Tree-ring δ^{13}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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Abstract:

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

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

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

1. Introduction

The stable carbon isotope composition ($\delta^{13}C$) of plant tissues depends on: (i) the inorganic carbon source used by the plants for their photosynthesis (*i.e*. atmospheric CO_2), (ii) the photosynthetic pathway used by plants (*i.e*. C_3, C_4 or CAM), and (iii) environmental conditions (Farquhar *et al.*, 1980; O'Leary, 1981; Leavitt and Long, 1983; Tieszen, 1991; McCarroll and Loader, 2004). A number of environmental parameters (*e.g*. irradiance, nutrient supply, temperature, water availability, etc.) may influence plant $\delta^{13}C$ values, water availability probably being one of the most important, in both arid and temperate climates (Farquhar *et al.*, 1982; Dawson *et al.*, 2002; Kress *et al*., 2010; Saurer *et al*., 2014). Applied to ancient plants, stable carbon isotope studies allowed reconstructions of past photosynthetic pathways (e.g. Bocherens *et al.*, 1993; Cotton *et al.,* 2012), of isotope composition of past atmospheric CO_2 (e.g. Gröcke, 1997), or of the water-stress experienced by plants in the past (Nguyen Tu *et al.*, 2002). Stable isotope characterization of tree-ring series from recent woods was established as an efficient approach to document detailed variations in temperature and rainfall over the last centuries (Leavitt and Long, 1991; Feng and Epstein, 1995; Treydte *et al.*, 2001; Danis *et al.*, 2006; Young *et al.*, 2012). Owing to their sensibility to temperature and/or precipitation, dendrometric patterns of woods, such as ring width or earlywood/latewood proportions (i.e. springwood/summerwood proportions) of a given growth ring, also constitute helpful paleoclimatic proxies (Nola, 1996; Zhang, 1997; Briffa *et al.* 2002; Dittmar *et al.* 2003; Büntgen *et al.* 2006). Combining ring width and stable isotope composition further provides better constrained paleoclimatic estimations, as they record complementary climatic signals (Ballantyne *et al.*, 2006; Weigl *et al.*, 2007). Indeed, ring-width characteristics are often considered as more sensitive to local factors than $\delta^{13}C$ which may give access to larger scale climatic signals (Andreu *et al.,* 2008). Stable isotope measurements are generally achieved on latewood/summerwood in dendroclimatology. Indeed, contrary to earlywood that is synthesized before bud break (Essiamah and Eschrich, 1985), latewood is expected to be little influenced by remobilization of the carbon stored the preceding years (Borella *et al.*, 1998; Barbaroux and Breda, 2002).

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

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

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

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

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

(ii) The Neolithic sedimentary sequence of Lake Chalain comprises several human occupation periods. Multidisciplinary studies (i.e. malacology, palynology, sedimentology and ^{14}C geochemistry) have shown that these occupation periods follow several decades without human occupation due to unfavorable climatic conditions for Neolithic farming societies. Two main phases were thus distinguished in this study: one without human occupation corresponding to cool and wet climatic conditions, followed by a phase comprising several human occupations and corresponding to favorable climatic conditions (low lake level due to relatively warmer and drier conditions; Damon *et al.*, 1989; Magny, 1993a; Richard, 1997; Mouthon, 1997).

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

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

2. The study site and its region

2.1. Regional setting

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

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

2.2. Archeological sites

Since the beginning of archeological research in 1904, 32 settlement sites localized in the Western lakeshore of Chalain have been found and dated between 5300 and 600 B.C. (Pétrequin, 2012). This study focuses on “Chalain 4” lake dwelling. “Chalain 4” is located on the North of the Western lakeshore of Chalain, on a peninsula of about 0.5 ha. The excavation area of 300 m^2 included a plank way connecting the village to the hinterland, and one of the rows of houses of the village, estimated at a dozen. Dendrochronological study of 350 timber pieces has shown that “Chalain 4” occupation had spanned from 3040 to 3000 B.C. (Lavier, 1996). The occupation is preceded by a lack of settlements between 3150 and 3040 B.C. corresponding to an important cultural change and a minor climatic deterioration phase with high water level, higher precipitation and lower temperatures (Pétrequin *et al.*, 2002; Magny, 2004).

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

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):

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

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 $\delta^{13}C$ 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

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

3.2. Raman microspectrometry

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

3.3. Stable carbon isotope analyses

Samples for isotope analyses were drilled under binocular objective magnification using a 0.9 mm tungsten carbide drill bit. This method allowed sampling separately earlywood and latewood, leading to approximately 0.5 mg of fine homogeneous powder per sample. Care was taken to avoid contamination between each sample: (i) the drill bit was washed with 96% ethanol and ultrasonicated in ultrapure water, and (ii) the sampled charcoal was cleaned with dry compressed air. The $^{13}C/^{12}C$ ratios were determined by isotope ratio mass spectrometry and expressed, in per mil (‰), as $\delta^{13}C$ values according to equation (1):

$$\delta^{13}C = [^{13}R_{sample}/^{13}R_{standard})-1]\times 1000 \qquad (1)$$

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

3.4. Acid or base pretreatment test

The minute size of samples for intra-ring isotope analyses did not allow systematic acid or base treatment as often recommended to avoid potential bias in isotope measurements due to adsorption of exogenous organic and inorganic carbon (Nissenbaum and Schallinger, 1974; DeNiro and Hastorf, 1985; Ascough *et al.*, 2011). Preliminary tests were thus conducted on 20 charcoal fragments, representative of layers A/B and layer G, to estimate the extent of this potential bias. The test protocol was adapted from DeNiro and Hastorf (1985), Midwood and Boutton (1998) and Bocherens and Mariotti (1999). The tested charcoals were ca. 3.5 mm width; they were divided into two equivalent parts by transversal sectioning (i.e. perpendicular to the axis of the fibers). One part was submitted to chemical treatment prior to isotope analysis, while the other part was directly analyzed. Ten charcoal fragments were tested for inorganic carbon adsorption with 0.1 M hydrochloric acid (HCl), while ten charcoal fragments were tested for organic carbon adsorption with 1M sodium hydroxide (NaOH). Charcoals were immersed for 30h in the mentioned solutions so as to evaluate the maximal deviation to be expected. The samples were then rinsed by three successive 1h ultrasonications in ultrapure water, changing water for each bath. After a final flushing with ultrapure water, the samples were further oven-dried at 40°C to constant weight and crushed prior to analyses.

3.5. Statistical analyses

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

4. Results and discussion

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

4.1. Dendro-anthracology

The studied charcoals appeared representative of the sequence as their dendro-anthracological features fall within the range of those previously obtained from a larger sample set of "Chalain 4" oak charcoals (121 from layer G and 111 from layers A/B; Dufraisse, 2005, 2008). Charcoal ring width averages at 0.7 mm for layers A/B, and at 1.1 mm for layer G (i.e. 37% more than layers A/B; Table 1, Fig. 3). Ring widths of charcoals in layer G are statistically higher than those of layers A/B (, $p < 0.05$). In deciduous oak, tree-ring widths are known to decrease when precipitation decreases and temperature increases (Michelot, 2011; Matisons *et al.*, 2013). The higher ring width in layer G when compared with layers A/B is thus consistent with paleoclimatic data (Magny, 1993a). Latewood proportion averages at 52% for A/B and G charcoals. It is statistically similar for the two data sets (p = 0.78), although it is often considered to decrease with decreasing precipitation (Zhang, 1997; Kern *et al.*, 2013).

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

4.2. Charcoalification temperatures

The studied charcoal fragments were characterized by Raman microspectrometry to control their degree of thermal alteration (e.g. Rouzaud *et al.*, 2015) as charcoal $\delta^{13}C$ may be affected by carbonization temperature (Ferrio *et al.*, 2006; Turney *et al.*, 2006; Ascough *et al.*, 2008). The H_D/H_G ratio gives an estimation of the highest combustion temperature undergone by charcoals, as established for pine and poplar woods carbonized at various temperatures in a muffle furnace (Deldicque *et al.*, 2016). The studied charcoal fragments exhibit Raman spectra typical for charred material, dominated by the usual G and D broad bands (Fig. 2). The two studied occupation layers led to statistically similar H_D/H_G ratios (p = 0.4), around 0.6 (Table 1). Deldicque *et al.* (2016) paleothermometer suggests combustion temperature around 700°C for the studied charcoals, substantially above the limit temperature for charcoals to reach $\delta^{13}C$ values representative of a single component, lignin (Ferrio *et al,* 2006; Turney *et al.*, 2006; Ascough *et al.* 2008). However, absolute temperature estimation should be taken with caution since the transfer function between H_D/H_G ratio and carbonization temperature was obtained (i) on woods that are less dense than deciduous oak wood, and (ii) in a muffle furnace for a carbonization duration of one hour (Deldicque *et al.*, 2016), which may not reflect the actual carbonization conditions undergone by the studied charcoals (*i.e*. in open fireplace). Nevertheless, although the calculated carbonization temperature may vary according to species or carbonization duration, the shape of the transfer function appeared stable whatever the conditions (Deldicque *et al.*, 2016). Accordingly, H_D/H_G ratios likely constitute reasonable parameters for comparative purposes within a single wood species. Hence, the H_D/H_G ratios obtained for layers A/B and G show that the charcoal fragments from these two layers were carbonized at similar temperature, thus implying that the isotope signature of the studied charcoals does not depend on their degree of charcoalification/carbonization.

4.3. Stable carbon isotopes

4.3.1. Potential contamination by exogenous carbon

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

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

4.3.2. General climatic patterns

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

$\delta^{13}C$ values of oak charcoal thus constitute a reliable record of climatic conditions along "Chalain 4" sequence. Climatic variations during Neolithic at Chalain were attested by several approaches (malacology, palynology, sedimentology and ^{14}C geochemistry; Damon *et al.*, 1989; Magny, 1993a; Mouthon, 1997; Richard, 1997). Nevertheless, these variations were rather subtle as (i) remaining within the range of mountain semi-continental temperate climate and (ii) the two layers studied were only 40 years apart (Lavier, 1996). As a result, subtle climatic variations can be recorded through stable carbon isotope ratios of oak charcoals, whereas this was not the case for latewood proportion (see section 4.1.). Growth-ring width and $\delta^{13}C$ values both depend on a number of genetic and physiological factors (position in the tree for example), environmental factors (such as light, soil) and human factors (clearings, woodland management). However, in the Jura Moutain at 500 m of altitude, temperature and precipitation are probably not a limiting factor for width growth while they markedly influence $\delta^{13}C$ values in deciduous oak. In any case, the present study thus extends the potential of isotope composition of archeological charcoals to record small climatic variations in temperate climate, while previous paleoclimatic studies based on charcoal isotope composition dealt with contrasted climates (Hall *et al.*, 2008; Aguilera *et al.*, 2009; Masi *et al*., 2013; Vaiglova *et al.*, 2014b).

4.3.3. Inter- and intra-year climatic variations

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

Although isotope dendroclimatology based on uncharred/recent wood favors analyses of latewood to avoid carbon contribution from previous year accumulates (Borella *et al.*, 1998), comparing $\delta^{13}C$ values of earlywood and latewood of a particular growth ring may bring information on seasonal contrasts (Livingston and Spittlehouse, 1996; Helle and Schleser, 2004; Li *et al.*, 2005; Eglin *et al.*, 2010; Kimak and Leuenberger, 2015). We propose to name $\Delta_{seasonality}$ the difference calculated by Livingston and Spittlehouse (1996), as in equation (3):

$$\Delta_{seasonality} = \delta^{13}C_{earlywood} - \delta^{13}C_{latewood} \qquad (3)$$

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

5. Conclusions

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

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

Parameter	**Layers A/B**[1]			**Layer G**[1]			**Difference**[2]
	Mean ± SD	Range	Size	Mean ± SD	Range	Size	(ANOVA)
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
$\delta^{13}C$ (‰) [6]	-25.9 ± 0.6	(-27.8; -21.9)	186	-26.5 ± 0.6	(-28.0; -25.0)	141	*
$\Delta_{seasonality}$ (‰) [7, 3]	-0.01 ± 0.61	(-2.84; +1.13)	86	+0.20 ± 0.28	(-0.78; +1.05)	57	*

[1] Mean ± standard deviation (minimum; maximum) and sample size (i.e. *n*); [2] significance of the difference between layers A/B and layer G based on ANOVA, *: statistically significant (*p*-value < 0.05), ns: non-significant (*p*-value > 0.05); [3] pooled values of sapwood and heartwood; [4] Proportion of sapwood charcoals among all the charcoals studied in the layer; [5] Height ratio of D and G bands in Raman microspectrometry; [6] pooled values of earlywood, latewood, sapwood and heartwood; [7] $\Delta_{seasonality} = \delta^{13}C_{earlywood} - \delta^{13}C_{latewood}$

Figure captions

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

Figure 2. Raman spectrum (1800-1000 cm^{-1}) typical for the studied charcoals.

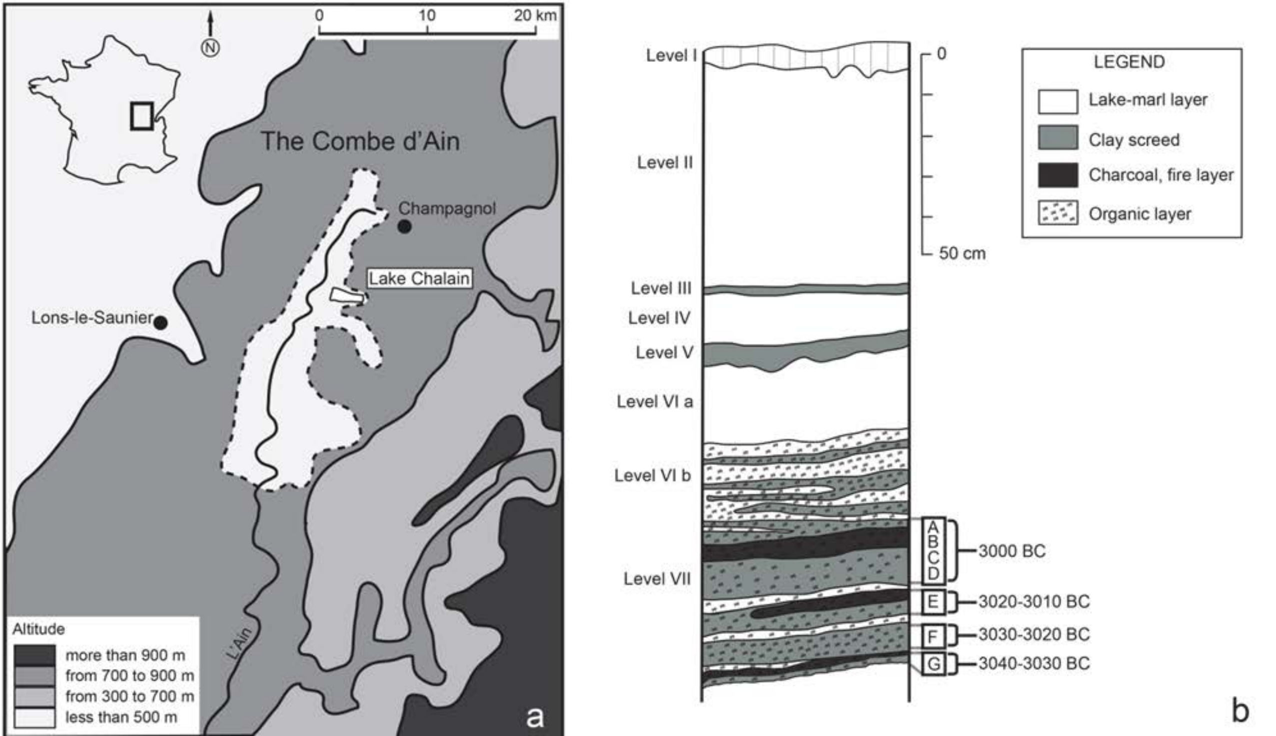
Figure 3. Ring width of charcoals from layers A/B and G. Boxplots show median (line), mean (circle) and minimum and maximum values (whiskers). The box displays the 25-75 percent quartile. Notches roughly indicate the 95% confidence interval of the median. Different letters above boxplots indicate statistically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

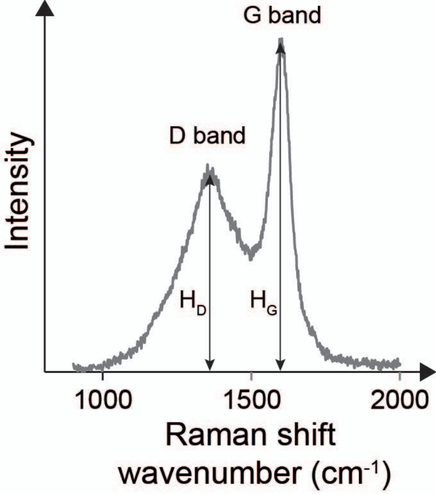
Figure 4. Relationship between acid (HCl, open symbols) and base treatment (NaOH, solid symbols) on charcoal $\delta^{13}C$ values. The 1/1 dotted line is given to facilitate comparisons between treatments. Blue ○: charcoals from layers A/B, Pink △: charcoals from layer G.

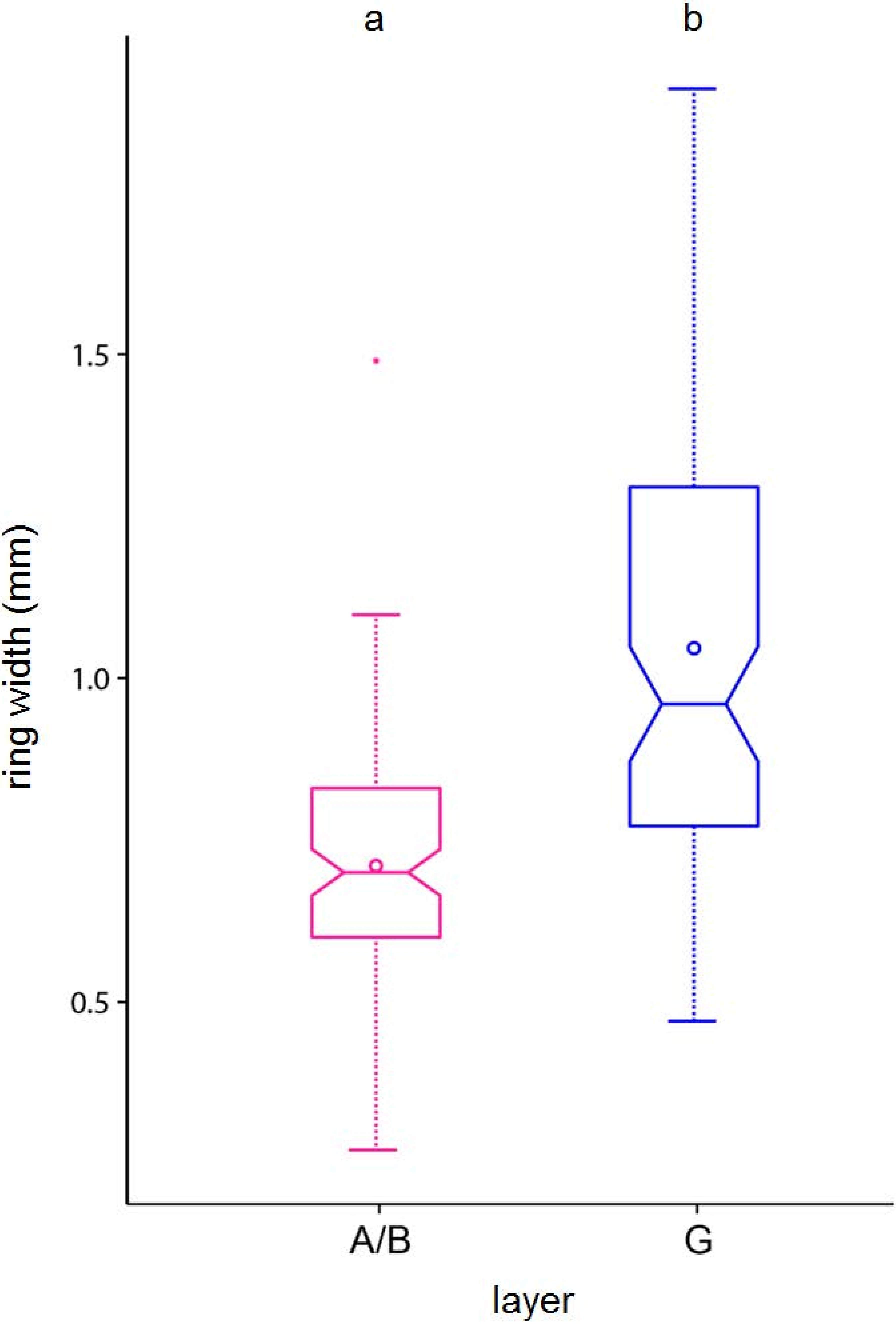
Figure 5. $\delta^{13}C$ values of charcoals from layers A/B and G (pooled values of earlywood and latewood) distinguish heartwood from sapwood. Boxplots show median (line), mean (circle) and minimum and maximum values (whiskers). The box displays the 25-75 percent quartile. Notches roughly indicate the 95% confidence interval of the median. Different letters above boxplots indicate statistically significant difference (ANOVA, p-value < 0.05).

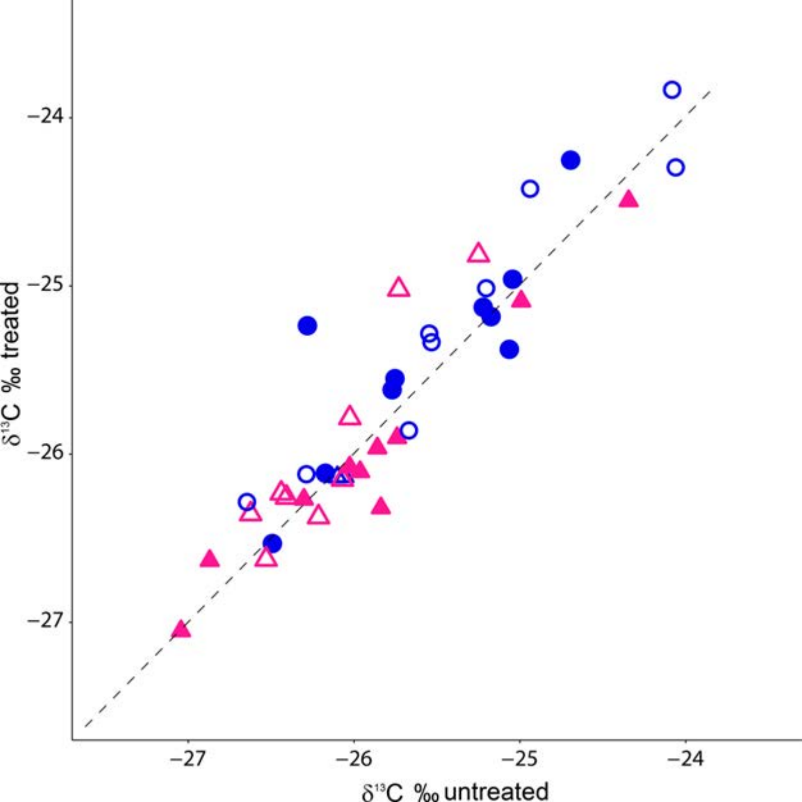
Figure 6. Relationship between latewood $\delta^{13}C$ and cambial age (ring number). For each charcoal fragment, the nearest ring to the pith was arbitrary numbered "1"; all rings "1" are not contemporaneous. Different charcoal fragments are represented by different symbols.

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

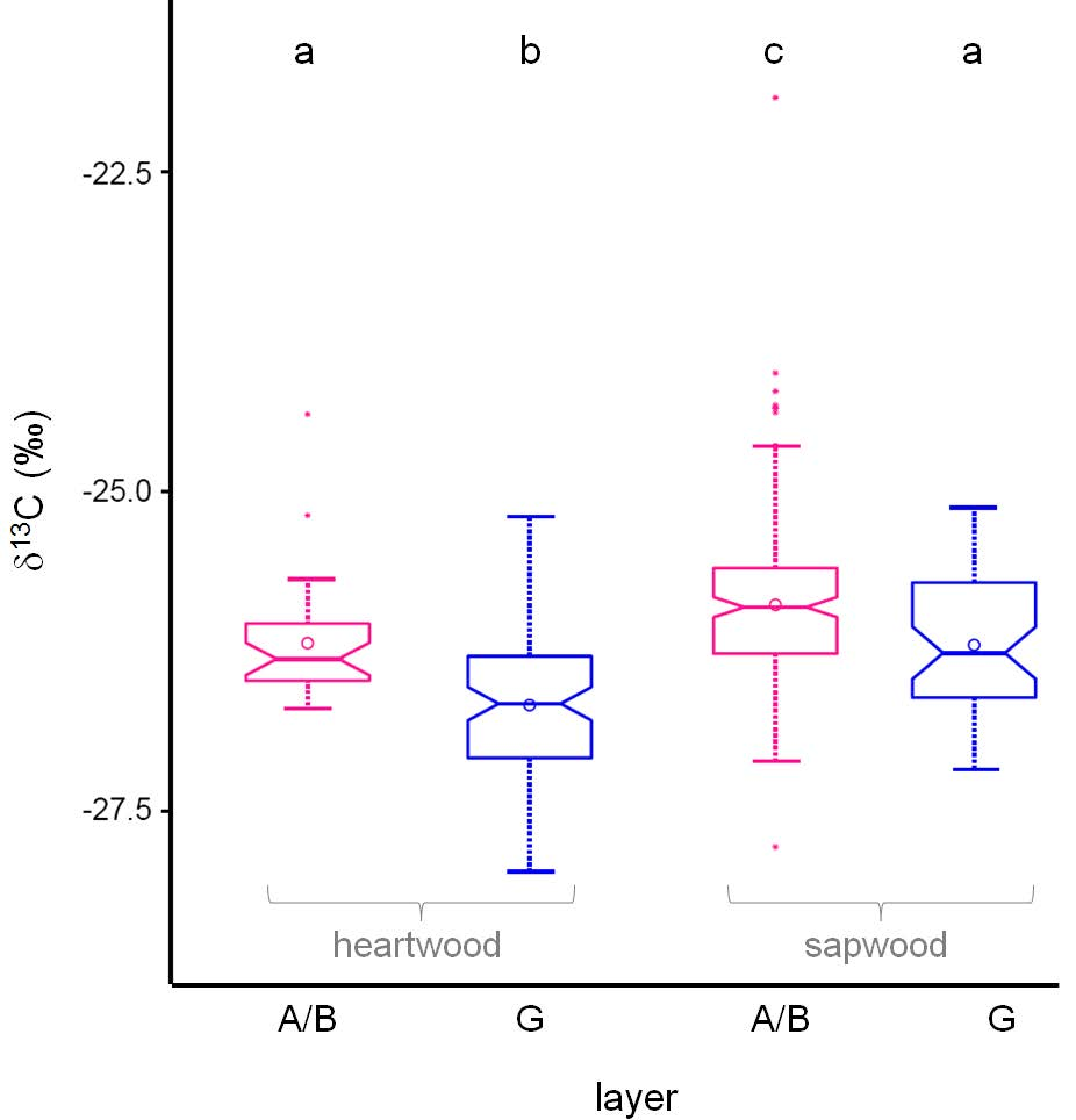

0
10
20 km
N
The Combe d'Ain
Champagnol
Lake Chalain
Lons-le-Saunier
L'Ain
Altitude
more than 900 m
from 700 to 900 m
from 300 to 700 m
less than 500 m
a
Level I
Level II
Level III
Level IV
Level V
Level VI a
Level VI b
Level VII
0
50 cm
LEGEND
Lake-marl layer
Clay screed
Charcoal, fire layer
Organic layer
A
B
C
D
E
F
G
3000 BC
3020-3010 BC
3030-3020 BC
3040-3030 BC
b

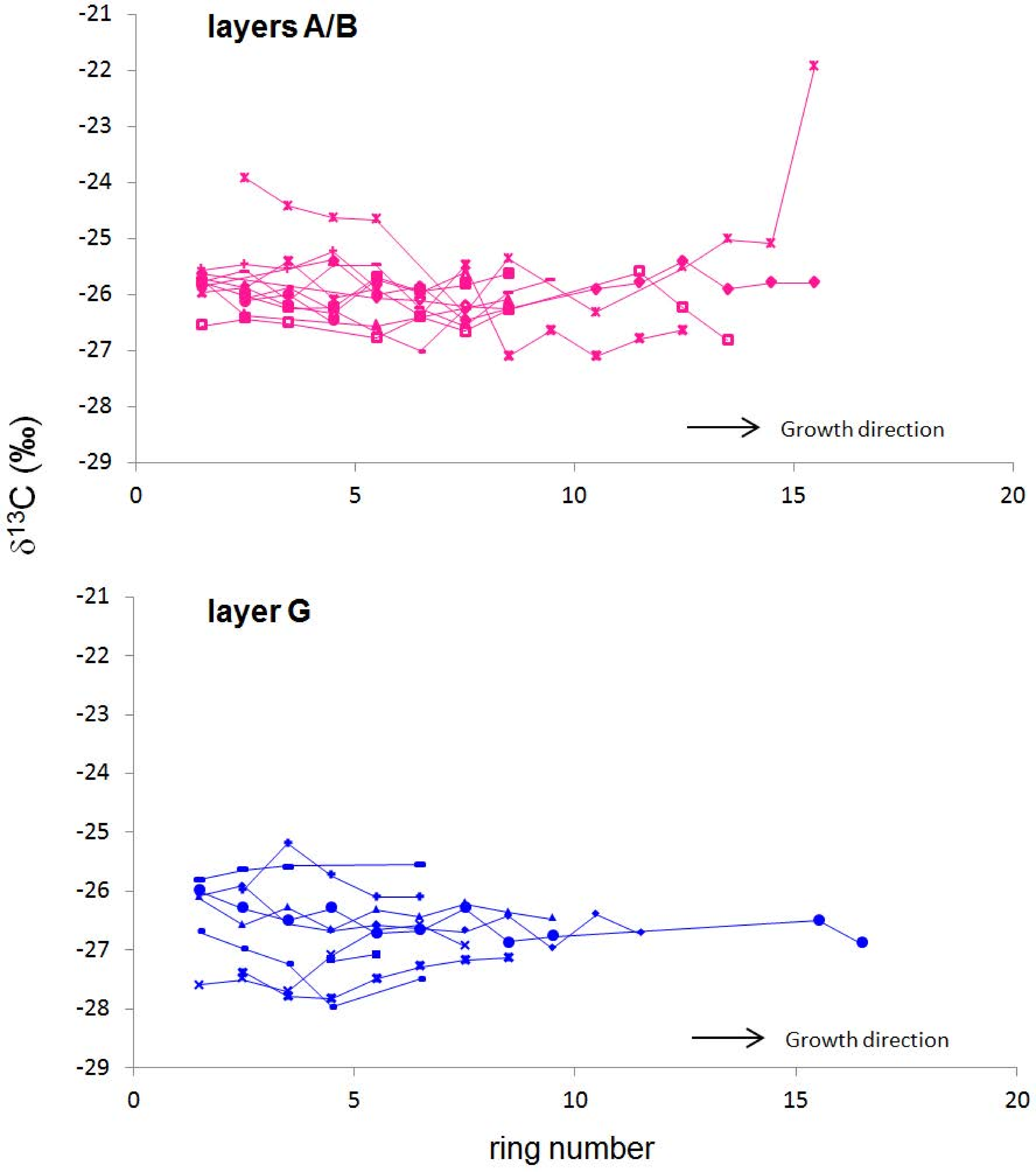

G band
D band
Intensity
H_D
H_G
1000
1500
2000
Raman shift
wavenumber (cm-1)


a
b
1.5
1.0
0.5
ring width (mm)
A/B
G
layer

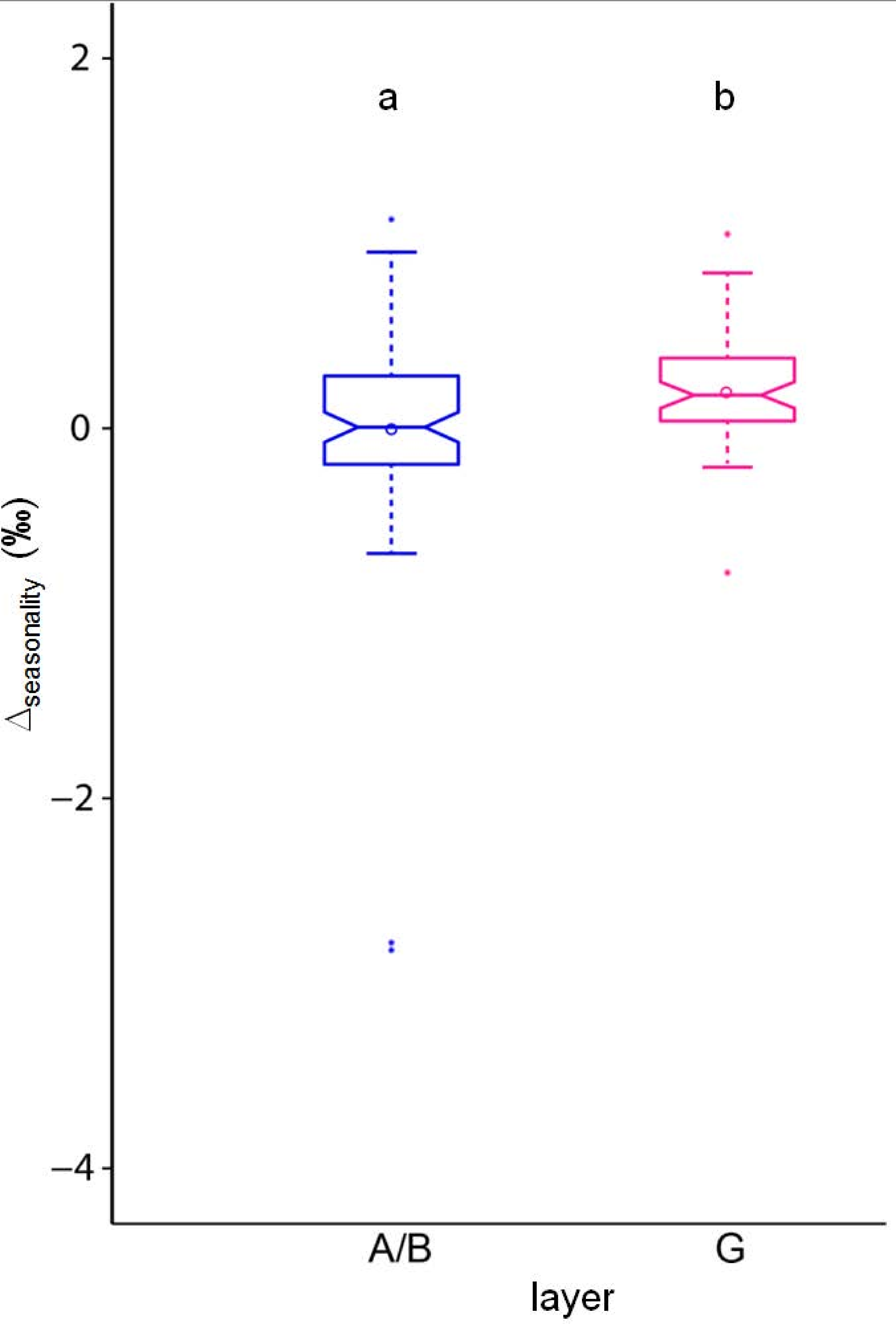


−24
−25
−26
−27
δ13C ‰ treated
−27
−26
−25
−24
δ13C ‰ untreated


a
b
c
a
-22.5
-25.0
-27.5
δ13C (‰)
heartwood
sapwood
A/B
G
A/B
G
layer



layers A/B
layer G
δ13C (‰)
ring number
Growth direction
Growth direction
-21
-22
-23
-24
-25
-26
-27
-28
-29
0
5
10
15
20



a
b
2
0
−2
−4
$\Delta_{seasonality}$ (‰)
A/B
G
layer