

Forest Ecosystems and Evolution of Cattle Husbandry Practices of the Earliest Central European Farming Societies

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Abstract

Stable isotope signatures of domesticates found on archaeology sites provide information about past human behaviour, such as the evolution and adaptation of husbandry strategies. A dynamic phase in cattle husbandry evolution is during the 6th millennium BCE, where the first cattle herders of central Europe spread rapidly through diverse forested ecological niches, where little is known about pasturing strategies. Here we investigate cattle pasturing and foddering practices using a multi-regional dataset of stable isotope values ($\delta^{13}\text{C}$ and $\delta^{18}\text{O}$; compound-specific stable isotopic analysis $\delta^{15}\text{N}$ -amino acids and $\delta^{13}\text{C}$ -dairy fats) measured from cattle bone and teeth, and pottery residues from early farming contexts, and palaeoenvironmental information. Our analysis reveals that farmers practiced different pasturing strategies with the intensive use of forested ecosystems in some areas for both graze and seasonal forage. We propose that the diversity of strategies is related to the adaptation of herding to new environments, which had a positive impact on cattle breeding and milk availability for human consumption.

Introduction

Early husbandry practices were an important step in the evolution of domesticated animals, either via conscious or unconscious selection, shaping animal morphology, phenotype, and genotypes. Subsistence practices, such as husbandry, are central components of human cultural activities¹⁻³. A dynamic phase in the evolution of cattle husbandry in Europe was during the rapid introduction of farming represented by the *Linearbandkeramik* (LBK) culture. These communities settled across a wide geographical range, initially in Hungary and eastern Austria between 5545-5360 cal BCE⁴ and then expanded into other regions to the north, east and west between 5380 to 5315 cal BCE⁵⁻⁸, during a period time characterised by numerous short-term and significant fluctuations in precipitation⁹. The traditional image of these communities is one of homogeneity⁸, partly reinforced by ancient DNA analysis where large-scale genomic studies suggest that LBK communities were the outcome of monolithic demic diffusion^{10,11}. However, regionally focused studies demonstrate that complex interactions between local hunter-gatherers and farmers existed¹² as well as subtle differences in animal husbandry strategies with greater exploitation of pigs in certain areas, such as Alsace^{6,13,14}. Moreover, detailed palaeodietary studies indicate significant differences in human diet between regional populations⁸.

Cattle (*Bos taurus*) were a central part of LBK economies and contributed to the success and rapid expansion of these communities^{3,15} providing an important source of both milk and meat^{15,16}. In comparison to other domesticates, cattle are slow to gain maximum weight with traditional land races reaching sexual maturity around 2-3 years with first parturition occurring between 3 and 4 years¹⁵. Maintaining herds of sub-adults and adults represents an investment in terms of pasture and fodder resources¹, particularly during winter and early spring when grazing may be restricted by snow or flooding¹⁷. The LBK communities inhabited diverse range of environments from the seasonally flooded marshes of the Carpathian Basin¹⁸ to the semi-open woodlands of the Polish lowlands¹⁹ and the

forested steppe in northern and central Bohemia, and southern Moravia²⁰ to the closed mixed oak forests of the Rhine valley²¹. European woodlands depending on their canopy structure and composition have always been important in traditional pasture systems^{17,22,23}. Branches and leaves (leafy hay), a fodder rich in nutrients and minerals²⁴, has been used since the Late Neolithic²⁵ as an alternative animal feed^{17,22}. Given the relative diversity in forested environments within LBK cultural distribution, husbandry practices, particularly foddering and pasturing strategies may have been shaped in part by these local ecosystems.

Without direct physical evidence of forest fodder consumption, carbon isotope ratios ($\delta^{13}\text{C}$) of animal bone and teeth can potentially identify the use of forest environments for pasture and forage²⁶⁻³⁰. This is based on the well-known 'canopy effect' principle³¹ (*see Methods*), where relative negative $\delta^{13}\text{C}$ values of bone collagen, dentine and bioapatite samples may indicate animals feeding under a dense forest canopy. Within the context of our study, an initial investigation observed a strong relationship between $\delta^{13}\text{C}$ values from LBK cattle bone collagen samples and geographical/paleoenvironmental proxies, with values become increasingly more negative moving westwards, not evident in sheep/goat samples³⁰. Another means to determine domesticated ruminant diet is via $\delta^{13}\text{C}$ values of dairy lipids trapped within the fabric of pottery. These are biosynthesised from dietary carbohydrates³², which in herbivores is directly related to consumed fodder. Dairy lipids are routinely detected using compound-specific stable isotope analysis (CSIA) of fatty acids (FA), providing direct evidence of milking^{33,34} and changes in pasture strategies overtime³⁵. Deciduous forest canopies by their nature change in density reaching their maximum during late summer³⁶. These changes in the canopy impact foliar $\delta^{13}\text{C}$ values, and thus seasonally collected fodder can be identified using $\delta^{13}\text{C}/\delta^{18}\text{O}$ values of sequential samples of the enamel following the tooth growth axis. This methodology has detected cattle winter foddering in central Europe: one individual at Chotěbudice (LBK, Czechia²⁸) and in both sheep and cattle from Bercy (Middle Neolithic, Paris Basin²⁶). While these results are promising, carbon isotope compositions cannot distinguish between plants growing within open canopy forests or forest edges, to those growing in open landscapes³⁷ nor can they determine the plant type. The recent development of a proxy based on CSIA of collagen/dentine amino acid (AA) $\delta^{15}\text{N}$ values provides an independent means of directly identifying the fodder source i.e., woody vs. herbaceous plants³⁸. Combining this proxy with $\delta^{13}\text{C}/\delta^{18}\text{O}$ values, within a single tooth, provides a powerful tool to assess the seasonal use of forest resources.

Stable isotope signatures of domesticated animals found on archaeology sites can be used to track the adaptation of husbandry strategies by early farmers to local ecosystems. Herein, we analyse $\delta^{13}\text{C}$, $\delta^{18}\text{O}$ and $\delta^{15}\text{N}$ values from bone and dentine collagen and bioapatite from cattle, and pottery dairy lipids from unpublished and published studies^{6,8,19,27,28,33,35,39-47} of early farming contexts characterised by a material culture with numerous shared elements across a wide geographical range (SI Table 1-5, 1,541 isotopic measurements, 61 sites; Figure 1). Together with site-specific paleoenvironmental data, we characterise pasture and forage strategies adopted by pioneer farming communities in central Europe. The paleoenvironmental proxies include mean forest cover (MFC), mean precipitation summer (PMS) and

winter (PMW); mean temperature summer (TMS) and winter (TMW) based on palynological data^{48,49}, and modern river information (river drainage systems (RDS); Strahler stream order (SSO); distance to nearest river (DNR)). The combination of these datasets offers unique insights into how local forested ecosystems influenced early cattle pasture management and seasonal foddering practices in central Europe.

Results

The global distribution of the $\delta^{13}\text{C}$ values for LBK cattle diet inferred from collagen and dental bioapatite, and for ruminant diet based on dairy lipids $\delta^{13}\text{C}$ values ranged from -31.2 to -19.8‰ . There was no significant difference between diet $\delta^{13}\text{C}$ values estimated from three datasets (ANOVA, $df=2$, $p=0.1$; Figure 2A: where each histogram represents a specific dataset). A significant correlation was found between diet $\delta^{13}\text{C}$ values and longitude (Pearson's correlation $r=0.6$, $p<0.001$, Figure 2B; SI Table 7). We compared the datasets to an average diet value for deer, a mainly forest-dwelling ruminant, based on published^{8,27,44,45} and unpublished bone collagen samples from LBK sites (SI Table 6; $-27.7 \pm 1\text{‰}$, $N=33$). The general trend observed for all datasets was values from sites in the west were within or below our forest dwelling ruminant baseline in contrast to those in the east (Figure 2B). This is further highlighted by the interpolated maps of diet $\delta^{13}\text{C}$ values, where values from collagen (Figure 2C) and dairy lipids (Figure 2D) become progressively more depleted in ^{13}C moving east to west.

Correlation analysis between all values and paleoenvironmental proxies found only PMW to have a correlation coefficient greater than 0.5 (SI Table 7; $r=-0.54$, $p<0.0001$). Within the dairy lipid dataset, other proxies were found to be significantly correlated with correlation coefficient greater than 0.5 (Dairy lipids diet $\delta^{13}\text{C}$ values \sim TMW: $r=-0.63$, $p<0.0001$; \sim MFC: $r=0.5$, $p<0.0001$). However, in both the collagen and bioapatite datasets, PMW and TMW were found to be significant but with r values < 0.4 which explains less than 16% of the variation in these datasets. Correlation between two variables is not causation, especially within datasets where other sources of variation exist and cannot be explored due to lack of information, which is often the case for archaeological data. We used Partial Least Square (PLS) regression analysis to further explore the relationship between diet-inferred $\delta^{13}\text{C}$ values and paleoenvironmental proxies (SI 1). For all datasets, the r^2 value for the PLS regression models were <0.4 . For both datasets, the variables TMW and PMW were within the top three components ranked for importance. However, the optimal models to explain diet $\delta^{13}\text{C}$ values based on bone collagen and dairy lipids used a single component (longitude) based on the RMSE scores of 1.24 and 3.15 respectively. For bioapatite diet $\delta^{13}\text{C}$ values, all three variables (longitude, TMW and PMW) were included in the optimal PLS model.

For cattle herders, it is important to be close to water sources since cattle are obligate drinkers. Most sites are situated near to streams with no tributaries (SSO 1) with the average distance of 1.5km to closest water source. The diet $\delta^{13}\text{C}$ values for each tissue from the major river drainage systems (RDS; Seine, Meuse, Rhine, Elbe, Danube, Vistula, and Oder; Figure 3) decreased from east to west. Furthermore,

samples from sites situated in the Seine, Meuse, and Middle/Upper Rhine River basins where the mean MFC ranged from 63.9 to 75.2% had lower $\delta^{13}\text{C}$ values in comparison to areas with higher MFC (>75%). This contrasts with the canopy effect, where we would expect negative values where the MFC was greatest.

Individual analysis

Incremental bioapatite samples of the third molar from 46 cattle exhibited $\delta^{13}\text{C}$ values between -12‰ to -10‰ , except at Bischoffsheim where all values were below -12‰ (Figure 4A, SI Table 4 and SI Figure 1). Individuals from Bischoffsheim, Balatonszárszó, Chotěbudice²⁸ and Apc-Berekalja I exhibited low $\delta^{13}\text{C}$ values that occur when the $\delta^{18}\text{O}$ values are low i.e. during cold months⁵¹ (SI Figure 1). Within individual teeth, the amplitude between the highest and lowest values ranged from 0.3‰ to 3.0‰ (SI Table 4; Figure 4C) and was negatively correlated with MFC ($r = -0.7, p < 0.05$). At sites where MFC was <75%, such as Bischoffsheim and Balatonszárszó, inter-tooth variation in amplitude was greatest. The diet β values based on CSIA-AA $\delta^{15}\text{N}$ values of sequential dentine samples from 12 cattle sampled here for $\delta^{13}\text{C}/\delta^{18}\text{O}$ analysis, ranged from -8.7‰ to -3.6‰ . Using modern references³⁸, we can identify the plant source i.e., herbaceous ($-5.4 \pm 2.1\text{‰}$) or woody ($-9.3 \pm 1.6\text{‰}$). The lowest values were found at Bischoffsheim while a single tooth from Balatonszárszó also exhibited values below the upper limit for diet based on woody plants (BAL 2, Figure 4B). Balatonszárszó individuals exhibited the greatest range in values (-7.7 to -3.6‰ ; SI Table 6) while largest intra-tooth variation between the highest and lowest β values was at Bischoffsheim (Figure 4D).

We integrated bioapatite ($\delta^{18}\text{O}/\delta^{13}\text{C}$) and dentine (CSIA- β) datasets (Figure 5A-F). For interpretation, a delay of around 6 months is taken into account between the formation of dentine and the mineralisation of enamel based on experimental analysis⁵². At Bischoffsheim, the $\delta^{13}\text{C}$ values fell within the range expected for the forest dwelling herbivore, with the lowest $\delta^{13}\text{C}$ values recorded when the $\delta^{18}\text{O}$ values were lowest i.e., winter. In the same teeth, β values showed variation over the annual cycle, with the lowest values indicating of woody plants in the diet (Figure 5A/B). These values occur, considering the delay in enamel mineralisation, when the $\delta^{18}\text{O}$ values were low indicating consumption of woody plants in winter. This is also observed in BAL3 (Figure 5E) where low $\delta^{13}\text{C}$ values and $\delta^{18}\text{O}$ values coincided with a decreasing trend in β values, suggesting leafy hay foddering or pasturing within forested environments during the winter months. Individuals LUD1, APC1 and BAL5 exhibit β values between -6.3 and -3.5‰ indicating a solely herbaceous plant diet (Figure 5C/D/F).

Discussion

The holistic analysis of $\delta^{13}\text{C}$ values from cattle teeth and bone, and ruminant dairy lipids provides a unique insight into early herding pasturing and foddering strategies. Our analysis shows the earliest evidence for seasonal winter foddering with leafy hay for cattle in central Europe and intensive use of forested areas for cattle and domesticated ruminant pasture west of the Rhine. We discuss the impact of

climate and other factors on these results, present an overall synthesis of early cattle land use and explore the potential long-term impact of forest pasture on early human societies and their cattle herds.

Impact of climate and other factors on carbon stable isotopes

Carbon stable isotope ratios of C₃ plants are sensitive to changes in their growing environments³⁷, these values are passed onto grazing herbivores and subsequently human consumers. Forested environments also impact foliar δ¹³C values^{31,37} due to the 'canopy' effect, a central principal of our study. We observed in areas where MFC was low that diet δ¹³C values were also low, which was unexpected. However, while the proxy MFC provides a unique measure for paleo-forest cover, it cannot characterise woodland composition. This is important for characterising canopy structure and density as it is a mosaic of different tree species³⁶. Moving from the paleoenvironment to paleoclimatic conditions, climate has an important impact on foliar δ¹³C values where plants growing in warm and dry environments exhibiting higher values than those growing in cool humid conditions, which is driven in principle by stomatal closure and its impact on ¹³C discrimination⁵⁴. Our analysis demonstrated a correlation between diet δ¹³C values, and winter precipitation (PMW) and temperatures (TMW), chiming with pervious large-scale palaeodietary analysis albeit using modern mean summer temperatures⁵⁵. However correlation analysis of sheep/goat bone collagen found no significant result between paleoclimate and δ¹³C values³⁰. It is difficult to assess the impact of local environments and climatic conditions on ancient plant foliar δ¹³C values and those of herbivores partly due to the poor temporal resolution as well as choice of paleoenvironmental proxies to accurately reflect past ecosystems.

The δ¹³C values of dairy FA, such as palmitic acid (C_{16:0}) reflect a dairy ruminant's diet at the time of lactation as they are largely biosynthesised *de novo* from dietary carbohydrates or incorporated from dietary FA³². Our results showed a clear decrease in dairy lipid δ¹³C values moving east to west. Pottery vessels can be used for multiple purposes, where mixtures of animal products within a single vessel can affect the dairy C_{16:0} FA δ¹³C values. Relatively high δ¹³C values (*ca.* - 26.5‰, equivalent to diet value of -25‰) could be explained by the mixing of animal products in vessels. For example, processing non-ruminant products, such as pig fat, with δ¹³C values *ca.* 3‰ more enriched than ruminant fats⁵⁶, would produce the observed isotopic shift. Pigs are more frequent in faunal assemblages to the west of the Rhine during the LBK⁵⁷, and pottery lipids⁶ and yet we still observe low dairy lipids δ¹³C values. Processing of dairy and freshwater aquatic products in the same vessel would lead to negative shifts in δ¹³C values. However, there is currently little evidence for the exploitation of freshwater resources in the LBK⁵⁷. Therefore, the east to west trend observed within dairy lipid δ¹³C values appears to mainly reflect ruminant fodder resources. The large variation observed in values in some regions (Paris Basin, Alsace, Carpathian Basin) may reflect changes in forage during the lactation period for example, provision of leafy hay.

Integrated perspectives of cattle pasturing practices and local ecological contexts

The lack of significant difference between $\delta^{13}\text{C}$ values from cattle skeletal material and dairy lipids, as well as the previous analysis of sheep/goat bone collagen²⁵ supports the hypothesis that cattle were the primary source of dairy lipids^{15,33}. The subsequent overview provides a synthesis of our results where we explore the evolution of cattle herding management strategies within forested ecosystems. Beginning in the Carpathian Basin, the region during the 6th millennium BC, was characterised by extensive marshland environments and gallery forests (MFC mean, 70.5%) strongly influenced by the Danube and its tributaries. Sites from this region represent some of the oldest in our dataset, Apc-Berekalja I and Balatonszárszó (5470-4950 cal BCE and 5335-4900 cal BCE respectively). In general, β and $\delta^{13}\text{C}$ values indicate the diet of cattle consisted of herbaceous plants from open environments. Cattle ranging between different ecological niches (marsh, forest, open steppe) may cause the high variation observed in bioapatite $\delta^{13}\text{C}$ values (Figure 4A). In addition, there are some indications of the use of woodland resources for forage. At Apc-Berekalja I, dairy lipids are depleted in ^{13}C ($-26.0 \pm 0.5\text{‰}$ ($N=9$)) in comparison to bioapatite samples ($-24.7 \pm 2.4\text{‰}$ $N_{teeth}=7$), which may be a result of leafy hay provisioned during milking. The β values from Balatonszárszó also suggest some contribution of woody plants in the cattle diet during winter. Overall, in this region we propose that cattle were managed extensively moving between different pastures with their fodder supplemented at specific times, for example, during lactation or when snow cover restricted pasture access.

In areas, such as Poland and Czechia, the ratio of open areas to forests was low (MFC, 80-85%). The Polish samples overall exhibit relatively higher diet $\delta^{13}\text{C}$ values than the reference for a forest-dwelling herbivore. CSIA and bulk values from teeth, bones and pottery suggest that cattle grazed on herbaceous plants from open or open canopy forest environments. The low variation in $\delta^{13}\text{C}$ values of bioapatite, collagen and dairy lipids from Polish sites suggested limited diversity in pasture types. Interestingly in subsequent TRB phase at Kopydłowo, dairy lipids and collagen indicate herders made greater use of densely forested areas^{19,35}. Moving south-west, where palynological investigations indicate the presence of open canopy steppe forest⁵⁸, one individual at Chotěbudice (Northern Czechia) had $\delta^{13}\text{C}$ values occurring in winter indicative of leafy hay foddering (CHO9, range -27.3‰ to -25.7‰)²⁸. Other cattle teeth from here, Černý Vůl and Těšetice-Kyjovice exhibit values that fall within the upper range of dietary values expected for a forest dwelling herbivore. We cannot rule out the use of forests in these regions for forage resources, but the absence of low $\delta^{13}\text{C}$ values may indicate cattle grazing in open areas surrounding the settlements, for example, river terraces and forest fringes.

In the western part of our study region, the MFC was less than 75% and forests typically consisted of mixed deciduous species. Focusing on the Alsace regions (Bas-Rhin and Haut-Rhin) bordering the Rhine, halophilic species, such as hazel were more frequent in the Haut-Rhin suggesting open canopy forested environments¹⁰. To the north, all our isotope datasets at Bischoffsheim (Bas-Rhin) support the use of forests as pasture with clear evidence of winter provision of collected leafy hay. The mean cattle diet values are found to be more enriched in ^{13}C at sites in the Haut-Rhin region, for example, Ensisheim-Ratfeld ($\delta^{13}\text{C}_{\text{lipid}}$: $-26.1 \pm 0.9\text{‰}$, $N=19$; $\delta^{13}\text{C}_{\text{coll}}$: $-26.8 \pm 1\text{‰}$, $N=14$). The variation in canopy structure

and species composition would explain the observed difference between north and south, while the use of forest pasture in the Bas-Rhin region may have been related to the increased presence of pigs^{6,13,14}, which encourage the development of undergrowth via rooting²³.

The Paris Basin was more open landscape (MFC: 65%). However, the average $\delta^{13}\text{C}$ values at Cuiry-lès-Chaudardes, from both cattle bone collagen ($-28.1 \pm 0.5\text{‰}$, $N=8$) and ruminant dairy lipids ($-27.7 \pm 1.2\text{‰}$ ($N=49$)) reflect forage depleted in ^{13}C , such as forest pasture and fodder. Further, comparison with Bischoffsheim (Student t-test, bone collagen $t=0.3$ $p=0.7$), supports this hypothesis. The significant presence of dairy lipids at the site suggests a strong emphasis on milk production and processing. Dairy lipids $\delta^{13}\text{C}$ values from Cuiry-lès-Chaudardes are not significantly different to cattle bone collagen samples from Bischoffsheim (T-test, bone collagen $t=1.1$ $p=0.7$). Cattle as the main dairy species should be approached with caution and various scenarios examined, because the slaughter profile analysis shows an orientation towards meat production¹⁶. Alternatively, the use of forest resources for cattle fodder and pasture may have fuelled dairy production while providing a by-product in the form of fatten male calves for meat production. It is also possible that dairy sheep/goat were pastured in woodland environments alongside cattle, winter provisioning of forest resources of both species is evident at more recent sites within the Paris Basin²⁶.

The impact of the use of forest resources on human societies and their herds

A recent study on cattle birth periodicity, predicted from bioapatite $\delta^{18}\text{O}$ values including individuals from our dataset, demonstrated that there was an increased frequency of out-of-season births in LBK⁵⁰ in comparison to other early Neolithic groups. The oestrous cycle in cattle is not governed by seasonal variation in temperatures as in sheep/goat⁵⁹. Provision of leafy hay, a rich source of minerals and nutrients²⁴, would have increased winter survival of females as well as improved their body condition, fertility and survival of offspring⁵⁹. A greater proportion in out-of-season births would have resulted in overlapping lactations and thus extended period of milk availability for human populations. Provision of feed, such as leafy hay, during milking increases the milk let-down⁶⁰ and certain tree species also improve milk quality²⁴. Processing milk into a variety of storable products, such as milk casein balls or hard cheeses, would have helped support them during lean months and food crises⁶¹. Pasturing animals within forests would have increased contact with local hunter-gatherer-fisher groups, with surplus dairy products as a medium of exchange⁶². In the Paris Basin, Middle Neolithic farming populations of the region exhibit both cultural and biological links with these communities^{7,12}. The forest and forest pasturing, which began in the LBK, may have acted as a catalyst stimulating interaction and exchange between groups.

Cattle were an important commodity to the first farmers of central Europe and this study reveals complex regional strategies that evolved in response to different forested ecosystems and resource

availability, and potentially lead to benefits to both humans and cattle populations. Supplementing cattle diets with leafy hay would have improved cattle health and influenced birth seasonality providing a direct benefit to communities with increased availability in milk. Pasturing and foddering of animals within forests contributed to the opening-up of these environments, facilitating the spread of prehistoric farming societies across Europe. The arrival of farmers and their herds to central Europe disrupted woodland environments marking an important point in the emergence of man-made ecosystems.

Methods

Stable isotope principles (Rosalind E. Gillis/ Iain P. Kendall)

The *canopy effect* is a result of atmospheric CO₂ under the canopy being ¹³C-depleted relative to the atmosphere due to the uptake of recycled CO₂ respired by decomposition of ¹³C-depleted organic matter³¹. This is coupled with decreased light intensity that reduces photosynthetic efficiency, discriminating against the transfer of ¹³C (ref.^{37,54}). These relatively depleted values are passed on to animals pastured and foddered on plants found under the canopy, and thus their milk and body tissues, such as carcass fats, collagen, and enamel, are expected to display relatively low δ¹³C values⁶³. Stable carbon and oxygen isotope values from sequential bioapatite samples can be used to investigate seasonal changes in diet. Oxygen isotopes of bioapatite precipitate in equilibrium with body water, influenced by the seasonal variability in stable isotopic composition of water⁵¹. Thus, δ¹⁸O values provide a seasonal reference for δ¹³C values (summer: high δ¹⁸O values; winter: low δ¹⁸O values).

Direct evidence of the type of plants (woody/herbaceous) consumed can be determined using the dietary β values based on δ¹⁵N CSIA of AA from incremental samples of dentine from cattle molars. These values represent the Δ¹⁵N_{Glx-Phe} values of the plants at the base of the food web, using a known trophic offset of -4.0‰ between cattle and their diet⁴⁹. The dietary β values can then be compared with established ranges of Δ¹⁵N_{Glx-Phe} values expected for herbaceous (-5.4 ± 2.1‰) and woody plants (-9.3 ± 1.6‰), based on modern references³⁸. This difference in values is likely due to the involvement of Phe in the phenylpropanoid pathway, by which lignin is produced, leading to isotopic fractionation and enrichment of the remaining Phe pool available for protein biosynthesis. This results in the more negative Δ¹⁵N_{Glx-Phe} values observed in woody plants relative to herbaceous plants, as the former are assumed to produce more lignin.

Diet values were calculated using the following enrichment values: Δ_{lipids-diet} is +1.5‰ based on Δ_{lipids-collagen} = 6.6 ‰ (ref. ⁶³) and Δ_{collagen-diet} = -5.1 ‰ (ref. ⁶⁴); Δ_{bioap-diet} = -14.5‰ (ref. ⁵³). The spacing between diet-inferred δ¹³C values and herbivore bone collagen δ¹³C values has been proposed to be between 5.1 to 5.3‰ (ref. ⁶⁴). Here we use 5.1‰ as to be comparable with previous stable isotope studies of LBK faunal material²⁸. The enrichment of bioapatite in ¹³C varies between species depending on the difference in physiology and size of the species⁴⁸. We have used an enrichment factor of 14.5‰

based on a recent synthesis of the spacing between diet, CO₂ breath and bioapatite in animals of different digestive systems⁵³. The spacing between collagen and fat δ¹³C values has been proposed to be -6.6‰ for consumers of terrestrial C₃ diets⁶³. The diet-inferred δ¹³C values are thus calculated by adding 1.5‰ to the δ¹³C values of the C_{16:0} fatty acid.

Lipid residue analysis of pottery vessels and determination of δ¹³C values from dairy lipids (Mélanie Roffet-Salque)

Lipid residue analyses and interpretations were based on established protocols⁶⁵. Briefly, 1 to 3 g samples were taken from potsherds and their surfaces cleaned with a modelling drill to remove exogenous lipids (e.g. soil or finger lipids arising from handling). The sherds were ground to a powder in a glass pestle with a mortar. The powdered sherd was transferred to a glass culture tube, internal standard added for quantification (*n*-tetratriacontane, 20 µg) and acidified methanol solution (H₂SO₄/MeOH, 4 % v/v, 5 mL, 70 °C, 1 h) added. The lipids were then extracted from the aqueous phase with *n*-hexane (4 x 3 mL). The solvent was evaporated under a gentle stream of nitrogen to obtain the total lipid extract (TLE). Aliquots of the TLE were trimethylsilylated using *N,O*-bis(trimethylsilyl)trifluoroacetamide containing 1 % trimethylsilyl chloride (20 µL, 70 °C, 1 h) and re-dissolved in *n*-hexane for analysis by gas chromatography (GC) and GC-combustion-isotope ratio mass spectrometry (GC-C-IRMS).

All GC analyses were performed on a Hewlett Packard 5890 series II chromatograph. Helium was used as carrier gas at constant flow (2 mL min⁻¹) and a flame ionization detector (FID) was used to monitor column effluent. Trimethylsilylated total lipid extracts (1 µL) were injected through an on-column injector, in track-oven mode onto a fused silica capillary column (50 m x 0.32 mm i.d.) coated with a dimethylpolysiloxane stationary phase (J&W Scientific, CP-Sil 5 CB, 0.1 µm film thickness). The oven temperature was programmed, after an isothermal hold at 50 °C for 2 min, to 300 °C at 10 °C min⁻¹, followed by a second isothermal hold at 300 °C for 10 min. Peaks were identified by comparison of retention times with those of an external standard and quantification was achieved by the internal standard method. Data acquisition and processing were carried out by the Clarity software.

GC-MS analyses of trimethylsilylated aliquots were performed using a Finnigan Trace MS quadrupole MS coupled to a Trace GC. Diluted samples were introduced using a PTV injector in the splitless mode onto a 50 m x 0.32 mm i.d. fused silica capillary column coated with a HP-1 stationary phase (100 % polymethylpolysiloxane, 0.17 µm film thickness; Agilent Technologies). The initial injection port temperature was 50 °C with an evaporation phase of 1 min, followed by a transfer phase from 50 °C to 300 °C at 14.5 °C s⁻¹, followed by an isothermal hold at 300 °C. The GC oven temperature was programmed as for the GC analyses. The MS was operated in the electron ionisation (EI) mode (70 eV) with a GC interface temperature of 300 °C and a source temperature of 200 °C. The emission current was 150 µA and the MS set to acquire in the range of *m/z* 50-650 Daltons at 8.3 scans per s. Data acquisition and processing were carried out using the XCalibur 1.2. software. Peaks were identified based on their mass spectra, GC retention times and by comparison with the NIST mass spectral library (version 2.0a).

Compound-specific $\delta^{13}\text{C}$ values of FA were determined using an Isoprime 100 GC-C-IRMS system. The same GC conditions were used as for the GC analyses (HP-1 column, 100 % dimethylpolysiloxane, 50 m x 0.32 mm x 0.17 μm , Agilent Technologies). Each sample was run at least in duplicate. Instrument stability was monitored by running a fatty acid methyl ester standard mixture every 2 or 4 runs. Results were calibrated against a CO_2 reference gas injected directly in the ion source as two pulses at the beginning of each run. Instrumental precision was 0.3 ‰.

Animal fats were identified as dairy lipids when their $\Delta^{13}\text{C}$ ($= \delta^{13}\text{C}_{18:0} - \delta^{13}\text{C}_{16:0}$) values were $\leq -3.1\text{‰}$ as proposed by Dunne et al.³⁴. We examined $\delta^{13}\text{C}$ values of $\text{C}_{16:0}$ fatty acids from a total of 352 extracts identified as originating from animal dairy lipids (of which 135 are published^{6,33,35,39-42}) from 44 sites. The species-specific identification of dairy species (cattle/sheep/goats) is not obtainable through the molecular or isotopic composition of the extracts and thus the dairy lipids from this study can come from any of these species.

$\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ analysis of bioapatite samples (Rosalind E. Gillis)

Cattle third molars (M3) were selected for stable isotopic analysis: 1) because the archaeozoological material was highly fragmented, making it difficult to distinguish between M1 and M2, and 2) to avoid effects from suckling and weaning. Each tooth sampled represents an individual except BIS3 and 4, which appear to come from the same individual. A minimum of eight M3s with early stages of occlusal wear were sampled from each site except at Těšetice-Kyjovice where only two teeth were sampled. Tooth surfaces were cleaned using an abrasive tungsten drill bit to remove dental calculus, cementum, and sediments. Enamel samples were removed by drilling with a diamond bit on the buccal side of the proximal lobe perpendicular to the crown growth axis. The purification protocol for stable isotopic analysis followed Balasse et al.⁶⁶. Purified enamel samples weighing between 551-650 μg were analysed on a Kiel IV device interfaced to a Delta V Advantage IRMS at the Service de Spectrométrie de Masse Isotopique du MNHN (SSMIM, Paris). The accuracy and precision of the measurements were verified using an internal laboratory calcium carbonate standard (Marbre LM normalized to NBS 19). Over the period of analysis, an average of six LM samples were analysed per run. These gave a mean $\delta^{13}\text{C}$ value of $2.13 \pm 0.03 \text{‰}$ (1σ) (theoretical value normalized to NBS 19 = 2.13‰) and a mean $\delta^{18}\text{O}$ value of $-1.66 \pm 0.15\text{‰}$ (1σ) (theoretical value = -1.83‰). Results are expressed relative to the VPDB standard.

Compound-specific stable isotope analysis of AAs from cattle dentine (Iain P. Kendall)

Cattle third molar (M3) teeth were sequentially sampled at six points along the growth axis of each tooth. Dentine was collected as a powder, using a modelling drill with a diamond abrasive drill bit. Once formed, dentine in teeth is not remodelled, and therefore the collagen preserves the isotopic composition of the period of formation. For each sample, the AA norleucine was added as an internal standard to ca. 15 mg of dentine. Demineralisation of the inorganic fraction and hydrolysis of the collagen was achieved in one step by heating with acid (6 M HCl, 5 mL; 100°C, 24 h), and the solution blown to dryness under nitrogen.

AA purification and derivatisation to *N*-acetyl isopropyl (NAIP) ester derivatives were prepared according to established protocols^{67,68}.

AAs were identified by GC-FID by comparison with AA standards and quantified by comparison with a known amount of norleucine internal standard. Their $\delta^{15}\text{N}$ values were determined by GC-C-IRMS as described in Styring, et al.⁶⁸ with a modified GC method, using DB-35 capillary column (30 m \times 0.32 mm internal diameter; 0.5 μm film thickness; Agilent Technologies, UK) and the oven temperature of the GC held at 40°C for 5 min before programming at 15°C min⁻¹ to 120°C, then 3°C min⁻¹ to 180°C, then 1.5°C min⁻¹ to 210°C and finally 5°C min⁻¹ to 270°C and held for 1 min. A Nafion drier removed water and a cryogenic trap removed CO₂ from the oxidised and reduced sample. Isotopic compositions are expressed using the delta scale as follows: $\delta^{15}\text{N} = R_{\text{sample}} / R_{\text{standard}} - 1$, where R is the ¹⁵N/¹⁴N ratio, and the standard is atmospheric N₂ (AIR). All $\delta^{15}\text{N}$ values are reported relative to reference N₂ of known isotopic composition, introduced directly into the ion source in four pulses at the start and end of each run. Each reported $\delta^{15}\text{N}$ value is the mean of triplicate determinations. A standard mixture of AAs of known $\delta^{15}\text{N}$ values was analysed every three runs to ensure acceptable instrument performance.

Paleoenvironmental variables (Marco Zanon)

The identification of past forest composition is hampered by the location of pollen cores as well as modelling uncertainties. Localised exploitation of forest resources may be underrepresented in traditional paleoecological investigations due to difficulties in capturing small-scale landscape dynamics. These difficulties may variously stem from a lack of targeted investigations, or from an absence of suitable archives. To sidestep these issues, and to proceed with a complete comparison of faunal and land cover data, we make use of interpolated reconstructions covering the whole study area. Such large-scale interpolated reconstructions may still be unable to fully resolve local dynamics, yet their use allows us to initiate a comparison between geographically spread-out datasets. The MFC data were generated from the interpolated Holocene reconstructions by Zanon et al.⁴⁸. We chose to use MFC values (%) sampled from the 7500, 7250, and 7000 cal BP (i.e. 5550, 5300, and 5050 cal BC) time slices at the location of every site in the faunal data set, and subsequently averaged.

Paleoclimate information (summer and winter temperature and precipitation) is based on the modelled values presented in Sánchez Goñi, et al.⁴⁹ and available for the time slice $\sim 7100 \pm 100$ years cal. BP (ca. 5150 \pm 100 BC). We applied inverse distance weighted interpolation to all data points using the R package *gstat* 2.0-6 (ref.⁶⁹). The optimal power value for each variable was selected via leave-one-out cross-validation, using the root-mean-square error as a metric to assess the model performance. We then sampled the interpolated climate values at the location of every site within the data set.

Statistical analysis (Marco Zanon/Rosalind E. Gillis)

The interpolated diet-inferred value maps (fig. 2C-D) were produced as follows: the median $\delta^{13}\text{C}$ values for each site were interpolated via Inverse Distance Weighted interpolation through the R package *gstat*

2.0-6 (ref.⁶⁹). The optimal power value for each variable was selected via leave-one-out cross-validation, using the root-mean-square error as a metric to assess the model performance. The size of the “bullseye” depends partly on purely graphical choices (number and width of the colour intervals) and partly on the parameters of the interpolation algorithm. Statistical analysis and graphic production were carried out using the free platform R program⁷⁰ (SI 1).

Declarations

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Figures



Figure 1

Site locations and mean modelled forest cover (MFC) calculated from past forest reconstructions⁴⁸ within the LBK distribution based on reference ⁷ (see *Methods*). All sites are LBK, except for Alföld Linear Pottery

(ALP, SI Table 1). N=number of sites. 1. Cuiry-lès-Chaudardes; 2. Maastricht-Cannerberg; 3. Maastricht-Klinkers; 4. Geleen-Janskamperveld; 5. Erkelenz-Kückhoven; 6. Konigshoven 14 (FR 5); 7. Langweiler 8⁴²; 8. Ensisheim-Ratfeld^{6,8}; 9. Colmar⁶; 10. Sierentz⁶; 11. Bischoffsheim^{6,8,50}; 12. Herxheim⁴⁵ 13. Kilianstädten; 14. Vaihingen an der Enz²⁷; 15. Heilbronn-Neckargartach⁸; 16. Dillingen-Steinheim; 17. Derenburg Meerestieg II⁴⁴; 18. Halberstadt Sonntagsfeld⁴⁴; 19. Karsdorf⁴⁴; 20. Altscherbitz; 21. Brodau⁴²; 22. Aiterhofen⁸; 23. Lerchenhaid⁸; 24. Stephansposching; 25. Rutzig/Haid⁸; 26. Płonia 2; 27. Brzezín 7; 28. Karwowo 1; 29. Żalęcino; 30. Żuków; 31. Černý Vůl^{28,47,50}; 32. Bylany^{39,41}; 33. Chotěbudice^{28,47,50} 34. Stroegen; 35. Těšetice-Kyjovice^{8,50}; 36. Brunn am Gebirge 37. Gnadendorf⁸; 38. Vedrovice-Sídliště⁸; 39. Asparn a. d. Zaya/Schletz⁸; 40. Blatné⁸; 41. Chabsko 24; 42. Žegotki; 43. Bozejewice 22/23; 44. Rożniaty 2; 45. Radojewice 29; 46. Kuczkowo 5; 47. Siniarzewo 1; 48. Kopydłowo 6^{19,35}; 49. Ludwinowo 7^{33,40,46,50}; 50. Bodzia 1; 51. Kruszyn 13; 52. Modlnica 5; 53. Vráble-Velké Lehembý⁴³; 54. Balatonszárszó-Kis-erdei-dűlő^{8,50}; 55. Štúrovo; 56. Tolna-Mözs-Községi-Csádés-földek; 57. Apc-Berekalja I⁵⁰; 58. Füzesabony-Gubakút (ALP)^{8,50}; 59. Polgár-Ferenci-hát (ALP)⁸; 61. Felsővadász-Várdomb (ALP); 60. Garadna-Elkerülő út (ALP).

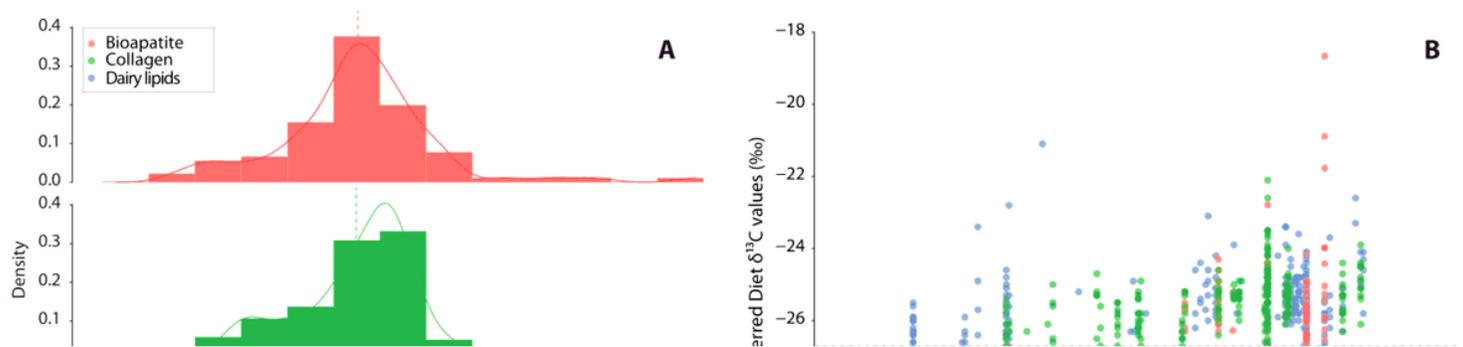


Figure 2

A. Histogram of the cattle/dairy ruminant diet $\delta^{13}\text{C}$ values based on unpublished and published $\delta^{13}\text{C}_{16:0}$ values from dairy lipids^{6,33,39-42} recovered from pottery vessels (blue), collagen $\delta^{13}\text{C}$ values^{8,27,43-45} (green) and, highest and lowest $\delta^{13}\text{C}$ values from the sequential bioapatite analysis of cattle teeth^{19,46} (red) (SI Table 2-4); B. Biplot of longitude and cattle diet values from the three datasets, (colour legend as in A). The dotted line with error margins in A and B represents the diet value of $-27.7 \pm 1\text{‰}$ for a forest dwelling ruminant based on the mean $\delta^{13}\text{C}$ value of published^{8,27,44,45} and unpublished contemporaneous deer bone collagen samples (SI Table 6); C. Interpolation map for cattle diet inferred from bone collagen $\delta^{13}\text{C}$ values; D. Interpolation map for dairy ruminant diet inferred from dairy lipid $\delta^{13}\text{C}$ values (colour legend as in C).

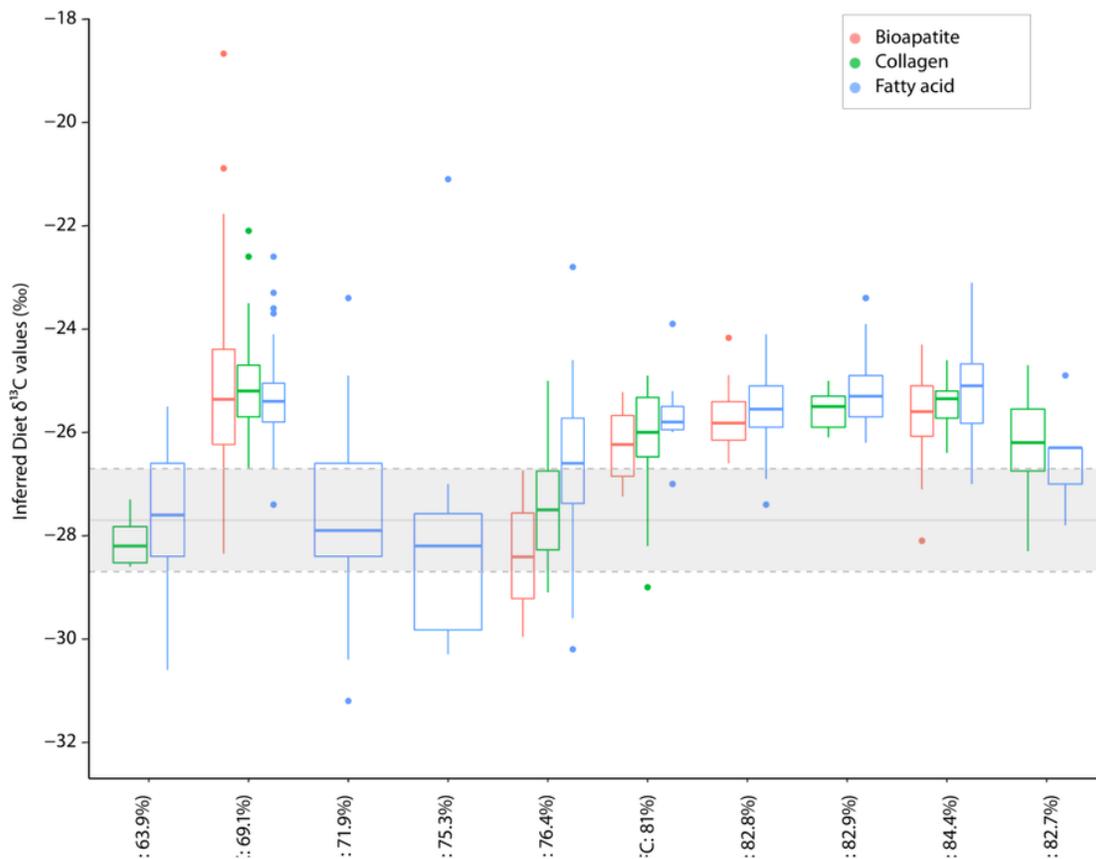


Figure 3

Boxplot of diet $\delta^{13}\text{C}$ values based on bone collagen, bioapatite (max and min) and dairy lipids for sites within each river basin, ordered according to the median MFC for the basin (in brackets).

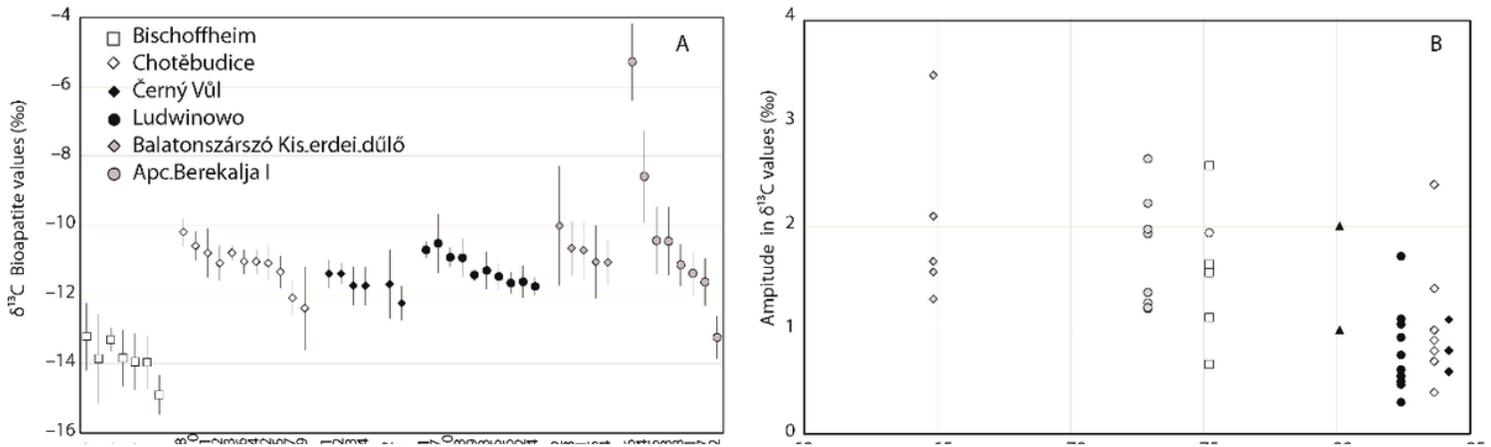


Figure 4

A. Mid-range, highest and lowest $\delta^{13}\text{C}$ values per tooth sampled from Bischoffsheim (BIS), Chotěbudice (CHO)^{28,47}, Černý Vůl²⁸ (CER), Těšetice-Kyjovice (TES), Ludwinowo 7 (LUD)⁴⁶, Apc-Berekalja I (APC) and Balatonszárszó (BAL). The grey box marks the corrected bioapatite value of -13.2‰ for a forest dwelling ruminant based on deer bone collagen data; B. Range in dietary β ($\Delta^{15}\text{N}_{\text{Glx-Phe}}$) values for each sampled cattle tooth, with the grey box marking the upper limit of the reference for woody plants ($-9.3 \pm 1.6\text{‰}$ ³⁸); C. Amplitude in $\delta^{13}\text{C}$ values ($\max \delta^{13}\text{C}_{\text{bioap}} - \min \delta^{13}\text{C}_{\text{bioap}}$) from individual sampled teeth in comparison to mean modelled forest cover (MFC); D. Amplitude in β values per cattle tooth sampled from Bischoffsheim (BIS), Ludwinowo 7 (LUD), Apc-Berekalja I (APC) and Balatonszárszó-Kis-erdei-dűlű (BAL) in comparison to MFC.

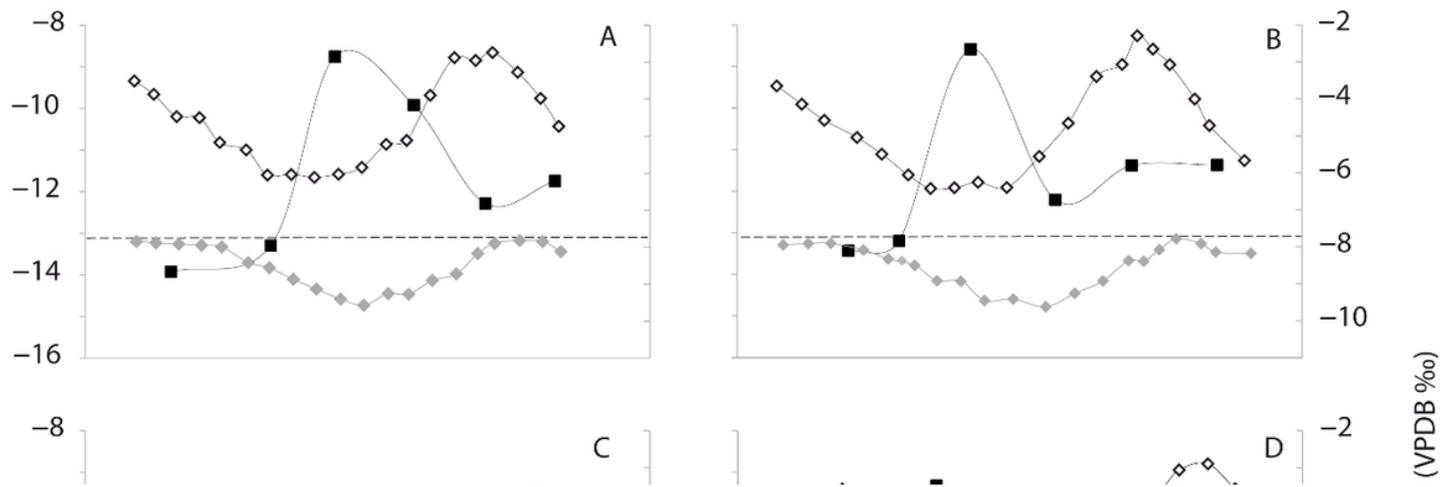


Figure 5

5A-F: Combined stable isotopic results of incremental analysis of bioapatite ($\delta^{13}\text{C}$ (grey diamonds) and $\delta^{18}\text{O}$ (white diamonds)) and dentine (dietary β values are black filled squares) from cattle teeth samples: A. BIS3; B. BIS4; C. LUD1; D. APC1; E. BAL3; F. BAL5. The black dotted line is the upper limit of both dietary β values for woody plants and hypothetical $\delta^{13}\text{C}$ value for forest based on contemporary deer collagen samples (-13.2‰ , based on -27.7‰ adjusted for $\Delta_{\text{bioap-diet}}$ by -14.5‰^{53}).

Supplementary Files

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