This study, by Brittingham et al, investigates the influence of transport and depositional processes on sedimentary lipid biomarker records (leaf-wax n-alkanes and their Hydrogen and Carbon isotopic composition) by analyzing soil and stream sediments across a 1000 m altitude gradient spanning the closed deciduous forest, treeline ecotone and alpine meadow vegetation belts in a first-order catchment located in the Areguni Mountains, Armenia. Main results show that, while there is a major difference in the soil and stream n-alkane and their isotopic values above and below the treeline, stream sediment biomarkers below the treeline predominantly reflect local vegetation rather than upstream contributions. This finding is important for the interpretation of sediment biomarker records, in that it shows that processes at the level of the catchment must also be accounted for and may critically influence the distribution of different biomarker compounds in sediment archives.

The manuscript reads very well, aims are clear, the topic approached is relevant and addresses an important knowledge gap in the field of biomarker-based palaeoenviromental and palaeoclimate reconstructions. Some further clarifications are needed in the overall design of the study and interpretation of the results (details below), but most of these are minor. The main recommendation for the authors is to describe the study area more thoroughly, in a separate section, and provide information on temperature and precipitation patterns, geology and soil types (for example, are soils acidic?) and dominant vegetation species for each of the vegetation belts, because in my opinion this information is relevant for interpreting biomarker distribution. Then, I think it is important to show at least some of the more representative chromatograms (these may be even placed in the supplementary material). Also, there is an issue that, in my opinion, needs to be further expanded in the discussion: if the top 10 cm of soil were removed prior to subsampling for biomarker analysis (L. 116), this means that the collected soil samples likely do not represent modern vegetation, as soil takes a long time to form. Conversely, stream bed sediments may be of a more recent age compared to the soil samples, as stream beds are highly dynamic environments. How could this potential age discrepancy impact the results?

I therefore recommend the manuscript for publication, provided that these clarifications are addressed.

- We thank the reviewer for their helpful comments on the draft of this paper. Detailed responses to the points addressed by reviewer 2 can be found below.

Minor comments

Title: could be adjusted a bit, because it suggests a generalized conclusion, while the results are study-case based, and it is not clear to what extent these findings can be extrapolated to all high-altitude catchments.

- We will alter the title as follows: “Locally Produced Leaf Wax Biomarkers in the High-Altitude Areguni Mountains Outweigh Downstream Transport”
Introduction:

L.45-48 A word is missing from this sentence: “and Iran, ‘that’ supports a wide variety…”?

- We will make this change as suggested

L.49-54 The link between interpretation of palaeoclimatic records in the study region and the environmental signal of biomarkers in sedimentary archives is unclear. I suggest adding a sentence to explain why understanding processes involved in the sedimentary integration of biomarkers, and the scale of biomarker environmental signals are relevant for palaeoclimatic reconstructions.

- As a similar suggestion was also made by reviewer 1, we will add a section referencing previous biomarker work done in the region to highlight the importance of this work for paleoclimate reconstruction

L.64-72 Why are the carbon isotope values of C3 vs C4 plants relevant for the study area or for the aims of this study? E.g., were there major shifts in the importance of C3 vs C4 plants in the vegetation history of the area? Is the proportion of the C4 plants in the current vegetation increasing?

- This area does have a vegetation history which includes C4 vegetation in the Holocene (Tornero et al 2016), and roughly 3% of plant species in Armenia are C4 today (Rudov et al 2020). We will add references to these publications in the revised manuscript.

L.73 Same as in the previous comment, it is hard to grasp the relevance of hydrogen isotope values in leaf-wax n-alkanes for the study area or for the aims of this study.

- Since we include hydrogen isotope value measurements in this study, we believe that it is important to include some background information on what influences those values. Primarily the physiological drivers of fractionation by different plant
species, which we observe in this study with the above and below treeline vegetation.

L.83-86 Could you, please, summarize some of the key findings of the referenced publications, that are also relevant for this study?

- We briefly describe the key findings of these referenced publications later in the manuscript (L255-L258), however at the suggestion of both reviewers we will include a larger paragraph in the introduction to summarize the findings of these papers.

L.105 What proxies were used to assess the relationships between the past treeline and climate? Are there knowledge gaps that remained unaddressed and that are addressed within the present study?

- These previous studies included analysis of pollen, carbon and oxygen isotope values of herbivore tooth enamel, and biomarkers. This study is designed to address some of the interpretive gaps in Malinsky-Buller et al, 2021, in which we analyzed biomarkers in fluvial sediments deposited between 60-45 ka in this catchment.

L.108 Regarding the potential of sediments at Kalavan to reconstruct the treeline-climate relationship, I assume it refers to a biomarker-based reconstruction, because it is not clear. But then, why would biomarkers be the preferred proxy instead of more established proxies, like for example plant macro-remains and pollen? Justification needs to be a bit stronger here.

- We will make it more clear that we are referring specifically to biomarker integration processes. Plant macro-remains and pollen also are likely to be subject to uncertainty in their transportation processes, which remain unstudied in this environment, and therefore we believe biomarkers will provide a good proxy for treeline relationship in the Pleistocene sediments present in this region.

Methods:

I recommend the authors to begin with a subsection which describes the study area in terms of climate, geology, soil types and dominant vegetation species for the two main vegetation belts and the treeline ecotone. I also recommend the authors to create another section, that could be placed last, that collates the description of the statistical methods used (significance tests and mixture models).

- We will expand the background information provided in the methods section. Descriptions of the dominant vegetation in the area from Joannin et al 2022 and Volodicheva 2002 and the geological background from Malinsky-Buller et al 2022, 2024.

L.119-120 Please provide a reference for the Soxhlet procedure used for lipid extraction. What intrigues me is the relatively high proportion of methanol in the solvent mixture and the long extraction time.

L.121 Please specify what solvent or solvent mixture was used for n-alkane separation.

L.121-125 Please add details on: oven temperature, use of blanks to test for lab contamination, standards used for n-alkane quantification, method used for integration of peak areas etc.

- For this study, we followed previously published extraction and quantification protocols from Brittingham et al 2017 (Organic Geochemistry), Brittingham et al 2019 (Scientific Reports) and Smolen and Hren 2023 (Chemical Geology). We will include the details on solvents used for separation and quantification, as well as references to these papers.
- “n-alkanes were separated from total liquid extract by passing samples through a column of activated silica gel (1.25 g) in baked Pasteur pipettes with 2 mL hexane (non-polar fraction), 4 mL dichloromethane (slightly polar fraction) and 4 mL methanol (polar fraction). n-alkanes were quantified through the analysis of the hexane fraction. We quantified n-alkanes using a BP-5 column (30 m × 0.25 mm i.d., 0.25 μm film thickness) with He as the carrier (1.5 ml/min). Oven temperature was set at 50 °C for 1 min, ramped to 180 °C at 12 °C/min, then ramped to 320 °C at 6 °C/min and held for 4 min.”
There should be a reference to Bush and McInerney (2013) here


It would be great to see some of the most illustrative chromatograms added to the supplementary file. This would help the reader understand better the n-alkane distribution in different sets of samples.

We will add illustrative chromatograms in the supplementary material

We used student’s t-tests for our significance tests, we will include this in the revised draft of this paper.

We will make this change as recommended and add “n-alkane” in this section

In the revised submission, we will include results and tables from the mixing model. This will include sample and vegetation composition of the stream samples, and the endmember constants for each of the variables.

We will increase the references to relevant figures, both here and throughout the manuscript

Consider moving these paragraphs in a separate section of the methods, where you could also include information about the statistical tests used. But overall, I very much like the idea of using a mixing model to compare expected and obtained biomarker compound values.
• As per recommendations from both of the reviewers, we will expand this section to include more details of the mixing model. This will include sample and vegetation composition of the stream samples, and the endmember constants for each of the variables.

L.200 What does the phrase ‘tree and grass sediment’ refer to?

• Here we refer to the areas covered by forest and grassland. We will change the wording of this sentences for clarity.

L.210-212 Please reference the relevant figure for this statement.

• We will add references to figure 6 here

L.212 I assume ‘deciduous-sourced n-alkanes’ refers to deciduous trees, but it’s a bit ambiguous, because there are also deciduous herbaceous plants. Could you also include what the dominant deciduous species in the forest are?

• In order to make this clearer to the reader, we will refer to these as “n-alkanes sourced from below-treeline vegetation”. We will add a section in the background describing the vegetation structure and dominant vegetation types in the forest (Oak/Beech/Hornbeam)

L.228 Please include the distance from the study site of the lacustrine core that was analyzed for pollen.

• The lacustrine core described here is ~5 km from the study area, at an altitude of 1912 meters. We will add this information to the manuscript.

L.233-234 This general statement needs a reference.

• This statement is in reference to this study. We will re-word this sentence to make it clear that we are referring to the specific results from ACL values and hydrogen and carbon isotopes in this study.

L.261-263 As an additional research direction, perhaps collecting water samples for lipid analysis could help clarifying the role of transport and depositional processes.

• We appreciate the suggestion from the reviewer, this would be an interesting direction for future research

Figures: most of them are blurry and should be uploaded in a better resolution.

• We will re-upload the figures in higher resolution
Fig. 1 Could you, please, specify the source of the satellite images? Also, I would find it more relevant if the figure included a close-up of the studied catchment with sampling points superposed on vegetation types.

- We will include a more zoomed in map of the sampling locations, so that the sample points are easier to distinguish.

Figures 3-5. I assume the green and red rectangles are soil samples (although it is not clear, and also not colorblind-friendly). But it should also be clarified which of the stream samples (blue triangles) were taken from above and from below the treeline respectively.

- Though we refer to these in the figure caption, we will also include the ‘sediment’ label in the legend. We will also change the color of these symbols.

Figure 6. Please make it larger, and also increase the resolution, because the labels are hardly visible.

- We will make this change as recommended.