1 **Locally Produced ~~Sedimentary~~ Leaf Wax Biomarkers in the High-Altitude ~~Catchments~~**

2 **Areguni Mountains Outweigh ~~Upstream~~ Downstream River TTransport ~~in Sedimentary~~**

3 ~~**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 into the sedimentary record, though the effect of these integration processes are not fully understood. ~~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~~shrubs found at lower elevations results in overprinting of stream signals by local vegetation. Though thes~~is~~e ~~latter~~ observations 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

42 hydrological changes rather than reflect fractionation changes due to turnover in upstream

43 vegetation structure. changes in upstream fractionation differences associated with vegetation

44 turnover.

45

1. Introduction

Mountain regions are important hubs for biodiversity and can provide refuge for a number of endemic species of flora and fauna (Antonelli et al., 2018). However, these high-altitude environments are often particularly vulnerable to climate change (Guisan and Theurillat, 2000). Therefore, gaining an understanding of sensitivity of these regions to past climate change is important for projecting the effects of future climate change on fragile ecosystems. The ~~so called~~ Caucasus Region in particular has been identified as a biodiversity hotspot covering the Republics of Armenia, Georgia, Azerbaijan, and parts of the Russian Federation, Türkiye, and Iran, ~~supports~~ that supports a wide variety of plant and animal species (Zazanashvili, 2009; Gasparyan and Glauberman, 2022). To better understand climate and environmental change in both the past and the present, it is necessary to refine our understanding and interpretation of paleoclimate records in this region. Plant wax biomarkers have been used in this region in both geological and archaeological contexts to reconstruct past climates, therefore understanding modern variability and transport processes will help refine these interpretations (Brittingham et al., 2019; Glauberman et al., 2020; Malinsky-Buller et al., 2021, 2024; Trigui et al., 2019). Specifically, we are interested in understanding the sedimentary processes involved in the formation, transport, recycling, and accumulation of organic biomarkers in sedimentary archives and assessing whether these archives record a local environmental signal or are a mix of local and transported organic material.

Normal alkanes (*n*-alkanes) are an important component of the epicuticular wax in terrestrial plants. This waxy coating on plants protects against ultraviolet damage, water loss and predation (Jetter et al., 2006). Specific compounds in this wax, such as *n*-alkanes, are a useful tool for reconstructing past environmental changes through the analysis of the distribution of alkane homologues as well as their stable hydrogen (δD) and carbon ($\delta^{13}C$) isotope values. Previous

research in the Greater and Lesser Caucasus Mountains has documented the applicability of the average chain length (ACL) of leaf wax biomarkers as a tool for differentiating between grassy and deciduous vegetation (Bliedtner et al., 2018; Trigui et al., 2019a), although on a global scale ACL does not differentiate well between vegetation types (Bush and McInerney, 2013a).

The biggest driver of tThe carbon isotope (δ^{13}C) values of plant tissue is primarily determined by the photosynthetic pathway of the plant (Diefendorf and Freimuth, 2017). C$_3$ plants, which thrive in areas with cooler growing season temperatures, have more negative δ^{13}C values than do C$_4$ plants, which thrive in warmer growing season temperatures (Ehleringer et al., 1977). C$_3$ vegetation is further influenced by water use efficiency, as water stress influences the c_i/c_a ratio of plants (Farquhar et al., 1982). δ^{13}C values in lipids generally follow the same trends, and C$_3$ plants have more negative δ^{13}C lipid values than C$_4$ plants (Diefendorf and Freimuth, 2017). However, carbon fractionation of lipids is not consistent in different classes of plants (Diefendorf et al., 2011; Pedentchouk et al., 2008; Sikes et al., 2013). Currently, C$_4$ vegetation makes up around 3% of plant species in Armenia (Rudov et al., 2020), and was present in the Kalavan region during the Holocene (Tornero et al., 2016).

The hydrogen isotope (δD) values of *n*-alkanes in terrestrial plants record the δD values of environmental water (Sachse et al., 2012). This is typically reflective of δD values in precipitation, though precipitation δD values can also undergo positive shifts due to soil evaporation. The δD values of plant waxes are also influenced by fractionation during biological synthesis of lipids, which imparts a strong negative fractionation on δD values, as well as transpiration of leaf water (Gamarra et al., 2016).The fractionation between meteoric water and lipids is typically larger in gymnosperms than in angiosperms (Oakes and Hren, 2016; Pedentchouk et al., 2008).

Despite the benefits in measuring δD and $δ^{13}C$ values in *n*-alkanes for understanding environmental and hydrological processes, not all the processes modifying isotope values from plant to *n*-alkane deposition are well understood. Sedimentary integration is one of the most poorly understood aspects of this process (Sachse et al., 2012). A number of studies on the integration of leaf waxes in catchments have been published in recent years which help clarify these processes (Alewell et al., 2016; Feakins et al., 2018; Häggi et al., 2016; Hemingway et al., 2016; Ponton et al., 2014; Suh et al., 2019). Previous studies on the integration of organic biomarkers have produced mix results, with some demonstrating spatial integration of catchment signals (Alewell et al., 2016; Feakins et al., 2018; Hemingway et al., 2016), whereas others did not observe this (Häggi et al., 2016; Ponton et al., 2014). However, these previous studies typically focused on very large river systems, which will undergo different transport processes than the first-order streams analyzed in this study. A number of these studies (Alewell et al., 2016; Feakins et al., 2018; Hemingway et al., 2016; Ponton et al., 2014) also observed seasonal differences in biomarker load in river sediments. Collecting seasonal samples in the Areguni Mountains, as well as testing these processes in other first-order streams, could further help clarify the transport processes measured in this setting.

However, most of these studies have focused on large river systems rather than first order streams. Thus, the sedimentary processes involved in the formation, transport, recycling, and accumulation of organic biomarkers in first and second order streams are not well understood. One challenge in assessing these processes in small streams is that the environment and plant communities are often homogenous, and thus it is not possible to differentiate between local and upstream transported organic material. To better understand transport processes affecting organic material in small catchments, we studied a set of streams in the Dany River, a tributary of the

Barepat River, located in the Areguni Mountains in the Lesser Caucasus Range. This stream system is divided into two distinct ecological regions by the treeline (at ~ 2000 masl), which separates alpine meadow above the tree line (2000 – 2500 masl) from deciduous forest below (1500 – 2000 masl). To evaluate the input of *n*-alkanes from upstream transported organic material relative to vegetation near the stream, we collected soil samples on the slopes of the mountains from both above- and below the treeline throughout the watershed and sediments deposited in the streambed along an elevation transect. ~~Comparing the hillslope sedimentary biomarkers and the streambed sedimentary~~Comparison of the hillside and streambed sedimentary *n*-alkanes ~~biomarkers~~ allows assessment of the input of *n*-alkanes locally produced by vegetation compared to those transported in stream sediments within the catchment.

An additional motivation of this research is that treelines are a vulnerable feature of higher altitude environments. Previous research in the Areguni Mountains study area has assessed the relationship between treeline dynamics and climate forcing in the past (Ghukasyan et al., 2010; Malinsky-Buller et al., 2021; Montoya et al., 2013; Tornero et al., 2016).~~, and the~~ Pleistocene sediments uncovered at archaeological sites at Kalavan village within this area have the potential to reconstruct this relationship through the analysis of plant wax biomarkers deposited in fluvial sediments. However, in order to reconstruct these systems in the past it is important to understand modern biomarkers integration processes in the first and second order streams and their potential effects on the sedimentary archives of the Areguni Mountains.

2. Methods

2.1 Sample Collection and Extraction

Hillslope soil samples were collected in September 2018 along an altitude transect (1500 – 2500 masl) above the Dany River watershed, a first order tributary of the Barepat River in the Areguni

137 Mountains, Armenia (Fig 1), which traverses the treeline at ~2000 masl. Forest vegetation is

138 predominantly oak (*Quercus macranthera*), beech (*Fagus orientalis*) and hornbeam (*Carpinus*

139 *orientalis*), while above treeline alpine meadow is comprised of *Hercleum sp.* and *Senecio sp.*

140 (Joannin et al., 2022; Volodicheva, 2002). Soil samples were collected by first clearing the top ~10

141 cm of soil to remove roots. Stream bed sediment samples were collected from the Dany River

142 throughout the altitude transect at intervals of ~100 m in altitude. In all cases, roughly 100 g of

143 sediment were collected for extraction of *n*-alkanes. ~~In order to extract *n*-alkanes, s~~Samples were

144 extracted using a Soxhlet apparatus with 2:1 dichloromethane:methanol for 48 hours. Following

145 lipid extraction, n-alkanes were separated from total liquid extract by passing samples through a

146 column of activated silica gel (1.25 g) in baked Pasteur pipettes with 2 mL hexane (non-polar

147 fraction), 4 mL dichloromethane (slightly polar fraction) and 4 mL methanol (polar fraction). n-

148 alkanes were quantified through the analysis of the hexane fraction. We quantified n-alkanes using

149 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).

150 Oven temperature was set at 50 °C for 1 min, ramped to 180 °C at 12 °C/min, then ramped to

151 320 °C at 6 °C/min and held for 4 min. (Brittingham et al., 2017; Smolen and Hren, 2023). We

152 measured a standard mixture of *n*-alkanes (C_{20}-C_{33}) of known concentration to correct for mass

153 dependent response decreases in longer chain *n*-alkanes. ~~*n*-alkanes were separated with silica gel~~

154 ~~column chromatography and quantified on a Thermo Scientific Trace GC Ultra (Manufacturer)~~

155 ~~fitted with a split-splitless (SSL) injector and flame ionization detector (FID) using a BP-5 column~~

156 ~~(30 m × 0.25 mm i.d., 0.25 μm film thickness) with He as the carrier (1.5 ml/min).~~ Odd over even

157 predominance (OEP) (Eq. 1) and average chain length (ACL) (Eq. 2) were used to evaluate

158 distributions of *n*-alkanes (Bush and McInerney, 2013b)~~(REF)~~. We also calculated P_{aq}, an *n*-

159 alkane proxy to evaluate the possible biomarker contribution of aquatic and emergent plants (Eq.

160 3) (Ficken et al., 2000).

161 $$OEP = \frac{C_{25} + C_{27} + C_{29} + C_{31} + C_{33}}{C_{24} + C_{26} + C_{28} + C_{30} + C_{32}}$$

162 $$ACL = \frac{25 * C_{25} + 27 * C_{27} + 29 * C_{29} + 31 * C_{31} + 33 * C_{33}}{C_{25} + C_{27} + C_{29} + C_{31} + C_{33}}$$

163 $$Paq = \frac{C_{23} + C_{25}}{C_{23} + C_{25} + C_{29} + C_{31}}$$

164 **2.3 Stable Isotope Analysis**

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

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

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

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

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

170 concentrations (5-30 V/s) to correct for size effects. Standard ~~deviations~~ errors were 0.~~5~~4‰ for

171 $\delta^{13}C$ and ~~4~~3‰ for δD. Isotope ratios (R) were converted to δX ($\delta^{13}C$ and δD) values (Eq. 3) and

172 are expressed in permill (‰).

173 $$\delta X = \left(\frac{R_{Sample}}{R_{Standard}} - 1\right) * 1000$$

174

175 **3. Results**

176 **3.1. Alkane abundances**

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

178   C_{29} or C_{31} alkane, which is typical for terrestrial plants (see S1-S4 for illustrative chromatograms).

179   Odd numbered alkanes are significantly more abundant than even numbered alkanes, and the OEP

180   of all samples ~~averages~~ is 11.2, with a range from 7.4-18.4. There is no significant difference

181   between the mean OEP of soil (11.1) and stream (11.3) samples in the watershed. These values

182   are similar to those previously measured in the Greater and Lesser Caucasus Mountains (Bliedtner

183   et al., 2018; Trigui et al., 2019).

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

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

186   31.8). In soils below the treeline, the ~~average~~ mean ACL value is 29.5 (range of 28.4-30.4). There

187   is a significant difference between the average ACL values of the *n*-alkanes in above treeline and

188   below treeline soils (Student's t-test, $p < 0.001$, n=30). Stream sediment above the treeline ha~~ve~~s an

189   average ACL value of 29.7 (range of 29.1-30.2) and stream sediments below the treeline have an

190   average ACL value of 29.3 (range of 28.6 ~~to~~ -30.0). The stream sediments from below the treeline

191   have a significantly (Student's t-test, $p < 0.001$, n=21 ~~p<0.001~~) lower average ACL value than those

192   above the treeline.

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

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

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

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

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

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

199   0.20, four stream and four soil samples. This indicates that there was not a significant contribution

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

201 origin.

3.2. δD and δ¹³C values

203 The $\delta^{13}C$ values in soils and stream sediments collected from the Areguni Mountains reflect

204 a C_3 landscape, which is typical in Armenia. $\delta^{13}C$ values in all samples ranged from -36.0 to -

205 32.3‰ (Fig 4). The range is similar for both soil samples (-35.9 to -32.3‰) and stream samples (-

206 36.0 to –32.5‰). However, there is a significant difference in the $\delta^{13}C$ values of above and below

207 treeline samples, both in the stream and soil samples collected. Above the treeline, $\delta^{13}C$ values in

208 soils average~~s~~ -34.9‰, and below the treeline soil alkanes average -33.3‰ (p<0.0001, student's t-

209 test, n=30). Stream sediment $\delta^{13}C$ values average -35.0‰ above the treeline and -33.6‰ below

210 the treeline (p<0.0001, student's t-test, n=21). ~~In both cases, these values are significant~~

211 ~~(p<0.0001, student's t test)~~. δ~~A~~^{13}C values in stream samples exhibit a step-like behavior, with ~2‰

212 shift to more negative values as the stream drops below the treeline.

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

214 185‰ (Fig 5). These values were significantly ~~(p<0.001, student's t test). M~~more negative in

215 above treeline sediments (-175‰) than in below treeline sediments (-156‰) (p<0.001, student's

216 t-test, n=30). δD values were also more negative in stream sediment samples collected above ~~This~~

217 ~~is also true in sediment collected from stream samples, which are significantly more negative~~

218 ~~above~~ the treeline (-175‰) than below the treeline (-158‰) (p<0.001, student's t-test, n=21). As

219 with the $\delta^{13}C$ values, the δD values of stream sediment samples show sudden change as the stream

220 drops below the treeline.

4. Discussion

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

223   The hillslope soil leaf wax ~~ACL (Fig 3), δD,~~ δ^{13}C (Fig. 4) and δD ~~ACL~~(Fig. 5) show a step-

224   like change at the treeline, indicating a significant separation between upstream (above treeline)

225   and downstream (below treeline) soils. Using this separation, it is possible to assess the

226   contributions and integration of upstream vs. downstream soils to the streambed sediments along

227   the altitude transect. The step-like transition in streambed δD and δ^{13}C values indicates an over-

228   printing of upstream alkane isotope values by input from deciduous vegetation. Thus, local

229   production largely outweighs upstream transport in this setting. However, to firmly evaluate the

230   upstream and downstream hillslope soil contribution to streambed sediments, there is a need to

231   quantitatively evaluate the area-weighted production of *n*-alkanes above and below the treeline.

232   **4.2. Modeling *n*-alkane production and estimating upstream transport and integration**

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

234   mixing model that calculates the expected δD, δ^{13}C and ACL values at each one of the sampling

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

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

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

238   The parameters we used for ~~this~~ our mixing model are: 1. Satellite images (Google Earth)

239   to map the areas ~~of tree and grass sediment~~covered by alpine meadow and forest vegetation

240   throughout the Dany River catchment. 2. An estimate of net primary productivity of organic

241   material production in grasses and trees (grams per area) (Brun et al., 2022). 3. Estimates of *n*-

242   alkane production in grasses and trees in the Greater and Lesser Caucasus Mountains (grams of *n*-

243   alkane per gram of organic material) (Bliedtner et al., 2018; Trigui et al., 2019). 4. End member

244   values of δD, δ^{13}C and ACL derived from the average hillslope soils above and below the treeline.

245   At each sample point within the catchment, we first calculated the upstream area covered by the

246 two dominant vegetation types within the catchment (deciduous forest and alpine meadow) (Figure

247 6). This area was then multiplied by the previously mentioned constants (Table 1). By multiplying

248 these terms (area x organic mass production x *n*-alkane production x end member soils value), we

249 created an *n*-alkane production map for the Dany River catchment. ~~Using this map, we~~Using this

250 method, we calculated~~, for each riverbed sampling location~~, the amount of grass and tree *n*-alkanes

251 produced on the hillslopes above the sampling locations and the expected δD, δ¹³C and ACL values

252 for each stream sampling location (Figure 7a, 7c, 7e).

253   We compared the results of ~~this~~ our mixing model with the measured δD, δ¹³C and ACL

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

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

256 ~~deciduous-sourced~~ *n*-alkanes sourced from below treeline vegetation. Measured δD, δ¹³C and ACL

257 values do not have a linear relationship with the expected values based on vegetation area (Fig 7b,

258 7d, 7f). These measured values would produce under-estimates of the upstream area of alpine

259 grasses, yielding incorrect reconstructions of paleo-vegetation in sedimentary records. Comparing

260 the mixing model with the observations indicates that an area-weighted mixing process is not an

261 adequate model for explaining the *n*-alkanes signal in the streambed sediments. A simple and

262 straightforward way to interpret this discrepancy is that an area-weighted quantitative integration

263 of *n*-alkanes is not a good model for describing this catchment system, and that local production

264 is much larger than transported organic material.

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

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

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

268 bed. Second, ~~though~~although production of *n*-alkanes in grasses and trees is not significantly

269 different in the Greater and Lesser Caucasus Mountains, concentrations are higher in soils in

270 deciduous areas (Bliedtner et al., 2018; Trigui et al., 2019). This retention of more biomarkers in

271 forest soils would also increase the contribution of deciduous alkanes into the stream bed. Third,

272 stream downcutting into older sediments has the potential to re-mobilize stored organic carbon,

273 which may contain a greater load of deciduous n-alkanes. However, analysis of pollen from a

274 ~~nearby~~ lake core nearby (~ 5km from the Dany catchment) ~~-~~in the Areguni Mountains shows a

275 gradual shift over the last 4000 years from a grass-dominated landscape to the deciduous forest

276 present today (Joannin et al., 2022b). Therefore, stored biomarkers are more likely to be grass-

277 dominant, and this is unlikely to explain the measured bias to deciduous alkanes.

278 Since n-alkanes in ~~this~~ the first order stream in this study do not quantitively integrate n-

279 alkanes based on the upstream area of different vegetation types, this likely precludes the use of

280 n-alkanes as a tool to reconstruct vertical treeline movement in this setting. However, this is a

281 benefit for attempts to reconstruct hydrological changes through the analysis of δD values in n-

282 alkanes. Given the ~20‰ difference in apparent fractionation (ε) values for above and below

283 treeline sediments, changes in upstream vegetation cover would alter measured δD values in n-

284 alkanes in sedimentary archives. Without this quantitative integration, n-alkanes measured in the

285 Pleistocene sediments found in this watershed are more likely to reflect changes in δD values of

286 precipitation, and therefore would serve to reconstruct hydrological cycles, rather than changes in

287 upstream vegetation cover. Since δ^{13}C and ACL of n-alkanes are also different in above and below

288 treeline sediments, these other analyses would also be useful to identify periods with large changes

289 in treeline that might complicate interpretation of δD values.

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

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

292   δD values. We use δD values of precipitation from water samples collected at the nearest

293   meteorological station with isotope data in Armenia (Dilijan, (Brittingham et al., 2019b) Given

294   the lack of quantitative integration in the catchment, a paleoclimate record from either above (A)

295   or below (C) treeline would record the shift in precipitation δD values. Below treeline sedimentary

296   records, with stream organic biomarker load overprinted by local vegetation production, would

297   likely provide a means to reconstruct the δD precipitation values. However, records near the

298   treeline (B) would be ~~heavily affected~~influenced by changes in apparent fractionation values

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

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

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

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

303   ~~some demonstrating spatial integration of catchment signals , whereas others did not observe this~~

304   ~~. However, these previous studies typically focused on very large river systems, which will~~

305   ~~undergo different transport processes than the first-order streams analyzed in this study. A number~~

306   ~~of these studies also observed seasonal differences in biomarker load in river sediments.~~

307   ~~Collecting seasonal samples in the Areguni Mountains, as well as testing these processes in other~~

308   ~~first-order streams, could further help clarify the transport processes measured in this setting.~~

309

310

311   **5. Conclusion**

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

313   Mountains in Armenia, demonstrate that there is a significant difference in hillslope soil δD, δ^{13}C

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

be used to differentiate between the above and below treeline sediments. However, *n*-alkanes extracted from stream sediments reflect their local area, rather than demonstrating transport from the higher-altitude alpine meadow. These results provide a complication for attempts to reconstruct changes in past treeline in this mountain range, given that the biomarker load in stream does not reflect the relative area of different upstream vegetation types. However, these results simplify interpretation of past *n*-alkane δD values, as apparent fractionation differences between grasses and trees are less likely to impart a significant influence on δD values in stream bed *n*-alkanes.

6. Competing interests

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

7. Acknowledgements

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	Conc[1]	NPP[2]	ACL[3]	$\delta^{13}C^{[3]}$	$\delta D^{[3]}$
Forest	7.69	1099.4	29.5	-33.3	-156
Alpine Meadow	3.03	719.1	30.6	-34.9	-175

Table 1: Constants used for mixing model. 1: *n*-alkane concentration in grasses and trees in the Caucasus Mountains (grams of *n*-alkane/gram of organic material) (Bliedtner et al., 2018; Trigui et al., 2019). 2: NPP for forest and grassland areas (grams per area) (Brun et al., 2022). 3: mean values for ACL, $\delta^{13}C$ and δD (this study)

555 Figure Captions

556 Figure 1: (Left) Topographic map (ASTER Global DEM) of Armenia with inset map of
557 sampling location (black box) (Right) Inset map of soil (yellow circles) and stream (blue circles)
558 samples collected in the Areguni Mountains, along with the limit of the Barepat (dashed line)
559 and Dany watersheds (solid line)

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

562 Figure 3: The average chain length (ACL) values of *n*-alkanes extracted from above treeline
563 (~~green~~ yellow squares) and below treeline (~~red~~ green squares) sediment ~~and stream (blue~~
564 ~~triangles) sediments~~ across the sampling elevation gradient

565 Figure 4: The δ¹³C values of *n*-alkanes extracted from above treeline (yellow squares) and below
566 treeline (green squares) sediment~~above treeline (green squares) and below treeline (red squares)~~
567 ~~and stream (blue triangles) sediments~~ across the sampling elevation gradient

568 Figure 5: The δD values of *n*-alkanes extracted from above treeline (yellow squares) and below
569 treeline (green squares) sediment ~~above treeline (green squares) and below treeline (red squares)~~
570 ~~and stream (blue triangles) sediments~~ across the sampling elevation gradient

571 Figure 6: Mixing model used to calculate expected values of stream sample points. Upstream
572 watershed area covered by deciduous forest (green) and alpine meadow (yellow) was calculated
573 at each sample location (blue dots). Point watershed for sample 24 (dashed line) is shown here as
574 an example.

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576 Figure 7~~6~~: Comparison of the measured ACL, δD and δ¹³C values against expected values of
577 stream sediments. Dashed line represents the range of expected values from stream sediments if
578 vegetation was integrated equally by area

579 Figure 8~~7~~: A photograph of the Dany watershed with hypothetical paleoclimate record from
580 three locations: (A, dashed line) Below treeline, (B, solid line) near treeline with fluctuations in
581 treeline altitude, and (C, dotted line) above treeline
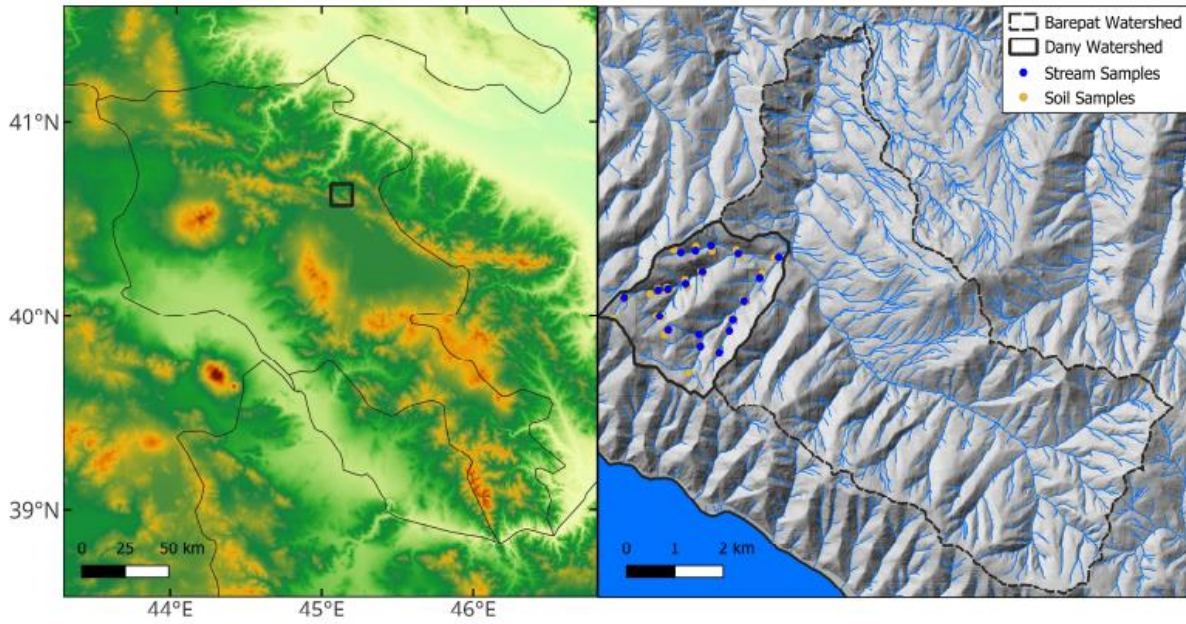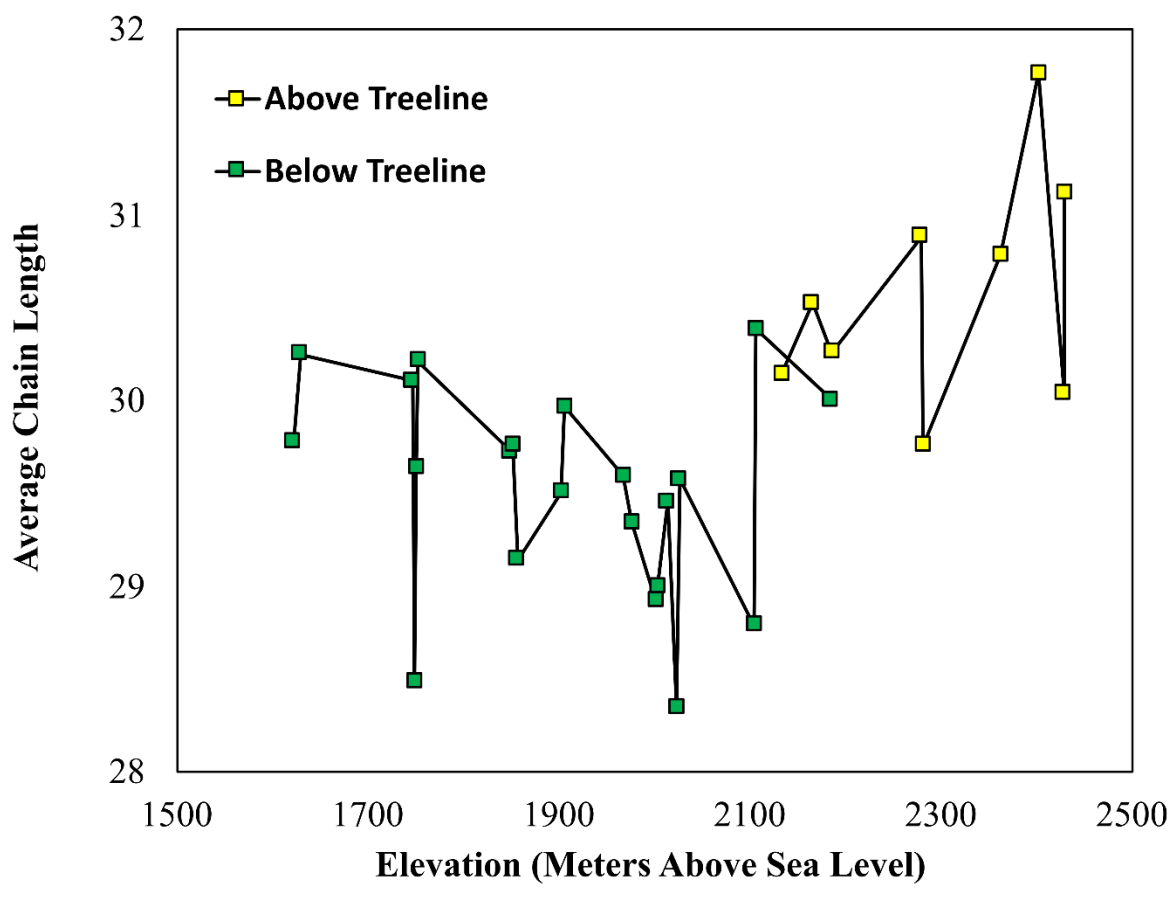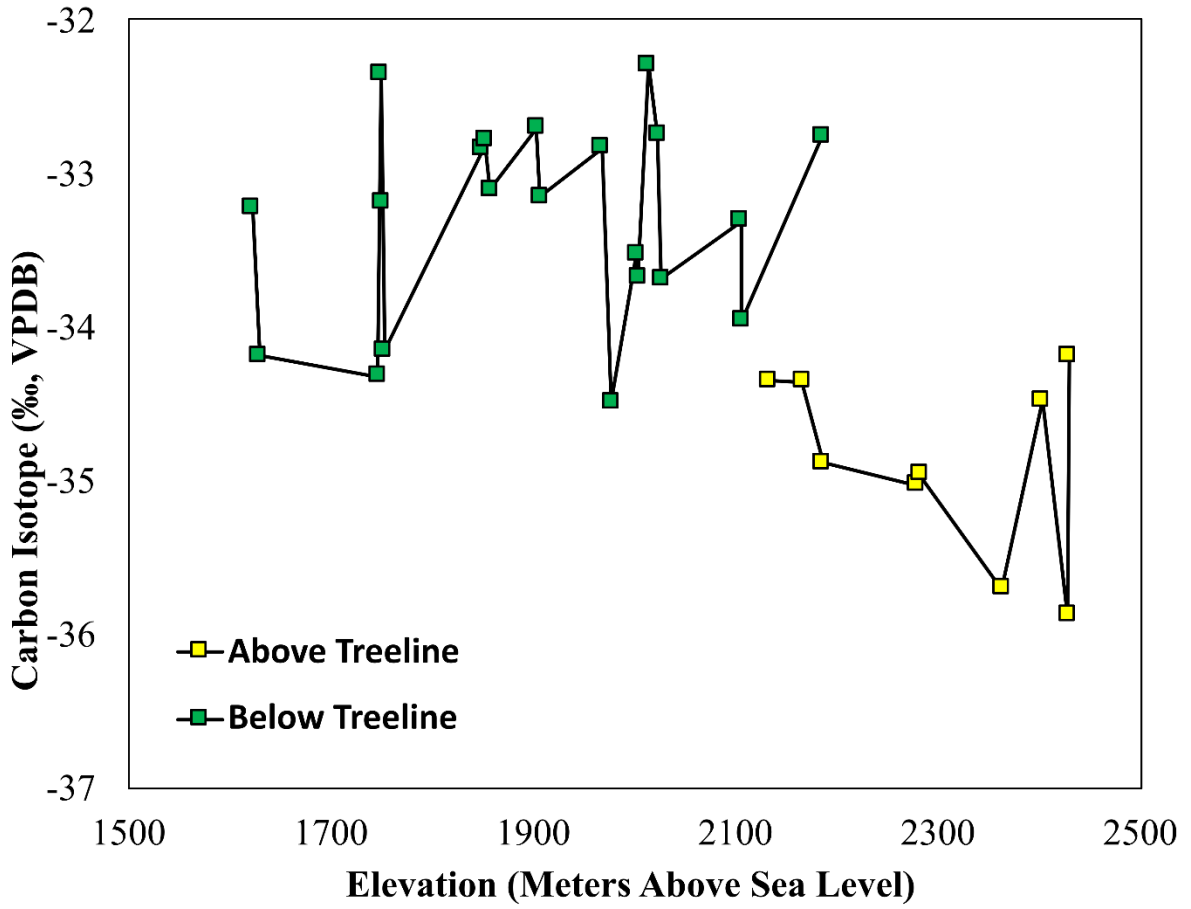
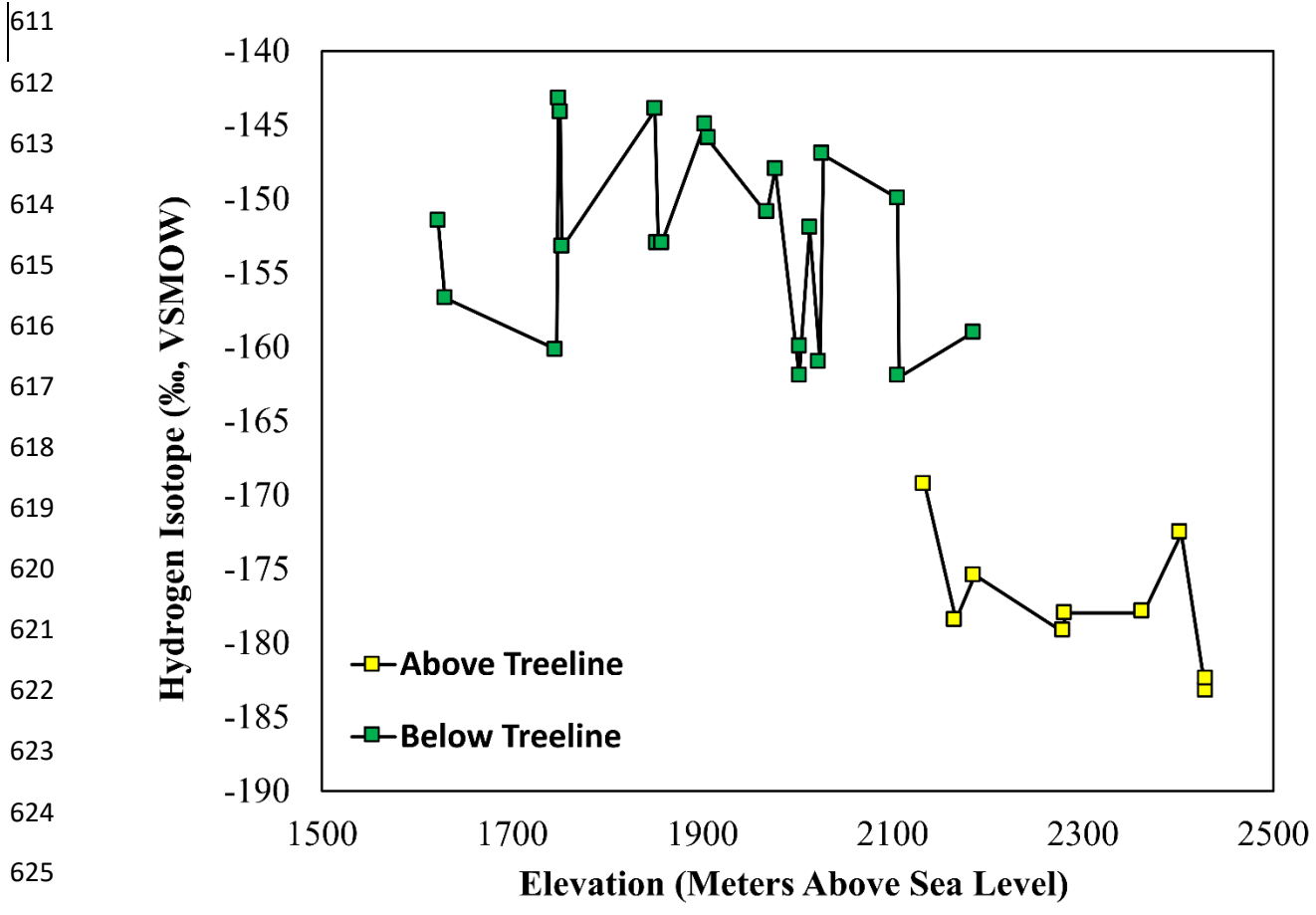582

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

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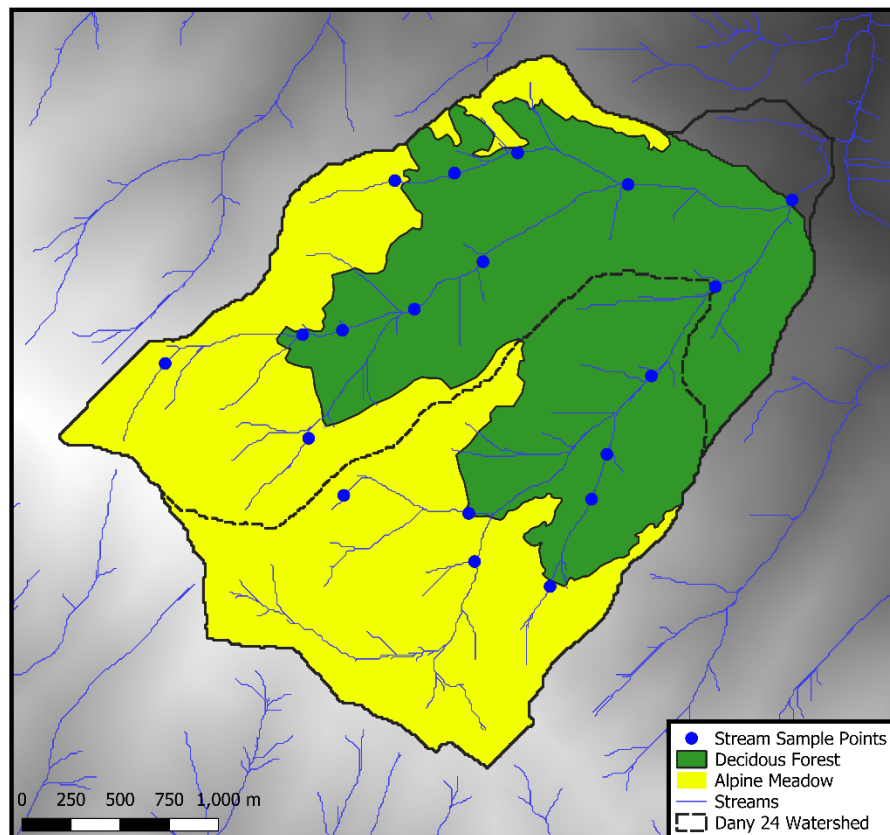624
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