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Variability in feeding habitats of red deer *sensu lato* in Eurasia in the Late Pleistocene and Holocene

Maciej Sykut[a]*, Sławomira Pawełczyk[b], Natalia Piotrowska[b], Krzysztof Stefaniak[c], Bogdan Ridush[d], Daniel Makowiecki[e], Pavel Kosintsev[f,g], Barbara Wilkens[h], Tomasz Borowik[a], Ralph Fyfe[i], Jessie Woodbridge[i], Magdalena Niedziałkowska[a]*

* corresponding authors: msykut@ibs.bialowieza.pl; mniedz@ibs.bialowieza.pl

[a]Mammal Research Institute, Polish Academy of Sciences, Stoczek 1c, 17-230 Białowieża, Poland

[b]Division of Geochronology and Environmental Isotopes, Institute of Physics – CSE, Silesian University of Technology, Konarskiego22 B, 44-100 Gliwice, Poland.

[c]Department of Palaeozoology, University of Wrocław, Sienkiewicza 21, 50-335 Wrocław, Poland

[d]Department of Physical Geography, Geomorphology and Paleogeography, Yuriy Fedkovych Chernivtsi National University, Kotsubynskogo 2, Chernivtsi 58012, Ukraine

[e]Nicolaus Copernicus University, Institute of Archaeology, Department of Historical Sciences, Szosa Bydgoska 44/48, 87-100 Toruń, Poland

[f]Institute of Plant and Animal Ecology, Ural Branch of the Russian Academy of Sciences, 8 Marta 202, Yekaterinburg 620144, Russia

[g]Ural Federal University, Prospekt Lenina, 51, Ekaterinburg, 620002, Russia

[h]Independent researcher, Alghero, Italy

[i]School of Geography, Earth and Environmental Sciences, University of Plymouth, Plymouth, Devon PL4 8AA, UK

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Highlights

- δ^{13}C and δ^{15}N values of ancient red deer and wapiti bones have been analysed
- We showed that δ^{13}C and δ^{15}N in deer fluctuated with environmental changes in Europe
- The values of δ^{13}C and δ^{15}N of red deer and wapiti overlapped
- Forest cover and July temperature influenced δ^{13}C values within Holocene deer bones
- Temperature, precipitation and altitude shaped δ^{15}N values within Holocene deer bones

Abstract

Red deer (*Cervus elaphus*) is one of the species that is rather wide spread and survived across Europe over the Holocene. The analyses of carbon and nitrogen stable isotopes in bone collagen of ungulate remains have been applied in paleoecological studies as environmental and dietary indicators. In this study we present the carbon and nitrogen stable isotope compositions of previously radiocarbon-dated red deer bone samples (N = 68) found in Central, Southern and Eastern Europe and Asia and aligned to one of two species: European red deer (*Cervus elaphus*) and wapiti (*Cervus canadensis*). We showed that the values of carbon and nitrogen stable isotope ratios of European red deer and wapiti overlapped. Among all analyzed independent factors (determined for the locality and time period relevant for each of the analysed samples), the variability of δ^{13}C values in European red deer dated to the Holocene is best explained by forest cover and mean July temperature, and variability of δ^{15}N values by the mean July temperature, annual precipitation and altitude. Additionally, combining the results of the present study with isotopic data on European red deer collected from published sources, we revealed that the values of δ^{13}C and of δ^{15}N in *C. elaphus* bones changed according to environmental oscillations that took place in Europe over the last 50 000 years. We concluded that red deer shifted their feeding habitats in relation to changing environmental conditions, for example, forest expansion during the climate warming, and in the mid to later Holocene in response to deforestation caused by human activity and the spread of agriculture. We also found out that red deer reacted in varied ways to changing local conditions in

47 different regions of Europe. Modern individuals of *C. elaphus* had the lowest $\delta^{13}C$ values among all
48 analysed specimens, so they probably inhabited the most densely forested areas in comparison to
49 other European red deer populations during the last 50 000 years.

50 1. **Introduction**

51 Red deer (*Cervus elaphus*) *sensu lato (s.l.)* is one of the most widely distributed ungulate
52 species in the Holarctic (Geist, 1998; Milner *et al.*, 2006; Apollonio *et al.*, 2010). It probably
53 evolved in central Asia about 7 million years ago (mya) (Ludt *et al.*, 2004; Pitra *et al.*, 2004) during
54 the spread of grasses over large areas of Eurasia (Cerling *et al.*, 1997). In the late Early Pleistocene,
55 red deer *s.l.* appeared in southwestern Siberia (Alekseeva, 1980; Foronova, 1999, 2001) and during
56 the mid-Pleistocene in Europe (van der Made *et al.*, 2014; Stefaniak, 2015; van der Made &
57 Dimitrijević, 2015). About 15 000 years (15 ka) ago the species' range expanded to North America
58 via the Bering Strait (Meiri *et al.*, 2018).

59 In accordance with current taxonomic consensus, red deer *s.l.* comprises three species:
60 European/West Asian red deer (*C. elaphus* Linnaeus, 1758), Central Asian red deer (*C. hanglu*
61 Wagner, 1844) and East Asian/North American wapiti or elk (*C. canadensis* Erxleben, 1777)
62 (Lorenzini & Garofalo, 2015; Meiri *et al.*, 2018). Hereafter, the term red deer *s.l.* is used when we
63 refer to individuals of both species: red deer and wapiti, or we were unable to assign the individual
64 to particular species.

65 During the last 50 ka, the range of red deer *s.l.* changed in response to climate oscillations,
66 contracting during colder period and expanding during warmer episodes (Sommer *et al.*, 2008;
67 Meiri *et al.*, 2013; Doan *et al.*, 2022; Niedziałkowska *et al.*, 2021). In the Late Pleistocene (54.0 ka
68 – 34.0 ka before present (BP)), red deer *s.l.* occurred across almost the entire Europe from the
69 Atlantic coast to the Urals. Due to the climatic cooling (33.0 – 26.5 ka BP), the range of the species
70 shrunk reaching its minimum during the Last Glacial Maximum (LGM, 26.5 – 20.0 ka BP) (Clark
71 *et al.*, 2009). Within this time period, red deer *s.l.* are likely to have survived not only on the

Iberian, Apennine and Balkan Peninsulas (Skog *et al.*, 2009; Sommer & Zachos, 2009) but also, as recent studies indicated, in western Europe, the Carpathians, surroundings of the Black Sea and the Urals (Queiros *et al.*, 2019; Niedziałkowska *et al.*, 2021). After the LGM when the climate became warmer, red deer *s.l.* populations recolonized the north and north-eastern parts of the continent (Sommer *et al.*, 2008; Niedziałkowska *et al.*, 2021).

In the Late Pleistocene (between approximately 50 to 26 ka BP) the ranges of *C. elaphus* (hereafter called "red deer") and *C. canadensis* (hereafter called "wapiti") partly overlapped in southeastern Europe and in the Urals (Stankovic *et al.*, 2011; Meiri *et al.*, 2018; Doan *et al.*, 2022). During this time, the wapiti inhabited vast areas of Eurasia from present-day Romania to northeastern Asia until the LGM (Doan *et al.*, 2022) or for even longer (Croitor & Obada, 2018). After the LGM, when the climate became warmer, their range moved to the east and it was limited to Asia. During the last 4 ka BP, *C. canadensis* disappeared from the Urals, western and northeastern Siberia (Doan *et al.*, 2022).

Cervus elaphus is a savanna-type deer with a mixed feeding strategy (Geist, 1998). Based on mitochondrial DNA (mtDNA) studies four or five main haplogroups of extant red deer (called A-E) have been identified (Ludt *et al.*, 2004; Skog *et al.*, 2009; Doan *et al.*, 2021). *Cervus canadensis* is a more cold-adapted open-country grazer which inhabits dry, cold, continental regions (Geist, 1998). Within wapiti, three main haplogroups (called X, Y and Z) have been described (Doan *et al.*, 2022). Modern *C. elaphus* and *C. canadensis* inhabit geographically separate areas and their ecological niches are slightly different (Geist, 1998; Brook *et al.*, 2018; Lovari *et al.*, 2018). In present time red deer are widely distributed throughout most of Europe except northern Fennoscandia and large areas of the European part of Russia (Lovari *et al.*, 2018). Modern wapiti occurs from the Tian-Shan and the Altai Mountains to the Far East including mountainous areas and lowland boreal forests (Stepanova, 2010; Brook *et al.*, 2018). Nowadays, due to climate warming, the wapiti has been recolonizing eastern Siberia (Stepanova, 2010).

Since the Late Pleistocene red deer *s.l.* occurrence in Europe was mostly associated with forest biomes (Niedziałkowska *et al.*, 2021). However, in the last 4 ka BP, the proportion of forests in red deer habitats decreased significantly (Niedziałkowska *et al.*, 2021) as a result of human-induced deforestation in Europe (Fyfe *et al.*, 2015; Roberts *et al.*, 2018). It is believed that forest is the most suitable habitat for contemporary red deer (Borowik *et al.*, 2013), although both species: red deer and wapiti occur in forest as well as in upland moors and open mountainous areas (Clutton-Brock & Albon, 1989; Mattioli, 2011). Also studies of the Pleistocene specimens (described by the authors as *C. elaphus*) from Western Europe showed that the species could have inhabited both forested and open areas as well (Saarinen *et al.*, 2016). The diet of red deer *s.l.* in Europe (Gebert & Verheyden-Tixier, 2001) and Asia (Chen *et al.*, 1998; Ohtsu & Takatsuki, 2021) may contain eatable parts of tree and shrubs as well as grasses and sedges. According to analyses of diet of red deer inhabiting different habitats in Europe, the main six major food items of the species were Calluna and Vaccinium, conifers, twigs and bark, leaves of deciduous trees, Rubus, forbs, seeds and fruits, which represented 59% of the diet. The variation in these food components was associated with habitat types. The only food items, which differ seasonally were seeds and fruits. Grass and sedges represented 29% of European red deer diet and their content did not varied between habitats and seasons (Gebert & Verheyden-Tixier, 2001). Key plant species for *C. elaphus* in moorland were Calluna and Vaccinium, in mixed-coniferous forests Calluna and Vaccinium and coniferous browse and in mixed-deciduous forests fruits, leaves of deciduous trees and shrubs, twigs and bark (Gebert & Verheyden-Tixier, 2001).

The analyses of carbon and nitrogen stable isotopes in bone collagen of ungulate remains have been applied in paleoecological studies as environmental and dietary indicators (e.g. Drucker *et al.*, 2003a; Drucker & Bocherens, 2009; Bocherens *et al.*, 2015). The isotope signatures of food are transferred up the food chain to animals and are recorded in their tissues (Ambrose & Norr, 1993). However, carbon and nitrogen stable isotopic compositions of plants can be affected by environmental factors (i.e. precipitation, temperature, salinity, altitude, forest cover) (Heaton, 1999;

Zhu *et al.*, 2010; Giroux *et al.*, 2015; Liu *et al.*, 2017). The combination of some of them leads to a phenomenon known as "canopy effect"- change in $\delta^{13}C$ values along a vertical gradient of forest trees and plants, with ^{13}C-enriched stable isotope values in the plants at the top of the canopy and ^{13}C-depleted plants on the forest floor. The "canopy effect" is thereby reflected in lower $\delta^{13}C$ values of plants growing under the canopy of dense forest stands compared with those grown in non-forested habitats (e.g. Van der Merwe & Medina, 1991; Stevens *et al.*, 2004; Drucker *et al.*, 2008; Bonafini *et al.*, 2013). The forest cover best explained the variability of $\delta^{13}C$ values in modern ungulates such as red deer, European bison (*Bison bonasus*) and moose (*Alces alces*) as well as in ancient cervids and large bovines (Drucker *et al.* 2008, Hofman-Kamińska *et al.*, 2018; Sykut *et al.*, 2021). Relatively low $\delta^{13}C$ values need to make us consider "canopy effect" and therefore, it is possible to distinguish if the animals used to feed in forested or more open habitats (Drucker *et al.*, 2003a; Sykut *et al.*, 2021). Furthermore, $\delta^{15}N$ values also differ among groups of plants, for instance, grasses, sedges, and forbs represent higher $\delta^{15}N$ values than shrubs and trees (Michelsen 1996, 1998; Amundson 2003). This allows grazing and browsing herbivore species to be distinguished (Drucker *et al.*, 2003a). Additionally, studies of modern red deer showed that the variability of $\delta^{15}N$ values of bone collagen are best explained by the percent of open area representing their food source. This is due to the fact that open areas are covered by plants with a higher $\delta^{15}N$ values e.g. grasses and thus $\delta^{15}N$ values can be also used as a proxy of habitat: closed (forested) or more open habitats (Sykut *et al.*, 2021). The $\delta^{15}N$ values of plants are associated with several different environmental factors such as local nitrogen soil pools, aridity levels, the mean annual temperature (Stevens *et al.*, 2006; Drucker *et al.*, 2011; Bocherens *et al.*, 2014).

Based on the conclusions of the previous studies, we assume that the past feeding habitats of red deer *s.l.* have been reflected in their bone collagen. As the range of the species changed in response to climate oscillations, we hypothesize that habitats, where the individuals of red deer *s.l.* used to forage, varied among different time periods and regions in Eurasia. We predict wapiti as open country grazer represents lower $\delta^{13}C$ and higher $\delta^{15}N$ values than red deer – mixed feeder.

Despite the unequal number of analysed red deer and wapiti samples, we expect that the values of $\delta^{13}C$ and $\delta^{15}N$ obtained for both deer lineages will not overlap. Due to the fact that the studied red deer and wapiti belonged to several mtDNA haplogroups inhabited various geographic areas (comp. Doan *et al*., 2022), it is possible that they fed also in different habitats and various isotopic signals will be obtained for individuals representing each of them. Furthermore, we hypothesise that environmental variables such as percentage of forest cover, temperature, precipitation and altitude will explain variability of $\delta^{13}C$ and $\delta^{15}N$ in European red deer during the Holocene. Finally, also human induced large-scale deforestation of Europe during the last 4 ka BP (Fyfe *et al.*, 2015) leading to red deer ecological niche transition can be reflected in changes of the isotopic composition of their bone collagen. We expected the increase of $\delta^{13}C$ and decrease of $\delta^{15}N$ values over that time period.

This paper seeks to: (a) reveal the feeding habitats of red deer in the Late Pleistocene and the Holocene in Europe, (b) investigate whether these habitats varied in time and space and if there are differences in $\delta^{13}C$ and $\delta^{15}N$ values among species and haplogroups within the species, (c) identify environmental variables that best explain variance in $\delta^{13}C$ and $\delta^{15}N$ values in European red deer in the Holocene.

2. Materials and methods

2.1. Sampling

Red deer (*s.l.*) subfossil fragments of bones were compiled from zoological and archaeological collections in Europe and Asia in agreement with the collection owners. The ancient sample set analysed in this study (N = 68, Tab. S1) covers northeastern Europe, the Carpathian Mountains region, the Eastern Alps, Italy and Corsica and southeastern Europe as well as the Ural Mountains, Eastern and Western Siberia from 41°W to 131° E (Fig. 1) and extends from the Late Pleistocene (48 ka cal BP) until historical times (ca. 200 cal BP). Species identification was based on comparative macroscopic and morphometric analyses and confirmed by sequencing of

cytochrome *b* of mitochondrial DNA (mtDNA), see Doan *et al.* (2017) for details. The results of genetic analyses (1131 bp long fragments of cytochrome *b* sequences) allowed us to assign the samples to one of two red deer species: European red deer (*Cervus elaphus*) and wapiti deer (*Cervus canadensis*) and further *C. elaphus* specimens into mtDNA haplogroups A-F, and *C. canadensis* specimens into mtDNA haplogroups X, Y and Z (Tab. S1; for more details see Doan *et al*., 2022).

The bone samples of red deer *s. l.* specimens were radiocarbon-dated using accelerator mass spectrometry (AMS) at the Division of Geochronology and Environmental Isotopes at the Institute of Physics, Silesian University of Technology (Gliwice, Poland). Dates used in this study have been published by Niedziałkowska *et al*. (2021) (50 samples), Doan *et al*. (2022) (10 samples), and Doan *et al*. (2017) (8 samples) (Tab. S1).

The literature searches were performed to include into the analyses additional isotopic data of red deer specimens dated to the Late Pleistocene and the Holocene from other regions of Europe: the Cantabria Mountains in Spain (Castaños *et al.*, 2014; Stevens *et al.*, 2014; Rofes *et al.*, 2015; Jones *et al.*, 2018, 2019, 2020), southwestern France (Bocherens *et al.*, 2014), northern France (Drucker *et al.*, 2020), the French Jura (Drucker *et al.*, 2011), the Western Alps in France (Drucker *et al.*, 2011), Sicily and southern Italy (Craig *et al.*, 2010; Mannino *et al.*, 2011a, 2011b; Di Maida *et al.*, 2019), (Fig. 1, Tab. S2). Data from the literature was generated using key word searches ("*stable isotope*" and "*deer*") in the Web of Science (Clarivate Analytics). We used records that have been radiocarbon-dated either directly or indirectly, i.e. dates derived from charcoal, humus or bones of other animals found in the same layer as red deer fossils. We did not include in our analyses localities, with less than 10 red deer records. Samples from the literature without radiocarbon-dates or without given collagen quality criteria (%C, %N, C/N ratio) were excluded from the analysis.

Stable isotopic data of modern European red deer samples were taken from Sykut *et al.*, (2021). We randomly selected 49 samples (Tab. S3) from the entire set of modern samples (n =

242). The number of modern samples has been matched, so that they were not overrepresented in the whole data set. These samples were collected from various habitats in Poland, Scotland, the Netherlands and Slovenia: (1) large woodlands, (2) mosaic of meadows, arable grounds and forest areas, and (3) grasslands (Fig. S1). The details concerning those habitats has been described in S1Table in Sykut *et al*. (2021). Due to anthropogenic CO_2 emissions, the $\delta^{13}C$ values of modern samples have been corrected for the shift in $\delta^{13}C$ values according to the formula proposed by Feng (1998) and provided in Sykut *et al.*, (2021).

All radiocarbon dates (obtained in the frame of this study and from the literature) were calibrated using OxCal v. 4.2 (Bronk Ramsey, 2009) and the IntCal20 calibration curve (Reimer *et al.*, 2020). Hereafter, the dates are provided as cal BP, i.e. calibrated age in years before AD 1950, using medians of the calibrated ages.

2.2. Sample preparation and analysis

The collected bone samples (approximately 1 g) were cleaned in an ultrasonic bath in demineralized water, then dried and ground in a ball mill. The powdered bone was demineralized in 0.5 M hydrochloric acid at room temperature in a glass vial. The acid was replaced several times, and the reaction was considered complete when pH stabilized at < 1 and no bubbles were observed. The insoluble residue was rinsed with demineralized water to neutral pH (Piotrowska & Goslar, 2002). The bone collagen was extracted according to the classical Longin method (Longin, 1971) with modification applied in the Gliwice Radiocarbon Laboratory (Piotrowska & Goslar, 2002). Gelatinization was performed as follows: the residue was acidified and maintained at 80°C for 12 hr in an acidic solution (HCl, pH = 3). The obtained supernatant was centrifuged, transferred to a glass vial and dried in an oven at 75°C. The subsample of gelatin was subjected to graphite preparation using an AGE-3 system equipped with a VarioMicroCube by elemental analyzer and automated graphitization unit (Němec *et al.*, 2010; Wacker *et al.*, 2010). The ^{14}C concentrations in graphite produced from blank samples, Oxalic Acid II standards, and coal blanks have been measured by the Direct AMS laboratory, Bothell, USA (Zoppi *et al.*, 2007; Zoppi, 2010). Another gelatin subsample

was assigned for analysis of carbon and nitrogen stable isotope composition ($\delta^{13}C$, $\delta^{15}N$), %C, %N, and C/N_{at}. The dried collagen was weighed into tin capsules. Three subsamples of each collagen sample were prepared for the measurements. The elemental and isotopic measurements were performed at the Division of Geochronology and Environmental Isotopes at the Institute of Physics, Silesian University of Technology (Gliwice, Poland) using an IsoPrime EA-CF-IRMS continuous flow isotope ratio mass spectrometer connected to the EuroVector elemental analyser. The obtained carbon and nitrogen isotope measurements were calibrated to VPDB and AIR standards, respectively (Coplen *et al.* 2006, Mariotti 1983). The stable isotope values were expressed in the isotope delta (δ) notation as follows:

‰)

and

‰)

The $\delta^{13}C$ and $\delta^{15}N$ values are presented in units of part per thousand and communicated in per mil shown as ‰ (Brand & Coplen, 2012). Samples of collagen were routinely calibrated to international standards. The $\delta^{13}C$ values were calibrated to values of IAEA–C8 ($\delta^{13}C$ = -18.31‰) and IAEA–C5 ($\delta^{13}C$ = -25.49‰). The $\delta^{15}N$ values are calibrated to values of IAEA–NO3 ($\delta^{15}N$ = 4.7‰) and IAEA–USGS34 ($\delta^{15}N$ = -1.8‰). C/N elemental ratio values were calibrated to values of UREA (elemental composition: C – 20%, H – 6,71%, N – 46,65% and O – 26,64%). The precision of these methods is lower or equal to 0.1‰ for $\delta^{13}C$ and 0.2‰ for $\delta^{15}N$. Samples with quality collagen extracts similar to those obtained from fresh bone (%C > 10, %N > 3, $2.9 \leq C/N \leq 3.6$) were considered a reliable source of isotopic signatures (DeNiro, 1985; Ambrose, 1990) and were used for further analyses. For samples with atomic C/N ratios above 3.6, which may indicate extraneous carbon contamination from humin acids, we applied NaOH treatment and ultrafiltration and again tested the collagen quality.

2.3. Climatic and environmental analyses

The climatic and biome data for each of the analysed samples have been taken from Niedziałkowska *et al.* (2021) and are presented in Table S1. These data (mean annual, mean January, mean July temperatures, annual, January and July precipitation, biome) were obtained from the FAMOUS database (FAst Met. Office and UK Universities Simulator) available online (Smith & Gregory, 2012) as described in Niedziałkowska *et al*. (2021). For the requirements of statistical analyses (to have enough number of samples in different biome categories), we merged the biomes from FAMOUS database into the four following categories: Forest (Cool conifer, Deciduous taiga/montane and Warm mixed forest), Mixed (Temperate xerophytic shrub and Temperate sclerophyll woodland), Open (Steppe tundra, Shrub tundra and Prostrate shrub tundra) and Desert.

Information on the relative proportion of forest cover in the European sites, where the deer samples dated to the Holocene (11– 0 ka BP) were recorded, was obtained from a pollen-inferred land cover change database after Fyfe *et al.* (2015) and the values for each of the studied samples have been taken from Niedziałkowska *et al.* (2021). This database contains pan-European land cover classification for the last 11 ka BP years at 200-year temporal resolution and was created as described in Niedziałkowska *et al.* (2021). The proportion of forest cover for modern sample locations was estimated as described in Sykut *et al.* (2021) based on CORINE Land Cover maps using ArcGIS 10.3.1 (ESRI, 2015) software.

2.4. Statistical analyses

The entire data set was divided based on the age of the samples: before the LGM and after the LGM (the Holocene) due to significantly different environmental and climatic conditions during these two periods (Markova *et al.*, 2008). Samples dated to the period before the LGM (47 857 – 26 813 cal BP) were genetically assigned to wapiti deer (n = 16) and European red deer (n = 4). The sample set dated to the period after the LGM (9 508 – 189 cal BP) consisted mainly of European red deer (n = 48) and only three samples were assigned to wapiti deer.

We tested the relationship between isotopic composition ($\delta^{13}C$, $\delta^{15}N$) and the age of the samples separately for the two time periods (before and after the LGM) using Pearson correlation. Due to low sample numbers and the different ecological niches of the two deer species (Geist, 1998), we excluded European red deer from the statistical analyses of samples dated to the time period before the LGM and wapiti deer from the analyses of samples dated to the period after the LGM.

To analyze changes in $\delta^{13}C$ and $\delta^{15}N$ values in *C. elaphus* bones during the last 50 ka cal BP, we combined isotopic data presented in this study with the literature data on ancient and modern European red deer. Data obtained in this study (n = 49) were divided according to the regions where the analysed ancient sample were recorded as follows: the Carpathian Mountains region (n = 12), the southeastern Europe (n = 14), the northeastern Europe (n = 14), Italy (including Sardinia) and Corsica (n = 8) and the Eastern Alps (n = 1), (Tab. S1). The data on ancient samples from the literature (n = 440) were divided as follows: the Cantabria Mountains (n = 292), the French Jura (n = 61), the Western Alps (n = 19), northern France (n = 13), southwestern France (n = 30) and Sicily and southern Italy (n = 25) (Tab. S2). For each of the regions, except the Eastern Alps (only one sample), we checked with regression analysis if $\delta^{13}C$ and $\delta^{15}N$ values of ancient samples had been significantly changing over time.

Furthermore, we used GAM (Generalized Additive Model) analysis to illustrate how the $\delta^{13}C$ and $\delta^{15}N$ values have been changing during the last 50 ka cal BP in all studied individuals of European red deer (ancient and modern) treated as one group. The GAM analyses were performed using the *mgcv* package (Wood, 2017) implemented in R version 4.0.2 (R Development Core Team, 2018).

The relationship between $\delta^{13}C$ and $\delta^{15}N$ and forest cover was analyzed separately for 41 ancient samples dated to the Holocene (9508 – 189 cal BP) (Tab. S4) and 49 modern samples (Tab. S3) in R version 4.0.2 (R Development Core Team, 2018).

Normal linear models (NLM) with a Gaussian error structure were used to test associations between the stable isotope composition (δ^{13}C or δ^{15}N) of 39 European red deer samples dated to the Holocene (9508 – 189 cal BP) and the following variables: median of calibrated age BP (hereafter called median cal BP), mtDNA haplogroup of the analysed samples, annual mean temperature, January mean temperature, July mean temperature, annual precipitation, January precipitation, July precipitation, altitude, biome, percentage of forest cover, where the analysed samples were recorded in certain time periods. Longitude and latitude were not included in the set of variables due to the low informative value of including this information and high autocorrelation with other environmental variables (Tab. S5). The median calibrated age BP of samples was included as a covariate to correct for the effect of the time period on the results of modelling. Due to the low number of samples each model consisted of one variable and the covariate. The homoscedasticity in distribution of final model residuals was checked by visual inspection of plots presenting model residuals against fitted values (estimated responses). Due to the lack of forest cover data for the samples dated to the Late Pleistocene, these samples were not included in these analyses. We also excluded the only red deer sample assigned to the mtDNA haplogroup D from these analyses due to the lack of group equality. We ran separate models with δ^{13}C and δ^{15}N values as the response variables. All LM models were performed using the *lmerTest* package (Kuznetsova *et al.*, 2015) implemented in R version 4.0.2 (R Development Core Team, 2018).

To test which set of variables best explained the observed variance in δ^{13}C and δ^{15}N values, we created two sets (one for δ^{13}C and one for δ^{15}N) of competing models. Next, the competing models were ranked with the Akaike Information Criterion (AIC) with the second-order correction for a small sample size (AICc) (Burnham & Anderson, 2002) using the *MuMin* package (Bartoń, 2013) implemented in R version 4.0.2 (R Development Core Team, 2018). All models close to the top performing model (lowest AICc), having ΔAIC < 2, were considered to have substantial empirical support. For the top models (or equal to the top) we created plots using the *effect* package (Fox & Weisberg, 2019) implemented in R version 4.0.2 (R Development Core Team, 2018).

3. Results

Variability of $\delta^{13}C$ and $\delta^{15}N$ in wapiti and red deer before the LGM

The set of samples within this study dated to the period before the LGM comprised of European red deer bones (n = 4) from Central and Southern Europe (from 19° to 34° E) and wapiti deer bones (n = 16) from Eastern Europe and Asia (from 52° to 131° E). The median age of the samples ranged from 47 857 to 26 813 cal BP. The values of $\delta^{13}C$ ranged from -21.2 to -18.6‰, and $\delta^{15}N$ from 4.5 to 10.3‰ (Fig.2 upper and lower panels). Due to the low number of European red deer samples dated to the period before the LGM, the correlation between $\delta^{13}C$, $\delta^{15}N$ and the median cal age of these samples were not tested. In wapiti samples no correlation of sample age (median cal BP) was observed with either $\delta^{13}C$ or $\delta^{15}N$ (Fig. 2 upper and lower panels). There was also no relationship between $\delta^{13}C$ and $\delta^{15}N$ values (Fig. S2 upper panel). The range of $\delta^{13}C$ and $\delta^{15}N$ values (-21.2 to -18.6‰ and 4.5 to 10.3‰ respectively) obtained from samples from the forest biome (n = 8) overlapped with the range of values obtained from samples from open (n = 7) and mixed biomes (n = 1) (Fig. S2 upper panel). Additionally, the values of $\delta^{13}C$ and $\delta^{15}N$ of red deer samples from mixed (n = 1) and forest (n = 3) biomes were in the range of these values for wapiti deer (Fig. 2 upper and lower panels).

Variability of $\delta^{13}C$ and $\delta^{15}N$ in European red deer and wapiti in the Holocene

The set of samples dated to the period after the LGM (the Holocene) comprised of European red deer bones (n = 45) from Central and Southern Europe (from 8° to 48° E) and wapiti bones (n = 3) from Asia (from 60° to 105° E). The median age of the samples ranged from 9508 to 189 cal BP. The values of $\delta^{13}C$ ranged from -23.3 to -19.3‰, and $\delta^{15}N$ from 2.9 to 10.6‰, (Fig. 3 upper and lower panels). In the case of the European red deer samples, no correlation was observed between $\delta^{13}C$ and the age of the samples (median cal years BP) (Fig. 3 upper panel), while the correlation between $\delta^{15}N$ and the age of the samples (median cal BP) was positive and statistically significant ($r = 0.5$, $p < 0.001$, Fig. 3 lower panel). The $\delta^{15}N$ values decreased with the median age. The

359 correlation between $\delta^{13}C$ and $\delta^{15}N$ values of red deer samples was positive and statistically
360 significant (r = 0.35, p = 0.02, Fig. S2 lower panel). Due to the low number of wapiti samples dated
361 after the LGM, the correlations between $\delta^{13}C$, $\delta^{15}N$ and age were not tested. The range of $\delta^{13}C$ and
362 $\delta^{15}N$ values (-23.2 to -19.3‰ and 2.9 to 10.6‰ respectively) of samples from the forest biome (n =
363 35) overlapped with the ranges of values obtained from red deer inhabiting the mixed biome (n =
364 10). Additionally, the values of $\delta^{13}C$ and $\delta^{15}N$ of wapiti samples from the forest biome (n = 3) were
365 in the range of values obtained for red deer (Fig. 3 upper and lower panels).

366 **Chronological changes in $\delta^{13}C$ and $\delta^{15}N$ of the European red deer bones during the last 50 ka**
367 **cal BP**

368 The relationship between $\delta^{13}C$ and $\delta^{15}N$ values and the median age of European red deer
369 samples (data sets from other studies are also included) varied depending on locality and time.
370 Among the isotopic results, the $\delta^{13}C$ values of samples from Cantabria (dated to 49.2 – 4.0 ka cal
371 BP), northern France (11.4 – 5.0 ka cal BP), the French Jura (14.2 – 6.8 ka cal BP), and Italy and
372 Corsica (9.5 – 0.6 ka cal BP) were positively correlated with median age (Tab. S6). The $\delta^{13}C$ values
373 decreased from older to more recent time periods (Fig. 4 upper panel).

374 The $\delta^{15}N$ values of samples from Cantabria (49.2 – 4.0 ka cal BP) and the Carpathian
375 Mountains region (6.0 – 0.2 ka cal BP) were positively correlated with median age and higher
376 values were recorded for samples dated to the older time periods (Fig. 4 lower panel, Tab. S6).
377 Negative correlations were recorded between $\delta^{15}N$ values and the median age of samples from
378 northern France (11.4 – 5.0 cal BP), the French Jura (14.2 – 6.8 ka cal BP) and Sicily and southern
379 Italy (18.5 – 9.3 ka cal BP). The $\delta^{15}N$ values of samples increased between older and younger time
380 periods (Fig. 4 lower panel, Tab. S6).

381 The $\delta^{13}C$ values of the European deer samples (all data sets pooled together) dated to the
382 period from about 50.0 to 14.0 ka cal BP oscillated mostly between -20 and -21‰. Over this time
383 period, mean $\delta^{13}C$ value hardly changed. Between 14.0 and 7.7 ka cal BP $\delta^{13}C$ mean value
384 decreased from -20.4 to -21.7‰. Over the period from 7.7 to 5.3 ka cal BP, mean $\delta^{13}C$ value hardly

385 changed and from 5.3 ka cal BP until modern times decreased from -21.7‰ to
386 -22.4‰ (Fig. 5 upper panel, Tables S1–S3).

387 The δ^{15}N values of the European deer samples (all data sets pooled together) for the period
388 from about 50.0 to 33.0 ka cal BP varied and ranged between 9.2 and 1.0‰. However, over this
389 period mean δ^{15}N value hardly changed. Between 33.0 and 15.5 ka cal BP the mean δ^{15}N value
390 decreased from 5.1 to 3.0‰. From 15.5 to 6.0 ka cal BP mean δ^{15}N values increased from 3.0 to
391 5.6‰. Over the period from 6.0 to modern times the mean δ^{15}N value decreased from 5.6 to 4.0‰
392 (Fig. 5 lower panel, Tables S1–S3).

393 **The influence of forest cover on δ^{13}C and δ^{15}N values in ancient and modern samples**

394 Forest cover in localities of the Holocene European red deer samples from this study ranged
395 from 31 to 80%, and for modern samples from 0 to 100% (Tables S1, S3). The values of δ^{13}C and
396 δ^{15}N within Holocene samples ranged from -23.3‰ to -19.3‰ and from 2.9‰ to 10.6‰,
397 respectively. The values of δ^{13}C and δ^{15}N of modern samples ranged from -24.1‰ to -20.9‰ and
398 from 0.6‰ to 8.5‰, respectively. The percentage of forest cover was negatively associated with
399 δ^{13}C values in both modern and ancient samples (r = -0.54, p = 0.002, and r = -0.49, p = 0.001,
400 respectively) (Fig. 6 upper panel). The percentage of forest cover was not associated with δ^{15}N in
401 ancient samples (r = 0.01, p = 0.96), while in modern samples a significant negative relationship
402 was observed (r = -0.56, p = 0.001) (Fig. 6 lower panel).

403 **Environmental factors explaining the variability of δ^{13}C and δ^{15}N in European red deer in the**
404 **Holocene**

405 Modeling was performed using the 39 Holocene *C. elaphus* samples (9.5 – 0.2 ka cal BP)
406 from Europe obtained in this study. Based on the AICc criteria, the best models explaining variation
407 in δ^{13}C values in bone collagen of red deer were the top-ranked models, which consisted of the
408 following variables – (i) median age and percentage of forest cover, (ii) median age and July
409 temperature (Tab. 1). The δ^{13}C values were negatively associated with forest cover percentage
410 (slope = -3.59 ± 0.93, t = -3.85, p < 0.001). With increasing percentage forest cover from 31 to

80%, δ^{13}C values decreased from -20.9‰ to -22.6‰ (Fig. 7, upper panel). The δ^{13}C values were positively associated with mean July temperature (slope = 1.38 ± 3.56, t = 3.87, p < 0.001). With increasing mean July temperature from ca. 10 to 26°C, δ^{13}C, values increased from -23.0‰ to -20.8‰ (Fig. 7, lower panel). Percentage of forest cover was negatively correlated with mean July temperature (r = -0.80, p < 0.05) (Tab. S5).

Based on the AICc criteria, the best models explaining variation in δ^{15}N values in bone collagen of red deer were the top-ranked models, which consisted of the following variables – (i) median age and mean July temperature, (ii) median age and annual precipitation, (iii) median age and altitude, (iv) median age and mean July precipitation, (v) median age and mean annual temperature (Tab. 2). However, models (iv) and (v) were not statistically significant (p = 0.125 and p = 0.129 respectively). The δ^{15}N values were positively associated with mean July temperature (slope = 0.12 ± 0.06, t = 2.07, p = 0.046). With increasing mean July temperature from ca. 10 to 26°C, δ^{15}N values increased from 4.1‰ to 5.9‰ (Fig. 8, upper panel). The δ^{15}N values were negatively associated with the annual precipitation (slope = -0.002 ± 0.001, t = -2.01, p = 0.052). With increasing annual precipitation from ca. 450 to 1500 mm, δ^{15}N values decreased from 5.7‰ to 3.5‰ (Fig. 8, upper panel). The δ^{15}N values tended to be negatively associated with the altitude (slope = -0.001 ± 0.001, t = -1.78, p = 0.084). With increasing altitude from ca. -100 to 1650 m, δ^{15}N values decreased from 5.6‰ to 3.6‰ (Fig. 8, lower panel). The decrease in δ^{15}N values per 1000 m amounted *c.* 1.14‰. Annual precipitation is negatively correlated with mean July temperature (r = -0.62, p < 0.05) and positively correlated with altitude (r = 0.53, p < 0.05) (Tab. S5). Mean July temperature is negatively correlated with altitude (r = -0.26, p < 0.05) (Tab. S5).

4. Discussion

Within the results of isotopic analyses of red deer samples from the present study and available in the literature, dated from 50.0 to 14.0 ka cal BP, δ^{13}C values were no lower than −22.5‰. Values lower than −22.5‰ can indicate the presence of the ‘canopy effect’ as indicated in

studies of ungulates inhabiting temporal and boreal ecosystems (Drucker *et al.*, 2008; Bocherens *et al.*, 2015, Sykut *et al.* 2021). So the obtained results revealed that during this time period the analysed individuals mainly fed on plants growing in more open habitats. However, the set of samples from this long period mostly came from the Cantabria region (Spain) and Southwestern France. Low variability of δ^{13}C values throughout this period indicate a lack of extreme changes in climate and vegetation in this region (Jones & Britton, 2019). A decrease in δ^{13}C values in the following postglacial period (14.0 – 6.4 ka cal BP) most likely corresponded to a climate-induced (Sommer, 2020) habitat shift from open or mixed areas to closed and densely forested habitats (Drucker *et al.*, 2011). Such decrease of δ^{13}C values in this time period was indicated also in local populations (Fig. 4) e.g. in red deer inhabiting French Jura, (Drucker *et al.*, 2003a) and the Northern France (Drucker *et al.*, 2020). It is in agreement with larger scale studies which indicated that red deer (*s.l.*) in Europe and the Urals inhabited open and mixed areas during the Late Pleistocene, while during the Holocene their habitats shifted from open and mixed areas to forests (Niedziałkowska *et al.*, 2021). The lowest values in this postglacial period were observed during the Holocene climatic optimum, which is coherent with the greatest extent of forested areas dated between 8.5 and 6.0 ka cal BP (Zanon *et al.*, 2018). After the Holocene climatic optimum, an increase in human activity was observed (Puhe & Ulrich, 2001; Gignoux *et al.*, 2011). A slight increase in red deer δ^{13}C values during the period from 6.4 to 3.6 ka cal BP may reflect environmental changes induced by the development of agriculture and demographic expansion at that time (Puhe & Ulrich, 2001). Archaeological studies revealed that agricultural practices and further population growth spread quickly across the Mediterranean regions of Europe (Gignoux *et al.*, 2011). The development of agriculture and demographic expansion resulted in increasing deforestation of European landscapes (Anderson *et al.*, 2007; Marquer *et al.*, 2017; Roberts *et al.*, 2018). In the last analysed period (from 3.6 ka cal BP to modern times) further decline in red deer δ^{13}C values was observed. In this time period deforestation of Europe, associated with human activities, intensified (Fyfe *et al.*, 2015). The observed decrease in δ^{13}C values of red deer bones can

463 be explained by a shift of the species' habitat to more forested areas to avoid human pressure
464 related to the spread of agriculture and increasing hunting activity (Lone *et al.*, 2015; Dixon *et al.*,
465 2021). A similar pattern of $\delta^{13}C$ changes were observed in the Holocene in other large ungulate
466 species, such as the European bison, aurochs (*Bos primigenius*), European moose (Hofman-
467 Kamińska *et al.*, 2019) and horse (*Equus ferus* and *Equus caballus)* (Stevens & Hedges 2004).
468 However, the explanation of those changes has been under debated. Stevens & Hedges (2004)
469 believed the faunal $\delta^{13}C$ mainly reflected changing plant $\delta^{13}C$ values due to an increase in
470 atmospheric CO_2 concentration, while Hofman-Kamińska *et al.* (2019) argued that variation of $\delta^{13}C$
471 values corresponded to a shift in foraging habitats and diet of large herbivores. The $\delta^{13}C$ values in
472 bones of contemporary red deer are generally lower among those dated to earlier time periods.
473 Modern red deer populations have probably inhabited the most densely forested areas in
474 comparison to populations of this species living in Europe over the last 50 ka. Nowadays red deer
475 may inhabit forest as a 'refuge areas' more often than before to avoid increasing human pressure, as
476 it was indicated for other large ungulate species occurring in contemporary times e.g. European
477 bison (Kerley *et al*., 2012).
478 As more factors influence bone nitrogen than carbon isotopic composition, we observed
479 higher variability in $\delta^{15}N$ values than $\delta^{13}C$ values in red deer bones during the last 50 ka cal BP.
480 Temperature, aridity, soil maturity, distance to the sea or type of consumed plants are the
481 parameters that drive significant changes in $\delta^{15}N$ values in herbivores (e.g. Iacumin *et al.*, 2000;
482 Drucker *et al.*, 2003a; Sykut *et al.*, 2021). The $\delta^{15}N$ values of red deer samples dated between
483 50 and 40 ka cal BP mostly from Cantabria region indicated, in agreement with the results of pollen
484 based analyses (Jones & Britton, 2019 and references therein) and carbon isotopic composition
485 analyses (this study), that the studied individuals used to feed in open steppe areas. An increase in
486 $\delta^{15}N$ values during the following period, with the maximum values obtained for samples dated to
487 about 40 – 35 ka cal BP, was observed in red deer from Cantabria and southwestern France as well
488 as in other large ungulate species such as reindeer (*Rangifer tarandus*), large bovine (*Bos*

primigenius or *Bison priscus*), and horse (*Equus ferus*) inhabiting southwestern France (Bocherens *et al.*, 2014). The ^{15}N enrichment in samples from this period is interpreted as a consequence of increasing aridity (Bocherens *et al.*, 2014). Subsequently, a significant decrease of red deer δ^{15}N values was observed after 26 ka cal BP, reaching the lowest values during the LGM. A similar pattern was observed in horse and reindeer from southwestern France (Drucker *et al.*, 2003b) and Switzerland (Reade *et al.*, 2020) at this time associated with the combination of prolonged low temperatures, limited bioavailable soil nutrients, and elevated nutrient demand from increasing vegetation cover. In contrast to southwestern France and Switzerland, collagen of red deer from Sicily and Southern Italy exhibited increase in δ^{15}N values during the LGM. This increase was linked with arid conditions (Drucker *et al*., 2003b). The significant increase in δ^{15}N values of deer collagen was observed during warmer episodes over postglacial times and the early Holocene. This may be explained by the effects of increased mean annual temperature (Davis *et al.*, 2003) on soil development, intense nitrogen turnover and to ^{15}N enrichment in soils and plants (Drucker *et al.*, 2003a). This trend was not observed in red deer from Cantabria region, which could indicate a lack of extreme changes in climate and vegetation in this region (Jones *et al.*, 2020). In the most recent period analysed (from 8 ka cal BP to modern) a significant decline in δ^{15}N values was observed. During this period human induced environmental changes occurred (i.e. agriculture development and deforestation). The decline in red deer δ^{15}N values can be explained by a shift in red deer diet to the edible parts of trees and shrubs available in inhabited forested areas.

Although biome analyses showed that the main habitats of red deer during the last 54 ka years were different types of forests (coniferous, mixed and deciduous) (Niedziałkowska *et al.*, 2021), the results of isotopic values (i.e. δ^{13}C values above the -22.5‰) of most analysed samples within the present study indicated that red deer often fed in more open habitats. The discrepancies are probably caused by the different resolution of isotopic and biome analyses and the fact that the "canopy effect" may be reflected through a threshold effect on the δ^{13}C values of collagen. Isotopic analyses provide data on foraging habitats at the level of individuals, whereas biomes provide less

detailed information about habitats of occurrence on a larger spatial scale. Moreover, as revealed by studies on modern red deer from the Białowieża Primeval Forest (Poland), even red deer inhabiting forested areas preferred foraging in forest gaps than in closed forest (Kuijper *et al.*, 2009).

The variability of δ^{13}C values in red deer dated to the Holocene is best explained by the percentage of forest cover (negative association) and mean July temperature (positive association). Plants growing under a closed canopy, in poorly ventilated, more humid and shaded conditions, showed depletion of ^{13}C abundance compared to those from open habitats (Tieszen, 1991; Bonafini *et al.*, 2013). These results are consistent with analyses conducted on modern red deer (Sykut *et al.*, 2021) and other ungulates (Hofman-Kamińska *et al.*, 2018) indicating that the percentage of forest cover is the most important factor explaining variability of δ^{13}C in bone collagen. The modeling included samples dated to last 10 ka, therefore different factors caused by natural processes and human activity had impact on the obtained results.

Considering the positive association between δ^{13}C values and mean July temperature, it must be acknowledged that there is no clear explanation for such relationship (Heaton, 1999). However, our results correspond with the findings of Van Klinken *et al.* (1994) who observed a positive correlation between July temperature and bone δ^{13}C values during the Holocene. In our study the Holocene red deer records with the highest July temperature were located in southern Europe, where forest cover was also lower than in more northern areas of the continent at this time (this study Tab. S4, comp. Fyfe *et al.*, 2015; Woodbridge *et al.*, 2018) due to climatic conditions and human impact. Red deer inhabiting these areas probably forage in more open habitats, and therefore they exhibited higher δ^{13}C values than individuals from other regions of Europe. Another explanation of high δ^{13}C values of red deer from southern Europe could be the abundance of C4 plants in this part of Europe (Pyankov *et al.* 2010). In European temperate and boreal ecosystems, woody and herbaceous C3 plants represent significantly higher δ^{13}C values than C4 plants (Dawson *et al.* 2002).

Among analyzed climatic and environmental factors, variability of δ^{15}N is best explained by mean July temperature (positive association), annual precipitation (negative association) and altitude (negative association). Temperature and aridity changes are climatic parameters driving significant oscillations in soil activity in modern as well as in ancient ecosystems and in consequence cause shift of δ^{15}N values in plants further foraged by herbivores (Drucker *et al.,* 2011 and references therein). A similar relationship with mean annual temperature was observed in studies on modern European bison (Hofman-Kamińska *et al.*, 2018). Additionally, higher δ^{15}N values are present in graminoids (grasses and sedges), and forbs than in shrubs and trees (Nadelhoffer *et al.*, 1996; Ben-David *et al.*, 2001). Such types of plants more often grow in open areas, such as grasslands, meadows and pastures, which explains why the percentage of open area is the most important factor determining variability of δ^{15}N in modern populations of European red deer (Sykut *et al.*, 2021).

In agreement with the results of present study, the altitude has been also proven to influence the δ^{15}N values of modern herbivores. Such relationships was observed in hair of ungulates pastured at the altitude ranging from 400 to 2500 m.a.s.l., the δ^{15}N values decreased *c.* 1.1‰ per 1000 m (Männel *et al.*, 2007). Although we analysed locations from -103 to 1646 m.a.s.l., we obtained similar results. The decreasing trend in δ^{15}N values are attributed to the lower mineralisation rate and net nitrification rate at higher altitude (Sah & Brumme, 2003). However, the highest impact of altitude on the δ^{15}N values can be expected in localities at high altitudes, above around 3200 m.a.s.l. (Zech *et al.,* 2011). Similar relationship was observed in the Holocene in Western Alps, where the decrease of δ^{15}N values in red deer bone collagen was related to the upward migration of the individuals (Drucker *et al.*, 2011).

The overlapping values of stable isotopic composition of European red deer and wapiti dated to the Late Pleistocene and the Holocene indicated in this study, may suggest that the foraging habitats of these two species were more similar in the past than in the modern times but further studies (including more wapiti samples) are needed to confirm this hypothesis. Additionally, we

found no association between the isotopic composition and haplogroups of red deer (Fig. S3). However, the wide range of $\delta^{13}C$ and $\delta^{15}N$ values in red deer bones and the variability of biomes, where they have occurred during the last 50 ka years (comp. Niedziałkowska *et al.,* 2021), revealed that they have had wide ecological niches and were able to adapt to different environmental conditions. Further studies are needed to confirm similar flexibility in case of wapiti.

5. Conclusions

During the Late Pleistocene and Holocene (last 50 ka) in Europe and Asia, red deer shifted their feeding habitats according to environmental changes (e.g. forest expansion related to climate warming), and also in response to landscape changes associated with human activity (i.e. deforestation and the spread of agriculture). Additionally, red deer feeding habitats analysed at the Eurasian scale differed regionally. According to isotopic analyses, contemporary red deer feed in the most densely forested areas in comparison with individuals over the past 50 ka. Surprisingly, the values of carbon and nitrogen stable isotopes of European red deer and wapiti overlapped. Among all analysed variables the variance of $\delta^{13}C$ in European red deer during the Holocene is best explained by forest cover and mean July temperature, while the variance of $\delta^{15}N$ is best explained by mean July temperature, annual precipitation and altitude. It's probably not altitude per se that is responsible for variance in $\delta^{15}N$, but soil types. Mountainous landscapes tend to have thin, nitrogen poor soils (often due to low organic content in the absence of rapid soil formation or addition of organic material). The results of this study have broadened understanding of the ecology of one of the most important game species in Eurasia in the last 50 ka. Moreover, presented in this study data can be useful in reveling human diet and environmental conditions since the Last Pleistocene in Europe.

572 Table 1. Multiple regression model selection (based on the AICc criteria) to investigate the effect of different factors on carbon ($\delta^{13}C$) stable
573 isotope compositions in bone collagen of the Holocene red deer *Cervus elaphus* from Europe. The first two models representing the highest
574 parsimony (the lowest AICc scores) have been selected as the best models; df - number of estimated parameters; AICc - Akaike's information
575 criterion with a second order correction for small sample sizes; *Δ*AICc - difference in AICc between a given model and the most parsimonious
576 model; *ωi* - weight of the model.

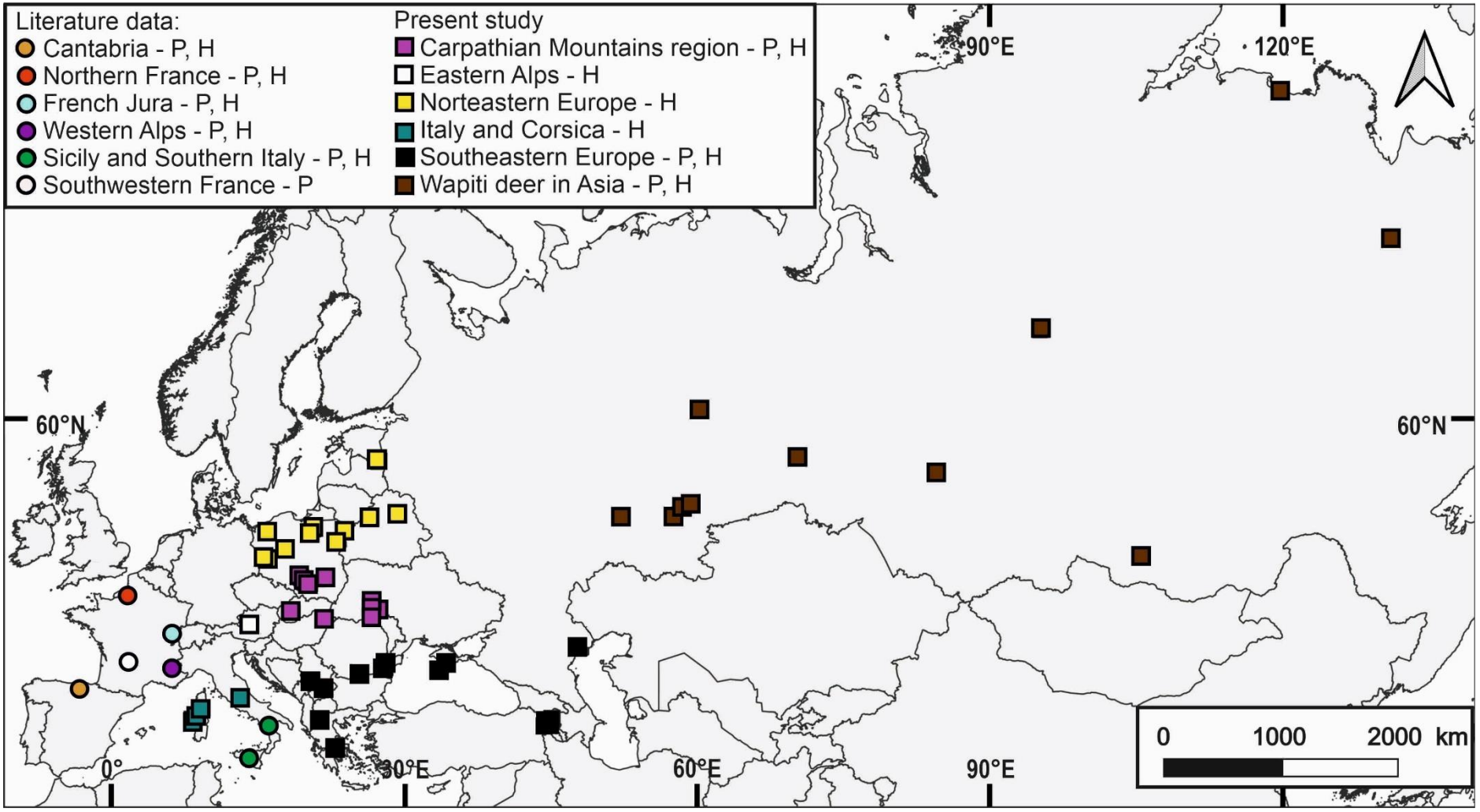
Variables	df	AICc	*Δ*AICc	*ωi*
Median age + Forest	4	91.3	0	0.4568
Median age + July temperature	4	91.8	0.50	0.3559
Median age + MtDNA haplogroup	5	95.5	4.14	0.0577
Median age + Annual mean temperature	4	95.7	4.41	0.0505
Median age + July precipitation	4	95.1	3.80	0.0682
Intercept	2	100.6	9.26	0.0045
Median age + January mean temperature	4	101.3	9.95	0.0031
Median age + Biome	4	102.9	11.52	0.0014
Median age + January precipitation	4	104.1	12.72	0.0008
Median age + Annual precipitation	4	104.6	13.30	0.0006
Median age + Altitude	4	105.3	13.96	0.0004

Table 2. Multiple regression model selection (based on the AICc criteria) to investigate the effect of different factors on nitrogen (δ^{15}N) stable isotope compositions in bone collagen of Holocene red deer *Cervus elaphus* from Europe. Three models representing the highest parsimony (the lowest AICc scores) have been selected as the best models; df -number of estimated parameters; AICc - Akaike's information criterion with a second order correction for small sample sizes; ΔAICc - difference in AICc between a given model and the most parsimonious model; ωi - weight of the model.

Variables	df	AICc	ΔAICc	ωi
Median age + July	4	129.0	0	0.2357
Median age + Annual	4	129.2	0.22	0.2108
Median age + Altitude	4	130.1	1.08	0.1373
Median age + July	4	130.8	1.79	0.0965
Median age + Annual	4	130.8	1.83	0.0943
Median age + MtDNA	5	131.7	2.72	0.0605
Median age + Forest	4	132.1	3.09	0.0503
Median age + January	4	132.4	3.38	0.0435
Median age + January	4	132.5	3.50	0.0409
Median age + Biome	4	133.1	4.14	0.0298
Intercept	2	141.5	12.49	0.0005

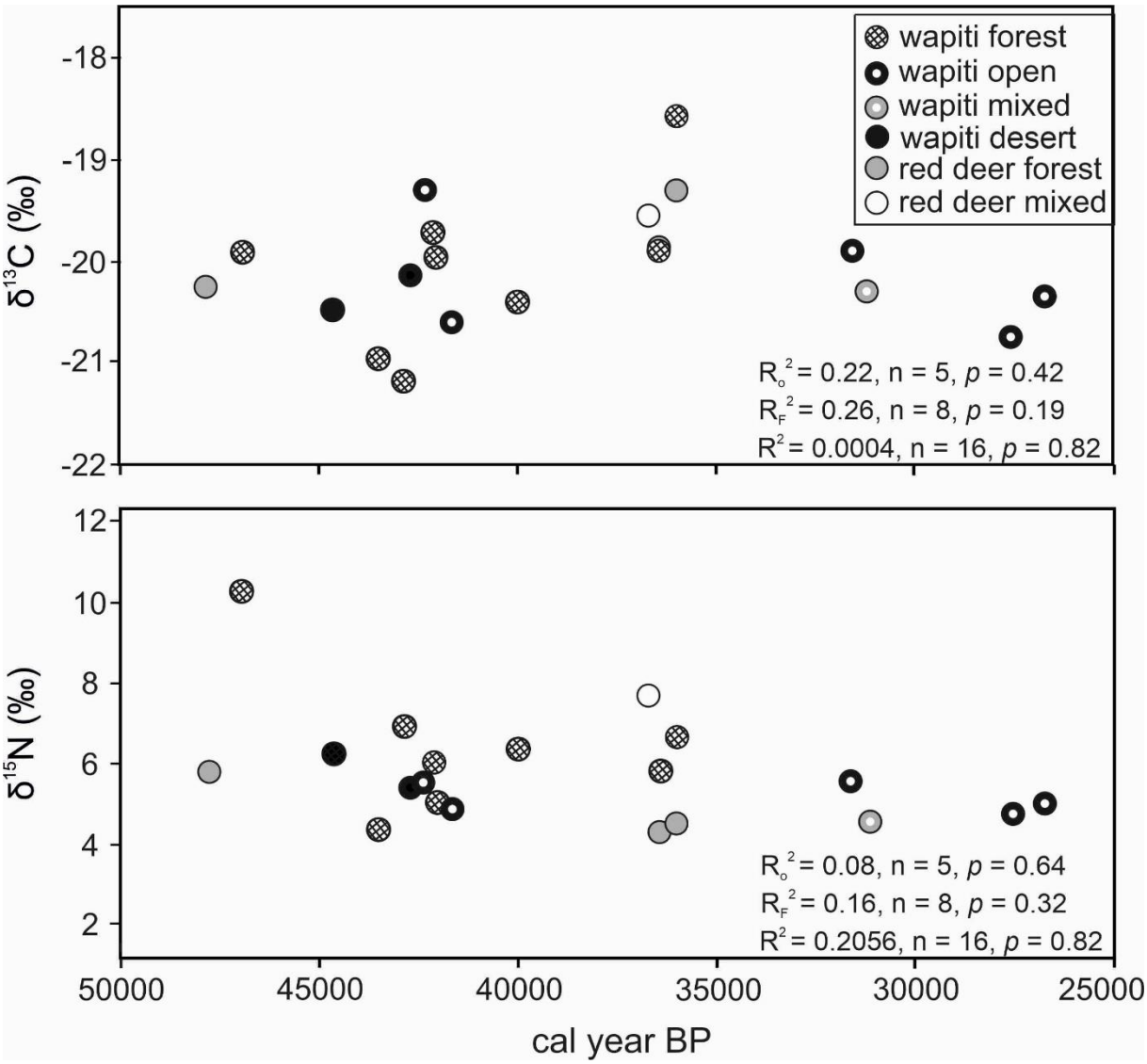
582

583


Literature data:
Cantabria - P, H
Northern France - P, H
French Jura - P, H
Western Alps - P, H
Sicily and Southern Italy - P, H
Southwestern France - P
Present study
Carpathian Mountains region - P, H
Eastern Alps - H
Norteastern Europe - H
Italy and Corsica - H
Southeastern Europe - P, H
Wapiti deer in Asia - P, H
90°E
120°E
60°N
60°N
0°
30°E
60°E
90°E
0
1000
2000 km

584

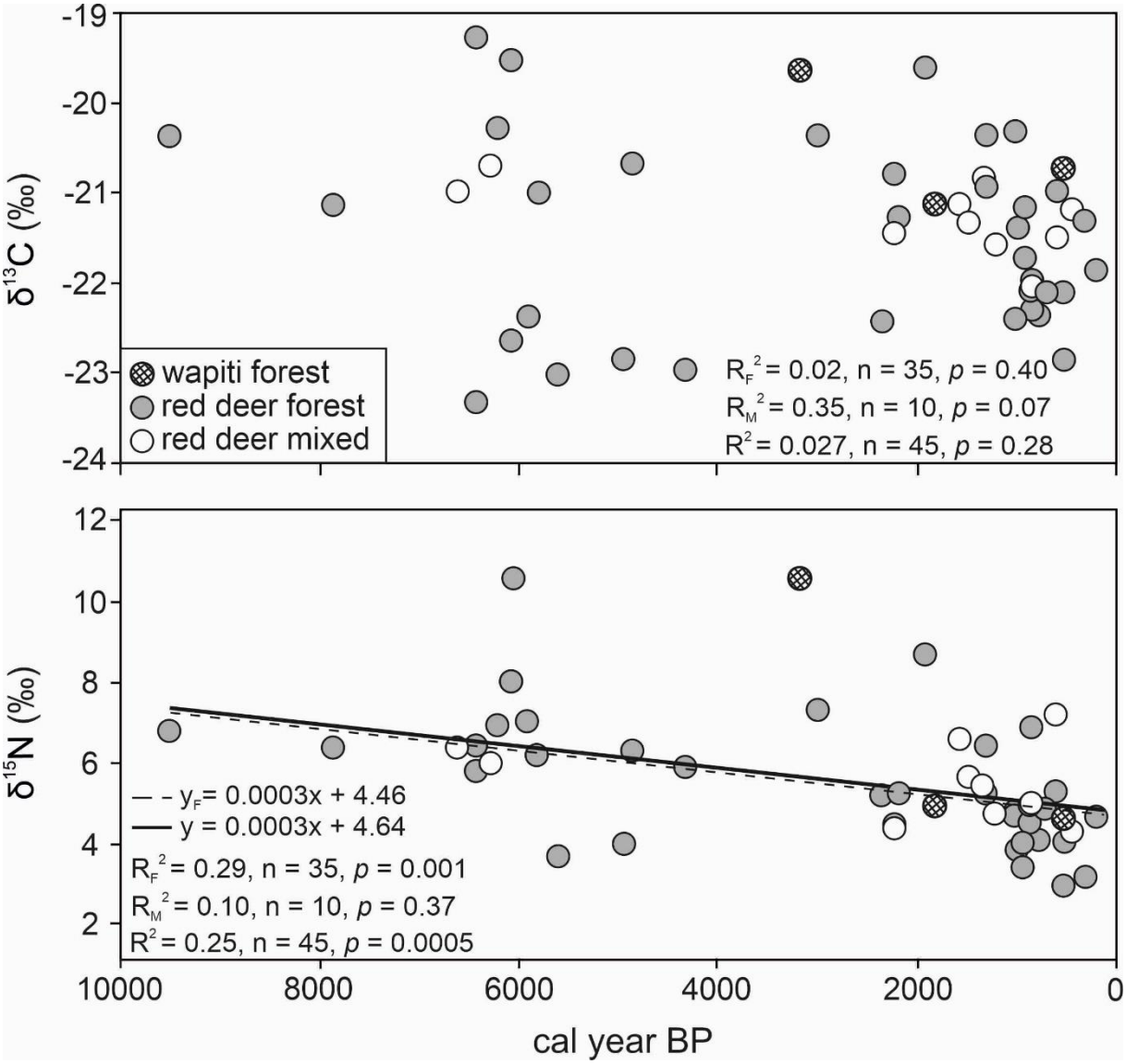
585 **Fig. 1**


wapiti forest
wapiti open
wapiti mixed
wapiti desert
red deer forest
red deer mixed
δ13C (‰)
-18
-19
-20
-21
-22
R0 2 = 0.22, n = 5, p = 0.42
RF 2 = 0.26, n = 8, p = 0.19
R2 = 0.0004, n = 16, p = 0.82
δ15N (‰)
12
10
8
6
4
2
R0 2 = 0.08, n = 5, p = 0.64
RF 2 = 0.16, n = 8, p = 0.32
R2 = 0.2056, n = 16, p = 0.82
50000
45000
40000
35000
30000
25000
cal year BP

586

587 **Fig. 2**

588



589

590 **Fig. 3**

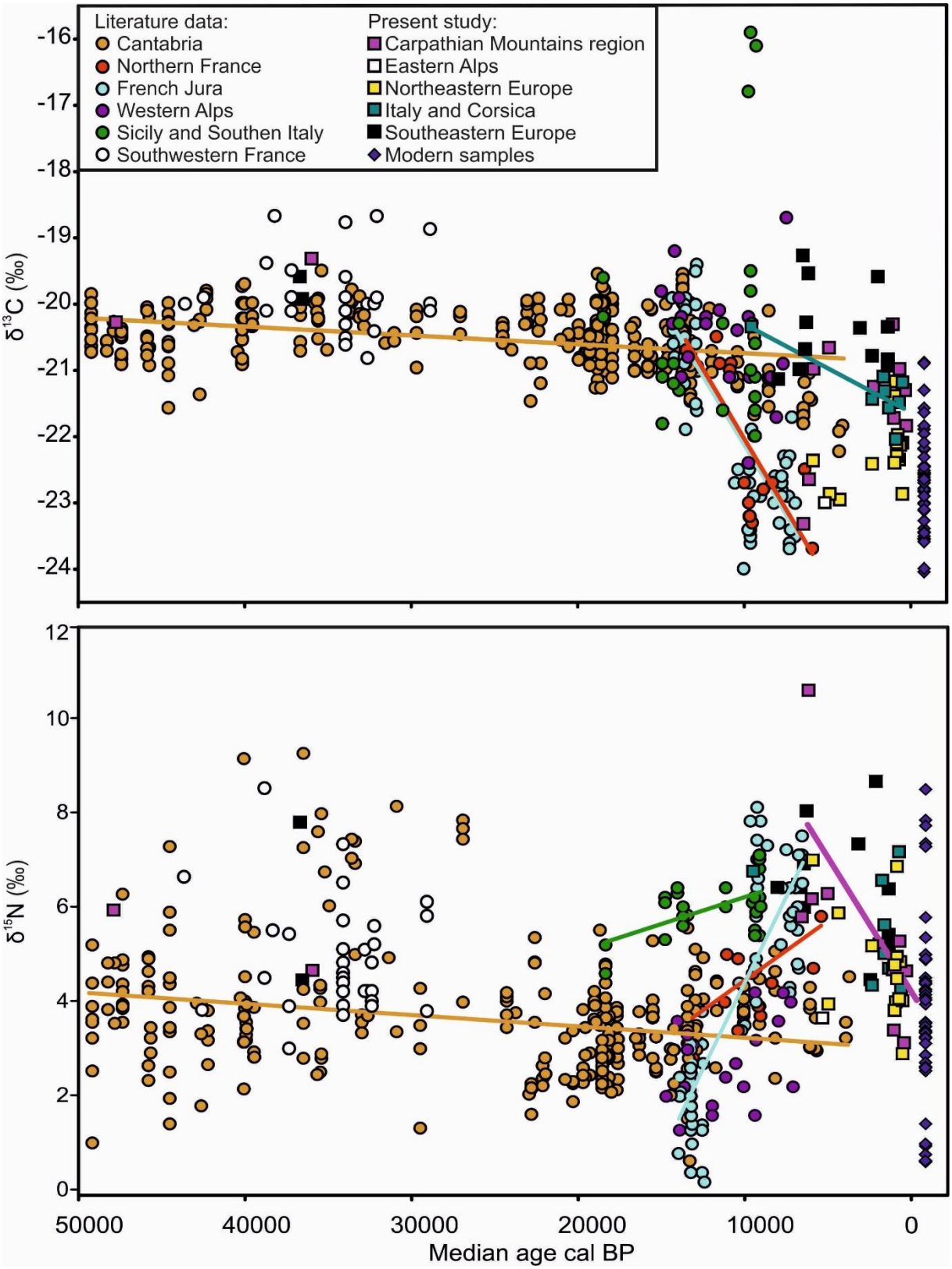

Literature data:
Cantabria
Northern France
French Jura
Western Alps
Sicily and Southen Italy
Southwestern France
Present study:
Carpathian Mountains region
Eastern Alps
Northeastern Europe
Italy and Corsica
Southeastern Europe
Modern samples
δ13C (‰)
δ15N (‰)
Median age cal BP

Fig. 4

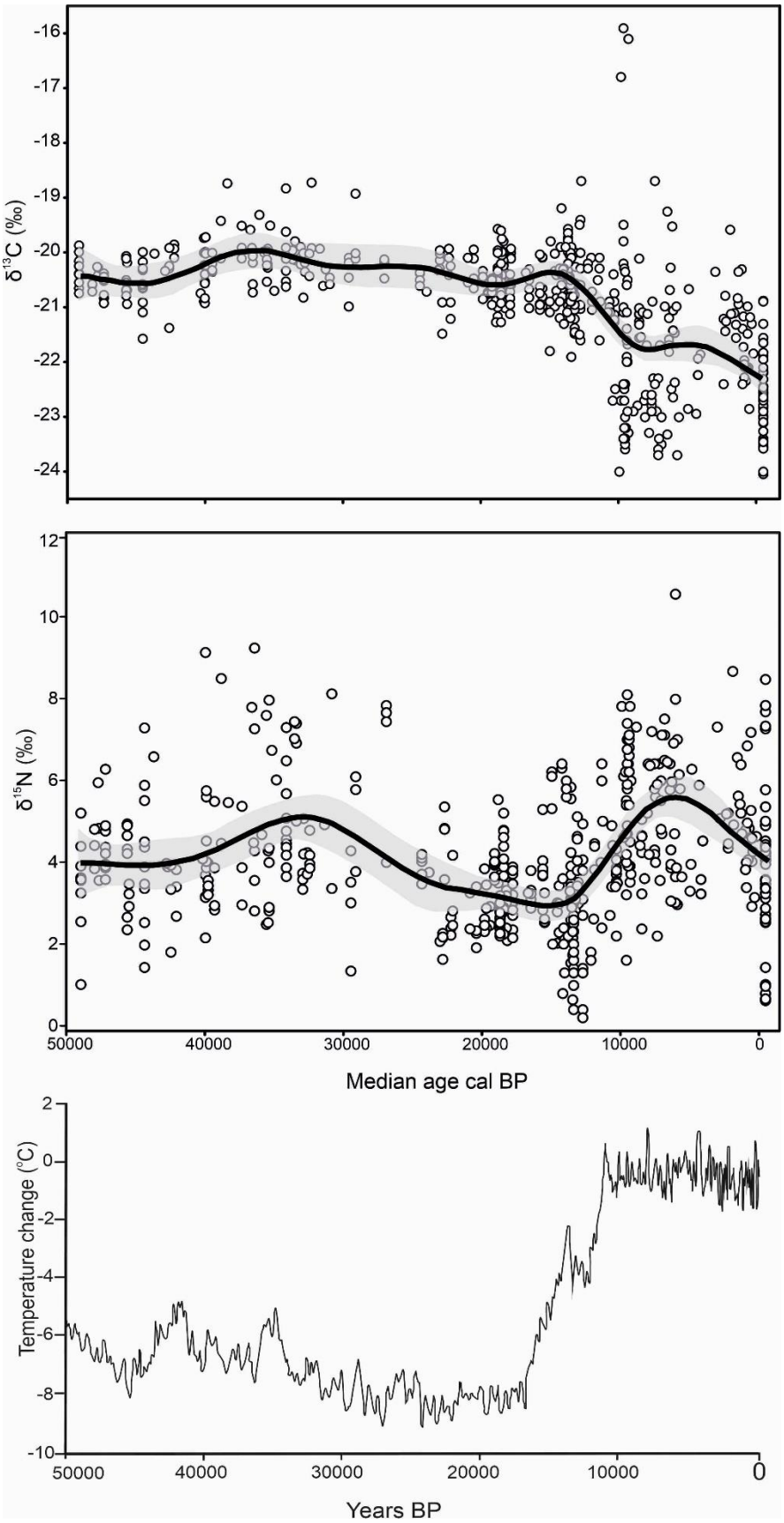

δ13C (‰)
-16
-17
-18
-19
-20
-21
-22
-23
-24
δ15N (‰)
12
10
8
6
4
2
0
50000
40000
30000
20000
10000
0
Median age cal BP
Temperature change (°C)
2
0
-2
-4
-6
-8
-10
50000
40000
30000
20000
10000
0
Years BP

Fig. 5

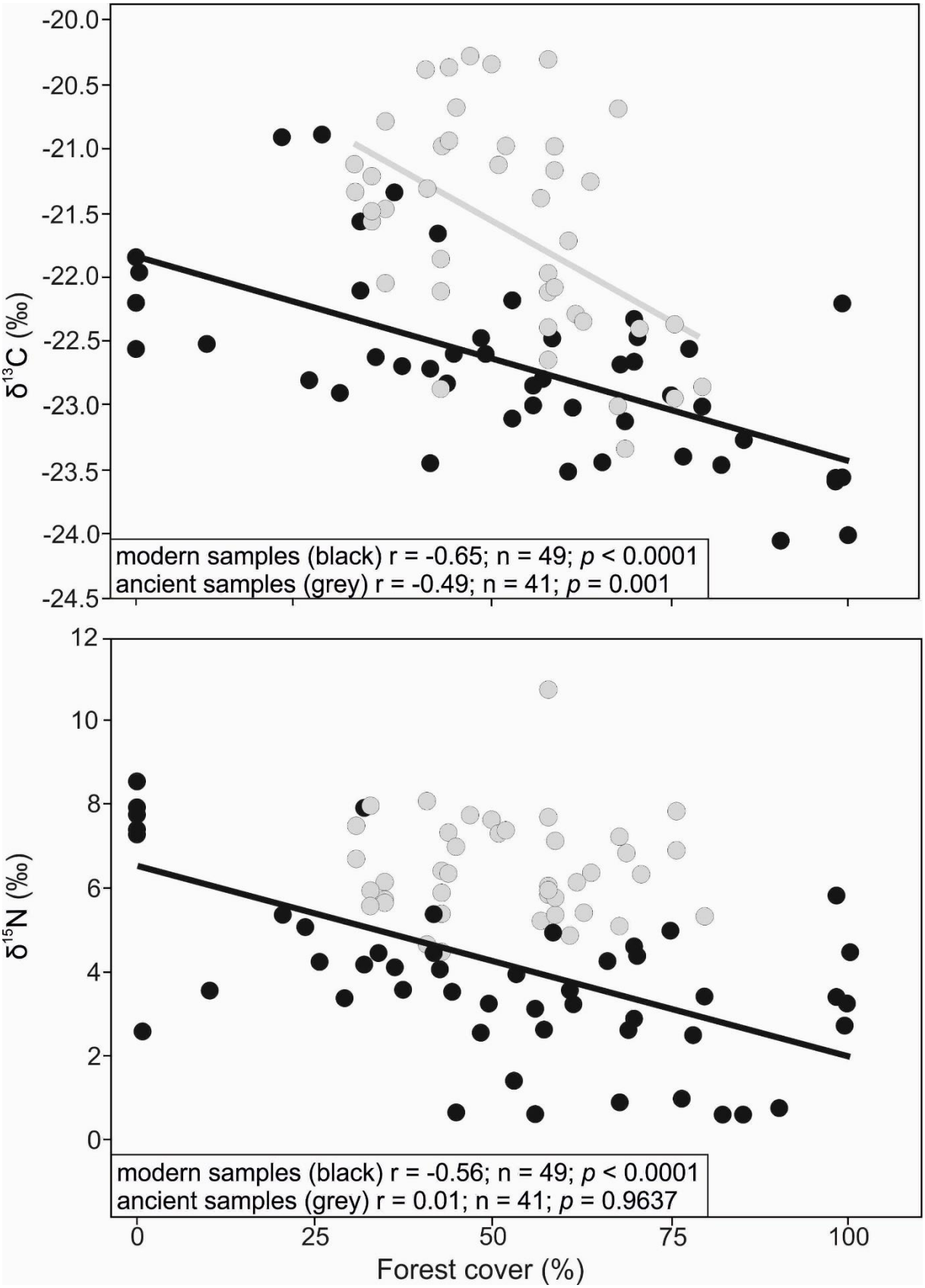

-20.0
-20.5
-21.0
-21.5
-22.0
-22.5
-23.0
-23.5
-24.0
-24.5
δ13C (‰)
modern samples (black) r = -0.65; n = 49; p < 0.0001
ancient samples (grey) r = -0.49; n = 41; p = 0.001
12
10
8
6
4
2
0
δ15N (‰)
modern samples (black) r = -0.56; n = 49; p < 0.0001
ancient samples (grey) r = 0.01; n = 41; p = 0.9637
0
25
50
75
100
Forest cover (%)

Fig. 6

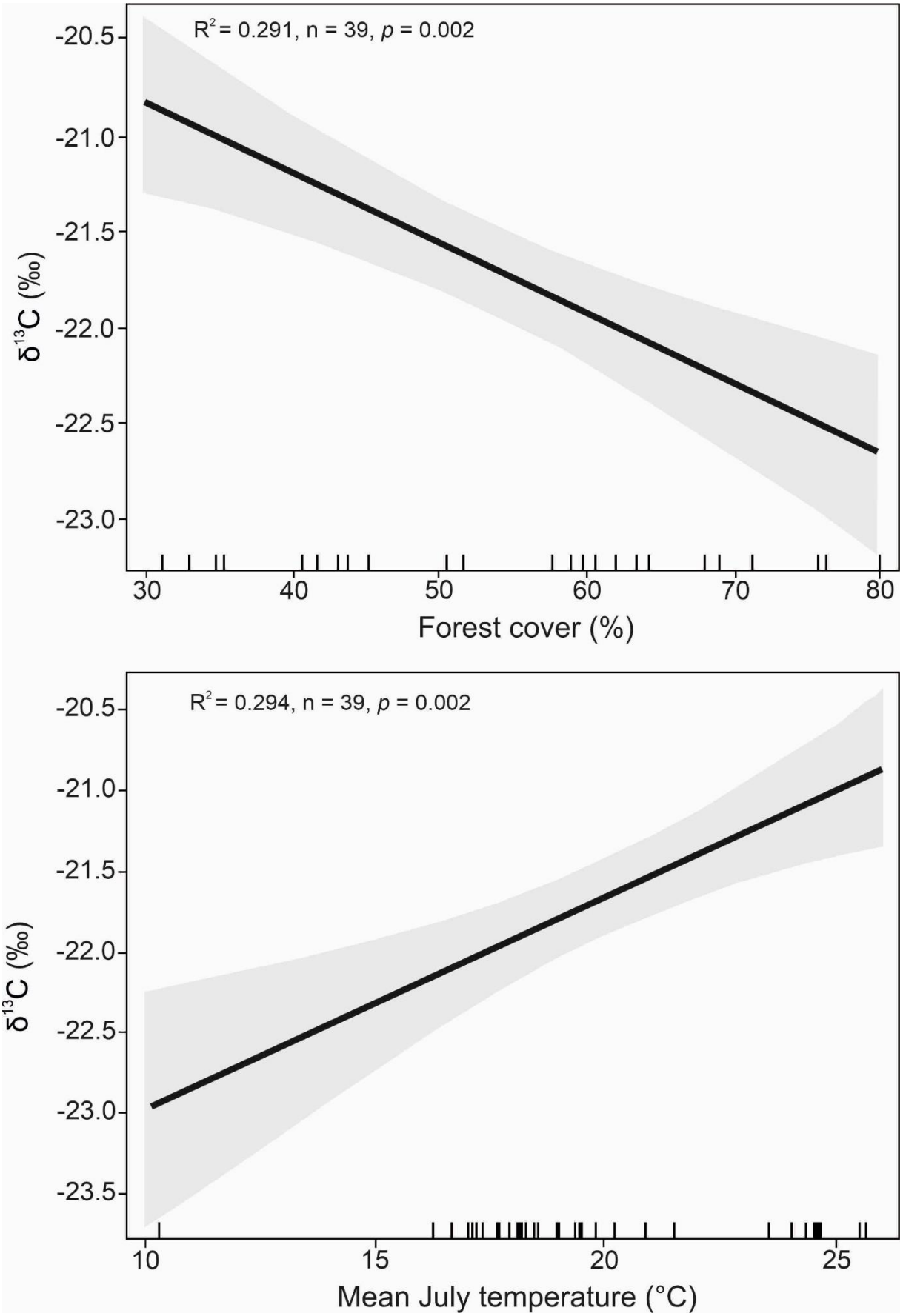
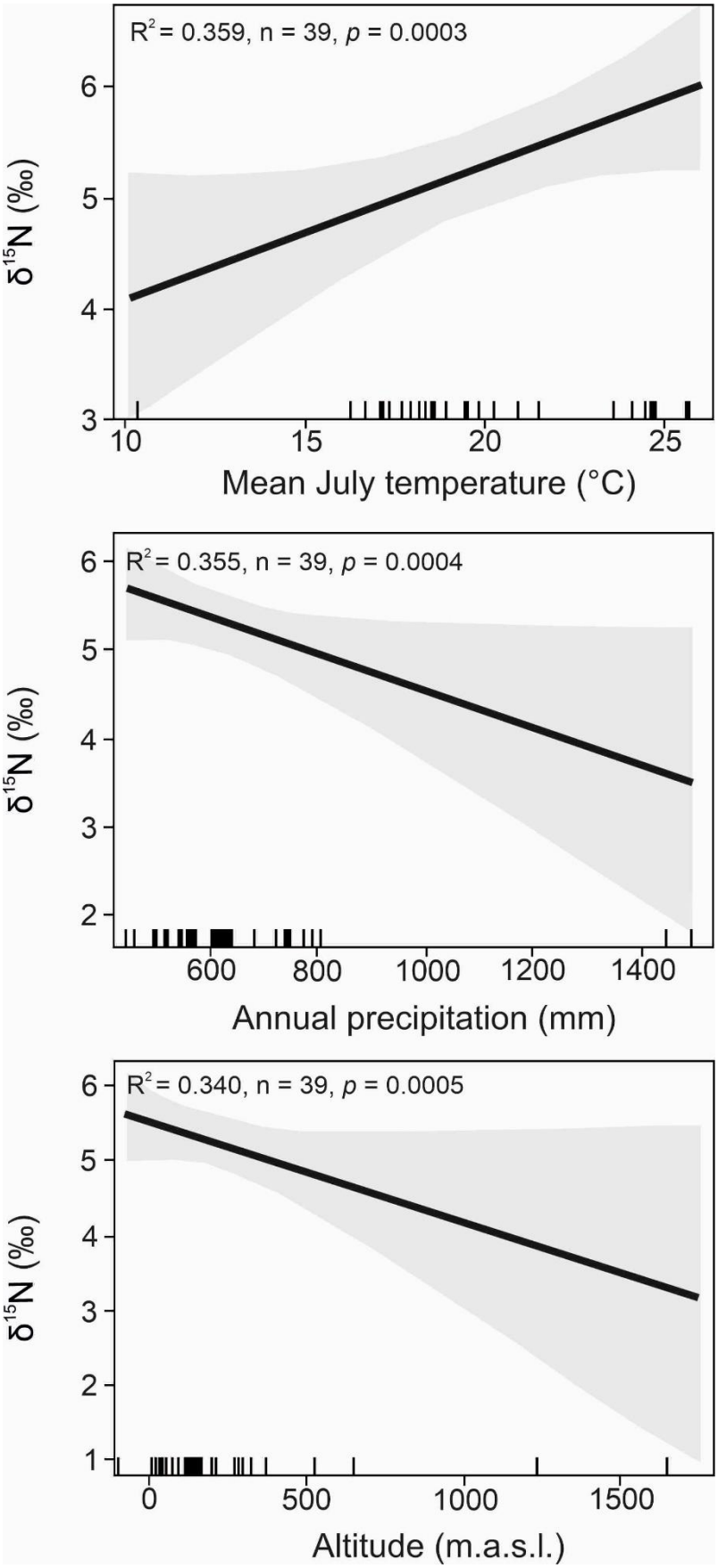

R² = 0.291, n = 39, p = 0.002
δ13C (‰)
-20.5
-21.0
-21.5
-22.0
-22.5
-23.0
30
40
50
60
70
80
Forest cover (%)
R² = 0.294, n = 39, p = 0.002
δ13C (‰)
-20.5
-21.0
-21.5
-22.0
-22.5
-23.0
-23.5
10
15
20
25
Mean July temperature (°C)

Fig. 7


R² = 0.359, n = 39, p = 0.0003
δ15N (‰)
Mean July temperature (°C)
R² = 0.355, n = 39, p = 0.0004
δ15N (‰)
Annual precipitation (mm)
R² = 0.340, n = 39, p = 0.0005
δ15N (‰)
Altitude (m.a.s.l.)

601

602 **Fig. 8**

Figures

Fig. 1. Spatial distribution of the ancient European red deer and wapiti deer samples analysed in the present study (Tab. S1) and location of sites where other studies (Tab. S2) on stable isotopic analyses including radiocarbon-dated subfossil bones of *Cervus elaphus* were performed. P – samples dated to the Late Pleistocene (50 000 - 26000 BP); H- samples dated to the Holocene (11700 – 0 BP).

Fig. 2. Chronological changes in $\delta^{13}C$ (upper panel) and $\delta^{15}N$ (lower panel) of red deer (n = 4) and wapiti (n = 16) during the Late Pleistocene. Wapiti biome categories: Forest - deciduous taiga or montane forest; Open - shrub or steppe tundra; Mixed - xerophytic shrub or sclerophyll woodland. The regression parameters were calculated for the wapiti samples only, including all *C. canadensis* samples and separately for wapiti inhabiting forest (F) and open (O) biomes. Wapiti from mixed and desert biomes were not included in the statistical analyses. Red deer biome categories: Forest – deciduous montane or coniferous forest, Mixed - sclerophyll woodland.

Fig. 3. Chronological changes in $\delta^{13}C$ (upper panel) and $\delta^{15}N$ (lower panel) and of red deer (n = 45) and wapiti (n = 3) during the Holocene. Red deer biome categories: Forest – coniferous or mixed forest; Mixed - xerophytic shrub or sclerophyll woodland. The regression parameters were calculated for the European red deer samples only, including all *C. elaphus* samples and separately for red deer inhabiting forest (F) and mixed (M) biomes. Wapiti biome categories: Forest – coniferous or deciduous taiga.

Fig. 4. Chronological changes in $\delta^{13}C$ (upper panel) and $\delta^{15}N$ (lower panel) in *Cervus elaphus* bones since the Late Pleistocene until the modern times in various localities in Europe. Data from the ancient European red deer individuals from this study (n = 49, Tab S1), data from the literature (n = 440, Tab. S2), and modern individuals (n = 49, Tab. S3, Sykut *et al*. 2021).

627 Fig. 5. Generalized Additive Model (GAM) plot showing changes in $\delta^{13}C$ (upper panel) and
628 $\delta^{15}N$ (middle panel) through time since the Late Pleistocene until modern times (the literature
629 data n = 440, this study n = 55, modern samples n = 49, for source of data see Tables S1–S3).
630 Lower panel: Antarctic (data from the Vostok ice core) temperature oscillations in the study
631 period (source: FAMOUS database; Smith & Gregory, 2012).
632 Fig. 6. Relationship between forest cover and $\delta^{13}C$ (upper panel), and $\delta^{15}N$ (lower panel) for
633 ancient (9 508 – 189 cal BP, n = 41) and modern red deer (n = 49) samples.
634 Fig. 7. Relationship between carbon stable isotope composition ($\delta^{13}C$) in bone collagen of
635 ancient European red deer (n = 39) and forest cover (upper panel), mean July temperature
636 (lower panel) based on estimates from multiple regression models. Grey areas – 95%
637 confidence intervals of the regression lines.
638 Fig. 8. Relationship between nitrogen stable isotope composition ($\delta^{15}N$) in bone collagen of
639 ancient European red deer samples (n = 39) and mean July temperature (upper panel), annual
640 precipitation (middle panel) and altitude (lower panel) based on estimates from multiple
641 regression models. Grey areas – 95% confidence intervals of the regression lines.

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658

659 **Conflict of interest**

660 The authors declare no conflict of interest

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Supplementary material

Supplementary material 1

Table S1 Database of stable isotopic values and direct radiocarbon dates of red deer *s. l.* samples and the climatic and environmental data determined for their localities and analysed in the frame of this study.

Table S2 Stable isotopic data and radiocarbon dates of red deer samples from different sites in Europe obtained from the literature sources.

Table S3 Results of isotopic analyses (δ^{13}C, δ^{15}N) on bone collagen of modern red deer, composition of the collagen (carbon/nitrogen ratio, percentage of nitrogen and carbon in a sample), year of death of sampled red deer individual, percentage of forest cover and open area calculated in buffers around the sample sites (see Sykut *et al.*, 2021 for more details). Study sites with abbreviated country name: PL-Poland, NL-Netherlands, UK-United Kingdom, SI-Slovenia,* samples with δ^{13}C and δ^{15}N values recalculated by the formulas described in the (Sykut *et al.*, 2020); δ^{13}C corr: δ^{13}C values corrected for the shift in δ^{13}C values (corr atm) caused by anthropogenic CO_2 emissions by the formula proposed by (Feng, 1998).

Table S4 List of the Holocene red deer samples, genetic, climatic and environmental data used in the Normal linear models (NLM). Abbreviations as in Tab. S1.

Supplementary material 2

Table S5 Pairwise correlation matrix of the following parameters: percentage of forest cover, percentage of open area, mean annual temperature, mean July temperature, mean January temperature, altitude, annual precipitation in each of the study sites, where the red deer Holocene fossils (n = 39, Tab. S4) were recorded and dated for certain time periods. Significant assays ($P \leq 0.05$) are given in bold.

Table S6. Parameters of regression between $\delta^{13}C$ and $\delta^{15}N$ values and the median of calibrated age BP of samples calculated in various localities of European red deer populations presented in Fig. S6. Significant assays ($P \leq 0.05$) are given in bold.

Fig. S1 Geographic distribution of modern red deer samples used in this study (n = 49) and published in Sykut *et al.* (2021).

Fig. S2 Stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope signatures of red deer *s.l.* from different habitats into two time periods before the LGM (47 857 – 26 813 cal BP) (upper panel) and after LGM (9508 – 189 cal BP) (lower panel). Wapiti biome categories: Forest - deciduous taiga or montane forest; Open - shrub or steppe tundra; Mixed - xerophytic shrub or sclerophyll woodland. The regression parameters were calculated for the wapiti samples only, including all *C. canadensis* samples and separately for wapiti inhabiting forest (F) and open (O) biomes. Wapiti from mixed and desert biomes were not included in the statistical analyses. Red deer biome categories: Forest – deciduous montane or coniferous forest, Mixed - sclerophyll woodland.

Fig. S3 Stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope signatures of red deer *s.l.* from different mitochondrial lineages and haplogroups into two time periods before the LGM (47 857 – 26 813 cal BP) (upper panel) and after LGM (9508 – 189 cal BP) (lower panel).