1   **Locally Produced Sedimentary Biomarkers in High-Altitude Catchments Outweigh**

2   **Upstream River Transport in Sedimentary Archives**

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Abstract: 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. The integration of biomarkers into the sedimentary record and the effects of integration processes on recorded environmental signals are complex and not fully understood. To determine the depositional constraints on biomarker records in a high-altitude small catchment system, we collected both soil and stream sediments along a 1000 m altitude transect (1500 – 2500 masl) in the Areguni Mountains, a subrange of the Lesser Caucasus Mountains in Armenia. We utilize the existence of a treeline at ~ 2000 masl, which separates alpine meadow above from deciduous forest below, to assess the relative contribution of upstream biomarker transport to local vegetation input in the stream. We find that average chain length (ACL), hydrogen isotope (δD) and carbon isotope ($\delta^{13}C$) values of *n*-alkanes are significantly different in soils collected above and below the treeline. However, samples collected from the stream sediments do not integrate these signals quantitively. As the stream drops below the treeline, the ACL, δD and $\delta^{13}C$ values of *n*-alkanes preserved in streambed sediments reflect a bias toward *n*-alkanes sourced from trees. This suggests that there is either 1) minimal transportation of organic matter from the more open vegetation in higher elevations, or 2) greater production of target biomarkers by trees and shurbs found at lower elevations results in overprinting of stream signals by local vegetation. Though this latter observation may preclude using *n*-alkanes to measure past treeline movement in these mountains, δD values of biomarkers in fluvial deposits in these settings are more likely to record local hydrological changes rather than changes in upstream fractionation differences associated with vegetation turnover.

1. Introduction

40

41 Mountain regions are important hubs for biodiversity and can provide refuge for a number

42 of endemic species of flora and fauna (Antonelli et al., 2018). However, these high-altitude

43 environments are often particularly vulnerable to climate change (Guisan and Theurillat, 2000).

44 Therefore, gaining an understanding of sensitivity of these regions to past climate change is

45 important for projecting the effects of future climate change on fragile ecosystems. The so called

46 Caucasus Region in particular has been identified as a biodiversity hotspot covering the Republics

47 of Armenia, Georgia, Azerbaijan, and parts of the Russian Federation, Türkiye, and Iran, supports

48 a wide variety of plant and animal species (Zazanashvili, 2009; Gasparyan and Glauberman, 2022).

49 To better understand climate and environmental change in both the past and the present, it is

50 necessary to refine our understanding and interpretation of paleoclimate records in this region.

51 Specifically, we are interested in understanding the sedimentary processes involved in the

52 formation, transport, recycling, and accumulation of organic biomarkers in sedimentary archives

53 and assessing whether these archives record a local environmental signal or are a mix of local and

54 transported organic material.

55 Normal alkanes (*n*-alkanes) are an important component of the epicuticular wax in

56 terrestrial plants. This waxy coating on plants protects against ultraviolet damage, water loss and

57 predation (Jetter et al., 2006). Specific compounds in this wax, such as *n*-alkanes, are a useful tool

58 for reconstructing past environmental changes through the analysis of the distribution of alkane

59 homologues as well as their stable hydrogen (δD) and carbon ($\delta^{13}C$) isotope values. Previous

60 research in the Greater and Lesser Caucasus Mountains has documented the applicability of the

61 average chain length (ACL) of leaf wax biomarkers as a tool for differentiating between grassy

62 and deciduous vegetation (Bliedtner et al., 2018; Trigui et al., 2019), though on a global scale ACL

63 does not differentiate well between vegetation types (Bush and McInerney, 2013).

64 The biggest driver of the carbon isotope (δ^{13}C) values of plant tissue is the photosynthetic

65 pathway of the plant (Diefendorf and Freimuth, 2017). C_3 plants, which thrive in areas with cooler

66 growing season temperatures, have more negative δ^{13}C values than do C_4 plants, which thrive in

67 warmer growing season temperatures (Ehleringer et al., 1977). C_3 vegetation is further influenced

68 by water use efficiency, as water stress influences the c_i/c_a ratio of plants (Farquhar et al., 1982).

69 δ^{13}C values in lipids generally follow the same trends, and C_3 plants have more negative δ^{13}C lipid

70 values than C_4 plants (Diefendorf and Freimuth, 2017). However, carbon fractionation of lipids is

71 not consistent in different classes of plants (Pedentchouk et al., 2008; Sikes et al., 2013; Diefendorf

72 et al., 2011).

73 The hydrogen isotope (δD) values of *n*-alkanes in terrestrial plants record the δD values of

74 environmental water (Sachse et al., 2012). This is typically reflective of δD values in precipitation,

75 though precipitation δD values can also undergo positive shifts due to soil evaporation. The δD

76 values of plant waxes are also influenced by fractionation during biological synthesis of lipids,

77 which imparts a strong negative fractionation on δD values, as well as transpiration of leaf water

78 (Gamarra et al., 2016).The fractionation between meteoric water and lipids is typically larger in

79 gymnosperms than in angiosperms (Pedentchouk et al., 2008; Oakes and Hren, 2016).

80 Despite the benefits in measuring δD and δ^{13}C values in *n*-alkanes for understanding

81 environmental and hydrological processes, not all the processes modifying isotope values from

82 plant to *n*-alkane deposition are well understood. Sedimentary integration is one of the most poorly

83 understood aspects of this process (Sachse et al., 2012). A number of studies on the integration of

84 leaf waxes in catchments have been published in recent years which help clarify these processes

85   (Alewell et al., 2016; Feakins et al., 2018a; Häggi et al., 2016a; Hemingway et al., 2016; Ponton

86   et al., 2014; Suh et al., 2019). However, most of these studies have focused on large river systems

87   rather than first order streams. Thus, the sedimentary processes involved in the formation,

88   transport, recycling, and accumulation of organic biomarkers in first and second order streams are

89   not well understood. One challenge in assessing these processes in small streams is that the

90   environment and plant communities are often homogenous, and thus it is not possible to

91   differentiate between local and upstream transported organic material. To better understand

92   transport processes affecting organic material in small catchments, we studied a set of streams in

93   the Dany River, a tributary of the Barepat River, located in the Areguni Mountains in the Lesser

94   Caucasus Range. This stream system is divided into two distinct ecological regions by the treeline

95   (at ~ 2000 masl), which separates alpine meadow above the tree line (2000 – 2500 masl) from

96   deciduous forest below (1500 – 2000 masl). To evaluate the input of *n*-alkanes from upstream

97   transported organic material relative to vegetation near the stream, we collected soil samples on

98   the slopes of the mountains from both above- and below the treeline throughout the watershed and

99   sediments deposited in the streambed along an elevation transect. Comparing the hillslope

100   sedimentary biomarkers and the streambed sedimentary biomarkers allows assessment of the input

101   of *n*-alkanes locally produced by vegetation compared to those transported in stream sediments

102   within the catchment.

103   An additional motivation of this research is that treelines are a vulnerable feature of higher

104   altitude environments. Previous research in the Areguni Mountains study area has assessed the

105   relationship between treeline dynamics and climate forcing in the past (Ghukasyan et al., 2010;

106   Montoya et al., 2013; Malinsky-Buller et al., 2021; Tornero et al., 2016), and the Pleistocene

107   sediments uncovered at archaeological sites at Kalavan village within this area have the potential

108   to reconstruct this relationship. However, in order to reconstruct these systems in the past it is

109   important to understand modern biomarkers integration processes in the first and second order

110   streams and their potential effects on the sedimentary archives of the Areguni Mountains.

111   **2. Methods**

112   **2.1 Sample Collection and Extraction**

113   Hillslope soil samples were collected in September 2018 along an altitude transect (1500 – 2500

114   masl) above the Dany River watershed, a first order tributary of the Barepat River in the Areguni

115   Mountains, Armenia (Fig 1), which traverses the treeline at ~2000 masl. Soil samples were

116   collected by first clearing the top ~10 cm of soil to remove roots. Stream bed sediment samples

117   were collected from the Dany River throughout the altitude transect at intervals of ~100 m in

118   altitude. In all cases, roughly 100 g of sediment were collected for extraction of *n*-alkanes. In order

119   to extract *n*-alkanes, samples were extracted using a Soxhlet apparatus with 2:1

120   dichloromethane:methanol for 48 hours. Following lipid extraction, *n*-alkanes were separated with

121   silica gel column chromatography and quantified on a Thermo-Scientific Trace GC Ultra

122   (Manufacturer) fitted with a split–splitless (SSL) injector and flame ionization detector (FID) using

123   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).

124   Odd over even predominance (OEP) (Eq. 1) and average chain length (ACL) (Eq. 2) were used to

125   evaluate distributions of *n*-alkanes (REF). We also calculated P_{aq}, an *n*-alkane proxy to evaluate

126   the possible biomarker contribution of aquatic and emergent plants (Eq. 3) (Ficken et al., 2000).

127    $$OEP = \frac{C_{25} + C_{27} + C_{29} + C_{31} + C_{33}}{C_{24} + C_{26} + C_{28} + C_{30} + C_{32}}$$

128    $$ACL = \frac{25 * C_{25} + 27 * C_{27} + 29 * C_{29} + 31 * C_{31} + 33 * C_{33}}{C_{25} + C_{27} + C_{29} + C_{31} + C_{33}}$$

129    $$\text{Paq} = \frac{C_{23} + C_{25}}{C_{23} + C_{25} + C_{29} + C_{31}}$$

130   **2.3 Stable Isotope Analysis**

131   δD and $\delta^{13}C$ values of individual *n*-alkanes were measured with a Thermo GC-Isolink coupled

132   with a Thermo Scientific MAT 253 (manufacturer) isotope ratio mass spectrometer with a BP-5

133   column (30 m × 0.25 mm i.d., 0.25 μm film thickness). Oven temperature was set at 50°C for 1

134   min, ramped to 180°C at 12°C/min, then ramped to 320°C at 6°C/min and held for 4 min. Internal

135   standards (Mix A5 from A. Schimmelman) were run every four samples across a range of

136   concentrations to correct for size effects. Standard deviations were 0.5‰ for $\delta^{13}C$ and 4‰ for δD.

137   Isotope ratios (R) were converted to δX ($\delta^{13}C$ and δD) values (Eq. 3) and are expressed in permill

138   (‰).

139    $$\delta X = \left(\frac{R_{Sample}}{R_{Standard}} - 1\right) * 1000$$

140

141   **3. Results**

142   **3.1. Alkane abundances**

143   The most abundant alkane homolog in samples collected in the Areguni Mountains is the

144   C_{29} or C_{31} alkane, which is typical for terrestrial plants. Odd numbered alkanes are significantly

145   more abundant than even numbered alkanes, and the OEP of all samples averages 11.2, with a

146   range from 7.4-18.4. There is no significant difference between the mean OEP of soil (11.1) and

147   stream (11.3) samples in the watershed. These values are similar to those previously measured in

148   the Greater and Lesser Caucasus Mountains (Trigui et al., 2019; Bliedtner et al., 2018).

149   The mean average chain length (ACL) of all samples averages 29.7, with a range from 28.4

150   to 31.8 (Fig 3). In soils above the treeline, the average ACL value is 30.6 (range of 29.8-31.8). In

151   soils below the treeline, the average ACL value is 29.5 (range of 28.4-30.4). There is a significant

152   difference between the average ACL values of the above treeline and below treeline soils

153   ($p < 0.001$). Stream sediment above the treeline has an average ACL value of 29.7 (range of 29.1-

154   30.2) and stream sediments below the treeline have an average ACL value of 29.3 (range of 28.6

155   to 30.0). The stream sediments from below the treeline have a significantly ($p < 0.001$) lower

156   average ACL value than those above the treeline.

157   The P_{aq} values of *n*-alkanes in these samples suggests a mostly terrestrial origin of the

158   organic matter. Higher P_{aq} values indicate contributions of floating and emergent macrophytes.

159   However, we do not find a significant difference between the P_{aq}values in the stream sediments

160   when compared to the soil samples, indicating that the organic load of the stream sediments is

161   mostly of terrestrial origin. Terrestrial plants have average P_{aq}values of 0.09, with emergent plants

162   averaging 0.25 (Ficken et al., 2000). Only eight of the 51 samples in this study had P_{aq}values above

163   0.20, four stream and four soil samples. This indicates that there was not a significant contribution

164   of aquatic plants in the Dany stream sediments, and the biomarker load is primarily terrestrial in

165   origin.

166   **3.2. δD and δ^{13}C values**

167   The δ^{13}C values in soils and stream sediments collected from the Areguni Mountains reflect

168   a C$_3$ landscape, which is typical in Armenia. δ^{13}C values in all samples ranged from -36.0 to -

169   32.3‰ (Fig 4). The range is similar for both soil samples (-35.9 to -32.3‰) and stream samples (-

170   36.0 to –32.5‰). However, there is a significant difference in the δ^{13}C values of above and below

171   treeline samples, both in the stream and soil samples collected. Above the treeline, δ^{13}C values in

172   soils averages -34.9‰, and below the treeline soil alkanes average -33.3‰. Stream sediment $\delta^{13}C$

173   values average -35.0‰ above the treeline and -33.6‰ below the treeline. In both cases, these

174   values are significant (p<0.0001, student's t-test). $\Delta^{13}C$ values in stream samples exhibit a step-

175   like behavior, with ~2‰ shift to more negative values as the stream drops below the treeline.

176   The δD values measured in soil samples collected in the catchment ranged from -144 to -

177   185‰ (Fig 5). These values were significantly (p<0.001, student's t-test). More negative in above

178   treeline sediments (-175‰) than in below treeline sediments (-156‰). This is also true in sediment

179   collected from stream samples, which are significantly more negative above the treeline (-175‰)

180   than below the treeline (-158‰). As with the $\delta^{13}C$ values, the δD values of stream samples show

181   sudden change as the stream drops below the treeline.

182   **4. Discussion**

183   **4.1 Integration of local and upstream soil *n*-alkanes into the river sediments**

184   The hillslope soil leaf wax δD, $\delta^{13}C$ and ACL show a step-like change at the treeline,

185   indicating a significant separation between upstream (above treeline) and downstream (below

186   treeline) soils. Using this separation, it is possible to assess the contributions and integration of

187   upstream vs. downstream soils to the streambed sediments along the altitude transect. The step-

188   like transition in streambed δD and $\delta^{13}C$ values indicates an over-printing of upstream alkane

189   isotope values by input from deciduous vegetation. Thus, local production largely outweighs

190   upstream transport in this setting. However, to firmly evaluate the upstream and downstream

191   hillslope soil contribution to streambed sediments, there is a need to quantitatively evaluate the

192   area-weighted production of *n*-alkanes above and below the treeline.

193   **4.2. Modeling *n*-alkane production and estimating upstream transport and integration**

194   To further evaluate the integration of *n*-alkanes above and below the treeline, we created a

195   mixing model that calculates the expected δD, δ¹³C and ACL values at each one of the sampling

196   locations based on the *n*-alkane production of hillslope sediments above each streambed sampling

197   point (Fig. 6). This mixing model assumes that the *n*-alkanes in the river are a function of the

198   weighted *n*-alkane production above the sampling location.

199   The parameters we used for this mixing model are: 1. Satellite images to map the areas of

200   tree and grass sediment throughout the Dany River catchment. 2. An estimate of net primary

201   productivity of organic material production in grasses and trees (grams per area) (Brun et al.,

202   2022). 3. Estimates of *n*-alkane production in grasses and trees in the Greater and Lesser Caucasus

203   Mountains (grams of *n*-alkane per gram of organic material) (Trigui et al., 2019; Bliedtner et al.,

204   2018). 4. End member values of δD, δ¹³C and ACL derived from the average hillslope soils above

205   and below the treeline. By multiplying these terms (area x organic mass production x *n*-alkane

206   production x end member soils value), we created an *n*-alkane production map for the Dany River

207   catchment. Using this map, we calculated, for each riverbed sampling location, the amount of grass

208   and tree *n*-alkanes produced on the hillslopes above the sampling locations.

209   We compared the results of this mixing model with the measured δD, δ¹³C and ACL in the

210   streams. Stream sediment samples collected above the treeline (from ~2000-2600 masl) fall within

211   the range of expected values, however, samples below the treeline consistently over-sample

212   deciduous-sourced *n*-alkanes. Measured values do not have a linear relationship with the expected

213   values based on vegetation area. These measured values would produce under-estimates of the

214   upstream area of alpine grasses, yielding incorrect reconstructions of paleo-vegetation in

215   sedimentary records. Comparing the mixing model with the observations indicates that an area-

216   weighted mixing process is not an adequate model for explaining the *n*-alkanes signal in the

217   streambed sediments. A simple and straightforward way to interpret this discrepancy is that an

218   area-weighted quantitative integration of *n*-alkanes is not a good model for describing this

219   catchment system, and that local production is much larger than transported organic material.

220   However, there are still other factors that may be driving this process that our mixing model

221   does not account for. First, the average slope of forested areas in the Dany watershed is higher than

222   those in grassy areas. These steeper slopes would cause more sediment transport into the stream

223   bed. Second, though production of *n*-alkanes in grasses and trees is not significantly different in

224   the Greater and Lesser Caucasus Mountains, concentrations are higher in soils in deciduous areas

225   (Trigui et al., 2019; Bliedtner et al., 2018). This retention of more biomarkers in forest soils would

226   also increase the contribution of deciduous alkanes into the stream bed. Third, stream downcutting

227   into older sediments has the potential to re-mobilize stored organic carbon, which may contain a

228   greater load of deciduous *n*-alkanes. However, analysis of pollen from a nearby lake core in the

229   Areguni Mountains shows a gradual shift over the last 4000 years from a grass-dominated

230   landscape to the deciduous forest present today (Joannin et al., 2022). Therefore, stored biomarkers

231   are more likely to be grass-dominant, and this is unlikely to explain the measured bias to deciduous

232   alkanes.

233   Since *n*-alkanes in this first order stream do not quantitively integrate *n*-alkanes based on

234   the upstream area of different vegetation types, this likely precludes the use of *n*-alkanes as a tool

235   to reconstruct vertical treeline movement in this setting. However, this is a benefit for attempts to

236   reconstruct hydrological changes through the analysis of δD values in *n*-alkanes. Given the ~20‰

237   difference in apparent fractionation (ε) values for above and below treeline sediments, changes in

238   upstream vegetation cover would alter measured δD values in *n*-alkanes in sedimentary archives.

239   Without this quantitative integration, *n*-alkanes measured in the Pleistocene sediments found in

240   this watershed are more likely to reflect changes in δD values of precipitation, and therefore would

241   serve to reconstruct hydrological cycles, rather than changes in upstream vegetation cover. Since

242     $δ^{13}C$ and ACL of *n*-alkanes are also different in above and below treeline sediments, these other

243   analyses would also be useful to identify periods with large changes in treeline that might

244   complicate interpretation of δD values.

245   In order to illustrate this point, we present hypothetical records of biomarker δD values

246   from three points in the Dany watershed (Fig. 7) documenting 20‰ and 30‰ shifts in precipitation

247   δD values. Given the lack of quantitative integration in the catchment, a paleoclimate record from

248   either above (A) or below (C) treeline would record the shift in precipitation δD values. Below

249   treeline sedimentary records, with stream organic biomarker load overprinted by local vegetation

250   production, would likely provide a means to reconstruct the δD precipitation values. However,

251   records near the treeline (B) would be heavily affected by changes in apparent fractionation values

252   associated with changes in vegetation around the stream. Co-occurring climate forcing of shifts in

253   δD values of precipitation and changes in treeline altitude would cause paleoclimate records in this

254   zone to over-estimate the magnitude of precipitation δD value shifts.

255   Previous studies on the integration of organic biomarkers has produced mix results, with

256   some demonstrating spatial integration of catchment signals (Alewell et al., 2016; Hemingway et

257   al., 2016; Feakins et al., 2018b), whereas others did not observe this (Häggi et al., 2016b; Ponton

258   et al., 2014). However, these previous studies typically focused on very large river systems, which

259   will undergo different transport processes than the first-order streams analyzed in this study. A

260   number of these studies (Alewell et al., 2016; Hemingway et al., 2016; Ponton et al., 2014; Feakins

261   et al., 2018b) also observed seasonal differences in biomarker load in river sediments. Collecting

262   seasonal samples in the Areguni Mountains, as well as testing these processes in other first-order

263   streams, could further help clarify the transport processes measured in this setting.

264

265

266   **5. Conclusion**

267   Sediment and stream samples from the Areguni Mountains, a subrange of the Lesser Caucasus

268   Mountains in Armenia, demonstrate that there is a significant difference in hillslope soil δD, $\delta^{13}C$

269   and ACL values above and below treeline. *n*-alkanes in sediments in the Areguni Mountains can

270   be used to differentiate between the above and below treeline sediments. However, *n*-alkanes

271   extracted from stream sediments reflect their local area, rather than demonstrating transport from

272   the higher-altitude alpine meadow. These results provide a complication for attempts to reconstruct

273   changes in past treeline in this mountain range, given that the biomarker load in stream does not

274   reflect the relative area of different upstream vegetation types. However, these results simplify

275   interpretation of past *n*-alkane δD values, as apparent fractionation differences between grasses

276   and trees are less likely to impart a significant influence on δD values in stream bed *n*-alkanes.

277   6. Competing interests

278   The contact author has declared that none of the authors has any competing interests

279   7. Acknowledgements

293

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294   Works Cited

295

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420   Figure Captions

421   Figure 1: (Left) Topographic map of Armenia with inset map of sampling location (black box)
422   (Right) Inset map of soil (yellow circles) and stream (blue circles) samples collected in the
423   Areguni Mountains, along with the limit of the Barepat (dashed line) and Dany watersheds (solid
424   line)

425   Figure 2: The δD and δ¹³C values of *n*-alkanes extracted from above treeline (green squares) and
426   below treeline (red triangles) sediments

427   Figure 3: The average chain length (ACL) values of *n*-alkanes extracted from above treeline
428   (green squares) and below treeline (red squares) and stream (blue triangles) sediments across the
429   sampling elevation gradient

430   Figure 4: The δ¹³C values of *n*-alkanes extracted from above treeline (green squares) and below
431   treeline (red squares) and stream (blue triangles) sediments across the sampling elevation
432   gradient

433   Figure 5: The δD values of *n*-alkanes extracted from above treeline (green squares) and below
434   treeline (red squares) and stream (blue triangles) sediments across the sampling elevation
435   gradient

436   Figure 6: Comparison of the measured ACL, δD and δ¹³C values against expected values of
437   stream sediments. Dashed line represents the range of expected values from stream sediments if
438   vegetation was integrated equally by area

439   Figure 7: A photograph of the Dany watershed with hypothetical paleoclimate record from three
440   locations: (A, dashed line) Below treeline, (B, solid line) near treeline with fluctuations in
441   treeline altitude, and (C, dotted line) above treeline

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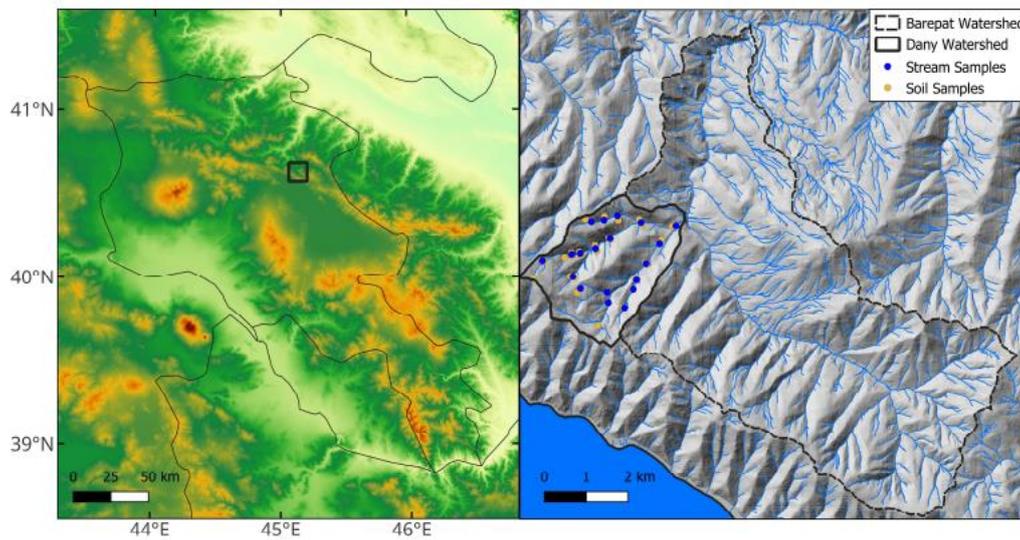
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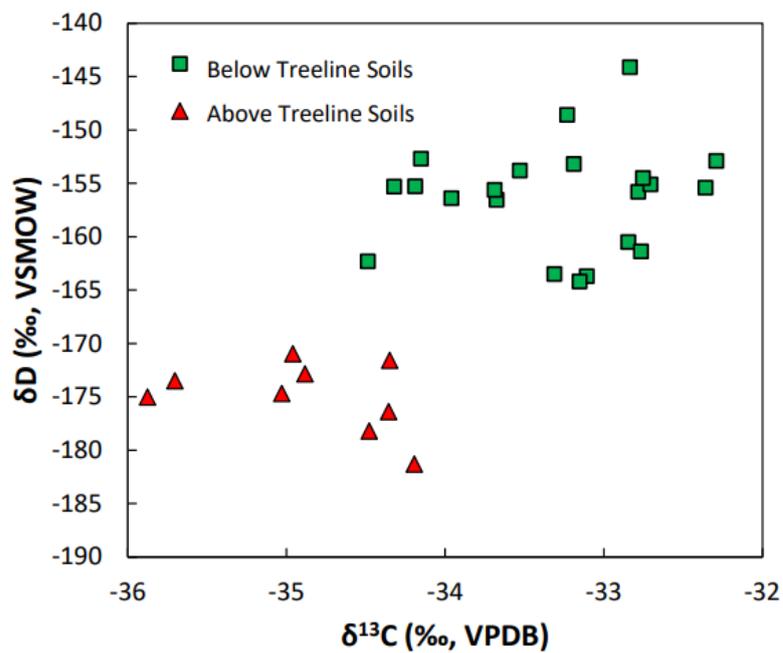
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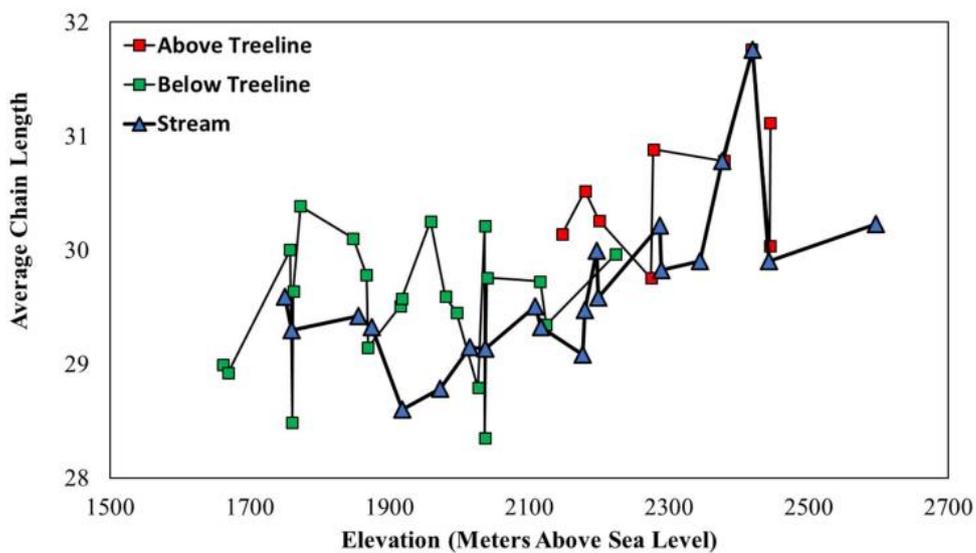
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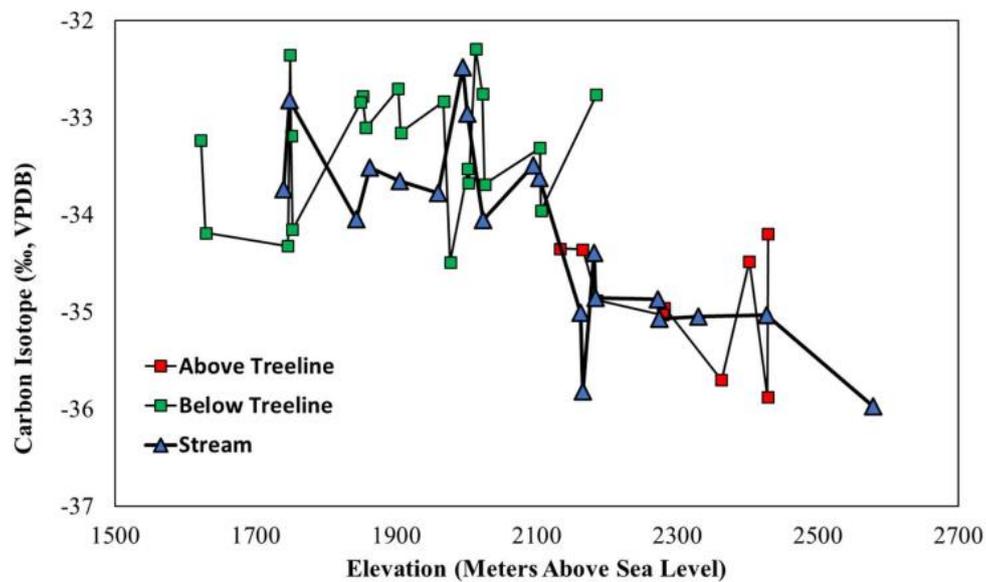
Figure 4: The $\delta^{13}C$ values of *n*-alkanes extracted from above treeline (green squares) and below treeline (red squares) and stream (blue triangles) sediments across the sampling elevation gradient

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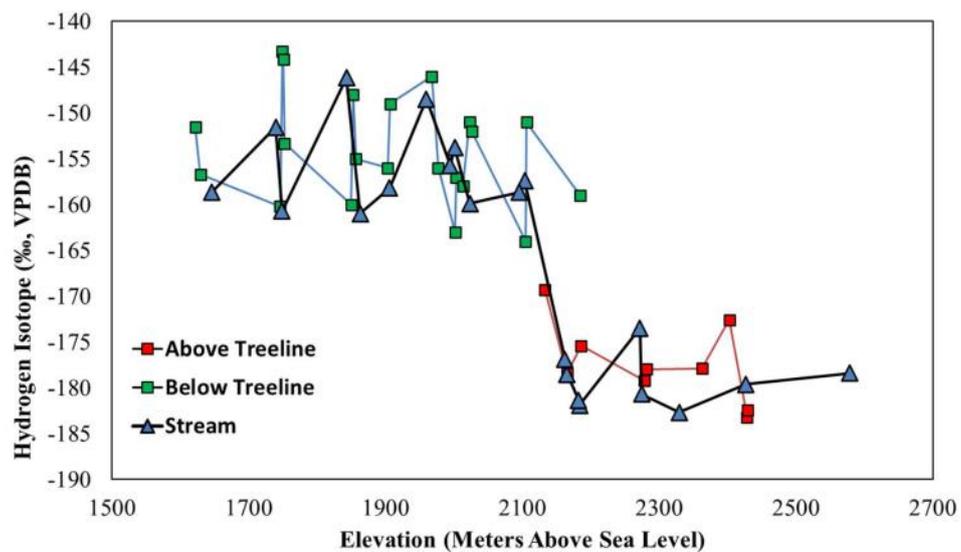
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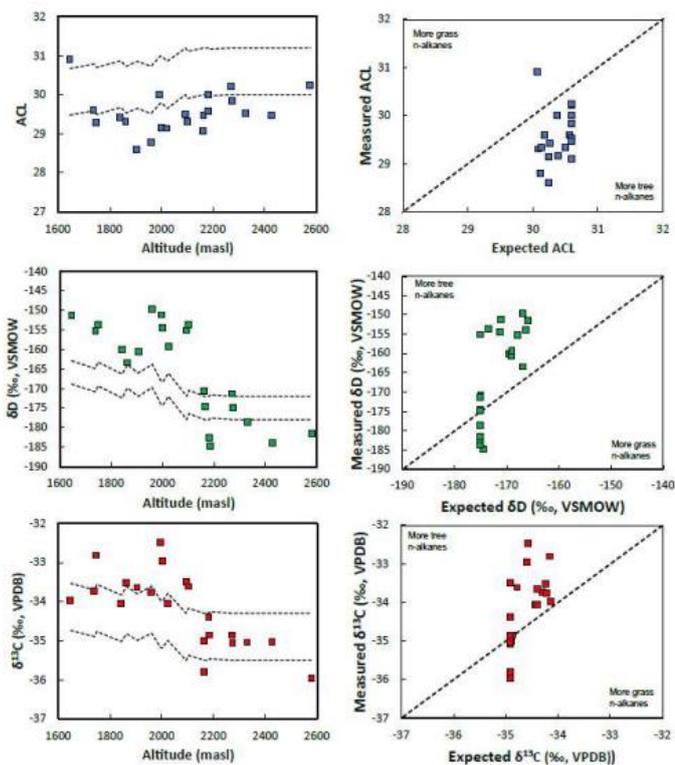
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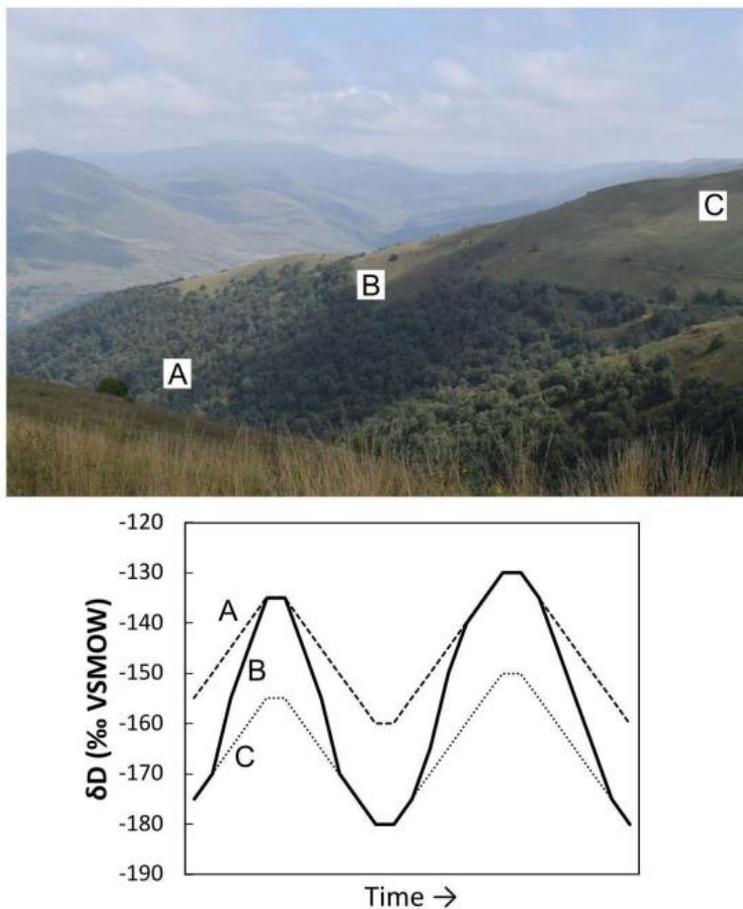
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