

# SUBSISTENCE SHIFT AND SOCIO-ECONOMIC RESPONSE TO CULTURAL AND CLIMATE CHANGES AMONG NORTH-CENTRAL IBERIAN MEGALITHIC GROUPS

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## ABSTRACT:

The insufficient attention traditionally paid to the complex mortuary biographies of megalithic graves has long obscured a significant amount of synchronic and diachronic information. The Rioja Alavesa region of north-central Iberia holds a number of megalithic graves with large skeletal assemblages that can generally be ordered by internal stratigraphy and/or relatively fine-grained radiocarbon chronologies, providing a rare opportunity to assess the potential of such information. Here, we undertake stable carbon and nitrogen isotope analyses of 125 human and 7 animal bone collagen samples from six late prehistoric megalithic graves (La Cascaja, El Sotillo, San Martín, Alto de la Huesera, Chabola de la Hechicera and Longar) which, together with 107 previously published Late Neolithic/Early Chalcolithic (ca. 3600-2900 cal. BC) bone collagen samples (100 humans and 6 animals) from the same tombs, are used to explore subsistence trends over time. This approach provides a means to address the socio-economic response of northern Iberian megalithic groups to the cultural and climate changes occurring in late prehistory. Isotopic data are contextualized using palaeoenvironmental and osteoarchaeological information. The results show a significant shift in diet between the late Middle Neolithic and the Late Neolithic/Early Chalcolithic that coincides with marked changes in burial practices that are likely related to the transition from mainly pastoral to mixed farming economies. There may also be a change in subsistence between the Late Neolithic/Early Chalcolithic and the following periods, suggested by differences in  $\delta^{15}\text{N}$  values between the layers of Alto de la Huesera megalithic grave, as well as in population and funerary dynamics. By contrast, an apparent continuity in subsistence is seen from the Middle Chalcolithic to the Bronze Age, despite clear changes in beliefs and socio-economic practices. These findings support the existence of substantial asynchronous changes in lifeways and ideology among Iberian megalithic groups and challenge the traditional idea of a long and uniform stability in late prehistoric northern Iberia.

**KEY WORDS:** Dietary change, diachronic variation, monumental tombs, late prehistory, Spain

## 1. INTRODUCTION

Abrupt environmental and/or cultural transformations have been suggested as an explanation for the appearance of megalithic graves around 4500 BCE along the Atlantic façade of Europe (Chapman 1977; Sherratt 1990; Carvalho 2015). By contrast, the generally long-lasting funerary activity found in these monuments and the features of the populations using them over time have long been understood as reflecting stability and continuity, possibly because their archaeological outcome is very often a jumble of bones and grave goods with no easy ordering. However, accumulating radiocarbon datasets have provided evidence of complex biographies with patterns of use typically based on relatively short bursts of initial mortuary activity followed by long periods of inactivity, punctuated by episodes of reuse (e.g. Bayliss and Whittle 2007; Delibes 2010; Scarre 2010; Schulting et al. 2012). Rather than stability, this suggests a lack of continuity in

the use of the tombs, accompanied by changes in funerary practices, in the meanings and understandings ascribed to monuments, and even in the human community that built, used and/or abandoned them. Major cultural and climate changes in western European late prehistory could have affected socioecological dynamics, and hence the lifeways of those buried in megalithic tombs over time (e.g. [Lillios et al. 2016](#)).

The Rioja Alavesa region of north-central Iberia serves here as an important case study. The region has a relatively extensive megalithic record, with a number of tombs showing internal stratigraphy (e.g. slabs placed horizontally or stone and earth pavings to create a new floor and/or to close the monument), supported with many radiocarbon dates, together providing a rare opportunity for synchronic and diachronic approaches. Such stratigraphic evidence has been suggested to be the result of ideological ruptures or reformulations ([Narvarte 2005](#)) that may be linked to climate and/or cultural transformations that occurred in Iberian late prehistory (e.g. [Andrés 2009](#)). These could have had a significant impact on the socioeconomic behavior and, particularly, the subsistence practices of the different prehistoric groups using megalithic graves over time.

In the virtual absence of other lines of evidence referring to dietary practices and lifeways – associated settlements are poorly documented for much of late prehistoric northern Iberia ([Fernández-Eraso et al. 2015a](#)) and plant and animal remains are scant in funerary contexts – the isotopic analysis of human remains can make a significant contribution to this issue. Here, carbon and nitrogen stable isotope data from 125 human and six faunal bone collagen samples are used, together with 107 previously published measurements (100 human and seven faunal samples), as a means to test whether any significant dietary shifts took place over time or, by contrast, continuity in subsistence and hence aspects of lifeways can be suggested for megalithic communities. Palaeoenvironmental, archaeological and anthropological data are used and discussed to understand the rationale behind the resulting evidence.

## 2. ARCHAEOLOGICAL CONTEXT

Rioja Alavesa is a confined region located in the mid-upper Ebro Valley (north-central Spain) with a total area of 400 km<sup>2</sup>. It is delimited by the steep Cretaceous limestone range of the Sierra de Cantabria-Toloño to the north and the course of the Ebro river to the south. The megalithic funerary record of the region includes eight passage tombs at present (El Montecillo, Layaza, El Sotillo, San Martín, Alto de La Huesera, Chabola de La Hechicera, El Encinal and Los Llanos) ([Fernández-Eraso and Mujika-Alustiza 2013](#)). There are two additional monuments (La Cascaja and Longar) that are located just outside of the limits of the region but still belong to the same natural and chrono-cultural environment ([Figure 1](#)). The megalithic graves are the earliest tombs documented in the region, in the absence of Early and Middle Neolithic open-air necropolises, like those documented in nearby Navarre, where a number of pit graves dating to the late 6<sup>th</sup> and 5<sup>th</sup> millennia cal. BC have been excavated, most notably at Los Cascajos and Paternanbidea ([Fernández-Crespo et al. 2019a](#)).

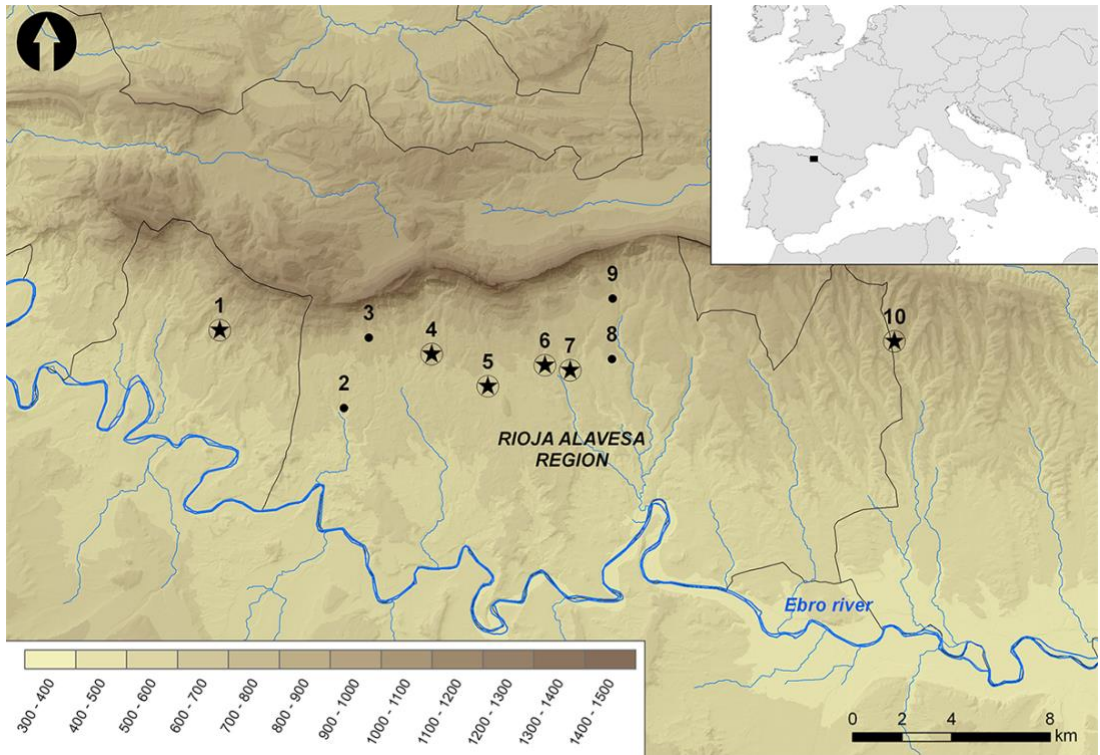
The megalithic graves included in this study (La Cascaja, El Sotillo, San Martín, Alto de la Huesera, Chabola de la Hechicera and Longar ([Text S1](#); [Figures S1-S10](#))) show complex, discontinuous biographies of funerary use that together span more than three millennia (ca. 4000-800 cal. BC), from the late Middle Neolithic to the Late Bronze Age ([Figure 2](#)), with especially intense episodes of funerary activity during the Late Neolithic/Early Chalcolithic transitional period (ca. 3600-2900 cal. BC). Unlike elsewhere in Spain ([García Sanjuán and Díaz-Gardamino 2015](#)), there is no evidence for protohistoric or historic funerary re-use of megalithic graves in the Rioja Alavesa.

The remaining four megalithic sites documented in the study region are not included in this work since either: a) they had been found looted and holding very limited or no human remains (i.e. El Montecillo and Layaza); or b) anthropological, radiocarbon dating and/or isotopic analyses are still pending (i.e. Los Llanos and El Encinal).

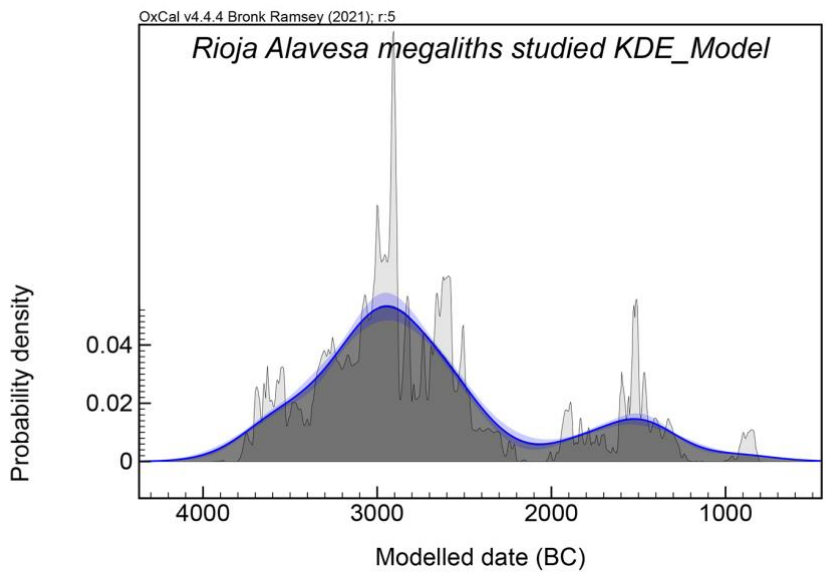
## 3. MATERIALS

A total of 125 human samples have been analysed for this study. They respectively come from the megalithic

115 graves of La Cascaja (n = 21), El Sotillo (n = 16), San Martín (n = 20), Alto de la Huesera (n = 19), Chabola  
 116 de la Hechicera (n = 27) and Longar (n = 22). Of these, 17 belong to the earliest phase of megalithic use in  
 117 the region, the late Middle Neolithic (first half of the 4<sup>th</sup> millennium cal. BC), 23 to the Late Neolithic/Early  
 118 Chalcolithic (second half of the 4<sup>th</sup> millennium and early 3<sup>rd</sup> millennium cal. BC) and 51 to the Middle-Late  
 119 Chalcolithic and Bronze Age (second half of 3<sup>rd</sup> millennium to early 1<sup>st</sup> millennium cal. BC). The remaining  
 120 34, in the absence of direct radiocarbon dates or stratigraphic data, cannot be attributed to any particular  
 121 period of funerary use.  
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124  
 125 **Fig 1. Map showing the location of the megalithic graves of the Rioja Alavesa region (north-central Iberia):** 1. La  
 126 Cascaja, 2. El Montecillo I, 3. Layaza; 4. El Sotillo, 5. San Martín, 6. Alto de la Huesera, 7. Chabola de la Hechicera, 8.  
 127 El Encinal, 9. Los Llanos, 10. Longar. The sites included in the study are those represented with stars.  
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130  
 131 **Fig 2. Kernel density model of the available radiocarbon dates on human bone from the megalithic graves**  
 132 **studied** (Armendáriz and Irigaray 1993-1994; Fernández Eraso and Mujika-Alustiza 2013; Fernández Eraso et al.  
 133 2015a; Fernández Eraso et al. 2016; Galilea 2009; Olalde et al. 2019; this study). The dates are modeled using OxCal  
 134 4.2.2 (Bronk Ramsey 2009; Reimer et al. 2013).

In addition, six faunal samples from terrestrial herbivores and omnivores were studied to ascertain the variability of baseline isotopic signatures in the region. They were closely associated with the human burials and come from La Cascaja (n = 1, from the Middle Chalcolithic) San Martín (n = 3, all from the late Middle Neolithic) and Alto de la Huesera (n = 2, both from the Middle-Late Chalcolithic and Bronze Age). The sample size is very small due to the scarcity of faunal remains in funerary contexts and the virtual absence of coeval settlements in the region.

Moreover, recently published isotope data from 100 Late Neolithic/Early Chalcolithic human and seven faunal samples from El Sotillo, Alto de la Huesera, Chabola de la Hechicera and Longar megalithic tombs (Fernández-Crespo and Schulting 2017a; Fernández-Crespo et al. 2018a) are used to complete the diachronic sequence and better assess regional dietary and cultural dynamics over time.

#### 4. METHODS

For an introduction to the basics of stable carbon and nitrogen isotope analysis, consult [Text S2](#).

**Sampling strategy.** Mandibular or cranial bone fragments weighing ca. 800 mg were sampled for the study, since these elements were instrumental in estimating the minimum number of individuals –thus ensuring that each sample corresponded to a unique individual– as well as providing age and sex data in most cases (Fernández-Crespo and de-la-Rúa 2015). Furthermore, the association with dentition provides the potential for high-resolution, sequential isotopic analyses of dentine and enamel for dietary and mobility investigations (e.g. Fernández-Crespo et al. 2020).

**Collagen preparation and analysis.** Extraction was carried out following Richards and Hedges (1999). The resulting ‘collagen’ was weighed into tin capsules and loaded into a SERCON 20/22 continuous flow ratio mass spectrometer coupled with an elemental analyzer at the Research Laboratory for Archaeology and the History of Art (RLAHA) of the University of Oxford. The stable carbon and nitrogen isotope ratios were measured in duplicate. Analytical precision is  $\pm 0.2\%$  ( $1\sigma$ ) for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  based on repeated analyses of internal (alanine, marine seal collagen, cow collagen) and international standards (IAEA 600, caffeine). In order to detect potentially degraded collagen that could affect the stable isotope values, the extracted collagen preservation was checked according to several preservation criteria: %col > 1; %C = 30-44; %N = 11-16; molar C:N = 2.9-3.6 (e.g. van Klinken 1999).

**Statistical testing.** Statistical analyses were performed using IBM SPSS software for Windows v17. Z-scores were initially calculated both to detect the presence of outliers and to evaluate data quality (e.g., results with anomalous collagen quality parameters). Shapiro-Wilk tests were used to assess whether or not the data were normally distributed. Since all the datasets analyzed were normally distributed with approximately equal variance (determined through the Levene test), Student’s *t*-tests were employed when comparing two samples, and one-way ANOVA tests were used when more than two groups were being compared (e.g. comparison between phases). Post-hoc Tukey’s HSD tests were also used to detect significant differences between samples. A significance level of  $\alpha = 0.05$  was used for all statistical tests.

#### 5. RESULTS

**Collagen preservation.** The majority of the samples provided collagen yields, carbon and nitrogen percentages and C:N ratios indicating well-preserved collagen. However, two human samples (SM.6D9.210 and ES.Sinsigla.1) exhibiting C:N ratios >3.6 were excluded from further analysis. In addition, 44 samples exhibited collagen yields, %C and/or %N marginally above or below the generally accepted limits but with C:N ratios within the accepted range. The effects of including or excluding these samples were considered and found not to affect the results, since they are consistent with other fully acceptable  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  measurements at each site (individual Z-scores generally < 1.0). Thus, these values do not appear to be diagenetically compromised and have been retained in subsequent analyses. Two samples have therefore been excluded, leaving 123 human and six fauna measurements for analysis. These have been combined for



subsequent analyses with the 100 Late Neolithic/Early Chalcolithic human and seven faunal samples previously published for the megalithic graves of the region (Fernández-Crespo and Schulting 2017a) (Tables S1 and S2).

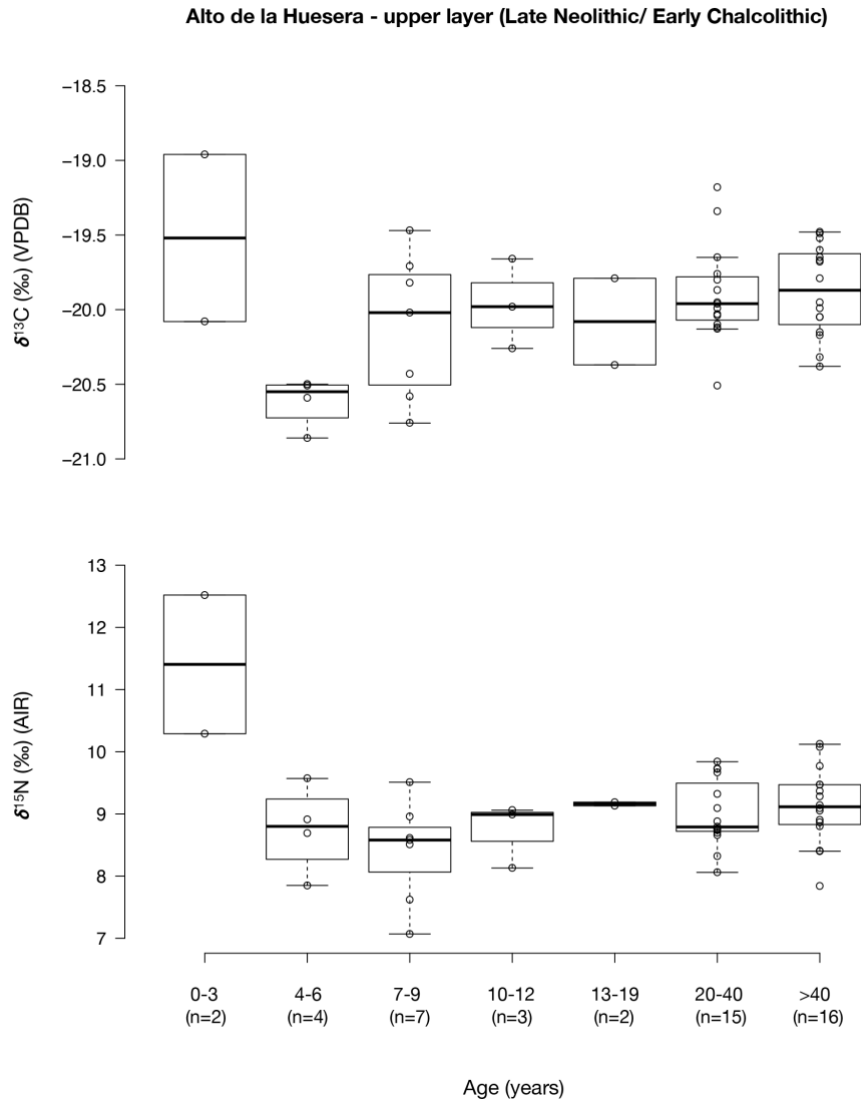
**Human isotope data.** The human  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values are consistent with diets focused on  $\text{C}_3$  plants and terrestrial animal resources, though there is some variability both between sites and between periods of use within sites (Tables 1 and 2). Since the main aim of paper is to test whether or not any significant subsistence shifts took place over time among megalithic people, a first analytical approach is to assess if differences in demographic profiles between sites and periods may affect statistical comparisons.

Table 1. Mean $\delta^{13}\text{C}$ values by site and phase/culture.																	
Site	Period	Yrs cal. BC	Total			Juveniles (<20)			Adults ( $\geq 20$ )			Adult males			Adult females		
			n	$\bar{x}$	$\sigma$	n	$\bar{x}$	$\sigma$	n	$\bar{x}$	$\sigma$	n	$\bar{x}$	$\sigma$	n	$\bar{x}$	$\sigma$
La Cascaja	Middle Chalcolithic	2840-2570	21	- 19.8	0.3	4	- 19.9	0.2	17	- 19.8	0.4	9	- 19.8	0.5	7	- 19.8	0.2
El Sotillo	Late Neolithic/ Early Chalcolithic	3090-2920	1	- 20.0	-	-	-	-	1	- 20.0	-	-	-	-	-	-	-
	Middle-Late Chalcolithic	2920-2350	2	- 20.4	0.5	-	-	-	2	- 20.4	0.5	2	- 20.4	0.5	-	-	-
	Bronze Age	2010-820	5	- 20.0	0.3	-	-	-	5	- 20.0	0.3	3	- 20.1	0.4	2	- 19.7	0.1
	Unknown	¿?	8	- 20.0	0.3	1	- 19.7	-	7	- 20.0	0.4	3	- 19.9	0.3	-	-	-
San Martín	Late Middle Neolithic	3760-3520	16	- 19.6	0.2	3	- 19.8	0.2	13	- 19.5	0.2	8	- 19.5	0.3	3	- 19.6	<0.1
	Middle-Late Chalcolithic	ca. 2700-2000	3	- 19.8	0.1	-	-	-	3	- 19.8	0.1	-	-	-	-	-	-
Alto de la Huesera	Late Neolithic/ Early Chalcolithic	3340-2880	55	- 20.0	0.4	18	- 20.1	0.5	37	- 19.9	0.3	17	- 19.9	0.3	12	- 19.9	0.2
	Middle-Late Chalcolithic and Bronze Age	ca. 2900-1200	18	- 20.0	0.4	3	- 20.3	<0.1	15	- 19.9	0.4	10	- 19.9	0.3	5	- 19.9	0.4
Chabola de la Hechicera	Late Neolithic/ Early Chalcolithic	3630-2890	6	- 20.3	0.4	2	- 19.9	0.3	4	- 20.5	0.3	2	- 20.6	0.4	2	- 20.4	<0.1
	Middle Bronze Age	1660-1450	1	- 20.0	-	1	- 20.0	-	-	-	-	-	-	-	-	-	-
	Unknown	¿?	26	- 20.2	0.2	6	- 20.2	0.2	20	- 20.1	0.2	10	- 20.2	0.1	6	- 20.1	0.3
Longar	Late Neolithic/ Early Chalcolithic	3500-3010	61	- 19.9	0.3	29	- 19.8	0.3	32	- 20.0	0.3	13	- 19.8	0.2	14	- 20.1	0.3

Table 2. Mean $\delta^{15}\text{N}$ values by site and phase/culture.																	
Site	Period	Yrs cal. BC	Total			Juveniles (<20)			Adults ( $\geq 20$ )			Adult males			Adult females		
			n	$\bar{x}$	$\sigma$	n	$\bar{x}$	$\sigma$	n	$\bar{x}$	$\sigma$	n	$\bar{x}$	$\sigma$	n	$\bar{x}$	$\sigma$
La Cascaja	Middle Chalcolithic	2840-2570	21	9.5	0.6	4	9.3	0.5	17	9.5	0.6	9	9.5	0.7	7	9.7	0.6
El Sotillo	Late Neolithic/ Early Chalcolithic	3090-2920	1	10.0	-	-	-	-	1	10.0	-	-	-	-	-	-	-
	Middle-Late Chalcolithic	2920-2350	2	9.3	0.7	-	-	-	2	9.3	0.7	2	9.3	0.7	-	-	-
	Bronze Age	2010-820	5	9.7	0.6	-	-	-	5	9.7	0.6	3	10.0	0.6	2	9.2	0.1
	Unknown	¿?	8	9.6	0.5	1	10.5	-	7	9.5	0.4	3	9.5	0.5	-	-	-
San Martín	Late Middle Neolithic	3760-3520	16	10.4	0.5	3	9.9	0.7	13	10.5	0.4	8	10.7	0.4	3	10.1	0.3
	Middle-Late Chalcolithic	ca. 2700-2000	3	9.7	0.6	-	-	-	3	9.7	0.6	-	-	-	-	-	-
Alto de la Huesera	Late Neolithic/ Early Chalcolithic	3340-2880	55	9.0	0.8	18	9.0	1.2	37	9.1	0.6	17	9.0	0.6	12	8.9	0.6
	Middle-Late Chalcolithic and Bronze Age	ca. 2900-1200	18	9.6	0.6	3	9.0	0.4	15	9.7	0.5	10	9.7	0.5	5	9.8	0.6
Chabola de la Hechicera	Late Neolithic/ Early Chalcolithic	3630-2890	6	9.1	0.6	2	8.8	0.1	4	9.2	0.8	2	9.7	0.1	2	8.7	0.9
	Middle Bronze Age	1660-1450	1	8.4	-	1	8.4	-	-	-	-	-	-	-	-	-	
	Unknown	¿?	26	9.3	0.7	6	9.7	1.4	20	9.2	0.4	10	9.2	0.3	6	9.4	0.5
Longar	Late Neolithic/ Early Chalcolithic	3500-3010	61	9.6	0.4	29	9.6	0.4	32	9.6	0.5	13	9.7	0.5	14	9.5	0.5

Considering age, the two burial contexts with a sufficient representation of juveniles (0-19 years), i.e. the Late Neolithic/Early Chalcolithic layers of Alto de la Huesera and Longar, do not show any difference between juveniles and adults either in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  mean values. However, variance should also be considered in detail. Juveniles generally show higher variability (as assessed by standard deviation) than adults whether within sites and periods (cf. Tables 1 and 2) or when combining individuals from all sites and periods (juveniles (n = 67):  $\delta^{13}\text{C} \pm 0.4\text{‰}$ ;  $\delta^{15}\text{N} \pm 0.8\text{‰}$ ; adults (n = 156):  $\delta^{13}\text{C} \pm 0.3\text{‰}$ ;  $\delta^{15}\text{N} \pm 0.6\text{‰}$ ), suggesting greater variability among juveniles especially in  $\delta^{15}\text{N}$ . This fact is probably influenced by several dietary, physiological and/or cultural factors (e.g. nursing, weaning, skeletal growth, stress) that have been demonstrated to notably increase or decrease their isotope values at different stages of life (Fernández-Crespo et al. 2018a). The case of Alto de la Huesera's Late Neolithic/Early Chalcolithic layer, which allows comparison of stable isotopes with more specific age categories (0–3, 4–6, 7–9, 10–12, 13–19, 20–39, and >40), is very illustrative in this regard, with infants and younger children showing elevated and depleted  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values due to nursing and weaning signals, respectively (Figure 3). Excluding these younger age cohorts from analyses and, therefore, selecting only individuals older than age 7 may be a solution. However, this approach does not solve the problem either, since then a statistically significant difference in mean  $\delta^{15}\text{N}$

224 values is observed between older juveniles ( $n = 48$ ,  $\bar{x} = 9.2 \pm 0.7\text{‰}$ ) and adults ( $n = 156$ ,  $\bar{x} = 9.5 \pm 0.6\text{‰}$ ) ( $t =$   
 225  $2.726$ ,  $df = 202$ ,  $p = 0.007$ ). Since the inclusion of different proportions of juveniles of different ages  
 226 between sites and periods may have a significant impact on the results, these have been excluded from any  
 227 diachronic comparison.  
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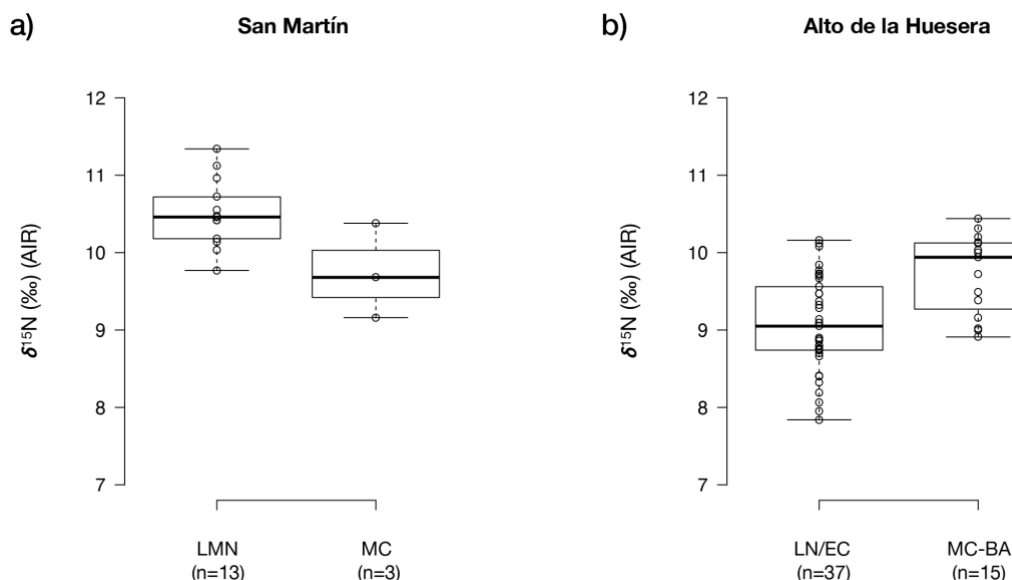


230  
 231 **Fig 3. Alto de la Huesera megalithic grave: boxplots showing comparison of Late Neolithic/ Early Chalcolithic**  
 232 **human bone collagen  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values with specific age categories (0-3, 4-6, 7-9, 10-12, 13-19, 20-40 and >40**  
 233 **years).**

234  
 235  
 236 Sex, by contrast, does not seem to have any clear statistical impact on the adult sample (sex is unknown for  
 237 most juveniles). Despite the overall predominance of males typical of megalithic graves ([Fernández-Crespo](#)  
 238 [and de-la-Rúa 2015](#)), no differences are seen between males and females either combining all the data or  
 239 within sites and periods (cf. Tables 1 and 2), except for the Late Neolithic monument of Longar, where males  
 240 show significantly higher mean  $\delta^{13}\text{C}$  values than females ( $t = 2.577$ ,  $df = 25$ ,  $p = 0.016$ ). This instance is not  
 241 expected to substantially bias the diachronic comparison pursued, since the difference only affects one site  
 242 that shows near-parity between sexes (13 males to 14 females) and both sexes are similarly represented in the  
 243 majority of contexts studied. Therefore, both male and female adult individuals are retained in subsequent  
 244 tests.  
 245

246 An intra-site diachronic comparison would be desirable to observe differences in subsistence over time in all  
 247 sites studied. However, only three of these (San Martín, Alto de la Huesera and El Sotillo) allow this  
 248 approach. San Martín shows statistically significant differences in  $\delta^{15}\text{N}$  values ( $t = 2.541$ ,  $df = 14$ ,  $p = 0.024$ )

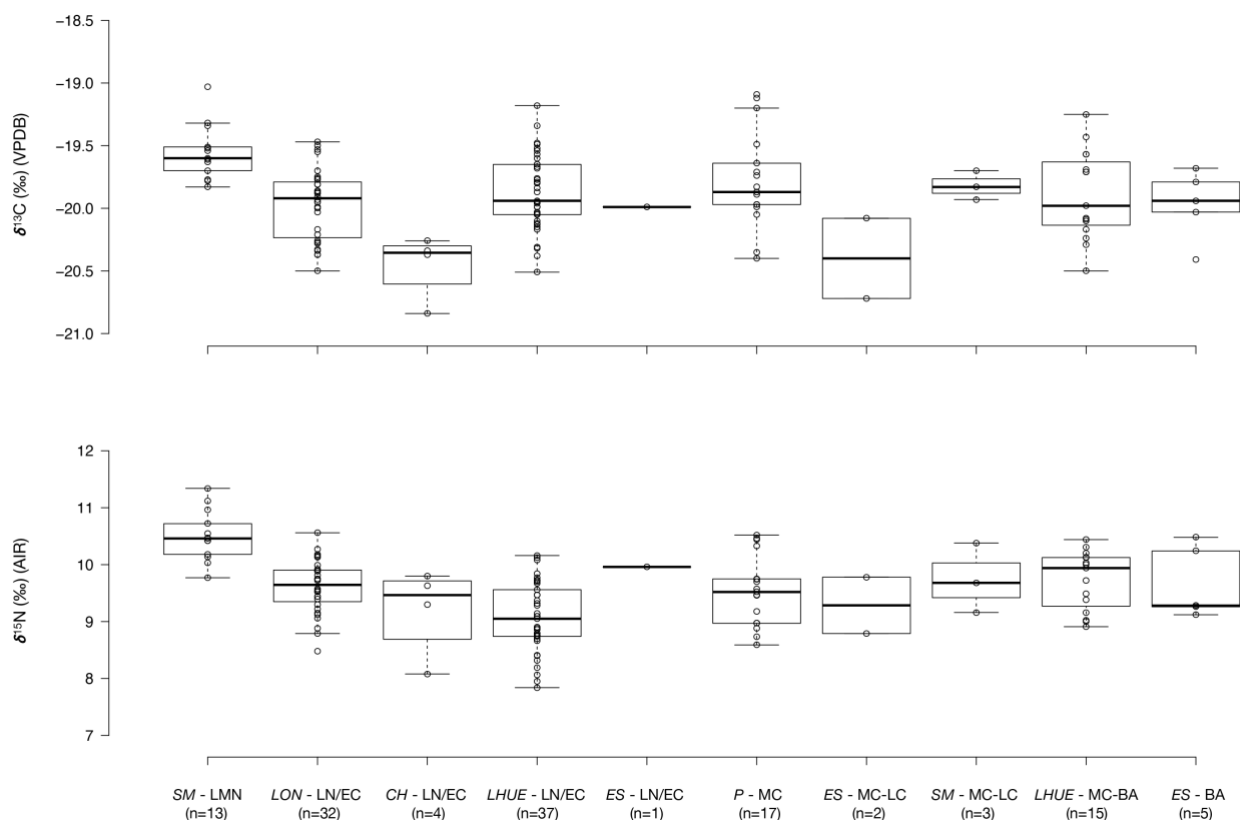
between the late Middle Neolithic and Middle Chalcolithic burials, with the former providing higher values (Figure 4a). Alto de la Huesera also shows significant differences in  $\delta^{15}\text{N}$  values between periods ( $t = 3.633$ ,  $df = 50$ ,  $p = 0.001$ ), but in this case between the Late Neolithic/Early Chalcolithic and the Middle-Late Chalcolithic and Bronze Age, whose burials show significantly higher values (Figure 4b). By contrast, El Sotillo shows no differences between the different periods represented, although smaller sample sizes must be acknowledged here.



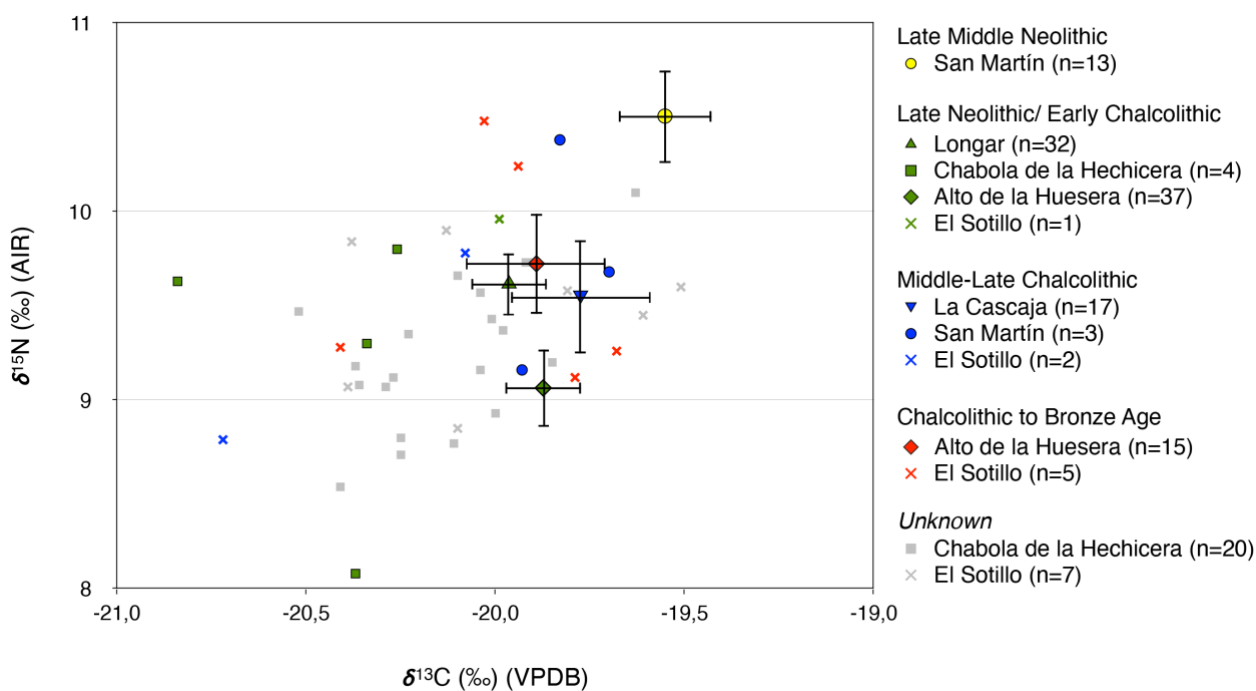
**Fig 4. Intracite comparison of human adult  $\delta^{15}\text{N}$  values by period of funerary use in San Martín (a) and Alto de la Huesera (b) megalithic graves.** LMN = late Middle Neolithic; LN/EC = Late Neolithic/ Early Chalcolithic; MC = Middle Chalcolithic; MC-BA = Middle Chalcolithic to Bronze Age.

To test the possibility that the differences observed between periods at San Martín and Alto de la Huesera extend to the region and to assess whether there may be other diachronic patterns that cannot be explored through an intra-site approach, a one-way ANOVA test was conducted between different contexts (considering layers of different periods and not the sites as different groups) (Tables 1 and 2). This shows the presence of significant differences between contexts in both  $\delta^{13}\text{C}$  ( $F_{(8,119)} = 5.0$ ,  $p < 0.001$ ) and  $\delta^{15}\text{N}$  ( $F_{(8,119)} = 8.8$ ,  $p < 0.001$ ), with no differences in variance (Levene's test:  $\delta^{13}\text{C}$ ,  $p = 0.258$ ;  $\delta^{15}\text{N}$ ,  $p = 0.551$ ). The application of a post hoc Tukey's HSD test (Table S3 and S4) reveals that inter-context heterogeneity is mainly driven by the late Middle Neolithic layer of San Martín. Its values are significantly higher than those of all the contexts corresponding to later periods both in  $\delta^{13}\text{C}$  and in  $\delta^{15}\text{N}$ , corroborating a meaningful pattern that needs to be explored. New findings are the significant differences detected in  $\delta^{13}\text{C}$  values between Chabola de la Hechicera's Late Neolithic/Early Chalcolithic layer and both Alto de la Huesera (regardless of period) and La Cascaja, and in  $\delta^{15}\text{N}$  values between Alto de la Huesera Late Neolithic/Early Chalcolithic layer and the broadly contemporary burial deposit of Longar. By contrast, no significant differences are seen between any of the contexts attributed to the Middle-Late Chalcolithic or the Bronze Age (Figure 5). In order to assess whether any of the aforementioned observations are influenced by the small sample sizes available for some contexts (e.g. Chabola de la Hechicera Late Neolithic/Early Chalcolithic layer, El Sotillo), the same test was repeated with the exclusion of those samples lower than 10 individuals. The results are consistent with those previously provided in both  $\delta^{13}\text{C}$  ( $F_{(4,109)} = 4.5$ ,  $p = 0.002$ ) and  $\delta^{15}\text{N}$  ( $F_{(4,109)} = 17.6$ ,  $p < 0.001$ ) and support a significant difference between San Martín and the later contexts studied and a difference in  $\delta^{15}\text{N}$  between Alto de la Huesera Late Neolithic/Early Chalcolithic layer and both the Middle-Late Chalcolithic and Bronze Age upper layer of the same site and Late Neolithic/Early Chalcolithic Longar megalith (Table S4 and S5) (Figure 6).





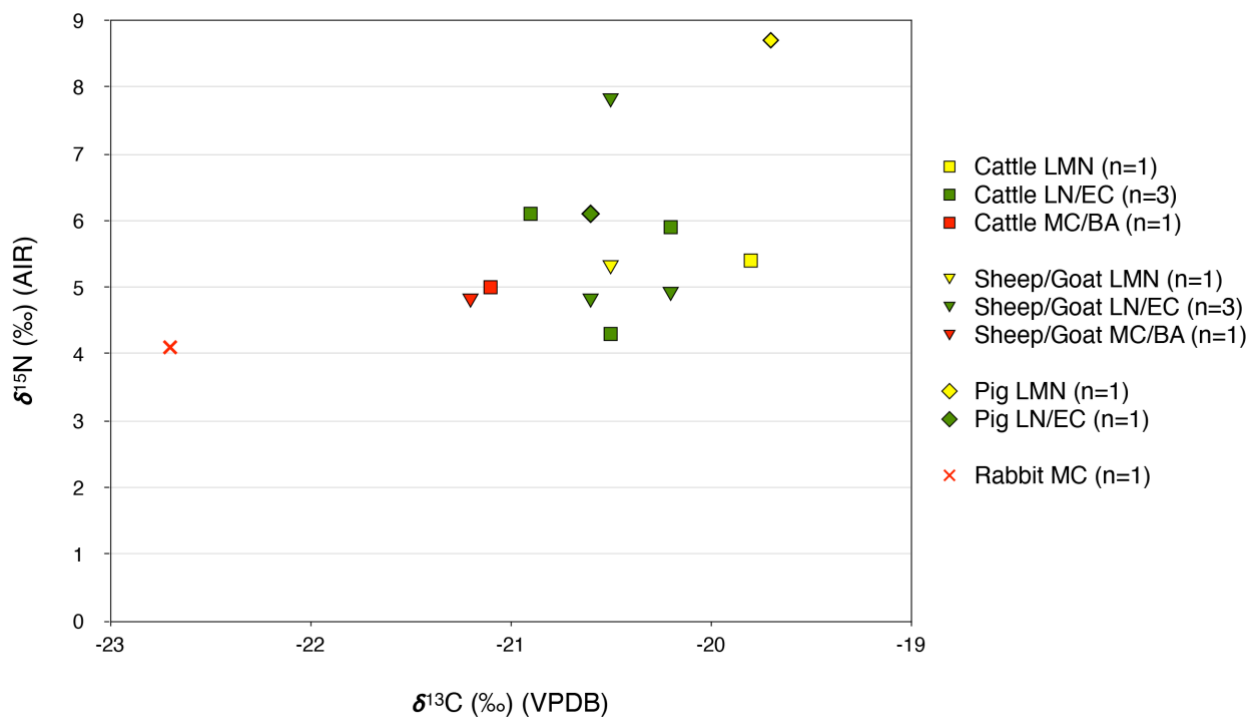
**Fig 5. Comparison of human adult  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values by context of funerary use (site/phase).** SM = San Martín; LON = Longar; CH = Chabola de la Hechicera; LHUE = Alto de la Huesera; ES = El Sotillo; P = La Cascaja // LMN = late Middle Neolithic; LN/EC = Late Neolithic/ Early Chalcolithic; MC = Middle Chalcolithic; MC-LC = Middle-Late Chalcolithic; MC-BA = Middle Chalcolithic to Bronze Age; BA = Bronze Age.



**Fig 6. Dispersion of human adult  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values by site and period of funerary use.** Bars represent 2 standard errors and are used for contexts with more than ten individuals.

**Faunal isotope data.** The  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  ratios for the animals (Table S2) are consistent with expectations for a temperate  $\text{C}_3$  ecosystem (DeNiro and Epstein 1978) regardless of chronology, ranging from -21.2 to -19.7‰ and from 4.3 to 8.7‰, respectively, if we exclude the single rabbit specimen available, which is clearly depleted in  $^{13}\text{C}$  (-22.7‰) (Figure 7). The higher nitrogen isotope values shown by two animals, a sheep/goat (CH.23123a) and a pig (SM.Sinsigla), may be reflecting a nursing signal since both are non-adult specimens, though in the latter case the enrichment might also be explained by an omnivorous diet.

The small sample size prevents the assessment of any differences in animal management either between periods of funerary use within each site or between sites, but the values seem to be consistent with the expectations for a trophic level shift in comparison to the mean human values (i.e. ca. 1‰ for  $\delta^{13}\text{C}$  and 3-5‰ for  $\delta^{15}\text{N}$ ), regardless of their chronology.



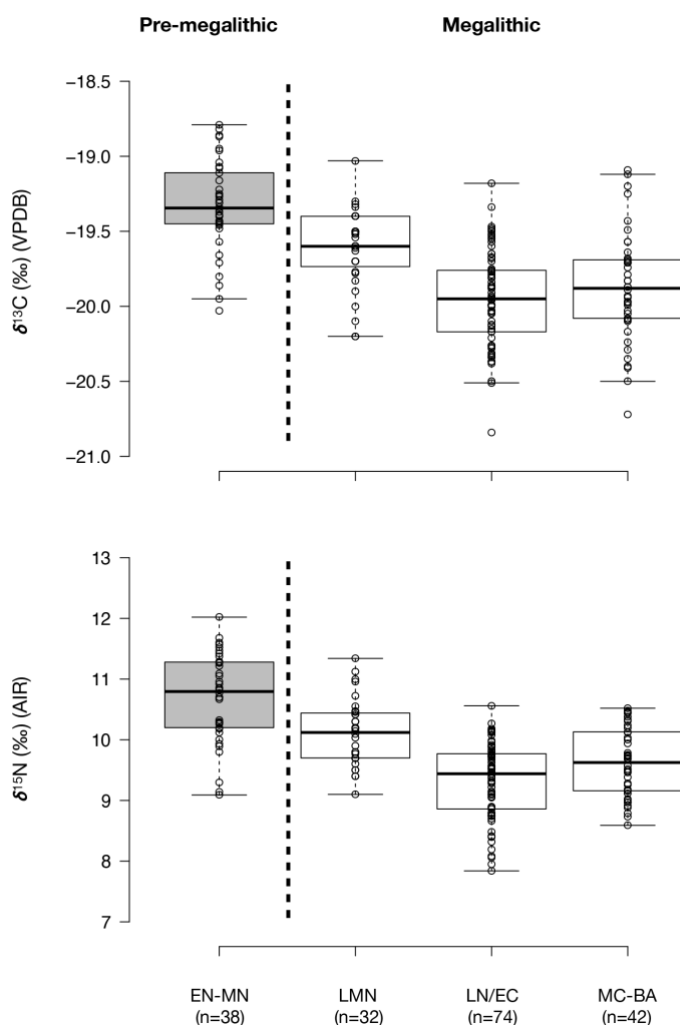
**Fig 7. Dispersion of faunal  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values by species and by period of funerary use.** LMN = late Middle Neolithic; LN/EC = Late Neolithic/ Early Chalcolithic; MC = Middle Chalcolithic; MC-BA = Middle Chalcolithic to Bronze Age.

## 6. DISCUSSION

The clearest trend to emerge is a marked shift in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values between the late Middle Neolithic and the subsequent periods of megalithic use (i.e. Late Neolithic/Early Chalcolithic, Middle-Late Chalcolithic and Bronze Age). The higher isotope values detected at the late Middle Neolithic layer of San Martín are consistent with those from the nearby coeval megalithic tomb of Alto de Reinoso in Burgos (Alt et al. 2016), supporting that there is a real shift between periods. The higher isotope values observed in the earlier phase may be interpreted as indicating a substantially greater contribution of terrestrial animal protein to diets at this time, whereas later populations appear to have had a greater contribution from plants (e.g.  $\text{C}_3$  cereals and legumes). Other sources of  $^{13}\text{C}$  and/or  $^{15}\text{N}$  enrichment (e.g. manuring,  $\text{C}_4$  plants, marine and freshwater resources) are not likely to have had a significant importance in north-central Iberia during the Neolithic (Fernández-Crespo and Schulting 2017a). This hypothesis does not necessarily mean that early megalithic communities (ca. 4000–3600 cal BC) had a fully pastoral economy, but may imply a key role of meat and perhaps dairy products in their subsistence that, ultimately, could have involved a greater commitment to a specialized herding economy (e.g. Carvalho 2015). Evidence of recurrent sheep/goat stalling in coeval rockshelters (i.e. fumiers) of the region (Polo and Fernández-Eraso 2008) and of anthropozoogenic flora and

coprophilous fungi forming part of pastures have been interpreted similarly (Pérez-Díaz et al. 2015). Unfortunately, the virtual absence of plant and faunal remains and the scarcity of coeval settlements in the study area make it difficult to know to what extent those early megalithic communities relied on this component of the economy (e.g. Fernández-Eraso et al. 2015a).

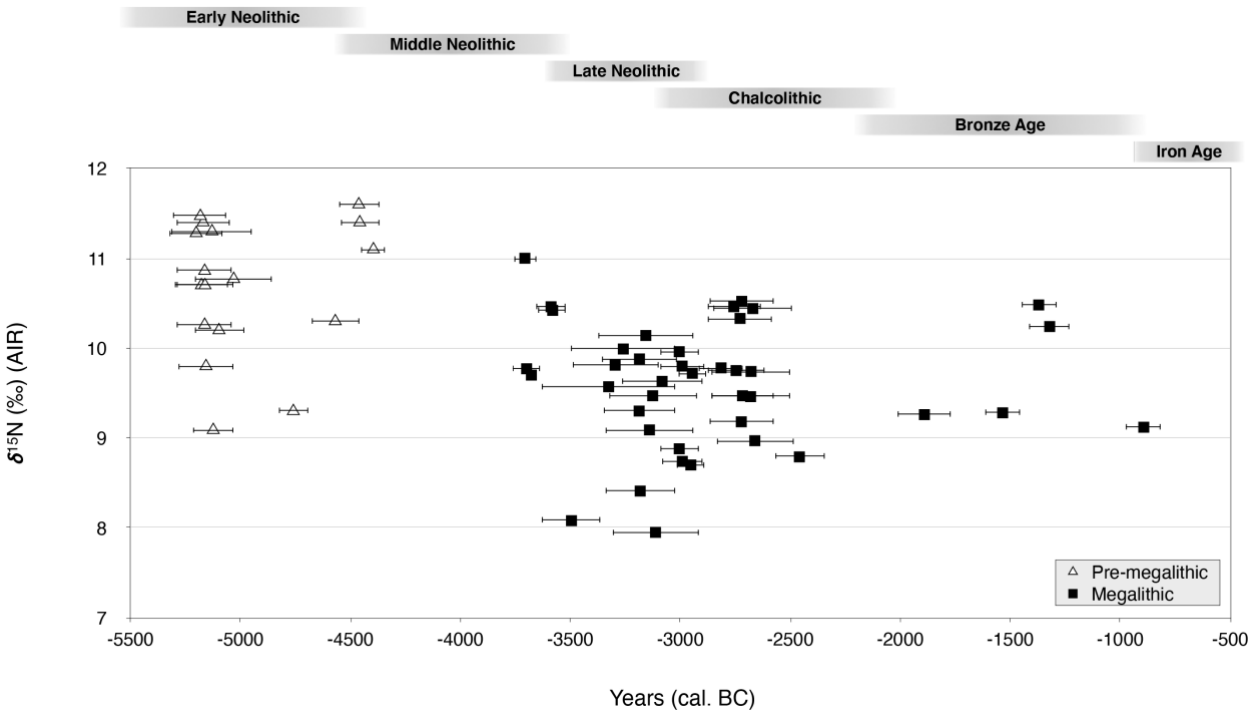
Dietary traditions characterized by comparatively high  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values have been identified among Early and Middle Neolithic communities of the Ebro valley and the Spanish plateau burying their dead in pit graves, as Los Cascajos, Paternanbidea and other smaller sites (Fernández-Crespo et al. 2019a) (Figure 8). This fact may be tentatively interpreted as evidence of a degree of continuity in subsistence practices (i.e. a model featuring an elevated terrestrial animal protein intake) between those first Neolithic communities and early megalithic groups, despite the deep cultural changes (e.g. ancestor veneration, astronomical interests, appearance of carved bone idol-palettes) and complex socio-economic responses assumed to be behind the appearance of the earliest monuments before 4000 cal. BC in northern Iberia (Andrés 2009; Carvalho 2015). This idea would be in keeping with the observation that increasing aridity and cooler temperatures attributed to the so-called 5.9 kyr cal BP (ca. 3900 cal BC) climatic event (Bond et al. 1997) did not have a significant impact on the environment of north-central Iberia, as both the palynological and the small mammal records support (e.g. Rofes et al. 2013; Pérez-Díaz et al. 2015), and that a consequent adaptive response may have not been necessary.



**Fig 8. Boxplot of human adult  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values obtained from late prehistoric pre-megalithic (i.e. Early and Middle Neolithic) and megalithic communities of the Rioja Alavesa region and nearby areas (Alt et al. 2016; Fernández-Crespo and Schulting 2017a, 2017b; Fernández-Crespo et al. 2019; this study), sorted by period. EN-MN = Early-Middle Neolithic (includes Los Cascajos, Paternanbidea, Llano del Montico and El Prado); LMN = late Middle Neolithic (San Martín and Alto de Reinoso); LN/EC = Late Neolithic/ Early Chalcolithic (El Sotillo, Alto de la Huesera, Chabola de la Hechicera and Longar); MC-BA = Middle Chalcolithic to Bronze Age (La Cascaja, El Sotillo, San Martín, Alto de la Huesera and Chabola de la Hechicera).**

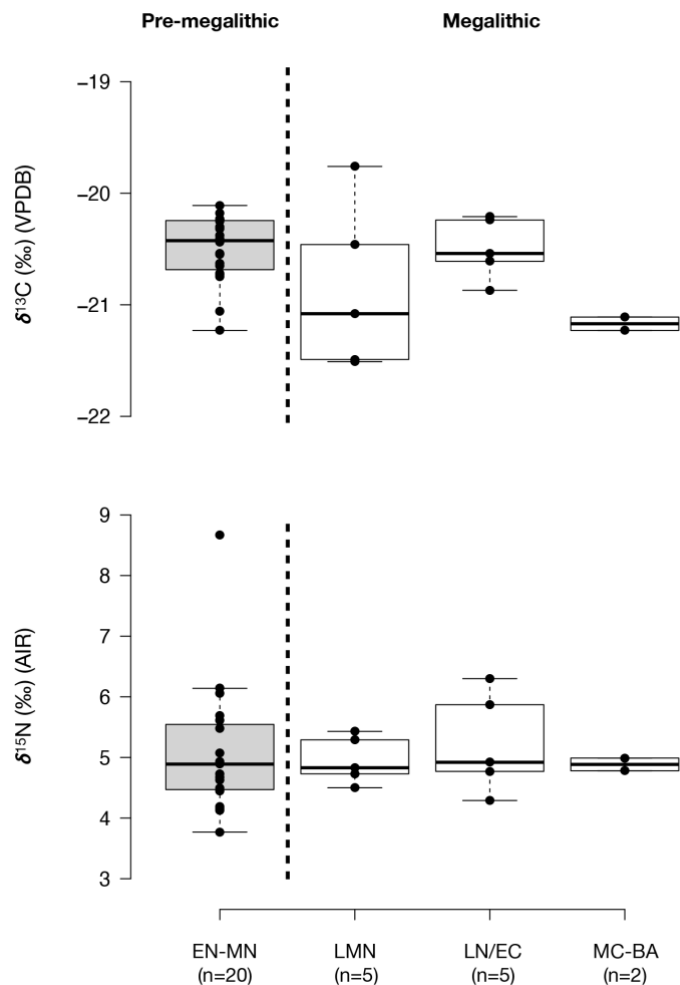
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The timing of the shift in subsistence can be identified reasonably precisely here thanks to a number of AMS <sup>14</sup>C dating programs undertaken within the study area (e.g. Armendáriz and Irigaray 1993-1994; Fernández-Eraso and Mujika-Alustiza 2013; Fernández-Eraso et al. 2015b; Fernández-Eraso et al. 2016; this study). It would have occurred between 3700 and 3500 cal. BC (Figure 9), at the onset of the Late Neolithic/Early Chalcolithic, which coincides with the beginnings of a period of social unrest linked to sharp population growth within the region (e.g. Fernández-Crespo et al. 2018a, 2018b). This demographic feature can provide a good explanation of the variation in resource use, as it facilitates the development of economic and social infrastructures. Theoretically, as population density increases, changes should occur in cropping techniques such as first expanding the area under cultivation or, when that is no longer possible, shortening fallow periods and increasing the labor input to meet the requirements of the population (Boserup 1965, 1981). In this scenario, the relative importance of animal resources could easily shift in favor of domestic cereals and perhaps legumes (although no evidence for pulses has been documented in the region so far). Indeed, the regional palynological record at this period shows a decline in the percentage of tree pollen and the appearance of ruderal and nitrophilic species that, together with the identification of ratios higher than 3% of domestic cereal pollen and cultivated seeds, have been interpreted as a progressive anthropogenic depletion of forest and the expansion of arable fields and pastures (Pérez-Díaz et al. 2015). These socio-economic changes could have also been promoted by the temperate climatic conditions pertaining at the beginning of Late Neolithic/Early Chalcolithic, in contrast with the less warm and slightly humid Early and Middle Neolithic periods (Rofes et al. 2013), which are reflected in lower ratios of riparian vegetation in the region (Pérez-Díaz et al. 2015). Unfortunately, plant and animal remains are very scarce in the region (e.g. Fernández-Eraso et al. 2015a), and it is not clear whether this shift was accompanied by a change from sheep and goat to cattle and pig keeping and a reduced crop spectrum, as usually occurs when diversified subsistence strategies are implemented (Halstead 1989). In any case, changes in herd management strategies do not seem to have had a major impact on the process or, at least, they are not detectable through stable isotope analysis, since  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of non-nursing domestic herbivores available for the region show no differences between periods (Figure 10), although larger sample sizes would be desirable to confirm this observation.



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**Fig 9.** Human bone collagen  $\delta^{15}\text{N}$  values of radiocarbon dated adult individuals of late prehistoric pre-megalithic (i.e. Early and Middle Neolithic) and megalithic communities of the Rioja Alavesa region and nearby areas (Alt et al. 2016; Fernández-Crespo and Schulting 2017a, 2017b; Fernández-Crespo et al. 2019; this study). Sites included are: Los Cascajos, Paternanbidea, Llano del Montico, El Prado, San Martín, Alto de Reinoso, El Sotillo, Alto de la Huesera, Chabola de la Hechicera, Longar and La Cascaja.



**Fig 10. Boxplot of non-nursing domestic herbivore  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values obtained from late prehistoric pre-megalithic (i.e. Early and Middle Neolithic) and megalithic communities of the Rioja Alavesa region and nearby areas (Alt et al. 2016; Fernández-Crespo and Schulting 2017a; Fernández-Crespo et al. 2019; this study), sorted by period. EN-MN = Early-Middle Neolithic (includes Los Cascajos and Paternanbidea); LMN = late Middle Neolithic (San Martín and Alto de Reinoso); LN/EC = Late Neolithic/ Early Chalcolithic (El Sotillo, Alto de la Huesera and Chabola de la Hechicera); MC-BA = Middle Chalcolithic to Bronze Age (Alto de la Huesera).**

The shift in subsistence coincides with meaningful changes in funerary practices, particularly in selection criteria for burial in a monument. Osteological evidence suggests that, while late Middle Neolithic burial in megalithic graves may have been primarily reserved for small groups of selected adults, mostly males, in the Late Neolithic/Early Chalcolithic the rules of access may have not been limited by age and sex, but perhaps instead related more to socio-economic parameters (e.g. acquired status) (Fernández-Crespo and de-la-Rúa 2015), suggesting the appearance of more flexible criteria for inclusion (Andrés 2009). Consequently, the presence of women and children increased during the Late Neolithic/Early Chalcolithic, despite adult and adolescent male predominance persisting in some megalithic tombs in central Iberia (e.g. Delibes 1995) and sex-based dietary differences being traced in some contexts (e.g. Longar, this study). In this regard, it is important to note that the isotopic distinction observed between periods is not biased by the different sex profiles being compared, as analyzed late Middle Neolithic males show significantly higher  $\delta^{15}\text{N}$  values than more recent males. Similarly, it is also possible that the type of burial changed from multi-stage to primary at this period, as both the finding of irregularities in bone representation in the region (Fernández-Crespo and de-la-Rúa 2015) and the existence of coeval ‘houses of the dead’ in nearby areas for the earlier phase may support (Alday et al. 2008; Alt et al. 2016). Moreover, it is probable that the role of megalithic grave goods evolved from communal (e.g. polished axes, carved bone idol-palettes) to individualized offerings (e.g. beads, pendants, arrowheads) during the Neolithic (e.g. Villalobos 2014). Unfortunately, the fact that the



dates available for the period fall within the late 4<sup>th</sup> millennium cal. BC plateau in the calibration curve prevents us from addressing the speed with which these funerary changes took place.

There may have been another shift in subsistence practices between the Late Neolithic/Early Chalcolithic and the following periods, as suggested by differences in  $\delta^{15}\text{N}$  values between the layers of Alto de la Huesera megalithic grave. However, the existence of a significant difference between the Late Neolithic/Early Chalcolithic isotope values from this site and those from the coeval megalithic tomb of Longar suggests a degree of synchronic variability in diet at this time that makes it difficult to assess the validity of this finding.

Surprisingly, our results do not show any isotopic variation across the later phases of megalithic use (i.e. Middle-Late Chalcolithic and Bronze Age, from ca. 2900 to 800 cal. BC) (Figure 8), despite encompassing the so-called 4.2 kyr BP event (ca. 2200 cal. BC) (Bond et al. 1997, 2001) characterized by a relatively cool and wet phase in the region (Rofes et al. 2013) and the major socio-economic and cultural changes (e.g. rise in complexity, appearance of metallurgy, emergence of new beliefs and religious ideas, introduction of new foods) that occurred during the 3<sup>rd</sup> and 2<sup>nd</sup> millennia cal. BC (e.g. Fernández-Eraso et al. 2015a). It is important to recognize, however, that, while these processes might be expected to have involved changes in the subsistence economy, they would not necessarily be detectable through stable isotope analysis. It may also be that, as burial in monuments is very infrequent from the Middle Chalcolithic onwards conversely to other forms of burial (e.g. single, double and triple pit graves), the sample size available is too small, and perhaps highly selective, to identify any dietary changes over time.

In contrast, the significant decline of evidence for funerary activity in the Middle Chalcolithic (Fernández-Crespo and de-la-Rúa 2015) confronts the image of stability conventionally assumed for late prehistoric Iberian groups, especially in the northern regions (e.g. Blanco-González 2018). Caves and rockshelters that saw extensive use for burial during the Late Neolithic/Early Chalcolithic are no longer used in the Rioja Alavesa, and megalithic graves only show punctuated episodes of mortuary activity (e.g. Fernández-Crespo and de-la-Rúa 2015; Fernández-Eraso and Mujika-Alustiza 2013). Signs of abandonment are so clear in the regional record that even natural disasters such as an earthquake have been proposed to explain the disruption in megalithic activity (Martínez-Torres 1997). However, evidence of fallen orthostates, stone pavings and earth or ochre layers sealing late Middle Neolithic or Late Neolithic/Early Chalcolithic burial activity, and of similar findings in other regions of Iberia, better support a social rather than a natural explanation for the phenomenon (Andrés 2005). Regional signs of environmental changes, as the expansion of humid meadows in detriment to grasslands and possible forest regeneration characterized by the development of evergreen forest (evergreen *Quercus*, *Pinus sylvestris*, *Buxus*) and the spread of *Fagus* (Rofes et al. 2013; Perez-Díaz et al. 2015) can also be seen as suggestive indicators for depopulation. The aforementioned high demographic pressure, resource scarcity and social unrest resulting into violent conflict detected in the region during the precedent Late Neolithic/Early Chalcolithic (Fernández-Crespo et al. 2018a, 2018b) may have all contributed to a decline in population and/or to population movements to other regions (Andrés 2005).

The rare burials that do occur within megalithic graves at this time, commonly accompanied by rich grave goods (e.g. decorated pottery, metallic weapons, rich adornments), have long been understood as representing the burials of chiefs or other socially prominent individuals (Andrés 2005). However, osteological research has recently shown that Middle-Late Chalcolithic and Bronze Age burials in north-central Iberian megalithic graves generally include not one but a few individuals and have asymmetries in bone representation attributable to secondary inhumation practices (Fernández-Crespo and de-la-Rúa 2015), challenging the traditional notion of single primary burials (e.g. Delibes 2010). Moreover, there is a notable representation of non-adult remains, despite the low number of individuals selected for burial in megaliths at that period. Unfortunately, there is little available information concerning sex because of the generally poor preservation of the skeletal material. It is plausible that requirements for burial from that time on relied on certain hereditary rights of prominent Bell Beaker and Bronze Age lineages (e.g. resource control and coercive power) (Andrés 2009; Bueno et al. 2005). However, no clear status-related isotope divergences have been detected between those individuals and others of similar ages from, supposedly less hierarchical, former mortuary phases, nor with contemporary individuals buried in non-megalithic funerary locations, like the nearby multiple pit of La Atalayuela (Fernández-Crespo et al. 2019b).

The aforementioned archaeological evidence suggesting depopulation of the region during the Middle Chalcolithic (Andrés 2005) makes it feasible to interpret the burial of certain individuals in old and ruined monuments as venues for maintaining and contesting social and political organization in the pursuit of rights within the territory (i.e., the so-called ‘geo-omphalic notion’) by new populations or the descendants of previous Late Neolithic/Early Chalcolithic groups (Andrés 2005; Bradley 1993). In this respect, it has been suggested that secondary burials in megalithic tombs may have been a particularly expedient method for the creation of genealogies to advance claims of political power (Holtorf 1998).

Contrary to some other European regions (Díaz-Guardamino et al. 2015), the funerary activity of the megalithic graves studied here seems to end in parallel to the latest Bronze Age burials. No mortuary afterlife has been detected in historic times so far, despite the monumentality of the tombs and their role in regional folklore as huts of witches or houses built by ‘jentillak’ (the legendary first inhabitants of the Basque Country characterized by supernatural height, strength and longevity, and magic powers, and to whom the adoption of the agriculture is attributed) (Barandiarán 1980).

## 7. CONCLUSION

This work demonstrates the importance of fine-grained chronological approaches for disentangling valuable social, economic and ecological information hidden in complex megalithic biographies. Consideration of both synchronic and diachronic variation in megalithic tombs should look beyond mono-causality and be integrated in a complete paleoenvironmental and cultural framework to understand the rationale behind different ritual and socio-economic behaviors. This study has shown discontinuity in subsistence associated with demographic and ritual changes among megalithic communities in north-central Iberia, despite archaeological literature insistence in underestimating the openness and adaptability of late prehistoric Iberian groups to change, especially of those from the northernmost area. In the light of these findings, further studies should assume that the reason for burial in megalithic graves and the lifeways of people buried in them may have substantially changed over time and recall the need of carrying out new approaches emphasizing the value of the socio-ecological information that is usually obscured in this palimpsest-like record.

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