

# Reviewer 1

## General comments:

*My main comment on the manuscript is a request that the authors include information about their mixing model, including necessary equations and values used for constants, as a subsection in the methods. The values for constants could be added as a table or in the text. The additional text and equations would substantially clarify the discussion in section 4.2. and, more importantly, are necessary for evaluating the comparison between measured and expected values shown in Figure 6. Much of the necessary text can be moved from lines 194–208 to the methods section.*

- We agree with the reviewer, an additional section will be added describing the model in detail. We will include a figure detailing the boundaries of the different vegetation types in the catchment. This section will also include a table describing the endmembers used and the expected isotope values at each point in the catchment.

*I also strongly recommend appending the supporting data for this study as supplementary tables or as files uploaded to a publicly accessible database. The data may prove useful for future paleoclimate or palaeoecological work in this region, so it is important to make sure the data is accessible to those who may need it in the future.*

- A supplementary table will be added to the final draft, including isotope values, n-alkane distributions and sampling locations.

*Lastly, I recommend inserting more references to the figures within the text. As is, each figure is only referenced once or twice, even in sections where the data is discussed in detail. More figure references will make the text easier to follow.*

- We will refer to the figures more in the final draft

## Specific comments

### *Title*

*I suggest amending the title to better reflect the findings and conclusions of the study. As is, the title appears to suggest that the findings here are applicable to all high-altitude river (and lake?) catchments. However, this assertion is not made in the manuscript.*

*Two other things to point: (1) n-alkanes measured in this study should really be referred to as leaf wax or vegetation biomarkers since they are produced by plants, not produced by sediments; (2) I believe you mean downstream river transport, as in away from the river's headwaters. As written, the word upstream implies transport of sediment in the opposite direction of the water flow, which I do not think is what you mean.*

*A potential alternative title could be:*

*“Locally Produced Leaf Wax Biomarkers in the High-Altitude Lesser Caucasus Outweigh Downstream Transport”*

- We will alter the title to be more general, and change it as follows: “Locally Produced Leaf Wax Biomarkers in the High-Altitude Areguni Mountains Outweigh Downstream Transport”

### ***Abstract***

*Lines 18–22: these first two sentences are a bit repetitive. I suggest removing the first sentence that reads, “Sedimentary records...signals.” and starting the abstract with the second sentence that reads “The integration of...”. This still introduces the study effectively while making the language more succinct.*

- We will revise the abstract to combine these sentences: “Sedimentary records of lipid biomarkers such as leaf wax n-alkanes are not only influenced by ecosystem turnover and physiological changes in plants, they are also influenced by earth surface processes integrating these signals into the sedimentary record, though the effect of these integration processes are not fully understood.”

*Line 25: I suggest removing “the existence of” and just stating “We utilize a treeline...”*

- We will make this change

*Lines 35–36: Please clarify the language here, I am unsure what you mean by the “latter observation” since the language in lines 32–35 makes it seem like you do not have sufficient evidence to distinguish which mechanism is driving the pattern you observe in the stream sediments (this is also what I took away from reading the rest of the manuscript).*

- We will remove the ‘latter observation’; since either (1) or (2) would prevent n-alkanes from quantitatively reflecting tree line movement. This sentence will read “Though both of these observations...”

*Line 38: I am unclear what “changes in upstream fractionation differences” means here. I suspect you are talking about fractionation of leaf wax hydrogen isotope composition caused by changes in landscape vegetation, please clarify the language as this confused me a bit.*

- The reviewer is correct that this is in reference to fractionation of leaf wax hydrogen isotope values, to clarify we will change the sentence to read “ $\delta D$  values of biomarkers in fluvial deposits in these settings are more likely to record local hydrological changes rather than reflect fractionation changes due to turnover in upstream vegetation structure”.

### ***Introduction***

Line 45: Is “so called” necessary here?

- We will remove this

Line 49: I suggest adding a sentence here briefly summarizing any paleoclimate records from this region that utilize leaf wax n-alkanes if there are any as that will clarify the importance of this study to your readers.

- We will add references to previous studies in Armenia that have used leaf wax n-alkanes. To the best of our knowledge, the following papers are those that use plant waxes in the region:
  - Brittingham, Alex, Michael T. Hren, Gideon Hartman, Keith N. Wilkinson, Carolina Mallol, Boris Gasparyan, and Daniel S. Adler. "Geochemical evidence for the control of fire by Middle Palaeolithic hominins." *Scientific Reports* 9, no. 1 (2019): 15368.
  - Malinsky-Buller, Ariel, Philip Glauberman, Vincent Ollivier, Tobias Lauer, Rhys Timms, Ellery Frahm, Alexander Brittingham et al. "Short-term occupations at high elevation during the Middle Paleolithic at Kalavan 2 (Republic of Armenia)." *PLoS One* 16, no. 2 (2021): e0245700.
  - Glauberman, Phil, Boris Gasparyan, Jennifer Sherriff, Keith Wilkinson, Bo Li, Monika Knul, Alex Brittingham et al. "Barozh 12: Formation processes of a late Middle Paleolithic open-air site in western Armenia." *Quaternary Science Reviews* 236 (2020): 106276.
  - Malinsky-Buller, Ariel, Lotan Edeltin, Vincent Ollivier, Sébastien Joannin, Odile Peyron, Tobias Lauer, Ellery Frahm et al. "The environmental and cultural background for the reoccupation of the Armenian Highlands after the Last Glacial Maximum: The contribution of Kalavan 6." *Journal of Archaeological Science: Reports* 56 (2024): 104540.
  - Triguí, Yesmine, Daniel Wolf, Lilit Sahakyan, Hayk Hovakimyan, Kristina Sahakyan, Roland Zech, Markus Fuchs, Tilmann Wolpert, Michael Zech, and Dominik Faust. "First calibration and application of leaf wax n-alkane biomarkers in loess-paleosol sequences and modern plants and soils in Armenia." *Geosciences* 9, no. 6 (2019): 263.

Line 62: I suggest replacing “though” with “although”

- We will make this change

Line 64: I suggest rewriting as “The carbon isotope ( $\delta^{13}\text{C}$ ) composition of plant tissue is primarily set by the photosynthetic pathway of the plant.

- We will make this change

Line 72: Could you please comment on which of these factors is important for your study site? For example, are there  $C_4$  plants in the Caucasus?

- We will add the following sentence in order to describe the importance of C4 vegetation in the study: “Currently, C4 vegetation makes up around 3% of identified species in Armenia (Rudov et al 2020), and was present in the Kalavan region during the Holocene (Torneró et al 2016). “
  - Rudov, Alexander, Marjan Mashkour, Morteza Djamali, and Hossein Akhani. "A review of C4 plants in southwest Asia: an ecological, geographical and taxonomical analysis of a region with high diversity of C4 eudicots." *Frontiers in plant science* 11 (2020): 546518.
  - Torneró, Carlos, Marie Balasse, Adrian Bălăşescu, Christine Chataigner, Boris Gasparyan, and Cyril Montoya. "The altitudinal mobility of wild sheep at the Epigravettian site of Kalavan 1 (Lesser Caucasus, Armenia): Evidence from a sequential isotopic analysis in tooth enamel." *Journal of Human Evolution* 97 (2016): 27-36.

*Lines 83–86: I suggest rewriting this sentence to summarize the common findings of the studies you cite here. For example, I recall that the Feakins et al. 2018a study cited here found that riverine n-alkanes approximated area-weighted vegetation in the Amazon River catchment (the opposite of your finding, which highlights what makes your study interesting). Perhaps these other studies have found the same thing, or mixed results?*

- We will expand this paragraph, in order to describe briefly the results of these previous studies. This is in the discussion section of the manuscript currently, but we will move it to the introduction and expand this section.

*Lines 99–102: I suggest rewriting as “Comparison of the hillside and streambed sedimentary n-alkanes allows...”. The current wording is a bit repetitive and clunky.*

- We will make this change as suggested

*Lines 104–108: I suggest splitting into two sentences, revising to: “...Torneró et al., 2016). Pleistocene sediments...”*

- We will make this change as suggested

*Lines 118–119: I suggest removing the phrase “In order to extract n-alkanes” as it is repeating the end of the previous sentence.*

- We will make this change as suggested

*Lines 120–121: please describe the solvent schedule, water content of the silica gel, and quantity of silica gel used for chromatographic separation.*

*Line 123: please describe any internal and external standards used for sample quantification. Please clarify whether and how the different response factors of the C<sub>25</sub> – C<sub>33</sub>n-alkanes were accounted for during sample quantification or in calculation of the OEP and ACL values. This is important because, while the peak area of each compound is proportional to concentration, that*

*relationship (response factor) is mass-dependent and so is slightly different for each of the n-alkanes.*

- 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.”
  - Smolen, J. D., & Hren, M. T. (2023). Differential effects of clay mineralogy on thermal maturation of sedimentary n-alkanes. *Chemical Geology*, 634, 121572.
  - Brittingham, Alex, Michael T. Hren, Gideon Hartman, Keith N. Wilkinson, Carolina Mallol, Boris Gasparyan, and Daniel S. Adler. "Geochemical evidence for the control of fire by Middle Palaeolithic hominins." *Scientific Reports* 9, no. 1 (2019): 15368.

*Line 125: Did you forget to insert a citation here?*

- There should be a reference to Bush and McInerney (2013) here
  - Bush, Rosemary T., and Francesca A. McInerney. "Leaf wax n-alkane distributions in and across modern plants: Implications for paleoecology and chemotaxonomy." *Geochimica et Cosmochimica Acta* 117 (2013): 161-179.

*Line 136: How exactly did you correct for size effects? Also, I think you want to use the standard error of the mean, not standard deviation. In general, Polissar and D'Andrea (2011) [<https://doi.org/10.1016/j.gca.2013.12.021>] is an excellent guide on how to calculate uncertainties associated with leaf wax stable isotope measurements, particularly hydrogen isotopes.*

- We analyzed a stable isotope standard (MixA6 from A. Schimmelman) at a variety of sizes (5-30 V/s) in order to determine the relationship between peak size and measured hydrogen isotope values. We will report standard errors, rather than standard deviation, for the final manuscript.

## **Results**

*Line 144: could you please provide some example chromatograms or histograms showing the n-alkane distributions in your samples? This could be as a supplementary figure if you prefer.*

- We will include example chromatograms from stream sediments and soil samples, both above and below treeline as a supplement to the final submission.

*Lines 144–145: I suggest reporting the range of carbon preference index (CPI) values of your samples to support this statement.*

- Both OEP and CPI calculations describe the relative distribution of odd and even n-alkanes in a sample, and these measurements will always have a linear correlation with one another. We believe that odd over even predominance (OEP) should be sufficient to describe the odd over even predominance of the samples, instead of describing a redundant measurement as well (CPI)

*Line 149: please replace “averages” with “is”*

- We will make this change as suggested

*Lines 150–151: Please replace “average” with “mean”*

- We will make this change as suggested

*Lines 151–153: Please specify what kind of statistical test was done here and the N samples or degrees of freedom. Same goes for the test mentioned in lines 155–156.*

- The statistical test performed here was a Student’s t-test (n=30), we will specify this in the text

*Line 161: please replace “average” with mean*

- We will make this change as suggested

*Lines 170–171: does the word “significant” imply a statistical test was done here? If so, please specify what kind, the p-value, and N samples or degrees of freedom*

- We will remove the word “significant” in this sentence

*Lines 173–174: I see, this is what is referred to in 170–171. Please consider combining and condensing into a single sentence to clarify the language / keep the text concise.*

- With the removal of “significant” in the previous sentence this should help the reader

*Line 177: Please specify the N samples or degrees of freedom*

- We will include the number of soil samples in this test (n=30)

*Lines 178–179: Please revise to: “ $\delta D$  values were also more negative in stream sediment samples collected above the treeline (-175‰) than those collected below the treeline (-158‰).”*

- We will make this change as suggested

*Please also specify the statistical test, p-value, and N samples / degrees of freedom*

- We will include the number of stream sediment samples in this test (n=21)

*Line 180: Please revise to “...stream **sediment** samples...”*

- We will make this change as suggested

### **Discussion**

*Lines 194–208: as said in my general comments, please move these lines to a new section of the methods that includes the equations and constants used in the mixing model. In addition, please specify where the satellite images used for your mapping came from with appropriate references.*

- The constants used in this section derive from the mean values provided in the results (L204), we will expand this section and repeat those values to clarify for the reader. We will also include here a map showing the different vegetation types present in the catchment, and the location of the treeline

*Line 209: I recommend beginning your revised section 4.2. here. Also, I suggest replacing “this mixing model” with “our mixing model”*

- We will make this change as suggested

*Line 211: range of expected values for which measurement(s)? Please clarify.*

- In this study we measured  $\delta D$ ,  $\delta^{13}C$ , and ACL values. We will mention these measurements in this sentence.

*Line 212: Please reference a figure to back up this assertion*

- We will include a reference to figure 6 here

*Lines 213–214: Please also reference a figure here. Additionally, this is not really how ACL values (I assume that is what you are talking about here, please clarify) are used in practice. They tend to be thought of as a more qualitative indicator of vegetation / ecosystem composition. I would suggest rewriting this sentence to discuss that the n-alkane distributions do not show an expected “mixed” signal but are rather indistinguishable from the endmember values.*

- We thank the reviewer for this comment, which highlights the importance of this paper. ACL values are generally ‘thought of’ as a qualitative indicator, which we set to test in this natural catchment.

*Line 223: I suggest replacing “though” with “although”*

- We will make this change as suggested

*Lines 236–241: Please clarify the language here. I had to reread this section several times to understand what you mean.*

*Lines 245–254: this is a good idea, but the implementation here could use some improvement. More information is needed about how the lines in Figure 7 were calculated, as is the “expected”  $\delta D$  timeseries and the relative timing of the vegetation shift. It may be useful to expand this exercise into its own section in the Discussion with an accompanying short section in the Methods, but this is really just a suggestion.*

- We will rewrite these two paragraphs in order to highlight the importance of this study for understanding  $\delta D$  values in plant wax sedimentary archives.
- We will include more details on the thought experiment provided here. The expected  $\delta D$  values were calculated using mean growing season  $\delta D$  values in precipitation from the nearby meteorological station in Dilijan (published in Brittingham et al 2019) and the differences in mean  $\epsilon$  values from the above and below treeline vegetation. The timing of the vegetation shifts in this is hypothetical, and we will indicate on figure 7 where these vegetation shifts will occur.
  - Brittingham, Alex, Zarmandukht Petrosyan, Joseph C. Hepburn, Michael P. Richards, Michael T. Hren, and Gideon Hartman. "Influence of the North Atlantic Oscillation on  $\delta D$  and  $\delta^{18}O$  in meteoric water in the Armenian Highland." *Journal of Hydrology* 575 (2019): 513-522.

*Line 251: I suggest replacing “heavily affected” with “influenced”*

- We will make this change as suggested

*Lines 255–258: I think this is better suited to the Introduction. Please see my comment regarding lines 83–86.*

- After expanding this section in the introduction, we will remove this from this section of the paper

## **Conclusions**

No comments

## **References**



*Line 294: why is this centered and not aligned to the left? Also, should it not be bolded and called “references”?*

- We will make this change as suggested

*In general, please check the formatting of your citations and ensure that all article titles have proper typesetting (subscripts, superscripts, Greek characters, etc.), that journal names are italicized, and check for typos.*

## **Figures**

*In general, please ensure to upload 300 DPI or higher images or vector files of the images. The current images are a bit fuzzy.*

- We will make this change as suggested

*Figure 1: the points showing your sample sites in the right panel are very difficult to see. Could they be larger or a different shape? Perhaps a shape with a black border would help.*

- We will increase the size of the points, and increase the size of the black border around the shapes

*Figure 2: in the figure caption the samples are referred to as sediments while on the figure they are referred to as soils. Please make these consistent with each other. I also recommend switching to a colorblind friendly color palette (red-green is particularly hard to distinguish for many colorblind people).*

- We will change ‘soil’ in the figure legend to ‘sediment’ to be consistent with the captions on other figures.

*Figure 3: Please remove the lines. They really clutter up the figure and make it difficult to see the pattern that is very clearly evident in the data (really nice result!). Also, I recommend making this figure square to match the style of figure 2. Finally, please consider using three different symbol shapes and a colorblind-friendly color palette.*

- We will re-plot the data with difference symbols, color palette and remove the lines

*Figure 4: please also remove the lines here. Same comments as figure 3 regarding the color palette and symbol shapes. Also, please use  $\delta^{13}\text{C}$  for your y-axis label for consistency with Figure 2.*

- We will re-plot the data with difference symbols, color palette and remove the lines, and change the axis label

*Figure 5: please also remove the lines here. Same comments as figure 3 regarding the color palette and symbol shapes. Also, please use  $\delta D$  for your y-axis label for consistency with Figure 2.*

- We will re-plot the data with difference symbols, color palette and remove the lines, and change the axis label

*Figure 6: Could this figure be made a little larger? It is very hard to read in this small format. Also, the low DPI of the image is really evident here. Please be sure to replace with a higher quality image. I also recommend switching to a colorblind friendly color palette and using different symbol shapes for the different panels.*

- We will increase the DPI of this figure and change the color palette

Figure 7: please see my comment regarding lines 245–254 of the text

### **Technical Corrections**

*Line 34: Typo, shrubs is misspelled as “shurbs”*

*Line 47: Typo here, “supports” should be “supporting”*

*Line 78: Missing space between period and first word of following sentence “...(Gamarra et al., 2016).The fractionation...”*

*Line 172: Typo, “averages” should be “average”*

*Line 174: lower case delta is needed, not an upper case delta. It does not matter that this is the beginning of a sentence, the upper case delta means something different than the lower case delta and is not applicable here.*

*Lines 177–178: Typo here, sentence is divided by a random period*

- We will make the above changes as suggested