# Bone stable isotopic signatures (15N, 18O) as tracers of temperature variation during the Late-glacial and early Holocene: case study on red deer Cervus elaphus from Rochedane (Jura, France)

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# Bone stable isotopic signatures ($^{15}$N, $^{18}$O) as tracers of temperature variation during the Late-glacial and early Holocene: case study on red deer *Cervus elaphus* from Rochedane (Jura, France)

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Stable isotopes in mammal bones are mainly used to reconstruct dietary preferences and their use as palaeoclimatic indicators is less developed. However, important variations in $^{15}$N abundances observed in the bone collagen of large mammals during the Late-glacial and early Holocene have been tentatively linked to a general increase in temperature. In order to test this hypothesis, we analysed nitrogen and oxygen isotopic abundances from bones of red deer (*Cervus elaphus*) from the Rochedane site (Jura, France). We observe a clear linear relationship between $^{15}$N and $^{18}$O that demonstrates the effect of temperature on the abundance of $^{15}$N in red deer bone collagen. These results suggest that an increase in soil maturation during global warming of the Late-glacial and early Holocene led to an increase of $^{15}$N in soils and plants that was passed on to their consumers. Red deer seem to be particularly suited for palaeoclimatic reconstruction based on the isotopic signatures of their bones. Copyright © 2009 John Wiley & Sons, Ltd.

## 1. INTRODUCTION

Biotic markers of the environment in a continental context are crucial for deciphering the impact of global climatic change on terrestrial ecosystems. Reconstruction of terrestrial palaeotemperature based on biotic tracers such as beetles, gastropods, micromammals, plant macrofossils and pollen has been relatively successful in the Late-glacial and early Holocene, because the occurrence of many key taxa is linked to a limited range of temperatures (e.g. Coope *et al*. 1998; Lemdahl 2000; Lotter *et al*. 2000; Magny *et al*. 2001; Ponel *et al*. 2005). A similar approach based on the uniformitarian principle that mammal species had the same biological requirements in the past as they do today has been proposed for herbivorous mammals associated with ancient human activities (e.g. Delpech 1973). However, the occurrence of non-analogous fossil communities that include species whose distribution do not overlap today raises the question of different ecological requirements for ancient representatives of modern species or the post-mortem mixing of fossils that lived under different ecological conditions. Several radiocarbon studies have ruled out taphonomic biases for many of these non-analogous assemblages (e.g. Woodmann *et al*. 1997; Bridault *et al*. 2000; Lyman 2008; Bridault and Chaix 2009). Stable isotopes in mammal bones have further

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demonstrated that some of these co-occurring taxa dwelled in the same type of environment, demonstrating more ecological flexibility than expected (e.g. Drucker *et al*. 2009). Therefore, the level of ecological flexibility for a given species of large mammals can be larger than in the modern world, due to interference of competition and human activities in the distribution of modern wild species; and to possible changes in the genetic diversity of a species through time (e.g. Shapiro *et al*. 2004; Dalen *et al*. 2007). Palaeoclimatic conclusions have also been attempted using phenotypic features that can be linked to climatic parameters, such as body size (e.g. Smith and Betancourt 2003). However, this type of approach requires large population sizes and morphological changes may be delayed relative to the climatic changes that may occur. Also, non-climatic factors may interfere with size, such as competition between taxa (e.g. Raia and Meiri 2006).

Stable isotopes in mammal bones have been mainly used as palaeodietary indicators during the Late-glacial and early Holocene (e.g. Lillie *et al*. 2003; Schulting *et al*. 2008), but there is no evidence for large isotopic variations within species that correlate broadly with known global climatic changes (e.g. Drucker *et al*. 2003a; Richards and Hedges 2003; Stevens and Hedges 2004). When properly calibrated and correlated with identified climatic factors, such variations could be used as proxies in palaeontological and archaeological sites. For instance, a clear relationship has been found between aridity and $^{15}$N levels in plants and animal bone collagen and have been used as a palaeoclimatic indicator (Heaton *et al*. 1986; Ambrose and DeNiro 1987; Sealy *et al*. 1987; Gröcke *et al*. 1997).

In the present study, the red deer (*Cervus elaphus*) was chosen since it is a ubiquitous species in northwestern Europe during the Late-glacial and early Holocene, in contrast to cold-adapted species such as the reindeer *(Rangifer tarandus)*. In previous work (Drucker *et al*. 2003b), collagen isotopic signatures ($\delta^{13}$C and $\delta^{15}$N) were measured for archaeological remains of red deer (*C. elaphus*) from the Late-glacial/early Holocene site of Rochedane in the French Jura. Important variations in $^{13}$C and $^{15}$N amounts were described, especially between samples dated to the Late-glacial and those corresponding to the early Holocene when the climate became warmer and vegetation cover more dense (Drucker *et al*. 2003b). The decrease in $\delta^{13}$C values in red deer collagen at Rochedane during the Late-glacial/early Holocene transition has since been confirmed in red deer and other species of large herbivores (horse, roe deer, large bovids) from other areas in western Europe (Richards and Hedges 2003; Stevens and Hedges 2004; Noe-Nygaard *et al*. 2005; Drucker *et al*. 2008). The lower $\delta^{13}$C values seen in the collagen of herbivores from the early Holocene relative to those from the Late-glacial was attributed to the 'canopy effect', which is a hypothesized depletion in $^{13}$C in plants growing under closed canopies which is reflected by the herbivores that consume them (Drucker *et al*. 2003b; Noe-Nygaard *et al*. 2005; Drucker *et al*. 2008). The increase in $\delta^{15}$N values in the red deer of Rochedane at the Late-glacial/early Holocene transition was also observed in horse collagen from Belgium and the British Isles (Stevens and Hedges 2004). Temperature is quoted among the different environmental parameters that could lead to variation in $\delta^{15}$N values in herbivores (Drucker *et al*. 2003a,b; Stevens and Hedges 2004). Indeed, decrease in $\delta^{15}$N values in soils and plants are observed with decrease in mean annual temperature in modern ecosystems (Amundson *et al*. 2003). These authors further recognize the importance of other parameters, such as soil age, topographic position, concentration of N in soils and perturbations by agriculture and or fire. However, they conclude that climate is the primary control of $\delta^{15}$N in soils and plants. Variation in $^{15}$N concentrations in plants is then reflected by $^{15}$N concentrations in the collagen of herbivores (Murphy and Bowman 2006). The $\delta^{15}$N values of herbivores may thus depend indirectly on the mean annual temperature through nitrogen-related processes in soils (see discussion in Drucker *et al*. 2003a).

To examine this hypothesis, we analysed the $\delta^{18}$O of bone phosphate, which has been directly linked to temperature. Indeed, the oxygen isotopic composition of bone phosphate depends mainly on the oxygen isotope composition of meteoric water and water contained in the food ingested by an animal (e.g. Longinelli 1984; Luz and Kolodny 1985; Iacumin *et al*. 1996). The oxygen isotope signature of some herbivorous species can also be influenced by relative humidity in more arid areas, as the water in leaves is subjected to evapotranspiration, and therefore can be $^{18}$O-enriched (e.g. Luz *et al*. 1990; Kohn 1996; Levin *et al*. 2006; Murphy *et al*. 2007). In the case of the European red deer, it has been suggested that relative humidity is not a significant parameter since this species exhibits oxygen isotope variations that reflect essentially those of local rainfall (D'Angela and Longinelli 1990). The $\delta^{18}$O value of meteoric water is a function of air temperature and humidity, but reflects essentially the temperature in boreal and temperate contexts (Fricke and O'Neil 1999). In the case of the red deer (*C. elaphus*), a linear relationship has been shown

between $\delta^{18}O$ in body water and the mean $\delta^{18}O$ value of local meteoric water (D'Angela and Longinelli 1990), as well as between the $\delta^{18}O$ of bone phosphate and the mean $\delta^{18}O$ values of local meteoric water (Iacumin *et al*. 1996).

If the variations of collagen $\delta^{15}N$ values observed in Late-glacial and early Holocene red deer from Rochedane are related to climate and more specifically, to temperature, then we expect a linear relationship to exist between collagen $\delta^{15}N$ and phosphate $\delta^{18}O$ measured from the same bones.

## 2. MATERIAL AND METHODS

The site of Rochedane ($47° 21'$ N, $6° 46'$ E) is located approximately 25 km south of the town of Montbeliard on the left bank of the Doubs river (Figure 1). It is a rock-shelter dug into an approximately 15 m high calcareous cliff, at an altitude of 355 m above sea level in an area where the neighbouring plateaux rise to 700 m above sea level. The excavation by André Thévenin and Jean Sainty (1968–1976) identified several archaeological layers ranging between from 12 500 to 8 000 years BP (e.g. Thévenin and Sainty 1998, Table 1). All layers yielded skeletal remains of red deer, *C. elaphus* (Bridault 1990).

Red deer bones were selected to represent different individuals using the following sampling protocol: whenever multiple samples were selected for one layer, the same bone was used, which guarantees that no individual is sampled twice in a given layer. Juvenile specimens with unfused epiphyses were discarded due to possible interferences from nursing on the nitrogen isotopic composition (Drucker *et al*. 2003b).

Most of the carbon and nitrogen isotopic values of the red deer from Rochedane discussed in this paper were published in Drucker *et al*. (2003b). Additional red deer samples were analysed from layer D1 ($n = 2$, including one tooth) and layer A1 ($n = 2$), which correspond to the base and the top of the stratigraphy, respectively. To obtain an isotopic signature recorded several years prior to the animal death, sampling was performed on compact bone from adult specimens. Tooth roots were avoided as they can be influenced by the consumption of milk during early ontogeny (Bocherens *et al*. 1994). One exception had to be made for one new sample from layer D1, a third molar, as this layer yielded few red deer remains. In this last case, the collagen $\delta^{15}N$ value was not considered in the palaeoenvironmental reconstruction.

Direct accelerator mass spectrometry (AMS) radiocarbon dating was performed on the collagen extracted from some of the bones selected for stable isotope analysis (Drucker *et al*. 2008). For a given stratigraphic layer, the obtained dates were slightly older than those previously published in Evin *et al*. (1978), which were obtained via conventional radiocarbon dating of mixed bones from the same layers. These new dates led to some slight revision in the chronological attribution of some of the material. For instance, bone isotopic results of layers C'1 and B were combined since their calibrated dating are very close, with ages from 13 605 to 13 270 cal BP and from 13 650 to 13 282 cal BP, respectively. Two bones from layer A3 resulted in differing dates, one (RCD6400) being similar to the date from layer A4 (from 12 917 to 12 815 cal BP), the other (RCD6300) corresponding to a more recent period (from 9772 to 9500 cal BP). Sample RCD6400 was eventually included in the same set of isotopic data as layer A4, while sample RCD6300 was considered independently. Due to the heterogeneity of radiocarbon dates, the other samples of layer A3 could not be attributed to a given chronozone. Hence, the isotopic signatures of these samples were only used in the intra individual comparison between collagen $\delta^{15}N$ and phosphate $\delta^{18}O$ values. The isotopic data obtained from the AMS radiocarbon dated bones could be related to pollen zones established in the French Jura and to the Greenland isotopic event chronology (Table 1).

The sample preparation for isotopic analyses was performed as follows. Chunks of about 200–500 mg were cut from the cortical part of each sampled bone and then ground to a powder sieved at less than 0.7 mm. Carbon and nitrogen isotopes were measured from the collagen that was extracted using the protocol presented in Bocherens *et al*. (1997). The new isotopic measurements of carbon and nitrogen were performed on a VG-Optima continuous-flow isotope-ratio mass spectrometer (CFIRMS) at the Institut des Sciences de l'Evolution of Montpellier 2 University (Montpellier, France). Elemental composition of the extracted collagen was measured on a CHN-elemental analyser (Eurovector) coupled to a VG-Optima mass spectrometer. For the oxygen isotope analysis, bone powder samples were treated according to the protocol presented by Crowson and Showers (1991)
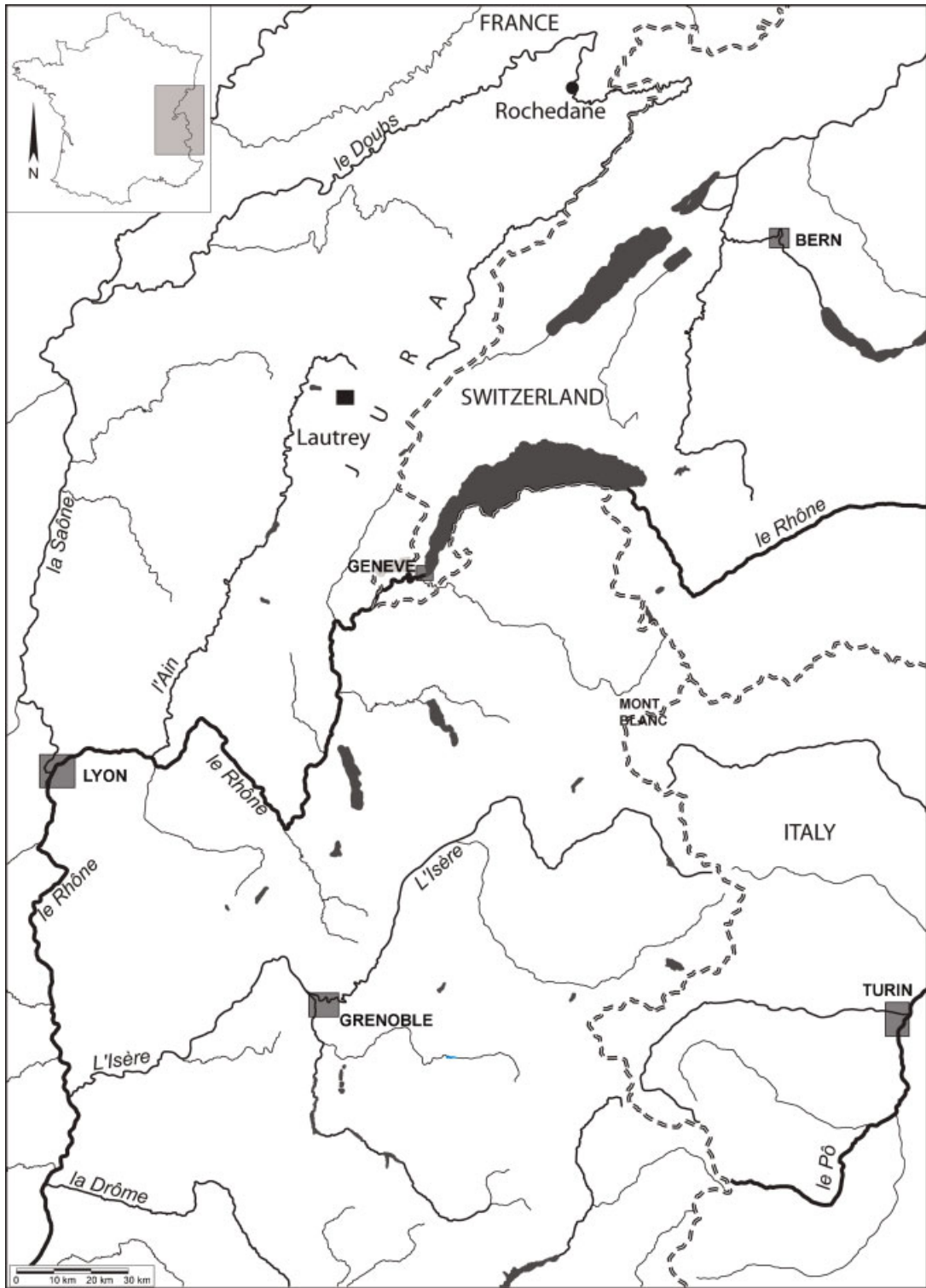
Figure 1.  Location of Rochedane site and Lake Lautrey in French Jura (eastern France).

Table 1. Cultural and chronological description of the stratigraphy of the site of Rochedane in French Jura

| Layer | Cultural attribution | Dated red deer bones | Conventional $^{14}$C age BP | Calibrated $^{14}$C age BP ($2\sigma$) | Pollen chronozone | GRIP stratigraphy | NGRIP stratigraphy |
|---|---|---|---|---|---|---|---|
| D1 | Late Magdalenian | L. radius | $12{,}250 \pm 70$ (GrA-21512) | 14 510–13 902 | Bölling | GI-1e and GI-1d | GI-1e and GI-1d |
| C′1 | Early Azilian | L. metatarsal | $11{,}570 \pm 70$ (GrA-21514) | 13 605–13 270 | Early Alleröd | GI-1c | GI-1c |
| B | Azilian | L. metatarsal | $11{,}600 \pm 80$ (GrA-21516) | 13 650–13 282 | Early Alleröd | GI-1c | GI-1c |
| A4 | Azilian | L. metatarsal | $10{,}880 \pm 50$ (GrA-23147) | 12 917–12 815 | Late Alleröd | GI-1a | Transition GI-1a/GS-1 |
| | | L. metatarsal | $10{,}830 \pm 70$ (GrA-21518) | 12 923–12 747 | Late Alleröd | GI-1a | Transition GI-1a/GS-1 |
| A3 | Early Mesolithic | L. metatarsal | $10{,}880 \pm 50$ (GrA-23150) | 12 917–12 815 | Late Alleröd | GI-1a | Transition GI-1a/GS-1 |
| | | L. metatarsal | $8640 \pm 60$ (GrA-21519) | 9772–9516, 9511–9500 | Boreal | | |
| A2 | Mesolithic | L. metatarsal | $6730 \pm 60$ (GrA-21520) | 7679–7490 | Early Atlantic | | |
| A1 | Neolithic | R. metacarpal | $6230 \pm 60$ (GrA-21522) | 7269–6976 | Early Atlantic | | |

Radiocarbon dates have been calibrated according to IntCal04 dataset (Reimer *et al*. 2004). Cultural attribution are based on Thévenin and Sainty (1998), pollen chronozone on Magny *et al*. (2006), GRIP stratigraphy on Björck *et al*. (1998) and NGRIP stratigraphy on Lowe *et al*. (2008).
L: Left.
R: Right.

and modified by Lécuyer *et al.* (1993). The oxygen obtained at the end of the process is converted to $CO_2$, which is then analysed using a Finnigan Delta S mass spectrometer at the Department of Earth Sciences at the University of Parma (Parma, Italy). Isotopic abundances are expressed as $\delta$ (delta) values, according to: $\delta^E X = (R_{sample}/R_{standard} - 1) \times 1000$ (‰), where X stands for C or N or O, $E$ for 13 or 15 or 18, and $R$ for the isotopic ratios, $^{13}C/^{12}C$, $^{15}N/^{14}N$ and $^{18}O/^{16}O$, respectively. The standards used are, internationally defined, and consisted of a marine carbonate (PDB) for carbon, atmospheric nitrogen (AIR) for nitrogen, and Vienna Standard Mean Ocean Water (VSMOW) for oxygen. Analytical error is 0.1‰ for $\delta^{13}C$ values, 0.2‰ for $\delta^{15}N$ values, and 0.2‰ for $\delta^{18}O$ values. Samples are calibrated to the d13C values of USGS 24 ($\delta^{13}C = -16{,}00$‰, relativ to UPDB) and to the d15N values of IAEA 305A ($\delta^{15}N = 39{,}80$‰, relativ to AIR).

## 3. RESULTS AND INTERPRETATION

The new collagen $\delta^{13}C$ and $\delta^{15}N$ ($\delta^{13}C_c$ and $\delta^{15}N_c$) values of red deer from layer D1 and A1 were consistent with those obtained for the same pollen chronozone. In layer D1, a higher $\delta^{15}N_c$ value was observed in the tooth root (RCD600). This enrichment in $^{15}N$ in the tooth relatively to bone was previously observed and attributed to milk consumption during growth that persists in the unremodelled collagen of the dentine (Bocherens *et al.* 1994). Altogether, the $\delta^{13}C_c$ and $\delta^{15}N_c$ values of the Rochedane red deer range from −23.7‰ to −19.5‰ and from 0.2‰ to 6.4‰, respectively (Table 2). Two distinct groups of collagen isotopic values are apparent between the Late-glacial (layers D1, C′1, B and RCD6400 from layer A3) and Early Holocene (layers A2, A1 and RCD6300 from layer A3) periods. During the Early Holocene, $\delta^{13}C_c$ values are lower and $\delta^{15}N_c$ values higher than those found during the Late-glacial, without any overlap between the periods (Table 2, Figures 2 and 3).

The phosphate $\delta^{18}O$ ($\delta^{18}O_p$) values of red deer bones from Rochedane varied from 14.0‰ to 17.2‰ (Table 2, Figure 4). The variation of $\delta^{18}O_p$ values through time follows the trend found in the corresponding $\delta^{15}N_c$ values from the same bones, including a slight decrease in values for layer A4 compared to layers B, C′1 and D. A linear regression could indeed be defined between $\delta^{15}N_c$ and $\delta^{18}O_p$ (Figure 5). The best-fit correlation gives the following equation: $\delta^{15}N_c = (1.62 \times \delta^{18}O_p) - 22.16$ ($n = 29$, $R^2 = 0.67$). This linear regression indicated a direct relationship between the amounts of $^{15}N$ in collagen and the amounts of $^{18}O$ in phosphate from the same bone.

Table 2. Isotopic results for red deer (*C. elaphus*) from Rochedane site in French Jura

| Layer | Lab No. | Sample | Excavation No. | $C_c$ (%) | $N_c$ (%) | $C/N_c$ | $\delta^{13}C_c$ (‰) | $\delta^{15}N_c$ (‰) | $\delta^{18}O_p$ (‰) |
|---|---|---|---|---|---|---|---|---|---|
| D1 | RCD500[a] | L. radius | D6 Roch70 no. 19 | 42.3 | 15.5 | 3.2 | −19.9 | 0.8 | 15.0 |
| D1 | RCD600 | Third molar | V/D no. 8 | 41.2 | 14.8 | 3.2 | −20.1 | (4.1)[b] | 14.9 |
| D1 | RCD10900 | Metacarpal | A3 | 41.7 | 16.0 | 3.0 | −20.4 | 2.0 | n.d. |
| D1 | RCD11000 | Metacarpal | A3 | 44.0 | 16.1 | 3.2 | −20.6 | 2.4 | n.d. |
| C′1 | RCD900 | L. metatarsal | | 40.3 | 14.6 | 3.2 | −20.1 | 1.8 | 15.0 |
| C′1 | RCD1000[a] | L. metatarsal | H9-G9 | 42.5 | 15.4 | 3.2 | −20.7 | 2.5 | 15.1 |
| C′1 | RCD1100 | L. metatarsal | H9-G9-225-230 | 42.0 | 15.3 | 3.2 | −21.9 | 3.0 | 15.3 |
| C′1 | RCD1200 | L. metatarsal | C10-D10 | 38.9 | 14.4 | 3.1 | −19.9 | 1.3 | 14.8 |
| C′1 | RCD1300 | L. metatarsal | G10 | 37.8 | 13.9 | 3.2 | −20.6 | 2.0 | 14.8 |
| C′1 | RCD1400 | L. metatarsal | H9-G9-220-225 | 41.0 | 15.0 | 3.2 | −20.7 | 0.4 | 14.9 |
| B | RCD2300 | L. metatarsal | B9 | 38.5 | 14.2 | 3.2 | −20.8 | 1.6 | 14.9 |
| B | RCD2400 | L. metatarsal | H8 Ro71-205-210 | 38.9 | 13.3 | 3.2 | −20.6 | 3.1 | 14.8 |
| B | RCD2500 | L. metatarsal | E8-200-207 | 40.0 | 14.6 | 3.2 | −21.2 | 2.6 | 14.6 |
| B | RCD2600[a] | L. metatarsal | | 38.8 | 14.0 | 3.2 | −20.0 | 2.3 | 15.1 |
| B | RCD2700 | L. metatarsal | E6-175 | 38.2 | 14.2 | 3.2 | −20.9 | 1.6 | 15.4 |
| B | RCD2800 | L. metatarsal | G7-195.5-204 | 40.3 | 14.7 | 3.2 | −20.5 | 1.3 | 14.8 |
| A4 | RCD4700[a] | L. metatarsal | F9-186 | 39.7 | 14.8 | 3.1 | −19.5 | 1.4 | 14.5 |
| A4 | RCD4800[a] | L. metatarsal | H9-190-195 | 40.7 | 14.7 | 3.2 | −20.6 | 1.3 | 14.9 |
| A4 | RCD4900 | L. metatarsal | F9-188-195 | 39.9 | 14.8 | 3.2 | −20.0 | 1.3 | 14.0 |
| A4 | RCD5000 | L. metatarsal | | 39.5 | 14.3 | 3.2 | −20.3 | 0.4 | 14.4 |
| A4 | RCD5100 | L. metatarsal | G6-185-190 | 39.8 | 14.7 | 3.2 | −20.0 | 0.2 | 14.1 |
| A3 | RCD6200 | L. metatarsal | | 38.8 | 14.2 | 3.2 | −22.5 | 3.5 | 16.9 |
| A3 | RCD6300[a] | L. metatarsal | H8 | 39.1 | 14.3 | 3.2 | −23.4 | 4.7 | 16.8 |
| A3 | RCD6400[a] | L. metatarsal | | 36.8 | 13.4 | 3.2 | −21.5 | 3.8 | 15.1 |
| A3 | RCD6500 | L. metatarsal | E11-E12-170-181 | 40.5 | 14.6 | 3.2 | −23.0 | 5.2 | 15.9 |
| A2 | RCD10100[a] | R. metatarsal | F11-12-135-140 | 37.4 | 13.5 | 3.2 | −22.8 | 5.8 | 15.6 |
| A2 | RCD10200 | R. metatarsal | G10-160-172 | 38.2 | 14.2 | 3.1 | −22.7 | 4.3 | 15.7 |
| A2 | RCD10300 | R. metatarsal | F11-136-139 | 38.1 | 14.0 | 3.2 | −22.9 | 6.4 | 17.2 |
| A2 | RCD10400 | R. metatarsal | D11 | 37.7 | 13.7 | 3.2 | −22.6 | 4.2 | 17.1 |
| A1 | RCD10500[a] | R. metacarpal | Ro72 F10-11 121 | 42.2 | 16.5 | 3.0 | −23.6 | 4.8 | 16.7 |
| A1 | RCD10600 | L. metacarpal | F11-10-124-129 | 40.7 | 15.1 | 3.1 | −22.3 | 6.0 | 16.8 |
| A1 | RCD10700 | Metacarpal | RA1 F10 | 40.6 | 14.9 | 3.2 | −23.7 | 5.5 | 16.5 |

$C_c$ and $N_b$ stand for collagen carbon amounts and collagen nitrogen amounts. The atomic ratio $C/N_c$ ranges between 3.0 and 3.2 which correspond to well-preserved collagen (DeNiro 1985).
[a]Radiocarbon dated samples (results reported in Table 1).
[b]Values in parenthesis were not included in the final study.
n.d.: not determined.
L: Left.
R: Right.

## 4. DISCUSSION AND CONCLUSIONS

The lower $\delta^{13}C_c$ values and higher $\delta^{15}N_c$ values found in Rochedane red deer from the Early Holocene compared to the Late-glacial were interpreted as the result of change in habitat and temperature, respectively (Drucker *et al.* 2003b). During the Early Holocene, red deer habitat in dense mature forests led to more negative $\delta^{13}C_c$ values and increased mean temperatures were reflected by more positive $\delta^{15}N_c$ values through direct and indirect mechanisms (see Drucker *et al.* 2003a, 2008).

The linear correlation evidenced between $^{15}N_c$ and $^{18}O_p$ amounts confirms a significant influence of temperature on the $\delta^{15}N$ values of Rochedane deer. The relationship between $\delta^{15}N_c$ and temperature is not a direct one and is possibly linked to soil maturation and nitrogen cycling activity (Amundson *et al.* 2003). Indeed, in the context of increase in temperatures and vegetation development of the Late-glacial, decomposition of organic matter and mineralization processes in soils were likely enhanced. In addition, loss of nitrogen due to volatilization and denitrification increases in warming conditions. The resulting increase of nitrogen turnover leads to higher
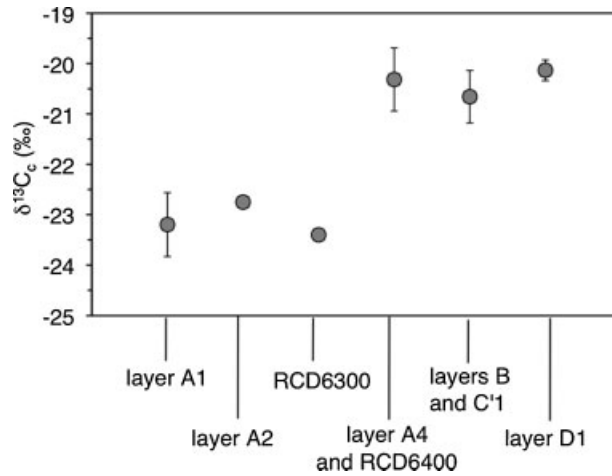
Figure 2. Mean $\delta^{13}C_c$ values of red deer from Rochedane according to stratigraphical layers. Vertical bars represent one standard deviation.
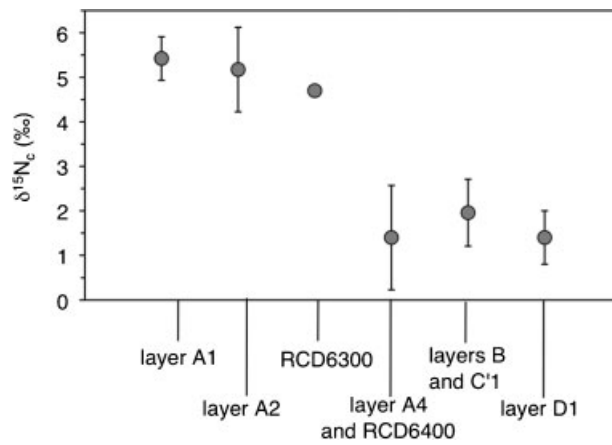


Figure 3. Mean $\delta^{15}N_c$ values of red deer from Rochedane according to stratigraphical layers. Vertical bars represent one standard deviation.
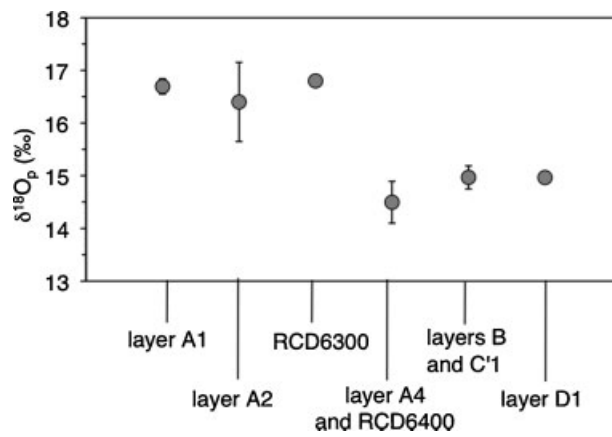


Figure 4. Mean $\delta^{18}O_p$ values of red deer from Rochedane according to stratigraphical layers. Vertical bars represent one standard deviation.
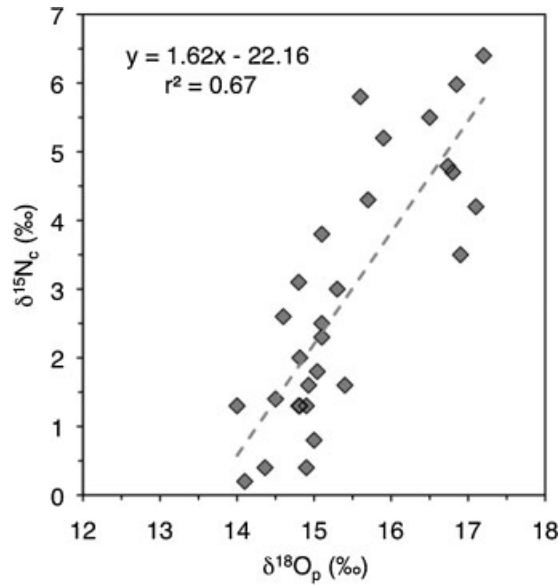
Figure 5. Relationship between the $\delta^{15}N$ values of collagen ($\delta^{15}N_c$) and the $\delta^{18}O$ values of phosphate ($\delta^{18}O_p$) measured on the same bones of red deer from Rochedane site.

$\delta^{15}N$ values in plants (Johannisson and Högberg 1994; Hobbie *et al*. 1998, 2005). Finally, we suggest that the $\delta^{15}N_c$ values of large herbivores such as red deer directly reflect the variation of $\delta^{15}N$ values of plants, which was primarily influenced by rapidly changing annual temperature during the Late-glacial and early Holocene.

Variations exhibited by $\delta^{18}O_p$ values of red deer from Rochedane are less pronounced than the corresponding $\delta^{15}N_c$ values from the same specimens. Thus, in red deer bone, the variation of annual temperature is more strongly reflected by the $\delta^{15}N_c$ values than by $\delta^{18}O_p$ values. The mathematical relationship that can be calculated between isotopic values and mean annual temperature for modern red deer is consistent with this observation. Indeed, the relationship between the $\delta^{18}O_p$ of modern red deer and the mean $\delta^{18}O$ values of local meteoric water ($\delta^{18}O_w$) based on data from Iacumin *et al*. (1996) and Stephan (1999) is: $\delta^{18}O_p = (1.07 \times \delta^{18}O_w) + 25.01$ ($n = 6$, $R^2 = 0.99$; Figure 6). On the other hand, a linear regression between mean $\delta^{18}O_w$ values and mean annual temperature (MAT) was calculated for Switzerland (Tütken *et al*. 2007, modified by Tütken 2008 personal communication) and gave the following result: $\delta^{18}O_w = (0.44 \times MAT) - 14.19$ ($n = 1230$, $R^2 = 0.68$). Thus, we suggest the following formula to relate the $\delta^{18}O_p$ values of red deer to the MAT: $\delta^{18}O_p = (0.47 \times MAT) + 9.82$.

A positive correlation between the $\delta^{15}N_c$ values of modern red deer populations and MAT was observed by Stevens *et al*. (2006): $MAT = (1.07 \times \delta^{15}N_c) + 3.37$ ($n = 5$, $R^2 = 0.66$). From this work, the following relation can be calculated: $\delta^{15}N_c = (0.93 \times MAT) - 3.15$. Hence, from these equations, a range of variation that is twice as high for $\delta^{15}N_c$ values than for $\delta^{18}O_p$ values can be predicted as a result of a similar variation in MAT. The results obtained in Rochedane are consistent with this prediction.

The decrease in $\delta^{15}N_c$ and $\delta^{18}O_p$ observed at level A4 compared to level B, C′1 and D1 can be interpreted as a drop in temperature. The linear regression between MAT and $\delta^{18}O_p$ on the one hand and $\delta^{15}N_c$ on the other hand allow us to tentatively calculate the possible temperature decrease witnessed by red deer of layer A4. Both calculations give a result of a maximum of 3°C decrease in mean annual temperature as reflected by the isotopic signature of red deer bones from Rochedane. The correlation of the A4 layer with a known cooling event is rather difficult. The dated red deer bones of the A4 level (and sample RCD6400) give a range of *ca*. 12 950–12 750 cal BP, which would either correspond to the event GI-1a (warmer episode) or the transition from GI-1a to GS-1 (colder episode) depending on the considered ice core stratigraphy (see Table 1). Despite the considerable effort put on the
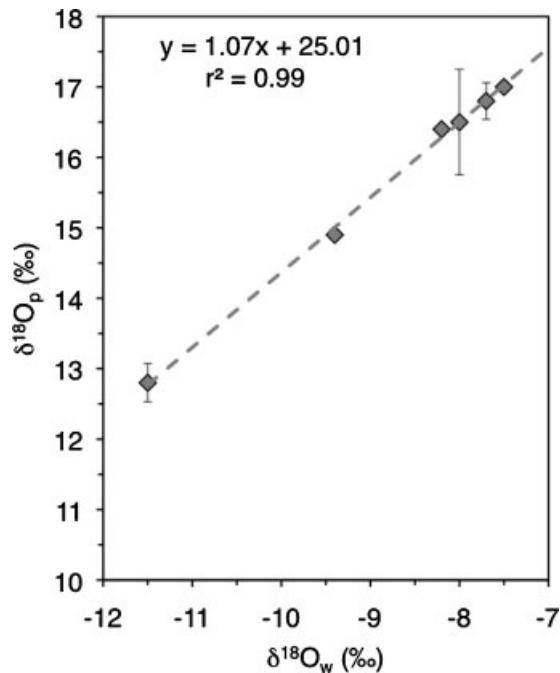
Figure 6. Relationship between the oxygen isotope values of bone phosphate ($\delta^{18}O_p$) of red deer (*Cervus elaphus*) and the mean oxygen isotope values of local meteoric water ($\delta^{18}O_w$) based on published data from Iacumin *et al.* (1996) and Stephan (1999).

refinement of the chronology of the Late-glacial, uncertainties remain on the dating of climatic events, especially for the transition period from the Alleröd to Younger Dryas (GI-1a to GS-1 transition) (e.g. Muscheler *et al.* 2008). In the late Alleröd, the Laacher See eruption represents a significant event and one of the most accurately dated references. The deposit of ashes from this volcanic event has been recorded in lake sediment in a large area surrounding the Eifel region. Laacher See Tephra (LST) is used as a major stratigraphic marker and was the centre of numerous radiocarbon dating studies, which place it at *ca.* 12 900 cal year BP (Brauer *et al.* 1999; Baales *et al.* 2002; Litt *et al.* 2003). Thus, the dated red deer from layer A4 would have immediately followed the Laacher See event. At that time, several proxies suggest a decrease in temperature and increase in precipitation at the scale of whole Europe. These changes are considered to be the climatic consequence of the Laacher See eruption (Friedrich *et al.* 1999; Schmincke *et al.* 1999; Baales *et al.* 2002). In the French Jura, the chironomid and pollen records in Lake Lautrey sediments suggest a short-term decrease in summer temperatures coeval to the LST deposition (Magny *et al.* 2006). The decrease in $\delta^{18}O_p$ values in red deer of Rochedane of layer A4 could correspond to this short-term cooling linked to the Laacher See event.

Red deer is a very promising species for tracing terrestrial palaeoclimatic changes through bone isotopic signatures. It is also present in Europe since the early Middle Pleistocene (Lister 1986). Other species could also be used, but caution is required. For instance, it would be tempting to use other taxa, such as reindeer, because it is very abundant in Europe during the colder climatic phases. However, it is questionable whether the isotopic signatures of this species could provide a reliable tracer, as it has been shown on modern material that a poor correlation exists between the $\delta^{18}O$ values in reindeer bone and those of local precipitation (Stephan 1999; Iacumin and Longinelli 2002; Longinelli *et al.* 2003).

Thus, though indirect, the relationship between air temperature and $\delta^{15}N_c$ values of red deer is confirmed in the Late-glacial and Holocene context of western Europe. Nitrogen isotope data on red deer collagen could be used to identify samples that could then be prepared for phosphate oxygen isotope analysis, a technique more challenging than nitrogen isotope analysis.

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