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Early Neolithic pastoral land use at Alsónyék-Bátaszék, Hungary (Starčevo culture): New insights from stable isotope ratios

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Abstract

The earliest introduction of livestock (cattle, goats, sheep, pigs) into the Carpathian Basin was an important step towards farming expansion into continental Europe. This spread beyond the environments of the southern Balkans was accompanied by a reduction in the spectrum of cultivated crops, changes in the relative representation of different domestic animals, and, most likely, adaptations of husbandry practices. How the earliest farmers in the Carpathian Basin kept their domestic stock is still understudied. We explored early animal management and land use strategies at the Starčevo settlement at Alsónyék-Bátaszék, Hungary (Early Neolithic, ca. 5800–5600 cal BC). Settled at the intersection of wide alluvial plains, waterlogged meadows and marshes to the east, and forested hills to the west, early farmers at Alsónyék had a wide variety of options for nourishing their livestock. We performed stable isotope ratio analysis of bone collagen ($n = 99$; $\delta^{13}C$, $\delta^{15}N$) and tooth enamel ($n_{teeth} = 28$, sequentially sampled for $\delta^{13}C$ and $\delta^{18}O$) from wild and domestic animals to locate them in the landscape and investigate herding practices on a seasonal scale. The bone collagen isotope ratios mostly indicate feeding in open environments. However, results from the sequential analysis of cattle and sheep enamel suggest diverse dietary strategies for winters, including consumption of forest resources, consumption of summer hay and grazing in an open environment. Most pigs appear to have had herbivorous diets, but several individuals likely supplemented their diet with animal protein. Stable isotope ratio results from the Lengyel phase at Alsónyék (ca. 4800–4300 cal BC) suggest more access to animal protein for pigs, and feeding in more open areas by wild boar, red deer and cattle compared to the Starčevo phase. This study's results demonstrate considerable variability in early animal husbandry practices at Alsónyék.

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Introduction

Domesticated animals and plants, initially originating from Southwest Asia and domesticated in the 10th-9th millennium BC [1], were introduced by Neolithic farmers to Thessaly and Aegean Macedonia in the early 7th millennium BC. The spread of agriculture to inland Europe took a decisive step at the turn of the 7th to the 6th millennium BC when settlers from the Starčevo-Çris-Körös cultural complex pioneered the northern Balkans and the Southern Carpathian Basin (modern northern Bulgaria, Serbia, Romania and Hungary; [2–4]). This northward diffusion of farming was accompanied by significant changes in the spectrum of plants cultivated and the species composition of animals kept [5–8]. In the sub-Mediterranean southern Balkans (modern-day Northern Greece and southern Bulgaria), a broad spectrum of crops was cultivated [8], which appears to have been the result of a strategy of diversification. Correlated with differences in environments and climatic conditions, expansion into the northern Balkans and the Great Hungarian Plain was generally accompanied by a reduction in the spectrum of crops cultivated, and the proportion of sheep, goats, and pigs diminished while cattle became dominant in many faunal assemblages (reviewed in [9]). Early Neolithic farmers in the Great Hungarian Plain settled preferentially on flat surfaces on hydromorphic meadow soils near freshwater [10]. Living in an environment of marshland islets and forested hills likely encouraged a large degree of adaptation by these early farmers [11]. Further investigations are needed to describe in more detail how these farming systems have adapted to the advantages and constraints of the landscape, including in terms of the respective–and not necessarily exclusive–spatial locations of crops and livestock at the site scale, as well as on a seasonal scale.

This study focuses on animal husbandry at Alsónyék-Bátaszék in southern Hungary (Fig 1; Lat. 46˚ 12’ N, Long. 18˚ 42’ E), which is particularly suited to such investigations of the place of animals in the landscape due to being located at an intersection of different ecosystems. Using stable isotope ratios ($\delta^{13}C$, $\delta^{18}O$, $\delta^{15}N$) of animal teeth and bones, the aim of this study is to research in what environments cattle and sheep (the dominant species) were herded and how pigs–rare but present–were kept in the Early Neolithic, to understand how herders utilised Alsónyék’s wider surrounding environment. Analysis of sequential tooth enamel samples also allows investigations of diets on a seasonal scale, through which it is possible to examine the use of woodlands and wetlands as potential seasonal food sources, as well as the proximity of pigs to dwellings. To construct a framework for the range of possibilities in animal husbandry practices, references are also made to ethnographic and historical accounts from Hungary from the 18th to 20th centuries. This work contributes to more wide-ranging research on understanding the environmental, biological and socio-cultural factors in the initial dispersal and adaptation of farming systems across Europe.

Alsónyék: Description of the site, environment, and subsistence practices

Alsónyék subsite 5603/1 (see Fig 38 in [12]), where most of the Starčevo features at Alsónyék were found, was occupied by people from the Starčevo cultural complex in the first half of the 6th millennium cal BC (beginning ca. 5800–5730 cal BC, and ending 5575–5505 cal BC, both 95% probability [13]). It appears as a substantial, clustered settlement [14]. Later occupation of the site included Neolithic communities from the Linearbandkeramik (LBK), Sopot and Lengyel cultures (Table 1). The village lies in the Carpathian Basin in the southwestern part of Tolna Sárköz in Southeast Transdanubia (see Fig 1) at the transition of hilly margins to marshy lowlands [15].

The Danube is 16 km to the east of Alsónyék-Bátaszék (hereafter: Alsónyék) today, and, together with its tributary Sárvíz, it shaped the landscape considerably [15]. Former Danube

Fig 1. Map showing the location of Alsónyék and other sites mentioned in this article. Map data: Natural Earth II from www.NaturalEarthData.com (public domain).

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channels left behind oxbow lakes and wide alluvial floodplains, dominated by (seasonally) waterlogged meadows and marshes, likely frequently inundated, and island-like plateaus above the floodplain [16, 17]. Gallery woods were likely present along rivers and oxbow lakes, and groves may have grown in the floodplains [18]. The settlement site of Alsónyék itself appears to have been outside the extensive flooding area [16], but compared to later groups, the Starčevo community at Alsónyék occupied the lower and wetter parts of the area [19]. Although crop cultivation to the east of the site was likely possible on the elevated islets dotted

Table 1. Sites mentioned in the text with their associated cultural groups and phases as relevant to this study.

Site name	Cultural group	Chronological phase	Dating cal BC	Reference
Alsónyék	Starčevo	Early Neolithic	ca. 5800–5600	this study
	LBK	Middle Neolithic	ca. 5500–5000	[25]
	Sopot	Middle/Late Neolithic	ca. 5100–4800	[25]
	Lengyel	Late Neolithic	ca. 4800–4300	[25]
Balatonszárszó	Early LBK	Middle Neolithic	ca. 5450–5250	[26]
Vinča-Belo Brdo	Vinča-Pločnik II	Late Neolithic	ca. 4850–4600	[27]
Bordușani-Popină	Gumelnița A2	Late Chalcolithic	ca. 4500–4250	[28]
Ecsegfalva	Körös	Early Neolithic	ca. 5800–5600	[29]
Endrőd 119	Körös	Early Neolithic	ca. 5800–5630	[30]
Füzesabony	ALP	Middle Neolithic	ca. 5550–5000	[26]
Halai	-	Early Neolithic	ca. 6400–5800	[31]
Hârșova-tell	Gumelnița A2	Chalcolithic	ca. 4350–4000	[28]
Kouphovouno	-	Middle Neolithic	ca. 5800–5400	[31]
Lánycsók-Égettmalom	Starčevo	Early Neolithic	ca. 5800–5600	[32]
Măgura—Boldul lui Moș Ivčnus	Starčevo-Criș I	Early Neolithic	ca. 6000–5800	[33]
Makriyalos II	-	Late Neolithic II	ca. 4950–4500	[31]
Stubline	Vinča-Pločnik II	Late Neolithic	ca. 4850–4600	[27]
Vitănești-Măgurice	Gumelnița A2	Chalcolithic	ca. 4450–4150	[28]
Zemunica	Impressed Pottery	Early Neolithic	ca. 6000–5800	[34]

References are to relevant studies on faunal stable isotope ratios (δ^{13}C and δ^{15}N). ALP = Alföld Linear Pottery, LBK = Linearbandkeramik.

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throughout the landscape, it would have been at high risk of harvest-loss due to flooding, likely making the cultivation of arable land to the west of the site more favourable [16].

To the west of Alsónyék, the Danubian floodplains are supplanted by skeletal soils and forested hills (Szekszárd Hills, 273 m; [14, 16]), which also provided arable land on the hilltops [16]. An open oak woodland would likely have been present which may have also included pine, elm, hazel, lime, beech and hornbeam [20]. Wild fauna during the Starčevo phase includes animal species consistent with both forested and open wetland environments, e.g. wildcats (*Felis silvestris*), beavers (*Castor fiber*) and multiple duck species (*Anatinae*) [21–23].

The faunal assemblage from the Starčevo phase at subsite 5603/1 in the southeastern part of Alsónyék is dominated by domestic animals (88%), with caprines (sheep and goats; for scientific names see Tables 2 and 3) and cattle being predominant, and only minor amounts of pigs (3%) and dogs (Table 2; data given as number of identifiable specimens (NISP) from [23]). Most cattle have been slaughtered as juveniles and adults, consistent with a focus on dairying, while pigs were mostly slaughtered as juveniles [23]. Hunting was not a dominant subsistence activity, with only ca. 12% of the identifiable mammal faunal assemblage being wild animals, mostly red deer and wild boar [23]. Freshwater fish remains were also present at the site (1.4% of $\text{NISP}_{\text{faunal remains}}$), including wels catfish, northern pike and carp [20, 21].

The recovered crops from the Starčevo phase at Alsónyék (einkorn, emmer and barley [24]) indicate that at this time a mixed farming economy was likely present, and people were somewhat locally bound due to the requirements of crop cultivation. Livestock herding could have taken place in the forested hills to the west of the site, in the wetlands and waterlogged humid meadows in the east along the river Sárvíz (pigs in particular; [16]), or on cropland after harvest.

Table 2. Mammal faunal spectrum at Alsónyék subsite 5603/1 from the Starčevo phase (identifiable mammals only).

Species	Common name	NISP	% of NISP$_{\text{identifiable mammal}}$
*Ovis aries/Capra hircus**	sheep/goat*	4688	42.5
Bos taurus	cattle	4633	42.0
Sus domesticus	pig	331	3.0
Canis familiaris	dog	46	0.4
Cervus elaphus	red deer	565	5.1
Sus scrofa	wild boar	525	4.8
Capreolus capreolus	roe deer	160	1.5
other (identifiable)		78	0.7
Total		**11026**	**100**

*Around six sheep per goat; data from [23].

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Stable isotope ratios for palaeodietary reconstruction

Bone collagen. Bone collagen stable carbon (δ^{13}C) and nitrogen (δ^{15}N) isotope ratios reflect those of consumed dietary protein (and other dietary components to a lesser extent) in the last years of an individual's life with a systematic diet-collagen offset (ca. 5–6‰ for δ^{13}C, and ca. 3–5‰ for δ^{15}N; [35–40]). Therefore, bone collagen δ^{13}C and δ^{15}N values can be used to determine what food groups were commonly consumed. Consumer δ^{15}N values are frequently used as indicators of trophic level and can be used to infer e.g. whether pig diets were likely supplemented with animal protein [28, 41–43], or consisted of fertilised crops [44], both elevating δ^{15}N values. In younger individuals, milk consumption puts the offspring at a higher trophic level than the mother leading to higher δ^{15}N values. However, when growth rates are high (e.g. in adolescence), δ^{15}N values tend to be lower [45]. This introduces some uncertainties in interpretations of δ^{15}N values of young animals.

Hungary's terrestrial vegetation is dominated by C_3-plants, with only minor amounts of C_4 plants being present in dry grasslands following recent colonisations [46], so that C_4 plants

Table 3. Bone collagen δ^{13}C and δ^{15}N stable isotope ratios from the Starčevo phase at Alsónyék, subsite 5603/1.

Species	common name	n	δ^{13}C (‰)			δ^{15}N (‰)		
			Min.	Median	Max.	Min.	Median	Max.
Bos taurus	cattle	10	−21.55	−20.71	−19.44	4.96	5.41	7.07
Ovis aries	sheep	18	−21.62	−20.76	−19.95	4.41	6.82	7.45
Sus domesticus	pig	13	−22.36	−20.43	−19.13	6.05	6.89	9.16
Sus scrofa	wild boar	19	−22.11	−21.08	−19.89	3.95	6.99	8.59
Canis familiaris	dog	6	−22.54	−21.27	−20.72	6.05	8.82	9.43
Cervus elaphus	red deer	10	−22.55	−21.46	−19.79	4.27	6.63	7.76
Capreolus capreolus	roe deer	10	−23.72	−21.20	−20.14	5.47	6.36	7.14
Vulpes vulpes	fox	2	−20.35	−19.64	−18.93	7.70	7.79	7.87
Abramis brama	bream	2	−24.37	−23.69	−23.01	8.00	8.63	9.25
Cyprinus carpio	carp	3	−27.18	−27.15	−25.97	6.86	8.37	8.64
Esox lucius	northern pike	2	−22.11	−21.41	−20.70	9.97	9.98	9.98
Sander lucioperca	zander	3	−21.77	−20.15	−19.13	9.47	9.81	10.18
Silurus glanis	wels catfish	1	−20.64	−20.64	−20.64	8.24	8.24	8.24

For additional information see Table A in S1 File; sample numbers exclude samples that failed the quality criteria.

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were likely not contributing to Early Neolithic diets at Alsónyék to a significant extent. Forest grazing/browsing tends to lead to lower consumer $\delta^{13}C$ values due to the canopy effect [47, 48]. Ground-level undergrowth was found to be depleted by 2–5‰ in dense forest compared to plants and trees in open environments [47, 49]. Bone collagen $\delta^{13}C$ values from modern forest-dwelling red and roe deer from dense deciduous forests in Dourdan (France) and Białowieża (Poland) were on average −23.7‰, with all except one below −22.5‰ (n = 29; corrected for the fossil fuel effect; [48]). Values approaching these thresholds are therefore considered to likely reflect a significant dietary contribution of forest resources (particularly ground-level undergrowth), especially in the case of herbivores. However, not all animals occupying forests necessarily have low $\delta^{13}C$ values, possibly in part due to feeding outside of the forest [50] and feeding on fruits (e.g. acorns) which are higher in $\delta^{13}C$, and can lead to higher $\delta^{13}C$ values than expected for forest-dwelling animals, particularly in the case of suids [51]. Conversely, low $\delta^{13}C$ values in suids may also occur due to consumption of freshwater fish [28].

Since water stress has been shown to elevate $\delta^{13}C$ values [52], and heavy watering leads to around 1‰ lower $\delta^{13}C$ values [53–55], it has been argued that freshwater wetlands may be expected to have lower $\delta^{13}C$ values than C_3 vegetation from drier areas [49, 56], lowering consumer $\delta^{13}C$ values. However, compared to e.g. the canopy effect, this is likely only a minor effect.

Since $\delta^{13}C$ and $\delta^{15}N$ values differ due to a large variety of different factors, it is important to establish site-specific baselines for different diets. To gain comparative references of the typical $\delta^{13}C$ and $\delta^{15}N$ values for the diets of the domesticated animals under study (cattle, pig, sheep), bones of wild fauna like wild boar, red and roe deer (primarily forest-dwelling), freshwater fish (potentially consumed by pigs), as well as dogs (consumers of higher trophic level foods) excavated from the same site and phase are also analysed.

Sequential analysis of tooth enamel

Sequential analysis of stable carbon and oxygen isotope ratios in tooth enamel gives access to dietary information on a seasonal scale. Bioapatite precipitates in oxygen isotopic equilibrium with body water [57], linked, through the ingestion of surface water, to local annual precipitation [58, 59], whose $\delta^{18}O$ values vary seasonally with air temperature in continental Europe [60]. Additionally, animal behavior and physiology in response to seasonal changes in temperature and air humidity also affect body water oxygen isotope composition [61]. All factors combine to create a seasonal signal in tooth enamel $\delta^{18}O$ values. Sequential sampling permits the retrieval of a one-year record from the third molar of sheep and cattle [62], or by combining measurements from the first and second incisors in suids [51], while the canines of male suids may provide a pluriannual record [51].

The stable carbon isotope ratios in bioapatite reflect those of the entire diet [35] as opposed to collagen, whose primary carbon source is proteins [39, 63]. A 14.1‰ isotope enrichment (ε) has been shown between diet and enamel bioapatite $\delta^{13}C$ values in a variety of large ruminant mammals [64], while this enrichment is 13.3‰ in experimental pigs fed C_3 diets [65]. Most C_3 plants have $\delta^{13}C$ values varying from −29‰ to −25‰ around an average value of −27‰ in open areas [66], or −25.5‰ in preindustrial times after correction for the fossil fuel effect [67]. This should lead to an average value of −11.8‰ in sheep and cattle enamel bioapatite, or −12.5‰ in pig enamel bioapatite–although a significant fruit component in suid diets [68, 69] would also elevate this value; the $\delta^{13}C$ values measured in predominantly open areas and under continental climate are globally comprised between −13‰ and −9‰ in cattle and sheep tooth enamel [33, 70]. By contrast, animals dwelling in closed forests have lower bone collagen $\delta^{13}C$ values (below −22.5‰, [48]), so that enamel bioapatite $\delta^{13}C$ values should tend towards −13,8‰ in large ruminants (applying a 5% spacing between collagen and diet [39] and a 14.1‰ isotope enrichment between diet and enamel bioapatite [64]), or towards −14.6‰ in pigs (applying a 13.3‰ isotope enrichment between diet and enamel bioapatite [65]) although a significant contribution of forest fruits would elevate this value. We consider values approaching these thresholds as reflecting a significant contribution of forest resources to animal diet.

Seasonal variations are expected in plant δ^{13}C values, in response to seasonal changes in the environmental factors affecting the stomatal aperture and therefore the carbon isotope discrimination during photosynthesis, and also possibly due to changes in plant physiology during the growing season. The highest δ^{13}C values are expected in the summer when the air temperature is the highest and air humidity and ground water are the lowest [71–73]. Consequently, δ^{18}O and δ^{13}C values are expected to follow the same pattern of seasonal variation. Indeed, in modern sheep grazing on the same pasture throughout the year, the δ^{18}O and δ^{13}C sequences in enamel were shown to vary in phase or very close to it [74] with amplitudes of variation of 1 to 3‰ in δ^{13}C [75]. Deviations from this pattern may result from the contribution of different food sources during the year due to foddering or mobility between areas where environmental factors affect plants differently.

Materials and methods

Bone collagen stable isotope ratio analysis

All bone and tooth samples in this study were excavated from Alsónyék subsite 5603/1, and date to the Early Neolithic, Starčevo phase (ca. 5800–5600 *cal* BC; [13]; stored at the Wosinsky Mór County Museum, Szekszárd). Around 2 g cortical bone samples of long bones were taken from domestic and wild terrestrial and aquatic fauna (n = 107; Table 3). Differentiation between wild boar and domestic pig was done according to size, following [76]. In the case of the mammals, the same part of the bone from the same side of the body (within each species group) was preferentially sampled, to prevent sampling the same individual twice. To enable this sampling strategy, in some cases, juvenile and subadult individuals were also sampled (see Table A in S1 File). This needs to be taken into account when interpreting the results as it introduces uncertainty when comparing between animals of differing ages, e.g. due to different nitrogen balances (see above). In the case of pigs, most samples were from younger individuals because pigs tend to be slaughtered as juveniles/subadults and few adult pig bones were available to sample. Previous studies have argued for including younger livestock in stable isotope ratio analyses (e.g. [77]) since this enables a more complete insight into husbandry practices without a bias toward adults.

Bone samples were cleaned by abrading the outer surface using a tungsten carbide drill bit. Collagen was extracted from 200–230 mg ground bone using 1 M HCl, followed by 0.125 M NaOH, following a modified Longin (1971) method [78] described in [79], but with the gelatinisation step at 70 ˚C instead of 100 ˚C. In the case of fish bones, the NaOH step was shortened to initially 15 minutes. If the solution coloured during this time, it was exchanged for fresh 0.125 M NaOH solution for another 15-minute immersion (following [80]).

Using an elemental analyser (EA; Thermo Flash 2000) interfaced with an isotope ratio mass spectrometer (IRMS, Thermo DeltaVAdvantage), coupled δ^{13}C and δ^{15}N measurements of 320-380 μg bone collagen were conducted. Within each run, multiple replicates of a secondary alanine standard were included (calibrated to primary standards IAEA-600 for δ^{13}C, and IAEA-USGS25, IAEA-N-1 and IAEA-N-2 for δ^{15}N). The alanine standards gave mean values of −21.47±0.09‰ for δ^{13}C (mean±σ; expected value: −22.16‰ with reference to VPDB) and 0.70±0.13% for δ^{15}N (expected value: +0.59‰ with reference to AIR), and 40.07±1.46% for C content (expected value: 40.44%) and 15.56±0.09% for N content (expected value: 15.72%) over the course of all measurements (n = 23), and were used to correct the measured data. Quality criteria to ensure data robustness were chosen such that results were rejected if the bone collagen yield was less than 1%, or if the collagen had a C/N (molar) ratio outside of 2.9–3.45, or if the C content was less than 13%, or the N content less than 4.8% (following suggestions in [81–83]).

Isotopic niche spaces were modelled as Bayesian ellipses with the R package SIBER [84].

Tooth enamel sequential stable isotope ratio analysis

The study material includes teeth from cattle (11 upper third molars-M3), sheep (9 lower M3), pig (1 lower M3), red deer (2 lower M3) and wild boar (1 upper canine, 1 upper M3, 1 lower M3, 2 lower incisors from the same individual; Table C in S2 File). The enamel surface was cleaned with a tungsten carbide drill bit. Teeth were sequentially sampled using a diamond-coated drill bit on the lingual side of the anterior lobe for the cattle M3; on the vestibular side of the middle lobe for the sheep and red deer M3; on the lingual side of the anterior cusp for the suid molars, and on the labial side for the suid incisors and the maxillary canine. The sampling procedure for the suid teeth was illustrated in [51]. These enamel powders were pre-treated to eliminate diagenetic carbonates (0.1 M acetic acid for 4 h at room temperature, 0.1 ml/mg). Pre-treated enamel samples weighing ~600 μg were reacted with 100% phosphoric acid at 70˚C in individual vessels in an automated cryogenic distillation system (Kiel IV device), interfaced with a DeltaVAdvantage isotope ratio mass spectrometer. The analytical precision for each run, estimated from 5 to 8 analyses of our laboratory carbonate standard (Marbre LM, calibrated to the NBS-19 international standard) was always less than or equal to 0.05‰ for δ^{13}C values and 0.04‰ for δ^{18}O values (both with reference to VPDB). For each run, the Marbre LM gave a mean δ^{13}C value comprised between 2.09‰ and 2.20‰ (expected value 2.13‰) and a mean δ^{18}O value comprised between −2.02‰ and −1.96‰ (expected value −1.83‰). The δ^{18}O values for sheep and cattle tooth enamel were previously published in [85, 86] respectively. In the sheep and cattle molars and in the wild boar canine, showing full annual cycles, the phase shift between the δ^{18}O and δ^{13}C sequences was determined using a sinusoidal model approach after [74]; see also Table H in S2 File.

Results

Bone collagen stable isotope ratios

During collagen extraction, four fish bone samples dissolved completely and could not be analysed, and the measured results from one sheep and four fish were excluded because they did not meet the quality criteria (see Table A in S1 File, where the complete set of data is also listed). Table 3 and Figs 2 and 3 show the δ^{13}C and δ^{15}N results for the samples that passed the quality criteria.

The results for most species overlap to a large extent (Figs 2 and 3). A one-way analysis of variance (ANOVA) was conducted to compare δ^{13}C values between pigs, wild boar, dogs, red deer, roe deer, cattle and sheep (F(6,79) = 2.53, p = 0.027). It revealed differences in the mean δ^{13}C values between species, but a post-hoc Tukey test showed no significant differences. A second one-way ANOVA (F(6,79) = 5.81, p = 0.00005) followed by a Tukey test indicated differences in mean δ^{15}N values between pigs and cattle, and between dogs and wild boar, red deer, roe deer, cattle and sheep. Domestic pigs had a smaller range of δ^{15}N values than wild boar (3.1‰ compared to 4.6‰; Fig 3, right). There was no statistically significant difference in δ^{15}N or δ^{13}C between juvenile (n = 6) and subadult pigs (n = 6), and no other age-based differences were identified.

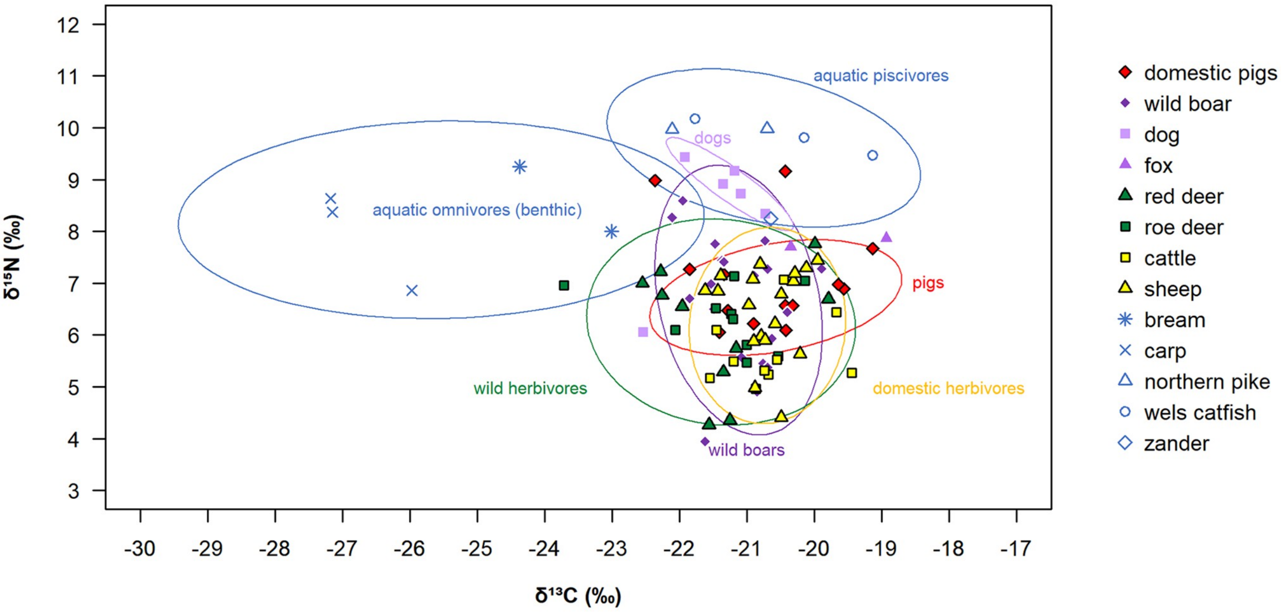

Fig 2. Stable carbon (δ^{13}C) and nitrogen (δ^{15}N) isotope ratio results for bone collagen from the Starčevo phase at Alsónyék, subsite 5603/1. The 90% prediction ellipses were modelled excluding an outlier dog and two pig datapoints (see S3 File).

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Red deer and roe deer yielded on average the lowest δ^{13}C values among terrestrial mammals (Fig 3), the lowest collagen δ^{13}C value being measured in a roe deer (−23.7‰, ALSCap9), followed by three red deer with δ^{13}C values between −23.0‰ and −22.0‰, which are close to and below the threshold value suggesting a significant contribution of forest resources to the animals' diets. The sampled freshwater fish exhibited a wide breadth of δ^{15}N and δ^{13}C values,

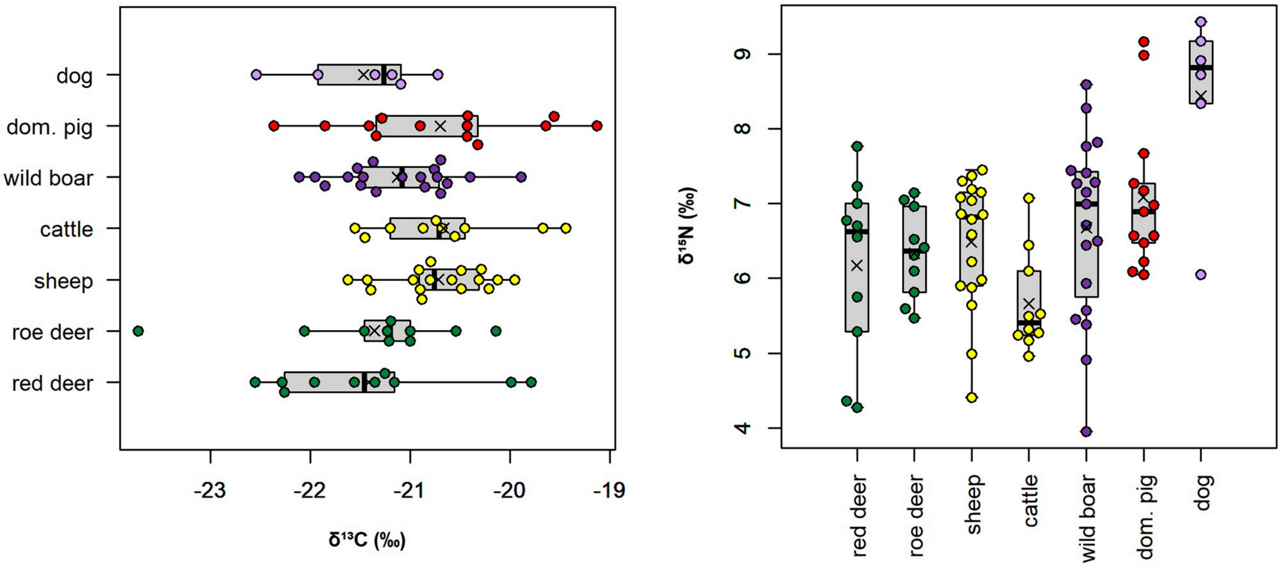

Fig 3. Stable carbon (δ^{13}C) and nitrogen (δ^{15}N) isotope ratio results for mammal bone collagen from the Starčevo phase at Alsónyék, subsite 5603/1. This figure shows the same data as in Fig 2, but visualised as boxplots. The line in the boxplot indicates the median, and the cross the mean. Whisker boundaries were chosen as 1.5 × interquartile range. Fish and foxes were excluded due to low sample numbers.

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with differences visible between dietary groups: benthic omnivores like carp and bream had the lowest $\delta^{13}C$ values (−27.2‰ to −23.0‰) of all measured samples, whereas piscivorous fish (northern pike, wels catfish and zander) had the highest $\delta^{15}N$ values (8.2‰ to 10.2‰).

Tooth enamel sequential stable isotope ratios

The results from the sequential analysis of tooth enamel are shown in Figs 4 and 5, Table 4, and Tables D-H in the S2 File (including phase shift modeling). Sheep enamel bioapatite $\delta^{13}C$ values vary overall between −13.8‰ and −10.0‰. All sheep have recorded seasonal changes in their diet $\delta^{13}C$ values (Fig 4). The amplitude of intra-tooth variation varies between 1.5‰ (ALS Ovis4) and 3.5‰ (ALS Ovis6). Different patterns of variations are observed. Most sheep show a sinusoidal variation in $\delta^{13}C$ values in phase with the seasonal changes in $\delta^{18}O$ values (phase shift comprised between 331 and 368˚, Table H in S2 File; Patterns A and B in Fig 4). Among those, three sheep (ALS Ovis2, Ovis6 and Ovis9) have lower winter $\delta^{13}C$ values (−13.8‰ to −13.6‰) tending towards the threshold indicating significant consumption of forest resources (Figs 4 and 5, Pattern B). ALS Ovis3 and ALS Ovis5 deviate from the sinusoidal pattern of variation and have a reduced amplitude of variation between $\delta^{13}C$ values recorded in winter and the consecutive summer (0.1‰ and 0.5‰ respectively; Pattern C, Fig 4).

Cattle enamel bioapatite $\delta^{13}C$ values vary between −14.0‰ and −9.7‰. All cattle have recorded seasonal variations in their diet $\delta^{13}C$ values (Fig 4) with amplitudes of intra-tooth variation of 1.0‰ to 2.2‰. As with sheep, most cattle follow Pattern A (ALS Bos4, Bos5, Bos8, Bos9, Bos10 and Bos11) or Pattern B (ALS Bos2 with a winter $\delta^{13}C$ value of −14.0‰) with phase shifts between the $\delta^{18}O$ and $\delta^{13}C$ sequences varying from 310˚ to 358˚; Table H in S2 File). ALS Bos1, Bos6 and Bos7 do not show a sinusoidal pattern of variation in $\delta^{13}C$ values (Pattern C) but rather have stable values over the summer and the preceding or consecutive winter recorded in the M3 (Fig 4).

The suid teeth have recorded seasonal variations in enamel bioapatite $\delta^{18}O$ values (Fig 5). Over a year is recorded in the wild boar canine (ALS Sus18 C) and a complete year may be reconstructed when combining the wild boar first and second incisors (ALS Sus20 I1 and I2) whose formations overlap in time [51]. The sequences recorded in the wild boar and pig M3s do not reflect complete annual cycles. Overall, the $\delta^{13}C$ values recorded in the suids teeth vary between −15.0‰ and −11.1‰. In the wild boar canine (ALS Sus18 C), the $\delta^{13}C$ values vary in phase with the $\delta^{18}O$ sequence (phase shift = 355˚) with an amplitude of variation of 2.3‰. The pattern of variation in ALS Sus20 I1&I2 also shows a trend for lower $\delta^{13}C$ values in winter (−15‰) and higher in the summer (−13.1‰). The short $\delta^{18}O$ sequences recorded in the three suid third molars are centered on the summer; in these teeth, the $\delta^{13}C$ values vary little around −14‰ and −13‰ in the wild boar M3s (ALS Sus19 and Sus21), although a steep gradient towards higher values (−11.1‰) is measured in the part of the tooth that was formed last, and corresponding to late summer, in ALS Sus21M3 (Fig 5). In the only pig's molar (ALS Sus22 M3), $\delta^{13}C$ values vary between −11.5‰ and −11‰.

The $\delta^{18}O$ sequences measured in the two red deer third molars show a steep decreasing trend suggesting a record over a summer-autumn-winter sequence. Over this period, these deer have recorded decreasing $\delta^{13}C$ values comprised between −12.5‰ and −12.9‰ in ALS Cervus1 and between −13.4‰ and −14.3‰ in ALS Cervus2. Fig 6 compares the range of variation in $\delta^{13}C$ values in all teeth. The wild fauna (red deer and wild boar) shows the lowest $\delta^{13}C$ values, in contrast to higher $\delta^{13}C$ values in domestic animals (pig, sheep, and cattle), although in some of the cattle and sheep teeth, lower $\delta^{13}C$ values are observed on a seasonal basis.

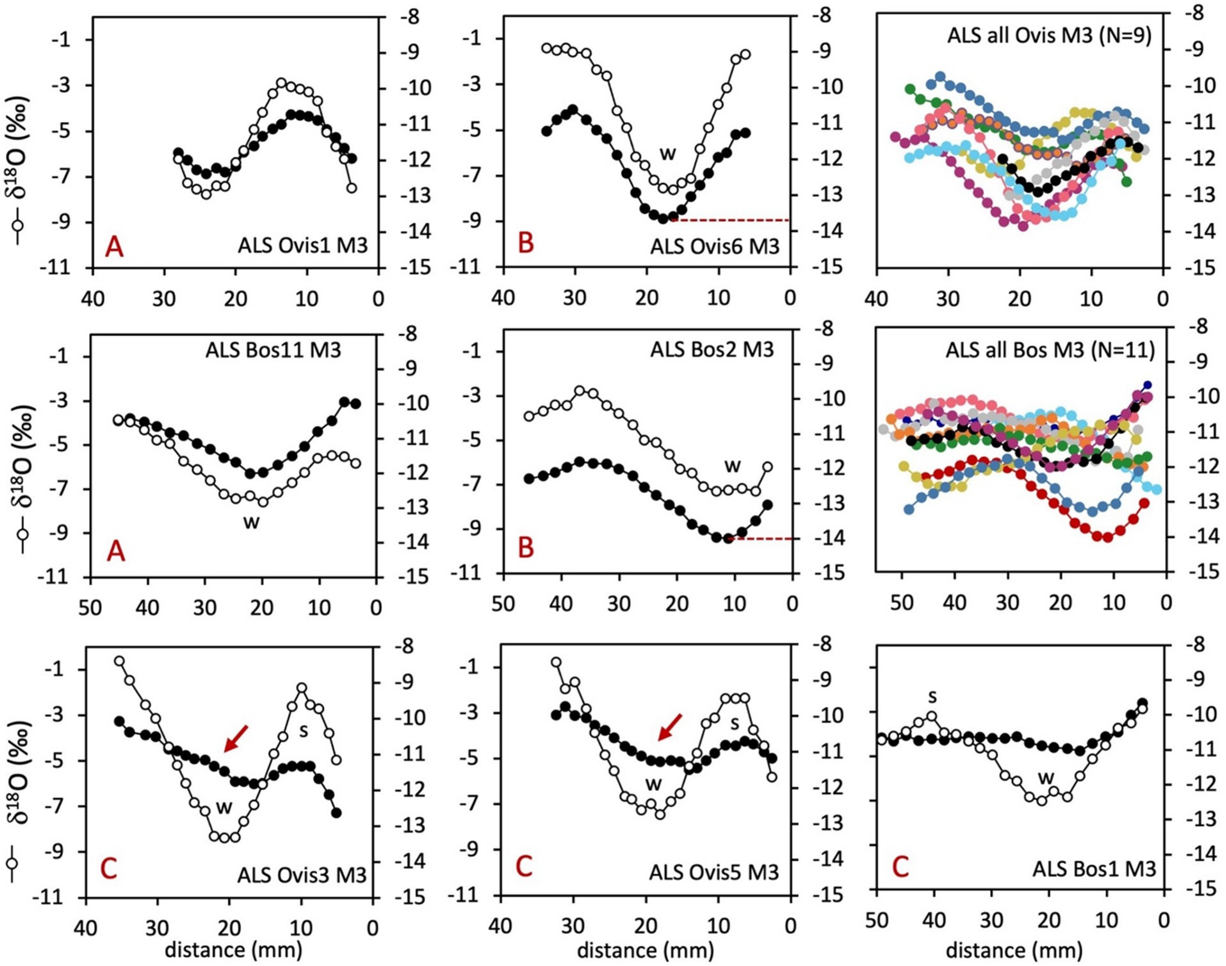

Fig 4. Results from the sequential analysis of stable carbon ($\delta^{13}C$) and oxygen ($\delta^{18}O$) isotope ratios in cattle (Bos) and sheep (Ovis) third molars from the Starčevo phase at Alsónyék (ALS). The figure shows some typical results for the different observed patterns. Patterns A, B and C: see main text. W = winter; S = summer.

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Discussion

Wild fauna: Setting Alsónyék's surrounding landscape

The bone collagen $\delta^{13}C$ values of benthic fish were lower and the bone collagen $\delta^{15}N$ values of the piscivorous fish were more elevated compared to nearly all terrestrial bone collagen samples (Fig 2), revealing a clear separation in stable isotope ratios between animals occupying the terrestrial and aquatic domains. Freshwater fish consumption would therefore lead to higher bone collagen $\delta^{15}N$ values, as well as lower $\delta^{13}C$ values, if benthic fish were consumed.

Feeding in forested areas likely explains the lower $\delta^{13}C$ values in some of the terrestrial animals, with e.g. roe deer ALS Cap9 having the lowest measured $\delta^{13}C_{\text{collagen value}}$ of −23.7‰. Some of the $\delta^{13}C_{\text{enamel}}$ values are similarly low (around −13.1‰ in ALS Cervus1 and as low as −14.3‰ in Cervus2). These low $\delta^{13}C$ values are consistent with year-round grazing/browsing in a forested area with plants subject to the canopy effect, possibly located on the hills to the west of the site. However, around two thirds of the 19 deer had $\delta^{13}C_{\text{collagen}}$ values between −21.5‰ and −19.8‰, likely reflecting feeding in predominantly open areas.

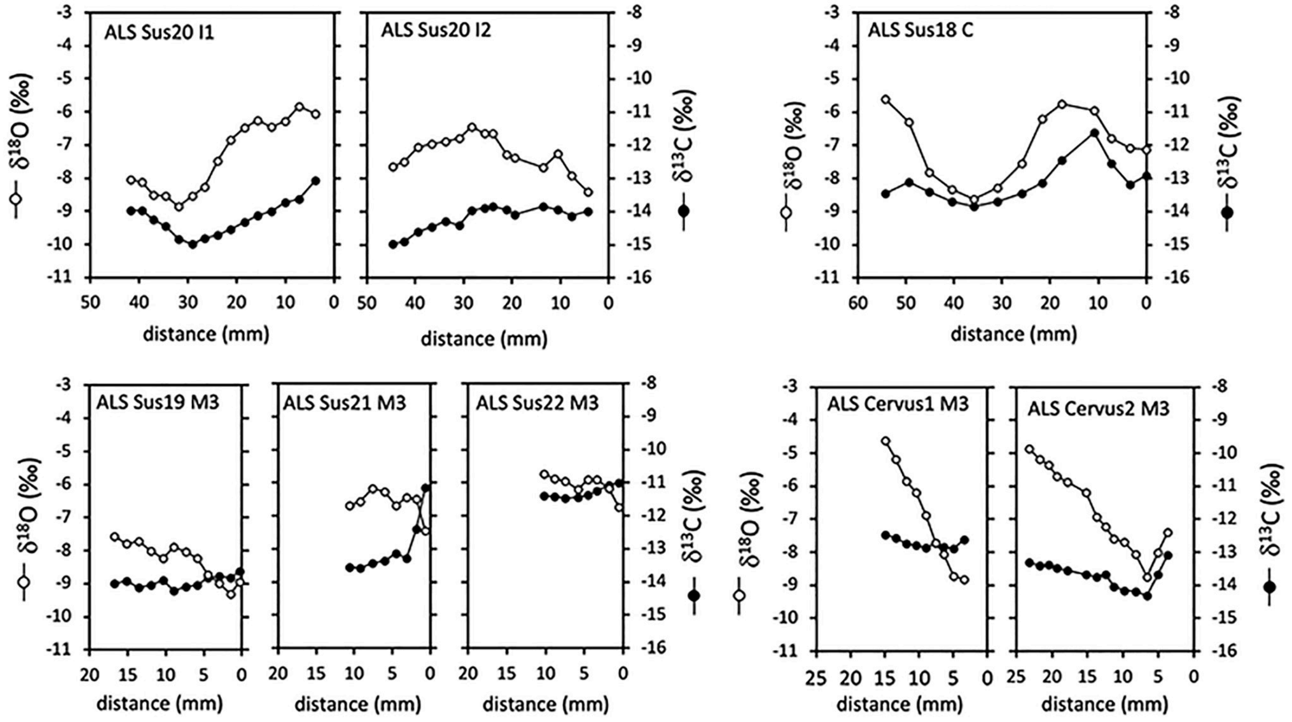

Fig 5. Results from the sequential analysis of stable carbon ($\delta^{13}C$) and oxygen ($\delta^{18}O$) isotope ratios in tooth enamel. Samples were wild boar (Sus18, 19, 20 and 21), pig (Sus22) and red deer (Cervus1 and 2) from the Starčevo phase at Alsónyék.

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Measured wild boar collagen $\delta^{13}C$ values between −21.1‰ and−19.9‰ suggest none of the wild boar sampled for bone collagen fed exclusively on undergrowth of dense forests, and may have moved frequently between forests and more open areas, possibly occupying similar habitats to wild and domestic herbivores. The sequential enamel samples give a more detailed picture: The low $\delta^{13}C_{enamel}$ values from the four sampled wild boar (Fig 6) indicate feeding in predominantly closed areas, with higher $\delta^{13}C_{enamel}$ values recorded in late summer/autumn in ALS Sus18C and ALS Sus21M3 (Figs 5 and 6) likely reflecting a greater contribution of forest fruits (e.g. acorns) at this time of the year for these individuals [51, 87].

The wild boar collagen $\delta^{15}N$ values showed very wide variability, from 4.0‰ to 8.6‰ (cf. a biological variability of ca. 1.5‰ for domestic pigs of differing ages and sex when consuming the identical diets; [45]). This suggests large differences in trophic levels between different wild boar individuals. Two individuals had similar $\delta^{15}N$ values to dogs (> 8‰: ALSSuss6, ALSSuss12), suggesting a large degree of animal protein consumption, possibly partly by feeding on wetland fish naturally trapped after flooding [88]. In the case of wild boars with low $\delta^{13}C$ and high $\delta^{15}N$ values, mushroom consumption may also have elevated the $\delta^{15}N$ values [89]. This large variability in the wild boar baseline $\delta^{15}N$ values complicates the interpretation of domestic pig diets.

Cattle and sheep husbandry

The cattle and sheep collagen $\delta^{13}C$ values suggest grazing in predominantly open areas, possibly also including wetlands. Ethnographic evidence from the Carpathian basin describes wetlands being used as pastures [90–92], although mainly for cattle and pigs. Historical accounts

Table 4. Tooth enamel carbon and oxygen stable isotope ratios from the Starčevo phase at Alsónyék.

Specimen	common name	Species	$\delta^{13}C$ (‰)				$\delta^{18}O$(‰)			
			Min	Max	M	Δ	Min	Max	M	Δ
ALS Cervus1 M3	red deer	*Cervus elaphus*	-12.9	-12.5	-12.7	0.4	-8.8	-4.6	-6.7	4.2
ALS Cervus2 M3	red deer	*Cervus elaphus*	-14.3	-13.1	-13.7	1.2	-8.7	-4.9	-6.8	3.9
ALS Sus20 I1	wild boar	*Sus scrofa*	-15.0	-13.1	-14.0	1.9	-8.8	-5.8	-7.3	3.0
ALS Sus20 I2	wild boar	*Sus scrofa*	-15.0	-13.8	-14.4	1.1	-8.4	-6.4	-7.4	2.0
ALS Sus18 C	wild boar	*Sus scrofa*	-13.9	-11.6	-12.7	2.3	-8.6	-5.6	-7.1	3.0
ALS Sus19 M3	wild boar	*Sus scrofa*	-14.2	-13.6	-13.9	0.6	-9.3	-7.6	-8.4	1.7
ALS Sus21 M3	wild boar	*Sus scrofa*	-13.6	-12.4	-13.0	1.2	-7.4	-6.2	-6.8	1.2
ALS Sus22 M3	dom. pig	*Sus domesticus*	-11.5	-11.0	-11.2	0.5	-6.7	-5.7	-6.2	1.0
ALS Ovis1 M3	sheep	*Ovis aries*	-12.4	-10.7	-11.5	1.6	-7.8	-2.9	-5.3	4.9
ALS Ovis2 M3	sheep	*Ovis aries*	-13.8	-11.4	-12.6	2.5	-9.6	-2.4	-6.0	7.3
ALS Ovis3 M3	sheep	*Ovis aries*	-12.6	-10.1	-11.4	2.6	-8.4	-0.6	-4.5	7.8
ALS Ovis4 M3	sheep	*Ovis aries*	-12.2	-10.7	-11.5	1.5	-6.8	-2.1	-4.4	4.7
ALS Ovis5 M3	sheep	*Ovis aries*	-11.5	-9.7	-10.6	1.8	-7.5	-0.8	-4.1	6.7
ALS Ovis6 M3	sheep	*Ovis aries*	-13.7	-10.6	-12.1	3.0	-7.6	-1.4	-4.5	6.2
ALS Ovis7M3	sheep	*Ovis aries*	-13.2	-10.8	-12.0	2.4	-9.6	-3.7	-6.7	5.9
ALS Ovis8 M3	sheep	*Ovis aries*	-12.9	-11.5	-12.2	1.4	-8.9	-3.5	-6.2	5.4
ALS Ovis9 M3	sheep	*Ovis aries*	-13.6	-11.6	-12.6	2.0	-7.7	-1.7	-4.7	5.9
ALS Bos1 M3	cattle	*Bos taurus*	-11.0	-9.7	-10.3	1.4	-7.0	-2.9	-4.9	4.1
ALS Bos2 M3	cattle	*Bos taurus*	-14.0	-11.8	-12.9	2.2	-7.3	-2.8	-5.0	4.6
ALS Bos3 M3	cattle	*Bos taurus*	-12.6	-10.4	-11.5	2.2	-8.2	-4.1	-6.1	4.1
ALS Bos4 M3	cattle	*Bos taurus*	-11.2	-10.1	-10.6	1.2	-9.1	-1.5	-5.3	7.6
ALS Bos5 M3	cattle	*Bos taurus*	-11.8	-10.2	-11.0	1.6	-8.7	-5.4	-7.1	3.2
ALS Bos6 M3	cattle	*Bos taurus*	-12.0	-10.6	-11.3	1.4	-8.5	-5.6	-7.0	2.9
ALS Bos7 M3	cattle	*Bos taurus*	-11.9	-10.9	-11.4	1.0	-7.8	-3.8	-5.8	4.1
ALS Bos8 M3	cattle	*Bos taurus*	-12.0	-10.0	-11.0	2.0	-6.8	-4.0	-5.4	2.9
ALS Bos9 M3	cattle	*Bos taurus*	-12.6	-10.8	-11.7	1.8	-6.2	-3.7	-5.0	2.5
ALS Bos10 M3	cattle	*Bos taurus*	-13.3	-11.8	-12.5	1.5	-7.0	-3.2	-5.1	3.8
ALS Bos11 M3	cattle	*Bos taurus*	-12.0	-9.9	-11.0	2.1	-7.6	-3.8	-5.7	3.8

For additional information see S2 File. Intra-tooth minimum (Min) and maximum (Max) value;
M = (Min+Max)/2; Δ = Max−Min. Sheep and cattle $\delta^{18}O$ data were published in [85, 86], respectively.

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from Hungary from the 18th to 20th centuries describe wetlands being seen as dangerous for sheep because they were liable to catch parasites (liver fluke), from which they could not be cured [93]. Floodplains and marsh edges were also described as having a predominance of unappealing, less nutritious grasses, which were still used as reserve pastures, particularly in dry years [93, 94]. Keeping sheep in the flat wetlands despite the disadvantageous environmental conditions has been suggested from lesions found on sheep remains from Körös settlements in eastern Hungary [95–97]. Nevertheless, caprine remains rather than pig remains are dominant in the faunal assemblages [96]. Cultivated fields could also have been used for grazing on a seasonal basis. In five of the twenty analysed sheep and cattle molars, the sequential enamel $\delta^{13}C$ values deviate from the expected sinusoidal pattern of variation with a reduced amplitude of variation between $\delta^{13}C$ values recorded in winter and the consecutive or preceding summer (Pattern C, Fig 4 and S2 File). In sheep (ALS Ovis3, Ovis5), this reduced amplitude appears to be due to a rise in the winter $\delta^{13}C$ values, possibly caused by summer hay being provided in

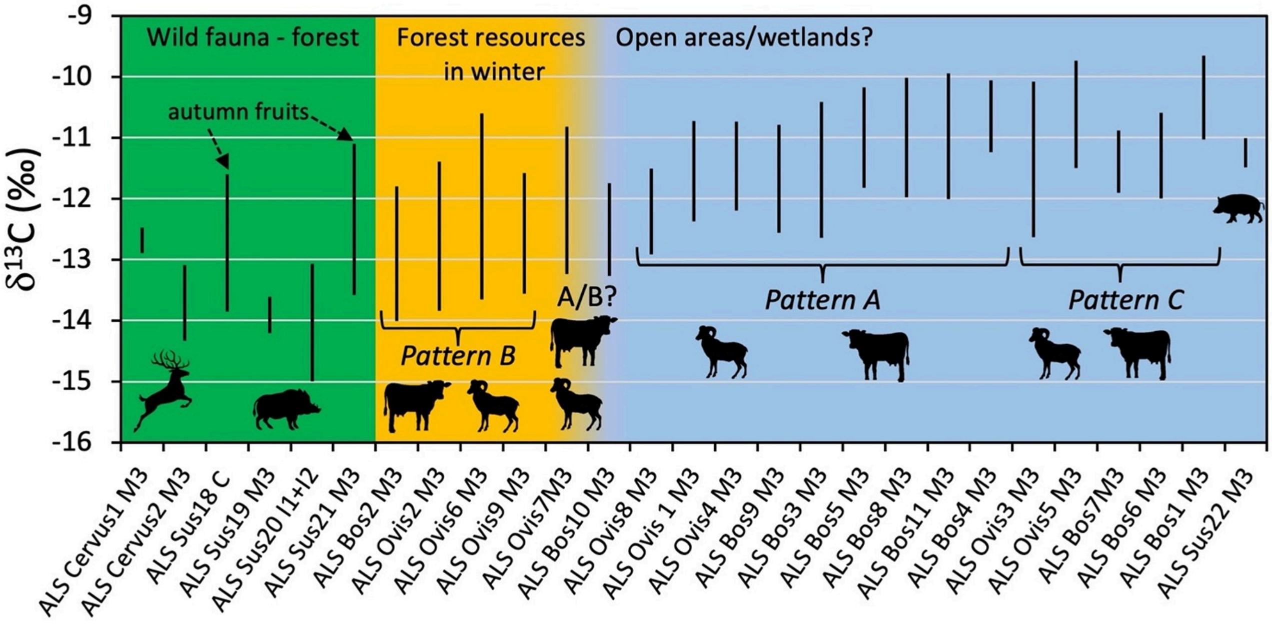

Fig 6. Range of variation of tooth enamel $\delta^{13}C$ values in the red deer (Cervus), wild boar (Sus18, 19, 20 and 21), cattle (Bos), sheep (Ovis) and pig (Sus22) from the Starčevo phase at Alsónyék. Patterns A, B and C: see main text.

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winter. Such hay could have consisted of crop wastes and grass (including wetland grasses; [93, 94]). In some cattle, the reduced amplitude of variation might rather be due to a lowering in summer $\delta^{13}C$ (ALS Bos 1 and Bos6), possibly caused by forest leaves provided in the summer. Ethnographic accounts report that tree-fodder can be particularly important for cattle and sheep in dry summers [93, 98, 99].

Forest resources also seem to have been used to supplement cattle and sheep diets during winter, as evidenced in four of the twenty analysed sheep and cattle molars showing significantly lower $\delta^{13}C$ values in winter (Pattern B, Figs 4 and 6). The phase shift between $\delta^{13}C$ and $\delta^{18}O$ sequences does not deviate from what is observed in other specimens, meaning that this contribution occurs mainly when temperatures are the lowest in winter. In the Alsónyék area of Hungary in the 20th century, snow cover in winter lasted an average of 35–40 days [100], reaching up to 25 cm [18]. Similar conditions appear to have prevailed in the past (within 1 ˚C of modern values; [101]), limiting the animals' foraging activities in winter. Ethnographic evidence from Central Europe in the 18th to 20th century indicates that domesticated animals were frequently kept in deciduous forests from spring until heavy snowfall in winter. Historical accounts from Hungary describe winter fodder being prepared by collecting and drying leaf-bearing twigs and branches, mistletoe, as well as acorns and beechnuts [93]. Most other sheep and four cattle had winter $\delta^{13}C_{enamel}$ values between −12‰ and −13‰, indicating that these individuals could also have consumed forest resources with low $\delta^{13}C$ values, but to a lower extent (or possibly in combination with larger amounts of acorns, which are higher in $\delta^{13}C$), or may have relied on freshwater wetland grasses.

There is little indication of forest resource use in the bone collagen $\delta^{13}C$ values, apparently contrasting with the enamel results. This discrepancy is likely due to the averaging effects in bone, whereby the effects of short-term consumption of foods with different $\delta^{13}C$ values are attenuated [102].

Pig husbandry

Historic sources from the 18th to 20th centuries show that pigs frequently had very varied diets, for example feeding in wetlands, where they were able to eat the vegetation, bird eggs and fish [93], and were frequently freely ranging in oak forests in autumn until they could not find acorns any more in winter and returned to where they were fed by humans [93].

The wide range of measured bone collagen $\delta^{15}N$ and $\delta^{13}C$ values shows large variability in pig diets. Eleven of the sampled pigs from Alsónyék had low $\delta^{15}N$ values, between 6.1‰ and 7.7‰, similar to most of the sampled wild boar, with $\delta^{13}C$ values mostly above −21.5‰ suggesting primarily herbivorous feeding in a predominantly open environment. However, the consumption of acorns (elevated $\delta^{13}C$ values) may mask feeding on forest undergrowth [51]. The high enamel $\delta^{13}C$ values (−11.5‰ to −11.0‰) for the sampled pig molar suggest feeding in an open environment for this individual. Since current ecological studies have shown wild boar diets to generally consist of around 86–96% plant matter (reviewed in [68]), and because the $\delta^{15}N$ values of wild and domesticated herbivores overlap with those of pigs, a highly plant-based diet can be inferred from these results for most of these domestic pigs.

Suids likely thrived in the environment surrounding Alsónyék, where acorn-bearing forests met with wet, marshy areas (cf. [23, 93]), so it is notable that pigs only made up 3% ($NISP_{identifiable\ mammal}$) of the mammal assemblage recovered from the Starčevo phase at Alsónyék [23]. This is comparable to the Starčevo site Lánycsók-Égettmalom and the Körös/Criş sites Ecsegfalva 23 and Endrőd 119, all in Hungary (Fig 1), where similarly wet conditions likely conducive to pig-keeping were present, but only low numbers of pigs were recovered (0.6% to 3% of animal remains by $NISP_{total}$; [95, 103–105]). This scarcity of pigs is also visible in the scarcity of non-ruminant adipose fats revealed by residue analysis of pottery sherds from both Alsónyék and Ecsegfalva 23 [106]. The near absence of pigs in favour of caprines and cattle has been suggested to be due to culturally driven motivations, possibly related to emotional reasons or taste preferences at other early Neolithic sites in eastern Hungary [94, 95].

Two of the 13 analysed pigs (ALS Susd5 and Susd8) had elevated $\delta^{15}N$ values of 9.0‰ and 9.2‰, similar to dogs and the lower end of the spectrum of human $\delta^{15}N$ values (Fig 7A; [25]), indicating consumption of fertilised crops or higher trophic level foods. Historical evidence from Hungary suggests these could have been, for example, meat, freshwater fish (stable isotope ratios suggest pike, catfish and zander in the case of dogs and ALS Susd5), whey, excess milk and leftover human food [93].

Ethnographic evidence from Greece shows that household pigs (i.e. 1–2 pigs stall-fed by one or more households) tended to be given dairying by-products, kitchen scraps and surplus products, whereas larger herds were taken to forage in fields and woods, and fed cereals in dry summers, or to encourage their return [107]. The bone collagen data from the Starčevo phase Alsónyék therefore suggest that a small number of pigs may have been kept as household pigs, intensely fed, whereas most pigs were kept extensively, grazing and foraging for themselves, with occasional dietary supplementation by humans.

Comparison to other phases at Alsónyék

As part of earlier work, faunal and human remains from Starčevo, Linearbandkeramik (LBK), Sopot and Lengyel phases (see Table 1; [14]) at Alsónyék were analysed for bone collagen $\delta^{13}C$ and $\delta^{15}N$ [25]. Comparison to this study indicates cattle, red deer and wild boar $\delta^{13}C$ values becoming higher in later phases at Alsónyék (Fig 8). This suggests feeding in more open areas and less reliance on forest resources after the Starčevo phase. As this trend appears not only for cattle (domesticated animals), but also for wild boar and red deer (wild animals), this suggests

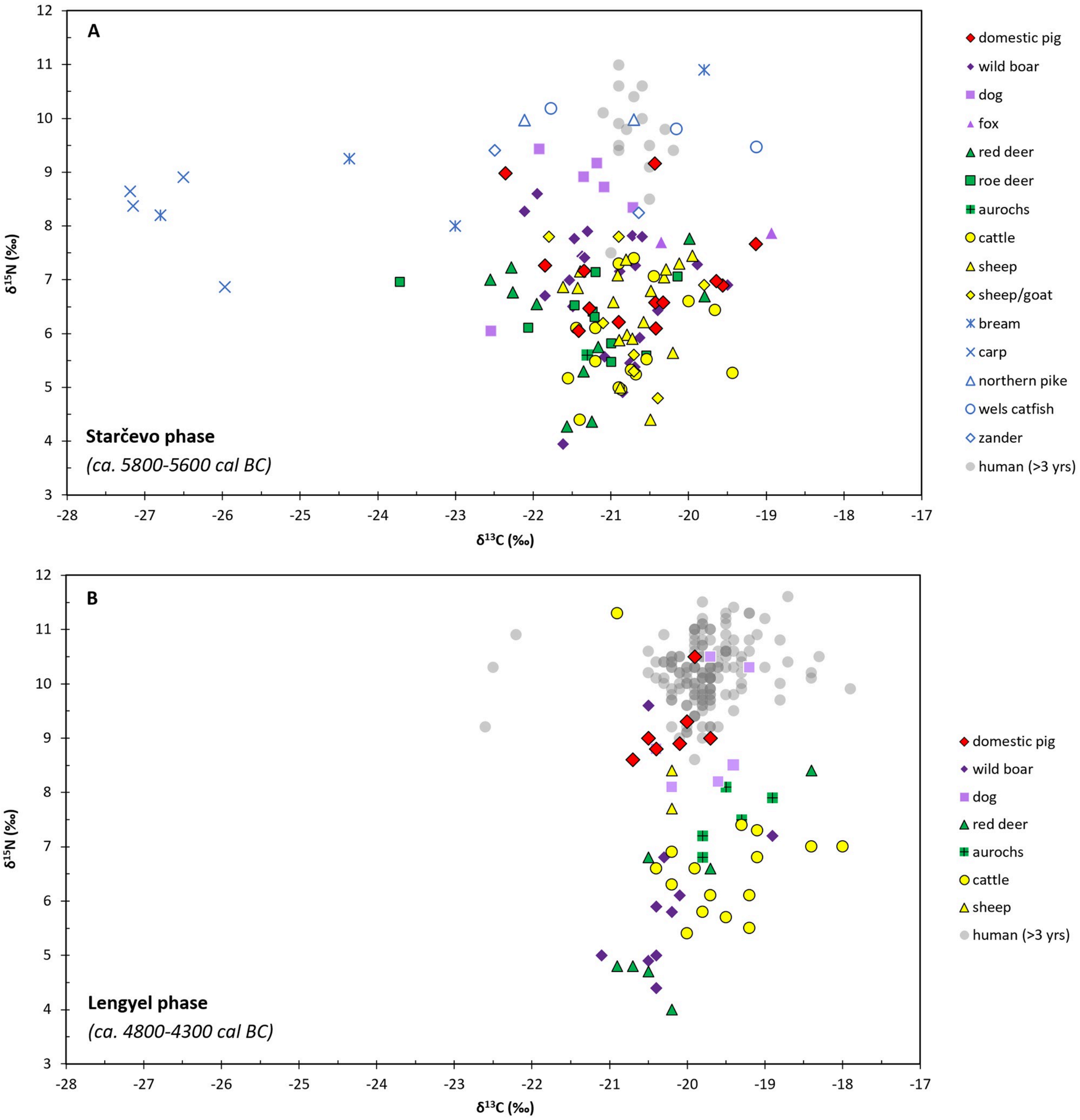

Fig 7. Stable carbon (δ^{13}C) and nitrogen (δ^{15}N) isotope ratio results for bone collagen from Alsónyék. (A) Starčevo phase, data from this study and [25]. (B): Lengyel phase, data from [25].

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deforestation from the Starčevo phase towards the Lengyel phase, likely as a result of increased grazing pressure, cutting down trees for wood, and/or intentional forest clearing.

Pigs from the Lengyel phase at Alsónyék had ca. 2–3 ‰ higher δ^{15}N values than wild boar, cattle and sheep (Fig 7B), suggesting better access to animal protein (or fertilised crops). Pig remains were around three times as common (as a proportion of domestic animals) at

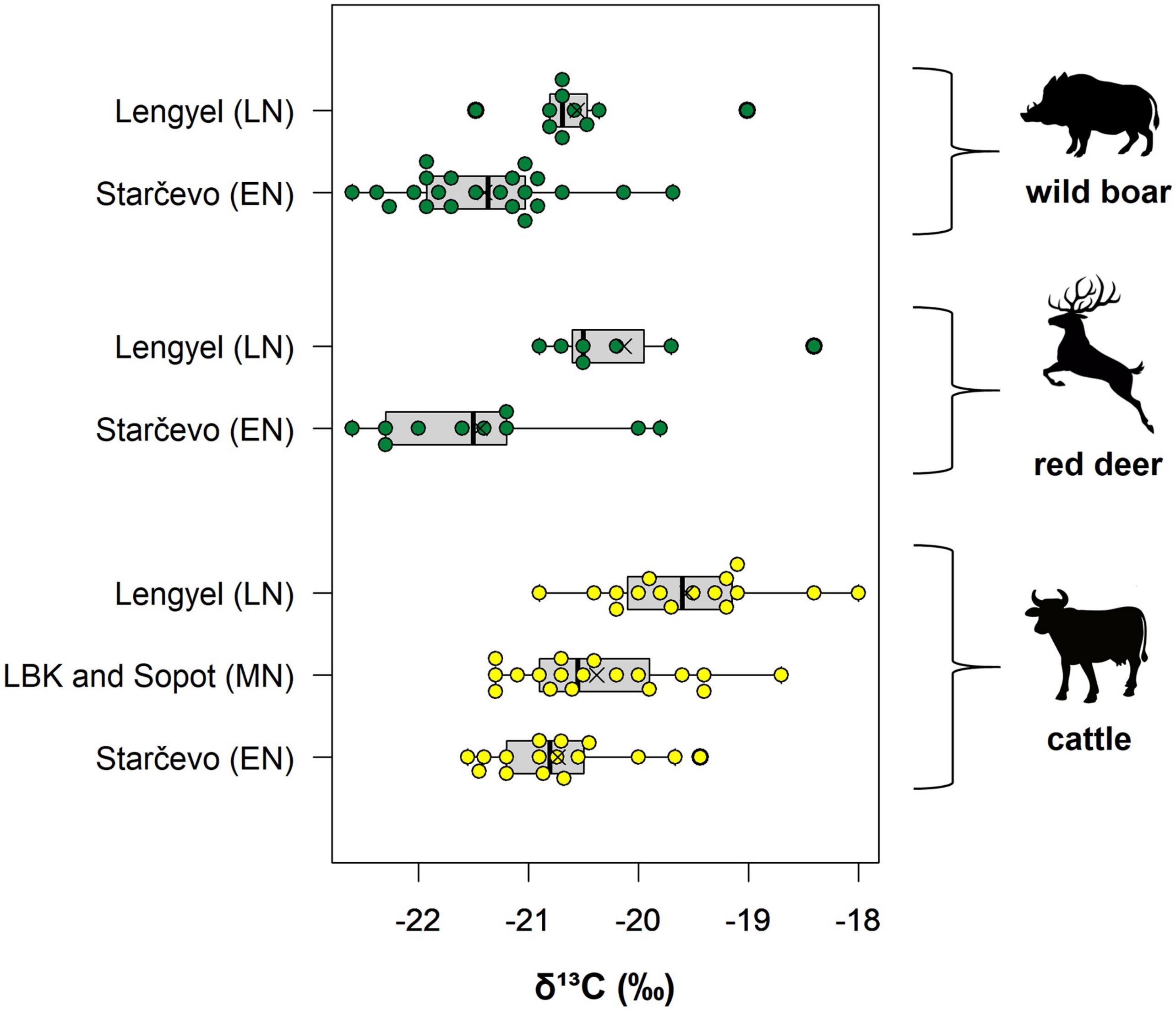

Fig 8. Stable carbon ($\delta^{13}C$) isotope ratio results for red deer, wild boar and cattle bone collagen from the Starčevo, LBK, Sopot and Lengyel phases at Alsónyék, shown as boxplots. The line in the boxplot indicates the median, and the cross the mean. Whisker boundaries were chosen as 1.5 × interquartile range. Data from this study and [25].

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Alsónyék during the Lengyel period compared to the Starčevo period [22], which, coupled with the difference in pig diets, could indicate a higher importance placed on pig keeping by the Lengyel than by Starčevo people at Alsónyék.

Comparison to other sites

To place the above results into a broader context, the literature was reviewed to identify numerically published faunal bone collagen $\delta^{13}C$ and $\delta^{15}N$ datasets with $n_{faunal} \geq 25$ and $n_{pigs} \geq 5$ from Hungary, Greece, Croatia, Serbia and Romania from the Early Neolithic to the Chalcolithic (Fig 1). Where data from multiple Neolithic phases was available, the earliest phase was compared to (cf. Table 1).

Analysis of remains from LBK phases at Füzesabony, Hungary, and from the Vinča phase at Stubline, Serbia, showed a large overlap between herbivore and pig $\delta^{13}C$ and $\delta^{15}N$ values [26, 27], suggesting pigs had little or no access to animal protein (e.g. whey, slaughter waste). (This trend is also consistent with data from Măgura—Boldul lui Moş Ivčnus, but only two domestic pigs have been analysed from this site so far [33]). By comparison, Early Neolithic remains from Zemunica, Croatia, show several pigs had low $\delta^{13}C$ and $\delta^{15}N$ values compared to cattle and sheep/goats [34], suggesting these pigs were kept primarily on forest resources, while cattle and sheep were less reliant on forest resources. At Zemunica, one analysed outlier individual had higher $\delta^{15}N$ values, and may have been a stall-fed pig [34].

Remains from Vinča-Belo Brdo (see Table 1 and Fig 1; [27]), Halai [31], Kouphovouno [108], Makriyalos [31, 109] and Balatonszárszó-Kis-erdei-dűlő [26] all showed an overlap between pig, cattle and sheep $\delta^{13}C$ and $\delta^{15}N$ values. However, at these sites, there was a tendency for pig $\delta^{15}N$ values to be on average slightly elevated compared to sheep and cattle, while pigs with $\delta^{15}N$ values toward the lower end of the spectrum displayed by cattle and sheep are missing. This suggests some (limited) availability of animal protein to pigs. Additionally, several outlier pigs at these sites had elevated $\delta^{15}N$ values, similar to the results seen for the Starčevo phase at Alsónyék, which appear to have had more access to animal protein (or fertilised crops), possibly as a result of being more intensely kept household pigs. From these data, a picture seems to be emerging, whereby pig husbandry strategies employed in Southeast Europe (cf. Fig 1) during the Early and Middle Neolithic mostly involved extensive pig herding (with occasional dietary supplementation) for the majority of individuals, while a few pigs were more intensively kept, possibly stall-fed in villages for at least part of their lives. Future data will confirm or deny this apparent trend.

In contrast, results from the late Chalcolithic (second half of 5th millennium BC) sites Vităneşti-Măgurice, Hârşova-tell and Borduşani-Popină, all in southeastern Romania, show only little overlap in $\delta^{15}N$ values between pigs and herbivores, suggesting pig diets at these sites to have comprised significantly larger amounts of animal protein [28]. Therefore, there appear to be clear spatial and temporal trends in pig keeping, the underlying affecting factors for which (whether environmental, cultural or other) remain to be discovered.

Conclusion

The results of this study showed a large extent of overlap in bone collagen $\delta^{13}C$ and $\delta^{15}N$ values for pigs, wild boar, domesticated and wild herbivores from the Starčevo phase at Alsónyék, suggesting that these animals may well have shared the same grazing/browsing areas for at least parts of the year. The spread of the data demonstrates variability between individuals of the same species, indicating that on an individual basis, different foods and habitats were used to different extents. Dense woodland or open environment "specialists" were the exception, and most of the studied animals from the Starčevo phase appear to have made use of the variability of diverse resources provided by the environment surrounding Alsónyék. The differences between individual cattle, sheep and pig diets could be due to metabolic variation, interannual variability in weather conditions, differing animal husbandry practices between households, and specific individuals being chosen to receive special treatment.

The collagen and enamel $\delta^{13}C$ data from the Starčevo phase at Alsónyék indicate that sheep and cattle tend to have fed in more open areas dominated by C_3 plants (possibly including grazing stubbles on croplands) compared to some of the roe deer and red deer. Sequential enamel analyses showed seasonal variability in the diets of sheep and cattle, whereby winter diets consisted of either grazing in open environments, consumption of forest resources, provision with summer hay, or a combination thereof. This variability in winter diets of domesticated herbivores suggests multiple strategies for coping with the challenges of wintery conditions, made feasible by the diverse environment around Alsónyék.

Most pigs appear to have mainly consumed plant matter, which, combined with the low numbers of pig remains at Alsónyék during the Starčevo phase, suggests pig husbandry at only very low intensity. Only a few pigs appear to have received greater amounts of dietary supplementation with animal protein or fertilised crops, emphasising the non-intensive character of most pig husbandry at Alsónyék during the Early Neolithic Starčevo phase.

Comparisons of the data in this study with published datasets from Neolithic sites in Greece, Serbia, Croatia and Hungary suggests that the large extent of overlap in bone collagen $\delta^{13}C$ and $\delta^{15}N$ values for pigs, wild boar, domesticated and wild herbivores observed in this study was not uncommon in this period. Sharing of the same grazing/browsing areas by different wild and domestic species may have been widely practiced during the 6th and 5th millennium in Southeast Europe.

The comparison of our data with those from later assemblages at the same site indicates a trend to higher $\delta^{13}C$ values for cattle, red deer and wild boar during the later phases at Alsónyék (Fig 8). This suggests a shift to less reliance on forest resources after the Starčevo phase, likely due to increased deforestation. These observations reinforce the importance of using animals from the same period as baselines for studies of human diets. Thus, in addition to elucidating past dietary management of domestic stock at Alsónyék, this study also provides baseline $\delta^{13}C$ and $\delta^{15}N$ values for future animal and human isotope ratio studies of Early Neolithic people in the Carpathian Basin.

Supporting information

S1 File. Bone collagen $\delta^{13}C$ and $\delta^{15}N$ results. Contains Tables A and B.
(XLSX)

S2 File. Tooth enamel $\delta^{13}C$ and $\delta^{18}O$ results. Contains Tables C-H.
(XLSX)

S3 File. Figures of bone collagen $\delta^{13}C$ and $\delta^{15}N$ results. Includes brief explanation of Bayesian ellipse modelling.
(PDF)

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References

1. Zeder MA. Domestication and early agriculture in the Mediterranean Basin: Origins, diffusion, and impact. Proc Natl Acad Sci U S A. 2008; 105(33):11597–604. https://doi.org/10.1073/pnas.0801317105 PMID: 18697943
2. Bánffy E. First Farmers of the Carpathian Basin: Changing patterns in subsistence, ritual and monumental figurines. Oxford & Philadelphia: Oxbow books; 2019.
3. Anders A, Siklósi Z. The First Neolithic Sites in Central/South-East European Transect. Volume III: The Körös Culture in Eastern Hungary. Oxford: BAR International; 2012.
4. Porčić M, Blagojević T, Pendić J, Stefanović S. The timing and tempo of the Neolithic expansion across the Central Balkans in the light of the new radiocarbon evidence. J Archaeol Sci Reports. 2020; 33(August):102528.
5. Halstead P. Like Rising Damp? An Ecological Approach to the Spread of Farming in South East and Central Europe. In: The Beginnings of Agriculture. 1989. p. 23–53.
6. Kreuz A, Marinova E. Archaeobotanical evidence of crop growing and diet within the areas of the Karanovo and the Linear Pottery Cultures: a quantitative and qualitative approach. Veg Hist Archaeobot. 2017; 26(6):639–57.
7. Benecke N. Animal husbandry and hunting in the early Neolithic of South-Eastern Europe—A review. Anim Husb Hunt early Neolit South-Eastern Eur. 2006;175–85.
8. Marinova E, Valamoti SM. Crop diversity and choice in the Prehistoric south-eastern Europe: cultural and environmental factors shaping the archaeobotanical record of northern Greece and Bulgaria. In: Plants and people: choices and diversity through time. 2013. p. 46–54.
9. Ivanova M, De Cupere B, Ethier J, Marinova E. Pioneer farming in southeast Europe during the early sixth millennium BC: Climate-related adaptations in the exploitation of plants and animals. PLoS One. 2018; 13(5):1–23.
10. Kempf M. Take a seed! Revealing Neolithic landscape and agricultural development in the Carpathian Basin through multivariate statistics and environmental modelling. Vol. 16, PLoS ONE. 2021. 1–34 p.
11. Bánffy E. Chapter 11 The first farmers in the Danube-Tisza interfluve. In: Bánffy E, editor. The Early Neolithic in the Danube-Tisza interfluve. BAR International; 2013. p. 155–90.
12. Rassmann K, Stevens F, Oross K, Marton T, Osztás A, Serlegi G, et al. Windows onto the landscape: Prospections on the prehistoric sites at Alsónyék, Fajsz-Kovácshalom, Fajsz-Garadomb and Tolna-Mözs in the Sárköz region. Confinia et Horizontes 1. 2020.

13. Oross K, Bánffy E, Osztás A, Marton T, Nyerges ÉÁ, Köhler K, et al. The early days of Neolithic Alsónyék: The Starčevo occupation. In: Bericht der Römisch-Germanischen Kommission. 2016. p. 93–121.
14. Bánffy E, Osztás A, Oross K, Zalai-Gaál I, Marton T, Nyerges ÉÁ, et al. The Alsónyék story: Towards the history of a persistent place. Bericht der Römisch-Germanischen Kommission. 2016; 94:283–361.
15. Osztás A, Bánffy E, Zalai-Gaál I, Oross K, Marton T, Somogyi K. Alsónyék-Bátaszék: Introduction to a major Neolithic settlement complex in south-east Transdanubia, Hungary. In: Bericht der Römisch-Germanischen Kommission. 2016. p. 7–21.
16. Depaermentier MLC, Osztás A, Bánffy E, Alt KW, Kempf M. Neolithic land-use, subsistence, and mobility patterns in Transdanubia: A multiproxy isotope and environmental analysis from Alsónyék–Bátaszék and Mórágy–Tűzkődomb. J Archaeol Sci Reports. 2020; 33(June):102529.
17. Nagy B, Gulyás S, Sümegi P. Paleoenvironmental Reconstruction of the Alsónyék-Bátaszék Starčevo Site Using Archeomalacological Data. Archeometriai Muh. 2019; 16(3):167–74.
18. Sümegi P, Náfrádi K, Jakab G, Törőcsik T, Bodor E, Molnár M, et al. Prehistoric environment of the Sárköz region in the Danube Valley, southern Hungary. Case studies from infilled oxbow lakes. Environ Hist Prehist Sárköz Reg South Hungary Confin horizontes 1. 2020;(Breasted 1916).
19. Bánffy E, Marton T, Osztás A. Early Neolithic settlement and burials at Alsónyék-Bátaszék. Neolit Carpathian Basin Northernmost Distrib Starčevo/Körös Cult. 2010;37–51.
20. Kreuz A, Pomázi P, Bánffy E. Hungarian Neolithic landscapes, crops and diet–Signs of cultural decisions? Quat Int. 2020; 560–561(February):102–18.
21. Gál E, Biller AZ, Nyerges ÉÁ, Osztás A. Bird remains from the Starčevo and Lengyel culture settlements of the site Alsónyék-Bátaszék (South-western Hungary). Mater şi Cercet Arheol (Serie nouă). 2021; 1(1):467–86.
22. Nyerges ÉÁ, Biller AZ. Neolithic Animal Husbandry in the Tolnai-Sárköz Region on the Basis of the Archaeozoological Finds From the Alsónyék-Bátaszék Archaeological Site. Hungarian Archaeol. 2015;1–7.
23. Biller AZ. Animal keeping and hunting habits of the Early Neolithic settlement of Alsónyék in the mirror of the animal bones. In: Bánffy E, Gramsch A, editors. The Neolithic of the Sárköz and adjacent regions in Hungary: bioarchaeological studies. Confinia et Horizontes 2. 2023. p. 387–417.
24. Kreuz A, Pomázi P, Osztás A, Oross K, Marton T, Petrasch J, et al. Investigation of the plant macroremains from four archaeological excavations at Fajsz-Garadomb and Alsónyék-Bátaszék in the Sárköz region and their comparison with the archaeobotanical record from other Hungarian Neolithic sites. In: The environmental history of the Prehistoric Sárköz region in southern Hungary. 2020. p. 187–220.
25. Bayliss A, Beavan N, Hamilton D, Köhler K, Nyerges ÉÁ, Ramsey CB, et al. Peopling the past: Creating a site biography in the Hungarian Neolithic. Vol. 94, Bericht der Römisch-Germanischen Kommission. 2016. p. 23–91.
26. Whittle A, Anders A, Bentley RA, Bickle P, Cramp L, Domboróczki L, et al. 3. Hungary. In: The first farmers of central Europe Diversity in LBK lifeways. 2013.
27. Gillis RE, Bulatović J, Penezić K, Spasić M, Tasić NN, Makarewicz CA. Herding and Hunting at Vinča–Belo Brdo and Stubline During the Late Neolithic, a Stable Isotopic Perspective. Anim Husb Hunt Cent West Balk Through Time. 2020;(Archaeopress):19–39.
28. Balasse M, Evin A, Tornero C, Radu V, Fiorillo D, Popovici D, et al. Wild, domestic and feral? Investigating the status of suids in the Romanian Gumelniţa (5th mil. cal BC) with biogeochemistry and geometric morphometrics. J Anthropol Archaeol. 2016; 42:27–36.
29. Pearson JA, Hedges REM. Stable carbon and nitrogen isotope analysis and the evidence for diet at Ecsegfalva and beyond. In: The Early Neolithic on the Great Hungarian Plain: Investigations of the Koros Culture Site of Ecsegfalva 23, County Bekes. 2007. p. 37–43.
30. Whittle A, Bartosiewicz L, Borić D, Pettitt P, Richards M. In the beginning: New radiocarbon dates for the early Neolithic in Northern Serbia and South-East Hungary. Antaeus. 2002; 25:63–117.
31. Vaiglova P, Coleman J, Diffey C, Tzevelekidi V, Fillios M, Pappa M, et al. Exploring Diversity in Neolithic Agropastoral Management in Mainland Greece Using Stable Isotope Analysis. Environ Archaeol. 2021; 0(0):1–24.
32. Kalicz N. Früh- und spätneolithische Funde in der Gemarkung des Ortes Lánycsók (Vorbericht). Janus Pannon Múzeum Évkönyve. 1977; 22:137–58.
33. Balasse M, Bălăşescu A, Janzen A, Ughetto-Monfrin J, Mirea P, Andreescu R. Early herding at Măgura-Boldul lui Moş Ivănuş (early sixth millennium BC, Romania): environments and seasonality from stable isotope analysis. Eur J Archaeol. 2013; 16(2):221–46.

34. Guiry E, Karavanić I, Klindžić RŠ, Talamo S, Radović S, Richards MP. Stable Isotope Palaeodietary and Radiocarbon Evidence from the Early Neolithic Site of Zemunica, Dalmatia, Croatia. Eur J Archaeol. 2017; 20(2):235–56.

35. Krueger HW, Sullivan CH. Models for carbon isotope fractionation between diet and bone. Stable Isot Nutr ACS Symp Ser No258. 1984;(258):205–20.

36. Lee-Thorp JA, Sealy JC, van der Merwe NJ. Stable carbon isotope ratio differences between bone collagen and apatite, and their relationship to diet. J Archaeol Sci. 1989; 16(6):585–99.

37. Blanz M, Mainland I, Richards M, Balasse M, Ascough P, Wolfhagen J, et al. Identifying seaweed consumption by sheep using isotope analysis of their bones and teeth: Modern reference δ13C and δ15N values and their archaeological implications. J Archaeol Sci. 2020; 118(April).

38. Schoeninger MJ, De Niro MJ, DeNiro MJ. Nitrogen and carbon isotopic composition of bone collagen from marine and terrestrial animals. Geochim Cosmochim Acta. 1984; 48(4):625–39.

39. Ambrose SH, Norr L. Experimental Evidence for the Relationship of the Carbon Isotope Ratios of Whole Diet and Dietary Protein to Those of Bone Collagen and Carbonate. In: Prehistoric Human Bone: Archaeology at the Molecular Level. 1993. p. 1–38.

40. Froehle AW, Kellner CM, Schoeninger MJ. FOCUS: Effect of diet and protein source on carbon stable isotope ratios in collagen: Follow up to Warinner and Tuross (2009). J Archaeol Sci. 2010; 37 (10):2662–70. Available from: https://doi.org/http%3A//dx.doi.org/10.1016/j.jas.2010.06.003

41. Barton L, Newsome SD, Chen FH, Wang H, Guilderson TP, Bettinger RL. Agricultural origins and the isotopic identity of domestication in northern China. Proc Natl Acad Sci U S A. 2009; 106(14):5523–8. https://doi.org/10.1073/pnas.0809960106 PMID: 19307567

42. Ervynck A, Lentacker A, Müldner G, Richards M, Dobney K. An investigation into the transition from forest dwelling pigs to farm animals in medieval Flanders, Belgium. In: Pigs and Humans: 10,000 years of interactions. 2007.

43. Madgwick R, Mulville J, Stevens RE. Diversity in foddering strategy and herd management in late Bronze Age Britain: An isotopic investigation of pigs and other fauna from two midden sites. Environ Archaeol. 2012; 17(2):126–40.

44. Bogaard A, Heaton THE, Poulton P, Merbach I. The impact of manuring on nitrogen isotope ratios in cereals: archaeological implications for reconstruction of diet and crop management practices. J Archaeol Sci. 2007; 34(3):335–43.

45. Webb EC, Stewart A, Miller B, Tarlton J, Evershed RP. Age effects and the influence of varying proportions of terrestrial and marine dietary protein on the stable nitrogen-isotope compositions of pig bone collagen and soft tissues from a controlled feeding experiment. STAR Sci Technol Archaeol Res. 2016; 2(1):54–66.

46. Kalapos T, Baloghné-Nyakas A, Csontos P. Occurrence and ecological characteristics of c4 dicot and Cyperaceae species in the Hungarian flora. Photosynthetica. 1997; 33(2):227–40.

47. Bonafini M, Pellegrini M, Ditchfield P, Pollard AM. Investigation of the "canopy effect" in the isotope ecology of temperate woodlands. J Archaeol Sci. 2013; 40(11):3926–35.

48. Drucker DG, Bridault A, Hobson KA, Szuma E, Bocherens H. Can carbon-13 in large herbivores reflect the canopy effect in temperate and boreal ecosystems? Evidence from modern and ancient ungulates. Palaeogeogr Palaeoclimatol Palaeoecol. 2008; 266(1–2):69–82.

49. Tieszen LL. Natural variations in the carbon isotope values of plants: implications for archaeology, ecology and palaeoecology. J Archaeol Sci. 1991; 18:227–48.

50. Stevens RE, Lister AM, Hedges REM. Predicting diet, trophic level and palaeoecology from bone stable isotope analysis: A comparative study of five red deer populations. Oecologia. 2006; 149(1):12–21. https://doi.org/10.1007/s00442-006-0416-1 PMID: 16628415

51. Frémondeau D, Cucchi T, Casabianca F, Ughetto-Monfrin J, Horard-Herbin MP, Balasse M. Seasonality of birth and diet of pigs from stable isotope analyses of tooth enamel (δ18O, δ13C): A modern reference data set from Corsica, France. J Archaeol Sci. 2012; 39(7):2023–35.

52. Wallace M, Jones G, Charles M, Fraser R, Halstead P, Heaton THE, et al. Stable carbon isotope analysis as a direct means of inferring crop water status and water management practices. World Archaeol. 2013; 45(3):388–409.

53. Jensen KB, Asay KH, Johnson DA, Waldron BL. Carbon isotope discrimination of tall fescue cultivars across an irrigation gradient. Can J Plant Sci. 2004; 84(1):157–62.

54. Zhao B, Kondo M, Maeda M, Ozaki Y, Zhang J. Water-use efficiency and carbon isotope discrimination in two cultivars of upland rice during different developmental stages under three water regimes. Plant Soil. 2004; 261(1–2):61–75.

55. Bloch D, Hoffmann CM, Märländer B. Impact of water supply on photosynthesis, water use and carbon isotope discrimination of sugar beet genotypes. Eur J Agron. 2006; 24(3):218–25.

56. Lynch AH, Hamilton J, Hedges REM. Where the wild things are: Aurochs and cattle in England. Antiquity. 2008; 82(318):1025–39.

57. Longinelli A. Oxygen isotopes in mammal bone phosphate: A new tool for paleohydrological and paleoclimatological research? Geochim Cosmochim Acta. 1984; 48(2):385–90.

58. Land LS, Lundelius EL, Valastro S. Isotopic ecology of deer bones. Palaeogeogr Palaeoclimatol Palaeoecol. 1980; 32(C):143–51.

59. D'Angela D, Longinelli A. Oxygen isotopes in living mammal's bone phosphate: Further results. Chem Geol Isot Geosci Sect. 1990; 86(1):75–82.

60. Rozanski K, Araguás-Araguás L, Gonfiantini R. Isotopic Patterns in Modern Global Precipitation. 2013;(July 2014):1–36.

61. Chen G, Schnyder H, Auerswald K. Model explanation of the seasonal variation of δ18O in cow (Bos taurus) hair under temperate conditions. Sci Rep. 2017; 7(1):1–15.

62. Balasse M, Ambrose SH, Smith AB, Price TD. The seasonal mobility model for prehistoric herders in the south-western Cape of South Africa assessed by isotopic analysis of sheep tooth enamel. J Archaeol Sci. 2002; 29(9):917–32.

63. Tieszen LLL, Fagre T. Effect of diet quality and composition on the isotopic composition of respiratory CO2, bone collagen, bioapatite, and soft tissues. In: Prehistoric human bone: archaeology at the molecular level. 1993. p. 121–55.

64. Cerling TE, Harris JM. Carbon isotope fractionation between diet and bioapatite in ungulate mammals and implications for ecological and paleoecological studies. Oecologia. 1999; 120(3):347–63. https://doi.org/10.1007/s004420050868 PMID: 28308012

65. Passey BH, Robinson TF, Ayliffe LK, Cerling TE, Sponheimer M, Dearing MD, et al. Carbon isotope fractionation between diet, breath CO2, and bioapatite in different mammals. J Archaeol Sci. 2005; 32 (10):1459–70.

66. Kohn MJ. Carbon isotope compositions of terrestrial C3 plants as indicators of (paleo)ecology and (paleo)climate. Proc Natl Acad Sci. 2010; 107(46):19691–5. https://doi.org/10.1073/pnas.1004933107 PMID: 21041671

67. Freyer HD, Belacy N. 13C/12C records in northern hemispheric trees during the past 500 years—Anthropogenic impact and climatic superpositions. J Geophys Res. 1983; 88(C11):6844–52.

68. Schley L, Roper TJ. Diet of wild boar Sus scrofa in Western Europe, with particular reference to consumption of agricultural crops. Mamm Rev. 2003; 33(1):43–56.

69. Ballari SA, Barrios-García MN. A review of wild boar Sus scrofa diet and factors affecting food selection in native and introduced ranges. Mamm Rev. 2014; 44(2):124–34.

70. Balasse M, Tornero C, Bréhard S, Ughetto-Monfrin J, Voinea V, Bălăşescu A. Cattle and Sheep Herding at Cheia, Romania, at the Turn of the Fifth Millennium cal BC. Early Farmers. 2014;(1).

71. Mole S, Joern A, O'Leary MH, Madhavan S. Spatial and temporal variation in carbon isotope discrimination in prairie graminoids. Oecologia. 1994; 97(3):316–21. https://doi.org/10.1007/BF00317320 PMID: 28313625

72. Smedley MP, Dawson TE, Comstock JP, Donovan L a, Sherill DE, Cook CS, et al. Seasonal carbon isotope discrimination in a grassland community. Oecologia. 1991; 85:314–20. https://doi.org/10.1007/BF00320605 PMID: 28312034

73. Heaton THE. Spatial, Species, and Temporal Variations in the $^{13}C/^{12}C$ Ratios of C_3 Plants: Implications for Palaeodiet Studies. J Archaeol Sci. 1999; 26(6):637–49.

74. Balasse M, Tresset A, Obein G, Fiorillo D, Gandois H. Seaweed-eating sheep and the adaptation of husbandry in Neolithic Orkney: new insights from Skara Brae. Antiquity. 2019; 370:919–32.

75. Balasse M, Mainland I, Richards MP. Stable isotope evidence for seasonal consumption of marine seaweed by modern and archaeological sheep in the Orkney archipelago (Scotland). Environ Archaeol. 2009; 14(1):1–14.

76. Bökönyi S. Problems With Using Osteological Materials of Wild Animals for Comparisons in Archaeology. Anthr Közl. 1995; 37:3–11.

77. Schulting RJ, Vaiglova P, Crozier R, Reimer PJ. Further isotopic evidence for seaweed-eating sheep from Neolithic Orkney. J Archaeol Sci Reports. 2017; 11:463–70.

78. Longin R. New method of collagen extraction for radiocarbon dating. Nature. 1971; 230(5291):241–2. https://doi.org/10.1038/230241a0 PMID: 4926713

79. Bocherens H, Fizet M, Mariotti A, Lange-Badre B, Vandermeersch B, Borel JP, et al. Isotopic biogeochemistry (13C,15N) of fossil vertebrate collagen: application to the study of a past food web including Neandertal man. J Hum Evol. 1991; 20(6):481–92.

80. Szpak P, Orchard TJ, Salomon AK, Gröcke DR. Regional ecological variability and impact of the maritime fur trade on nearshore ecosystems in southern Haida Gwaii (British Columbia, Canada): Evidence from stable isotope analysis of rockfish (Sebastes spp.) bone collagen. Archaeol Anthropol Sci. 2013; 5(2):159–82.
81. Guiry EJ, Szpak P. Improved quality control criteria for stable carbon and nitrogen isotope measurements of ancient bone collagen. J Archaeol Sci. 2021; 132(October 2020):105416.
82. Ambrose SH. Preparation and characterization of bone and tooth collagen for isotopic analysis. J Archaeol Sci. 1990; 17(4):431–51.
83. Van Klinken GJ. Bone collagen quality indicators for palaeodietary and radiocarbon measurements. J Archaeol Sci. 1999; 26(6):687–95.
84. Jackson AL, Inger R, Parnell AC, Bearhop S. Comparing isotopic niche widths among and within communities: SIBER—Stable Isotope Bayesian Ellipses in R. J Anim Ecol. 2011; 80(3):595–602. https://doi.org/10.1111/j.1365-2656.2011.01806.x PMID: 21401589
85. Balasse M, Tresset A, Bălăşescu A, Blaise E, Tornero C, Gandois H, et al. Animal Board Invited Review: Sheep birth distribution in past herds: A review for prehistoric Europe (6th to 3rd millennia BC). Animal. 2017; 11(12):2229–36. https://doi.org/10.1017/S1751731117001045 PMID: 28532521
86. Balasse M, Gillis R, Živaljević I, Berthon R, Kovačiková L, Fiorillo D, et al. Seasonal calving in European Prehistoric cattle and its impacts on milk availability and cheese-making. Sci Rep. 2021; 11 (1):8185. https://doi.org/10.1038/s41598-021-87674-1 PMID: 33854159
87. Frémondeau D, Nuviala P, Duval C. Pigs and Cattle in Gaul: The Role of Gallic Societies in the Evolution of Husbandry Practices. Eur J Archaeol. 2017; 20(3):494–509.
88. Dardaillon M. Seasonal feeding habits of the wild boar in a mediterranean wetland, the Camargue (Southern France). Acta Theriol (Warsz). 1987; 32(1976):389–401.
89. O'Regan HJ, Lamb AL, Wilkinson DM. The missing mushrooms: Searching for fungi in ancient human dietary analysis. J Archaeol Sci. 2016; 75:139–43.
90. Biró M, Molnár Z, Öllerer K, Lengyel A, Ulicsni V, Szabados K, et al. Conservation and herding co-benefit from traditional extensive wetland grazing. Agric Ecosyst Environ. 2020; 300(January):106983.
91. Biró M, Molnár Z, Babai D, Dénes A, Fehér A, Barta S, et al. Reviewing historical traditional knowledge for innovative conservation management: A re-evaluation of wetland grazing. Sci Total Environ. 2019; 666:1114–25. https://doi.org/10.1016/j.scitotenv.2019.02.292 PMID: 30970477
92. Babai D, Molnár K, Biró M. Changing year-round habitat use of extensively grazing cattle, sheep and pigs in East-Central Europe between 1940 and 2014: Consequences for conservation and policy. Agric Ecosyst Environ. 2016; 234:142–53.
93. Andrásfalvy B, Balassa I, Égető M, Gráfik I, Gunda B, Kotics J, et al. Magyar Néprajz Vol. 2. Budapest: Akadémiai Kiadó; 2001.
94. Molnár Z. Perception and management of spatio-temporal pasture heterogeneity by Hungarian herders. Rangel Ecol Manag. 2014; 67(2):107–18.
95. Bartosiewicz L. Mammalian bone. In: The early neolithic on the Great Hungarian Plain: Investigations of the Körös culture site of Ecsegfalva 23, County Békés. 2007. p. 287–325.
96. Bartosiewicz L. Mammalian remains from Körös Culture sites in Hungary. In: The Körös Culture in Eastern Hungary. 2012. p. 195–204.
97. Gál E, Bartosiewicz L. Integrating New Prehistoric Palaeopathological Finds from Hungary. Animals. 2023; 13:1–15. https://doi.org/10.3390/ani13121994 PMID: 37370504
98. Haas JN, Karg S, Rasmussen P. Beech Leaves and Twigs used as Winter Fodder: Examples from Historic and Prehistoric Times. Environ Archaeol. 1996; 1(1):81–6.
99. Halstead P. Ask the Fellows who Lop the Hay: Leaf-Fodder in the Mountains of Northwest Greece. Rural Hist. 1998; 9(02):211.
100. Mayer A. Bátaszék természetföldrajzi viszonyai. In: Bátaszék Története: A kezdetektől 1539-ig. 1997. p. 1–23.
101. Davis BAS, Brewer S, Stevenson AC, Guiot J, Allen J, Almqvist-Jacobson H, et al. The temperature of Europe during the Holocene reconstructed from pollen data. Quat Sci Rev. 2003; 22(15–17):1701–16.
102. Berthon R, Kovačiková L, Tresset A, Balasse M. Integration of Linearbandkeramik cattle husbandry in the forested landscape of the mid-Holocene climate optimum: Seasonal-scale investigations in Bohemia. J Anthropol Archaeol. 2018; 51(November 2017):16–27.
103. Bökönyi S. The Early Neolithic vertebrate fauna of Endrőd 119. In: Bökönyi S, editor. Cultural and Landscape Changes in South-East Hungary I Reports on the Gyomaendrőd Project. Cambridge: Cambridge University Press; 1992. p. 195–300.

104. Bökönyi S. Early Neolithic vertebrate fauna from Lánycsók-Égetthalom. Acta Archaeol Acad Sci Hungaricae Budapest. 1981; 33:21–34.

105. Pike-Tay A, Bartosiewicz L, Gal E. Body part representation and seasonality: sheep/goat, bird and fish remains from early Neolithic Ecsegfalva 23, SE Hungary. J Taphon. 2004; 2(4):221–46.

106. Ethier J, Bánffy E, Vuković J, Leshtakov K, Bacvarov K, Roffet-Salque M, et al. Earliest expansion of animal husbandry beyond the Mediterranean zone in the sixth millennium BC. Sci Rep. 2017; 7(1):1–10.

107. Halstead P, Isaakidou V. A pig fed by hand is worth two in the bush: Ethnoarchaeology of pig husbandry in Greece and its archaeological implications. Ethnozooarchaeology Present Past Human-Animal Relationships. 2011;160–74.

108. Vaiglova P, Bogaard A, Collins M, Cavanagh W, Mee C, Renard J, et al. An integrated stable isotope study of plants and animals from Kouphovouno, southern Greece: A new look at Neolithic farming. J Archaeol Sci. 2014; 42(1):201–15.

109. Triantaphyllou S. A bioarchaeological approach to prehistoric cemetery populations from Western and Central Macedonia. University of Sheffield; 1999.