



HAL
open science

Tree-ring $\delta^{13}\text{C}$ of archeological charcoals as indicator of past climatic seasonality. A case study from the Neolithic settlements of Lake Chalain (Jura, France)

Franck Baton, Thanh Thuy Nguyen Tu, Sylvie Derenne, Alexandre Delorme, Frédéric Delarue, Alexa Dufraise

► To cite this version:

Franck Baton, Thanh Thuy Nguyen Tu, Sylvie Derenne, Alexandre Delorme, Frédéric Delarue, et al. Tree-ring $\delta^{13}\text{C}$ of archeological charcoals as indicator of past climatic seasonality. A case study from the Neolithic settlements of Lake Chalain (Jura, France). *Quaternary International*, 2017, 457, pp.50-59. <10.1016/j.quaint.2017.03.015>. <hal-01503090>

HAL Id: hal-01503090

<https://hal.sorbonne-universite.fr/hal-01503090v1>

Submitted on 6 Apr 2017

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



HAL Authorization

1 **Tree-ring $\delta^{13}\text{C}$ of archeological charcoals as indicator of past climatic seasonality.**
2 **A case study from the Neolithic settlements of Lake Chalain (Jura, France).**

3

4 Franck Baton^{1,2}, Thanh Thuy Nguyen Tu^{1,*}, Sylvie Derenne¹, Alexandre Delorme^{1,2},
5 Frédéric Delarue³, Alexa Dufraisse²

6

7 ¹ Sorbonne Universités, UPMC, Univ Paris 06, CNRS, EPHE, UMR 7619 Metis, 4, place
8 Jussieu, 75252 Paris cedex 05, France

9 ² Sorbonne Universités, CNRS, Muséum national d'Histoire naturelle, UMR 7209,
10 Archéozoologie, Archéobotanique: sociétés, pratiques et environnements, CP56, 55 rue
11 Buffon, 75 005 Paris, France

12 ³ Sorbonne Université, UPMC, Univ Paris 06, CNRS, Muséum national d'Histoire naturelle,
13 UMR 7590 IMPMC,IRD, 75 231 Paris, France

14 * Corresponding author. *E-mail address*: thanh-thuy.nguyen_tu@upmc.fr

15

16 **Abstract:**

17 Charcoal fragments from the Neolithic settlements of Lake Chalain (Jura Mountains, France)
18 were characterized by dendro-anthracology (charcoal-pith distance, tree-ring width,
19 earlywood/latewood proportion) and ring-scale isotope geochemistry (^{13}C) to assess the
20 relevance of this combined approach for paleoclimate reconstructions. Two differing climatic
21 periods were investigated: (i) a climatic deterioration period characterized by cool and moist
22 conditions and (ii) a climatic improvement period characterized by slightly less precipitation
23 and warmer temperature. Latewood proportion in charcoal tree-rings was similar for the two
24 studied climatic periods. However, the charcoal tree-rings exhibited width and ^{13}C -content
25 significantly different between the two studied periods, in agreement with previously inferred
26 climatic difference. Monitoring ring-to-ring ^{13}C variation within each charcoal fragment
27 revealed no noticeable climatic trend, for none of the studied periods. However, calculation of
28 the difference in ^{13}C -content between earlywood and latewood of a given tree-ring suggested

29 that the cool and moist climatic period also corresponded to higher seasonal contrast than
30 the dryer climatic period. Although this exploratory study needs further confirmation, it opens
31 promising developments for paleoclimatic reconstructions based on the stable carbon
32 isotope composition of archaeological charcoals: the potential for recording subtle
33 paleoclimatic variations and seasonal contrasts.

34

35 **Keywords:** domestic firewood, ^{13}C , growth-ring width, earlywood, latewood, seasonality

36

37 **1. Introduction**

38 The stable carbon isotope composition ($\delta^{13}\text{C}$) of plant tissues depends on: (i) the
39 inorganic carbon source used by the plants for their photosynthesis (*i.e.* atmospheric CO_2),
40 (ii) the photosynthetic pathway used by plants (*i.e.* C_3 , C_4 or CAM), and (iii) environmental
41 conditions (Farquhar *et al.*, 1980; O'Leary, 1981; Leavitt and Long, 1983; Tieszen, 1991;
42 McCarroll and Loader, 2004). A number of environmental parameters (*e.g.* irradiance,
43 nutrient supply, temperature, water availability, etc.) may influence plant $\delta^{13}\text{C}$ values, water
44 availability probably being one of the most important, in both arid and temperate climates
45 (Farquhar *et al.*, 1982; Dawson *et al.*, 2002; Kress *et al.*, 2010; Saurer *et al.*, 2014). Applied
46 to ancient plants, stable carbon isotope studies allowed reconstructions of past
47 photosynthetic pathways (*e.g.* Bocherens *et al.*, 1993; Cotton *et al.*, 2012), of isotope
48 composition of past atmospheric CO_2 (*e.g.* Gröcke, 1997), or of the water-stress experienced
49 by plants in the past (Nguyen Tu *et al.*, 2002). Stable isotope characterization of tree-ring
50 series from recent woods was established as an efficient approach to document detailed
51 variations in temperature and rainfall over the last centuries (Leavitt and Long, 1991; Feng
52 and Epstein, 1995; Treydte *et al.*, 2001; Danis *et al.*, 2006; Young *et al.*, 2012). Owing to
53 their sensibility to temperature and/or precipitation, dendrometric patterns of woods, such as
54 ring width or earlywood/latewood proportions (*i.e.* springwood/summerwood proportions) of a
55 given growth ring, also constitute helpful paleoclimatic proxies (Nola, 1996; Zhang, 1997;
56 Briffa *et al.* 2002; Dittmar *et al.* 2003; Büntgen *et al.* 2006). Combining ring width and stable

57 isotope composition further provides better constrained paleoclimatic estimations, as they
58 record complementary climatic signals (Ballantyne *et al.*, 2006; Weigl *et al.*, 2007). Indeed,
59 ring-width characteristics are often considered as more sensitive to local factors than $\delta^{13}\text{C}$
60 which may give access to larger scale climatic signals (Andreu *et al.*, 2008). Stable isotope
61 measurements are generally achieved on latewood/summerwood in dendroclimatology.
62 Indeed, contrary to earlywood that is synthesized before bud break (Essiamah and Eschrich,
63 1985), latewood is expected to be little influenced by remobilization of the carbon stored the
64 preceding years (Borella *et al.*, 1998; Barbaroux and Breda, 2002).

65 Charcoals are rather frequent in the sedimentological and archeological records as
66 charcoalification confers wood a crystalized structure with higher chemical stability and
67 resistance to degradation processes, when compared with uncharred woods (Figueiral,
68 1999; Bird and Ascough, 2012). Charcoalification generally preserves wood anatomy
69 allowing taxonomic identification and dendrological studies (Couvert, 1970; Marguerie and
70 Hunot, 2007). Archeological sites commonly yield numerous charcoal fragments produced
71 either by fire events or domestic fires that are associated with heating, lighting and cooking
72 activities. Dendrological characterization of charcoals (*i.e.* dendro-anthracology) from
73 domestic firewood allows reconstructing variations in past woodland structure as well as
74 firewood and woodland management (*e.g.* Lundström-Baudais, 1986; Ludemann and Nelle,
75 2002; Dufraisse, 2005, 2006; Marguerie and Hunot, 2007; Deforce and Haneca, 2015). As
76 far as it has not been significantly affected by combustion and post-depositional processes,
77 isotope composition of archeological charcoals potentially constitutes an efficient
78 paleoenvironmental proxy. Selectively working on charcoals from a single type of fire (*i.e.*
79 domestic fire), isotope composition was proven useful in archeological context to reconstruct
80 paleoenvironmental parameters, particularly those related to water availability (February and
81 Van der Merwe, 1992; Vernet *et al.*, 1996; Ferrio *et al.*, 2006; Vernet, 2006; Hall *et al.*, 2008;
82 Aguilera *et al.*, 2009; Drake *et al.*, 2012; Masi *et al.*, 2013; Fiorentino *et al.*, 2014). For
83 example, systematic isotope characterization of charcoals dated from Bronze to Iron Ages
84 allowed spatial paleoclimate reconstruction for the Iberian Peninsula, showing that

85 precipitation was significantly higher during the so-called Iron Age Cold Epoch than present-
86 day values (Aguilera *et al.*, 2009).

87 Different plant components have different isotope composition (Park and Epstein,
88 1961; Gleixner *et al.*, 1993). For example, among the most abundant wood components,
89 cellulose is systematically ^{13}C -enriched with respect to lignin (Benner *et al.*, 1987; Ehleringer,
90 1991). Therefore, the isotope composition of a given plant tissue corresponds to the
91 weighted average of the isotope composition of each of its constituents. As a consequence,
92 the analysis of a single plant constituent is often favored in isotope dendroclimatology, so as
93 to avoid biases due to variations in the relative proportions of different wood components
94 (Mazany *et al.*, 1980; Leavitt and Danzer, 1993). Although the isotope composition of lignin
95 was shown to accurately record climate, α -cellulose has been the preferred sample material
96 as its synthesis and deposition in wood are considered synchronous of ring formation
97 (Roberston *et al.*, 2004; Loader *et al.*, 2011). Nevertheless, bulk wood $\delta^{13}\text{C}$ was proven to
98 accurately record past climatic trends; this is notably the case for woods devoid of resin since
99 resins are among the main components that can bias wood isotope signature (Borella *et al.*,
100 1998; Loader *et al.*, 2003; Verheyden *et al.*, 2005). Cellulose extraction is not possible for
101 charcoals as charcoalification mostly corresponds to carbonization, a thermal process
102 leading to the alteration of the chemical structure of cellulose. A subsequent enrichment in
103 aromatic moieties results from both cellulose degradation and selective preservation of
104 lignin-derived compounds (Ishimaru *et al.*, 2007). The effects of charcoalification on the
105 isotope composition of wood are not well documented in domestic open fireplaces. However,
106 muffle furnace experimentations suggested that carbonization either (i) leads to no significant
107 isotope effect, at least at moderate temperatures (i.e. up to 300-400°C; DeNiro and Hastorf,
108 1985, Turekian *et al.*, 1998; Czimczik *et al.*, 2002; Ascough *et al.*, 2008) or (ii) tends to shift
109 whole wood $\delta^{13}\text{C}$ values down to that of lignin, especially at temperatures higher than 500°C
110 (Czimczik *et al.*, 2002; Turney *et al.*, 2006, Ferrio *et al.*, 2006; Ascough *et al.*, 2008). Above
111 500°C, carbonization thus eventually gives access to an isotope signal close to that of a

112 single plant component (i.e. lignin) as recommended for isotope dendroclimatology on extant
113 wood.

114 Isotope studies of archeological charcoals were so far achieved on bulk charcoals
115 although a ring-scale approach may provide further paleoclimatic details. The present study
116 thus constitutes a first approach for assessing the relevance of ring-scale isotope study, in
117 combination with dendro-anthracology, for paleoclimatic reconstructions based on
118 archeological charcoals. The charcoals recovered in archeological sites generally comprise
119 less than 10 growth rings so that deriving long term climatic trends would require particularly
120 large charcoals and/or important sample sets. Alternatively, charcoal isotope study at ring
121 scale can provide information on short term environmental variations as well as inter-
122 seasonal variations. Indeed, although the isotope signature of earlywood (i.e. springwood) is
123 markedly influenced by previous year accumulates, comparing $\delta^{13}\text{C}$ values of earlywood and
124 latewood of a given growth-ring may bring information on seasonal contrasts (Livingston and
125 Spittlehouse, 1996; Helle and Schleser, 2004; Li *et al.*, 2005). This study thus investigated
126 inter- and intra-ring isotope composition of archeological charcoals as an attempt to
127 document detailed inter-annual and inter-seasonal paleoclimatic/paleoenvironmental
128 variations.

129 To test the response of these isotope proxies to environmental variations, charcoals
130 from the Neolithic sites of Lake Chalain (French Jura Montains) were characterized with an
131 integrated approach coupling dendro-anthracology and isotope geochemistry. The lake-
132 shore settlements of "Chalain 4" offer a unique opportunity to test new paleoclimatic proxies
133 since:

134 (i) The charcoal fragments come from trees located in a limited area (Dufraisse *et al.*, 2008)
135 so that variations in their isotope composition are likely mainly influenced by regional/global
136 environmental variations, with limited influence of variations in site conditions.

137 (ii) The Neolithic sedimentary sequence of Lake Chalain comprises several human
138 occupation periods. Multidisciplinary studies (i.e. malacology, palynology, sedimentology and
139 ^{14}C geochemistry) have shown that these occupation periods follow several decades without

140 human occupation due to unfavorable climatic conditions for Neolithic farming societies. Two
141 main phases were thus distinguished in this study: one without human occupation
142 corresponding to cool and wet climatic conditions, followed by a phase comprising several
143 human occupations and corresponding to favorable climatic conditions (low lake level due to
144 relatively warmer and drier conditions; Damon *et al.*, 1989; Magny, 1993a; Richard, 1997;
145 Mouthon, 1997).

146 (iii) “Chalain 4” settlements yielded thousands of charcoal fragments from domestic firewood.
147 An extensive dendro-anthracological study of these charcoals showed that they all come
148 from small woods having grown under unfavorable or favorable climatic conditions,
149 depending on the considered occupation level (Dufraisse, 2008).

150 In order to minimize interspecific $\delta^{13}\text{C}$ variations (Leavitt, 2010), the present study focuses on
151 the European deciduous oak (*Quercus* sp.). Although charcoals from deciduous oak species
152 are difficult to distinguish, this taxon can only correspond to two species in the studied area:
153 *Quercus robur* and *Quercus petraea*. *Quercus* sp. was chosen for its abundance in
154 temperate forests, its anatomy with clearly identifiable growth rings, its representativity in
155 anthracological spectra and the potential of its tree-ring $\delta^{13}\text{C}$ to record climatic variations
156 under temperate climate (Michelot, 2011; Young *et al.*, 2012), even at the bulk wood level
157 (Loader *et al.*, 2003).

158

159 **2. The study site and its region**

160 *2.1. Regional setting*

161 The small lake of Chalain is located on the left bank of the Ain River at an altitude of
162 500 meters in the Combe d’Ain (French Jura Mountains). The Combe d’Ain is an alluvial
163 valley bordered to the west by the first Jura plateau of Lons-le-Saunier at an altitude of 450–
164 560 m, and to the east by the upper Jura plateau of Champagnole from 800 to 1,100 m (Fig.
165 1a). The Lake Chalain region is characterized by a semi-continental climate. The mean
166 annual temperature is 10°C. Protected from the westerly winds, the Combe d’Ain receives

167 1300-1400 mm of precipitation, and is characterized by late freezing, moderated by the
168 frequency of fogs.

169

170 *2.2. Archeological sites*

171 Since the beginning of archeological research in 1904, 32 settlement sites localized in
172 the Western lakeshore of Chalain have been found and dated between 5300 and 600 B.C.
173 (Pétrequin, 2012). This study focuses on “Chalain 4” lake dwelling. “Chalain 4” is located on
174 the North of the Western lakeshore of Chalain, on a peninsula of about 0.5 ha. The
175 excavation area of 300 m² included a plank way connecting the village to the hinterland, and
176 one of the rows of houses of the village, estimated at a dozen. Dendrochronological study of
177 350 timber pieces has shown that “Chalain 4” occupation had spanned from 3040 to 3000
178 B.C. (Lavier, 1996). The occupation is preceded by a lack of settlements between 3150 and
179 3040 B.C. corresponding to an important cultural change and a minor climatic deterioration
180 phase with high water level, higher precipitation and lower temperatures (Pétrequin *et al.*,
181 2002; Magny, 2004).

182 The sediment sequence of “Chalain 4” is especially thick and shows seven
183 stratigraphic levels (named level I to VII; Fig.1b). Seven archeological layers were further
184 distinguished in level VII (from layer G at the bottom to layer A at the top); they evidenced a
185 succession of four periods of occupation briefly interrupted by high water level (brief flooding
186 episode characterized by calcareous deposit). The oldest occupation phase (corresponding
187 to layer G) corresponds to high lake-level at the end of a minor climatic deterioration. The
188 following phases (corresponding to layers F, E and ABCD) correspond to lower lake level
189 within a regional increase in temperature and decrease in precipitation, leading to a climate
190 with higher summer temperature and lower precipitation during occupation layers A/B than
191 occupation layer G (Magny, 1993a). Summer temperature difference between the two
192 climatic periods probably did not exceed 1.6°C (Magny *et al.*, 1993b, 2009). For the present
193 study, charcoals from layer G and layers A/B, 40 years apart, were selected for testing the
194 potential of isotope dendroclimatology to distinguish the two climatic phases.

195

196 **3. Materials and methods**

197 Each charcoal fragment was first examined under a macroscope for dendrometric
198 measurements, prior to micro-drill sampling and geochemical analysis. In addition to stable
199 carbon isotope analysis, the charcoals were characterized by Raman microspectrometry to
200 estimate their carbonization degree and temperature (e.g. Rouzaud *et al.*, 2015).

201

202 *3.1. Dendro-anthracology*

203 Sampling was exclusively performed (i) in zones where the succession of cultural
204 layers is clear and not mixed with burnt layers and (ii) in scattered deposits to avoid short-
205 term events such as a single domestic fire (Dufraisse, 2008). Charcoal fragments were
206 washed with water on a 2 mm mesh sieve and air-dried. The largest oak charcoal fragments
207 were selected for geochemical analysis: 10 charcoals from layers A/B and 9 charcoals from
208 layer G. The selected charcoals comprised from 5 to 19 growth rings (11 growth rings per
209 fragment in average).

210 Measurements of ring and latewood widths were conducted with a Nikon AZ100
211 macroscope using the “NIS Element[®]” software. Shrinkage effects during charcoalification
212 being probably heterogeneous between earlywood and latewood, the proportion of latewood
213 was expressed according to equation (2) (Dufraisse *et al.*, accepted a):

$$214 \text{ Latewood Proportion} = \text{Latewood Width} / \text{Ring Width} * 100 \quad (2)$$

215 Minimal wood diameter was determined by calculating the charcoal-pith distance, which was
216 obtained from trigonometric tools (Dufraisse and Garcia Martinez, 2011).

217 Duraminisation, the changeover of sapwood to heartwood, may influence wood $\delta^{13}\text{C}$
218 values (Borella *et al.*, 1998). In some species the coloration of heartwood due to the
219 deposition of lignins and polyphenols makes heartwood easily recognizable (Hillis, 1987).
220 However, charcoalification process erases such a color difference. Although tyloses can form
221 in response to fungal attack, the abundant formation of tyloses in earlywood vessels was
222 recently shown diagnostic for distinguishing sapwood from heartwood in deciduous oak

223 (Dufraisse *et al.*, accepted b). In *Quercus*, less than 65% of vessels sealed by tyloses are
224 characteristic for sapwood while more than 85% indicate heartwood. Vessels with and
225 without tylosis were thus counted to assess the wood type of the studied charcoal fragments.

226

227 3.2. Raman microspectrometry

228 The Raman study was performed using a Renishaw Invia microspectrometer
229 equipped with a 514.5 nm argon laser at 20 mW. The laser power at the sample surface was
230 set below 1 mW to prevent thermal alteration of charcoals leading to artifactual Raman
231 spectra. The spectrometer was calibrated using a silicon standard before each session. For
232 each sample analyses, the laser was focused using a DMLM Leica microscope with a 100X
233 objective and the spectra were recorded in the 900–2000 cm^{-1} wavenumber range. Focusing
234 on the 1200 to 1700 cm^{-1} wavenumber, two bands are detected (Fig. 2). They correspond to
235 the defect (“D”) and graphite (“G”) bands of thermally altered carbonaceous matter occurring
236 at ca. 1350 and 1580 cm^{-1} respectively. While the G band is known to probe polyaromatic
237 layers, the signification of the D band is still debated (Rouzaud *et al.*, 2015). Nonetheless,
238 the relative evolution of the D band in comparison to the G band is tightly associated with
239 temperature (Rouzaud *et al.*, 2015). Hence, the recent study by Deldicque *et al.* (2016)
240 demonstrates that the maximum temperature of carbonization/charcoalification can be
241 estimated from the ratio between the heights of the D and G bands (H_D/H_G ratio; Fig. 2). In
242 order to compare the maximal thermal alteration underwent by charcoals, the H_D/H_G ratio
243 was then determined in duplicate on the most external ring of each charcoal fragment.

244

245 3.3. Stable carbon isotope analyses

246 Samples for isotope analyses were drilled under binocular objective magnification
247 using a 0.9 mm tungsten carbide drill bit. This method allowed sampling separately
248 earlywood and latewood, leading to approximately 0.5 mg of fine homogeneous powder per
249 sample. Care was taken to avoid contamination between each sample: (i) the drill bit was
250 washed with 96% ethanol and ultrasonicated in ultrapure water, and (ii) the sampled charcoal

251 was cleaned with dry compressed air. The $^{13}\text{C}/^{12}\text{C}$ ratios were determined by isotope ratio
252 mass spectrometry and expressed, in per mil (‰), as $\delta^{13}\text{C}$ values according to equation (1):

$$253 \quad \delta^{13}\text{C} = \left[\frac{{}^{13}\text{R}_{\text{sample}}}{{}^{13}\text{R}_{\text{standard}}} - 1 \right] \times 1000 \quad (1)$$

254 where ^{13}R stands for the ratio $^{13}\text{C}/^{12}\text{C}$, the standard being V-PDB (the international reference
255 Vienna-Pee Dee Belemnite). Aliquots of approximately 0.2 mg were combusted using an
256 elemental analyzer (Thermo Fisher Scientific Flash 2000) coupled to an isotope ratio mass
257 spectrometer (Thermo Fisher Scientific Delta V advantage). Over the period of analysis,
258 more than 100 of our laboratory internal standards gave an analytical precision of 0.16‰ for
259 $\delta^{13}\text{C}$ values. Duplicates were prepared from 30 charcoal samples to test the full analytical
260 uncertainty. Difference between charcoal duplicates ranged from 0.01‰ to 0.06‰ with a
261 mean difference of 0.03‰.

262

263 3.4. Acid or base pretreatment test

264 The minute size of samples for intra-ring isotope analyses did not allow systematic
265 acid or base treatment as often recommended to avoid potential bias in isotope
266 measurements due to adsorption of exogenous organic and inorganic carbon (Nissenbaum
267 and Schallinger, 1974; DeNiro and Hastorf, 1985; Ascough *et al.*, 2011). Preliminary tests
268 were thus conducted on 20 charcoal fragments, representative of layers A/B and layer G, to
269 estimate the extent of this potential bias. The test protocol was adapted from DeNiro and
270 Hastorf (1985), Midwood and Boutton (1998) and Bocherens and Mariotti (1999). The tested
271 charcoals were ca. 3.5 mm width; they were divided into two equivalent parts by transversal
272 sectioning (i.e. perpendicular to the axis of the fibers). One part was submitted to chemical
273 treatment prior to isotope analysis, while the other part was directly analyzed. Ten charcoal
274 fragments were tested for inorganic carbon adsorption with 0.1 M hydrochloric acid (HCl),
275 while ten charcoal fragments were tested for organic carbon adsorption with 1M sodium
276 hydroxide (NaOH). Charcoals were immersed for 30h in the mentioned solutions so as to
277 evaluate the maximal deviation to be expected. The samples were then rinsed by three

278 successive 1h ultrasonications in ultrapure water, changing water for each bath. After a final
279 flushing with ultrapure water, the samples were further oven-dried at 40°C to constant weight
280 and crushed prior to analyses.

281

282 3.5. Statistical analyses

283 Differences in geochemical and dendro-anthracological characteristics between
284 charcoals from layers A/B and from layer G, and between sapwood-charcoals and
285 heartwood-charcoals, were tested by analysis of variance (ANOVA). As the sample size was
286 limited for the acid or base treatment, a Wilcoxon signed rank test was applied to assess the
287 effect of pre-treatment on charcoal $\delta^{13}\text{C}$. Hypotheses were tested on the basis of a 5%
288 significance level. All analyses were conducted with R (Rcmdr version 2.2-3 of the 11/11/15,
289 R Foundation for Statistical Computing, Vienna, Austria).

290

291 4. Results and discussion

292 19 charcoal fragments and their 212 growth rings were characterized in “Chalain 4”
293 settlements by a combined dendro-anthracology and geochemistry approach. The results are
294 synthesized in Table 1 and detailed in Supplementary Table 1.

295

296 4.1. Dendro-anthracology

297 The studied charcoals appeared representative of the sequence as their dendro-
298 anthracological features fall within the range of those previously obtained from a larger
299 sample set of “Chalain 4” oak charcoals (121 from layer G and 111 from layers A/B;
300 Dufraisse, 2005, 2008). Charcoal ring width averages at 0.7 mm for layers A/B, and at 1.1
301 mm for layer G (i.e. 37% more than layers A/B; Table 1, Fig. 3). Ring widths of charcoals in
302 layer G are statistically higher than those of layers A/B ($p < 0.05$). In deciduous oak, tree-
303 ring widths are known to decrease when precipitation decreases and temperature increases
304 (Michelot, 2011; Matisons *et al.*, 2013). The higher ring width in layer G when compared with
305 layers A/B is thus consistent with paleoclimatic data (Magny, 1993a). Latewood proportion

306 averages at 52% for A/B and G charcoals. It is statistically similar for the two data sets ($p =$
307 0.78), although it is often considered to decrease with decreasing precipitation (Zhang, 1997;
308 Kern *et al.*, 2013).

309 Estimated mean charcoal-pith distances, (47 mm for A/B and G charcoals with a
310 maximum value of 65 mm), did not vary between layers (Table 1). Since the outlying part of
311 the wood generally disappears during carbonization, this maximum value of 65 mm leads to
312 an estimation of 13 cm for the minimum diameter of exploited wood. Therefore, the studied
313 charcoal fragments come from relatively small and young woods. Determination of the
314 proportion of vessels sealed by tylosis showed that 82% and 33% of the studied charcoals
315 correspond to sapwood for layers A/B and G, respectively. Sapwood growth rings
316 corresponding to the last 20-25 years of tree life, A/B charcoal fragments had thus grown
317 under the paleoclimate inferred for the embedding archeological layers, a climate relatively
318 warmer and dryer than the one preceding the occupation layers. The lower proportion of
319 sapwood in charcoals from layer G indicates that these charcoals come from trees relatively
320 older than in layers A/B. Additionally, layer G is the oldest occupation layer of “Chalain 4” and
321 it corresponds to the end of a cool and moist climatic period that lasted 60 years. Taking into
322 account the minimum diameter and the tree-ring width of G charcoal fragments, we can thus
323 confidently consider that the trees from which they come, grew during these unfavorable
324 conditions. As a result, the charcoal fragments selected for the present study indeed
325 correspond to different climatic periods, a cool and moist climate for layer G and a relatively
326 warmer and drier climate for layers A/B.

327

328 4.2. Charcoalification temperatures

329 The studied charcoal fragments were characterized by Raman microspectrometry to
330 control their degree of thermal alteration (e.g. Rouzaud *et al.*, 2015) as charcoal $\delta^{13}\text{C}$ may be
331 affected by carbonization temperature (Ferrio *et al.*, 2006; Turney *et al.*, 2006; Ascough *et*
332 *al.*, 2008). The $\text{H}_\text{D}/\text{H}_\text{G}$ ratio gives an estimation of the highest combustion temperature
333 undergone by charcoals, as established for pine and poplar woods carbonized at various

334 temperatures in a muffle furnace (Deldicque *et al.*, 2016). The studied charcoal fragments
335 exhibit Raman spectra typical for charred material, dominated by the usual G and D broad
336 bands (Fig. 2). The two studied occupation layers led to statistically similar H_D/H_G ratios ($p =$
337 0.4), around 0.6 (Table 1). Deldicque *et al.* (2016) paleothermometer suggests combustion
338 temperature around 700°C for the studied charcoals, substantially above the limit
339 temperature for charcoals to reach $\delta^{13}C$ values representative of a single component, lignin
340 (Ferrio *et al.*, 2006; Turney *et al.*, 2006; Ascough *et al.* 2008). However, absolute temperature
341 estimation should be taken with caution since the transfer function between H_D/H_G ratio and
342 carbonization temperature was obtained (i) on woods that are less dense than deciduous oak
343 wood, and (ii) in a muffle furnace for a carbonization duration of one hour (Deldicque *et al.*,
344 2016), which may not reflect the actual carbonization conditions undergone by the studied
345 charcoals (*i.e.* in open fireplace). Nevertheless, although the calculated carbonization
346 temperature may vary according to species or carbonization duration, the shape of the
347 transfer function appeared stable whatever the conditions (Deldicque *et al.*, 2016).
348 Accordingly, H_D/H_G ratios likely constitute reasonable parameters for comparative purposes
349 within a single wood species. Hence, the H_D/H_G ratios obtained for layers A/B and G show
350 that the charcoal fragments from these two layers were carbonized at similar temperature,
351 thus implying that the isotope signature of the studied charcoals does not depend on their
352 degree of charcoalification/carbonization.

353

354 4.3. Stable carbon isotopes

355 4.3.1. Potential contamination by exogenous carbon

356 Adsorption of exogenous organic and inorganic carbon may bias the isotope
357 composition of charcoals (e.g. Nissenbaum and Schallinger, 1974; Ascough *et al.*, 2011),
358 although this contamination effect was not systematically detected (e.g. DeNiro and Hastorf,
359 1985; Vaiglova *et al.*, 2014a). Acid (HCl) or base (NaOH) treatment of a representative sub-
360 sample set of “Chalain 4” charcoal fragments showed non-significant differences in $\delta^{13}C$
361 values between untreated and treated samples ($< 0.2\%$, Fig. 4; Wilcoxon test, p -value $>$

362 0.05, Supplementary Table 2). Hence, the stable carbon isotope composition of studied
363 charcoal fragments was not substantially affected by adsorption of exogenous organic and
364 inorganic carbon. As a consequence, acid or base pre-treatment was removed from the
365 charcoal preparation procedure allowing direct analysis of sub-ring samples.

366

367 4.3.2. General climatic patterns

368 The $\delta^{13}\text{C}$ values measured on sub-ring samples (i.e. earlywood and latewood) of
369 charcoals from “Chalain 4” settlement average at -25.9‰ and -26.5‰ for layers A/B and
370 layer G, respectively (Tab.1). These $\delta^{13}\text{C}$ values fall within the range of previously analyzed
371 archeological charcoals from plants using the C_3 photosynthetic pathway (Hall *et al.*, 2008;
372 Aguilera *et al.*, 2009; Masi *et al.*, 2013; Vaiglova *et al.*, 2014b). Within a given layer, sapwood
373 charcoals are systematically ^{13}C -enriched with respect to heartwood charcoals (statistically
374 significant; $p < 0.05$; Fig. 5). This is in agreement with (i) the general ^{13}C -depletion of lignin
375 with respect to bulk plant tissues (Benner *et al.*, 1987; Macko *et al.*, 1987) and (ii) the
376 increase in lignin content of wood during duraminisation (Hillis, 1987). Charcoals from layers
377 A/B exhibit $\delta^{13}\text{C}$ values significantly higher than those from layer G ($p < 0.05$). This difference
378 remains significant whatever the sample set, either all charcoals pooled together or
379 considering exclusively earlywood samples, latewood samples, heartwood samples or
380 sapwood samples. The ^{13}C -enrichment of A/B charcoals when compared with G charcoals is
381 in agreement with previously inferred paleoclimate since (i) layers A/B were shown to
382 correspond to a drier and warmer climate than layer G (e.g. Magny, 1993a) and (ii)
383 decreasing precipitation and increasing temperature are known to generally increase plant
384 $\delta^{13}\text{C}$ values (e.g. Farquhar *et al.*, 1982; Dawson *et al.*, 2002) and specially oak ring $\delta^{13}\text{C}$
385 values (Michelot, 2011).

386 $\delta^{13}\text{C}$ values of oak charcoal thus constitute a reliable record of climatic conditions
387 along “Chalain 4” sequence. Climatic variations during Neolithic at Chalain were attested by
388 several approaches (malacology, palynology, sedimentology and ^{14}C geochemistry; Damon

389 *et al.*, 1989; Magny, 1993a; Mouthon, 1997; Richard, 1997). Nevertheless, these variations
390 were rather subtle as (i) remaining within the range of mountain semi-continental temperate
391 climate and (ii) the two layers studied were only 40 years apart (Lavier, 1996). As a result,
392 subtle climatic variations can be recorded through stable carbon isotope ratios of oak
393 charcoals, whereas this was not the case for latewood proportion (see section 4.1.). Growth-
394 ring width and $\delta^{13}\text{C}$ values both depend on a number of genetic and physiological factors
395 (position in the tree for example), environmental factors (such as light, soil) and human
396 factors (clearings, woodland management). However, in the Jura Mountain at 500 m of
397 altitude, temperature and precipitation are probably not a limiting factor for width growth while
398 they markedly influence $\delta^{13}\text{C}$ values in deciduous oak. In any case, the present study thus
399 extends the potential of isotope composition of archeological charcoals to record small
400 climatic variations in temperate climate, while previous paleoclimatic studies based on
401 charcoal isotope composition dealt with contrasted climates (Hall *et al.*, 2008; Aguilera *et al.*,
402 2009; Masi *et al.*, 2013; Vaiglova *et al.*, 2014b).

403

404 4.3.3. *Inter- and intra-year climatic variations*

405 Ring-scale sampling enabled the evaluation of the potential of stable carbon isotope
406 composition of archeological charcoals to record short-term climatic trends. A plot of $\delta^{13}\text{C}$
407 values vs. ring-number revealed no noticeable trend during the growth period covered by the
408 charcoals. Similar results were obtained when only taking into account layers A/B, layer G,
409 latewood (Fig. 6) or earlywood (data not shown). Hence, monitoring isotope pattern of
410 charcoal rings through growth does not appear relevant here for documenting short-term
411 climatic variations, even though relatively large fragments were selected (*i.e.* comprising at
412 least 5 growth rings). The restricted sample set of this exploratory study is probably too
413 limited to go beyond natural climate variability.

414 Although isotope dendroclimatology based on uncharred/recent wood favors analyses
415 of latewood to avoid carbon contribution from previous year accumulates (Borella *et al.*,

416 1998), comparing $\delta^{13}\text{C}$ values of earlywood and latewood of a particular growth ring may
417 bring information on seasonal contrasts (Livingston and Spittlehouse, 1996; Helle and
418 Schleser, 2004; Li *et al.*, 2005; Eglin *et al.*, 2010; Kimak and Leuenberger, 2015). We
419 propose to name $\Delta_{\text{seasonality}}$ the difference calculated by Livingston and Spittlehouse (1996),
420 as in equation (3):

$$421 \quad \Delta_{\text{seasonality}} = \delta^{13}\text{C}_{\text{earlywood}} - \delta^{13}\text{C}_{\text{latewood}} \quad (3)$$

422 The $\Delta_{\text{seasonality}}$ calculated for “Chalain 4” charcoals varies between -2.84‰ and +1.13‰ and
423 averages at -0.01‰ and +0.20‰ for layers A/B and G, respectively (Tab. 1, Fig. 7). These
424 values fall in the range of those reported by previous authors for uncharred wood (Livingston
425 and Spittlehouse, 1996; Helle and Schleser, 2004; Li *et al.*, 2005; Eglin *et al.*, 2010; Kimak
426 and Leuenberger, 2015). Within a given layer, $\Delta_{\text{seasonality}}$ is not significantly different between
427 heartwood and sapwood ($p = 0.15$), suggesting that duraminisation does not influence this
428 parameter (*i.e.* equally influences earlywood and latewood $\delta^{13}\text{C}$ values). The difference in
429 $\Delta_{\text{seasonality}}$ between layers A/B and layer G is statistically significant ($p < 0.05$) and may reflect
430 higher seasonal contrast in layer G with respect to layers A/B. $\Delta_{\text{seasonality}}$ was proposed to be
431 controlled by the combined effect of (i) the extent of remobilization of ^{13}C -enriched stored
432 carbon, mainly during spring, and (ii) differences in cumulative transpiration between spring
433 and summer, both factors being notably driven by water availability/precipitation (Livingston
434 and Spittlehouse, 1996; Helle and Schleser, 2004; Li *et al.*, 2005; Eglin *et al.*, 2010; Kimak
435 and Leuenberger, 2015). Therefore, the colder and wetter climate of layer G (high lake-level)
436 when compared with layers A/B (low lake-level) e.g. Magny, 1993a) was also possibly
437 characterized by enhanced variations in precipitation between spring and summer. This
438 finding converges with reconstruction of the “growing degree days” index (GDD5, the sum of
439 daily temperatures above 5°C) calculated from lake-level and pollen data of Lake Le Bourget
440 and Lake Annecy, 100 km South of Lake Chalain (Magny *et al.* 2003, 2009). Lower lake
441 levels were associated with higher GDD5 indicating longer growing seasons and thus lower

442 seasonal contrasts. $\Delta_{\text{seasonality}}$ can thus be used in charcoals to derive further information for
443 paleoclimatic reconstructions.

444

445 **5. Conclusions**

446 19 archeological charcoals from two different climatic periods, 40 years apart, of the
447 Neolithic settlement of Lake Chalain (Jura, France) were characterized by dendro-
448 anthracology and ring-scale isotope geochemistry to assess the relevance of this combined
449 approach for documenting paleoclimates. While the two studied periods differed only by
450 slight differences in precipitation and temperature, the tree-ring width and stable carbon
451 isotope composition of charcoals did record the investigated climatic variation. Within a given
452 charcoal fragment, ring-to-ring isotope pattern exhibited no specific trend through growth, for
453 none of the climatic period studied. Although relatively large charcoal fragments (i.e.
454 comprising 5-19 growth-rings) were selected for the study, they did not allow documenting
455 inter-annual climatic variations. However, comparing earlywood and latewood $\delta^{13}\text{C}$
456 suggested that the wettest and coldest period studied was also characterized by the highest
457 seasonal contrast. The rather limited dataset of this exploratory study calls for more
458 extensive sampling to confirm the obtained conclusions. Nevertheless, this study opens new
459 prospects for paleoclimatic reconstructions based on archeological charcoals as it shows the
460 potential of (i) stable carbon isotopes to record subtle climatic variations under temperate
461 climate and (ii) difference in isotope composition between earlywood and latewood to further
462 document seasonal contrasts.

463

464 **Acknowledgments**

465 We are indebted to Marie Balasse for mass spectrometry facilities and to Denis
466 Fiorillo for isotope measurements. We warmly thank Pierre Pétrequin for providing the
467 charcoal fragments from “Chalain 4” dwelling. Thanks are also due to Damien Deldicque and
468 Jean-Noël Rouzaud for providing access to Raman microspectrometry facility and for
469 valuable advice. Michel Lemoine is acknowledged for help in sample preparation. We owe
470 special thanks to Michel Magny for guidance on climatic interpretations. We are grateful to
471 Véronique Vaury, Anun Martinez, Elise Dufour and Karyne Debue for technical assistance.
472 We also thank Olivier Girardclos, Jean-Luc Dupouey, Marie Alexis and Valérie Daux for
473 helpful discussions. This work was funded by grants from Agence Nationale de la Recherche
474 (ANR JCJC 200101 DENDRAC directed by A. Dufraisse), Sorbonne Universités (CARESE)
475 and Cluster of Excellence MATISSE (Investissements d'Avenir programme ANR-11-IDEX-
476 0004-02 Accompagnement Doctoral Axe Biomineralisation) which were greatly appreciated.
477 Thanks are also due to the three anonymous referees who provided constructive reviews of
478 the manuscript.

479

480 **References**

- 481 Aguilera, M., Espinar, C., Ferrio, J.P., Pérez, G., Voltas, J., 2009. A map of autumn precipitation for the
482 third millennium BP in the Eastern Iberian Peninsula from charcoal carbon isotopes. *Journal of*
483 *Geochemical Exploration, Isoscapes: Isotope mapping and its applications. Isoscapes 2008* 102,
484 157–165.
- 485 Andreu, L., Planells, O., Gutiérrez, E., Helle, G., Schleser, G.H., 2008. Climatic significance of tree-ring
486 width and $\delta^{13}\text{C}$ in a Spanish pine forest network. *Tellus B* 60, 771–781.
- 487 Ascough, P.L., Bird, M.I., Wormald, P., Snape, C.E., Apperley, D., 2008. Influence of production
488 variables and starting material on charcoal stable isotopic and molecular characteristics.
489 *Geochimica et Cosmochimica Acta* 72, 6090–6102.

490 Ascough, P.L., Bird, M.I., Francis, S.M., Thornton, N., Midwood, A.J., Scott, A.C., Apperley, D., 2011.
491 Variability in oxidative degradation of charcoal: Influence of production conditions and
492 environmental exposure. *Geochimica et Cosmochimica Acta* 75, 2361-2378.

493 Ballantyne, A.P., Rybczynski, N., Baker, P.A., Harington, C.R., White, D., 2006. Pliocene Arctic
494 temperature constraints from the growth rings and isotopic composition of fossil larch.
495 *Palaeogeography Palaeoclimatology Palaeoecology* 242, 188–200.

496 Barbaroux, C., Bréda, N., 2002. Contrasting distribution and seasonal dynamics of carbohydrate
497 reserves in stem wood of adult ring-porous sessile oak and diffuse-porous beech trees. *Tree*
498 *Physiology* 22, 1201–1210.

499 Benner, R., Fogel, M.L., Sprague, E.K., Hodson, R.E., 1987. The depletion in ¹³C of lignin and its
500 applications for stable carbon isotope studies. *Nature* 329, 708-710.

501 Bird, M.I., Ascough, P.L., 2012. Isotopes in pyrogenic carbon: A review. *Organic Geochemistry*,
502 *Organic Isotopes in Soil* 42, 1529–1539.

503 Bocherens, H., Friis, E.M., Mariotti, A., Pedersen, K.R., 1993. Carbon isotopic abundances in Mesozoic
504 and Cenozoic fossil plants: Palaeoecological implications. *Lethaia* 26, 347–358.

505 Bocherens, H., Mariotti, A., 1999. Carbon stable isotope analysis of fossil plants. In *Fossil plants and*
506 *spores* (T P Jones & N P Rowe. éd.), The Geological Society, 139-142.

507 Borella, S., Leuenberger, M., Saurer, M., Siegwolf, R., 1998. Reducing uncertainties in δ¹³C analysis of
508 tree rings: Pooling, milling, and cellulose extraction. *Journal of Geophysical Research*.
509 *Atmospheres* 103, 19519–19526.

510 Briffa, K.R., Osborn, T.J., Schweingruber, F.H., Jones, P.D., Shiyatov, S.G., Vaganov, E.A., 2002. Tree-
511 ring width and density data around the Northern Hemisphere: Part 1, local and regional climate
512 signals. *The Holocene* 12, 737–757.

513 Büntgen, U., Franck, D.C., Schmidhaller, M., Neuwirth, B., Seifertn M., Esper, J., 2006. Growth/climate
514 response shift in a long subalpine spruce chronology. *Trees* 20, 99-110.

515 Cotton, J.M., Sheldon, N.D., Strömberg, C.A.E., 2012. High-resolution isotopic record of C4
516 photosynthesis in a Miocene grassland. *Palaeogeography Palaeoclimatology Palaeoecology* 337–
517 338, 88–98.

518 Couvert, M., 1970. Études des charbons préhistoriques: préparation des coupes minces et analyse
519 des structures. AMG, Arts et métiers graphiques.

520 Czimczik, C.I., Preston, C.M., Schmidt, M.W.I., Werner, R.A., Schulze, E.-D., 2002. Effects of charring
521 on mass, organic carbon, and stable carbon isotope composition of wood. *Organic Geochemistry*
522 33, 1207–1223.

523 Damon, P.E., Cheng, S., Linick, T.W., 1989. Fine and hyperfine structure in the spectrum of secular
524 variations of atmospheric ^{14}C . *Radiocarbon* 31, 704–718.

525 Danis, P.A., Masson-Delmotte, V., Stievenard, M., Guillemin, M.T., Daux, V., Naveau, P., von
526 Grafenstein, U., 2006. Reconstruction of past precipitation $\delta^{18}\text{O}$ using tree-ring cellulose $\delta^{18}\text{O}$ and
527 $\delta^{13}\text{C}$: A calibration study near Lac d’Annecy, France. *Earth and Planetary Science Letters* 243, 439–
528 448.

529 Dawson, T.E., Mambelli, S., Plamboeck, A.H., Templer, P.H., Tu, K.P., 2002. Stable Isotopes in Plant
530 Ecology. *Annual Review of Ecology and Systematics* 33, 507–559.

531 Deforce, K., Haneca, K., 2015. Tree-ring analysis of archaeological charcoal as a tool to identify past
532 woodland management: The case from a 14th century site from Oudenaarde (Belgium).
533 *Quaternary International, Charcoal: resource and ubiquitous proxy* 366, 70–80.

534 Deldicque, D., Rouzaud, J.-N., Velde, B., 2016. A Raman – HRTEM study of the carbonization of wood:
535 A new Raman-based paleothermometer dedicated to archaeometry. *Carbon* 102, 319–329.

536 DeNiro, M.J., Hastorf, C.A., 1985. Alteration of $^{15}\text{N}/^{14}\text{N}$ and $^{13}\text{C}/^{12}\text{C}$ ratios of plant matter during the
537 initial stages of diagenesis: Studies utilizing archaeological specimens from Peru. *Geochimica et*
538 *Cosmochimica Acta* 49, 97–115.

539 Dittmar, C., Zech, W., Elling, W., 2003. Growth variations of Common beech (*Fagus sylvatica* L.) under
540 different climatic and environmental conditions in Europe—a dendroecological study. *Forest*
541 *Ecology and Management*. 173, 63–78.

542 Drake, B.L., Hanson, D.T., Boone, J.L., 2012. The use of radiocarbon-derived $\Delta^{13}\text{C}$ as a paleoclimate
543 indicator: applications in the Lower Alentejo of Portugal. *Journal of Archaeological Science* 39,
544 2888–2896.

- 545 Dufraisse, A., 2008. Firewood management and woodland exploitation during the late Neolithic at
546 Lac de Chalain (Jura, France). *Vegetation History and Archaeobotany* 17, 199–210.
- 547 Dufraisse, A., 2006. Charcoal anatomy potential, wood diameter and radial growth. *BAR International*
548 *Series* 1483, 47.
- 549 Dufraisse, A., 2005. Économie du bois de feu et sociétés néolithiques : analyses anthracologiques
550 appliquées aux sites d'ambiance humide des lacs de Chalain et de Clairvaux (Jura, France). *Gallia*
551 *préhistoire* 187–233.
- 552 Dufraisse, A., Garcia-Martinez, M., 2011. Mesurer les diamètres du bois de feu en anthracologie.
553 Outils dendrométriques et interprétation des données. *Anthropobotanica* 1–18.
- 554 Dufraisse, A., Coubray, S., Girardclos, O., Garcia-Martinez, M.S., Nocus, N., Dupin, A., Lemoine, M.,
555 Dupouey, J.L., Marguerie, D., accepted a. Anthraco-typology as a key approach of firewood
556 exploitation and woodland management. Part I: dendro-anthracological tools and reference
557 dataset modelling in the frame of ANR DENDRAC project. *Quaternary International*, this volume.
- 558 Dufraisse, A., Coubray, S., Girardclos, O., Dupin, A., Lemoine, M., accepted b. Contribution of tyloses
559 quantification in earlywood oak vessels to archaeological charcoal analyses: estimation of a
560 minimum age and influences of physiological and environmental factors. *Quaternary*
561 *International*, this volume.
- 562 Eglin, T., Francois, C., Michelot, A., Delpierre, N., Damesin, C., 2010. Linking intra-seasonal variations
563 in climate and tree-ring $\delta^{13}\text{C}$: A functional modelling approach. *Ecological Modelling* 221, 1779–
564 1797.
- 565 Ehleringer J.R. (1991) $^{13}\text{C}/^{12}\text{C}$ fractionation studies and its utility in terrestrial plant fractionation. In
566 *Carbon isotope technique* (Coleman D.C. & Fry B. éd.), Academic Press, 187-200.
- 567 Essiamah, S., Eschrich, W., 1985. Changes of Starch Content in the Storage Tissues of Deciduous Trees
568 During Winter and Spring. *IAWA Journal* 6, 97–106.
- 569 Farquhar, G.D., Caemmerer, S. von, Berry, J.A., 1980. A biochemical model of photosynthetic CO_2
570 assimilation in leaves of C_3 species. *Planta* 149, 78–90.

- 571 Farquhar, G., O'Leary, M., Berry, J., 1982. On the Relationship Between Carbon Isotope
572 Discrimination and the Intercellular Carbon Dioxide Concentration in Leaves. *Functional Plant*
573 *Biology* 9, 121–137.
- 574 February, E., Van der Merwe, N., 1992. Stable carbon isotope ratios of wood charcoal during the past
575 4000 years: anthropogenic and climatic influences. *South African Journal of Science* 88, 291–292.
- 576 Feng, X., Epstein, S., 1995. Carbon isotopes of trees from arid environments and implications for
577 reconstructing atmospheric CO₂ concentration. *Geochimica et Cosmochimica Acta* 59, 2599–2608.
- 578 Ferrio, J.P., Alonso, N., López, J.B., Araus, J.L., Voltas, J., 2006. Carbon isotope composition of fossil
579 charcoal reveals aridity changes in the NW Mediterranean Basin. *Global Change Biology* 12, 1253–
580 1266.
- 581 Figueiral, I., 1999. Lignified and charcoaled fossil wood. In: Jones, T.P., Rowe, N.P. (Eds.), *Fossil*
582 *plants and spores: modern techniques*. The Geological Society, London, 92–96.
- 583 Fiorentino, G., Ferrio, J.P., Bogaard, A., Araus, J.L., Riehl, S., 2014. Stable isotopes in archaeobotanical
584 research. *Vegetation History and Archaeobotany* 24, 215–227.
- 585 Gleixner, G., Danier, H.J., Werner, R.A., Schmidt, H.L., 1993. Correlations between the ¹³C Content of
586 Primary and Secondary Plant Products in Different Cell Compartments and That in Decomposing
587 Basidiomycetes. *Plant Physiology* 102, 1287–1290.
- 588 Gröcke, D.R., 1997. Distribution of C₃ and C₄ Plants in the Late Pleistocene of South Australia
589 Recorded by Isotope Biogeochemistry of Collagen in Megafauna. *Australian Journal of Botany* 45,
590 607–617.
- 591 Hall, G., Woodborne, S., Scholes, M., 2008. Stable carbon isotope ratios from archaeological charcoal
592 as palaeoenvironmental indicators. *Chemical Geology* 247, 384–400.
- 593 Helle, G., Schleser, G.H., 2004. Beyond CO₂-fixation by Rubisco – an interpretation of ¹³C/¹²C
594 variations in tree rings from novel intra-seasonal studies on broad-leaf trees. *Plant, Cell &*
595 *Environment* 27, 367–380.
- 596 Hillis, W.E., 1987. *Heartwood and tree exudates*. Springer Verlag, Berlin.

597 Ishimaru, K., Hata, T., Bronsveld, P., Meier, D., Imamura, Y., 2007. Spectroscopic analysis of
598 carbonization behavior of wood, cellulose and lignin. *Journal of Materials Science* 42, 122-129.

599 Kern, Z., Patkó, M., Kázmér, M., Fekete, J., Kele, S., Pályi, Z., 2013. Multiple tree-ring proxies
600 (earlywood width, latewood width and $\delta^{13}\text{C}$) from pedunculate oak (*Quercus robur* L.), Hungary.
601 Quaternary International, Advancing Pleistocene and Holocene climate change research in the
602 Carpathian-Balkan region 293, 257–267.

603 Kimak, A., Leuenberger, M., 2015. Are carbohydrate storage strategies of trees traceable by early–
604 latewood carbon isotope differences? *Trees* 29, 859–870.

605 Kress, A., Saurer, M., Siegwolf, R.T.W., Frank, D.C., Esper, J., Bugmann, H., 2010. A 350 year drought
606 reconstruction from Alpine tree ring stable isotopes. *Global Biogeochemical Cycles* 24, GB2011.

607 Lavier, C., 1996. Dendrochronologie appliquée à l'archéologie: élaboration d'une chronologie du
608 chêne (*Quercus* sp.) pour le Néolithique à partir des sites lacustres de Clairvaux-les-Lacs et de
609 Chalain (Jura, France). Université de Franche-Comté, Faculté des Lettres et Sciences Humaines,
610 Besançon. Besançon.

611 Leavitt, S.W., 2010. Tree-ring C–H–O isotope variability and sampling. *Science of the Total*
612 *Environment* 408, 5244–5253.

613 Leavitt, S.W., Long, A., 1983. An atmospheric $^{13}\text{C}/^{12}\text{C}$ reconstruction generated through removal of
614 climate effects from tree-ring $^{13}\text{C}/^{12}\text{C}$ measurements. *Tellus B* 35B, 92–102.

615 Leavitt, S.W., Long, A., 1991. Seasonal stable-carbon isotope variability in tree rings: possible
616 paleoenvironmental signals. *Chemical Geology: Isotope Geoscience section* 87, 59–70.

617 Leavitt, S.W., Danzer, S.R., 1993. Method for batch processing small wood samples to holocellulose
618 for stable-carbon isotope analysis. *Analytical Chemistry* 65, 87–89.

619 Livingston, N.J., Spittlehouse, D.L., 1996. Carbon isotope fractionation in tree ring early and late
620 wood in relation to intra-growing season water balance. *Plant Cell & Environment* 19, 768–774.

621 Li, Z.-H., Leavitt, S.W., Mora, C.I., Liu, R.-M., 2005. Influence of earlywood–latewood size and isotope
622 differences on long-term tree-ring $\delta^{13}\text{C}$ trends. *Chemical Geology* 216, 191–201.

- 623 Loader, N.J., Robertson, I., McCarroll, D., 2003. Comparison of stable carbon isotope ratios in the
624 whole wood, cellulose and lignin of oak tree-rings. *Palaeogeography Palaeoclimatology*
625 *Palaeoecology* 196, 395–407.
- 626 Loader, N.J., Mccarroll, D., Gagen, M., Robertson, I., Jalkanen, R., 2011. Extracting climatic
627 information from Stable Isotopes in Tree Rings. In *Stable Isotopes as indicators of ecological*
628 *change* (Todd E. Dawson and Rolf T.W.Siegwolf. éd.), Academic Press, 27-48.
- 629 Ludemann, T., Nelle, O., 2002. Die Wälder am Schauinsland und ihre Nutzung durch Bergbau und
630 Köhlerei. Forstliche Versuchs-und Forschungsanst. Baden-Württemberg.
- 631 Lundström-Baudais, K., 1986. Étude paléoethnobotanique de la station III de Clairvaux. In : P.
632 Pétrequin (éd.). Les sites littoraux néolithiques de Clairvaux-les-Lacs (Jura). I, Problématique
633 générale, l'exemple de la station III, Paris : Maison des Sciences de l'Homme. Archéologie et
634 culture matérielle, p. 311-391.
- 635 Macko, S.A., Fogel, M.L., Hare, P.E., Hoering, T.C. (1987) Isotopic fractionation of nitrogen and carbon
636 in the synthesis of aminoacids by microorganisms. *Chemical Geology* 65, 79-92.
- 637 Magny, M., 2004. Holocene climate variability as reflected by mid-European lake-level fluctuations
638 and its probable impact on prehistoric human settlements. *Quaternary International, The record*
639 *of Human /Climate interaction in Lake Sediments* 113, 65–79.
- 640 Magny, M., 1993a. Un cadre climatique pour les habitats lacustres préhistoriques? *Comptes-rendus*
641 *de l'Académie des sciences. Série 2, Mécanique, Physique, Chimie, Sciences de l'univers, Sciences*
642 *de la Terre* 316, 1619–1625.
- 643 Magny, M., 1993b. Une nouvelle mise en perspective des sites archéologiques lacustres : Les
644 fluctuations holocènes des lacs jurassiens et subalpins. *Gallia Préhistoire* 35, 253-282.
- 645 Magny, M. Bégeot, C., Guiot, J., Peyron, O., 2003. Contrasting patterns of hydrological changes in
646 Europe in response to Holocene climate cooling phases. *Quaternary Science International* 22,
647 1589-1596.
- 648 Magny, M., Peyron, O., Gauthier, E., Rouèche, Y., Bordon, A., Billaud, Y., Chapron, E., Marguet, A.,
649 Pétrequin, Vannière, B., 2009. Quantitative reconstruction of climatic variations during the Bronze

650 and early Iron age based on pollen and lake-level data in the NW Alps, France. *Quaternary*
651 *International* 200, 102-110.

652 Marguerie, D., Hunot, J.-Y., 2007. Charcoal analysis and dendrology: data from archaeological sites in
653 north-western France. *Journal of Archaeological Science* 34, 1417–1433.

654 Masi, A., Sadori, L., Baneschi, I., Siani, A.M., Zanchetta, G., 2013. Stable isotope analysis of
655 archaeological oak charcoal from eastern Anatolia as a marker of mid-Holocene climate change.
656 *Plant Biology* 15, 83–92.

657 Matisons, R., Elferts, D., Brūmelis, G., 2013. Pointer years in tree-ring width and earlywood-vessel
658 area time series of *Quercus robur*—Relation with climate factors near its northern distribution
659 limit. *Dendrochronologia* 31, 129–139.

660 Mazany, T., Lerman, J.C., Long, A., 1980. Carbon-13 in tree-ring cellulose as an indicator of past
661 climates. *Nature* 287, 432–435.

662 McCarroll, D., Loader, N.J., 2004. Stable isotopes in tree rings. *Quaternary Science Reviews, Isotopes*
663 *in Quaternary Paleoenvironmental reconstruction* 23, 771–801.

664 Michelot, A., 2011. Croissance et $\delta^{13}\text{C}$ des cernes de trois essences forestières tempérées (*Fagus*
665 *sylvatica*, *Quercus petraea* et *Pinus sylvestris*) face aux variations climatiques à l'échelle
666 interannuelle et saisonnière. Ph.D. Thesis, Université Paris-Sud 11, Orsay, France.

667 Midwood, A.J., Boutton, T.W., 1998. Soil carbonate decomposition by acid has little effect on $\delta^{13}\text{C}$ of
668 organic matter. *Soil Biology and Biochemistry* 30, 1301–1307.

669 Mouthon, J., 1997. Paléoenvironnement du lac de Chalain vu par la malacologie (de l'Atlantique
670 ancien au Subboréal). Sites Littoraux Néolithiques Clairvaux--Lacs Chalain Jura 3200-2900 Av J-C 3,
671 87.

672 Nguyen Tu, T.T., Kvaček, J., Uličný, D., Bocherens, H., Mariotti, A., Broutin, J., 2002. Isotope
673 reconstruction of plant palaeoecology. Case study of Cenomanian floras from Bohemia.
674 *Palaeogeography, Palaeoclimatology, Palaeoecology* 183, 43–70.

675 Nissenbaum, A., Schallinger, K.M., 1974. The distribution of the stable carbon isotope ($^{13}\text{C}/^{12}\text{C}$) in
676 fractions of soil organic matter. *Geoderma* 11, 137–145

677 Nola, P., 1996. Climatic signal in earlywood and latewood of deciduous oaks from northern Italy. *Tree*
678 *Rings, Environment, and Humanity. Radiocarbon. The University of Arizona, Department of*
679 *Geosciences, Tucson* 249–258.

680 O’Leary, M.H., 1981. Carbon isotope fractionation in plants. *Phytochemistry* 20, 553–567.

681 Park, R., Epstein, S., 1961. Metabolic fractionation of C13 & C12 in plants. *Plant Physiology* 36, 133–
682 138.

683 Petrequin P., 2012.- Réflexions sur dix années de recherche sur le Néolithique dans la Région
684 Franche-Comté (1995-2005), in : 1995-2005. Dix ans d’archéologie en Franche-Comté. Bilan
685 scientifique hors-série, Besançon, Direction Régionale des Affaires Culturelles : 102-112.

686 Pétrequin P., Arbogast R.-M., Bourquin-Mignot C., Duplaix A., Martineau R., Pétrequin A.-M., Viellet
687 A., 2002. Le mythe de la stabilité : déséquilibres et réajustements d’une communauté agricole
688 néolithique dans le Jura français, du 32^{ème} siècle au 30^{ème} siècle av. J.-C. In : Richard, H., Vignot, A.
689 (Eds.), *Equilibres et ruptures dans les écosystèmes depuis 20000 ans en Europe de l’Ouest*
690 *(septembre 2000), Besançon : Collection Annales Littéraires de l’Université de Franche-Comté.*
691 *Série Environnement, Sociétés et Archéologie n°3, pp. 175-190.*

692 Pétrequin P., Pétrequin A.-M., 2000. Chalain 2000. Quatre millénaires d’habitat lacustre mis en
693 question. Report, Besançon et Doucier : Laboratoire de Chrono-Ecologie, UFR des Sciences et le
694 Centre de Recherche Archéologique de la Vallée de l’Ain.

695 Richard, H., 1997. Analyse pollinique d’un sondage de 7,5 m. In : Pétrequin, P. (Ed.). *Les sites*
696 *littoraux néolithiques de Clairvaux-les-Lacs et de Chalain (Jura). III, Chalain station 3, 3200-2900*
697 *av. J.-C., Paris : Maison des Sciences de l’Homme. Archéologie et culture matérielle, p. 101-112.*

698 Robertson, I., Loader, N.J., McCarroll, D., Carter, A.H.C., Cheng, L., Leavitt, 2004. $\delta^{13}\text{C}$ of tree-ring
699 lignin as an indirect measure of climate change. *Water, Air and Soil Pollution: Focus* 4, 531-544.

700 Rouzaud, J.-N., Deldicque, D., Charon, É., Pageot, J., 2015. Carbons at the heart of questions on
701 energy and environment: A nanostructural approach. *Comptes Rendus Geoscience* 347, 124–133.

702 Saurer, M., Spahni, R., Frank, D.C., Joos, F., Leuenberger, M., Loader, N.J., McCarroll, D., Gagen, M.,
703 Poulter, B., Siegwolf, R.T.W., Andreu-Hayles, L., Boettger, T., Dorado Liñán, I., Fairchild, I.J.,
704 Friedrich, M., Gutierrez, E., Haupt, M., Hiltunen, E., Heinrich, I., Helle, G., Grudd, H., Jalkanen, R.,

- 705 Levanič, T., Linderholm, H.W., Robertson, I., Sonninen, E., Treydte, K., Waterhouse, J.S., Woodley,
706 E.J., Wynn, P.M., Young, G.H.F., 2014. Spatial variability and temporal trends in water-use
707 efficiency of European forests. *Global Change Biology* 20, 3700–3712.
- 708 Tieszen, L.L., 1991. Natural variations in the carbon isotope values of plants: implications for
709 archaeology, ecology, and paleoecology. *Journal of Archaeological Science* 18, 227–248.
- 710 Treydte, K., Schleser, G.H., Schweingruber, F.H., Winiger, M., 2001. The climatic significance of $\delta^{13}\text{C}$ in
711 subalpine spruces (Lötschental, Swiss Alps). *Tellus B* 53.
- 712 Turekian, V.C., Macko, S., Ballentine, D., Swap, R.J., Garstang, M., 1998. Causes of bulk carbon and
713 nitrogen isotopic fractionations in the products of vegetation burns: laboratory studies. *Chemical*
714 *Geology* 152, 181–192.
- 715 Turney, C.S.M., Wheeler, D., Chivas, A.R., 2006. Carbon isotope fractionation in wood during
716 carbonization. *Geochimica et Cosmochimica Acta* 70, 960–964.
- 717 Vaiglova, P., Snoeck, C., Nitsch, E., Bogaard, A., Lee-Thorp, J., 2014a. Impact of contamination and
718 pre-treatment on stable carbon and nitrogen isotopic composition of charred plant remains.
719 *Rapid Communications in Mass Spectrometry* 28, 2497–2510.
- 720 Vaiglova, P., Bogaard, A., Collins, M., Cavanagh, W., Mee, C., Renard, J., Lamb, A., Gardeisen, A.,
721 Fraser, R., 2014b. An integrated stable isotope study of plants and animals from Kouphovouno,
722 southern Greece: a new look at Neolithic farming. *Journal of Archaeological Science* 42, 201–215.
- 723 Verheyden, A., Roggeman, M., Bouillon, S., Elskens, M., Beeckman, H., Koedam, N., 2005.
724 Comparison between $\delta^{13}\text{C}$ of α -cellulose and bulk wood in the mangrove tree *Rhizophora*
725 *mucronata*: Implications for dendrochemistry. *Chemical Geology* 219, 275–282.
- 726 Vernet, J.-L., 2006. History of the *Pinus sylvestris* and *Pinus nigra* ssp. *salzmanni* forest in the Sub-
727 Mediterranean mountains (Grands Causses, Saint-Guilhem-le-Désert, southern Massif Central,
728 France) based on charcoal from limestone and dolomitic deposits. *Vegetation History and*
729 *Archaeobotany* 16, 23–42.
- 730 Vernet, J.-L., Pachiardi, C., Bazile, F., Durand, A., Fabre, L., Heinz, C., Solari, M.-E., Thibeault, S., 1996.
731 Le $\delta^{13}\text{C}$ de charbons de bois préhistoriques et historiques méditerranéens, de 35 000 BP à l'actuel.

- 732 Premiers résultats. Comptes rendus de l'Académie des sciences. Série 2. Sciences de la terre et
733 des planètes 323, 319–324.
- 734 Weigl, M., Grabner, M., Helle, G., Schleser, G.H., Wimmer, R., 2007. Variability of latewood-widths
735 and $\delta^{13}C$ -stable isotope ratios in a sessile oak tree (*Quercus petraea* (Matt.) Liebl.).
736 Dendrochronologia, EuroDendro 2005: Humans and EnvironmentEuroDendro 2005: Humans and
737 Environment 24, 117–122.
- 738 Young, G.H.F., Bale, R.J., Loader, N.J., Mccarroll, D., Nayling, N., Vousden, N., 2012. Central England
739 temperature since AD 1850: the potential of stable carbon isotopes in British oak trees to
740 reconstruct past summer temperatures. Journal of Quaternary Science 27, 606–614.
- 741 Zhang, S.Y., 1997. Variations and correlations of various ring width and ring density features in
742 European oak: implications in dendroclimatology. Wood Science and Technology 31, 63–72.

743 **Table 1.** Dendro-anthracological and geochemical characteristics of the charcoals studied in "Chalain
 744 4" sequence.

Parameter	Layers A/B ¹			Layer G ¹			Difference ² (ANOVA)
	Mean ± SD	Range	Size	Mean ± SD	Range	Size	
Ring width (mm) ³	0.7 ± 0.1	(0.4; 1.0)	103	1.1 ± 0.3	(0.5; 1.9)	87	*
Latewood proportion (%) ³	52 ± 13	(23; 84)	103	52 ± 11	(24; 82)	72	ns
Charcoal-pith distance (mm) ³	47 ± 11	(36; 65)	10	47 ± 5	(40; 53)	8	ns
Sapwood proportion (%) ⁴	81.8			33.3			*
H _D /H _G ^{5, 6}	0.6 ± 0.1	(0.5; 0.8)	10	0.6 ± 0.1	(0.5; 0.8)	9	ns
δ ¹³ C (‰) ⁶	-25.9 ± 0.6	(-27.8; -21.9)	186	-26.5 ± 0.6	(-28.0; -25.0)	141	*
Δ _{seasonality} (‰) ^{7, 3}	-0.01 ± 0.61	(-2.84; +1.13)	86	+0.20 ± 0.28	(-0.78; +1.05)	57	*

745
 746 ¹ Mean ± standard deviation (minimum; maximum) and sample size (i.e. *n*); ² significance of the
 747 difference between layers A/B and layer G based on ANOVA, *: statistically significant (*p*-value <
 748 0.05), ns: non-significant (*p*-value > 0.05); ³ pooled values of sapwood and heartwood; ⁴ Proportion of
 749 sapwood charcoals among all the charcoals studied in the layer; ⁵ Height ratio of D and G bands in
 750 Raman microspectrometry; ⁶ pooled values of earlywood, latewood, sapwood and heartwood; ⁷
 751 Δ_{seasonality} = δ¹³C_{earlywood} - δ¹³C_{latewood}

752 **Figure captions**

753

754 **Figure 1.** Lake Chalain setting. a) Geographical location of Lake Chalain (46° 40' 30" N, 5°
755 46' 40" E), b) Sedimentary sequence of "Chalain 4" dwelling (modified from Pétrequin and
756 Pétrequin 2000).

757

758 **Figure 2.** Raman spectrum (1800-1000 cm⁻¹) typical for the studied charcoals.

759

760 **Figure 3.** Ring width of charcoals from layers A/B and G. Boxplots show median (line), mean
761 (circle) and minimum and maximum values (whiskers). The box displays the 25-75 percent
762 quartile. Notches roughly indicate the 95% confidence interval of the median. Different letters
763 above boxplots indicate statistically significant difference (ANOVA, *p*-value < 0.05).

764

765 **Figure 4.** Relationship between acid (HCl, open symbols) and base treatment (NaOH, solid
766 symbols) on charcoal δ¹³C values. The 1/1 dotted line is given to facilitate comparisons
767 between treatments. Blue ○: charcoals from layers A/B, Pink △: charcoals from layer G.

768

769 **Figure 5.** δ¹³C values of charcoals from layers A/B and G (pooled values of earlywood and
770 latewood) distinguish heartwood from sapwood. Boxplots show median (line), mean (circle)
771 and minimum and maximum values (whiskers). The box displays the 25-75 percent quartile.
772 Notches roughly indicate the 95% confidence interval of the median. Different letters above
773 boxplots indicate statistically significant difference (ANOVA, *p*-value < 0.05).

774

775 **Figure 6.** Relationship between latewood δ¹³C and cambial age (ring number). For each
776 charcoal fragment, the nearest ring to the pith was arbitrary numbered "1"; all rings "1" are
777 not contemporaneous. Different charcoal fragments are represented by different symbols.

778

779 **Figure 7.** $\Delta_{\text{seasonality}}$ of charcoals from layers A/B and G ($\Delta_{\text{seasonality}} = \delta^{13}\text{C}_{\text{earlywood}} - \delta^{13}\text{C}_{\text{latewood}}$).
780 Boxplots show median (line), mean (circle) and minimum and maximum values (whiskers).
781 The box displays the 25-75 percent quartile. Notches roughly indicate the 95% confidence
782 interval of the median. Different letters above boxplots indicate statistically significant
783 difference (ANOVA, p -value < 0.05).













