

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)

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Introduction

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

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

In recent decades, studies focusing on the $\delta^{13}C$ isotopic signals of plants have provided a reliable method to reconstruct past climatic and environmental conditions. During plant absorption of atmospheric CO_2, a discrimination (Δ) of carbon isotopes occurs. It is defined, in C3 metabolism, as the result of discrimination against ^{13}C induced by stomatal conductance (a = 4.4‰) and the RuBisCO enzyme during carboxylation (b $\simeq$ 30‰), with an intensity that depends on the ratio of intracellular CO_2 to atmospheric CO_2 content (ci/ca) : $\Delta \simeq a + (b-a)\ ci/ca$ (1) (Farquhar and Lloyd, 1993; Farquhar et al., 1982). Thus, changes in stomatal opening, controlled by environmental and climatic parameters (including atmospheric parameters), constrain the $\delta^{13}C$ record in the plant (Farquhar et al., 1989). Correlation between the $\delta^{13}C$ wood signal and different stress factors (drought, high temperatures, etc.) can be observed over scales at various resolutions (seasonal wood, tree-rings, tree-ring clusters) (Cernusak and English, 2015; McCarroll and Loader, 2004). In particular, correlations between the wood $\delta^{13}C$ signal and water availability parameters were observed in a major study, which corresponds to the first recurrent limiting factor (Battipaglia et al., 2014; Dawson et al., 2002; Farquhar and Richards, 1984; Gessler et al., 2014; Panek and Goldstein, 2001; Warren et al., 2001). In the presence of optimal levels of atmospheric moisture or in the global warming, temperature or Irradiation also dominate the relationship between isotopic and climatic signals (Gagen et al., 2007; McCarroll and Pawellek, 2001; Seftigen et al., 2011).

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

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

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

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

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

Archaeological context and previous data

General context

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

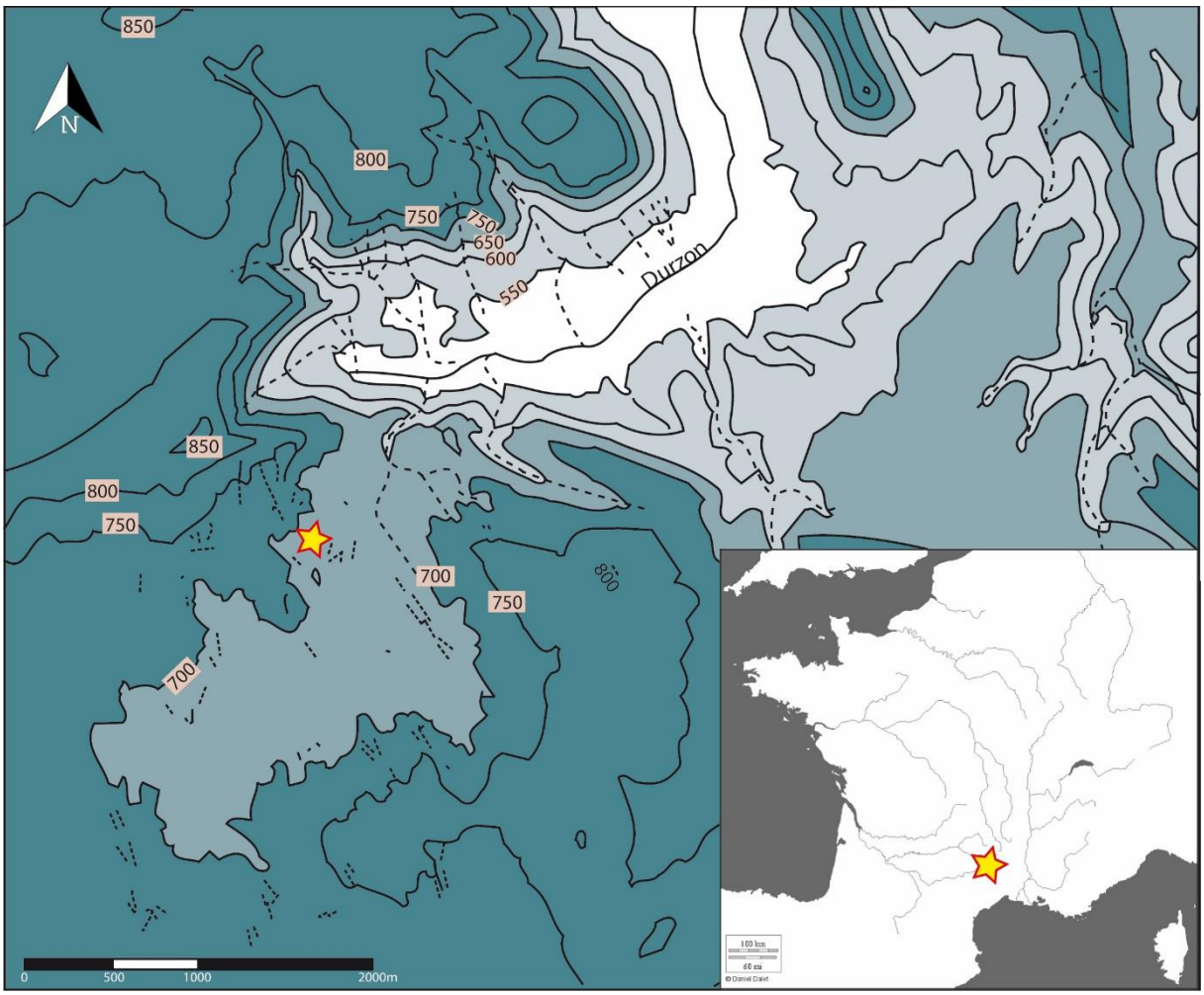


Figure 1: Location of the Middle Paleolithic site of Les Canalettes rock shelter (yellow star), on the south-east of the Larzac Causse Plateau (South-East France). Modified after Meignen, 1993.

The site presents a two-meter-thick stratigraphic sequence where numerous lithic remains assigned to the Mousterian have been found along the deposit (Figure 2). The sedimentary dynamics of the shelter are fairly homogeneous over the entire thickness of the stratigraphy, with a sedimentological composition of loose dolomitic sands more or less rich in heterometric gravel. The sequence has been divided into 4 stratigraphic units, mainly on changes in color of the sandy matrix 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

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

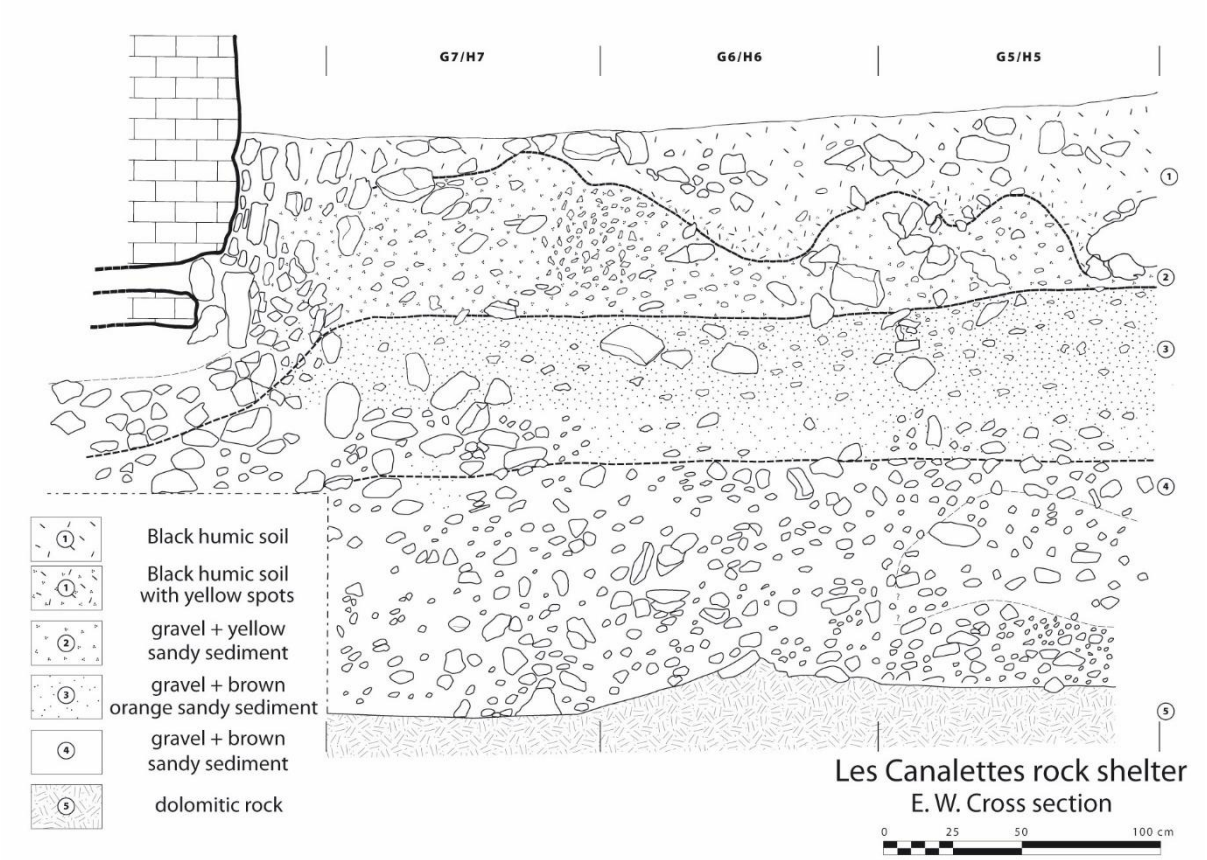


Figure 2: East-West stratigraphic cross section of Les Canalettes rock shelter. Modified after Meignen, 1993.

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

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

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

Previous results of Paleoenvironmental studies

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

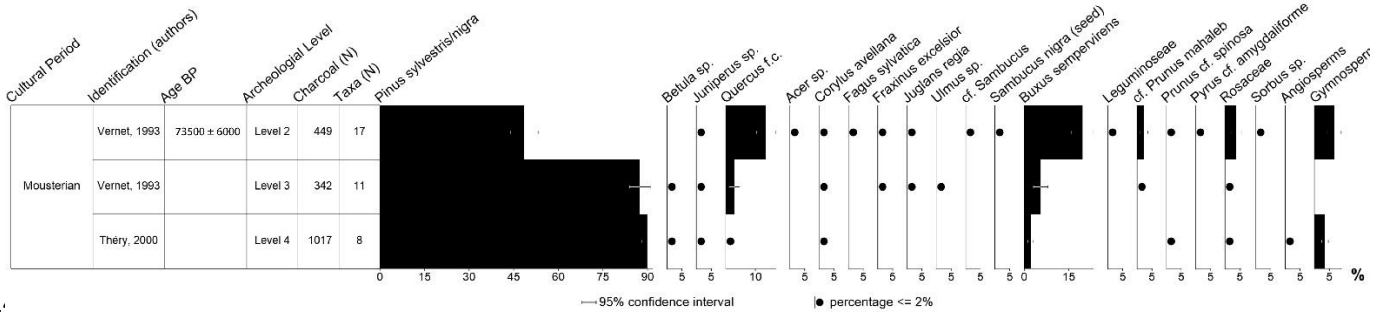


Figure 3: Anthracological diagram of the mousterian site Les Canalettes (modified according to Théry-Parisot, 1998)

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

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

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

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

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

Materials and Methods

Materials

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

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

Pre-treatment of samples

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

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

Isotopic analysis

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

Data processing

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

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

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

Results

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

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

Stratigraphic Level	N	Mean $\delta^{13}C$ (‰)	$\delta^{13}C$ standard deviation (‰)	$\delta^{13}C$ 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
US 2	**30**	**-25.3**	**1.0**	**3.8**
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
US 3	**38**	**-25.0**	**0.6**	**2.8**
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
US 4	**50**	**-24.7**	**0.6**	**2.8**
Mean	**9**	**-24.9**	**0.5**	**1.6**

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

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

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

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

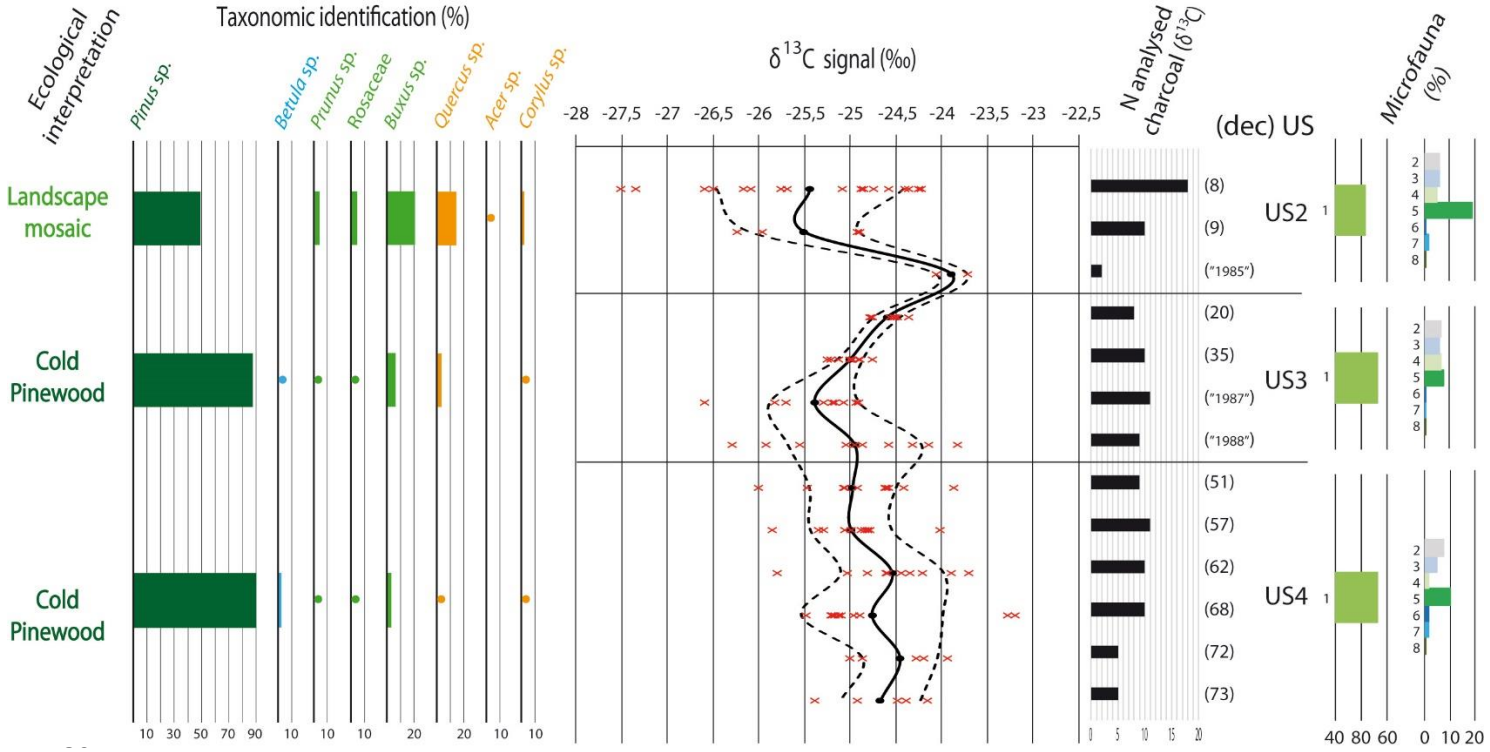


Figure 4: Summary of the isotopic data obtained from charcoal remains, supplemented by previous paleoenvironmental data from Les Canalettes rock shelter. We observed, from left to right: (i) the anthracological identification by US and the ecological interpretations (Vernet, 1993; Théry-Parisot, 1998);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

Interpretation

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

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

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

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

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

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

Discussion

Isotopic signal variability

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

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

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

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

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

399

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 $\delta^{13}C$ 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-Oesschger" (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 Moula-
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 $\delta^{13}C$ 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).

443

The techno-cultural system

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

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

Conclusion

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

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

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

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

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