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An annual-resolution stable isotope record from Swiss subfossil pine trees growing in the Late Glacial --Manuscript Draft--

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Corresponding Author:	Maren Pauly Bath Spa University Bath, UNITED KINGDOM					
First Author:	Maren Pauly					
Order of Authors:	Maren Pauly					
	Gerhard Helle					
	Ulf Büntgen					
	Lukas Wacker					
	Kerstin Treydte					
	Frederick Reinig					
	Chris Turney					
	Daniel Nievergelt					
	Bernd Kromer					
	Michael Friedrich					
	Adam Sookdeo					
	Ingo Heinrich					
	Frank Riedel					
	Daniel Balting					
	Achim Brauer					
Abstract:	Previous studies have suggested that the Late Glacial period (LG; ~ 14,600 – 11,700 cal BP) was characterised by abrupt and extreme climate variability over the European sector of the North Atlantic. The limited number of precisely dated, high-resolution proxy records, however, restricts our understanding of climate dynamics through the LG. Here, we present the first annually-resolved tree-cellulose stable oxygen and carbon isotope chronology (δ 18Otree, δ 13Ctree) covering the LG between ~14,050 – 12,795 cal BP, generated from a Swiss pine trees (P. sylvestris; 27 trees, 1255 years). Comparisons of δ 18Otree with regional lake and ice core δ 18O records reveal that LG climatic changes over the North Atlantic (as recorded by Greenland Stadials and Inter-Stadials) were not all experienced to the same degree in the Swiss trees. Possible explanations include: (1) LG climate oscillations may be less extreme during the summer in Switzerland, (2) tree-ring δ 18O may capture local precipitation and humidity changes and/or (3) decayed cellulose and various micro-site conditions may overprint large-scale temperature trends found in other δ 18O records. Despite these challenges, our study emphasises the potential to investigate hydroclimate conditions using subfossil pine stable isotopes.					

An annual-resolution stable isotope record from Swiss subfossil pine trees growing in the Late Glacial

Maren Pauly^{1,2,3*}, Gerhard Helle^{1,2}, Ulf Büntgen^{4,5,6}, Lukas Wacker⁷, Kerstin Treydte⁵, Frederick Reinig⁵, Chris Turney⁸, Daniel Nievergelt⁵, Bernd Kromer⁹, Michael Friedrich¹⁰, Adam Sookdeo^{7,11}, Ingo Heinrich¹, Frank Riedel², Daniel Balting^{1,12}, Achim Brauer^{1,13}

*<u>m.pauly@bathspa.ac.uk</u>. Present address: Bath Spa University, Newton Park, Bath UK BA2 9BN

1. GFZ German Research Centre for Geosciences, Section 43 'Climate Dynamics and Landscape Evolution' (Potsdam, Germany)

2. Free University of Berlin, Department of Earth Sciences, Section of Palaeontology (Berlin, Germany)

3. Bath Spa University, School of Science (Bath, United Kingdom)

4. University of Cambridge, Department of Geography (Cambridge, United Kingdom)

5. Swiss Federal Institute for Forest, Snow and Landscape Research WSL, Dendrosciences Group (Birmensdorf, Switzerland)

6. Global Change Research Centre and Masaryk University (Brno, Czech Republic)

7. ETH Zürich, Department of Physics, Ion Beam Physics Laboratory (Zürich, Switzerland)

8. University of New South Wales, Chronos ¹⁴Carbon-Cycle Facility Changing, and Earth Research Centre, School of Biological, Earth and Environmental Sciences (Sydney, Australia)

9. University of Heidelberg, Institute of Environmental Physics (Heidelberg, Germany)

10. University of Hohenheim, Institute of Botany (Stuttgart, Germany)

11. University of New South Wales, Chronos ¹⁴Carbon-Cycle Facility (Sydney, Australia)

12. Alfred Wegener Institute, Climate Sciences/Paleo-climate Dynamics (Bremerhaven, Germany)

13. University of Potsdam, Institute for Earth and Environmental Sciences (Potsdam, Germany)

1 <u>Abstract</u>

Previous studies have suggested that the Late Glacial period (LG; ~ 14,600 – 11,700 cal BP) was characterised by abrupt and extreme climate variability over the European sector of the North Atlantic. The limited number of precisely dated, high-resolution proxy records, however, restricts our understanding of climate dynamics through the LG. Here, we present the first annually-resolved tree-cellulose stable oxygen and carbon isotope chronology ($\delta^{18}O_{tree}$, $\delta^{13}C_{tree}$) covering the LG between ~14,050 – 12,795 cal BP, generated from a Swiss pine trees (*P. sylvestris*; 27 trees, 1255 years).

9 Comparisons of $\delta^{18}O_{tree}$ with regional lake and ice core $\delta^{18}O$ records reveal that LG climatic changes over the North Atlantic (as recorded by Greenland Stadials and Inter-10 Stadials) were not all experienced to the same degree in the Swiss trees. Possible 11 explanations include: (1) LG climate oscillations may be less extreme during the 12 summer in Switzerland, (2) tree-ring δ^{18} O may capture local precipitation and humidity 13 14 changes and/or (3) decayed cellulose and various micro-site conditions may overprint large-scale temperature trends found in other δ^{18} O records. Despite these challenges, 15 our study emphasises the potential to investigate hydroclimate conditions using 16 17 subfossil pine stable isotopes.

18 <u>Keywords</u>: Tree-ring cellulose, δ^{13} C, δ^{18} O, Late Glacial, Switzerland, Climate

19 <u>Highlights</u>

Millennial-length stable isotope chronologies of δ^{13} C and δ^{18} O developed from 20 -27 Swiss subfossil pine trees, covering the Late Glacial (~14,050 – 12,795 cal 21 22 BP); Two periods of extreme $\delta^{18}O_{tree}$ depletions parallel known North Atlantic 'cool 23 periods' recorded in Greenland $\delta^{18}O_{NGRIP}$ (GI-1c, GS-1), while another LG 24 oscillation (GI-1b) is not clearly expressed in $\delta^{18}O_{tree}$ 25 Trees with less extensive decay have significantly higher inter-isotope 26 - $(\delta^{18}O_{tree}: \delta^{13}C_{tree})$ correlations, and decay seemed to impact the stability of 27 $\delta^{13}C_{tree}$ more strongly over $\delta^{18}O_{tree}$ 28

29 **1. Introduction**

30 <u>1.1 The Late Glacial & climate proxy records</u>

31 The Late Glacial (LG; ~ 14,600 – 11,700 cal BP) in the North Atlantic (NA) region is characterised by extreme and abrupt climate change, including at least four prominent 32 "cold periods". In Greenland, these events are defined by ice core $\delta^{18}O$ downturns, i.e. 33 substages within the Greenland Stadials (GS) and Inter-Stadials (GI); GI-1d 34 (Rasmussen et al. 2006), GI-1c (Rasmussen et al. 2014), GI-1b (Björk et al. 1998) and 35 GS-1 (Mayle et al. 1999, Rasmussen et al. 2006, Steffensen et al. 2008). Greenland 36 ice core δ^{18} O records are regarded as key datasets for comparison of hydroclimate 37 indices across the NA region (Figure 3) (Alley et al. 1993, Rasmussen et al. 2014, 38 39 Steffensen et al. 2008), due to their unrivalled temporal resolution (annual in parts) and extent (tens of thousands of years) as well as its sensitivity to NA precipitation and 40 temperature variability. 41

In central Europe, δ^{18} O in carbonates from several lake sediments offer sequences 42 that can be directly compared to Greenland and portray broadly consistent signals of 43 44 inferred temperature change (δ^{18} O drop) in the low frequency domain (e.g. Lauterbach 45 et al., 2011, Schwander et al. 2000, Siegenthaler et al. 1984, van Raden et al. 2012, 46 2013, Verbruggen et al. 2010, von Grafenstein et al. 2000). Pronounced excursions in lake carbonate $\delta^{18}O$ (negative) and speleothem $\delta^{18}O$ (positive) records have 47 pinpointed similar cold periods across central Europe including Germany (von 48 Grafenstein et al. 1999, Rach et al., 2014), Switzerland (Lotter et al. 1992, 1999, 2000, 49 50 Verbruggen et al. 2010) and Austria (Lauterbach et al. 2011). The characterisation and 51 drivers of these temperature fluctuations, however, remains highly uncertain (Rach et al. 2014, Rasmussen et al. 2014). The low frequency climate variability inherent in lake 52

records provides information about decadal-scale variability on local to regional
scales. However, the inter-annual features of such events are indistinguishable.

55 In this respect, tree-rings offer an annually resolved archive not only in terms of dating like varved lake sediments, but also with respect to proxy information from parameters 56 (e.g. ring width, maximum latewood density, stable isotope ratios) measured on 57 58 sequences of individual tree rings. However, due to the relatively short lifespans of 59 individual trees, it is challenging to maintain low frequency climate information within millennial records (Cook et al. 1995, Esper et al. 2002), as some records have 60 61 revealed limited power at multi-centennial time scales (e.g. Saurer et al. 2012, Cook et al. 2019). Fortunately, it has been demonstrated that subfossil tree-ring δ^{18} O 62 records can capture large-scale climate deterioration, revealed through increased 63 variability and distinct negative excursions (Pauly et al. 2018). 64

65 <u>1.2 Climate proxies in tree-ring stable isotopes</u>

The growth increments (ring width), wood density and stable isotope ratios of numerous modern species in Europe have been correlated significantly to relevant climatic parameters (temperature, precipitation, humidity) in numerous studies (e.g. Saurer et al. 2012, Büntgen et al. 2011, Esper et al. 2014). Signal strengths were found depending on the local limiting factors. Nevertheless, tree-ring widths, density and stable isotopes have the capacity to trace inter-annual variability and extremes, which are characteristic of climate change (e.g. drought, wet periods, storms).

 δ^{13} C of tree-ring cellulose (δ^{13} C_{tree}) is based on the δ^{13} C of the atmospheric CO₂ source which contains no direct climatic signal. Its climate signature originates from fractionations during photosynthesis at the leaf or needle level (Farquhar et al., 1982). In contrast, δ^{18} O of tree-ring cellulose (δ^{18} O_{tree}) is related to the δ^{18} O of the

precipitation source via soil water. δ^{18} O of soil water constitutes the δ^{18} O input to the 77 arboreal system and represents an average δ^{18} O over several precipitation events 78 modified by partial evaporation from the soil (depending on soil texture and porosity) 79 and by a possible time lag, depending on rooting depth (Saurer et al. 2012). $\delta^{18}O_{\text{tree}}$ is 80 further dependent on two tree-internal processes: evaporative ¹⁸O-enrichment of leaf 81 or needle water via transpiration, as well as biochemical fractionations and isotopic 82 exchange of δ^{18} O with trunk water during cellulose biosynthesis (e.g. Roden et al., 83 2000, Barbour, 2007, Kahmen et al. 2011, Treydte et al., 2014 and citations therein). 84

85 <u>1.2.1 Climate signals in stable isotopes of modern Swiss tree-ring records</u>

Modern tree-ring stable isotope chronologies in Switzerland have been found to have 86 87 relatively strong correlations with a variety of summer (JJA) meteorological parameters. For example, $\delta^{18}O_{tree}$ has been correlated with summer precipitation 88 extremes (various species: Saurer et al. 2008), relative humidity and sunshine duration 89 90 (Pinus sylvestris: Saurer et al. 1997, Larix decidua: Kress et al. 2010, Pinus sylvestris, *Picea abies, Fagus sylvatica, Fraxinus excelsior:* Saurer et al. 2012) and $\delta^{13}C_{tree}$ with 91 92 summer temperature (various species: Saurer et al. 2008) and drought conditions 93 (Larix decidua: Kress et al. 2010). Synoptic patterns and associated indices (e.g. cyclone and anticyclone activity, air pressure) have also been reconstructed from 94 95 Swiss tree-ring $\delta^{18}O_{tree}$, with stronger correlations revealed during extreme years or periods (Saurer et al. 2012). Despite the success in tree-ring isotopes reflecting 96 climate parameters, the relationships have been found to be unstable through time 97 (Kress et al. 2010, Reynolds-Henne et al. 2007, Seftigen et al. 2011) as 98 interdependency between meteorological variables also often changes depending on 99 100 the climate state. As a result, Saurer et al. (2012) has suggested the integration of 101 multiple climate parameters, with focus on reconstructing acute and widespread

synoptic deviations, rather than individual meteorological parameters for long tree-ringstable isotope records.

104 <u>1.2.2 Subfossil trees and stable isotope records</u>

105 The availability of robust tree-ring chronologies is limited prior to the Holocene and 106 only a few subfossil records have been established for the LG (e.g. Reinig et al. 2018 107 and references therein). Absolutely dated tree-ring chronologies begin in 12 325 cal BP with the Preboreal Pine Chronology (PPC) from Germany followed by the 108 Holocene Oak Chronology (HOC) (Friedrich et al. 2004). Beyond 12 325 cal BP, 109 floating tree-ring chronologies exist for Switzerland (Reinig et al. 2018), Germany (e.g. 110 111 Friedrich et al. 1999, 2001, 2004), France (Miramont et al. 2000a, 2000b, 2011), Italy 112 (Casadoro et al. 1976), USA (Leavitt et al. 2006, Panyushkina and Leavitt 2007, 2013), New Zealand (Hogg et al. 2013, 2016) and Tasmania (Barbetti et al. 2004, Hua et al. 113 2009). Scots pine trees from a floating tree-ring chronology (Reinig et al. 2018) was 114 115 used for this study.

Some stable isotope records exist from subfossil tree-ring sequences from the 116 117 prehistoric Holocene (Aguilera et al. 2011, Boettger, et al. 2003, Edvardsson, et al. 118 2014, Frumkin 2009, Helama, et al. 2015, 2018, Helle 1994, Helle and Schleser 1998, 119 Hunter, et al. 2006, Leavitt, et al. 2006, Mayr et al. 2003) and very few from the LG 120 (Pauly et al. 2018, Wagner 2010, Becker et al. 1991). Many of these records lacked 121 the sample replication to build a chronology (Aguilera et al. 2011, Frumkin 2009, Hunter et al. 2009), or developed a chronology with a limited temporal resolution or 122 123 scale (Becker et al. 1991, Evardsson et al. 2013, Helama et al. 2015, Leavitt et al. 124 2006, Mayr et al. 2003,). Only a few records exist with robust chronologies at annual (Pauly et al. 2018, Wagner et al. 2010) or decadal (Helama et al. 2018) resolution 125

during this time. Higher sample replication ($n_{trees} > 5$) has been possible at decadal resolution compared to lower sample replication ($n_{trees} = 1-4$) at annual resolution due to the limited availability and quality of subfossil wood material (See Supplementary Information 1).

130 2. Material and Methods

131 <u>2.1 Stable Isotopes from pine trees in Switzerland</u>

132 To develop a new tree-ring stable isotope chronology (hereafter referred to as CH-133 ISO) wood material from the improved Swiss Late Glacial Master Chronology, SWILM 134 (Kaiser et al. 2012, Schaub et al. 2008, Friedrich et al. 1999) was used. While still floating, SWILM is the most robust and oldest LG tree-ring chronology currently 135 136 available in the Northern Hemisphere, representing a very well-preserved LG forest. It covers 1500 years (~14 200 - 12 635 cal BP, Reinig et al. 2018) and comprises of 137 trees from four pine stands in the Zürich region: Gaenziloo (ntrees=55), Landikon 138 (n_{trees}=28), Daettnau (n_{trees}=46) and Binz (n_{trees}=187). The tree-ring chronology is 139 plotted according to a local timescale "Zürich Scale" (representing floating positions 140 prior to radiocarbon dating) as well as under cal BP. In conjunction with several 141 hundred new ¹⁴C measurements of high quality - not only in terms of replication, but 142 also resolution (annual) (Reinig et al. 2020) - the relative positioning of the SWILM 143 with respect to IntCal13 (Reimer et al. 2013) was greatly improved (Reinig et al. 2018), 144 145 resulting in a dating uncertainty of \pm 8 years (2 σ error). By independent interhemispheric ¹⁴C wiggle matching with New Zealand's subfossil Kauri (Hogg et al. 146 2016), the consolidated SWILM used in this study was shifted by +35 years with 147 respect to IntCal13 (Sookdeo et al. 2019). 148

149 A subset of 27 trees was chosen from the SWILM wood material to establish a pair of oxygen and carbon stable isotope chronologies from tree-ring cellulose at annual 150 resolution (CH-ISO, Figure 1), covering approximately 14 050 - 12 795 cal BP. 151 152 Cellulose was extracted from wholewood material of individual tree-rings (Wieloch et al. 2011, Schollaen et al. 2017), homogenised and freeze-dried prior to being weighed 153 154 and packed (silver capsules (ø3.3x4mm) for stable isotope measurement (Delta V, Thermofisher Scientific Bremen; coupled with TC/EA HT at 1400°C). Results were 155 compared against international and lab-internal reference material (IAEA-CH3, IAEA-156 CH6 and Sigma-Aldrich Alpha-Cellulose) using two reference standards with 157 widespread isotopic compositions for a single-point normalisation (Paul, Skrzypek, 158 159 and Forizs 2007). Final isotope ratios are given in δ value, relative to VSMOW (δ^{18} O) and VPDB (δ^{13} C), with replication reproducibility of ±0.3‰ (δ^{18} O) and ±0.15‰ (δ^{13} C). 160

161 2.2 Data Analysis

162 Tree-ring records (δ^{13} C and δ^{18} O) were z-scored, as outlined in *method one* from Hangartner et al. (2012) due to the high variability in absolute levels (‰) of individual 163 trees. Pearson's correlations between (1) intra-tree isotope series of δ^{13} C and δ^{18} O 164 (C:O) and (2) inter-tree δ^{18} O were completed to investigate whether carbon and 165 oxygen varied in tandem (thus controlled by similar environmental conditions) and to 166 what degree δ^{18} O was consistent across concurrently growing trees (i.e. the 167 population signal). Inter-tree δ^{18} O correlations were compared with sample replication 168 using a linear model to explore the potential chronological bias. 169

170



Figure 1. Subfossil trees in Switzerland: (a) Temporal distribution of a subset of subfossil tree-ring chronologies from 4 Swiss sites that make up the SWILM chronology: (1) Landikon (green, $n_{trees} = 28$), (2) Gaenziloo (blue, $n_{trees} = 55$), (3) Binz (orange, $n_{trees} = 170$) and (4) Daettnau ($n_{trees} = 9$). Additional groups of Binz and Daettnau unrelated to CH-ISO are not pictured. Site locations in (c) map, coloured according to (a). Trees were dated using high resolution radiocarbon (NH SWILM), wiggle-matched with decadal Southern Hemisphere Towai chronology. (b) Displays a subset of highest resolution dating results (timespan highlighted in a). (d) Tree replication for stable isotope chronology (CH-ISO). The 'full tree' represents the entire length of sample where tree-ring widths have been measured and cross-dated (Reinig et al. 2018, Kasier et al., 2012) and 'subset' indicates rings from full tree used for stable isotope analysis. Resulting individual stable isotope chronologies of carbon (e) and oxygen (f) with individual annual resolution in grey, 20-year individual means in black and chronology mean in green (carbon) and purple (oxygen).

171 **3. Results**

172 <u>3.1 Tree-ring stable isotopes</u>

173 The isotope chronologies are a bit shorter than the SWILM (Figure 1d) due to limitations in cutting tracks and rings from the delicate subfossil wood material and 174 avoidance of tree piths (to reduce any juvenile effects), with a total extent of ~14 050 175 - 12 795 cal BP. The trees were chosen based on various selection criteria, including 176 clearly expressed wood structure (allowing dissection of rings; ring-width ≥0.3mm), a 177 178 relatively long lifespan (>150 years), suitability for tree-ring cross-dating, and detailed radiocarbon series data. Despite this, many of the tree disks exhibited signs of 179 degradation and decay, which became apparent during the dissection process; 180 181 particularly along the outer rings comprising the sapwood which is softer than the inner heartwood and where the tree stumps were likely exposed to aqueous conditions. 182 Approximate level of decay was noted during the sample preparation (Table 1). 183 184 however, sections of very clearly visible decay and crumbled wood were avoided where possible during sample collection. The wood texture varied greatly between 185 trees of all decay states - from brittle and dry to hard and resinous - but this was not 186 quantitatively measured for this study. The sample replication varies throughout CH-187 ISO, with a maximum of 7, a minimum of 1 and a mean of 4. 188

189 <u>3.2 Time Series Analysis</u>

The inter-tree correlation of $\delta^{18}O_{tree}$ was highly variable (Figure 2a), with a mean of approximately 0.18, with average values ranging from 0.01 to 0.41. A significant relationship was found between the average inter-tree $\delta^{18}O$ correlation (Figure 2e) and the sample replication (r, Figure 2f) with an error of ± 0.01:





Figure 2. CH-ISO Chronology parameters: average series correlation of δ 18O (a) boxplot and (b) compared to sample replication; (c) average individual (intra-tree) correlations between stable isotopes (δ 18O: δ 13C), (d) mean tree-ring δ 18O (z-scored) with standard deviation shaded, (e) average chronology (inter-tree) δ 18O correlation, shaded from high correlation (green) to low correlation (yellow), and (f) chronology sample replication.

CH-ISO δ^{18} O correlation = 13.9 + 0.92*r ± error

195 This relationship demonstrates that sample replication is positively related to inter-tree 196 δ^{18} O corrections. However, this linear relationship may be biased by more data points and spread in lower replication values (e.g. rep = 2 & 3, Figure 2b). For much of the 197 chronology, the inter-tree δ^{18} O corrections average between 12-20% (Figure 2b,e). 198 The highest average inter-tree δ^{18} O correlations (>25) occur in two distinct periods 199 200 (~13 820 - 13 690 cal BP and 12 870 - 12 800 cal BP) with sample replications 201 between 2-3 trees; demonstrating instances when outliers contradict the 202 abovementioned linear relationship.

203 On the other hand, inter-tree correlation of $\delta^{18}C_{tree}$ were generally very low (<0.10), 204 and therefore further analysis will focus more on the suitability of $\delta^{18}O_{tree}$ as a 205 paleoproxy.

The covariance between the mean δ^{18} O and δ^{13} C is insignificant for the majority of trees within the chronology (Figure 2c). Only 9 of the 27 trees (33%) in CH-ISO demonstrated a δ^{18} O: δ^{13} C correlation R² value >0.10.

15 trees are strongly decayed (Table 1 "high": 56% CH-ISO), 9 trees show moderate 209 210 decay (Table 1 "medium": 33% CH-ISO) and 3 trees have little decay (Table 1 "low": 11% CH-ISO). Of those trees with significant inter-isotope correlations ($R^2 > 0.10$), one 211 212 had "high" decay, 5 had "medium" decay and 3 had "low" decay. Therefore, 100% of the trees that exhibited low levels of decay, 56% with moderate decay and 7% with 213 high decay showed strong inter-isotope correlations. Of these trees, the highest 214 correlations were found in low decay trees (mean $R^2 = 0.39$, n=3) compared to 215 moderately decayed (mean $R^2 = 0.16$, n=5) and highly (mean $R^2 = 0.15$; n=1) decayed 216 217 trees. In addition, the vast majority of the highly decayed trees (93%) had low inter-

isotope correlations (mean $R^2 = 0.03$, n=14). When comparing individual tree $\delta^{18}O$ to overlapping trees in the chronology, there was no significant difference in the average cross-correlation of different decay states – with a mean of 17.6% for low decay, 18.2% for moderate decay and 19.6% for high decay. The cross-correlations varied significantly, between 2% to 75% between tree pairs, averaging 7% to 40% for the population compared with each individual (Table 1, Figure 2e).

Sample	R ² (O:C)	P (O:C)	Decay	Average inter-tree cross correlation (%)	Timespan (cal BP)	Zurich Scale
LAN10	0.15	<0.001	High	18	13355 - 13241	1740-1854
GAE28	0.06	<0.05	High	20	13330 - 13183	1765-1912
GAE67	0.16	<0.001	Medium	14	13303 - 13045	1792-2050
K353	0.15	<0.001	Medium	24	13185 - 12991	1910-2104
K352	0.15	<0.001	Medium	7	13165 - 13005	1930-2090
GAE59	0.02	-	High	11	13091 - 12967	2004-2128
GAE60	0.01	-	High	5	12984 - 12842	2111-2253
GAE25	0.06	<0.05	High	32	12883 - 12795	2212-2309
GAE5	0.001	-	High	32	12904 - 12795	2192- 2365
LAN44	0.001	-	High	30	13904 - 13781	1191-1314
BIN138	0.19	<0.001	Medium	29	13838 - 13768	1257-1327
BIN137	0.01	-	High	40	13767 - 13697	1328-1398

LAN21	0.16	<0.001	Medium	11	13743 - 13555	1352-1540
GAE68	0.05	<0.05	Medium	17	13670 - 13535	1425-1560
LAN16	0.07	<0.001	Medium	17	13667 - 13489	1428-1606
GAE74	0.015	-	High	13	13647 - 13 509	1448-1586
GAE48	0.02	0.1	High	19	13589 - 13413	1506-1682
BIN150	0.42	<0.001	Low	14	13565 - 13 507	1530-1588
BIN75	0.14	<0.001	Low	18	13558 - 13320	1537-1775
GAE45	0.01	-	High	11	13546 - 13466	1549-1629
GAE51	0.001	-	High	16	13417 - 13232	1678-1863
GAE31	0.6	<0.001	Low	21	13369 - 13238	1726-1857
BIN155	0.01	-	Medium	7	14022 - 13855	1073-1240
BIN162	0.06	<0.001	High	14	14033 - 13874	1062-1221
BIN191	0.07	<0.001	High	17	14030 - 13879	1065-1216
BIN207	0.003	-	High	16	14050 - 13944	1040-1151
LAN37	0.001	-	Medium	38	13930 - 13772	1165-1323

224 <u>Table 1</u>: Individual tree correlations between isotopes ($\delta^{18}O:\delta^{13}C$) within a single tree,

225 average inter-tree δ^{18} O cross-correlations and preliminary qualitative decay assessment of

each tree. Significant inter-isotope correlations are highlighted.



Figure 3. An overview of Late Glacial climate oscillations in the Northern Hemisphere from (A) Austria (Lake Mondsee, Lauterbach et al. 2011), (B) Germany (Lake Ammersee, von Grafenstein et al. 1999) and (D) Greenland (Rasmussen et al. 2014), compared to the Swiss tree-ring stable oxygen isotope record (CH-ISO, this study). Four NGRIP cool substages indicated (GI-1d, GI-1c_{1,2}, GI-1b and GS-1) matched with comparable climate oscillations in other archives. Blue bars represent periods of relatively high population signal within the CH-ISO stable oxygen series.

227 **4. Discussion**

228 <u>4.1 Considerations in CH-ISO chronology development</u>

229 <u>4.1.1. Cellulose decay and stable isotopes</u>

Due to wood and cellulose decay considerations (Supplementary Material 1), in addition to the fact that cellulose is non-labile and does not exchange oxygen isotopes with xylem water following formation (Wright et al. 2008), cellulose was extracted for the development of CH-ISO. Similar to Nagavciuc et al. (2018), there were divergences in both δ^{13} C and δ^{18} O between trees, with weaker population signals in δ^{13} C despite the use of standard sample replication. As a result of the low correlation of δ^{13} C_{tree} between trees, this discussion will focus on the implications of δ^{18} O_{tree}.

Evidence of decay can be seen around bark and sapwood in most CH-ISO trees 237 (Figure 4), where the wood material is relatively discoloured and brittle, indicating 238 variable levels of rot. Based on other studies (Savard et al. 2012; Blanchette 2000), 239 240 we assume this is a result of bacteria from water-saturated soil, preferentially attacking the outer trunk portions through water infiltration. Yet, due to the expectation that 241 stable oxygen in cellulose is less vulnerable to diagenetic alteration (Leavitt et al. 242 243 1993), the varying decay states were not considered when developing the CH-ISO chronology. In CH-ISO, the subfossil trees exhibit highly variable states of visual decay 244 (Table 1), as demonstrated by a qualitative assessment. While preliminary, it appears 245 246 that trees with less extensive decay have significantly higher inter-isotope (δ^{18} O: δ^{13} C) correlations than those with moderate to high levels of decay. In cases where the 247 248 stable isotopes do not exhibit similar trends, it is assumed that climatological variables 249 which impact both isotopes through vapor pressure deficit and leaf stomatal conductance (e.g. relative humidity, precipitation) are not strongly recorded within the 250 tree-rings. As a result, it can be assumed that trees with relatively high levels of decay 251



Figure 4. Swiss subfossil pine wood quality (a) disk of tree demonstrating variable levels of decay: (1) low, (2) moderate and (3) high. Higher levels of wood decay were generally found closer to the bark, with best preserved wood material closer to the pith (b).



Figure 5. Hypothesised sequence of Late Glacial air mass teleconnections and migrations: (a) general Allerød conditions with oscillating polar front; the Allerød representing the Interstadial in the Late Glacial between 13 900 - 12 900 cal BP, followed by (b) the Younger Dryas (GS-1) cold reversal, with increased zonal westerlies and migrated polar front. Study site indicated (white circle).

lead to more extensive chemical alteration of $\delta^{13}C_{tree}$ over $\delta^{18}O_{tree}$, causing the greater divergence between the two measures and between $\delta^{13}C_{tree}$ of different trees.

Inter-tree $\delta^{18}O_{tree}$ correlations did not significantly differ as a function of wood decay, 254 further suggesting that $\delta^{18}O_{tree}$ may be less prone to fractionation during wood decay, 255 256 or at least wood decay that is clearly visible. The still relatively low cross-correlation between inter-tree $\delta^{18}O_{\text{tree}}$ (2% - 75%; mean: 15%) implies that chemical wood decay 257 258 may be present but indistinguishable under light microscope. This is within the range, if not slightly lower than the inter-tree $\delta^{18}O_{tree}$ correlations for modern pine trees in the 259 260 region; a cross-correlation of 29% (n_{trees} = 4) was found by Reynolds-Henne et al. 261 (2007) in the Swiss alps.

In accordance to our findings, Savard et al. (2012) established divergences in $\delta^{18}O$ between trees in the absence of visual decay, proving further processes of aqueous decay impacting subfossil wood are yet to be discovered. Indeed, while strongly decayed portions of tree disks were avoided where possible for stable isotope analysis, some differences in the structural integrity of the wood (in the absence of colouration change) was noted. The impact of textural variability on tree-ring isotopes was not taken into consideration and requires further investigation.

269 <u>4.1.2 Local site and tree-level considerations</u>

Local site conditions, which may impact tree-ring isotopic signatures, are difficult to decipher for CH-ISO. Trees from SWILM grew along an unstable slope, impacting root development and potentially uptake of sourcewater. Furthermore, varying depths of the active soil layer from (potential) permafrost conditions during cold episodes could have caused distinct variability in subsurface hydrology and rooting depth. Fluctuating thaw depth could have provided inconstant amounts of melted permafrost water to

individual trees, and it can well be assumed that the soil water δ^{18} O with a large 276 contribution from permafrost melt had a much different δ^{18} O signature (more depleted) 277 than summer rain. Moreover, the presence or absence of permafrost could have 278 279 influenced the seasonal uptake of different water sources with various isotopic signatures (e.g. relatively δ^{18} O-enriched liquid rain vs. δ^{18} O-depleted snow). For 280 example, meltwater and/or permafrost thaw could lead to depleted δ^{18} O signatures 281 despite high summer temperatures. Potentially drier and warmer summer conditions 282 may lower soil water δ^{18} O values, i.e. altering tree water source composition due to 283 increased permafrost or snowpack meltwater. This can lead to inverse climate-δ¹⁸O_{cel} 284 relationships as indeed reported for larch trees in Siberia (Saurer et al 2016). 285 286 Furthermore, during very dry summers meltwater may have served as the most 287 important water source for the trees, potentially compensating increased ¹⁸O enrichment of needle water due to increased transpiration rates during drought years. 288

As a result of these potential differences in microsite-conditions, trees within the same 289 vicinity could theoretically have divergence sourcewater signals. For example, more 290 stable soil may have longer precipitation water residence times and trees may be less 291 prone to take up water from event precipitation or subsequent surface runoff (which 292 293 have different isotopic values) (Genereux & Hooper 1998). It is therefore expected that trees growing in unstable subsites may track short-term hydrological events more 294 295 effectively (higher inter-annual δ^{18} O) than those in more stable subsites. At present, it is impossible to tell which trees diverge from a population signal due to slope instability 296 specifically without significantly increasing the sample replication. 297

298 Scenario-specific responses of tree-rings to meteorological drivers may be an 299 important factor moderating the stability between subfossil tree-ring δ^{18} O and regional

300 δ^{18} O correlations at high frequency over the LG. For example, during dry periods, trees 301 tend to reduce stomata conductance to limit increasing water loss by transpiration due to increased leaf-to-air vapour pressure difference. Dry summers usually lead to 302 increased ¹⁸O enrichment of leaf water and correspondingly higher $\delta^{18}O_{tree}$ values 303 compared to sourcewater values (e.g. Kahmen et al. 2011). Conversely, during humid 304 periods, ¹⁸O enrichment is reduced leading to reduced $\delta^{18}O_{tree}$ values being closer to 305 δ^{18} O of source (assuming oxygen isotope exchange between xylem water and sugars 306 during cellulose biosynthesis in the trunk is constant during dry and wet years). 307 Therefore, in times of high precipitation, regional δ^{18} O precipitation tends to be 308 309 incorporated more readily into tree-ring cellulose, with less overprinting due to 310 physiologically driven fractionation, which may be the case during the periods of strong δ^{18} O population signals and associated δ^{18} O depletions within CH-ISO. 311

312 <u>4.1.3 Temporal stability of CH-ISO</u>

313 Time series analysis revealed high variability in the strength of the δ^{18} O population 314 signal, with a linear model suggesting higher sample replication leads to higher inter-315 tree $\delta^{18}O_{\text{tree}}$ correlations (Results 3.2). However, numerous outliers (Figure 2a) 316 suggest this relationship is limited. For example, relatively high correlations (>25%) exist only during periods of 2-3 tree replication (Figure 2b) and strong $\delta^{18}O_{tree}$ 317 depletions (Figure 2d), despite moderate wood decay. During these extremes, intra-318 tree $\delta^{18}O_{\text{tree}}$: $\delta^{13}C_{\text{tree}}$ correlations are generally quite low (Figure 2c, R² < 0.20), with 319 exception to the $\delta^{18}O_{tree}$ extreme at ~13 765 cal BP. This reveals that when the CH-320 ISO trees exhibit significantly similar $\delta^{18}O_{tree}$ values, the $\delta^{18}O_{tree}$ is generally not well 321 322 correlated to $\delta^{13}C_{\text{tree}}$. Therefore, during these periods, the environmental factors that 323 have the potential to modulate both isotopes (e.g. relative humidity, temperature) are

not strongly recorded in the tree-rings; but rather, factors that only control $\delta^{18}O_{tree}$ 324 325 (precipitation source, type and amount) are possibly dominating the population signal and/or wood decay is preferentially altering the $\delta^{13}C_{tree}$. Note, temporal instability of 326 327 climate-isotope relationships are also observed in modern stable isotope records from Switzerland (Reynolds-Henne et al. 2007). Potentially, uncertainties in climate-proxy 328 329 relationships can be detected by comparing independent proxy records from different archives, provided that they generally reflect the same climate variable at similar time 330 331 and spatial resolution.

332 <u>4.2. The potential of subfossil tree-ring δ^{18} O to record hydroclimate variability</u>

Swiss Plateau sourcewater (δ^{18} O) is broadly driven by precipitation originating from 333 North Atlantic air masses but can also receive other air masses (sub-tropical, 334 continental; Figure 5, Kozel and Schotterer, 2003) and local hydrological variations 335 influenced by geomorphological (local hillslope) processes. This demonstrates an 336 337 element of non-stationarity to the tree-ring isotope relationship to climate variables over time, which has also been found in modern tree-ring isotope records (Kress et al. 338 2010, Reynolds-Henne et al. 2007, Seftigen et al. 2011). Shorter-term fluctuations in 339 340 δ^{18} O precipitation correlate to synoptic patterns in precipitation-bearing air masses over temperature (Gat & Carmi 1987), overriding the general temperature-precipitation 341 relationships and are further affected by leaf- and soil- level evaporative enrichment 342 during warm/dry periods. As a result, rapid changes in precipitation isotopes may be 343 strong, yet independent of temperature, creating significant deviations from the classic 344 345 precipitation-temperature Daansgard relationship (Edwards et al. 1996, Teranes and McKenzie 2001, Hammarlund et al. 2002). 346

347 Swiss water isotopes recorded in tree-rings are theoretically sensitive to summer (growing season; JJA) precipitation, whereas Greenland water isotopes ($\delta^{18}O_{NGRIP}$) 348 are more strongly driven by winter precipitation and temperature (DJF). Similarly, 349 350 water isotopes from LG southern France speleothems reflect winter (recharge season) precipitation; the signal of which is also moderated by summer evaporation and 351 352 deviations in storm track pathway (e.g. Genty et al. 2006). Furthermore, regional lake water isotopes are sensitive to annual temperature variations and subject to reservoir 353 effects (e.g. von Grafenstein 2000). These isotopic differences demonstrate the 354 355 inherent seasonality of these archives, which makes regional climate change comparison challenging. Moreover, circulation controls the $\delta^{18}O$ signal in the 356 357 atmosphere more strongly during the winter (due to stronger temperature gradients 358 and less extreme continental moisture cycling; Baldini et al. 2008), making winter δ^{18} O a more sensitive recorder of changes in atmospheric circulation. Accordingly, annual 359 or winter driven proxy records (e.g. lakes, ice cores, speleothems) may have more 360 361 success reconstructing such hemispheric variability compared to summer proxies (e.g. trees), unless the circulation change is extreme and/or prevails for a few consecutive 362 years (Saurer et al. 2012). Modern $\delta^{18}O_{tree}$ reveals the strongest climate correlations 363 during circulation changes (Saurer et al. 2012), suggesting that widespread climate 364 extremes could be reconstructed from LG $\delta^{18}O_{tree}$. 365

<u>4.3 Challenges in reconstructing LG climate oscillations from the CH-ISO subfossil</u> <u>tree-rings</u>

368 Due to complex and dynamic factors influencing the propagation of δ^{18} O from the 369 atmosphere into tree-ring cellulose, long-term and widespread climate interpretation 370 from subfossil dendroisotope records is challenging; particularly when sample

selection is limited. For example, while two drops in $\delta^{18}O_{tree}$ seem to approximately 371 parallel NGRIP 'cool periods' ($\delta^{18}O_{NGRIP}$ depletions; Figure 3), the inter-annual 372 373 variability as well as low frequency trends of the rest of the dataset show limited visual 374 resemblance between the records. The fluctuating similarity between tree-ring $\delta^{18}O$ chronologies (e.g. Pauly et al. 2018 and this study) and other regional LG δ^{18} O records 375 prove climate-proxy relationships in subfossil dendroisotope records are not stable 376 through time - shifting between recording regional and local weather conditions as 377 378 well as being influenced by tree- and population- level factors.

379 <u>4.4 Low frequency δ^{18} O similarity between LG archives</u>

Negative excursions ('cool periods') present in both European and Greenland $\delta^{18}O$ records (GI-1C, GS-1c) are evident in $\delta^{18}O_{tree}$ (Figure 3) as significant depletions. These occur when inter-tree $\delta^{18}O_{tree}$ correlations are high, and thus the $\delta^{18}O_{tree}$ chronology is more likely to be recording regional climate conditions over local effects.

This connection between $\delta^{18}O_{tree}$ and other records (Lauterbach et al. 2011, 384 Rasmussen et al. 2014, von Grafenstein et al. 1999) could be due to an increased 385 prevalence of North Atlantic air masses in mainland Europe as a result of a southerly 386 moving polar front (e.g. Kageyama et al. 1999, Schenk et al. 2018). Models have 387 388 predicted a winter amplification of this process due to sea ice expansion in winter and 389 atmospheric blocking of westerlies in summer during the Late Glacial (e.g. Schenk et al. 2018 and references therein), which may explain its muted signature in the summer 390 tree-ring archive for CH-ISO in comparison to the lake and ice core records. 391

On the other hand, one other Greenland isotope substage (GI-1b) - demonstrated as
the Gerzensee Oscillation across Europe (e.g. Eicher & Siegenthaler 1976,
Lauterbach et al. 2011, Lotter et al. 1992, 2012) - is not identifiable in CH-ISO. This

395 could be due to the relatively low tree correlation (5-15%) in $\delta^{18}O_{tree}$ over this interval 396 as a result of local site conditions overriding regional climate signals.

397 δ^{18} O records display a distinct excursion beginning between ~12 900 – 12 600 cal BP 398 (Figure 3), ascribed as the onset of the GS-1 / Younger Dryas. A short tree-ring 399 sourcewater reconstruction from southern France (Barbiers: ~12 900 – 12 600 cal BP, 400 Pauly et al. 2018) revealed an onset at 12740 cal BP, reflected by an increasing influence of both Mediterranean (+ $\delta^{18}O_{tree}$) and North Atlantic (- $\delta^{18}O_{tree}$) storms, 401 402 attributed to an oscillating polar front and more extreme weather locally. In contrast, 403 SWILM trees exhibit high inter-annual variability along a low frequency decline (-5.5%) over 40 years), providing further evidence of unstable weather conditions during the 404 405 cooling onset (Steffenson et al. 2008). Subfossil forests from both Barbiers (Pauly et al. 2018) and SWILM (Reinig et al. 2018; Figure 2a) demonstrate a distinct decline in 406 excavated trees at approximately ~12 600 cal BP, suggesting a tree die off concurrent 407 with the sustained drop in all δ^{18} O records. 408

409 **5. Conclusions**

The subfossil tree-ring δ^{18} O and δ^{13} C records developed in this study reflect a complex set of interacting climate variables modulated by local factors (microclimate, wood degradation); all of which must be carefully identified and investigated prior to a comprehensive paleoclimate interpretation.

414 Detailed research in the calibration of cellulose decay and related isotopic fractionation 415 is required as a prerequisite to further developing this chronology in order to increase 416 the population signal of both δ^{13} C and δ^{18} O. In particular, experiments on present-day 417 wood samples infected with certain fungi and/or bacteria targeting the isotopic effects 418 of cellulolytic enzymes are recommended. Furthermore, a suite of pre-treatment

419 protocols would have to be completed, including cellulose content weighing (to 420 measure mass loss of whole wood), wood strength tests (to determine texture) as well 421 as wood anatomy and colorimetry (to distinguish decay), prior to selecting optimal 422 subfossil samples for further analysis. Also, additional isotope data from wholewood, cellulose and/or lignin methoxyl groups will potentially allow to better identify 423 424 problematic sequences in multi-parameter approach (e.g. Mischel et al. 2015). As Swiss subfossil trees continue to be dated (Reinig et al., 2018), a revised subfossil 425 426 dendroisotope protocol and continued expansion of CH-ISO will follow.

427 The strongly variable (visual) correlation between NGRIP, Mondsee, Ammersee and 428 CH-ISO across millennia demonstrates that proxy-climate relationships are complex and non-stationary through time, beyond the differences in temporal resolution and 429 430 dating uncertainties. Despite having the same variable (δ^{18} O), each dataset records very different aspects of environmental conditions across space and time. A multi-431 432 proxy approach with regional inter-archive comparisons is vital in detangling the relative contribution of different factors in the overall local isotopic signal. Nonetheless, 433 this new stable isotope record here demonstrates the potential to hydroclimate 434 435 conditions in the Late Glacial using subfossil pine trees.

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- 444 Competing Interests Statement
- 445 We declare no competing interests in this work

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Supplementary Material

Pauly et al. 2020

1. Wood decay and tree-ring stable isotopes

Wood decay and decomposition have been shown to significantly impact intra- and inter-tree-ring stable isotopes, particularly in subfossil wood (e.g. Ziehmer et al. 2016). Diagenetic effects, in terms of degradation from biotic (bacteria, fungi) and abiotic (hydrological) decay, impact the structural and therefore the chemical nature of wood (Björdal et al. 1999, Daniel 1994, Benner et al. 1987, Daniel & Nilsson 1998, Staccioli et al. 1997, Schleser et al. 1999). It has been well established that cellulose is preferentially decayed over lignin and other wood components, impacting their relative ratios in wholewood over time (Park & Epstein 1961, Spiker & Hatcher 1987, Schleser 1999). This differential decay is a crucial consideration, as various wood components have divergent isotopic signatures, which can significantly impact the resultant wholewood stable isotopic signature. For example, a consistent enrichment of $\delta^{13}C$ (Loader et al. 2003; Lukens et al. 2019) and inconsistent δ^{18} O contained in lignin compared to cellulose (Park & Epstein 1961, Wilson & Grinsted 1977, Barbour 2002, McCarroll & Loader 2004, Borella et al. 1999). During burial and preservation of subfossil wood, infiltration of sediment-laden water leads to the reduction of specific acidic groups (native carboxyls and salt-derived carboxylates), and production of others (e.g. catalysation of esters) (Menchi et al. 1997, Staccioli et al. 1994). Hence, in this study cellulose was extracted in an attempt to avoid isotopic artefacts arising from the use of wholewood.

Apart from cellulose decay (due to burial post-death spread fungi and maybe more importantly bacteria), oxygen and carbon ions can be redistributed between tree-rings (English et al. 2011), significantly impacting inter-annual variability and particularly isotopic trends from pith to bark (and/or heartwood to sapwood). Decay has shown

instances of δ^{13} C depletion proportional to diminishing cellulose content to a specific

threshold (Schleser et al. 1999), significant decreases in δ^{18} O (Savard et al. 2012), as

well as generally unreliable stable isotope values (Savard et al. 2012; Nagavciuc et al.

2018; Savard et al. 2012) within the same tree and between trees at the same site.

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