yamaguchi et al., 2017). To clarify this, we have rewritten the original section 4.2 with a new section 4.3 on "Preservation of AA-specific isotope signals" that outlines our arguments for the preservation of primary $\delta^{15}N$-AA signals.
Third, regarding the use of end-member data from the literature: unfortunately, the analytical demands of CSIA made it logistically and financially impossible to reproduce endmember $\delta^{15}N$-AA signatures for different geographic areas of interest. However, our use of literature data (which is now better explained in response to comment 2 above) follows an established precedent (Doherty et al. 2021; Wojtal et al. 2022; Golombek et al. 2024). Specifically, normalization to the baseline proxy $\delta^{15}N$-Phe accounts for regional differences in baseline $\delta^{15}N$. The Phe-normalized $\delta^{15}N$-Ala and $\delta^{15}N$-Thr signatures arise from differences in metabolic pathways and are independent of any region-specific biogeochemistry. Note also that our direct measurements of copepods and previous measurements of sea ice algae and pelagic algae (Fig. 7d) verified the accuracy of the model that was built on literature data. Nevertheless, we recognize that an expanded library of endmember data would indeed by desirable and have acknowledged this in the revised manuscript on LN 606.

4. Overall, some of the data in this paper does not support the conclusions very strongly, and some of the less definitive conclusions considered can be left out of the discussion. For example, the analysis of organic composition in 4.2 is merely inferential and does not require a great deal of space for extensive discussion of a less than robust conclusion.
RESPONSE: We thank the reviewer for the constructive comments. Section 4.2 has been redrafted to make our arguments clearer. Unfortunately, we could not significantly shorten it without losing valuable contextual information.

Others:

Line 143-146: What is the depth of collecting the zooplankton with a multi-net plankton sampler? And '200-0 m layer' means from 200 to 0 m or at the depth of 200m?
RESPONSE: It means from 200 to 0 m. The net was opened at 200 m and pulled up all the way to the surface. This is now clarified on LN 144-145.

Line 257: It will be better to add ' 2017 ' to the left side of the abscissa in Figure 2, which is consistent with the following figure. The same applies to Figure 8.
RESPONSE: We made this change.

Line 309-311: I don't fully understand the sentence. The δ13C-EAA patterns of sinking particles were more similar to sea ice algae than to pelagic algae? Maybe you can express it in detail.
RESPONSE: We tested the difference in individual EAA between sinking particles and sea ice algae and pelagic algae. Between sinking particles and sea ice algae, only one EAA showed significantly different δ13C, whereas three EAAs were significantly different between sinking particles and pelagic algae. We elaborated the details in the revised sentence (LN 331-333).

Line 627: Please check your reference format again. For example, the name of the journal (e.g., Annual review of marine science, Limnology and oceanography, Global change biology) should be capitalized, consistent with other references in this article. And the format of cited reference in line 672-673 is wrong. You need to modify and check again.
RESPONSE: Thank you for pointing this out. We double checked and corrected the reference list throughout.