European Bison as a Refugee Species? Evidence from Isotopic Data on Early Holocene Bison and Other Large Herbivores in Northern Europe

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Abstract

According to the refugee species concept, increasing replacement of open steppe by forest cover after the last glacial period and human pressure had together forced European bison (Bison bonasus)—the largest extant terrestrial mammal of Europe—into forests as a refuge habitat. The consequent decreased fitness and population density led to the gradual extinction of the species. Understanding the pre-refugee ecology of the species may help its conservation management and ensure its long time survival. In view of this, we investigated the abundance of stable isotopes (δ13C and δ15N) in radiocarbon dated skeletal remains of European bison and other large herbivores—aurochs (Bos primigenius), moose (Alces alces), and reindeer (Rangifer tarandus)—from the Early Holocene of northern Europe to reconstruct their dietary habits and pattern of habitat use in conditions of low human influence. Carbon and nitrogen isotopic compositions in collagen of the ungulate species in northern central Europe during the Early Holocene showed significant differences in the habitat use and the diet of these herbivores. The values of the δ13C and δ15N isotopes reflected the use of open habitats by bison, with their diet intermediate between that of aurochs (grazer) and of moose (browser). Our results show that, despite the partial overlap in carbon and nitrogen isotopic values of some species, Early Holocene large ungulates avoided competition by selection of different habitats or different food sources within similar environments. Although Early Holocene bison and Late Pleistocene steppe bison utilized open habitats, their diets were significantly different, as reflected by their δ15N values. Additional isotopic analyses show that modern populations of European bison utilize much more forested habitats than Early Holocene bison, which supports the refugee status of the species.
Introduction

The transition between the Pleistocene and the Holocene witnessed in Europe the extinction of several large mammalian herbivores, such as steppe bison *Bison priscus*, woolly mammoth *Mammuthus primigenius*, woolly rhinoceros *Coelodonta antiquitatis* and giant deer *Megaloceros giganteus*, although these species had survived there previously several similar climatic and vegetational oscillations during the Pleistocene [1], [2], [3]. At the end of the Pleistocene (c.a. 13,000 yrs BP) the area now forming Denmark, northern Germany and southern Sweden was already de-glaciated and about three thousand years later it was inhabited by survivors of the mega-herbivore community: European bison (*Bison bonasus*), aurochs (*Bos primigenius*), moose (*Alces alces*) and reindeer (*Rangifer tarandus*) [1], [4]. The earliest Holocene fossil remains of European bison, from between 12,000±600 and 10,022 ± 229 cal BP, were found in southern Scandinavia and northern Germany and this species is considered the likely niche successor (ecological replacement) of the extinct steppe bison *Bison priscus*, which was widespread in the Pleistocene [2] and exhibited high morphological similarities with modern European bison [5]. Later in the Holocene, bison and other large herbivores faced severe environmental changes, first in effect of Early Holocene forest expansion and later due to deforestation and the demographic explosion of human population related to Neolithic agriculture spread, which began around 7500 years ago [6], [7], [8]. Infrequent records dating to the following millennia show that the bison became extinct in southern Scandinavia but still occurred in Central Europe [5]. Over the last thousand years it has become a very rare species in the western part of the Central European lowlands, and by the 19th century the species survived only in a few isolated Eastern European pockets. European bison eventually became extinct in the wild at the beginning of the 20th century [9], but were restored to the wild from captive survivors and today occur in over 30 isolated locations in Eastern Europe [10], together with one population in Western Europe [11]. Aurochs, the second largest herbivore to survive in Europe until the Holocene, became extinct in 1627 [12]. European moose populations also experienced numerous range reductions and fragmentations in the past, but this species has returned to most of its original range in Europe during the 20th century [13], [14].

The habitat of the European bison, the largest wild mammal living in Europe today, is traditionally considered to be forest. This notion comes from its survival in this type of environment during historical times [15], [16]. However, several lines of evidence suggest that European bison probably evolved in grasslands or mixed habitats [17]. These include some morphological features of the species, such as a wide muzzle, hypsodont teeth, and length of the anterior part of the jaw, which would confirm its adaptation to graze in open environments [18], as well as its dietary habits [19]. It has been proposed that bison is a refugee species and that its survival in the forest habitats may be a reaction to environmental changes and anthropogenic pressure rather than a reflection of the natural habits and food preference of the species [17], [20].

Stable isotopes next to tooth microwear analysis are powerful tools because as showed recently, morphology is not always the best indicator of the type of feeding strategies of ungulates [21], [22], [23]. It is known that carbon and nitrogen isotopic compositions of an animal body reflect the isotopic compositions of its diet and the type of foraging habitat, with a shift (isotope fractionation) whose value depends on the considered tissue [24], [25], [26], [27]. This approach has been used to document recent ecological changes in a range of species and also for the reconstruction of their diet and habitat use in the past [28], [29]. In addition, stable isotope analyses provide an unprecedented opportunity to explore the relationship between different species at present, as well as in the past, to identify interspecific resource partitioning or food and niche competition [30], [31], [32], [33], [34]. Comparison of the isotopic composition of fossil and modern material will not only provide similar information on the European bison
and other large herbivores, but also may indicate the pre-refugee ecology of this large herbivore. Such insights would certainly help to improve the strategies for the conservation of this endangered species.

Investigations of herbivores’ habitats in temperate and boreal ecosystems of Europe are based on the differences in $\delta^{13}C$ of C3 plants growing in different environmental conditions. Since the dense understory is poorly ventilated, the decomposition of leaf litter provides depleted CO$_2$, and the intensity of light reaching the forest floor is decreased, plants growing under a shade-crown dense canopy exhibit depleted $\delta^{13}C$ abundance [35], [36], in comparison to plants growing in more open habitats [37], [38], [39]. This canopy effect was observed in plants and its impact on the carbon isotopic composition in animal tissues was confirmed by investigations of modern populations of ungulates [40]. It can also be applied to the reconstruction of habitat use by animals in the past [34], [40], [41], [42], [43], [44], [45].

Both carbon and nitrogen isotopic compositions are essential for the interpretation of feeding strategy and for the tracking of competitive behavior between animals, because plant groups differ in the isotopic ratios of both chemical elements in a specific manner. As previously stated, $\delta^{13}C$ values depend essentially on the density of vegetation cover. In addition, more arid conditions tend to increase the $\delta^{13}C$ values of C3 plants [46]. Higher $\delta^{15}N$ values in graminoids (grasses and sedges) than in shrubs and trees [30], [47], [48] allow the separation of grazing and browsing species. In boreal forest ecosystems, the $\delta^{15}N$ values are higher in non-mycorrhizal plants such as graminoids, herbs, forbs, clubmosses, than in ectomycorrhizal and ericoid plants, such as trees and shrubs. Intermediate values of $\delta^{15}N$ are found in mosses which, unlike most vascular plants, can rely on different sources of nitrogen, as well as in lichens [49], [50], [51], [52] (see also S1 Text). Even after standardization to a common mean annual temperature, non-mycorrhizal plants had the highest average $\delta^{15}N$ [53]. The same patterns were found in open peatland, sub-arctic and arctic tundra [47], [54], [55]. Because nitrogen isotopic composition of plants may also vary due to a range of factors, such as aridity, temperature, precipitation, soil activity, maturity and acidity [48], [56], [57], investigation of herbivorous species other than bison co-occurring in the study area are needed to track differences in the diet and differentiate between browsing and grazing among herbivores.

Different studies, including those which exploit isotopic composition, have shown that the moose is today mainly a browser consuming leaves of trees and shrubs [43], [58], [59]. In turn, reindeer is known as a grazer in arctic-alpine ecosystems and also as a lichen eating species [60], [61]. Although European bison is traditionally recognized as a forest species and was re-introduced into such habitats [17], its diet is mixed, including leaves and grass, and may change seasonally [19], [62]. Extinct aurochs has been described as a grazer, especially for the Preboreal period [12], [63], [64]. Therefore, having carbon and nitrogen isotope data from coeval aurochs and moose, representing the end-members of grazing and browsing behavior respectively, will allow us to establish the level of browsing versus grazing for the bison in which we are interested.

The current study aims to reconstruct the diet and habitat utilization of the earliest Holocene European bison and accompanying species from northern Central Europe, using carbon and nitrogen isotopic compositions. We wanted to test whether European bison was originally a grazer inhabiting open habitats, as predicted by its morphological adaptations and evolutionary history, and can be considered a refugee in forest habitats.
Materials and Methods

Sample collection

The material used for the present study was collected from museum collections in The Natural History Museum of Denmark, University of Copenhagen (Denmark), Centre for Baltic and Scandinavian Archaeology (Germany) and Museum of Zoology, Lund University (MZLU) (Sweden), Carpathian Wildlife Research Station at Ustrzyki Dolne (Poland) and from the literature. In total five bones belonging to *Bison bonasus* were sampled for analysis of carbon and nitrogen stable isotope ratios of their bone collagen (Table 1). Four of them (two from Schleswig-Holstein in northern Germany and two from Denmark), have previously been radiocarbon dated between 10,070±50 and 8970±75 BP (11,642±229–10,022±229 cal BP) [65], [66], [67], [68]. The fifth bison bone from Denmark has been indirectly dated, from its stratigraphic context, to the same period [66] (Table 1, Fig. 1). This material represents most of the skeletal material available for the European bison in north central Europe during the early Holocene [5].

Data obtained for bison were compared with those from two other large ungulates: moose and aurochs. These bone samples were collected from Denmark and southwestern Sweden (Fig. 1) and dated from 11,702±446 cal BP to 10,160±387 cal BP 14C BP (Table 1). Some data on aurochs remains from the same age range (N = 5) were additionally taken from a paper by Noe-Nygaard et al. [69]. Carbon isotopic data from 16 early Holocene reindeer antlers from Denmark, published by Aaris-Sørensen et al. [4], together with seven dated moose and one dated aurochs from Denmark, published by Jessen et al. [70], have been used to provide an additional proxy of the environment (S1 Table). Reference material also included previously published isotopic data obtained from steppe bison (N = 14) and aurochs (N = 9) from Paleolithic sites located in river valleys in Belgium [71] and France [72], dated from the Late Pleistocene (S2 Table).

Early Holocene bison were also compared with material from two modern European bison populations in Poland, from the Bieszczady Mountains and Białowieża Primeval Forest, where they live in different types of forested landscape, as well as with one American bison (*Bison bison bison*) population from Prince Albert National Park in Saskatchewan, Canada (S3 Table). Original data from Bieszczady Mountains (N = 5) represent the European bison population with the highest forest utilization (70–90% of home ranges utilized) [73], [74] and low exploitation of winter supplementation. The bison population from Białowieża Primeval Forest receives fodder in the winter in the form of hay provided at feeding stations spread in the forest [9], [19], [75]. Isotopic data published already by Drucker et al. [40], for six individuals of European bison from Białowieża Primeval Forest and for nine American plains bison from Prince Albert National Park, were also used (S3 Table). The range of the Canadian plains bison includes forests (85%), meadows (10%) and water sources (5%), but bison from this population forage mainly in open meadows [76], [77].

Sample preparation and analysis

Small samples (< 1 g) were sawn from previously identified bone remains. After cleaning with acetone and water in an ultrasound bath, the pieces were crushed to a powder of 0.7 mm grain size with a mortar and pestle and sieved. Preparation of collagen was performed following the method published by Bocherens et al. [78].

The elemental and isotopic measurements were performed at the Department of Geosciences at the University of Tübingen (Germany), using an elemental analyzer NC 2500 connected to a Thermo Quest Delta+XL mass spectrometer. The isotopic ratios are expressed
### Table 1. Results of the isotopic analysis of the collagen extracted from bones of bison, aurochs and moose, selected from different sites in northern central Europe with information on chronology and direct datings.

| Lab-no | Species            | Country     | Site          | Chronozone | Age (14C yr BP) | Calibrated age (cal yr BP) | δ13C (‰) | δ15N (‰) | C/N | %C | %Nc | δ13C (%) | δ15N (%) |
|--------|--------------------|-------------|---------------|-------------|----------------|--------------------------|----------|----------|-----|-----|-----|----------|----------|
| BIS-12 | Bison bonasus      | Germany     | Stellmoor     | Boreal      | 8970 ± 75     | 10,022 ± 229            | -20.7    | 4.0      | 3.4 | 41.6 | 14.4 | -20.7    | 4.0      |
| BIS-13 | Bison bonasus      | Germany     | Stellmoor     | Younger Dryas | 10,070 ± 50  | 11,642 ± 229          | -19.7    | 3.3      | 3.3 | 41.0 | 14.3 | -19.7    | 2.8      |
| 73 BP  | Bison bonasus      | Denmark     | Akkerup Mose  | Preboreal   | 9540 ± 85     | 10,882 ± 286           | -19.6    | 2.9      | 3.3 | 39.4 | 13.8 | -19.6    | 2.9      |
| 74 BP  | Bison bonasus      | Denmark     | Harmdrup      | Younger Dryas | 12,000 ± 600  |                      | -19.6    | 3.3      | 3.3 | 39.4 | 13.9 | -19.6    | 3.5      |
| 75 BP  | Bison bonasus      | Denmark     | Jarmsted Mose | Preboreal   | 10,000 ± 80   | 11,528 ± 288           | -20.0    | 3.5      | 3.4 | 37.5 | 12.7 | -20.0    | 3.5      |
| 77 TP  | Bos primigenius    | Sweden      | Frörum Mosse  | Younger Dryas | 10,120 ±120  | 11,702 ± 446          | -22.6    | 6.0      | 3.2 | 40.8 | 14.8 | -22.6    | 6.0      |
| 78 AP  | Alces alces        | Denmark     | Lunggard      | Preboreal   | 9050 ± 125    | 10,160 ± 387          | -10.9    | 2.0      | 3.2 | 30.2 | 10.9 | -10.9    | 2.0      |
| 79 AP  | Alces alces        | Denmark     | Tved          | Preboreal   | 9460 ± 145    | 10,743 ± 442          | -10.7    | 2.0      | 3.3 | 30.0 | 10.7 | -10.7    | 2.0      |
| 80 AP  | Alces alces        | Denmark     | Anhøj Myr     | Preboreal   | 9190 ± 130    | 10,343 ± 395          | 9.7      | 1.5      | 3.3 | 27.0 | 9.7  | -9.7     | 1.5      |
| 81 AP  | Alces alces        | Denmark     | Rutsker, Bornholm | Preboreal | 9720 ± 135    | 11,134 ± 468          | -21.7    | 3.2      | 3.3 | 38.7 | 13.6 | -21.7    | 3.2      |

*a*The following chronozones are used Younger Dryas (12,900–11,600 cal BP), Preboreal (11,600–10,640 cal. BP).

*b*The information about datings was previously published by Hedges et al. [65], Bratlund [67], Aaris-Sørensen et al. [4] and Aaris-Sørensen [66].

*Radiocarbon dates were calibrated using OxCal v4.2.3 with IntCal13 atmospheric curve [83], [84].

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using the “δ” (delta) value as follows:

\[ \delta^{13}C = \left( \frac{^{13}C/^{12}C}_{\text{sample}} \right) - 1 \times 1000 (\%o) \]

and

\[ \delta^{15}N = \left( \frac{^{15}N/^{14}N}_{\text{sample}} \right) - 1 \times 1000 (\%o) \]
with the international reference being V-PDB for \(\delta^{13}C\) values and atmospheric nitrogen (AIR) for \(\delta^{15}N\) values. Samples were calibrated to \(\delta^{13}C\) values of USGS24 (\(\delta^{13}C = -16.00\)%o) and to \(\delta^{15}N\) values of IAEA 305A (\(\delta^{15}N = 39.80\)%o). The reproducibility was \(\pm 0.1\)%o for \(\delta^{13}C\) measurements and \(\pm 0.2\)%o for \(\delta^{15}N\) measurements, based on multiple analysis of purified collagen from modern bones.

The reliability of the isotopic signatures of the collagen extracts was addressed using their chemical composition. Only extracts with %C, %N, and C/N similar to those of collagen extracted from fresh bone should be considered reliable for isotopic measurements. Several studies have shown that collagen with atomic C/N ratios lower than 2.9 or higher than 3.6 are altered or contaminated, and should be discarded [79], [80]. Extracts with 2.9 < C/N < 3.6 and %N < 5% may also be problematic [80] and should be excluded from further palaeobiological interpretations as well.

The \(\delta^{13}C\) values measured on modern American and European bison material have been corrected for the shift due to anthropogenic CO2 emissions using the formula \(\delta^{13}C_{\text{atm}} = -6.429 - 0.0060 \times \exp \left(0.0217(t-1740)\right)\) from Feng [81] and set to a \(\delta^{13}C\) value of atmospheric CO2 of -6.429%o, according to the actual date of death of the modern individuals of bison. No correction were made between Holocene and Pleistocene carbon isotopic values since the \(\delta^{13}C\) values of atmospheric CO2 have been shown recently to be comparable through the last 25,000 years until industrial development in the 19th century AD [82].

All radiocarbon dates were calibrated using OxCal v4.2.3 [80] using the IntCal13 atmospheric curve [83], [84]. All dates were calibrated to BP dates with 2\(\sigma\) (95.4%) probability. We used non-parametric U Mann-Whitney test to verify the statistical significance of carbon and nitrogen isotopic differences (STATISTICA, StatSoft Software, Version 9.0).

Results

The Early Holocene European bison isotopic composition ranged from -20.7 to -19.6%o for \(\delta^{13}C\) and from 2.8 to 4.0%o for \(\delta^{15}N\) values (Table 1, Fig. 2). For the moose, the \(\delta^{13}C\) values ranged from -22.6 to -20.1%o, while the \(\delta^{15}N\) values ranged from 1.5 to 3.2%o (Table 1, S1 Table, Fig. 2). In the case of the aurochs, the \(\delta^{13}C\) values ranged widely from -22.6 to -19.0%o, while the \(\delta^{15}N\) values ranged from 4.0 to 6.1%o (Table 1, S1 Table, Fig. 2). The largest data scatter, from -21.7 to -17.8%o in \(\delta^{13}C\), occurred in Holocene reindeer (S1 Table, Fig. 3). Late Pleistocene Bovinae from Belgium and France had the smallest variation in \(\delta^{13}C\) concentration, ranging from -19.8 to -20.9 %o (Fig. 4, S2 Table). Early Holocene bison and aurochs did not differ significantly in their \(\delta^{13}C\) values, but there were significant differences in their \(\delta^{15}N\) (Table 2, Fig. 3). While European bison and moose \(\delta^{15}N\) patterns did not differ, both species showed significant differences in concentration of \(\delta^{13}C\) isotopes. Early Holocene European bison and reindeer did not differ in \(\delta^{13}C\) values, but the latter species showed significantly higher values than aurochs and moose (Table 2, Fig. 3). Early Holocene aurochs and moose differed in \(\delta^{15}N\), being lower in bison, but not in \(\delta^{13}C\) (Table 2, Fig. 3).

The \(\delta^{15}N\) values for Holocene European bison were significantly lower than those of Late Pleistocene steppe bison but there was little, if any, difference in their \(\delta^{13}C\) values (Table 2, Fig. 4). In the case of aurochs, significantly lower abundance of both isotopes was recorded in Early Holocene in comparison to Pleistocene specimens from France (Table 2, Fig. 4). In contrast, no statistical differences in \(\delta^{13}C\) and \(\delta^{15}N\) isotope abundance were found between bison and aurochs from the Pleistocene, either in Belgium or France (Table 2, Fig. 4).

Early Holocene bison showed significantly higher values of \(\delta^{13}C\) than the contemporary populations of European and American bison (Table 2, Fig. 5).
Fig 2. Stable $\delta^{13}C_{\text{coll}}$ and $\delta^{15}N_{\text{coll}}$ isotope values for moose (Alces alces), aurochs (Bos primigenius) and European bison (Bison bonasus) in Early Holocene in northern Europe. Shaded area denotes range of carbon isotope values characteristic for forest use. Ellipses are plotted in a way to include the extreme points of the range of measured isotopic values and to improve readability of the figure. The data used to generate the figure can be found in Table 1 and S1 Table.

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Fig 3. Box plots of stable $\delta^{13}C_{\text{coll}}$ and $\delta^{15}N_{\text{coll}}$ isotope values measured for Early Holocene moose, aurochs, European bison and reindeer. (* denote statistically significant differences for $p$ between 0.05 and 0.01, **—for $p < 0.01$). Boxes show the median, upper and lower quartiles while, the whiskers show the range of the data. The data used to generate the figure can be found in Table 1 and S1 Table.

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None of the carbon isotopic measurements performed on European bison, aurochs and moose indicate a dense forest habitat for these species during the Early Holocene in northern Europe.

**Discussion**

None of the carbon isotopic measurements performed on European bison, aurochs and moose indicate a dense forest habitat for these species during the Early Holocene in northern Europe.
Germany and most of Denmark. However a few $\delta^{13}$C values slightly lower than -22‰ found in one aurochs from southern Sweden and two moose from Denmark reflect some early stages of forest succession. This is consistent with vegetational reconstructions that indicate relatively open landscape in Denmark and northern Germany during this period [70], [85], [86], [87]. Geological, pollen-botanical and zoological investigations have shown that northern Germany and the south of Denmark was a park tundra (steppe-tundra and forest-steppe with birch and pine), while the north-west of Denmark was covered by open tundra dominated by dwarf shrubs and willows [88]. Tundra and steppe animals such as reindeer, wild horse, aurochs and bison grazed in open conditions, which persisted throughout the first two millennia of the Holocene, especially on fine grained soils, where seasonal dryness prevented the establishment of deciduous trees [89]. However, the open forest vegetation had become attractive to forest animals including giant deer, which has been recorded from Denmark at that time [66], [88], and moose, as reflected in two of the most negative carbon isotope values (-22.6 and -22.2‰) that were measured for this species from the Preboreal, i.e. the Early Holocene period. Indeed, the beginning of the Holocene sees a global warming trend after the cold spell of the Younger

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**Table 2. Pairwise differences for $\delta^{13}$C carbon and $\delta^{15}$N nitrogen isotope values between species in Early Holocene and Pleistocene and between samples of modern Bison bonasus (Poland) and Bison bison (Prince Albert National Park, Canada) as well as between modern and Early Holocene Bison.**

| Species Comparison | N:N | $\delta^{13}$C | $\delta^{15}$N |
|--------------------|-----|----------------|----------------|
| **EARLY HOLOCENE SPECIES COMPARISONS** | | | |
| Bos vs. Alces | 6:11 | 0.182 | 0.014 |
| Bison vs. Alces | 5:11 | 0.005 | 0.064 |
| Rangifer vs. Alces | 16:11 | 0.001 | - |
| Bos vs. Bison | 6:5 | 0.061 | 0.010 |
| Rangifer vs. Bos | 16:6 | 0.008 | - |
| Bison vs. Rangifer | 5:16 | 0.098 | - |
| **PLEISTOCENE SPECIES COMPARISONS** | | | |
| Bos vs. Bison (Belgium) | 3:6 | 0.897 | 0.699 |
| Bos vs. Bison (France) | 6:8 | 0.366 | 0.061 |
| Bos (Belgium) vs. Bos (France) | 3:6 | 1.000 | 0.028 |
| Bison (France) vs. Bison (Belgium) | 8:6 | 0.366 | 0.107 |
| Bison (France) vs. Bison (Early Holocene) | 8:5 | 0.048 | 0.004 |
| Bison (Belgium) vs. Bison (Early Holocene) | 6:5 | 0.171 | 0.014 |
| Bos (France) vs. Bos (Early Holocene) | 6:6 | 0.045 | 0.027 |
| Bos (Belgium) vs. Bos (Early Holocene) | 3:6 | 0.137 | 0.305 |
| **INTER-REGION MODERN BISON COMPARISONS** | | | |
| Bieszczady Mountains vs. Prince Albert National Park | 5:9 | 0.003 | - |
| Białowieża Forest vs. Bieszczady Mountains | 6:5 | 0.068 | - |
| Prince Albert National Park vs. Białowieża Forest | 9:6 | 0.289 | - |
| **MODERN WITH HOLOCENE BISON COMPARISONS** | | | |
| Bieszczady Mountains vs. Early Holocene Bison | 5:5 | 0.012 | - |
| Białowieża Forest vs. Early Holocene Bison | 6:5 | 0.008 | - |
| Prince Albert National Park vs. Early Holocene Bison | 9:5 | 0.009 | - |

Insignificance at $p > 0.05$ is indicated in normal font for Mann-Whitney—Wilcoxon test. Bold indicates significant differences. Abbreviations: N = number of specimens.

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Dryas and an afforestation of the former tundra landscape in the northern part of central Europe [90]. The pattern of isotopic variation among the ungulate species in northern central Europe during the Early Holocene indicates that were significant differences in habitat use and diet between the bison and the other ungulates. In contrast with the Pleistocene, when aurochs and bison strongly overlapped in their patterns of habitat use and diet, these bovines diversified ecologically as a result of the successional stages of habitats and vegetation during the early Holocene climate warming, which created a mosaic habitat with diversified vegetation. This probably allowed them to avoid competition by a separation of their food or habitat niches. Holocene bison and aurochs occupied quite similar habitats, with a wider range of habitat types for aurochs as suggested by carbon isotopes, but they differed in their respective diets. Bison and moose utilized different habitats as indicated by their δ^{13}C values, but their diet as reflected in δ^{15}N was quite similar, although more woody in moose. Bison may therefore be characterized as an intermediate feeder during the Early Holocene, as also observed in forest habitats now [19]. According to nitrogen isotopes, Holocene moose might be

![Box plots of stable δ^{13}C_core values for Early Holocene Bison bonasus, compared to modern populations of European (Bison bonasus) and American bison (Bison bison bison) living in forest habitats. (* denote statistically significant differences for p between 0.05 and 0.01, **— for p < 0.01). Boxes show the median, upper and lower quartiles, while the whiskers show the range of the data. The data used to generate the figure can be found in Table 1 and S3 Table.](https://doi.org/10.1371/journal.pone.0115090.g005)
characterised as a typical browser, as also shown by microwear analyses performed on Pleistocene specimens [91]. Isotopic results are consistent with investigations of contemporary populations of these two species, as well as with δ¹³C depletion in the shrub and woody diet preferred by moose [47], [48], [58] when compared to the mixed diet of bison [62], [92]. Interestingly, when aurochs, moose and bison did not differ in the isotope concentration of one element, carbon or nitrogen, they differed in the second one. Our results show that, despite the partial overlap in carbon and nitrogen isotopic values in Early Holocene ungulates, they avoided competition by selecting different habitats or different food sources within similar habitats, a phenomenon that has also been observed among other Pleistocene ungulates [33], [34], as well as in modern populations of herbivores studied with stable isotopes [32], [93].

As expected, reindeer exhibited the least negative δ¹³C values, as reported for this species during the late Pleistocene and recent times, due to its foraging on lichen [45], [94], [95], a plant with δ¹³C values higher than those of vascular plants [96], [97], [98]. The best explanation for the scatter in Holocene reindeer being the largest among the studied herbivores is variability and seasonality in the diet of this species. At present, reindeer summer diet is composed of shrubs and includes a wide range of vascular plants and a lower share of lichen, while in winter, their diet is dominated by lichens, evergreen shrubs and mosses [94], [95], [99], [100]. The present study also confirms previously detected niche partitioning between A. alces and R. tarandus [30], [43]. Although Early Holocene bison exhibit on average higher δ¹³C values than those of most other ungulates from the same period, the fact that the δ¹³C values of bison are not significantly different from the δ¹³C values of reindeer may indicate that these bison did consume some lichens, unlike moose and aurochs. Similar food habits are observed in modern American bison in boreal forests [101], [102].

A comparison of European bison with aurochs and steppe bison can help us to better define the pre-refugee ecology of European bison. During the Pleistocene, aurochs and steppe bison occurred together at many sites, but their skeletal similarity makes them difficult to distinguish [103], [104], [105], so that remains of the two species are very often grouped together as Bos/Bison. In two cases, it was nevertheless possible to obtain isotopic values for steppe bison and aurochs in the same area at the same time, once in Southwestern France [72] and once in Belgium [71], both from times around 30,000 to 40,000 years ago. The δ¹³C values are directly comparable with those of Early Holocene bovines since recent investigations of changes in isotopic composition of atmospheric CO₂ revealed that they are very similar between the Holocene and Pleistocene, in contrast to previous evaluations [82]. In contrast with the observed difference in δ¹⁵N values between bison and aurochs in the Holocene, there were no differences in their δ¹⁵N values when steppe bison and aurochs occurred together in the Late Pleistocene, reflecting a grazing diet for both large bovine species [71], [72]. Collagen δ¹³C values for both species were in the ranges of values characteristic for grazing on graminoids and forbs, when measured for these plants in arctic tundra [47], [48]. However, aurochs preference for a grazing diet differ from the results obtained by microwear analysis for Pleistocene specimens (45,000–34,000 BP, North Sea, Brown Bank), where this species has been recognized as a browse-dominated mixed feeder or mixed feeder, while the same study partly confirms the domination of grasses in the steppe bison diet and characterizes it as a grass-dominated mixed feeder [91]. A relatively homogeneous ecosystem, with no dense canopy, was present in the periglacial area [34] and the narrow choice of different habitats probably forced Bison and Bos to share the same dietary niche. Distinctive environmental conditions between glacial (Pleistocene) and interglacial (Holocene) periods probably reflect changing competitive relationships between co-occurring Bovinae species over the millennia.

Differences in δ¹⁵N values between European bison and Pleistocene steppe bison from Belgian and French sites suggest a higher content of shrubs in the diet of Holocene bison than in
Pleistocene steppe bison. However, the diet of steppe bison was probably not totally free of woody vegetation, since some individuals, such as the bison bull mummy "Blue Babe" from Alaska (which lived in the Mid-Wisconsin warming interstadial around 36,425 + 2575/-1974 BP (QC-891)), had 7% of woody material among the plant fragments trapped in its teeth [106]. The diet of the steppe bison in our study was probably exclusively composed of grasses or sedges and forbs, due to the limitation of browse vegetation during the glacial period.

When compared with modern bison populations living in forested landscapes in Europe and North America, the Early Holocene bison exhibit the highest $\delta^{13}C$ values. During the Early Holocene, the habitat use of bison was significantly different from that of all the extant forest bison populations, including those from boreal forest or those supplemented by hay during the winter. This supports the refugee status of the European bison as proposed by Kerley et al [17]. For both bison and aurochs, forested areas were probably marginal or sub-optimal habitats that allowed them to survive only at lower densities and led to a declining fitness as predicted by Fretwell’s model [107]. Indeed, a progressive disappearance of both species from large areas of Europe was observed during the Holocene [5], [108]. However, wide range of stable isotopes values recorded in aurochs and bison (combined Early Holocene and modern populations of the species) indicate plasticity of both species that probably allowed them to adapt to the environmental changes that took place over thousands of years and survive much longer than other species of mega herbivores.

Conclusions

This study is one of the first examples of the application of conservation palaeobiology in Europe. As stated by Dietl and Flessa [109], the fossil record can be used to understand ecological and evolutionary responses of species to changes in their environment. Thus, comparison of the isotopic composition of early Holocene and modern material for European bison provides evidence essential for bison conservation management.

According to results of this study, the European bison of early Holocene north central Europe was living in a relatively open tundra-like environment. It consumed some browse and probably also lichen and it can therefore be defined as a mixed feeder. The study demonstrates also that the species exhibited some niche partitioning with aurochs and moose, but at the same time avoided competition by different use of resources and space. In the course of the Holocene, the spread of more densely forested landscape did not prevent this species, which was pre-adapted to consume browse, from surviving. However, it did increase the risk of its extinction, as predicted by the refugee species concept [17]. Understanding the pre-refugee ecology of bison and its ability to live in relatively open habitats may open new perspectives for reintroducing this endangered species in habitats other than forests, as suggested recently [17], [20]. It is questionable whether bison would be able to survive in forests in larger populations without human support. Forest habitats offer sufficient biomass of graze in summer, however, mainly browse in winter. In a forested area the bison needs access to highly productive open river valleys or meadows which offers a high biomass of dry fodder in winter. Without such an access, or without human support as currently offered [17], the species probably would not survive in forest habitats, or at least those that occur today in Europe. The introduction of bison to forest habitats sooner or later results in an expansion of their range to involve more open habitats [110] and concomitant damage to farm crops in surrounding areas [75]. In the light of this study, bison conservation management and reintroduction programme require improvement to take into account pre-refugee ecology of the species and its response to environmental changes in course of the Holocene. Inclusion of geohistorical data as proposed by Dietl and Flessa [109] in decision making process may provide a more scientifically robust basis for
bison conservation policies than those dependent on short-term observations recognizing bison as a forest specialist.

Supporting Information

S1 Table. Isotopic analysis data of the collagen extracted from bones of moose, aurochs, and reindeer, selected from published studies. Radiocarbon dates were calibrated using OxCal v4.2.3 with IntCal13 atmospheric curve [83], [84]. The following chronozones are used: Younger Dryas (12,900–11,600 cal BP), Preboreal (11,600–10,640 cal. BP). The information about datings was previously published by Aaris-Sørensen et al. [4], Noe-Nygaard [69] and Jessen et al. [70].

S2 Table. List of isotopic measurements of Bison priscus and Bos primigenius from Pleistocene. The data have been published previously by Bocherens et al. [71], [72].

S3 Table. Description of modern ecosystems inhabited by European bison Bison bonasus and American bison Bison bison and measured isotopic values for these species with corrections of δ13C values for the shift due to anthropogenic CO2 emissions. Corrections were made using the equation from Feng [81]. Δ13C atm - the difference in the atmospheric CO2 δ13C value according to the date of the animal death, δ13C corrected - values correspond to the δ13C coll values set to the same atmospheric CO2 δ13C of -6.429‰. Values of hair from Bison bison have been adjusted for collagen-hair carbon isotopic fractionation [40].

S1 Text. Paleobiological tracking of herbivorous mammal paleoecology using carbon and nitrogen isotopes in bone collagen.

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Author Contributions

Conceived and designed the experiments: EHK RK HB DGD. Performed the experiments: EHK HB DGD RK. Analyzed the data: EHK HB RK. Contributed reagents/materials/analysis tools: EHK HB RK US DGD. Wrote the paper: EHK HB RK.

References

1. Stuart AJ (1991) Mammalian extinctions in the Late Pleistocene of northern Eurasia and North America. Biological Review of the Cambridge Philosophical Society 66: 453–562. PMID: 1801948
2. Stuart AJ (1999) Late Pleistocene megafaunal extinctions: a European perspective. In: Extinctions in Near Time: Causes, Contexts, and Consequences (ed. MacPhee R. D. E.). New York: Kluwer Academic/Plenum. pp. 257–269.

3. Turvey ST (2009) In the shadow of the megafauna: prehistoric mammal and bird extinctions across the Holocene. In: Turvey ST, editor. Holocene Extinctions. Oxford University Press, Oxford. pp. 17–39.

4. Aaris-Sørensen K, Mühldorff R, Petersen EB (2007) The Scandinavian reindeer (Rangifer tarandus L.) after the last glacial maximum: time, seasonality and human exploitation. Journal of Archaeological Science 34: 914–923.

5. Benecke N (2005) The Holocene distribution of European bison—the archaeozoological record. Munibe (Antropologia-Arkeologia) 57: 421–428.

6. Kowalczyk R, Taberlet P, Coissac E, Valentini A, Miquel C, et al. (2011) Predicting potential European bison habitat across its former range. Ecological Applications 21: 830–843. PMID: 21639048

7. Kumerle T, Perzanowski K, Chaskovsky K, Halda L, et al. (2010) European bison habitat in the Carpathian Mountains. Biological Conservation 143: 908–916.

8. Deinet S, Ieronymidou C, McRae L, Burfield IJ, Foppen RP, et al. (2013) Wildlife comeback in Europe: The recovery of selected mammal and bird species. Zoological Society of London. 312 p. doi: 10.1007/s12070-013-0687-x PMID: 25621273

9. Kowalczyk R, Taberlet P, Coissac E, Valentini A, Miquel C, et al. (2011) Influence of management practices on large herbivore diet—case of European bison in Białowieża Primeval Forest (Poland). Forest Ecology and Management 261: 821–828.

10. Kowalczyk R, Taberlet P, Coissac E, Valentini A, Miquel C, et al. (2011) 3D dental microwear texture analysis of feeding habits of sympatric ruminants in the Białowieża Primeval Forest, Poland. Forest Ecology and Management 328: 262–269.

11. Mendoza M, Palmqvist P (2008) Stable isotopes, hypsodonty, and the paleodiet of Hemiauchenia (Mammalia: Camelidae): a morphological specialization creating ecological generalization. Paleobiology 29: 230–242.

12. Sponheimer M, Lee-Thorp JA, Deruiter DJ, Smith JM, Van Der Merwe NJ, et al. (2003) Diets of Southern African Bovidae: Stable Isotope Evidence. Journal of Mammalogy 84: 471–479.

13. Mecerson G, Hofman-Kamińska E, Kowalczyk R (2014) 3D dental microwear texture analysis of feeding habits of sympatric ruminants in the Białowieża Primeval Forest, Poland. Forest Ecology and Management 328: 262–269.

14. DeNiro MJ, Epstein S (1978) Influence of diet on the distribution of carbon isotopes in animals. Geochimica Et Cosmochimica Acta 42: 495–506.
25. DeNiro MJ, Epstein S (1981) Influence of diet on the distribution of nitrogen isotopes in animals. Geochimica Et Cosmochimica Acta 45: 343–351.

26. Teeri JA, Schoeller DA (1979) Delta-C-13 values of an herbivore and the ratio of C-3 to C-4 plant carbon in its diet. Oecologia 39: 197–200.

27. Schoeninger MJ, DeNiro MJ (1984) Nitrogen and carbon isotopic composition of bone collagen from marine and terrestrial animals. Geochimica Et Cosmochimica Acta 48: 625–639.

28. Pushkina D, Bocherens H, Chaimanee Y, Jaeger JJ, (2010) Stable carbon isotope reconstructions of diet and paleoenvironment from the late Middle Pleistocene Snake Cave in Northeastern Thailand. Naturwissenschaften 97: 299–309. doi: 10.1007/s00114-009-0642-6 PMID: 20127068

29. Crowley BE, Godfrey LR, Guilderson TP, Zermeño P, Koch PL, et al. (2012) Extinction and ecological retreat in a community of primates. Proceedings of the Royal Society B-Biological Sciences 279: 3597–3605. PMID: 22628463

30. Ben-David M, Shochat E, Adams LG (2001) The utility of stable isotope analysis in studying the foraging ecology of herbivores: Examples from moose and caribou. Aces 37: 421–434.

31. Stewart KM, Bowyer RT, Kie JG, Dick BL, Ben-David M (2003) Niche partitioning among mule deer, elk, and cattle: Do stable isotopes reflect dietary niche? Écoscience 10: 297–302.

32. Feranec RS (2007) Stable carbon isotope values reveal evidence of resource partitioning among ungulates from modern C-3-dominated ecosystems in North America. Palaeogeography Palaeoclimatology Palaeoecology 252: 575–585.

33. Feranec RS, Hadly EA, Paytan A (2009) Stable isotopes reveal seasonal competition for resources between late Pleistocene bison (Bison) and horse (Equus) from Rancho La Brea, southern California. Palaeogeography Palaeoclimatology Palaeoecology 271: 153–160.

34. Pushkina D, Bocherens H, Ziegler R (2014) Unexpected palaeoecological features of the Middle and Late Pleistocene large herbivores in southwestern Germany revealed by stable isotopic abundances in tooth enamel. Quaternary International 339: 164–178. http://dx.doi.org/10.1016/j.quaint.2013.12.033

35. Van der Merwe NJ, Medina E (1991) The canopy effect, carbon isotope ratios and foodwebs in Amazonia. Journal of Archaeological Science 18: 249–259.

36. Broadmeadow MSJ, Griffiths H, Maxwell C, Borland AM (1992) The carbon isotope ratio of plant organic material reflects temporal and spatial variations in CO2 within tropical forest formations in Trinidad. Oecologia 89: 435–441.

37. Francey RJ, Farquhar GD (1982) An explanation of C-13/C-12 variations in tree rings. Nature 297: 28–31.

38. Gebauer G, Schulze ED (1991) Carbon and nitrogen isotope ratios in different compartments of a healthy and a declining Picea abies forest in the Fichtelgebirge, NE Bavaria. Oecologia 87: 198–207.

39. Bonafini M, Pellegrini M, Ditchfield P, Pollard AM (2013) Investigation of the ‘canopy effect’ in the isotope ecology of temperate woodlands. Journal of Archaeological Science 40: 3926–3935.

40. Drucker DG, Bridault A, Hobson KA, Szuma E, Bocherens H (2008) Can carbon-13 abundances in large herbivores track canopy effect in temperate and boreal ecosystems? Evidence from modern and ancient ungulates. Palaeogeography Palaeoclimatology Palaeoecology 266: 69–82.

41. Bocherens H, Billiou D, Patou-Mathis M, Otte M, Bonjean D, et al. (1999) Palaeoenvironmental and palaeoecological implications of isotopic biogeochemistry of late interglacial Neandertal and mammal bones in Scladina Cave (Belgium). Journal of Archaeological Science 26: 599–607.

42. Drucker DG, Bocherens H (2009) Carbon stable isotopes of mammal bones as tracers of canopy development and habitat use in temperate and boreal contexts. In: Creighton JD, Roney PJ editors. Forest Canopies: Forest Production, Ecosystem Health, and Climate Conditions Nova Science Publishers. Inc. pp. 103–108.

43. Drucker DG, Hobson KA, Ouellet JP, Courtois R (2010) Influence of forage preferences and habitat use on C-13 and N-15 abundance in wild caribou (Rangifer tarandus caribou) and moose (Alces alces) from Canada. Isotopes in Environmental and Health Studies 46: 107–121. doi: 10.1007/10256010903388410 PMID: 20229388

44. Drucker DG, Bridault A, Cupillard C, Hujic A, Bocherens H (2011) Evolution of habitat and environment of red deer (Cervus elaphus) during the Late-glacial and early Holocene in eastern France (French Jura and the western Alps) using multi-isotope analysis (C-13, N-15, O-18, S-34) of archaeological remains. Quaternary International 245: 268–278.

45. Drucker DG, Kind CJ, Stephan E (2011) Chronological and ecological information on Late-glacial and early Holocene reindeer from northwest Europe using radiocarbon (C-14) and stable isotope (C-13, N-15) analysis of bone collagen: Case study in southwestern Germany. Quaternary International 245: 218–224.
46. Diefendorf AF, Mueller KE, Wing SL, Koch PL, Freeman KH (2010) Global patterns in leaf C-13 discrimination and implications for studies of past and future climate. Proceedings of the National Academy of Sciences of the United States of America 107: 5738–5743. doi: 10.1073/pnas.0910513107 PMID: 20231481

47. Barnett B (1994) Carbon and nitrogen isotopes in caribou tissue, vascular plants, and lichens from northern Alaska. M.Sc. Thesis, The University of Alaska. Fairbanks. Alaska. U.S.A.

48. McLeman CIA (2006) Determining the relationships between forage use, climate and nutritional status of barren ground caribou, Rangifer tarandus groenlandicus, on Southampton Island, Nunavut, using stable isotopes analysis of C-13 and N-15. M.Sc. Thesis in Biology, The University of Waterloo. Ontario. Canada. Available: https://uwspace.uwaterloo.ca/bitstream/handle/10012/2957/clamclem2006.pdf?sequence=1. Accessed 06 June 2014.

49. Schulze ED, Chapin FS, Gebauer G (1994) Nitrogen nutrition and isotope differences among life forms at the northern treeline of Alaska. Oecologia 100: 406–412.

50. Emmerton KS, Callaghan TV, Jones HE, Leake JR, Michelsen A, et al. (2001) Assimilation and isotopic fractionation of nitrogen by mycorrhizal and nonmycorrhizal subarctic plants. New Phytologist 151: 513–524.

51. Hobbie JE, Hobbie EA, Drossman H, Conte M, Weber JC, et al. (2009) Mycorrhizal fungi supply nitrogen to host plants in Arctic tundra and boreal forests: N-15 is the key signal. Canadian Journal of Microbiology 55: 84–94. doi: 10.1139/W08-127 PMID: 19190704

52. Kristensen DK, Kristensen E, Forchhammer MC, Michelsen A, Schmidt NM (2011) Arctic herbivore diet can be inferred from stable carbon and nitrogen isotopes in C3 plants, faeces, and wool. Canadian Journal of Zoology-Revue Canadienne De Zoologie 89: 892–899.

53. Craine JM, Elmore AJ, Aidar MPM, Bustamante M, Dawson TE, et al. (2009) Global patterns of foliar nitrogen isotopes and their relationships with climate, mycorrhizal fungi, foliar nutrient concentrations, and nitrogen availability. New Phytologist 183: 980–992. doi: 10.1111/j.1469-8137.2009.02917.x PMID: 19563444

54. Asada T, Warner BG, Aravena R (2005) Nitrogen isotope signature variability in plant species from open peatland. Aquatic Botany 82: 297–307.

55. Michelsen A, Quarmby C, Sleep D, Jonasson S (1998) Vascular Plant N-15 Natural Abundance in Heath and Forest Tundra Ecosystems Is Closely Correlated with Presence and Type of Mycorrhizal Fungi in Roots. Oecologia 115:406–418.

56. Högberg P (1997) N-15 natural abundance in soil-plant systems. New Phytologist 137: 179–203.

57. Amundson R, Austin AT, Schuur EAG, Yoo K, Matzek V, et al. (2003) Global patterns of the isotopic composition of soil and plant nitrogen. Global Biogeochemical Cycles 17: 1031. doi: 10.1029/2002GB001903

58. Morow K (1976) Food habits of moose from Augustow Forest. Acta Theriologica 21: 101–116.

59. Wam HK, Hjeljord O (2010) Moose summer and winter diets along a large scale gradient of forage availability in southern Norway. European Journal of Wildlife Research 56: 745–755. doi: 10.1007/s10344-010-0370-4

60. Drucker DG, Bridault A, Cupillard C (2012) Environmental context of the Magdalenian settlement in the Jura Mountains using stable isotope tracking (C-13, N-15, S-34) of bone collagen from reindeer (Rangifer tarandus). Quaternary International 272: 273–322. doi: 10.1016/j.quaint.2012.3459227345922.Feasibility PMID: 23365883

61. Pape R, Löffler J (2012) Climate Change, Land Use Conflicts, Predation and Ecological Degradation as Challenges for Reindeer Husbandry in Northern Europe: What do We Really Know After Half a Century of Research? Ambio 41: 421–434. doi: 10.1007/s13280-012-0257-6 PMID: 22451266

62. Gębężyńska Z, Gębężyński M, Martynowicz E (1991) Food eaten by the free-living European bison in Białowieża Forest. Acta Theriologica 36: 307–313.

63. Hofmann RR (1989) Evolutionary steps of ecophysiological adaptation and diversification of ruminants: a comparative view of their digestive system. Oecologia 78: 443–457.

64. Schulz E, Kaiser TM (2007) Feeding strategy of the Ursus Bos primigenius BOJANUS, 1827 from the Holocene of Denmark. Courier Forschungsinstitut Senckenberg 259: 155–164.

65. Hedges REM, Housley RA, Bronk Ramsey C, van Klinken GJ (1993) Radiocarbon dates from the Oxford AMS system: archaeometry datelist 16. Archaeometry 35: 145–167. doi: 10.1111/j.1475-4754.1993.tb01030.x

66. Aaris-Sørensen K (2009) Diversity and dynamics of the mammalian fauna in Denmark throughout the last glacial-interglacial cycle, 115–0 kyr BP. Fossils and Strata 57: 1–59.
67. Bratlund B (1999) A revision of the rarer species from the Ahrensburgian assemblage of Stellmoor. In: Benecke N, editor. The Holocene History of the European Vertebrate Fauna. Archäologie in Eurasien 6. Rahden/Westf. pp. 39–42.
68. Benecke N, Heinrich D (2003) Neue Daten zur Entwicklung der Huftierfauna im Tieflandgebiet zwischen Elbe und Oder im Spätglazial und Altholozän. Archeozoologia 21: 19–36.
69. Noe-Nygaard N, Price TD, Hede SU (2005) Diet of aurochs and early cattle in southern Scandinavia: evidence from N-15 and C-13 stable isotopes (vol 32, pg 855, 2005). Journal of Archaeological Science 32: 1432–1433.
70. Jessen CA, Pedersen KB, Christensen Ch, Olsen J, Mortensen MF, et al. (2014) Early Maglemosian culture in the Preboreal landscape: Archaeology and vegetation from the earliest Mesolithic site in Denmark at Lundby Mose, Sjælland. Quaternary International (In press). http://dx.doi.org/10.1016/j.quaint.2014.03.056
71. Bocherens H, Drucker DG, Bonjean D, Bridault A, Conard NJ, et al. (2011) Isotopic evidence for diet-ecology of cave lion (Panthera spelaea) in North-western Europe: prey choice, competition and implications for extinction. Quaternary International 245: 249–261.
72. Bocherens H, Drucker DG, Billiou D, Patou-Mathis M, Vandermeersch B (2005) Isotopic evidence for diet and subsistence pattern of the Saint-Césaire I Neanderthal: Review and use of a multi-source mixing model. Journal of Human Evolution 49: 71–87. PMID: 15869783
73. Perzanowski KA, Wołoszyń-Gałęza A, Januszczak M (2008) Indicative factors for European bison refuges in the Bieszczady Mountains. Annales Zoologici Fennici 45: 347–352.
74. Perzanowski KA, Januszczak M, Wołoszyń-Gałęza A (2011) The use of open and forested areas by wisents of western subpopulation in the Bieszczady Mts. Roczniki Bieszczadzkie 19: 191–206. (Polish with English summary)
75. Hofman-Kamińska E, Kowalczyk R (2012) Farm crops depredation by European bison (Bison bonasus) in the vicinity of forest habitats in northeastern Poland. Environmental Management 50: 530–541. doi: 10.1007/s00267-012-9913-7 PMID: 22842710
76. Fortin D, Fryxell JM, Pilote R (2002) The temporal scale of foraging decisions in bison. Ecology 83: 970–982.
77. Fortin D, Fryxell JM, O’Brodovich L, Frandsen D (2003) Foraging ecology of bison at the landscape and plant community levels: the applicability of energy maximization principles. Oecologia 134: 219–227. PMID: 12647163
78. Bocherens H, Billiou D., Patou-Mathis M., Bonjean D., Otte M, et al. (1997) Paleobiological Implications of the Isotopic Signatures (C-13, N-15) of Fossil Mammal Collagen in Scladina Cave (Sclayn, Belgium). Quaternary Research 48: 370–380.
79. DeNiro MJ (1985) Postmortem preservation and alteration of in-vivo bone collagen isotope ratios in relation to palaeodietary reconstruction. Nature 317: 806–809.
80. Ambrose SH (1990) Preparation and characterization of bone and tooth collagen for isotopic analysis. Journal of Archaeological Science 17: 431–451.
81. Feng XH (1998) Long-term c(i)/c(a) response of trees in western North America to atmospheric CO2 concentration derived from carbon isotope chronologies. Oecologia 117: 19
82. Schmitt J, Schneider R, Elsig J, Leuenberger D, Lourantou A, et al. (2014) Early Maglemosian culture in the Preboreal landscape: Archaeology and vegetation from the earliest Mesolithic site in Denmark at Lundby Mose, Sjælland. Quaternary International (In press). http://dx.doi.org/10.1016/j.quaint.2014.03.056
83. Reimer PJ, Bard E, Bayliss A, Beck JW, Blackwell PG, et al. (2013) IntCal13 and Marine13 Radiocarbon Age Calibration Curves 0–50,000 Years cal BP. Radiocarbon 55: 1869–1887.
84. Björck J, Andrén T, Wastegård S, Ponsnett G, Schoning K (2002) An event stratigraphy for the Last Glacial-Holocene transition in eastern middle Sweden: results from investigations of varved clay and terrestrial sequences. Quaternary Science Reviews 21: 1489–1501.
85. Eriksen BV (2002) Reconsidering the geochronological framework of Lateglacial hunter-gatherer colonization of southern Scandinavia. In: Eriksen BV, Bratlund B, editors. Recent studies in the Final Palaeolithic of the European plain. Proceedings of a U.I.S.P.P. Symposium. Stockholm, 14.–17. October 1999. Jutland Archaeological Society Publications Vol. 39. Helbjerg, pp. 25–41.
86. Usinger H (2005) Vegetation and climate of the lowlands of northern Central Europe and adjacent areas around the Younger Dryas-Preboreal transition—with special emphasis on the Preboreal oscillation. Internationale Archäologie 5: 1–27. doi: 10.1016/j.acvd.2014.03.005 PMID: 24997733
87. Jensen J (2003) The prehistory of Denmark. Routledge. Taylor & Francis Group. London and New York. 331 p. PMID: 29057699
89. Theuerkauf M, Bos JAA, Jahns S, Janke W, Kupari A, et al. (2014) Corylus expansion and persistent openness in the early Holocene vegetation of northern central Europe. Quaternary Science Reviews 90: 183–198.

90. Bos JAA (2001) Lateglacial and Early Holocene vegetation history of the northern Wetterau and the Amöneburger Basin (Hessen), central-west Germany. Review of Palaeobotany and Palynology 115: 177–212. doi: 10.1016/S0034-6667(01)00069-0 PMID: 11440769

91. Rivals F, Mihlbachler MC, Solounias N, Mol D, Semperebon GM, et al. (2010) Palaeoecology of the mammoth steppe fauna from the late Pleistocene of the North Sea and Alaska: separating species preferences from geographic influence in paleoecological dental wear analysis. Palaeogeography Palaeoclimatology Palaeoecology 286: 42–54.

92. Borowski S, Kossak S (1972) The natural food preferences of the European bison in seasons free of snow cover. Acta Theriologica 17: 151–169.

93. Cerling TE, Hart JA, Hart TB (2004) Stable Isotope Ecology in the Ituri Forest. Oecologia 138: 5–12. PMID: 14530961

94. Boertje RD (1984) Seasonal diets of the Denali Caribou Herd, Alaska. Arctic 37: 161–165.

95. Nieminen M, Heiskari U (1988) Diets of freely grazing and captive reindeer during summer and winter. Rangifer 9: 17–34.

96. Brooks JR, Flanagan LB, Buchmann N, Ehleringer JR (1997) Carbon isotope composition of boreal plants: functional grouping of life forms. Oecologia 110: 301–311.

97. Fizet M, Mariotti A, Bocherens H, Lange-Badré B, Vandermeersch B, et al. (1995) Effect of diet, physiology and climate on carbon and nitrogen isotopes of collagen in a late Pleistocene anthropic paleoecosystem- Marillac, Charente, France). Journal of Archaeological Science 22: 67–79.

98. Drucker DG, Bocherens H, Billiou D (2003) Evidence for shifting environmental conditions in southwestern France from 33 000 to 15 000 years ago derived from carbon-13 and nitrogen-15 natural abundances in collagen of large herbivores. Earth and Planetary Science Letters 216: 163–173.

99. Martin MLSt (2012) Ecological development a management plan for reindeer (Rangifer tarandus tarandus) on St. George Island, Alaska. McGill-Queen’s University Press. Montreal, Quebec. 585 p. PMID: 25506952

100. Guthrie RD (1990) Frozen Fauna of the Mammoth Steppe: The story of Blue Babe. The University of Chicago Press, Chicago and London. 323 p. PMID: 25144095

101. Fretwell SD (1972) Populations in a seasonal environment. Princeton University Press. 225 p. PMID: 4417484

102. Weniger G (1999) Archäologie und Biologie des Auerochsen. Archaeology and Biology of the Au-rochs. Neanderthal Museum, Mettmann. 200 p.

103. Dietl GP, Flessa KW (2011) Conservation paleobiology: putting the dead to work. Trends in Ecology & Evolution 26: 30–37. doi: http://dx.doi.org/10.1016/j.tree.2010.09.010

104. Kowalczyk R, Krasinska M, Kamiński T, Gómy M, Stru P, et al. (2013) Movements of European bison (Bison bonasus) beyond the Białowieża Forest (NE Poland): range expansion or partial migrations? Acta Theriologica 58: 391–401. doi: 10.1007/s13364-013-0136-y PMID: 24244043