1 Tree-ring isotopes from the Swiss Alps reveal non-climatic fingerprints of cyclic insect

2 population outbreaks over the past 700 years

3 Short title: Isotopes as non-climatic fingerprints

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58 59	Abstract
60	Recent experiments have underlined the potential of $\delta^2 H$ in tree-ring cellulose as a physiological
61	indicator of shifts in autotrophic versus heterotrophic processes (i.e. the use of fresh versus stored non-
62	structural carbohydrates). However, the impact of these processes has not yet been quantified under

63 natural conditions. Defoliator outbreaks disrupt tree functioning and carbon assimilation, stimulating 64 remobilization, therefore providing a unique opportunity to improve our understanding of changes in 65 δ^2 H. By exploring a 700-year tree-ring isotope chronology from Switzerland, we assessed the impact of 66 79 larch budmoth (LBM, *Zeiraphera griseana*) outbreaks on the growth of its host tree species, *Larix* 67 *decidua*.

LBM outbreaks significantly altered the tree-ring isotopic signature, creating a ²H-enrichment and an ¹⁸O- and ¹³C-depletion. Changes in tree physiological functioning in outbreak years are shown by the decoupling of δ^2 H and δ^{18} O (O–H relationship), in contrast to the positive correlation in non-outbreak years. Across the centuries, the O–H relationship in outbreak years was not significantly affected by

- temperature, indicating that non-climatic physiological processes dominate over climate in determining
- δ^2 H. We conclude that the combination of these isotopic parameters can serve as a metric for assessing
- 74 changes in physiological mechanisms over time.

75 **1. Introduction**

76 The isotopic ratio of the non-exchangeable carbon-bound hydrogen (δ^2 H) has rarely been studied in tree-77 ring cellulose (C₆H₁₀O₅), while carbon (δ^{13} C) and oxygen (δ^{18} O) isotopes in tree rings have been used extensively to investigate the effects of past climatic conditions (Saurer et al., 1995; Saurer, Aellen & 78 Siegwolf, 1997; Loader et al., 2007; Andreu-Hayles et al., 2017; Sakashita et al., 2018; Loader et al., 79 2020; Shestakova & Martínez-Sancho, 2021; Field et al., 2022; Sano et al., 2022). The climatic signal 80 81 of ²H has been found to be inconsistent and systematically lower than the signal recorded by the two other isotopes (Vitali *et al.*, 2021). The relationship between the two water isotopes (δ^{18} O and δ^{2} H) in 82 83 hydrological cycles is largely accepted to follow the Global Meteoric Water Line (GMWL; (Dansgaard, 84 1964; Voelker et al., 2014). However, this O-H relationship barely holds true in the year-to-year variation of tree-ring cellulose, which exhibits site-specific patterns ranging from the expected positive 85 relationship to negative ones (O-H relationship; Vitali et al., 2021). This evidence indicates that 86 87 complex interactions between hydrological and physiological components affect tree-ring cellulose δ^{18} O and δ^2 H signals differently. However, disentangling the effects of these processes has proven 88 challenging. 89

90 Recent experimental results have pointed to a different perspective for understanding the signal stored 91 Ain δ^2 H. Specifically, a strong link has been found between the δ^2 H value of plant material and the relative 92 proportions of autotrophic and heterotrophic processes (Yakir & Deniro, 1990; Cormier et al., 2018; 93 Cormier et al., 2019). This can be explained by changes in metabolic pathways of plant leaves' carbon primary metabolism in response to climatic conditions, such as drought (Wieloch, Augusti & 94 95 Schleucher, 2022; Wieloch & Grabner et al., 2022), low light conditions and low CO₂ concentrations (Cormier *et al.*, 2018; Cormier *et al.*, 2019). On the other hand, changes in the δ^2 H of tree-ring cellulose 96 might also be caused by shifts in the relative use of fresh and older stored non-structural carbohydrates 97 98 (NSCs) for growth, as these storage NSCs differ in their isotopic ratios (Kimak, 2015; Nabeshima et al., 3

99 2018). For instance, ²H-enrichment has been found under conditions where cellulose biosynthesis relies 100 largely on NSC remobilization (e.g. earlywood, early leaf development Nabeshima et al., 2018). These

101 studies suggest the potential of $\delta^2 H$ to be an indicator of physiological mechanisms related to the C

- storage dynamics of trees (Lehmann et al., 2021), in addition to serving as a record of the hydrological 102
- 103
- signal from source water shared with δ^{18} O (Allen *et al.*, 2022). Therefore, a significant change in δ^{2} H values is to be expected in the case of sudden needle loss, when fresh assimilates become largely 104
- unavailable and growth has to rely on stored NSCs (Lovett et al., 2002; Peters et al., 2017). 105
- 106 In this context, defoliation events induced by insect outbreaks can be used as a non-climatic stressor, to 107 investigate the signal recorded with $\delta^2 H$ and the decoupling of the O–H relationship. Defoliator 108 outbreaks induce major canopy desiccation, playing an important role in forest nutrient cycling 109 (Berryman, 2002), but also significantly affect forest functioning and biomass production (Lovett et al., 2002; Peters et al., 2017). At the tree level, insect-induced defoliation events impact tree functioning by 110 (i) reducing photosynthetic C uptake (Baltensweiler et al., 2008), (ii) inducing remobilization of NSCs 111 112 (Li et al., 2002), and (iii) causing a significant decrease in soluble sugars at the leaf level (Peters et al., 2020) while preserving stem and root xylem starch levels (Kosola et al., 2001; Saffell et al., 2014; Peters 113 et al., 2020). These results indicate the prioritization of C allocation to storage over other C-dependent 114 processes in outbreak years (Sala et al., 2012). The sum of these processes influences tree-ring cell 115 production and therefore can be expected to induce significant shifts towards heterotrophic processes, 116 which are reflected in the isotopic signals. Such shifts likely limit the climatic signal detectable in the 117

isotopic signatures, in favour of non-climatic signals. 118

119 Tree-ring stable isotopes provide unique insights into the effects of insects on host tree physiology 120 (Ulrich *et al.*, 2022). Tree-ring C isotopic ratios (δ^{13} C) reflect leaf-level changes in photosynthesis and 121 stomatal conductance, whereas oxygen isotopic ratios (δ^{18} O) record changes in source water and 122 evaporative conditions. In outbreak years, δ^{13} C values have been shown to be only slightly enriched 123 (Ellsworth et al., 1994; Haavik et al., 2008; Simard et al., 2008; Kress & Saurer et al., 2009; Weidner et al., 2010; Simard et al., 2012) and δ^{18} O values only decrease slightly or show no change (Kress & 124 Saurer *et al.*, 2009; Weidner *et al.*, 2010). Meanwhile, $\delta^2 H$ values have been barely explored in the 125 context of insect outbreaks. 126

127 Extensive research on larch budmoth (LBM) population outbreaks in the European Alps has yielded 128 chronologies of both tree-ring width and maximum latewood density dating back to 800 CE (Esper et 129 al., 2007), posing a unique opportunity to further our understanding of tree physiological reactions to 130 this non-climatic stressor and the resulting tree-ring signals. LBM infestations are caused by foliage-131 feeding Lepidopteran insects (Zeiraphera griseana [Hübner]). In outbreak years, LBM hatches in large 132 quantities and feeds on needle clusters of *Larix decidua* (European larch), typically peaking by the end of June or the beginning of July (Baltensweiler et al., 2008; Peters et al., 2020). In this way, 133 134 the mass and quality of larch foliage is reduced (Baltensweiler & Fischlin, 1988; Asshoff & 4

Hättenschwiler, 2006), and re-flushing may occur within 3-4 weeks of the end of larval feeding
(Baltensweiler *et al.*, 2008). The feeding cycle effectively impacts the majority of the vegetative
season, which spans from the start of June to the end of September. The intensity of defoliation is

- temporally and spatially variable, as a critical mass of larvae needs to be present to have a visible
- 140 European distribution (Bjørnstad *et al.*, 2002), cyclically affecting the subalpine valleys of the

impact in an outbreak year. Further, the wave of LBM outbreaks travels across their Central

- 141 European Alps (Rolland *et al.*, 2001; Nola *et al.*, 2006; Esper *et al.*, 2007; Büntgen *et al.*, 2009; Kress
- 142 & Saurer *et al.*, 2009; Hartl-Meier *et al.*, 2017; Saulnier *et al.*, 2017; Büntgen *et al.*, 2020). An LBM
- 143 history reconstruction spanning 1200 years showed a remarkable regularity of the outbreak's recurrence,
- happening on average every 9 years (Esper et al., 2007), and their link to cool summer temperatures
- 145 (Kress & Saurer *et al.*, 2009). Climate warming has disrupted this pattern, with no extensive outbreaks
- 146 occurring from the 1980s (Wermelinger & Gossner et al., 2018) until 2018 when a new major outbreak
- 147 occurred in Switzerland (Büntgen et al., 2020). However, the interaction between LBM outbreaks and
- 148 climate is still poorly understood.

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149 This long-standing host-pathogen co-existence has indicated that larch trees cope with LBM infestations 150 through physiological and morphological adaptations. The production of a second set of needles in the 151 outbreak season, with traits and physiology similar to that of the first set (Peters et al., 2020), comes at 152 the expense of C reserves. Although the photosynthetic potential is restored, NSCs are not fully 153 replenished (Wermelinger & Gossner et al., 2018). The lack of C uptake due to defoliation and the use of C reserves to build the second set of needles is also reflected in a reduction in total ring width 154 (Arbellay et al., 2018). These processes result in dramatically narrower tree-ring widths and thinner 155 156 latewood cell walls, which are commonly used to identify these major outbreak years (Baltensweiler et al., 2008; Büntgen et al., 2009; Hartl-Meier et al., 2017; Arbellay et al., 2018). Furthermore, these 157 reductions have long-term effects lasting for up to 7 years (Peters et al., 2017). The C-demanding cell-158 159 wall thickening process stops early during an LBM outbreak year (Peters et al., 2020). Further, cell walls 160 are thinner along the entire ring (Rolland et al., 2001) and especially in the latewood (Castagneri et al., 161 2020). The different structure of the density of outbreak rings, as shown by Castagneri et al. (2020), opens the question of whether annually resolved isotope measurements are suitable to investigate the 162 163 impact of LBM outbreaks, as we would expect a proportionally larger share of biomass in the earlywood 164 (EW) than in the latewood (LW). Further, in deciduous broadleaf species (*Quercus*), δ^{18} O and δ^{2} H have 165 been shown to have lower values in LW compared with EW (Kimak, 2015; Nabeshima et al., 2018). 166 However, in deciduous conifers (Jahren & Sternberg, 2008) and for δ^{13} C (Kress & Young *et al.*, 2009) 167 no such difference has been shown. Therefore, to answer this question separate EW and LW isotopic 168 analyses are needed to identify whether a mass-balance issue should be accounted for in annually 169 resolved measurements. Nonetheless, defoliator outbreaks clearly have a severe impact on trees' 170 assimilation and allocation strategies, potentially overwriting the climatic signal. This offers the

171 opportunity to evaluate the "physiological signal" recorded by tree-ring isotopic ratios and $\delta^2 H$ in 172 particular.

173 In this study, we investigated the impact of identified LBM outbreaks on physiological signals in trees

in the Swiss Lötschental valley. Specifically, we studied the signals stored in chronologies of tree-ring

- width, maximum latewood density, and the stable isotopes δ^2 H, δ^{18} O and δ^{13} C (Esper *et al.*, 2007) in
- 176 connection to the centennial changes in temperature. We investigated the following hypotheses (Hp):
- 1771. LBM-induced changes in tree physiology create significant shifts in δ^{18} O, δ^{13} C and δ^{2} H178in tree rings.
 - 2. LBM causes changes in the variability of intra-annual EW and LW isotopic ratios.
- Befoliation events lead to a decoupling of the O–H relationship, due to an increase in
 the recorded non-climatic signal connected to the use of stored C reserves.
- 1824. The isotopic signature in outbreak years is consistent across the seven centuries of183outbreaks and is independent of temperature change.
- 184 **2. Methods**

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185 **2.1. Site and data sources**

Both living trees and timber from buildings were pooled to construct the chronologies for this study, 186 187 and a combination of published (Büntgen et al., 2006; Esper et al., 2007; Kress & Saurer et al., 2009; 188 Hangartner et al., 2012) and unpublished data were used. The tree-ring width (TRW), maximum latewood density (MXD), δ^{13} C and δ^{18} O data utilized in this study were previously published (Büntgen 189 et al., 2006; Kress et al., 2014). The Larix decidua Mill. samples were collected from the Lötschental, 190 Valais, in southwestern Switzerland (46°26'N, 7°48'E). The vegetation in the sampling area is 191 192 dominated by the subalpine belt of spruce-larch forests, which are gradually mixed with larch-Swiss 193 stone pine forests towards the upper timberline, occurring at 2100 to 2200 m a.s.l. (for further details 194 see Büntgen et al., 2006 and Esper et al., 2007). Samples of living trees from the same valley covered 195 the "recent period" (1650-2004), while relict material originating from buildings in the same valley 196 covered the "earlier period" (1256-1700; (Kress et al., 2014). Both historic and living tree samples were 197 combined into a composite chronology for further analyses (see Hangartner et al., 2012). Further 198 samples from five additional living dominant trees in the Lötschental (2000 m a.s.l., 46°23'40"N, 7°45'35"E) were used for the measurement of earlywood (EW) and latewood (LW) for selected LBM 199 200 events from the 20th century or "current period" (for further details see Peters et al., 2020).

201 **2.2. LBM events**

LBM defoliation events have been studied extensively to identify past outbreaks, and they correspond
to severe and unambiguous alterations in the wood structure, with narrow annual tree rings containing
thin latewood cell walls (Rolland *et al.*, 2001), i.e. "budmoth rings" (Baltensweiler *et al.*, 2008; Büntgen *et al.*, 2009; Hartl-Meier *et al.*, 2017; Arbellay *et al.*, 2018). For the present study, LBM events were

taken from Esper *et al.* (2007), yielding 79 events for the 1256–2004 CE (748-year) period (Table S.1).

- The LBM years were identified through the analysis of tree-ring density variations, and a threshold of $<0.005 \text{ g cm}^{-3}$ was set for classification as an LBM mass outbreak year (Esper *et al.*, 2007). Furthermore,
- 209 we verified the identified years with field observations for several outbreaks in the 20th century by
- 210 Baltensweiler *et al.* (2008) in another Swiss region (Engadin). These events were synchronised with
- 210 Baltensweiler *et al.* (2008) in another Swiss region (Engadin). These events were synchronised with
- 211 delays of one or two years, and congruent with the spatial "wave pattern" spreading of LBM outbreaks
- 212 (Bjørnstad *et al.*, 2002). Nonetheless. variation in annual TRW and MXD values in outbreak years was
- expected and could be attributed to variability in the intensity of outbreak events, early arrival or longer
- 214 permanence of the budmoth in the years before and after the year of "critical mass" outbreak. Further,
- in this study, we analysed a composite chronology, which might have contributed to the variability in
- annual TRW and MXD values, especially in low-intensity LBM years (Esper *et al.*, 2007).

217 2.3. Dendrochronological methods

218 As reported by Kress and Saurer et al. (2009) and Hangartner et al. (2012), TRW was measured and 219 cross-dated following standard procedures (Cook & Kairiukstis, 1990). TRW and MXD data were given 220 as RCS-standardized chronologies (regional curve standardization, as described by Büntgen et al., 221 2006). For isotopic analysis, five trees were selected for each period, and the cores were manually split by year using a scalpel. Each tree was analysed separately regarding the calibration period (A.D. 1900-222 2004). For the remaining period, all cores were pooled prior to the analysis of each annual ring, but 223 224 single-tree measurements were retained every tenth year (Kress & Saurer et al., 2009; Hangartner et al., 225 2012). The first 50 years of each core were not used, to avoid any age effects from juvenile growth 226 stages (McCarroll & Loader, 2004; Arosio et al., 2020b; Arosio et al., 2020a). Alpha-cellulose was 227 extracted from all samples and isotopic ratios were measured (Filot et al., 2006; Boettger et al., 2007). 228 All isotope chronologies from the different wood sources were merged and indexed according to 229 Hangartner et al. (2012).

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231 2.4. Earlywood and latewood measurements

232 After a 2018 LBM outbreak event, five affected trees (~200 years old) were cored in the study area at 2000 m a.s.l. (46°23'40"N, 7°45'35"E; Peters et al., 2020). A core-microtome was used to obtain a plane 233 234 surface suitable for TRW measurements and isotopic analysis while avoiding dust carryover between 235 rings (Gärtner & Nievergelt, 2010). For this study, rings were dated, three years with LBM outbreaks 236 were identified (1972, 1981, 2018), and EW and LW were visually identified and hand-split following 237 standard procedures (Holmes, 1983). Cellulose was extracted using standard Teflon filter bags (Ankom 238 Technology, Macedon, NY, USA), homogenized, and packed into silver capsules for the measurement of δ^{13} C, δ^{18} O and δ^{2} H as described below. 239

240 **2.5. Isotopic analysis**

241 The isotope data from the long-term LBM chronologies and detailed methodological descriptions have

- been given in previous publications (Hangartner *et al.*, 2012; Kress *et al.*, 2014)). In brief, δ^{13} C was measured by combustion in an elemental analyser and δ^{18} O using a high-temperature pyrolysis system 243
- coupled to an isotope ratio mass spectrometer (IRMS, Delta Plus XP; Thermo Finnigan MAT, Bremen, 244
- 245 Germany), with a precision of ca. $\pm 0.1\%$ for δ^{13} C and $\pm 0.3\%$ for δ^{18} O. Corrections for past changes in
- the δ^{13} C of atmospheric CO₂ were applied to the raw δ^{13} C data (Leuenberger, 2007). For measuring non-246
- 247 exchangeable carbon-bound δ^2 H, the cellulose samples were equilibrated and subsequently converted
- 248 to H₂ by high-temperature pyrolysis (TC/EA) and analysed by IRMS with a precision of ca. $\pm 2\%$ (Filot
- 249 et al., 2006).

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- 250 For EW and LW analyses, cellulose samples were prepared and subsequently treated with the hot watervapour equilibration method described by Schuler et al. (2022). In brief, samples were packed in silver 251 capsules, equilibrated at 130°C with water of known isotopic composition for 2 hours, and dried with 252 253 dry nitrogen gas for 2 h. The samples were then transferred to the autosampler of a high-temperature 254 pyrolysis system at 60°C (PYROcube, Elementar, Hanau, Germany). The shielded autosampler was flushed with argon, and the samples were kept there for 2 h to re-equilibrate with this environment. The 255 256 samples were then thermally decomposed at 1420°C and the isotopic ratios of C, O and H were measured by mass spectrometry (IRMS; Loader et al., 2015; Weigt et al., 2015). The hot water vapour 257 equilibration made it possible to correct for the O-bound H isotopes (which readily exchange with water 258 vapour and thus reflect a bias) in cellulose and to determine the non-exchangeable carbon-bound $\delta^2 H$ 259 260 (Schuler et al., 2022).
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262 2.5 Data analyses

The differences, extracted from the indexed annual chronologies, between years with LBM outbreak 263 264 (event years) and those without such an event (non-event years), as well as differences between EW and 265 LW values, were tested with the Welch t-test (Welch, 1947), which does not assume the same variance 266 between groups. Pearson correlation matrices were used to evaluate the relationships between tree-ring 267 parameters and to compare correlations in event and non-event years.

To compare the impact of the outbreak events across the 748-year chronology, an "event-based" 268 269 normalization was carried out. For each event year, a time window of ±5 years was considered, and the 270 values were normalized by subtracting the 5-year pre-outbreak mean (R-code adapted from Rao et al., 271 2019). This subtraction reduces the impact of low-frequency variability, i.e. the likelihood that a cluster 272 of low-growth years biases the classification of low or high TRW or MXD values (Adams et al., 2003), 273 and it enhances the high-frequency response signal of interest while minimizing noise. Event-based 274 normalized data were used to investigate the effects of the 79 LBM outbreaks identified by Esper et al. (2007; Table S.1). A Welch t-test was performed on the normalized data ($\delta^2 H_{norm}$, $\delta^{18}O_{norm}$, $\delta^{13}C_{norm}$) to 275

evaluate differences between LBM defoliation outbreak years (event years), and the 5 years prior to (pre-event years) or after the event (post-event years), singularly and as a group. P<0.05 was taken as

the threshold of statistical significance.

279 The relationship between $\delta^2 H$ and $\delta^{18} O$ isotopic ratios (O–H relationship) was examined with linear

280 model fitting, for all event years and for each of the 5 years before and after outbreak years to assess 281 any lag effect on the relationship. The century-wise linear models for the non-event years were built on

any lag effect on the relationship. The century-wise linear models for the non-event years were built on a randomly selected subset of years, applying 1000 iterations to ensure a balanced sample size between

the two groups (Table S.2). The century-wise linear models of event years were fitted using all event

- vears (Table S.2).
- Temperature reconstructions based on tree growth have been shown to have limitations (Wilmking et 285 286 al., 2020). However, they are valuable tools when alternative temperature information is not available. 287 The temperature reconstruction used here is based on the MXD data; however, the LBM effects were regarded as noise, removed and replaced with statistical estimates (Büntgen et al., 2006), showing ~60% 288 accuracy in the validation between the reconstruction and the common period for the "recent period" 289 290 (see Table 1 in Büntgen *et al.*, 2006). The summer temperature reconstruction ($T_{recon JJAS}$) completed by 291 Büntgen et al. (2006) was used as a reference to calculate the average temperature for each century for event and non-event years (Table S.2), and their difference was tested with a two-way repeated-measures 292 ANOVA. Century averages of T_{recon JJAS} were then related to the slope and R² value of the O-H 293 294 relationship to assess the impact of changing temperature on tree functioning and consequently on the isotopic signature of tree rings. All computations were performed in R version 4.0.3 (R Core Team, 295 296 2020).
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298 3. Results

299 **3.1. Chronologies of tree-ring parameters**

The 79 LBM events described by Esper *et al.* (2007) in the indexed chronologies for tree-ring δ^2 H, δ^{18} O 300 and δ^{13} C and the RCS chronologies for TRW and MXD are presented in Fig. 1a–e. Relatively low values 301 can be observed for δ^{18} O, TRW and MXD in many outbreak years, whereas δ^{2} H values were generally 302 303 higher than normal. The highest value in the δ^2 H chronology occurred during the 1352 outbreak year. All 79 events are superimposed in Fig. 1f-l to illustrate the characteristic responses in the isotopic 304 signatures. A significant ²H-enrichment is clearly noticeable in the year of the event, while the 2 years 305 306 after the outbreak show no significant differences from the average δ^2 H values (Fig. 1f). On the contrary, 307 ¹⁸O values in outbreak years and in the first year following the outbreaks were significantly depleted compared with non-event years. $\delta^{13}C$ did not change detectably in response to outbreak years. MXD 308 309 was significantly lower and TRW significantly smaller in the outbreak years and the first year following 310 the outbreaks. The effect of outbreaks on TRW was significant up to 2 years after the events. 9

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313 3.2. Earlywood and latewood differences in event years

314 To test whether annual isotopic ratios were potentially influenced by changes in the EW/LW mass

315 balance, we separated EW and LW of the three most recent known budmoth outbreaks (1972, 1981,

316 2018), and also considered 1 year before and after each event. No significant differences were observed

317 between EW and LW for any of the measured isotopic ratios in the year before or after an outbreak event or in the outbreak year itself (Fig. 2). δ^2 H had the largest between-tree variability (maximum)

 $\pm 20\%$) in the event year, whereas δ^{13} C and δ^{18} O had low variability (<1‰). The increase in δ^{2} H and 319

decrease in δ^{18} O in LBM years observed in the overall analysis (Fig. 1) was only apparent during the 320

321 outbreak in 1972.

322 **3.3. Relationships between tree-ring parameters**

In the analysis of the full chronologies, a strong correlation between δ^{18} O and δ^{13} C values was observed 323 in both outbreak years (r=0.41) and non-event years (r=0.36; Table 1). On the contrary, the correlation 324 325 between δ^2 H and δ^{18} O was significantly stronger in non-event years (r= 0.50) than in outbreak years 326 (r=0.15). This was observed to a lesser extent for the correlation between $\delta^2 H$ and $\delta^{13}C$ (r=0.18 in non-327 event years, r=0.08 in event years). TRW was only weakly correlated with all other parameters except MXD (r=0.59). MXD was correlated similarly with δ^{18} O and δ^{13} C in non-event years (r=0.32), but 328 poorly with $\delta^2 H$ (r=0.08). In outbreak years the correlations between MXD and the other variables were 329 330 much weaker.

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3.4. Event-based normalization 332

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3.4.1. Isotopic signatures

334 The event-based normalization involved an absolute assessment of the impact of LBM events on tree growth, independent from long-term trends, and highlighted the effects of LBM even more clearly than 335 a simple standardization, as shown in section 3.1. In the event-based normalization, the isotopic ratios 336 of the three elements were on average significantly different in event years compared with non-event 337 years, with lower δ^{13} C_{norm} and δ^{18} O_{norm} and higher δ^{2} H_{norm} (Fig. 3). 338

Considering the 5 years before and after the events individually (Fig. S.1), δ^{18} O had the faster recovery 339 340 to pre-event values, with only 1 year after the event being non-significantly different from the event 341 year. Both pre-and post-event $\delta^2 H$ values were significantly different from the event year, although less strongly than for δ^{18} O (Fig. S.1 a). δ^{13} C showed a prolonged effect, with less significant differences 342 343 between the event year and the 5 years after (Fig. S.1 c).

344 The normalized values were used to further investigate the decoupling between δ^{18} O and δ^{2} H during the

- event and surrounding years. We observe a consistent positive relationship between δ^{18} O and δ^{2} H, with
- 346 the lowest strength in the event year. The 2 years after the event also show low R^2 values (~0.08),
- 347 indicating a lasting effect before the system achieved a pre-disturbance balance ($R^2 \sim 0.3$). Further, in
- event years the slopes declined from ~4 to ~2, similarly indicating a loss of relationship between $\delta^2 H$
- 349 and δ^{18} O (Figs 4, S.2).

350 3.4.2.Centennial temperature effect

Based on reconstructed average June to September temperatures (Büntgen *et al.*, 2006), summer temperature in LBM event years was consistently significantly lower than the century's average reconstructed temperature for all eight centuries analysed, except for the 1600s when the temperature reached an overall minimum and was not different between event and non-event years (Fig. 5). Event years and the following 2 years were consistently cooler than non-event or pre-event years.

- The O-H relationship was investigated for each century in our chronology, separating the non-event 356 357 years and the LBM event years, after the precautionary removal of the 2 years preceding and following 358 the event years. The non-event years showed the expected positive relationship between δ^{18} O and δ^{2} H, although the variation in the slopes and R^2 values was large (slope = 1.9 to 8, $R^2 = 0.11$ to 0.73; Figs 6, 359 7). The event years showed a wider range of O-H relationship in terms of slope and strength (slope = -360 4.5 to 8.9, $R^2 = 0.039$ to 0.82; Figs 6, 7), with negative relationships occurring in the first part of the 361 362 chronology (i.e. the 1300s to 1500s) and positive relationships occurring from the 1600s to 1900s. No LBM outbreak was recorded in the 2000s. 363
- We then compared the strengths of these correlations with the estimated average centennial temperatures (Fig. 7). Higher summer temperatures corresponded to a more positive O–H relationship in non-event years and improved the goodness-of-fit for linear regression models for each century, with a peak in the 1900s when R² reached 0.58, indicating a strong influence of temperature. Average summer (JJAS= June, July, August, September) temperatures did not correspond to the slope or R² of the O–H relationship in event years across the centuries (Fig. 7), indicating a large between-century variability in event years and a decoupling from summer temperature.
- 371

372 **4. Discussion**

374

373 This study is among the first to investigate $\delta^2 H$ from tree-ring cellulose, and it demonstrates the

375 trees. We found δ^2 H changes particularly informative when considered in relation to δ^{18} O, as the

usefulness of this parameter for assessing past physiological changes in the metabolic performance of

- decoupling of the two water isotopes indicates an overwriting of the hydrological signal originating from
- 377 the source water by physiological processes, with the use of stored NSCs leading to an ¹⁸O-depletion
- and ²H-enrichment of cellulose. The distinct processes involved in LBM outbreaks, which interfere with
- 379 the recording of the climatic signal by hindering leaf-level functions and triggering resource relocation,
- 380 enabled us to isolate outbreak-specific non-climatic patterns of enrichment or depletion of the stable
- isotopic ratios in cellulose. Here we discuss the processes which likely influence the isotopic signals
- 382 stored in tree rings, from disruption of the functioning of the photosynthetic system to stimulation of the
- 383 heterotrophic mechanism, resulting in loss of strength of the O–H relationship in outbreak years.

4.1. Processes modifying isotopic ratios during outbreak events

385 Our analyses demonstrated the consistent and significant impact of LBM outbreaks on the isotopic 386 composition of tree-ring cellulose over the last seven centuries. Outbreak years showed ²H-enrichment, contrasting the ¹⁸O- and ¹³C-depletion, in comparison to non-event years, confirming our Hp1 (Figs 1, 387 388 3). Isotope patterns in the years after the outbreaks showed only minor prolonged effects and were 389 overall not significantly different from the values in pre-event years (Figs 3, S.1). This finding indicates 390 that relationship trends involving isotopes recovered from LBM disturbance faster than TRW and MXD, 391 which have a shown an impact for up to 7 years after an outbreak (Peters et al., 2017; Castagneri et al., 2020). This suggests that the isotopic signal recorded in outbreak years is directly connected to the LBM-392 393 induced defoliation processes and can therefore be used to trace the numerous cascade effects of the defoliation outbreaks on a tree's physiological reaction as a whole. Two major processes can be related 394 395 to the shift in the recorded isotopic signals: (i) disruption of the gas exchange mechanisms at the foliar 396 level and (*ii*) changes in the autotrophic and heterotrophic processes (i.e. NSCs remobilization).

397 Regarding the first process, caterpillars perforate needle surfaces as they feed, disrupting stomatal 398 regulatory functions and water fluxes over the course of several weeks (Kress & Saurer et al., 2009; 399 Weidner et al., 2010). The disrupted tissues of the damaged needles allow a free flow of source water, 400 which is now in direct contact with the atmosphere, resulting in higher evaporation and ultimately 401 desiccation of the needles (Weidner et al., 2010). The changes in stomatal conductance, transpiration 402 and leaf temperature drastically affect evapotranspiration processes and thus the isotopic fractionations 403 and enrichment levels of leaf water (Kress & Saurer et al., 2009; Weidner et al., 2010). The defoliation-404 induced changes in needle water are then transferred to the tree rings if enough carbohydrates are still produced for cell formation and assuming that δ^{18} O and δ^{2} H values in tree-ring cellulose are (at least 405 partially) the result of the leaf and source-water isotopic signals (Roden & Ehleringer, 2000). However, 406 no clear difference between LBM-affected trees and non-affected ones was observed for $\delta^{18}O$ values of 407 12

408 leaf assimilates over the course of the growing season (Peters et al., 2020), suggesting that disruption of 409 the gas-exchange system plays a smaller role than generally assumed. Similar investigations of δ^2 H in assimilates are still lacking. On the contrary, δ^{13} C is strongly connected to even small changes in 410 photosynthesis and stomatal conductance, which can result in a modification of the δ^{13} C in tree rings 411 412 (Farquhar et al., 2007; Kress & Saurer et al., 2009). However, considering the loss of stomatal regulation described above, it is interesting that the changes in δ^{13} C in outbreak years were much smaller than the 413 shifts observed for the water isotopes. This again suggests a minor impact of leaf-functioning changes 414 415 on the isotopic signal recorded in tree rings (Figs 1, 3). The duration, timing and intensity of a defoliation 416 event might play an important role in the strength of the isotopic signal; however, no significant 417 difference in the intensity of the outbreaks was found (data not presented). Further, defoliation can occur 418 at different stages of EW production, and if it occurs at a later growth stage it could allow a "normal-419 functioning" canopy signal to be recorded in the tree rings. Nevertheless, research indicates that wood 420 production remains low after defoliation (Peters et al., 2020), suggesting that regardless of the timing of the outbreak, the "budmoth rings" (Baltensweiler et al., 2008; Büntgen et al., 2009; Hartl-Meier et al., 421 422 2017; Arbellay et al., 2018) characteristic changes should be visible both in wood anatomy and isotopic 423 ratios.

Regarding the second process, defoliation has a major and two-fold influence on the C-use strategy. 424 First, it hinders the production of fresh assimilates, and second, it triggers reserve remobilization for the 425 secondary flushing and formation of new needles (Baltensweiler et al., 1977; Handa et al., 2005; 426 427 Wermelinger, Forster & Nievergelt, 2018). Further, it has previously been observed that cell production is not fully arrested during an LBM outbreak, even in strongly defoliated trees. This indicates a 428 429 continuous use of reserves for tree-ring production, even though new needle production is the reserve's 430 main sink (Peters et al., 2020). It has been shown that the use of C reserves causes a ²H-enrichment in 431 tree-ring cellulose (Kimak et al., 2015; Cormier et al., 2018; Lehmann et al., 2021), and it is clear that 432 at least part of the significant ²H-enrichment recorded during LBM outbreaks indicates processes of 433 NSC remobilization and changes in the carbohydrate dynamics in the needles (Peters et al., 2020). The depleted ¹⁸O values could also partially reflect the increased use of NSCs, as metabolites become 434 increasingly 18 O depleted when travelling in the stem, due to exchange with isotopically depleted water 435 (Gessler et al., 2014). On the contrary, we would have expected a ¹³C-enrichment effect connected to 436 437 the use of starch reserves (Le Roux et al., 2001; Helle & Schleser, 2004), as observed for spruce budworm (Simard et al., 2008). This does not seem to be the case for LBM outbreaks, but it is in line 438 439 with recent leaf-level observations that gas exchange and photosynthesis drive δ^{13} C variation when post-440 photosynthetic ¹³C fractionations are low, and only small differences exist between the δ^{13} C in sugars 441 and starch reserves (Lehmann et al., 2019).

442 To summarize, the triple isotope signatures of defoliated larch trees are the result of a mixture of the 443 above-mentioned leaf-level processes and C-use strategies, which explains why the interpretation of the impact of LBM on δ^{18} O and δ^{13} C variations has been ambiguous in the past (Kress & Saurer *et al.*, 2009; Weidner *et al.*, 2010). Only a minor ¹⁸O-depletion in tree-ring cellulose as a result of LBM outbreaks was reported by Kress and Saurer *et al.* (2009), who studied the same chronologies as used in the present

- 447 study, albeit on a shorter time scale. The authors speculated that lower temperatures influenced the
- 448 source-water signal while also triggering outbreaks. However, the reliability of the δ^{18} O climatic signal
- 449 during outbreak events was questioned by Weidner *et al.* (2010), who showed relatively low δ^{18} O in 450 outbreak years. Similarly, a low impact of LBM on δ^{13} C values was observed by both Kress and Saurer
- 451 *et al.* (2009) and Weidner *et al.* (2010). However, thanks to the extremely long time-scale investigated
- 452 in our study (i.e. 97 outbreaks), in combination with the event-based normalization, an overall significant
- 453 ¹⁸O- and ¹³C-depletion connected to outbreak years was apparent (Fig. 3). Thus, our findings support
- the questioning of the climatic signal recorded by tree-ring isotopes in outbreak years. Therefore, we
- 455 recommend the removal of outbreak years for high-frequency climate reconstruction studies using larch.

456 **4.2. Lack of intra-annual differences in isotopic ratios**

For some deciduous species, EW ¹⁸O- and ²H-enrichment compared with LW has been observed and 457 linked to post-photosynthetic processes associated with the remobilization of stored starch and a lack of 458 fresh photo-assimilates for cellulose synthesis during the early vegetative season (Wilson et al., 1978; 459 Epstein, 1995; Kimak, 2015; Nabeshima et al., 2018). These observations drove our Hp2 that 460 differences in isotopic ratios in LBM years could be attributed to a strong early-season remobilization 461 bias due to the imbalance between the thickness of EW and LW (as our long-term reconstruction was 462 463 based on annual tree-ring values). However, contrary to our expectations, there was no significant 464 difference between the EW and LW isotopic ratios in event years or the pre- and post-event years for 465 the three outbreaks in the 1900s (Fig. 4). This difference from earlier findings might be explained by the 466 fact that most previously tested species were also deciduous angiosperm species, for which tree growth 467 starts before leaf flush and therefore must reflect storage use (Nabeshima et al., 2018). Although larch 468 is also a deciduous conifer species, it does not appear to apply this strategy. Instead, C assimilation for 469 the formation of tree rings happens as an overflow mechanism of the fresh assimilates of the current year, as indicated by Peters *et al.* (2020). Intra-annual δ^{18} O and δ^{2} H measurements indicated very little 470 471 variation between EW and LW, suggesting that annually resolved measurements of LBM years (i.e. 472 whole rings) are not significantly impacted by the mass-balance effect of the narrow LW. The 473 particularly large variation during the LBM event in 1972 compared with that in 1981 and 2018 is 474 explained by the fact that the outbreaks were characterized by different defoliation intensities (high in 475 1972, low in 1981 and moderate in 2018), which has been shown to dramatically impact the strength of 476 the resulting signals stored in the tree rings (Castagneri et al. 2020). Further, between-tree variations in 477 the intensity of defoliation at the same site during the same outbreak event have been observed (Peters 478 et al., 2020), and are another source of variation influencing the signal strength in average tree-ring 479 chronologies. On the contrary, the lack of difference between δ^{13} C in EW and LW is in line with the

481 and attributed a lack of ¹³C difference in EW and LW to high C turnover rates at these locations.

482 **4.3. LBM outbreaks impact the O–H relationship**

483 The striking outbreak-induced changes we observed in the relationship between $\delta^2 H$ and $\delta^{18}O$ support

- 484 our Hp3. They suggest that uneven shares of climatic, hydrological and physiological signals are
- recorded as water isotopes. In non-event years, the correlation between δ^{18} O and δ^{2} H was strong (r=0.5;
- 486 Table 1), suggesting a coherent dominance of the hydrological signal in the tree-ring isotopic ratios.
- 487 However, their correlation dropped dramatically in outbreak years (r=0.15), and the O–H relationship
- deteriorated significantly in the outbreak years and recovered only in the third year after the outbreaks
- 489 (Fig. 4).

490 Given that δ^{18} O and δ^{2} H share the same hydrological pathways (i.e. soil water and evaporation 491 enrichment, and temperature dependence; Iannone et al., 2010), a strong connection between the 492 isotopic ratios of the two elements in tree-ring cellulose would be expected (Dansgaard, 1964; Edwards 493 & Fritz, 1986; Brooks *et al.*, 2010). At a continental scale, it has indeed been reported that δ^{2} H and δ^{18} O 494 stored in tree-ring cellulose are correlated and reflect, at least partially, hydrological and temperature signals (Gray & Song, 1984; Saurer, Borella & Leuenberger, 1997; Vitali et al., 2021; Allen et al., 2022; 495 Lehmann et al., 2022). Nonetheless, recent studies showed that O and H isotopic responses of leaf water 496 497 can be different due to their different sensitivity to relative humidity and the isotopic composition of 498 water vapour (Cernusak et al., 2022), which might also influence the isotopic response of plant organic 499 matter. The O-H relationship in tree-ring cellulose has been found to be weak over 100-year 500 chronologies for several sites across Europe, indicating both sites- and species-specific variation (Vitali et al., 2021). In experimental settings, a decoupling of the two water isotopes during biochemical 501 502 processes and post-photosynthetic fractionations has been observed repeatedly (Yakir & Deniro, 1990; 503 Luo & Sternberg, 1992; Roden & Ehleringer, 2000; Cormier et al., 2018; Lehmann et al., 2019; 504 Lehmann *et al.*, 2022). The opposite patterns of δ^{18} O and δ^{2} H in outbreak years are likely due to the imprinting of storage used during stem wood formation, with longer-term storage compounds 505 exchanging with plant water, leading to an ¹⁸O-depletion and ²H-enrichment before cellulose synthesis. 506 At least for δ^{18} O, this would be in line with recent insights that ¹⁸O-depletion occurs as a result of post-507 photosynthetic isotope fractionation during the transport of sugars to sink tissues and during tree-ring 508 509 cellulose synthesis (Gessler *et al.*, 2014). While similar investigations have not been completed for $\delta^2 H$, 510 there is growing evidence of additional ²H-enrichment connected to the use of C reserves (Kimak et al., 511 2015; Cormier et al., 2018; Lehmann et al., 2021). Our results of an O-H decoupling clearly support 512 these concepts, highlighting the important impact of resource remobilization on the $\delta^2 H$ signature. 513 Further, the O-H relationship was still not fully restored during the 2 years after the outbreaks (Fig. 4), 514 which might indicate a longer reliance of larch trees on reserves. This suggests that a larger share of the

515 isotopic signal can be attributed to reserve remobilization, or to trailing canopy-level defoliation-induced

- 516 processes after the main outbreak year.
- 517

518 **4.4. Centennial changes in temperature affect the O–H relationship but not in outbreak years**

519 Our study included not only the long-term variation in environmental conditions, ranging from cooler 520 periods associated with the Little Ice Age (1350–1850) to warmer ones in the 1900s and 2000s (Büntgen 521 et al., 2006) but also short-term temperature variation around each LBM event year. During the past, seven centuries, LBM outbreaks occurred in years that were colder than non-event years (Fig. 5). As 522 suggested by Kress and Saurer et al. (2009), below-average temperatures of the vegetative season peak 523 (corresponding to July to August in the Lötschental) appear to trigger LBM outbreaks, as they aid in the 524 synchronization of larvae development and needle maturation (Baltensweiler et al., 1977; Asshoff & 525 526 Hättenschwiler, 2006). Although the LBM cycle is quite regular on average, there is still variability by 527 several years in the recurrence time, which could be related to this temperature influence (Esper et al., 2007). Further, uncertainties in the identification of outbreak years should be acknowledged, as small 528 529 rings with a low density could be related to climatic conditions rather than to outbreak events. 530 Nonetheless, the overall consistent lower temperatures in outbreak years are in line with the resulting ¹⁸O-depleted source water. In combination with the ¹⁸O- depletion due to the use of remobilized reserves 531 (Gessler *et al.*, 2014), ¹⁸O-depleted source water could explain the strong change in the δ^{18} O signal in 532 533 event years. Further, the lower temperature in LBM outbreak years could also explain the ¹³C-depletion, which is known to decrease with higher stomatal conductance under cool conditions (Barbour et al., 534 535 2004; Kress & Saurer et al., 2009). On the contrary, the ²H-depletion connected to cooler summer conditions appears to be lost in the δ^2 H signal, which likely indicates a masking of the hydrological 536 537 signal by metabolic processes compared with δ^{18} O.

The centennial shifts in temperature over our study period were reflected in the O-H relationship of 538 non-event years, which was strongest in warmer centuries. The slope and R² of the O-H relationship in 539 540 non-event years clearly increased with increasing average centennial temperature (Figs 6, 7), indicating 541 a strong coupling between the two water isotopes, ergo a clear hydrology- and evaporation-driven signal. 542 On the contrary, temperature had no impact on the O-H relationship in LBM outbreak years, as shown 543 by the variable and non-significant relationships between the reconstructed summer temperatures and 544 the O–H slopes or R² values (Fig. 6, Table S.2). This supports our Hp4 that the isotopic signal recorded 545 in outbreak years is more influenced by physiological aspects that are largely independent of summer 546 temperature.

547 **5.** Conclusions

548 LBM outbreaks produce a typical triple isotope signature composed of ¹⁸O-depletion, ¹³C-depletion, ²H-

549 enrichment, and an unusual decoupling between δ^2 H and δ^{18} O. Our results show that: (*i*) isotopic ratios 16

are sensitive indicators of the effects of defoliation and, by extension, of changes in autotrophic vs heterotrophic processes; (*ii*) non-climatic stressors cause a weakening of the hydrological relationship

- between the two water isotopes, and (*iii*) across the centuries the hydrological relationship between δ^{18} O
- and $\delta^2 H$ is temperature-dependent except in outbreak years, indicating a loss of the climatic signal.
- 554 Therefore, we conclude that when the O–H relationship is weak, the use of stored reserves contributes
- more to the isotopic signal. In the future, combined assessments of $\delta^2 H$ and $\delta^{18}O$ chronologies will
- enable the retrospective analysis of physiological signals in long tree-ring chronologies. Such analyses
- 557 will provide new information on tree functioning, especially in contexts where the climatic signal is not
- the main signal driving isotopic ratios, for example during defoliator outbreak events. Further, tree-ring
- stable isotopes have the potential to improve our understanding of tree responses to past insect outbreaks,
- 560 due to their annual resolution and long chronologies, and could prove key to identifying stressors which
- 561 impact NSC use.
- 562

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574 Author contributions

- 575 MS, MML and VV conceptualized the study. VV carried out the analyses with support from RLP. VV
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577 Data storage

- 578 The data used in this article that has not been previously published will be stored on the EnviDat
- 579 (www.envidat.ch) data portal of the Swiss Federal Institute for Forest, Snow and Landscape Research
- 580 WSL.

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Fig. 1 (a–e) Chronologies of the tree-ring parameters δ^2 H, δ^{18} O, δ^{13} C, tree-ring width (TRW) and indexed maximum latewood density (MXD) for the investigated period 1256–2004 (Büntgen *et al.*, 2006; Esper *et al.*, 2007; Kress & Saurer *et al.*, 2009; Hangartner *et al.*, 2012). Red dots highlight values during LBM events. (f–l) Mean LBM responses (with standard errors) of the super-imposed 79 events of tree-ring δ^2 H, δ^{18} O, δ^{13} C, TRW index and MXD index. Significance levels were determined using a t-test comparing each variable and year around the outbreak event to 0 for the isotopes and to 1 for TRW and MXD. Blue bars represent 2 pre-event years, red bars the event year, and orange bars 2 post-event years. * P<0.05.





Fig. 2 Earlywood (EW) and latewood (LW) isotope values for the three measured larch budmoth outbreak
events (1972, 1981, 2018; shaded areas), including the year before and after the outbreak. In each year EW
and LW isotope values were not significantly different (P>0.05).





Fig. 3 Boxplots of event-based normalized differences in δ^2 H, δ^{18} O and δ^{13} C between the 5 years before (preevent years) a larch bud moth outbreak, the year of the outbreak (event year), and the 5 years after (postevent years) the outbreak, for each of the 79 larch budmoth outbreaks. Points indicate annual measurements. Significant differences between groups are indicated by asterisks (* P<0.05; ** P<0.01; *** P<0.001; **** P<0.001).

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Fig. 4 Linear relationships between $\delta^{18}O_{norm}$ and $\delta^{2}H_{norm}$ in the 4 years before (pre-event) and after (postevent) each of the 79 larch budmoth outbreaks. The fitted linear model equations and explained variance (R²) are given for each year. Colours indicate the years before or after the outbreak events. The remaining combinations of the relationships between $\delta^{18}O$ and $\delta^{13}C$ are given in the Supplementary Material (Figs S.2, S.3, S.4).





905Fig. 5 Reconstructed mean summer (JJAS = June, July, August, September) temperatures for the last seven906centuries (from Büntgen *et al.*, 2006), separated into event and non-event years, and ± 1 and ± 2 years around907the outbreak events (pre- and post-event years). The mean summer temperature of event years was908significantly different from the mean summer temperature of non-event years in the centuries 1300, 1400909and 1700.



912 Fig. 6 O–H relationship and the respective fitted linear model for each century (indicated by different

913 colours) for event years (a), and non-event years (b). The non-event years exclude the ± 2 years around

914 each LBM event. The equations, explained variance (R²) and significance (P values) for the fitted linear

915 model are given in Table S.2. No event years were recorded in the 2000s.



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average summer (JJAS= June, July, August, September) temperatures (from Büntgen *et al.*, 2006). For
values see Table S.2.

922Table 1 Pearson correlation matrices (r) between the tree-ring parameters in event years (a) and in non-923event years (b) for the period 1256–2004. Significant values (a. P<0.001 if r>0.36; b. P<0.001 if r>0.16) are924indicated in bold.

⁹²¹

	a.	Event years					
			$\delta^{18}O$	$\delta^{13}C$	$\delta^2 H$	TRW index	MXD index
		δ^{18} O		0.41	0.15	-0.15	0.06
		δ^{13} C			0.08	-0.14	0.14
		$\delta^2 H$				0.07	0.14
		TRW index					0.24
	b.	Non-event yea	rs				
		U	$\delta^{18}O$	$\delta^{13}C$	$\delta^2 H$	TRW index	MXD index
		δ^{18} O		0.36	0.50	0.16	0.32
		$\delta^{13}C$			0.18	0.12	0.32
		$\delta^2 H$				0.01	0.08
		TRW index					0.59
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