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**New climatic approaches to the analysis of the middle Paleolithic sequences: Combined taxonomic and isotopic charcoal analyses on a Neanderthal settlement, Les Canalettes (Aveyron, France)**

Benjamin Audiard, Liliane Meignen, Thierry Blasco, Giovanna Battipaglia,  
Isabelle Théry-Parisot

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## 1 Introduction

2 The Upper Pleistocene is a long period marked by climate and environmental changes in  
3 Europe, and it is heavily influenced by the last phase of glaciation. Understanding the way in which  
4 ancient societies faced environmental conditions is critical issues for prehistorians. Charcoal analysis  
5 is a reliable method to reconstruct the regional changes in forests (see previous proceedings from the  
6 4th, 5th, and 6th International Anthracology Meetings) (Badal et al., 2011; Damblon, 2008; Ludemann  
7 and Nelle, 2015). The frequency of taxa and their changes over time are interpreted by analogy with  
8 current floristic compositions, as a diachronic history of the structure and ecology of forest stands.  
9 Furthermore, anthracology provides complementary information on the wood supply and its  
10 management by ancient societies (Olive and Taborin, 1987; Théry-Parisot, 1998, 2001).

11 However, different limits in anthracology can be identified for Upper Pleistocene sequences.  
12 Indeed, these sequences sometime exhibit anthracologic assemblages with low taxonomic diversity,  
13 mainly represented by *Pinus* species as woody refuges in a steppe landscape or as broad forest cover.  
14 Moreover, anthracology does not provide information on forest density. Most of the time, the lack of  
15 taxonomic diversity does not allow us to specify whether it comes from a monospecific environment  
16 or is attributable to human practices (i.e., wood selection). In addition, Pleistocene anthracological  
17 assemblages are exposed to significant post-depositional processes, which reduce the number, size,  
18 and quality of the charcoal (Chrzasvez et al., 2014; Théry-Parisot et al., 2010). Methodological studies  
19 from anthracology suggest a relatively significant number of charcoal remains from relatively long  
20 human occupations are required to provide a reliable quantitative signal of past environments (Chabal,  
21 1992, 1997). Thus, low numbers of charcoal fragments in Pleistocene sequences are sometime limited  
22 to a qualitative interpretation with a low stratigraphic resolution. To overcome this limitation,  
23 complementary approaches have recently been developed.

24 In recent decades, studies focusing on the  $\delta^{13}\text{C}$  isotopic signals of plants have provided a  
25 reliable method to reconstruct past climatic and environmental conditions. During plant absorption of  
26 atmospheric  $\text{CO}_2$ , a discrimination ( $\Delta$ ) of carbon isotopes occurs. It is defined, in C3 metabolism, as the  
27 result of discrimination against  $^{13}\text{C}$  induced by stomatal conductance ( $a = 4.4\text{‰}$ ) and the RuBisCO  
28 enzyme during carboxylation ( $b \approx 30\text{‰}$ ), with an intensity that depends on the ratio of intracellular  
29  $\text{CO}_2$  to atmospheric  $\text{CO}_2$  content ( $c_i/c_a$ ) :  $\Delta \approx a + (b-a) c_i/c_a$  (1) (Farquhar and Lloyd, 1993; Farquhar et  
30 al., 1982). Thus, changes in stomatal opening, controlled by environmental and climatic parameters  
31 (including atmospheric parameters), constrain the  $\delta^{13}\text{C}$  record in the plant (Farquhar et al., 1989).  
32 Correlation between the  $\delta^{13}\text{C}$  wood signal and different stress factors (drought, high temperatures,  
33 etc.) can be observed over scales at various resolutions (seasonal wood, tree-rings, tree-ring clusters)  
34 (Cernusak and English, 2015; McCarroll and Loader, 2004). In particular, correlations between the  
35 wood  $\delta^{13}\text{C}$  signal and water availability parameters were observed in a major study, which corresponds  
36 to the first recurrent limiting factor (Battipaglia et al., 2014; Dawson et al., 2002; Farquhar and  
37 Richards, 1984; Gessler et al., 2014; Panek and Goldstein, 2001; Warren et al., 2001). In the presence  
38 of optimal levels of atmospheric moisture or in the global warming, temperature or Irradiation also  
39 dominate the relationship between isotopic and climatic signals (Gagen et al., 2007; McCarroll and  
40 Pawellek, 2001; Seftigen et al., 2011).

41 The analysis of the isotopic signal ( $\delta^{13}\text{C}$ ) in charcoal remains from archaeological contexts is an  
42 additional climatic proxy, which raises new perspectives. Pioneering research have attested to its  
43 potential to reconstruct long-term climatic trends, in both the Holocene (Aguilera et al., 2012; Baton

44 et al., 2017; Ferrio et al., 2006; Fiorentino et al., 2012a; Masi et al., 2013; Voltas et al., 2008) and the  
45 Pleistocene archeological context (Audiard et al., 2019; Hall et al., 2008; Vernet et al., 1996). However,  
46 the resolution and application of isotope analyses in Pleistocene sequences is an important issue.  
47 Indeed, certain issues are common for all isotopic studies on charcoal. For example, the effect of  
48 charring on isotopic signal evolution is now well documented. The measurement of the carbon content  
49 (%C) as an indicator of the carbonization state allow for isotopic signal corrections (Audiard et al., 2018;  
50 Ferrio et al., 2006; Resco et al., 2011; Steinbeiss et al., 2006; Voltas et al., 2008).

51 In contrast, major interpretative and methodological challenges must be investigated,  
52 according to specificity of the context and chronology or different anthracological assemblage  
53 features. Thus, the method needs to be adapted for each archeological site. In particular, the type of  
54 charcoal deposit (spread, hearth), the sampling method (manual, or sifting), the intensity of the  
55 taphonomic processes and fragmentation (size, mass and number of charcoal samples; reliability of  
56 taxonomic identification) can restrict the potential of the isotopic study (Audiard et al., 2019). In  
57 addition, the taphonomic processes and the nature of the sediment can lead to exogenous carbon  
58 contamination and necessitate pretreatment, which can decrease the mass of charcoal (Vaiglova et  
59 al., 2014). Moreover, interpretation of the  $\delta^{13}\text{C}$  signal presents several challenges, particularly for the  
60 Pleistocene period, for which we lack understanding of the signal response over longer periods and  
61 under different environmental conditions from those of the present day. Indeed, we are only able to  
62 analyze a mean signal from isolated charcoals. Thus, the resulting values have automatically integrated  
63 the intra-individual variability, the intra-species variability (inter-individual variability) and the inter-  
64 species variability (Francey, 1981; Leavitt, 2002, 2007, 2010; Leavitt and Lara, 1994; Leavitt and Long,  
65 1986; Ramesh et al., 1985; Robertson et al., 1997; Tans and Mook, 1980). Consequently, the use of  
66  $\delta^{13}\text{C}$  from charcoal of Pleistocene sequences raises questions about (i) the nature of the isotopic signal  
67 obtained and (ii) the possibility to use it as a climatic and environmental proxy of low frequency  
68 changes, despite significant natural variability and a limited amount of charcoal for analyses. Lastly,  
69 since the variability of  $\delta^{13}\text{C}$  is taxon-dependent, it is crucial to analyse the same taxon for the entire  
70 sequence and to base the interpretations on current studies on the same taxon.

71 In addition to environmental and methodological issues, the relationship between the isotopic  
72 signal of charcoal and the wood collected environment raises questions about the impact of the  
73 diversification of collection areas on the isotopic signal. The integration of the anthracological data and  
74 the isotopic signal could then allow to discuss the Neanderthal behaviours in relation to the wood  
75 harvesting and environmental evolutions.

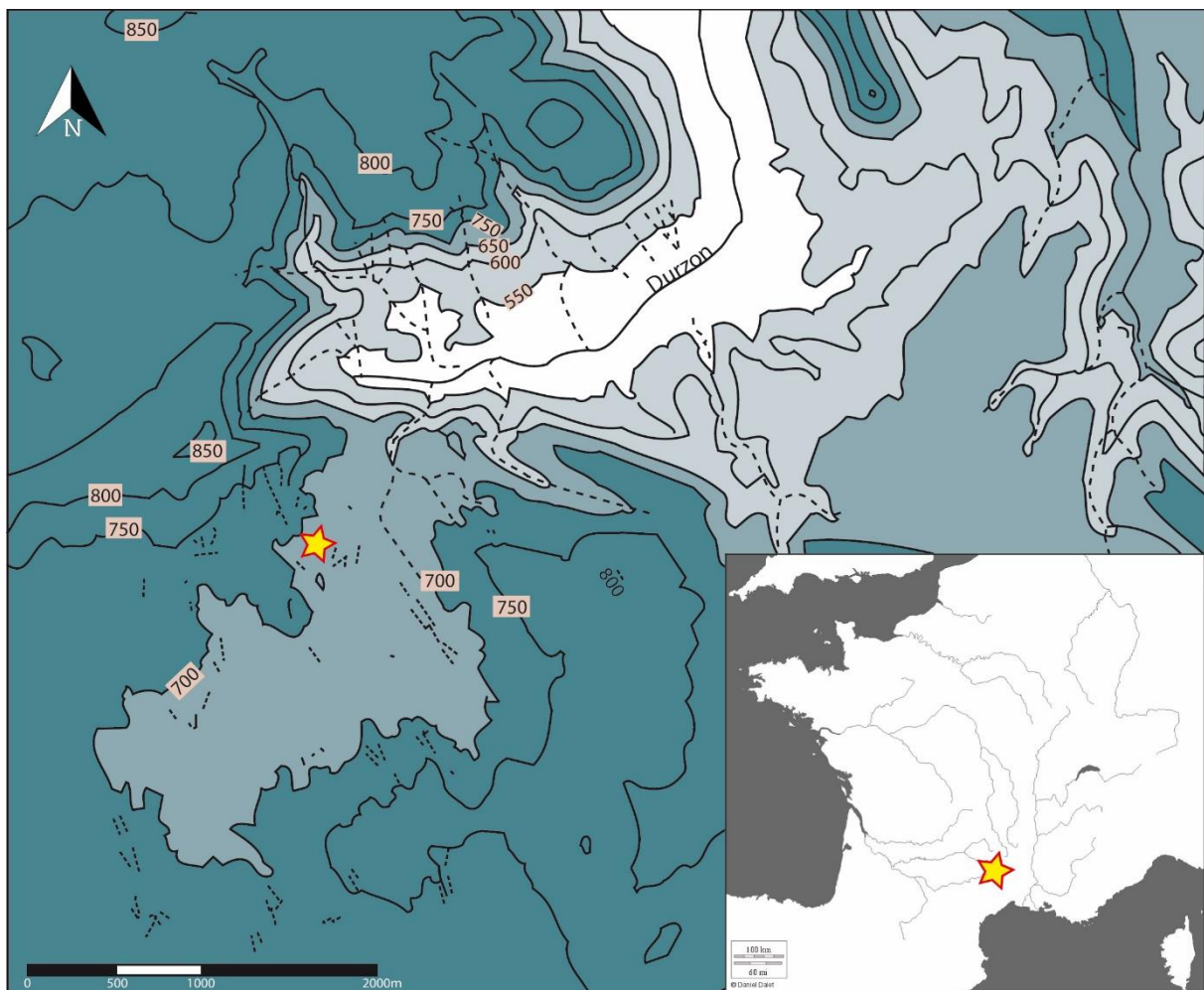
76 The methodological bases of the application of  $\delta^{13}\text{C}$  to Pleistocene can be found in (Audiard et  
77 al., 2018) and a first application to the study of the Middle Paleolithic sequence of La Combette  
78 (Vaucluse, France) has shown the potential of  $\delta^{13}\text{C}$  as a paleoclimatic proxy (Audiard et al., 2019). In  
79 the continuation of this work, we present here, the first application of the method to charcoal, with  
80 individual analyses from a major Middle Paleolithic settlement: Les Canalettes rock shelter (Aveyron,  
81 France). The results of previous paleoenvironmental studies from les Canalettes (fauna, microfauna,  
82 geoarcheology and anthracology) lead to divergent interpretations of the evolution of climatic  
83 conditions over the sequence (Brugal, 1993; Kervazo et al., 1995; Marquet, 1993, 1995; Théry-Parisot,  
84 1998; Vernet, 1993). Our aim was to enrich the sequence of new data increasing the resolution in order  
85 to document the paleoenvironmental context and to discuss human/environment interactions at the  
86 local scale. The variability and the climatic resolution of the isotopic signal will be discussed with  
87 respect to previous, complementary paleoenvironmental results. In addition, methodologic issues will  
88 be addressed regarding previous isotopic works in paleolithic sequences with charcoal grouping  
89 analyses (Audiard et al., 2019).

90

## 91 **Archaeological context and previous data**

### 92 General context

93 Les Canalettes site is a rock shelter (680 m in altitude) located in a wide alveolus (extended  
94 Polje) of the Larzac Causse Plateau (700 to 900 m) in the Aveyron region (Figure 1). Oriented south-  
95 eastward in a wider karstic depression (Canaule) the shelter is separated by a tectonic escarpment of  
96 the Vallée du Durzon (550 m in altitude, tributaries of the Dourbie) (Ambert, 1993). The Les Canalettes  
97 rock shelter holds a strategic position in a natural passage between the Causse plateau and the valley,  
98 which is particularly favorable for raw material and environmental resources (Lebegue, 2012; Meignen,  
99 1993b).



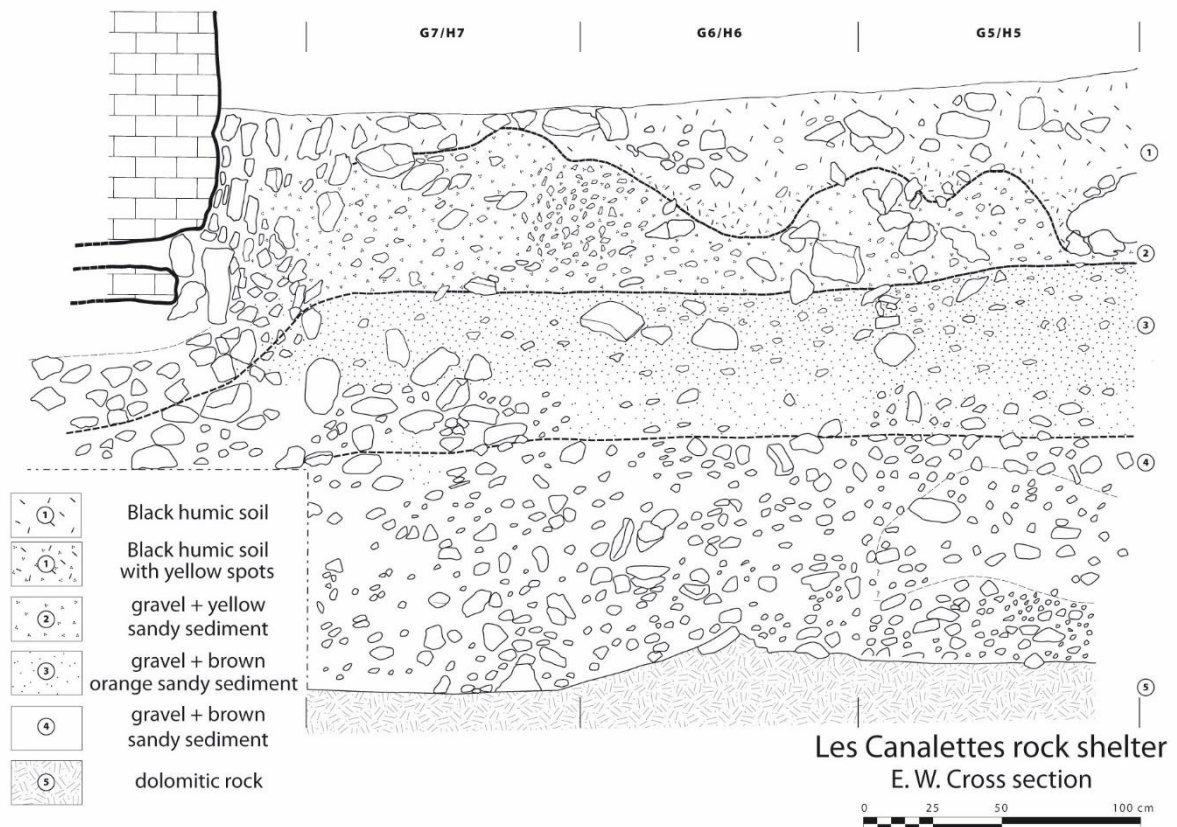
100

101 Figure 1: Location of the Middle Paleolithic site of Les Canalettes rock shelter (yellow star), on the south-east of  
102 the Larzac Causse Plateau (South-East France). Modified after Meignen, 1993.

103

104 The site presents a two-meter-thick stratigraphic sequence where numerous lithic remains  
105 assigned to the Mousterian have been found along the deposit (Figure 2). The sedimentary dynamics  
106 of the shelter are fairly homogeneous over the entire thickness of the stratigraphy, with a  
107 sedimentological composition of loose dolomitic sands more or less rich in heterometric gravel. The  
108 sequence has been divided into 4 stratigraphic units, mainly on changes in color of the sandy matrix  
109 and a decrease of coarser fraction in the middle of the stratigraphy. However, US 1 (black silty-sandy  
sediment, with coarser fraction mainly composed of 15-25 cm blocks) corresponds to a humic level of

110 variable depth. The humic development is responsible of the uneven limit with US 2 (25-60 cm thick,  
 111 sandy, yellow sediment with coarser fraction from small gravel to 25-30 cm blocks). The US 1 and 2 are  
 112 impacted by runoff and percolation, principally close the wall and at the shelter limit. US 1 is  
 113 represented by heterogeneous archeological material (Mousterian, Neolithic, Middle Age, Historic  
 114 Period), and US 2 is mostly represented by Mousterian material with a few exceptions against the wall.  
 115 In contrast, US 3 (30-60 cm thick, sandy, yellow-orange sediment with coarser fraction from gravel to  
 116 block) and US 4 (25-60 cm thick, sandy, brown sediment with coarser fraction from little gravel to  
 117 block) are well preserved with horizontal limits observed during excavation (US 2/3 and US 3/4) and  
 118 rich Mousterian material (Ambert, 1993; Kervazo et al., 1995; Meignen, 1993a).



119  
 120 Figure 2: East-West stratigraphic cross section of Les Canalettes rock shelter. Modified after Meignen, 1993.

121 The geomorphologic dynamic and the lithic material indicate a relatively short and continuous  
 122 deposit phase (Ambert, 1993; Meignen, 1993a). The single dating study was realized by  
 123 thermoluminescence on the upper part of US 2 and indicates occupation during the MIS5a and MIS4  
 124 transition at 73500 ± 6000 years BP (Valladas et al., 1987).

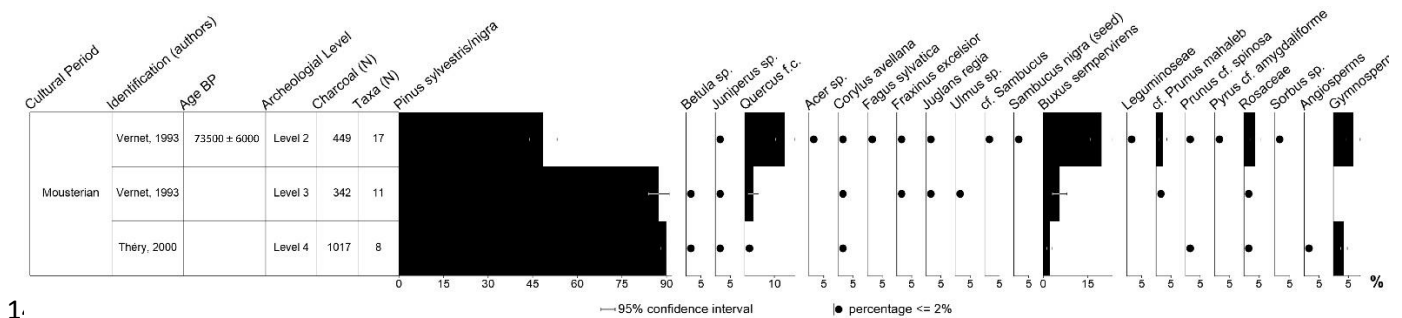
125  
 126 The well-preserved archeological material and the multidisciplinary approaches enabled  
 127 reconstruction of the behavior of the Neanderthals present in Les Canalettes, in a landscape long  
 128 considered austere. It seems to be a long-term and recurrent occupation camp, undoubtedly seasonal  
 129 (during the good season, late spring to early fall), and partly linked with easy access to abundant  
 130 herbivore fauna from the plateau and forest of the alveolus (extended polje) as well as its proximity to  
 131 the raw material (Brugal, 1993; Lebegue, 2012; Meignen, 1993b; Meignen et al., 2001). Moreover, Les  
 132 Canalettes is also known as the site with the oldest use of the lignite as fuel (Théry-Parisot and  
 133 Meignen, 2000; Théry et al., 1995, 1996), found in the same relative proportions in US 4 to 2. This  
 134 material, accessible in the valley, was used by Neanderthal either for a specific use (long fire without

135 flames), (ii) or for its ease of use (harvesting, storage and transport easier than healthy wood) (Théry-  
 136 Parisot and Meignen, 2000).

137

138 Previous results of Paleoenvironmental studies

139 The multidisciplinary program at Les Canalettes has provided numerous and complementary  
 140 paleoenvironmental results. But, the palynologic tests were negative and the homogeneity of the  
 141 deposit only allows to define deposit conditions in temperate to cold conditions (Ambert, 1993).  
 142 Similarly, the low number of identified faunal remains prevented the authors from obtaining reliable  
 143 information on environmental evolution (Brugal, 1993). However, the recurrence of horse and deer  
 144 throughout the sequence indicates a relatively open and temperate to cold environment. Moreover, a  
 145 slight increase in hare, mammoth and marmot (one to a few remains), between US 3 and 2, allowed  
 146 the author to propose a climatic degradation.



1. Figure 3: Anthracological diagram of the mousterian site Les Canalettes (modified according to Théry-Parisot, 1998)

150 The high number of charcoal remains identified (1811) provided reliable information on the  
 151 paleoenvironment (Théry-Parisot, 1998; Théry-Parisot and Meignen, 2000; Théry et al., 1996; Vernet,  
 152 1993). The anthracological diagram (Figure 3) presents a consistent trend supporting progressive  
 153 transformation of the environment under the effect of climate change (Théry-Parisot, 1998; Vernet,  
 154 1993) with a diversification of taxa, from 8 taxa in US 4 to 17 taxa in US 2. US 4 is characterized by the  
 155 presence of mountain taxa (*Pinus*, *Betula* and *Juniperus*), which largely dominate the assemblage, with  
 156 a few occurrences of mesophilic (*Quercus* f.c., *Corylus*) together with xeric heliophilic taxa (*Buxus*,  
 157 *Rosaceae*). US 3 is still largely dominated by pine, but mesohygrophilic taxa are diversified (*Ulmus*,  
 158 *Fraxinus*). US 2 shows a significant increase in mesophilic elements (notably *Quercus*), and is enriched  
 159 by new taxa (*Fagus*, *Acer*) and a marked decrease in mountain taxa.

160 Thus, the charcoal assemblages of US 4 and 3 are quite similar, whereas US 2 shows a marked  
 161 change in the composition of the anthracological spectrum. This could suggest a change in wood  
 162 harvesting behavior at US 2, but this assumption seems weak as the ecological dynamics are very  
 163 consistent with changing climatic conditions. At the base of the sequence (US 4), charcoal remains  
 164 suggest the existence of an open environment of pine groves that had developed under rather cold  
 165 and dry conditions. Gradually, a very slight improvement in the climate is reflected in the localized  
 166 development of mesophilic elements, while the frequency of mountain taxa begins to decrease. US 3  
 167 then appears to be a transition level. US 2 shows a marked change in climatic conditions and a more  
 168 forest-like environment with a mixed series of pine and oak in which pine dominates but the mesophilic  
 169 elements remain well represented, suggesting humid temperate conditions that were slightly cooler  
 170 than current conditions.

171 The anthracological assemblage thus reflects a consistent dynamic in the evolution of taxa  
172 frequencies, arguing for environmental closure related to less severe conditions and higher humidity  
173 at the top of the sequence.

174 Microfaunal studies have also yielded a sufficient number of identifications in each of the lower  
175 three US (145 to 492) to provide reliable paleoenvironmental information. The data correlated with  
176 the anthracological study and support the hypothesis of a relatively temperate period with a mosaic  
177 landscape around the site (temperate forests, watering place) dominated by an open landscape (not  
178 very arid steppes with wet grasslands). Given their higher resolution, the microfaunal results show a  
179 slight progressive reduction in dryland species through the stratigraphy (Marquet, 1993, 1995).

180 All paleoclimatic studies (Brugal, 1993; Marquet, 1993, 1995; Théry-Parisot, 1998; Vernet,  
181 1993) suggest a predominantly open environment with colder temperatures than at present,  
182 connected to more forested and/or a humid environment (e.g. Canalettes alveolus, valley) which  
183 certainly would have fostered human settlement. However, the authors were divided on the possible  
184 climate change between the layers. While sedimentary and large fauna studies provide data in favor  
185 of climatic degradation (US 4 to US 2), microfauna and anthracological studies propose a more humid  
186 climate, which would favor the increase in a forest environment. The stratigraphic homogeneity and  
187 the low number of fauna identifications are put forward as a possible explanation for this opposition  
188 in climate signals.

189

## 190 **Materials and Methods**

### 191 Materials

192 The isotopic study focused on charcoal identified as *Pinus* type *sylvetsris/nigra* (including  
193 *mugo/cembra*). Indeed, pines not only composed the majority of the charcoal remains of Paleolithic  
194 sites but are also one of the most represented species in the isotopic studies of wood. Nevertheless,  
195 *Pinus* species cannot be anatomically discriminated. However, several studies on isotopic variability  
196 between species of the same genus reveal that it is possible to combine their signals (same response)  
197 (Gagen et al., 2006; Marshall and Monserud, 1996). Moreover, in a previous study we demonstrated  
198 that the interspecific variability tested on 3 species of pine (*P. nigra*, *P. sylvestris* and *P. halepensis*) is  
199 lower than the intra- and inter-individual variability before and after carbonization (same effects)  
200 (Audiard et al., 2018). These analyses suggested a limited and not significant supplementary intra-  
201 genus variability in a  $\delta^{13}\text{C}$  *Pinus* signal.

202 In order to obtain an isotopic signal at high stratigraphic resolution, the study focused on  
203 charcoal from sediment sifting of the center square G5 (low post-depositional impact). Pine charcoal  
204 from both new identifications and previous studies (I. Thery-Parisot) were isolated under a  
205 stereomicroscope. We collected 98 charcoals spread across 13 artificial sub-stratigraphic levels  
206 (décapages), with 2 to 18 charcoal samples for each one, the majority of which contained more than  
207 10. The dominant charcoal size comprised 1 to 5 mm, followed by less than ¼ of charcoal total between  
208 5 mm and 1 cm and only few charcoals bigger. Considering, this size heterogeneity, the difficulty to  
209 have enough material to analyze with smaller ones and the difficulty to distinguish tree-ring due to  
210 numerous surface charcoal alteration, we used each entire charcoal as a sample without subsampling.  
211 This protocol should avoid the loss of material, with a lower number of analyses. In addition, the  
212 analysis of charcoal large enough to contain several tree-rings should lead to mean isotopic values  
213 closer to a low frequency averaged isotopic signal (long time).

214

215

216 Pre-treatment of samples

217 In order to study the stable carbon isotope ratios of charcoal, possible contamination of other  
218 carbon sources (e.g., carbonates, humic acid and fine rootlets) must be removed. However, the usual  
219 decontamination procedure, consisting of an acid-base-acid treatment, decreases the charcoal mass  
220 that is essential for individual analysis. Thus, we applied the method proposed by Vaiglova et al. (2014).  
221 It consists in an evaluation of the level of humic acid contamination by analyzing charcoal remains with  
222 a FTIR spectrometer. Then, if the contamination is lower than 10%, the effect on  $\delta^{13}\text{C}$  is considered to  
223 be negligible and the base-acid treatment can be avoided (Vaiglova et al., 2014). In this respect, a  
224 selection of charcoal samples from each level of the stratigraphy was analyzed by infra-red  
225 spectrometry with a PerkinElmer Frontier FTIR spectrometer coupled to the Spectrum software. No  
226 significant humic contamination was observed and, thus, only the pre-treatment for carbonates was  
227 applied.

228 The decarbonation method is based on (DeNiro and Hastorf, 1985), which had previously been  
229 applied to burnt archaeological remains (Aguilera et al., 2012; Ferrio et al., 2006). Charcoal samples  
230 were soaked individually (sufficient mass) with 1 M HCl for 45 min at 80°C, then rinsed repeatedly with  
231 distilled water to a neutral pH.

232 Isotopic analysis

233 An isotopic and elemental analysis ( $\delta^{13}\text{C}$  and %C) was performed with an Elementar Vario  
234 Pyrocube coupled to continuous flow Isoprime 100 isotope ratio mass spectrometer at the  
235 Oceanography Laboratory of Villefranche (LOV, CNRS/SU). The usual internal calibration procedures  
236 with standards and blanks were applied, with a caffeine standard (isotopically certified, AIEA-600)  
237 calibrated relative to V-PDB, and a secondary standard calibrated with the caffeine (acetanilide from  
238 Merck, CAS 103-84-4). Standard variability and error analyses were in the range of 0.1‰.  $\delta^{13}\text{C}$  values  
239 were corrected using a monopoint standard correction (Paul et al., 2007) and were reported per mille.

240 Data processing

241 First, we observed that all charcoal samples recorded the same carbon content (%C) of around  
242 60%. Correlation observed between the decrease in  $\delta^{13}\text{C}$  and the increase in %C allowed to propose a  
243 relative same carbonization impact on the charcoal (Audiard et al., 2018; Ferrio et al., 2006). Since our  
244 samples record the same degree of carbonization, no correction was applied.

245 With regard to atmospheric parameters, Les Canalettes are dated around  $73500 \pm 6000$  years  
246 BP (Valladas et al., 1987) with relatively fast site filling. The average  $\delta^{13}\text{C}_{\text{atm}}$  for this period is relatively  
247 stable (<0.1‰ for the range  $\delta^{13}\text{C}_{\text{atm}}$  between 80ka and 66ka) and precedes the relatively strong  
248 decrease around 60ka ( $\delta^{13}\text{C}_{\text{atm}}$  from -6.92‰ to 58 ka for -6.47‰ to 66 ka) (Eggleston et al., 2016). At  
249 the same time, the maximum evolution of the  $\text{CO}_2_{\text{atm}}$  concentration does not exceed 25 ppmv  
250 (between 82.8 and 68.5 ka) with gradual variations compared to the current conditions (234 ppmv to  
251 82.8 ka; 219.5 ppmv to 76.8 ka; 243 ppmv to 70.7ka; 226 ppmv to 68.5 ka) (Barnola et al., 1987).  
252 However, without abrupt changes, we expect plants to adapt their physiology and maintain a constant  
253 ca/ci ratio and negligible isotopic impact (Ehleringer and Cerling, 1995). Hence, no atmospheric  
254 correction was applied to the isotopic signal. One of the most important variations of the Würm (which  
255 remains slow compared to the present one) is observed between 70.7ka and 62 ka, with a decrease of  
256 50 ppmv (Barnola et al., 1987). Thus, a slight negative impact can occur on the  $\delta^{13}\text{C}$  during possible  
257 contemporaneous occupation. This possible impact has to be considered in the interpretation of the  
258  $\delta^{13}\text{C}_{\text{atm}}$  from charcoal of the upper part of US 2.



259 Lastly, thanks to the high sampling resolution, a  $\delta^{13}\text{C}$  mean and standard deviation for each  
 260 artificial sub-stratigraphic unit are calculated.

261

## 262 Results

263 The raw results are synthesized below by artificial stratigraphic sub-unit (décapage, Dec) and  
 264 US in [Table 1](#), and are then compared with the charcoal taxonomic and microfaunal data in [Figure 4](#).

265 Firstly, we observed relative low variability by décapage. The isotopic standard deviation  
 266 between charcoal from the same décapage does not exceed 1‰ and is mainly lower than 0.5‰.  
 267 Moreover, except for Dec 8 in the US 2 (3.3‰), no décapage shows isotopic deviation above 2.5‰,  
 268 and more than 2/3 of the  $\delta^{13}\text{C}$  max deviation stayed lower than 2‰. Comparatively, we observed a  
 269 higher  $\delta^{13}\text{C}$  max deviation by US, up to 3.8‰ for US 2 and up to 2.8‰ for US 3 and 4. However, the  
 270 standard deviations remain relatively low (0.5 and 0.6‰, respectively), whereas those from US 2 reach  
 271 1.0‰ (equal to maximum standard deviation by décapage).

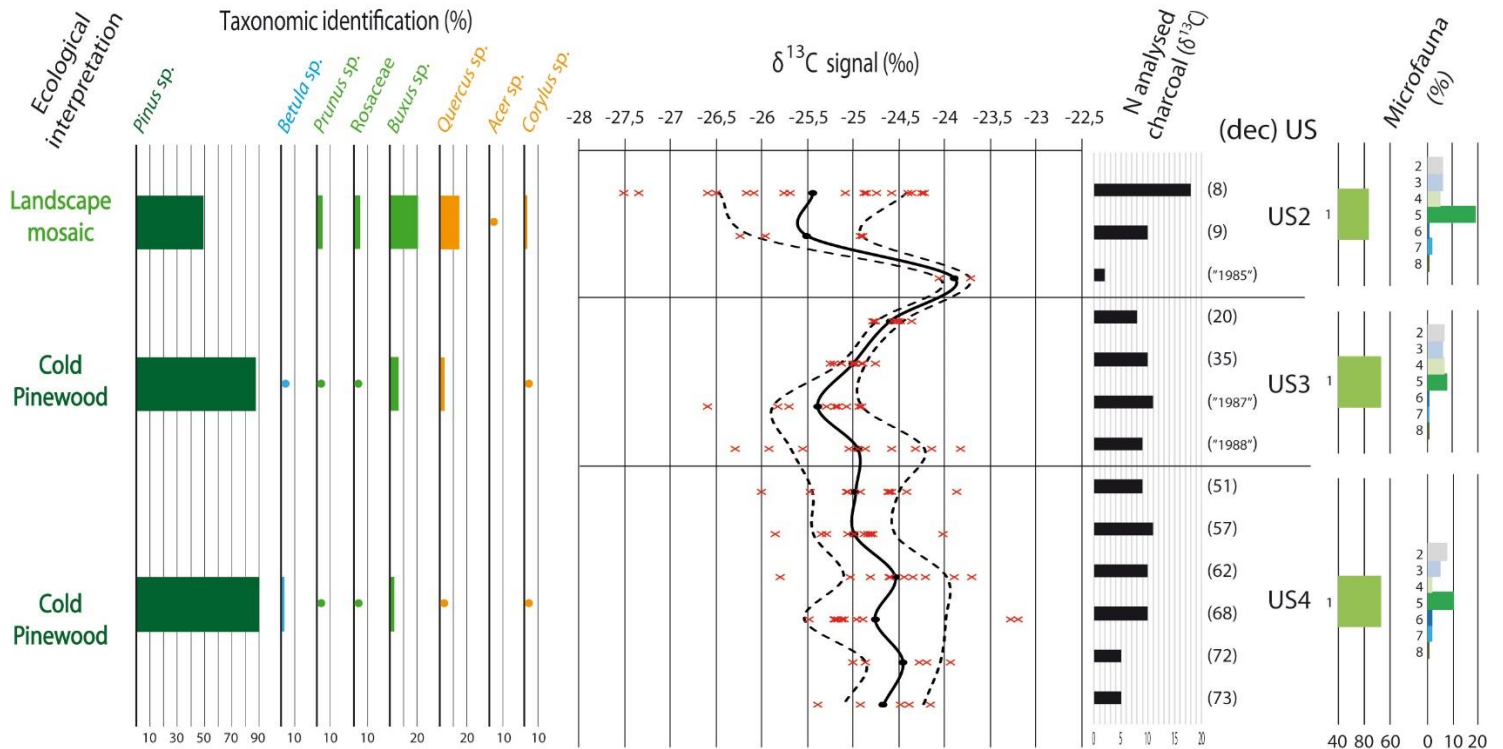
Stratigraphic Level	N	Mean $\delta^{13}\text{C}$ (‰)	$\delta^{13}\text{C}$ standard deviation (‰)	$\delta^{13}\text{C}$ max deviation (‰)
Dec 8	18	-25.4	1.1	3.3
Dec 9	10	-25.5	0.7	1.3
Dec de 1985	2	-23.9	0.2	0.3
<b>US 2</b>	<b>30</b>	<b>-25.3</b>	<b>1.0</b>	<b>3.8</b>
Dec 20	8	-24.6	0.2	0.4
Dec 35	10	-25.0	0.2	0.5
Dec of 1987	11	-25.4	0.5	1.7
Dec of 1988	9	-24.9	0.8	2.5
<b>US 3</b>	<b>38</b>	<b>-25.0</b>	<b>0.6</b>	<b>2.8</b>
Dec 51	9	-25.0	0.5	1.6
Dec 57	11	-25.0	0.5	2.0
Dec 62	10	-24.5	0.6	2.1
Dec 68	10	-24.8	0.8	2.3
Dec 72	5	-24.5	0.5	1.1
Dec 73	5	-24.7	0.5	1.2
<b>US 4</b>	<b>50</b>	<b>-24.7</b>	<b>0.6</b>	<b>2.8</b>
<b>Mean</b>	<b>9</b>	<b>-24.9</b>	<b>0.5</b>	<b>1.6</b>

272 Table 1: Summary of isotopic data obtained from Les Canalettes charcoal remains, with N being the number of  
 273 charcoal samples analyzed individually.

274 Furthermore, a mean  $\delta^{13}\text{C}$  variation up to 0.5‰ for US 4, up to 0.8‰ for US 3 and up to 1.6‰  
 275 for US 2 is observed between different décapages from the same US. In general, we observed a  $\delta^{13}\text{C}$   
 276 decrease during the sequences, with a difference of 0.6‰ between the lowest and uppermost  
 277 décapage (Dec 73 – US 4 with -24.7‰ and Dec 8 – US 2 with -25.3‰, respectively).

278 On a finer scale, we first observed low fluctuation of the isotopic mean until « Dec 68 » of US 4  
 279 (between -24.8 et -24.5‰), then a decrease to the end of US 4 (-25.0‰) and which continues in US 3  
 280 until the Dec of 1987 (-25.4‰). From Dec 3, the mean  $\delta^{13}\text{C}$  increases in the last décapages (up to

281 24.6‰). This increase continues with the first décapage in US 2 (Dec of 1985; up to -23.9‰). However,  
 282 it is important to note that this mean value was obtained with only two charcoal samples. Lastly, the  
 283 last two décapages of US 2 are marked by a low mean  $\delta^{13}\text{C}$  (-25.5 and -25.4‰), with an abrupt decrease  
 284 at Dec 9 (-23.9 to -25.5‰). However, we noted that the final décapage (Dec 8) has the highest standard  
 285 deviation and maximum deviation of  $\delta^{13}\text{C}$  for the site (1.1‰ and 3.3‰, respectively).



287 Figure 4: Summary of the isotopic data obtained from charcoal remains, supplemented by previous  
 288 paleoenvironmental data from Les Canalettes rock shelter. We observed, from left to right: (i) the anthracological  
 289 identification by US and the ecological interpretations (Vernet, 1993; Théry-Parisot, 1998); (ii) the synthetic graph  
 290 of the isotopic data with, according to the stratigraphy, the mean  $\delta^{13}\text{C}$  (continuous black line), the standard  
 291 deviations by level of the  $\delta^{13}\text{C}$  (dotted black line) and the individual isotopic values obtained for each charcoal  
 292 sample (red cross); (iii) N the number of charcoal samples analyzed per stripping; (iv); décapage and stratigraphic  
 293 unit (v) microfaunal data represented by percentage of typical ecological groups, with the following numbers:  
 294 arctic steppes (1), boreal swampy open spaces (2), very arid continental steppes (3), not very arid open spaces  
 295 (4), grasslands and wetlands (5), waterfronts (6), heliophilic rock groups (7) and temperate forests (8) (Marquet,  
 296 1995).

297

## 298 Interpretation

299 The variability of the mean isotopic signal between décapages is relatively large (for the most  
 300 pronounced changes) compared to the standard deviation of the  $\delta^{13}\text{C}$  for each décapage. The  
 301 evolutions of the mean  $\delta^{13}\text{C}$  are considered to be significant (correlated to the climatic and  
 302 environmental signal) and not only due to the restricted sampling and the variability of the signal (intra-  
 303 individual, inter-individual, seasonal/annual variations; highlighted by maximum isotopic deviation).  
 304 However, even if the  $\delta^{13}\text{C}$  mean of Dec of 1985 follows the isotopic increase observed in US 3, the fact  
 305 that only two charcoal samples were analyzed raises question about the representativity of the signal.  
 306 However, despite a high number of analyzed charcoal samples, the significant mean  $\delta^{13}\text{C}$  variability in  
 307 Dec 8 (US 2) deserves to be highlighted too.

308 The climatic interpretations of the isotopic signal evolution are based on current knowledge of  
309 the wood  $\delta^{13}\text{C}$  (Cernusak and English, 2015; McCarroll and Loader, 2004). However, it is possible that  
310 the low atmospheric carbon content (<243 ppmv) amplified plant sensitivity to climate change  
311 (particularly factors linked to water availability) and thus the isotopic signal (Beerling, 1994; Beerling  
312 and Woodward, 1995; Ehleringer and Cerling, 1995). Therefore, independently of atmospheric  
313 temperature, decreasing trends of the  $\delta^{13}\text{C}$  signal are interpreted as a wet and/or favorable  
314 environment for plant growth (e.g. soil development) and conversely for an increasing trend (dry  
315 and/or unfavorable environment for growth). Nevertheless, in relative wet conditions and/or  
316 significant warming, temperature can also trigger variation of isotope signal (positive trend  
317 corresponding to higher temperature and vice versa).

318 Consequently, the general decrease of the isotopic mean signal from the base to the top of the  
319 sequence can be interpreted as an increase in moisture, better edaphic conditions and/or possible  
320 colder climate. Nevertheless, this general trend is not linear but is interrupted by an increasing isotopic  
321 phase between US 3 and US 2. Based on what we know about the response of the isotope signal, two  
322 hypotheses can be proposed: (i) a dry episode and/or (ii) a warm episode.

323 In this context, human occupations in Les Canalettes were contemporaneous, first in a wet  
324 increase phase in a cold environment ( $\delta^{13}\text{C}$  decrease; US 4 to US 3), then a significant temperature  
325 increase or dry episode ( $\delta^{13}\text{C}$  increase; US 3/2), to finish with a return to cool and relatively wet  
326 conditions (lowest  $\delta^{13}\text{C}$  ; top of US 2), i.e. continuous occupation independent of climatic variations.  
327 The significant isotopic variability in the last décapage (Dec 8) may correspond to higher inter-tree  
328 variability, which can be explained by (i) an expansion of the wood harvesting area to include different  
329 environments (forest or plateau), (ii) a mix of charcoal from different climatic periods, or (iii) a  
330 difference in the physiology of the collected trees (resulting from differential adaptation to the  
331 extreme climatic event).

332  
333 Furthermore, the possibility to crossing isotopic and taxonomic data from the same charcoals  
334 allow us to developp the interpretation. The global environnement information gived by  
335 anthracological study is used to determine the different hypothesis probability. Moreover, the  
336 micromorphologic study provides additional data on the local environmental and climatic signal which  
337 consolidates this multi-proxy analysis.

338 In that respect, the general increase in mesophilic wood species, contemporaneous of a  
339 climatic improvement observed through the microfaunal study (principally between US 3 and US 2)  
340 (Marquet, 1993, 1995; Théry-Parisot, 1998; Vernet, 1993), can allow us to assume that the mean  $\delta^{13}\text{C}$   
341 more likely corresponds to an increase in humidity (general trend, US4 to US2). In addition, the climatic  
342 improvement that was identified in the microfaunal and anthracologic assemblage study supports the  
343 second hypothesis (Marquet, 1993, 1995; Théry-Parisot, 1998; Vernet, 1993): the occurrence of a  
344 warmer episode may have favored the establishment of mesophilic taxa (among charcoal and  
345 microvertebrates) observed in US 2. Moreover, considering the relatively slow changes in the forest  
346 cover (over one to several centuries), the absence of a visible hiatus in the stratigraphy and the relative  
347 rapid deposit establishment, it is unlikely that a dry event occurred.

348

## 349 **Discussion**

### 350 Isotopic signal variability

351 One of the most important issues that arises from this study is related to the  
352 climatic/environment significance of the mean isotopic signal from isolated charcoal analysis in the  
353 Pleistocene context. Nevertheless, the constant carbon content of about 60% that was found for all

354 samples reveals the same degree of carbonization and allowed us to avoid a "carbonization correction"  
355 of the values in  $\delta^{13}\text{C}$  (Audiard et al., 2018; Ferrio et al., 2006). However, this observation had already  
356 been made in a previous study of Paleolithic charcoal (Audiard et al., 2019). This phenomenon could  
357 be a response to differential resistance to the post-deposition process. Insufficient carbonization  
358 (charred wood) could lead to natural degradation, whereas excess carbonization (400-450°C; C%  $\approx$ 60%)  
359 could lead to loss of cellulose (Czimczik et al., 2002). This would subsequently lead to a decrease in the  
360 robustness of the charcoal structure in the face of taphonomic processes, which is particularly  
361 important in paleolithic sequences (Chrzaszew et al., 2014; Théry-Parisot et al., 2010). Thus, given the  
362 limited impact of carbonization, the observed variability in  $\delta^{13}\text{C}$  must mainly be related to natural  
363 variability and climatic/environmental recording.

364           Given the relatively large size of the milled charcoal (over 2mm), the  $\delta^{13}\text{C}$  value corresponds  
365 to the mean of the isotopic signal recorded across several tree rings, which resulted in the loss of the  
366 high-frequency (annual) isotopic resolution to yield a low-frequency (multi-year) recording. The  
367 variability is thus should mainly represented by the inter-tree variability.

368           In this study, the maximum deviation of the isotopic signal, for one décapage, usually does not  
369 exceed 2‰, with an average of 1.6‰. The standard deviation is consistently less than 1.0‰, with an  
370 average of 0.5‰. These values are similar to those obtained for inter-individual (intra-genre) variability  
371 in current wood studies (maximum variability from 1 to 3‰ between contemporaneous tree-rings)  
372 (Francey, 1981; Leavitt, 2010; Leavitt and Long, 1984, 1986; Mazany et al., 1980; McCarroll and  
373 Pawellek, 1998). In particular, our values correspond to those obtained from a study of 100 Scots pine  
374 (95% margin of the mean of 2.02‰ and standard deviation of 0.56‰) (Loader et al., 2013). In another  
375 study, a minimum number of trees, between 15 and 30, is proposed to obtain a representative mean  
376  $\delta^{13}\text{C}$  of the low-frequency climatic signal (Esper et al., 2015). Thus, considering the variability of  $\delta^{13}\text{C}$   
377 mostly represents inter-individual variability, we can assume an optimal sampling to obtain low  
378 frequency signal between 15-30 charcoals. Nevertheless, depending on the charcoal size, we can  
379 readily obtain a mean  $\delta^{13}\text{C}$  from a low number of charcoal samples that correlate with a low frequency  
380 climatic signal. Here, our charcoal analyzed number remain relatively low, by comparison, and show  
381 the importance of increasing the sampling as much as possible. Unfortunately, the study on one square  
382 meter (to avoid stratigraphic evolution) limits the number of *Pinus* charcoal remains compared to the  
383 need for more robust data.

384           Nevertheless, the larger change in the mean isotopic signal compared to the standard  
385 deviation of the  $\delta^{13}\text{C}$  for each décapage (for the most pronounced changes), and the correlation with  
386 other paleoenvironmental proxies (microfauna, anthracology) suggest correlation with a low  
387 frequency climatic signal. Therefore, the isolated  $\delta^{13}\text{C}$  value can be interpreted as a possible isolated  
388 tree (different micro-climatic conditions, inter-tree variability) and the increase in the  $\delta^{13}\text{C}$  variability  
389 could be the result of a larger harvest area (creating different micro-environmental conditions) or a  
390 population split (different physiological adaptation to the climate or atmospheric parameters). In this  
391 context, our results are consistent with those of La Combette (MIS5-4, Vaucluse, France) (Audiard et  
392 al., 2019). Indeed, in this study, isotopic analyses on pooled charcoal samples (by artificial  
393 substratigraphic unit) revealed the strong potential for  $\delta^{13}\text{C}$  to be used as a climatic proxy, with the  
394 main obtained values being lower than the inter-tree variability (error margin of the isotopic wood  
395 study). However, this study highlights the loss of information in the case of charcoal pooling. Indeed,  
396 by using the mean  $\delta^{13}\text{C}$  value of several charcoal samples, we mitigate the real isotopic variability  
397 (variability with individual value). Thus, it does not provide information on the extent of inter-tree  
398 variability or the reliability of the signal.

400 Paleoclimatic and paleoenvironmental context

401 Crossing the isotopic signal and anthracologique (and macrofaunal observations), allowed us  
402 to confirm the hypothesis of climatic/environnement improvement throught the Canalettes's  
403 sequences (Marquet, 1993, 1995; Théry-Parisot, 1998; Vernet, 1993), with a general humidity increase  
404 and the likely passage from relatively cold conditions in the basis of the sequence (US4-3), then a  
405 warming episode (US 3 to 2), and finally a return to cooler conditions.. Thus, the hypothesis of climatic  
406 deterioration, which was proposed based on the faunal study, seems less likely (Brugal, 1993, 1995).  
407 However, the presence of taxa from cold environment observed in the faunal study of US 2 could  
408 correlate with a decrease in temperature, which could explain, in part, the low  $\delta^{13}\text{C}$  at the top of US 2  
409 (high humidity and temperature decrease).

410 In a general context, Les Canalettes is contemporaneous of the MIS 5/4 transition known in ice  
411 core as the beginning of temperature decreasing and the glacial period (Johnsen et al., 2001). However,  
412 about 20 episodes of rapid warming have been recorded in the ice core data and are distributed along  
413 the last glacial phase. These warming episodes can reach more than 5 to 15°C in Greenland within  
414 about 10 years and are followed by slow cooling: "Dansgaard-Oeschger" (D/O) interstages (Cacho et  
415 al., 1999; Dansgaard et al., 1989; Schulz, 2002). Thus, the first beginning of MIS 4 is then marked by a  
416 succession of cold events (Herinrich 7-a and 7-b) and thaws (D/O events 20 and 19) before the onset  
417 of glacial conditions sensu stricto (*ibid*). In France, similar variations are observed in the pollen diagram  
418 of La grande Pile with more humid episodes in a cold context (forest recovery of Pinus and Betula ;  
419 Ognon I and II event) (de Beaulieu and Reille, 1992; Helmens, 2014; Woillard, 1978). This phenomenon  
420 is also known in the Echets sequences by an considerable increase of Isoetes (de Beaulieu and Reille,  
421 1984), or in the Massif Central (Lanterne II pollen period; Bouchet/Ribains) by fluctuations in Pinus  
422 reconquest (Reille and Beaulieu, 1988, 1990). More locally, in the south-east France, similar  
423 improvements were observed in climatic archive (geomorphology, microfauna, anthracology) of  
424 several other archeological sites dated from the beginning of the MIS5/4 transition : Baume de Moulau-  
425 Guercy, Saint-Marcel, Abri des Pêcheurs and Abri Maras in Ardèche (Defleur and Desclaux, 2019;  
426 Desclaux and Defleur, 1997; Hardy et al., 2013; Kalaï, 1998; Moncel et al., 2008; Moncel et al., 2010;  
427 Moncel et al., 2004) or les Calmettes in the Gard (Bazile-Robert, 1979) and la Combette in the Vaucluse  
428 (Audiard et al, 2019).

429 In that context, the general increase of humidity through the Canalette's sequences could  
430 correspond to the transition between a cold and dry environment during the very beginning of MIS4  
431 to wetter conditions contemporary of the climatic events mentioned above. Moreover, while it seems  
432 difficult to correlate our local isotopic episode with a global-scale climate event (recorded in ice cores),  
433 a less intense climate event can still be envisaged in the region. The rapid temperature evolution would  
434 have been such that plant physiology would not have been able to adapt. In contrast, if the decrease  
435 of the mean  $\delta^{13}\text{C}$  corresponds to a temperature decrease with relative humid conditions, it is possible  
436 to consider plant adaptation to higher temperatures. Some authors propose that climate rapidly  
437 affects the phenotypic and genotypic selection of individuals over only a few generations (Anderson  
438 et al., 1996; Ferrio et al., 2003b). Furthermore, this adaptation could explain, in part, the highest  
439 variability of the isotopic signal in the top sequence. Nevertheless, without complementary data, this  
440 increase in the variability most likely corresponds to a larger harvest zone (different environmental  
441 conditions) or a mix of charcoal from different climatic periods (climate change record by one tree  
442 population or different tree populations collected during several occupations).

444 The techno-cultural system

445 One of the aims of this study is to shed light on Neanderthal behavior by comparing taxonomic  
446 and isotopic data. Here, correlations between the general trend of the mean  $\delta^{13}\text{C}$  and the charcoal  
447 analysis support the hypothesis that the taxonomic assemblage is representative of the forest  
448 composition over time. This hypothesis is consistent with a non-selective wood harvest by the  
449 Neanderthals of Les Canalettes. The presence of a relatively large forest in the alveolus of Les  
450 Canalettes, dominated by *Pinus* sp. (with significant natural pruning) certainly favored this practice  
451 (Théry-Parisot and Meignen, 2000).

452 Furthermore, our study shows that the different groups of Neanderthals experienced climatic  
453 changes during the different occupation periods. In contrast, the technological organization of the  
454 lithic production (raw material acquisition, tool production and maintenance) and the subsistence  
455 strategies in terms of animal resource acquisition and treatment are constant through the stratigraphic  
456 sequence (Brugal, 1993; Lebegue, 2012; Meignen, 1993b; Meignen et al., 2001; Pathou-Mathis, 1993).  
457 Moreover, the settlement patterns (long-term and repetitive human occupations corresponding to  
458 base camps, concentrated in the good season) remained the same throughout this period (Brugal,  
459 1993; Lebegue, 2012; Meignen, 1993a; Pathou-Mathis, 1993; Roussel et al., 2019). This opposition  
460 between environmental/climatic changes and the technical and cultural continuity (e.g. lithic  
461 traditions, subsistence strategies, lignite gathering) that characterizes the occupations of the site,  
462 reflects the persistence of strong cultural traditions independent of climatic conditions (hunter-  
463 gatherer mobility organized around seasonal occupations of this rock shelter on the Causse). The  
464 transmission of the knowledge regarding such a strategic supply area in a relatively vast environment  
465 (Causse, valleys, etc.), has most certainly favored this cultural continuity.

466

467 **Conclusion**

468 This is the first isotopic study ( $\delta^{13}\text{C}$ ) of isolated charcoal (individual values) in a Pleistocene  
469 sequence in Europe. The use of this method on the charcoal remains of Les Canalettes shelter has thus  
470 been able to show:

- 471 - Relatively low variability of the isotopic signal by *décapage* (artificial sub-stratigraphic  
472 unit) compared to the evolution of the mean  $\delta^{13}\text{C}$  signal along the sequence.
- 473 - A correlation between the general isotope signal data and other paleoenvironmental  
474 data (anthracology, microfauna). A general decrease in aridity is thus observed during  
475 the different successive occupations (general trend).
- 476 - The possibility to obtain an isotopic signal representative of environment/climatic with  
477 relatively small number of charcoal (ideally 15-30 charcoals but less big charcoals can  
478 be already indicative). It provides a high-resolution signal, complementary of the  
479 anthracological data. Thus, we observe global warming between layers 3 and 2 (not  
480 observable with the other climate markers). However, interpretation without an  
481 anthracological taxonomic background is still complicated.
- 482 - Finally, the cross-referencing of isotope and taxonomic analyses support the  
483 hypothesis of climate change and dismissed the assumption of a change in wood  
484 harvesting behavior in US 2.
- 485 - Cultural sustainability of technical knowledge and pattern of territory exploitation,  
486 despite observed climate change

487 Based on the study of a lower number of charcoal samples than a classical charcoal analysis,  
488 isotopic analysis provides a higher resolution stratigraphic signal, which is rarely possible in Pleistocene  
489 sequences. Applying isotopic analyses to individual charcoal samples from Pleistocene sequences  
490 allows us to obtain a reliable climatic proxy with relatively low variability. Our results are in agreement  
491 with those of La Combette (MIS5-4, Vaucluse, France) (Audiard et al., 2019) but with a higher  
492 resolution. Indeed, in La Combette, isotopic analyses on charcoal pooling (by artificial stratigraphic  
493 unit) highlights the loss of information by attenuating the actual isotope variability due to the  $\delta^{13}\text{C}$   
494 mean values from several charcoal samples.

495 This study also highlights the importance of increased numbers of charcoal samples in order  
496 to obtain the most reliable signal possible. Moreover, questions about plant adaptation to climatic (or  
497 atmospheric) changes arise. Thus, further studies on the variability and responses of the isotope signal  
498 to climate change and on complementary Pleistocene sequences are required to consolidate this  
499 approach.

500

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