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# 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)

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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

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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 ( $^{13}\text{C}$ ) 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  $^{13}\text{C}$ -content  
25 significantly different between the two studied periods, in agreement with previously inferred  
26 climatic difference. Monitoring ring-to-ring  $^{13}\text{C}$  variation within each charcoal fragment  
27 revealed no noticeable climatic trend, for none of the studied periods. However, calculation of  
28 the difference in  $^{13}\text{C}$ -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,  $^{13}\text{C}$ , 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  $\text{CO}_2$ ),  
40 (ii) the photosynthetic pathway used by plants (*i.e.*  $\text{C}_3$ ,  $\text{C}_4$  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  $\text{CO}_2$  (*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  $^{13}\text{C}$ -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,  $\alpha$ -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  $^{14}\text{C}$  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 m<sup>2</sup> 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.



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**3. Materials and methods**

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

*3.1. Dendro-anthracology*

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

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

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

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

Duraminisation, the changeover of sapwood to heartwood, may influence wood  $\delta^{13}\text{C}$  values (Borella *et al.*, 1998). In some species the coloration of heartwood due to the deposition of lignins and polyphenols makes heartwood easily recognizable (Hillis, 1987). However, charcoalification process erases such a color difference. Although tyloses can form in response to fungal attack, the abundant formation of tyloses in earlywood vessels was 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  $\text{cm}^{-1}$  wavenumber range. Focusing  
234 on the 1200 to 1700  $\text{cm}^{-1}$  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  $\text{cm}^{-1}$  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  $^{13}\text{R}$  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  $H_D/H_G$  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  $\text{C}_3$  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  $^{13}\text{C}$ -enriched with respect to heartwood charcoals (statistically  
374 significant;  $p < 0.05$ ; Fig. 5). This is in agreement with (i) the general  $^{13}\text{C}$ -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  $^{13}\text{C}$ -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  $^{14}\text{C}$  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  $^{13}\text{C}$ -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

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479

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

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

745

746 <sup>1</sup> Mean ± standard deviation (minimum; maximum) and sample size (i.e. *n*); <sup>2</sup> 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); <sup>3</sup> pooled values of sapwood and heartwood; <sup>4</sup> Proportion of  
749 sapwood charcoals among all the charcoals studied in the layer; <sup>5</sup> Height ratio of D and G bands in  
750 Raman microspectrometry; <sup>6</sup> pooled values of earlywood, latewood, sapwood and heartwood; <sup>7</sup>  
751 Δ<sub>seasonality</sub> = δ<sup>13</sup>C<sub>earlywood</sub> - δ<sup>13</sup>C<sub>latewood</sub>

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<sup>-1</sup>) 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 δ<sup>13</sup>C 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.** δ<sup>13</sup>C 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 δ<sup>13</sup>C 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).















