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Mobility of interred individuals at Neolithic tombs in Wales using sulfur ($\delta^{34}S$) bone collagen isotope values and a predictive archaeological sulfur isoscape for the UK

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We measured human bone collagen sulfur isotope values of 27 Neolithic humans from five early Neolithic (fourth millennium cal BC) tombs in south Wales, UK (Heston Brake, Parc le Breos Cwm, Penywyrlod, Tinkinswood and Ty Isaf). To determine whether individuals interred within these tombs were 'local' to the area or 'non-local', their values were compared to a random forest collagen $\delta^{34}S$ isoscape map that was developed from archaeological fauna from across the UK using the R package 'AssignR'. All individuals within the sample are probably local to Wales, but four of five sites had individuals that may be considered 'non-local' to the region where the tomb was located. We then compared the $\delta^{34}S$ collagen isoscape to a $\delta^{34}S$ modern plant isoscape. In some regions, there are large differences between the collagen isoscape and modern plant isoscape that may be due to modern pollution or anthropogenic changes to the landscape that may impact bacteria in soil or the local sulfur cycle.

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1. Introduction

Neolithic communities probably reached southern Wales and the Marches in the early fourth millennium cal BC, in the thirty-ninth or possibly the fortieth century BC [1]. The early Neolithic in Britain is characterized by the introduction of agriculture, domesticated fauna (sheep, goats, cattle and pigs) and pottery, substantial dietary changes and the construction of megalithic and other structures [2–6]. The construction of megalithic tombs probably began a century or so after Neolithic settlers appeared [1]. Genetic evidence now strongly indicates that the dramatic shift in diets and material culture between the Mesolithic and the Neolithic was due to the arrival of new people [7,8], although there remains much debate on the details of this issue [9].

In this study, we explore the potential origins and mobility of Neolithic individuals from five Neolithic tombs in south Wales. This work builds upon the earlier strontium isotope studies of mobility at a number of these tombs by Neil *et al.* through the use of sulfur isotope measurements of bone collagen [10,11]. Similar to strontium, sulfur isotopes can be used as a mobility indicator and specifically can point to the geographical origin of foods that were consumed by past humans [12–14]. Strontium isotopes reflect mobility during childhood, whereas sulfur can reflect later adult mobility if analysed on bone collagen, so the two methods are complementary. Also similar to strontium, interpreting sulfur isotopes for mobility requires a good understanding of the baseline sulfur isotope values of a region.

We have produced a baseline sulfur map (a random forest δ^{34}S isoscape) based on collagen sulfur isotope measurements from both previously published and new δ^{34}S faunal values from across the UK. We then used this isoscape as a baseline to compare with the sulfur isotope values of 27 individuals from Heston Brake, Parc le Breos Cwm, Penywyrlod, Tinkinswood and Ty Isaf Neolithic tombs using previously published δ^{34}S collagen values [15]. The δ^{34}S collagen isoscape is also compared to a sulfur isoscape for the UK based on modern plants measured by Evans *et al*. [16] to find potential δ^{34}S baseline shifts across regions of the UK.

2. The study sites

These five sites (figure 1) are a select few of the numerous Neolithic burial cairns in south Wales [6,17–19]. All five sites have previously published human isotope values, including carbon, nitrogen, sulfur and strontium [6,10,15]. Further information on each of the sites is given below. For further information on these sites, including site layouts and radiocarbon chronologies, please see [6], and the chapters therein.

Heston Brake is close to the upper Severn estuary in southeast Wales. The surviving remains suggest an unusually elongated chamber, which has defied easy classification [17–20]. The tomb was first explored in antiquarian excavations in the late 19th century, when a number of human remains were uncovered [21]. It has not been radiocarbon dated.

Parc le Breos Cwm is located on the Gower peninsula. It is a substantial trapezoidal cairn with forecourt and transepted chambers, in Severn-Cotswold style [22]. First investigated in 1869 by Sir John Lubbock, it was re-excavated in 1960–1961 by Richard Atkinson. Its primary use probably dates to the thirty-seventh century cal BC [1,22]; bone from the passage may have been reburied from nearby Cat Hole Cave [23]. Stable isotope analysis (carbon and nitrogen) showed terrestrial diets for all individuals [15].

Penywyrlod (Talgarth) is one of the distinctive inland Black Mountains groups of Neolithic long cairns [6,17]. Its large, carefully built stone cairn probably contains several chambers; three have been investigated, first in excavations in 1972 by Hubert Savoury, and more recently in 2015–2016 by Bill Britnell [6,24]. Its style suggests strong resemblances to Cotswold lateral-chambered tombs. The monument was probably begun in the thirty-eighth century cal BC, with pre-cairn occupation probably going back to the thiry-ninth century [1]. As such, this site is probably one of the first Neolithic monuments in south Wales [6].

Tinkinswood is located in the Vale of Glamorgan in south-east Wales. Tinkinswood had a prominent terminal chamber approached by a forecourt; another cist within the fabric of the cairn may have been added later. The monument was excavated in 1914 by John Ward [25]. Radiocarbon dating indicates construction and primary use probably in the thirty-eighth or early thirty-seventh century cal BC [1]. Early research demonstrated that the materials used to construct the tomb were sourced locally [25].

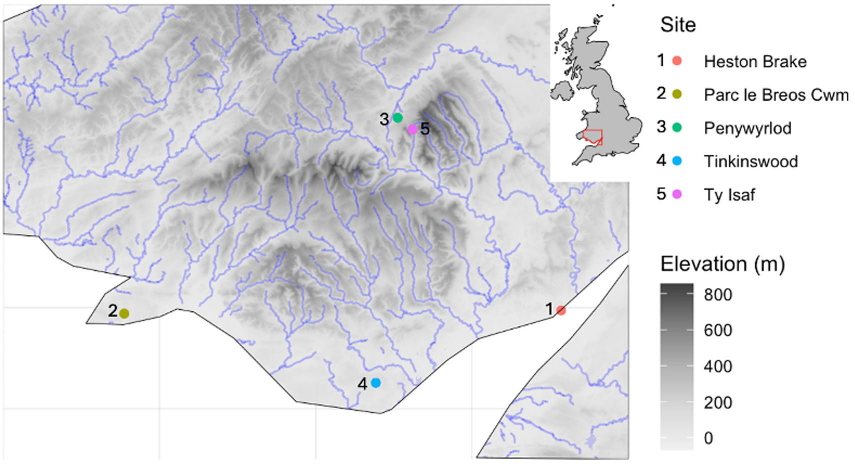

Figure 1. Map of south Wales and the study sites. Administrative maps from naturalearth.com

Ty Isaf is located near Penywyrlod in the Black Mountains. A round cairn with passage and opposed chambers is incorporated within a wedge-shaped cairn with opposed lateral chambers and a forecourt; partially preserved remains also suggest another chamber at the distal end of the cairn. It is unlikely that all these features were built at the same time. The site was excavated by W.F. Grimes in 1938 [17,26]. Radiocarbon dating has not produced a clear picture of construction and use but could suggest first use from the 37th or 36th centuries cal BC [1].

3. Sulfur in the biosphere

Sulfur enters the ecosystem in a variety of ways, such as precipitation, weathering from bedrock geology and dry deposition such as dust [12,27]. Once in the soils of an ecosystem, sulfur isotopes can experience a wide range of potential fractionation [12,27–29]. In wetlands or flood lands where anaerobic conditions occur in soils, bacteria can reduce sulfate to sulfide, and in doing so, cause large isotope fractionations [29]. In these regions, it can be expected that values may be very low (approx. −20‰ δ^{34}S).

Plants will incorporate sulfur in most circumstances as sulfate; in wetland conditions, plants may incorporate the δ^{34}S-depleted sulfide [27–35]. Often, plants are approximately 1.5‰ depleted relative to local soils. From here, plants will be consumed by fauna, with a small and possibly variable trophic fractionation [12]. Sulfur in animals and humans comes directly from diet; for collagen, it is the protein portion [36]. Thus, sulfur may tell us information not only about the diet, but also where the food was grown or raised.

Sulfur within mammal bone collagen is found only in the amino acid methionine [13]. Methionine cannot be formed by humans and therefore is incorporated from dietary methionine. However, within individuals, methionine may experience further fractionation during the potential formation of cysteine. Therefore, cysteine-sufficient diets may experience less methionine fraction between diet and consumer. This internal individual fractionation may explain the variance in fractionation studies [12]. Populations that share similar diets will then likely have similar dietary fractionation from food consumed and similar bone collagen methionine δ^{34}S values.

δ^{34}S spatial variation in plants and animals can generally be grouped into three types of environments, namely wetland, coastal or inland. In wetland environments, it is expected that sulfur isotope values in plant and animal tissues can have low values (δ^{34}S = approx. −25‰ to 0‰). Regions that are inundated with marine sulfate from rain and sea spray often have sulfur isotope values that are much higher (δ^{34}S = approx. 14‰ to 21‰). Interior regions that are not wetlands often have middle range values between these extremes (δ^{34}S = approx. 0‰ to 14‰).

For more details and reviews of sulfur in archaeology and in the biosphere, see [12–14,27–30,32,33,35,37–43].

3.1. Sulfur isoscapes for mobility

As δ^{34}S values vary spatially and undergo little trophic fractionation, it is possible to use sulfur isotopes in tissues for mobility and migration studies [14,44–46]. Recent research has sought to determine the utility of modern plants as potential matrices for the development of sulfur isoscapes [27,29,47]. It is ideal to matrix-match the isoscape and the tissues under study; in archaeology, this is commonly bone or dentine collagen. Furthermore, it is ideal to keep the isoscape data temporally similar; however, much larger datasets will be required before this is a possibility. As any isotope value or ratio in human/animal tissues is ultimately a reflection of the broader bioavailable isotope systems within their local ecosystem, 'non-local' determinations ultimately reflect that the individual is different from the 'local' baseline. Therefore, 'non-local' determinations can capture both the mobility of an individual during life and whether their food was produced/raised away from the archaeological context they were excavated from.

Relative to oxygen or strontium, sulfur is less often applied in archaeology due to the technical difficulty in measuring sulfur isotopes in collagen [12,48–51]. Bataille *et al.* were the first to develop a sulfur isoscape using archaeological collagen for western Europe [14]. This paper seeks to build upon this work using only faunal data from the UK to develop a UK-specific random forest model. Sulfur isoscapes of the UK have also been developed using soils [52], animal hair [45], insects [53] and modern plants [16].

4. Methods

4.1. Human bone collagen sulfur isotope analysis

Carbon, nitrogen and sulfur isotope measurements on the humans were made on previously extracted and dried collagen that was re-hydrated and then ultrafiltered [15]. Isotope measurements were made at the Archaeology Isotope Lab at the Department of Archaeology, Simon Fraser University, Canada. All samples had acceptable collagen preservation [44,54]. The isotope data are presented below in table 1, and previously published radiocarbon dates on individual samples (where applicable) are given in table 2. Age and sex for these individuals are presented in table 1; importantly, many of the human remains are from mixed and fragmentary assemblages; thus, complete biological profiles cannot always be done. Furthermore, due to the nature of the assemblages, it is not always possible to directly compare the results of this study with previous [11].

4.2. Faunal bone collagen sulfur isotope analysis

Faunal sulfur isotope data for the UK was compiled from unpublished and published datasets, and the mean and other descriptive statistics are given in table 3. All mammalian fauna with $\delta^{34}S$ values from each site were used to produce the data presented in table 3. In total, this sample represents 735 faunal analyses. This sample is predominantly *Bos*, *Sus* and *Ovis*/*Capra*, with smaller numbers of Equidae, Canidae, Cervidae and unidentified herbivores.

Preparation and measurements methods for $\delta^{34}S$ values for published values are given within those publications. For the Madgwick *et al.* 2021–2026, (unpublished data), publication is forthcoming as part of the Feeding the Roman Army in Britain Project [67], and preparation and measurement methods are the same as used in [57]. The extracted collagen was analysed for $\delta^{34}S$ using a Thermo Finnigan EA IsoLink coupled to a Delta V Plus isotope ratio mass spectrometer via a Conflo IV interface at the British Geological Survey. The Jay *et al.* 2007–2009, unpublished data methods are given in [66].

The descriptive statistics of minimum, maximum, mean, median and s.d. were collected for each site. Thirty-eight sites were included in the study.

4.3. Random forest regression

Random forest regression was selected because it generates high-resolution isoscapes and permits us to consider what variables may be influencing the sulfur values within tissues [14,68–70]. The list of variables used can be found in the supplementary material. For a complete review of random forest isoscapes and other mapping methods, see the following papers [68,70,71]. This paper follows the statistical methods outlined in [14,69,70]. At each site, the median value of all taxa is used [72].

Only sulfur isotope measurements on archaeological fauna were used for the development of the $\delta^{34}S$ isoscape. Human sulfur isotope values were not used to avoid possible circular arguments for human mobility and migration using human data that were pre-defined as 'local'. Due to this, the

Table 1. Isotope data for humans from Welsh Neolithic tombs [15].

S-SFU	site	sample ID I	sample ID 2	element	age and sex	δ^{13}C ‰ V-PBD	δ^{15}N ‰ AIR	δ^{34}S ‰ V-CDT	%C	%N	%S	C:N	C:S	N:S
2653	Penywyrlod	P-1	74.23M/95	L humerus		−21.3	8.4	16.4	41.9	15.0	0.2	3.3	701	171
2654	Penywyrlod	P-2	74.23M/95	L femur		−20.9	8.8	14.5	40.3	15.1	0.1	3.1	800	345
2655	Penywyrlod	P-3	74.23H/9.1	L humerus		−21.3	8.4	15.8	31.0	11.9	0.1	3.0	652	272
2656	Penywyrlod	P-4	74.23H/9.1	R humerus		−21.4	9.7	15.5	34.9	13.0	0.1	3.1	676	297
2657	Penywyrlod	P-5	74.23H/9.7	L femur		−21.3	9.9	15.8	31.1	11.9	0.1	3.1	753	272
2658	Penywyrlod	P-6	74.23H/9.14/NEII.Y.2	L femur		−20.9	8.7	14.4	39.1	14.7	0.2	3.1	649	168
2659	Penywyrlod	P-7	74.23M/95	L femur		−20.9	8.1	15.7	41.9	15.4	0.2	3.2	653	176
2660	Tinkinswood	Ti-1				−20.7	8.1	13.7	43.2	15.7	0.2	3.2	692	179
2661	Tinkinswood	Ti-2	TI-2/Tink.Fe.8	femur	Adult F	−20.7	8.8	11.5	41.1	15.2	0.2	3.2	710	174
2662	Tinkinswood	Ti-3	Ti-3/TINK.FE.12	femur	Adult M	−20.7	9.6	12.4	35.3	13.2	0.2	3.1	552	151
2663	Tinkinswood	Ti-4				−20.8	9.6	14.1	39.1	14.2	0.1	3.2	699	325
2664	Tinkinswood	Ti-5	TI-3/TINK.FE.9	femur	Adult	−21.0	9.8	16.3	44.2	16.0	0.1	3.2	860	366
2665	Tinkinswood	Ti-6				−20.7	9.1	13.9	45.8	16.3	0.2	3.3	689	186
2666	Tinkinswood	Ti-7				−20.7	9.7	13.9	45.5	16.4	0.2	3.2	775	187
2667	Tinkinswood	Ti-8	Ti-8/TINK.FE.6	femur	Adult M	−20.7	9.4	14.1	41.6	15.1	0.2	3.2	707	172
2670	Ty Isaf	TY-3	39-190/138	femur		−20.8	9.9	13.9	43.6	15.3	0.2	3.3	669	175
2671	Ty Isaf	TY-4	39-190/108	L femur		−21.8	10.0	14.3	38.7	14.9	0.2	3.0	501	170
2672	Ty Isaf	TY-5	39-190/81	R femur		−22.2	8.6	16.4	44.2	15.0	0.2	3.4	668	171
2673	Ty Isaf	TY-6	39-190/170	R humerus	Adult F	−22.5	9.5	13.7	42.4	15.2	0.2	3.2	736	174
2676	Ty Isaf	TY-9	39-190/99	L femur		−22.0	8.6	16.5	43.7	15.4	0.2	3.3	729	176
2678	Heston Brake	H-2	31.78/106.1	L femur		−20.9	11.2	14.9	46.2	15.0	0.2	3.6	756	171
2679	Heston Brake	H-3	31.78/106.1	L femur		−21.2	11.0	14.1	22.6	8.6	0.1	3.1	443	196
2680	Heston Brake	H-4	31.78/106.1	L femur		−21.5	10.9	13.7	21.3	8.0	0.1	3.1	526	183
2683	Parc le Breos Cwm	PA7925	PC#4	L humerus	Adult	−21.1	9.7	17.7	27.6	10.5	0.1	3.1	714	240

(*Continued.*)

Table 1. (*Continued.*)

S-SFU	site	sample ID I	sample ID 2	element	age and sex	δ^{13}C ‰ V-PBD	δ^{15}N ‰ AIR	δ^{34}S ‰ V-CDT	%C	%N	%S	C:N	C:S	N:S
2684	Parc le Breos Cwm	PA7926	PC#5	L humerus	Adult	−21.5	10.1	12.7	24.3	9.8	0.1	2.9	490	224
2686	Parc le Breos Cwm	PA7928	PC#7	L humerus		−22.0	8.9	11.2	15.1	5.9	0.1	3.0	390	135
2688	Parc le Breos Cwm	PA7932	PC#11	L humerus		−21.9	9.8	13.2	22.8	9.1	0.1	2.9	478	208

Table 2. Radiocarbon dates for humans with isotope data from this study from Welsh Neolithic tombs from various sources [22,55] as discussed in [1]. Calibration was undertaken using the calibration curve in Stuiver and Reimer *et al.* using the CALIB program [56].

S-SFU	site	radiocarbon age BP	cal CE age range (95%)	relative area under probability distribution	14C lab no.	reference
2653	Penywyrlod	4937 ± 39	3790–3642	1.000	OxA-16608	[1]
2654	Penywyrlod	4512 ± 40	3363–3092	0.996	OxA-16494	[1]
			3050–3045	0.004		
2655	Penywyrlod	4726 ± 39	3631–3552	0.363	OxA-16525	[1]
			3542–3492	0.233		
			3463–3375	0.404		
2657	Penywyrlod	4611 ± 39	3518–3451	0.949	OxA-16526	[1]
			3215–3189	0.037		
			3150–3134	0.014		
2658	Penywyrlod	4823 ± 39	3653–3522	1.000	OxA-16527	[1]
2659	Penywyrlod	4713 ± 38	3628–3557	0.247	OxA-16569	[1]
			3536–3488	0.242		
			3470–3373	0.511		
2661	Tinkinswood	4916 ± 41	3780–3638	1.000	OxA-16528	[55]
2662	Tinkinswood	4859 ± 39	3710–3600	0.734	OxA-16529	[55]
			3590–3528	0.266		
2664	Tinkinswood	4907 ± 39	3773–3636	1.000	OxA-16530	[55]
2667	Tinkinswood	4970 ± 39	3932–3922	0.011	OxA-16531	[55]
			3915–3877	0.075		
			3804–3648	0.914		
2683	Parc le Breos Cwm	4445 ± 60	3339–3205	0.354	OxA-6489	[22]
			3199–3000	0.509		
			2998–2925	0.137		
2684	Parc le Breos Cwm	4660 ± 60	3631–3552	0.113	OxA-6490	[22]
			3541–3339	0.882		
			3206–3197	0.005		
2686	Parc le Breos Cwm	4805 ± 55	3702–3682	0.200	OxA-6492	[22]
			3655–3499	0.899		
			3434–3379	0.081		
2688	Parc le Breos Cwm	4850 ± 65	3783–3512	0.975	OxA-6496	[22]
			3426–3408	0.015		
			3397–3383	0.010		

isoscape is not suited for the study of mobility and migration of archaeological fauna. While it is likely that some of the fauna used in the model themselves were non-local to their site, by using the median $\delta^{34}S$ value to represent the local baseline, there is reduced risk that non-locals significantly impact the local baseline prediction. The median value is robust against outliers, which in this case would reflect non-local fauna, and thus the faunal median value should reflect the bioavailable $\delta^{34}S$ baseline of the given site. Therefore, no faunal measurements were selectively removed from the sample.

A number of assumptions were required in developing this isoscape. First, the median value of each site represents the 'local' value assuming the majority of fauna lived at these sites in the years leading to their death. Second, that there are no, to little, fractionation effects between the environment and different dietary niches (e.g. herbivore, omnivore and carnivore). Controlled feeding studies have

Table 3. Descriptive statistics of faunal $\delta^{34}S$ values from both literature and published in this study.

site	*n*	minimum	maximum	mean	s.d.	median	period	ref.
All Cannings Ccross	15	−4.2	14.5	9.4	5.5	12.1	Bronze Age	[57]
Barrow Hills, Radley	7	−13.1	−0.6	−5.0	4.9	−2.6	Roman	[58]
Beckery	12	−10.6	11.8	0.4	7.4	−0.5	Post-Roman	[29]
Burtle Priory	12	2.4	13.8	6.8	3.0	7.3	Post-Roman	[29]
Caerleon	18	−18.2	21.7	12.5	6.2	13.7	Roman	Madgwick, Lamb, Mion 2021–2026, unpublished data
Cardiff Castle	18	8.8	16.2	12.8	2.4	13.0	Roman	Madgwick, Lamb, Mion 2021–2026, unpublished data
Carding Mill Bay	10	18.7	21.0	20.2	0.7	20.2	Neolithic	[59]
Danebury Hillfort	43	0.8	18.6	14.6	4.3	15.8	Iron Age	[43]
Durrington Walls	98	−1.6	19.6	11.0	3.9	11.9	Neolithic	[60]; Jay and Richards 2007–2009, unpublished data
East Chisenbury	38	−7.4	17.5	12.8	4.3	13.8	Bronze Age	[57]
Eye	10	−13.4	1.4	−6.6	4.9	−7.7	Bronze Age	[29]
Garrowby Wold 32	10	8.7	15.0	12.1	2.1	12.5	Beaker	[61]
Gayhurst	5	−0.9	5.2	2.8	2.3	3.3	Bronze Age	[62]
Irthlingborough	11	−5.5	4.9	−1.6	3.0	−1.4	Bronze Age	[62]
Langtoft	7	−15.2	6.5	−1.5	7.5	1.2	Roman	[29]
Links of Noltland	15	16.8	20.8	18.7	1.1	18.7	Neolithic	[59,63]
Loughor	24	8.3	17.6	14.3	2.4	14.5	Roman	Madgwick, Lamb, Mion 2021–2026, unpublished data
Marden	6	6.5	13.0	9.4	2.7	9.1	Neolithic	[60]
Mount Pleasant	16	2.8	18.8	14.1	4.3	15.2	Neolithic	[60]
Muchelney	12	−12.7	8.3	−4.3	6.5	−6.4	Roman	[29]

(*Continued.*)

Table 3. (*Continued.*)

site	*n*	minimum	maximum	mean	s.d.	median	period	ref.
Navan Fort	28	13.1	17.1	15.2	1.1	15.5	Iron Age	[64]
Over	12	−19.7	−3.5	−11.9	4.8	−12.7	Iron Age	[29]
Pool	8	17.7	20.4	19.1	0.8	19.1	Neolithic	[59]
Potterne	60	−17.1	16.7	−1.5	10.3	−3.3	Bronze Age	[57]
Risga	6	18.9	19.8	19.4	0.3	19.4	Mesolithic	[59]
Runnymede	49	−16.1	16.3	3.0	5.8	3.3	Bronze Age	[57]
St. Patrick's Chapel	13	4.7	18.4	16.3	3.6	17.3	Medieval	[65]
Stanton St. Bernard	34	0.9	15.4	9.2	4.0	9.4	Bronze Age	[57]
Suddern Farm	28	4.1	19.9	15.5	4.0	16.9	Iron Age	[43]
Thornwell Farm	18	−7.1	15.8	11.0	5.9	12.8	Roman	Madgwick, Lamb, Mion 2021–2026, unpublished data
Thwing	19	5.8	15.7	12.1	2.6	12.7	Bronze Age	Jay and Richards 2007–2009, unpublished data
Tubney (Oxford)	7	−13.5	0.5	−7.3	5.3	−8.7	Roman	[58]; Nehlich and Richards 2005–2007, unpublished data
Ulva	2	19.6	20.6	20.1	0.7	20.1	Neolithic	[59]
Usk	4	14.2	16.4	15.1	1	14.9	Roman	Madgwick, Lamb, Mion 2021–2026, unpublished data
Wallingford	23	−16.9	10.8	1.0	6.2	2.1	Bronze Age	[57]
West Kennet Palisade Enclosures	15	6.9	17.5	13.2	3.3	14.6	Neolithic	[60]
Wetwang Slack	7	13.6	15.7	14.6	0.7	14.6	Iron Age	[66]
Whitton	15	4.3	17.6	12.6	3.1	12.4	Roman	Madgwick, Lamb, Mion 2021–2026, unpublished data

demonstrated that there is probably little fractionation between consumer and diet for δ^{34}S values [13]. Krajcarz *et al*. [73] demonstrated that the δ^{34}S values of carnivorous foxes represent the average of their local diet with little fractionation between diet and consumer. Therefore, it can be assumed that using all samples regardless of dietary niche across the UK to construct a faunal isoscape is appropriate and should not impact disproportionately the accuracy of the predictive δ^{34}S across space. More research, and importantly, larger samples are required to better understand the mechanisms behind sulfur isotopic incorporation into tissues, and the potential impact of protein quality on cysteine formation and its potential impacts on δ^{34}S values [27,37].

Finally, that there were no major environmental or (human-influenced) cultural baseline shifts in archaeological fauna throughout time is another assumption. In this study, there have been no major climatic changes in the recent Holocene, and thus changes to the sulfur cycle would not be due to climate. The δ^{34}S values of individuals are directly related to their diet, and therefore cultural or environmental factors can substantially change the local faunal and human δ^{34}S collagen baseline [74]. have demonstrated how human feeding decisions can impact the δ^{34}S baseline of hair of modern domesticated animals. In their study, cattle, horses and cats consuming local flora or fauna have comparable δ^{34}S hair values regardless of dietary niche and reflect the local δ^{34}S baseline, while dogs fed pet food had very different δ^{34}S values.

This example demonstrates how human agency can impact the δ^{34}S values of fauna, selective feeding, importing of food products and location where animals and food are grown/raised can all influence the δ^{34}S baseline of the local food web and subsequently may impact our determination of local or non-local. Therefore, it is important to consider how local animal husbandry practices may shape collagen baselines, and whether modern matrices used to construct isoscapes are comparable to animal husbandry practices of the temporal period under study, such as animals living in salt marshes, wetlands, and so on, and whether the modern plants sampled capture the δ^{34}S baseline of the regions where animals were raised. The selection of samples for the comparability of modern matrixes is significant and has the potential to result in correct determinations of locality versus nonlocality in archaeological samples.

4.4. Geographic assignment

Assessing whether these individuals lived near the tombs prior to death was done using AssignR [75]. Prior probability 'iso-surfaces' are made for each individual. Then, the locality assessment is done qualitatively, considering potential limitations due to sampling density. We have chosen to present our results in the non-absolute terms of 'more likely' and 'less likely' due to the limitations of equifinality and site density in isoscape development. The results of this analysis for each individual are given in electronic supplementary material.

5. Results

5.1. Sulfur collagen isoscape

The random forest model predicting sulfur collagen values of fauna across the UK had an R^2 of 0.82, a mean average error of 3.93, and a root square mean error of 4.88 (figure 2). Of all the auxiliary variables (electronic supplementary material), mean annual precipitation, potential evapotranspiration and mean age of bedrock geology were selected (figure 2). These variables were determined to be the most important variables for the highest accuracy prediction of δ^{34}S values in collagen across the UK. However, there are likely many other factors that have an influence on geospatial variation in δ^{34}S values other than these, especially when examining specific matrixes (such as soil δ^{34}S values). The most significant variable for the prediction of δ^{34}S values in collagen was mean annual precipitation.

Figure 3A demonstrates the predicted spatial variation of δ^{34}S values of faunal collagen across the UK, while figure 3B shows the predicted error on the δ^{34}S faunal collagen isoscape mapped in figure 3A.

This isoscape builds upon the work of Bataille *et al.* [14], which has generally similar values, but our isoscape had much lower values for south-central England, which was also observed by Lamb *et al.* [29]. With a larger sample size and more spatially representative sampling, this isoscape would

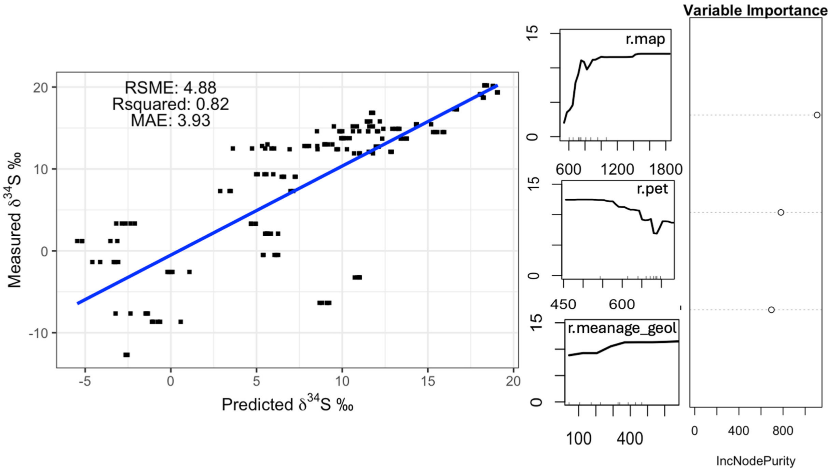

Figure 2. Variable importance plot and model accuracy scatter plot.

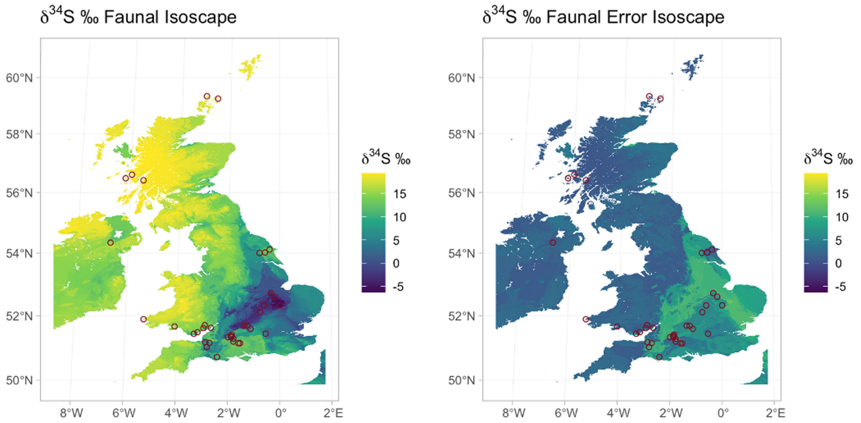

Figure 3. (A) δ3^{4}S isoscape of the UK using faunal collagen. (B). δ3^{4}S faunal error isoscape of the UK. Circles represent site locations for the collagen sulfur data.

have greater accuracy and precision. However, the region under study in this paper (south Wales) is well represented. Should researchers use this isoscape for their own mobility assessments, it should be considered that local environmental factors may change the local baseline beyond what is presented here. In unique regions that lack comparative fauna, the impacts to the local baseline may be dramatically different from what is captured in the predicted collagen isoscape (such as conditions that change the bacterial baseline or precipitation). Local regional sampling continues to be required to increase the accuracy and validity of mobility studies.

5.2. Sulfur isotope interpretation

The humans from the five study sites all have similar collagen δ^{34}S values, ranging from about 12‰ to 16 ‰. As outlined and discussed in Richards, the carbon and nitrogen isotope values of all these individuals (table 1; figure 4B) indicate terrestrial diets with no clear input of marine foods [15]. So, we are confident that the sulfur isotope values reflect the local terrestrial δ^{34} values of foods consumed and are not influenced by marine sulfur in diets.

The two inland Black Mountain sites of Ty Isaf and Penywyrlod have elevated sulfur values relative to the three near-coastal sites. As these two sites are located inland within the Black Mountains, it may be possible that marine sulfate is present in greater abundances due to orographic precipitation. Neil *et al.* suggest that the individuals found in the Penywyrlod monument did not grow their food near the site, which may also explain why the sulfur values of these individuals are enriched beyond what is expected [10]. Therefore, it is unlikely that these individuals will appear local to their respective sites;

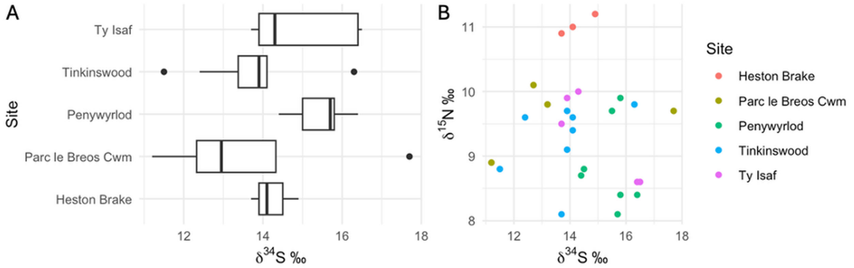

Figure 4. (A) Boxplot of δ^{34}S collagen values from individuals at Heston Brake, Parc le Breos Cwm, Penywyrlod, Tinkinswood and Ty Isaf. (B) δ^{34}S and δ^{15}N scatterplot of collagen isotope values.

however, without faunal samples from these regions, it is not possible to know whether the baseline here is naturally elevated.

5.3. Geographic assessment

The probability 'iso-surface' generated for each individual can be found in the supplementary material, figure 5 demonstrates how these data are presented using the example of one individual from each site. As discussed previously, we have chosen to use more general terms to describe our interpretation. Single isotope studies of mobility have a high degree of equifinality and at times lack spatial representation of bioavailable δ^{34}S measurements (such as collagen or plants). Therefore, these general terms reflect that more research and isotopic analysis is required to have greater confidence in the results. The mobility considered more broadly for each site is considered in greater detail in the following discussion. Importantly, we have limited the scope of our assignments to Wales. Due to equifinality, much of the UK may also be possible, and if extrapolated further, much of continental Europe. Further isotope analysis from other isotopic systems (strontium, oxygen, hydrogen, lead, etc.) will be needed for robust mobility studies that suggest broader mobility outside of Wales. We have limited our analysis to Wales as it is a more conservative scale, especially with only one isotope system under study.

6. Discussion

6.1. Evidence for mobility at each study site

6.1.1. Heston Brake

At Heston Brake, all individuals appeared local to the site. It does appear that these individuals may have sourced their food further north; however, these results may also reflect local regional mobility as well. None of the individuals within the sample have been radiocarbon dated, and we note that the sample size is very small ($n = 3$). Individuals from Heston Brake have a δ^{15}N value approximately 1‰–1.5‰ higher than the rest of this study's sample. This could be due to the consumption of higher trophic level proteins relative to the other Neolithic communities in this study; however, as suggested by Richards *et al.* [15], Heston Brake is situated on a salt marsh, which may be increasing the δ^{15}N baseline of this site without the need for higher trophic level food. Further research and radiocarbon dating from this site is necessary for more nuanced interpretation of the isotope data.

6.1.2. Parc le Breos Cwm

Considering the sample distribution (figure 4A) using box plots from Parc le Breos Cwm ($n = 4$), one individual appears non-local to the site (ID: 2683; 3199–3000 cal BC 0.509 area under 95% probability). However, considering the geographic assessment and their δ^{34}S value of 17.7‰, we suggest that this individual is the only one local to Parc le Breos Cwm. Individuals 2684 (3541–3339 cal BC, 0.882 area under 95% probability), 2686 (3655–3499 cal BC, 0.889 area under 95% probability) and 2688 (3783–3512 cal BC, 0.975 area under 95% probability) are probably non-local to the site and lived further east

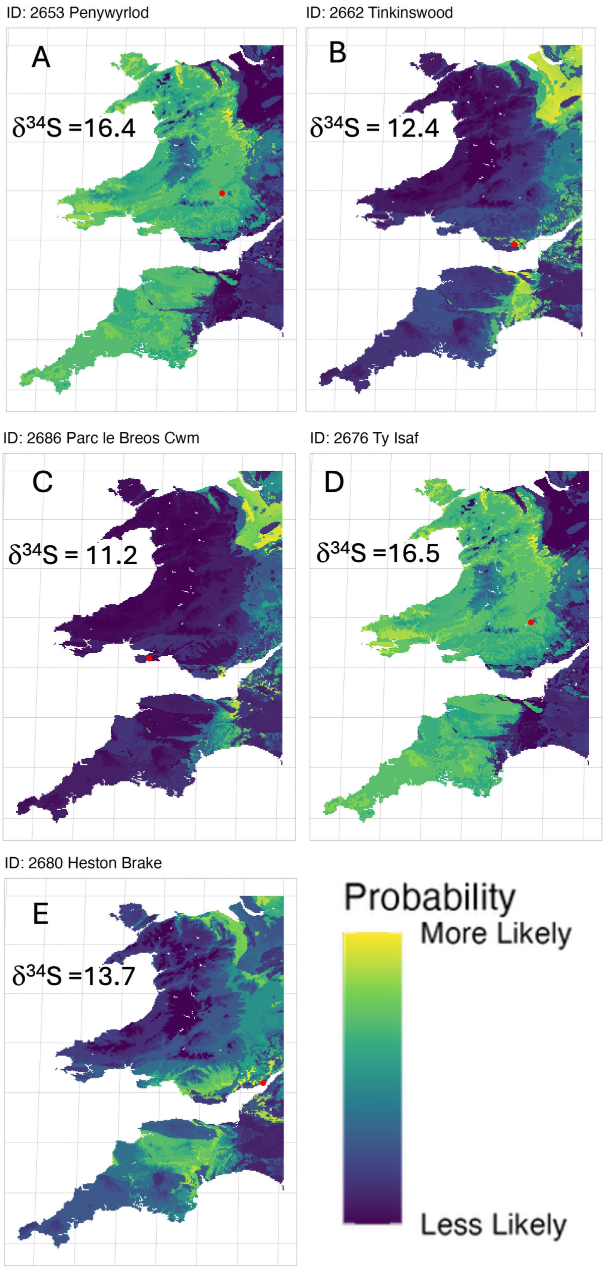

Figure 5. Geographic assessment 'iso-surfaces' for one individual from each site within the study.

in the years prior to their death, or consumed food grown further inland. As this site was probably constructed and first used in the thirty-seventh century cal BC, these results indicate that these three individuals may represent mobility and movement over the first centuries of the use of Parc le Breos Cwm.

6.1.3. Penywyrlod

The Black Mountains lack comparative δ^{34}S values from fauna. It is clear that the sulfur values of the individuals in this region are above what is expected for inland Wales (median δ^{34}S = 15.7‰). This may be due to the specific environmental factors involved with being near the Black Mountain range. These high δ^{34}S values could be explained if Neolithic agricultural land exists within watersheds that receive significant marine sulfate from orographic precipitation. The isoscape assessment does suggest that these individuals lived in Wales more broadly. With more evidence, such as from oxygen isotope analysis, there may be greater confidence in their assignment to the area around the cairn. Without a more spatially representative faunal sample, it is not possible to confidently determine whether these individuals reflect the local biosphere, or all lived near the coast and were transported or moved to the cairn after or near death.

The individuals from this site were previously studied using strontium isotopes in enamel, and results indicate that these individuals did not regularly grow or raise their food near the monument [10,11]. Considering the assessment from the isoscape, it does appear that these individuals lived locally, but with much of Wales also being a possible region. These results may further support Neil *et al.* that these individuals did not source their food from directly near the long cairn [10]. However, sulfur and strontium isotope analyses represent different temporal periods of life. Neil *et al.* have demonstrated that during their childhood formative years, the individuals at this site did not consume food from the region of the cairn, and our sulfur isotope analysis (which represents later life) may suggest that they did not consume food from the region in the years before death [10,11]. Archaeologically, this may tentatively suggest that this tomb was not intended for 'locals'. However, further research and more robust sampling will be needed to assess this hypothesis. It should be noted here that as these tombs generally contain scattered and mixed bone, it is not possible to determine if the sulfur samples taken from long bones are from the same individuals with tooth strontium isotope measurements. Future work that perhaps measures collagen isotope values in bone from mandibles or maxillae directly associated with teeth will allow us to better explore the lifetime mobility of these individuals.

6.1.4. Ty Isaf

The $\delta^{34}S$ values from Ty Isaf (median = 14.3‰) are slightly lower than those from Penywyrlod. The isoscapes themselves suggest that individuals from Ty Isaf probably lived in either south-east or far south-west Wales during the years before their death. It could be possible that, as suggested by Neil *et al.*, these results indicate that they consumed non-local food [10]. Non-local food in this circumstance may reflect food grown in regions with contrasting bioavailable strontium isotope baseline, as well as a contrasting bioavailable sulfur baseline. Individuals 2672 ($\delta^{34}S$ = 16.4‰), and 2676 ($\delta^{34}S$ = 16.5‰) have values that are higher than expected for inland locations. Considering the whole sample, these two are the most likely to be non-local and are more similar to those from Penywyrlod. However, given the current limitations of this analysis, we suggest that all individuals from Ty Isaf are local, and potential non-local determination from the geographic assessment is due to variations in the $\delta^{34}S$ baselines in this region that are currently uncaptured by our isoscape. Future faunal sulfur isotopic analysis from the Black Mountains (and Wales more generally) will increase the validity of mobility assessments.

6.1.5. Tinkinswood

Tinkinswood falls within a region of Wales that is well represented in the isoscape. Considering the sample from Tinkinswood, two individuals appear to be outliers: 2661 (3780–3638 cal BC, 1.0 under 95% probability) and 2664 (3773–3636 cal BC, 1.0 under 95% probability). The first use of Tinkinswood probably occurs in the thirty-eighth or earlier thirty-seventh century cal BC [1]. Therefore, these two individuals were presumably deposited early in the history of the tomb. The low $\delta^{34}S$ value of individual 2661 of 11.5‰ suggests that they lived further east prior to death, while the value for individual 2664 of 16.3‰ indicates western coastal living. From these results, there are (at least) three possible conclusions: first, that the community that lived near the cairn was constituted from a regionally diverse population; second, that human remains from across south Wales were brought to be interred at the cairn; or third, that the population regularly interred at the site moved throughout Wales during their lives.

6.2. Sulfur isoscape comparability

Figure 6 is an isoscape that demonstrates the difference between animal collagen sulfur values and plant sulfur isotope values [16,47]. This plot was created by taking the sulfur isoscape of modern plants [47] and contrasting it with the results from the archaeological collagen isoscape. As there should be low fractionation between diet and consumer, this plot may demonstrate how baselines have changed between archaeological fauna and modern plants. There are several caveats. First, the $\delta^{34}S$ values of archaeological fauna may be influenced heavily by cultural practices such as selective feeding, manuring, deforestation, mobility and so on. Therefore, the difference between modern plant and archaeological collagen may not be due to environmental baseline sulfur shifts but instead reflect how humans influenced baseline over time. Second, plant tissues measure the bulk sulfur in the plant, while collagen only measures sulfur from methionine, which has been demonstrated to fractionate between consumer and diet [12]. Third, plants reflect their extremely local environment and can be greatly influenced by climate, precipitation, underlying lithology and soil conditions. Therefore, there is probably a broader spatial variation in plants than animals, and therefore faunal collagen will represent the average of a larger space and context. Finally, it is not yet known which plant types or root depths are best for sulfur isoscape development. Research for strontium isoscape development suggests that grasses tend to capture the most influence for anthropogenic changes to bioavailable baseline [76]. As suggested by Sengeløv *et al.*, shallow-rooted plants, particularly grasses, are more impacted by strontium pollution by atmospheric deposition [76]. As the primary expected source of anthropogenic sulfur is atmospheric SO_2, it is likely that shallow-rooted plants will also reflect atmospheric pollution more readily. The incorporation of strontium isotopes and sulfur isotopes in plants is different, but both are affected by atmospheric pollution. While more research on this topic is needed, it is likely that root depth and its relationship to anthropogenic sulfur will be important variables to consider during research design.

Therefore, it can be expected that there will be a few ‰ difference between animal collagen and plant tissues. It is hypothesized that plants are approximately 5‰ lower in value than animal collagen in most environments [16]. This difference is probably due in part to fractionation between diet and consumer, but also because plant tissues are a bulk value reflecting all S compounds within the sample. In regions where there is sufficient cysteine between trophic levels, plants and animals may be fairly similar (but more research on inter-amino acid fractionation, and the bulk plant isotope values and their relationship with methionine, is required). In figure 6 values between δ^{34}S 0‰ and –5‰ have been coloured as white. These regions are assumed to fall within the expected variation between plant tissues and animal collagen. Therefore, in these regions, it appears that modern plant sulfur values are comparable to the collagen sulfur values and a potentially useful matrix for isoscape development for sulfur. In regions outside this 0‰ to −5‰ range, it is likely that there has been a baseline shift.

The cause of this baseline shift may be due to modern sulfur pollution and/or anthropogenic changes to the landscape. While modern pollution has probably influenced the sulfur values of plants in the UK, we do not believe that it is the only causal factor. The range of δ^{34}S differences between the modern plant isoscape of Tarrant *et al*. [47] and archaeological collagen is quite large (δ^{34}S: −29.5‰ to 7.5‰), especially considering that only the values around 0 to −5‰ fall within what is assumed to be an acceptable offset between modern plants and archaeological collagen. In regions outside of this δ^{34}S range in the UK, modern plants may not then be an appropriate matrix for isoscape development for archaeology. However, greater research in these regions, and on what variables are causing the discrepancy in δ^{34}S values through time, is required.

It is also important to recognize that there is not sufficient research on what plant tissues, species or root depth are best for isoscape development. Sulfur isotopes and their application for mobility studies continue to be understudied. Particularly, the theory behind the use of sulfur isotopes as mobility indicators requires greater empirical consideration. Preliminary studies such as this suggest that modern plants may be a useful and readily available matrix for the construction of isoscapes, but each region must be considered independently. While in some locations modern plants and archaeological collagen appear to be an appropriate matrix for mobility studies, in other regions they do not.

It was expected that the southeast and eastern UK would be the regions with the greatest difference between modern plants and archaeological collagen [77]. However, figure 6 suggests that these regions are more comparable. Some possible explanations for this may be that while the total range of δ^{34}S values of modern plants has shifted due to anthropogenic sources, the median value has remained fairly consistent through time, or that environmental conditions (such as bacterial reduction) are so great that anthropogenic sulfur signals are too weak to cause a real change in the δ^{34}S of plants. Ultimately, more research is required to understand why in some regions archaeological collagen and modern plants are dissimilar (and similar in others). Other plants, different tissues and different depths may all have resulted in a plant isoscape that is more comparable to archaeological tissues. More research into plant baselines and their comparability to collagen is necessary.

While these results suggest a baseline shift in the UK, it is also necessary to consider to what degree the matrices selected (both archaeological and modern plants) construct this narrative. If shallow-rooted plants do indeed capture anthropogenic sources of sulfur more readily, then a different plant isoscape than those of Tarrant *et al*. [47] and Evans *et al*. [16] may be constructed that has significantly

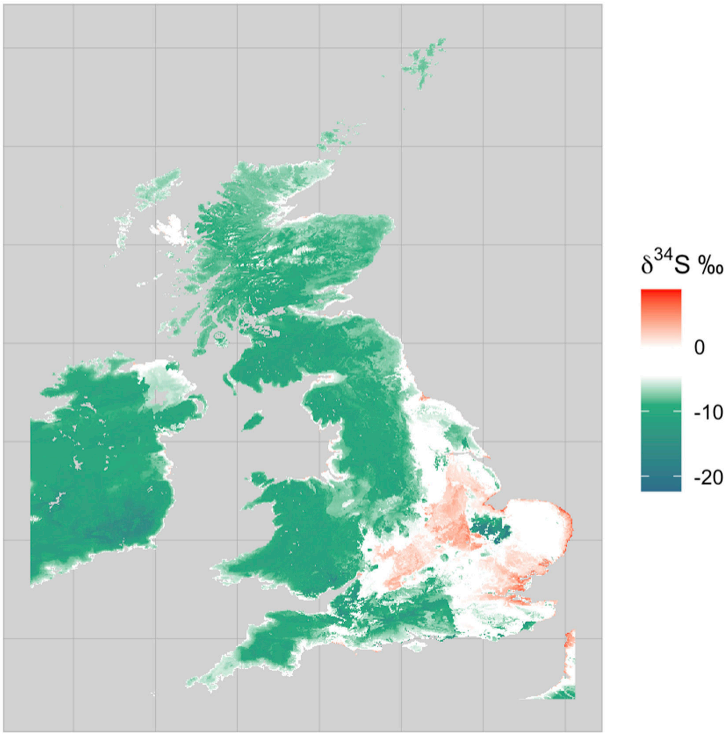

Figure 6. Difference between sulfur isotope values between random forest predicted modern plants [47] and random forest predicted archaeological faunal collagen.

less difference between archaeological collagen and modern plants. Furthermore, only 38 sites in the UK have faunal δ^{34}S values, many in the south of England. With a greater sample from a greater spatial representation, a more accurate collagen isoscape can be generated. For example, this study suggests that Ty Isaf and Penywyrlod in the Black Mountains have higher human collagen δ^{34}S than is expected for this region of Wales, possibly due to orographic precipitation and local watersheds relative to where domesticates were raised. However, without δ^{34}S values of animal collagen from these sites, this cannot be confirmed, nor could the collagen isoscape predict values that may be equally due to the local environment, and where Neolithic farmers chose to raise their domesticates.

6.3. Diachronic changes to δ^{34}S values

As sulfur is a light element that appears to be predominantly influenced by precipitation and bacterial flora in soil, it is likely that the δ^{34}S baseline will change through time due to climate. However, to what degree δ^{34}S values will change in plants, fauna, and humans is currently unknown. This study has chosen not to consider the potential diachronic changes through time because of the spatial variability involved with this sample. Further research that considers how sulfur values change in a single matrix through time at a single site (or in a single individual if possible) is needed to understand to what degree we can expect diachronic impacts to shift sulfur isotope values, and whether this isotope system may also be useful as a climatic indicator.

Furthermore, cultural choices can also impact the sulfur cycle in a given environment, which may also shift the isotopic baseline [30,78]. Future research is needed in regions where anthropogenic change to the landscape is well understood and how it impacts δ^{34}S baselines. A further question is whether animal husbandry and the location chosen to raise domesticates can significantly impact the sulfur baseline of animals and humans relative to the broader environment.

7. Summary and conclusion

This paper analysed 27 individuals from five early Neolithic burial tomb sites in south Wales. Our research indicates that all individuals were probably local to Wales in general, but the majority of the monuments appear to have a number of non-local interred individuals. A δ^{34}S isoscape was generated using the median $\delta^{34}S$ values of archaeological fauna from 36 sites. This isoscape was then compared to spatial variation of $\delta^{34}S$ values from modern plants [16]. We identified that some regions of the UK have experienced large shifts in their $\delta^{34}S$ baseline comparing the archaeological to the modern isoscapes. It may be possible that these differences are due to modern pollution or anthropogenic changes to the landscape (and subsequent impacts to the sulfur cycle), but more research is needed to understand the total range of potential variables that may explain the differences between modern plants and archaeological collagen. We suggest caution in using modern plant sulfur isoscapes for archaeological samples in areas that have high historic and current industrial pollution and anthropogenic landscape changes.

Ethics. This work did not require ethical approval from a human subject or animal welfare committee.

Data accessibility. The data for the interred individuals from Neolithic Wales can be found in Richards *et al*. [15], and the data used to construct the isoscape can be found in the associated references of table 3. The code used is the same developed and published by Clement Bataille and copies of the code can be found in previous publications [14,70].

Supplementary material is available online [79].

Declaration of AI use. We have not used AI-assisted technologies in creating this article.

Authors' contributions. D.T.: conceptualization, data curation, formal analysis, investigation, methodology, validation, visualization, writing—original draft, writing—review and editing; R.M.: conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, validation, visualization, writing—original draft, writing—review and editing; L.M.: data curation, formal analysis, investigation, methodology, writing—original draft, writing—review and editing; A.L.: data curation, formal analysis, investigation, methodology, writing—original draft, writing—review and editing; A.W.R.W.: data curation, formal analysis, methodology, visualization, writing—original draft, writing—review and editing; M.P.R.: conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, project administration, resources, supervision, validation, visualization, writing—original draft, writing—review and editing.

All authors gave final approval for publication and agreed to be held accountable for the work performed therein.

Conflict of interest declaration. We declare we have no competing interests.

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