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Stable carbon isotopes in archaeobotanical remains and palaeoclimate

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Resum

Es descriu una metodologia recent per a inferir la precipitació en el passat basada en l'anàlisi de la composició isotòpica del carboni ($\delta^{13}C$) en restes arqueobotàniques. Un cop descrita la base fisiològica de la tècnica, s'il·lustra l'aplicabilitat de $\delta^{13}C$ mitjançant un exemple referent al NE peninsular. Hom pretén proporcionar una estimació quantitativa de l'evolució de la precipitació estacional (primavera) i anual al llarg dels darrers quatre mil anys basada en $\delta^{13}C$. Les mostres analitzades comprenen carbons (pi blanc) i llavors carbonitzades (blat i ordi), i s'obtenen estimes pluviomètriques superiors en el passat que actualment, amb una tendència gradual cap a condicions progressivament més àrides. No obstant això, aquesta tendència no esdevé uniforme, i es detecten dues fases de major precipitació (1800-900 aC; 300 aC - 300 dC) alternadament amb períodes relativament secs (900-300 aC; 900 dC - present). Dels resultats presentats també es desprèn que la importància relativa de la pluja primaveral en el passat fou variable. Des d'aproximadament el 300 aC en endavant, el període primaveral subministrà una major proporció de pluja anual que actualment. Contràriament, durant el període 1800-800 dC la seva contribució va esdevenir inferior, i va aparèixer una fase transitòria (800-300 aC) que mostra una recuperació sobtada en aportació primaveral. Posteriorment a aquesta fase la sincronia de canvis en $\delta^{13}C$ en granes i carbons suggereix l'arribada del clima mediterrani a la regió.

Paraules clau: arqueologia · fustes carbonitzades · granes carbonitzades · Holocè tardà · precipitació · disponibilitat hídrica · nord-oest de la Mediterrània

Abstract

The present report describes a novel approach to infer the amount of precipitation in antiquity based on the analysis of carbon isotope composition ($\delta^{13}C$) in archaeobotanical remains. After discussing the physiological background of the technique, we illustrate the usefulness of $\delta^{13}C$ as palaeoclimate proxy by means of a case study from the NE Iberian Peninsula. The goal of the study was to quantitatively reconstruct the evolution of seasonal (spring) and annual precipitation during the last 4000 years based on $\delta^{13}C$ evidence. The samples analysed were charcoals of Aleppo pine and charred grains of barley and wheat. Our findings indicate that estimated past precipitation was consistently higher than today, with a gradual trend towards a drier climate. This increase in aridity, however, did not develop uniformly; instead, two main phases of greater precipitation (1800–900 BCE; 300 BCE–300 CE) alternated with drier periods (900–300 BCE; 900 CE–present). The relative significance of spring rainfall in the past was variable. From approximately 300 BCE onwards, spring accounted for a higher proportion of annual rainfall than is the case today. In contrast, during the period 1800–800 BCE, the contribution of spring rainfall to annual precipitation was less. A transition phase occurred from ca. 800 to 300 BCE, a period marked by a sudden recovery in spring precipitation. Subsequently, the synchrony of $\delta^{13}C$ changes in grains and charcoal points to the installation of the Mediterranean climate in the region.

Keywords: archaeology · wood charcoal · charred grains · late Holocene · precipitation · water availability · NW Mediterranean

Beyond contributing to our knowledge of the Earth's climate system, palaeoclimate studies provide a key tool for assessing future climate change in response to external forcing factors, such as greenhouse gases and the concomitant increase in atmospheric CO_2 concentrations. Understanding the dynamics of climate variability on all relevant time scales and in adequate spatial resolution requires careful application of palaeoreconstruction methods. This applies particularly to the recent past, since the signal-to-noise ratio is lower than for glacial climate variability [17]. In arid and semi-arid areas, precipitation is the most relevant climate element determining the impact of climate fluctuations on ecosystems. Unlike temperature, precipitation regimes show relatively poor spatial correlations and can exhibit contrasting associations with global climate trends in

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different areas [44]. The Holocene, the period of largest climate stability in almost half a million years, has featured the emergence and development of large, complex societies, following the retreat of the Pleistocene ice and the discovery of agriculture some 10000 years ago. Evidence of past changes in aridity over the Holocene is still scarce and mainly of a qualitative nature [29]. This hold true for vast areas of the world, such as the Iberian Peninsula (NW Mediterranean Basin), where the climate is defined by complicated interactions between Atlantic, continental, Mediterranean, and subtropical influences. According to Shulmeister and Lees [45], the increase in aridity in the Mediterranean during the Holocene has probably been triggered by changes in atmospheric global circulation, especially by the North Atlantic Oscillation. However, regional factors, such as those related to anthropogenic activities (e.g. agriculture and animal husbandry), may also play an important role in accelerating aridification in specific regions, such as Eastern Spain (one of the most eroded and dry areas in Mediterranean Europe). Reliable Holocene climate proxies for this region should allow for high time-frequency and spatial resolutions. Indeed, a broad spatial heterogeneity in response to global climate trends in the Western Mediterranean Basin is evidenced by present climatic data [44] and by the palaeoenvironmental record [14,31,38,43].

In this review, we describe a novel methodology in which past precipitation is inferred from the analysis of carbon isotope composition ($\delta^{13}C$) in charred plant remains (wood charcoal and charred grains) routinely recovered in the course of archaeological excavations. After a general introduction describing the physiological background of the technique, the relevant methodological aspects and the properties of archaeobotanical remains, the use of the carbon isotopes in this material as palaeoclimate proxy is illustrated by means of a case study. Thus, the methodology was applied to analyse a series of archaeobotanical samples from the Mid-Ebro Depression (NE Iberian Peninsula) in order to reconstruct the evolution of aridity in this area during the last 4000 years [21,22]. The Mid-Ebro Depression is among the most arid zones in Europe (mean annual rainfall between 350 and 400 mm), but it remains unclear whether present conditions are due to recent environmental changes or to an ongoing aridification that began in prehistoric times. Palaeoenvironmental records for the Holocene are still scarce for this area and are mostly derived from pollen and geomorphological studies [14,25,43]. The analysis of stable carbon isotopes in ancient plant remains provides complementary (and quantitative) data on the time-course of water availability in the NW Mediterranean Basin during the Late Holocene. The samples analysed herein consisted of charcoals of Aleppo pine (*Pinus halepensis* Mill.) and charred grains of barley (*Hordeum vulgare* L.) and wheat (*Triticum aestivum/durum*, after [50]).

1 Archaeobotany or the study of archaeological plant remains

During excavations of archaeological sites, different kinds of plant remains may be recovered. In temperate and semi-arid climates, most plant material has been preserved through carbonisation [41,51]. When plant materials are combusted under poor oxygen availability they become charred, which prevents fungal or bacterial decay while retaining identifiable morphological traits. During this process, however, soft plant parts (e.g. leaves or stems) are usually lost; thus, most archaeobotanical remains are wood charcoal and charred grains. There was little interest in the plant fossil remains of the Mediterranean region until the studies of Helbaek [28] in the Near East. Since then, and due to its particular relevance in investigations into the origin of Mediterranean agriculture, the Near East is still the most extensively surveyed region in the field of archaeobotany [41,51,40].

1.1 Recovery of Plant Remains

Small charred grains and charcoal fragments are not easily recognised by the naked eye; rather, specialised means are necessary to recover them. The most common method by which macrobotanical fragments are concentrated and recovered is through the technique known as flotation. All flotation systems are based on the same principle: when archaeological sediment is released into a water-filled container, the sediment sinks and the grains and charred plant remains float [51]. The floating grains and charcoal fragments are retrieved with a submerged sieve and, once dried, stored and identified.

1.2 Sample Dating

The dating of archaeobotanical remains involves several strategies. Radiocarbon dating, based on the rate of decay of the unstable isotope ^{14}C, is currently the most widely used dating technique for the Late Pleistocene and Holocene periods [48]. However, ^{14}C must be calibrated externally to account for past changes in atmospheric ^{14}C composition, and the final accuracy ranges from several hundred years to a few decades, depending on the period considered [47]. Also, the high cost of a single analysis strongly limits the number of samples that can be dated. Consequently, most plant macroremains are assigned an age according to the dating obtained from other charcoals, grains, or bones from the same stratigraphic layer or archaeological phase. In most cases, a combination of stratigraphic methods and typological analyses of archaeological materials (e.g. based on ceramic or tool styles) provides more accurate dating than radiocarbon analyses alone.

2 Physiological Basis of Carbon Isotope Studies in Plants

The two stable carbon isotopes (^{13}C and ^{12}C) occur in the molar ratio of 1:89 in the atmosphere. However, plants with a C3 photosynthetic pathway (e.g., most tree species and winter cereals) generally contain proportionally less ^{13}C than does the air [18]. Indeed, their typical $^{13}C/^{12}C$ molar ratio is about 1:99. Nowadays, $^{13}C/^{12}C$ ratios are usually determined by mass spectrometry and referred to the PeeDee Belemnite (PDB) standard as $\delta^{13}C$ values:

$$\delta^{13}C(‰) = \left[\left(\frac{R_{sample}}{R_{standard}}\right) - 1\right] \times 1000 \qquad \text{Eq. 1}$$

where R is the $^{13}C/^{12}C$ ratio. On this basis, the present carbon isotope composition ($\delta^{13}C$) in air is about -8.5‰, whereas typical C3 leaf values range between -35‰ and -22‰. In plant studies, carbon isotope discrimination (Δ) is often calculated as a means of quantifying the magnitude of discrimination events against ^{13}C from the carbon dioxide source used by plants during photosynthesis [18]:

$$\Delta^{13}C\ (‰) = \frac{\delta^{13}C_a - \delta^{13}C_p}{1 + \delta^{13}C_p} \qquad \text{Eq. 2}$$

where $\delta^{13}C_a$ and $\delta^{13}C_p$ refer to air and plant composition, respectively. In fact, $\Delta^{13}C$ integrates the ratio of intercellular to atmospheric concentrations of CO_2 (ci/ca) during the period in which carbon atoms were fixed, and thus reflects the balance between assimilation rate (*A*) and stomatal conductance (g_s), the so-called intrinsic water-use efficiency (WUEi). Plants typically react to a decrease in water availability by stomatal closure; although *A* may also decline, g_s is usually affected to a larger extent, leading to a reduction in $\Delta^{13}C$ [18]. The rate of evaporation from the leaf also determines the stomatal responses that subsequently affect $\Delta^{13}C$. Indeed, an increase in the leaf-to-air vapour pressure deficit (VPD, the driving force for transpiration) will also cause lower $\Delta^{13}C$ values [8]. This is the basis for the extensively reported relationships between $\Delta^{13}C$ and the environmental variables related to water availability, such as precipitation, relative humidity, and potential evapotranspiration (see references in [19]).

3 Stable Carbon Isotopes, Plant Remains and Palaeoenvironmental Reconstruction

The major interest in using stable isotope techniques in palaeoenvironmental reconstruction lies in their ability to integrate plant processes over time, which may range from a few days to thousands of years. In seasonally dry climates, $\delta^{13}C$ is related to changes in several environmental variables that influence plant water status, but the magnitudes of these relationships are species-dependent, varying according to the particular adaptive mechanisms exhibited by the plants to cope with drought. In particular, species with a high reliance on stomatal control tend to show large $\delta^{13}C$ variations in response to changes in aridity (Fig. 1). These species include winter cereals, such as wheat and barley (in the form of charred grains), and Aleppo pine (in the form of wood charcoal), both of which are commonly found in archaeological contexts in the Mediterranean. In cereal grains, $\delta^{13}C$ is mainly determined by water availability during grain filling, a period of about 45 days from anthesis to maturity usually occurring during the second half of April and throughout May, either in the past or in the present (see references in [20]). In this context, our team has pioneered the use of $\delta^{13}C$ in charred grains to gain insight into the environmental conditions that prevailed in the early agriculture of the Iberian Peninsula [4,5] and the Near East [6,7].

While tree-ring variability in wood $\delta^{13}C$ allows for continuous high-resolution climate reconstructions back to ca. 11000 BP (see references in [27]), such studies have been restricted to the few global regions where long-term chronologies are available, but usually they cannot be carried out at a local scale. The short-living Aleppo pine, a Mediterranean conifer of wide distribution, only exceptionally lives for more than 150 years and is therefore unsuitable for the reconstruction of extended chronologies. However, its abundance in the form of archaeo-

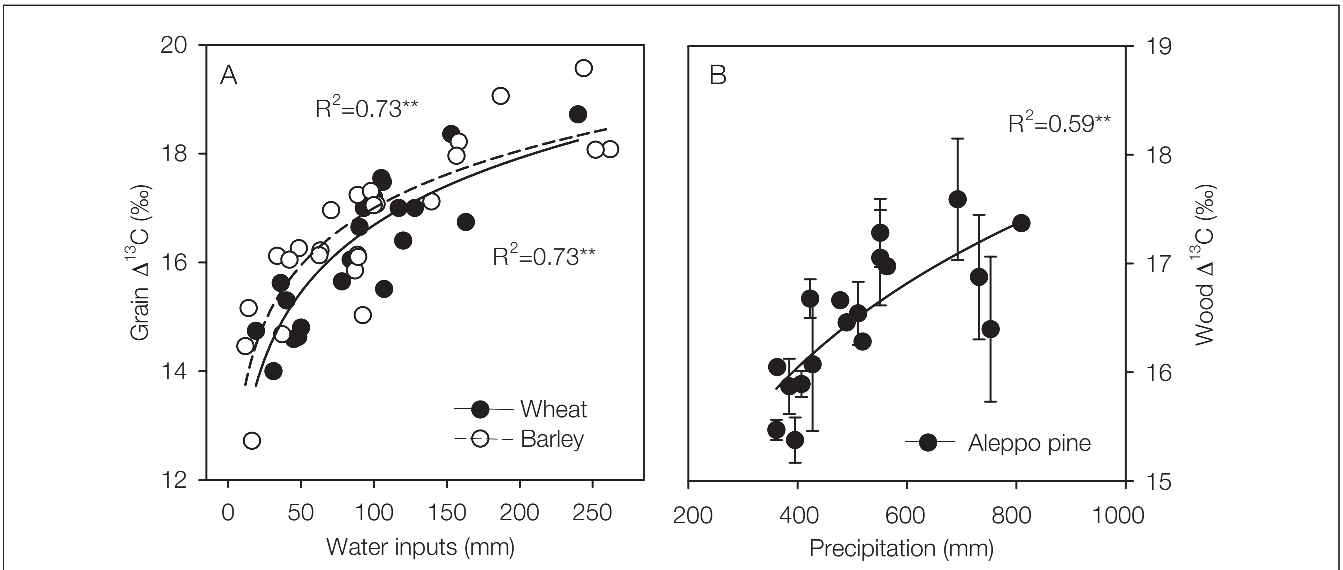

Figure 1. Relationships between (**A**) water inputs (WI, rainfall plus irrigation, if applied) during grain filling and the $\Delta^{13}C$ of barley and wheat grains. Data from Araus et al. (1997) for barley and from Araus et al. (1999) for wheat; $\Delta^{13}C_{barley}$= 9.99 + 1.52×ln (WI); $\Delta^{13}C_{wheat}$= 8.50 + 1.78×ln (WI). (**B**) Mean annual precipitation (25-year period) and $\Delta^{13}C$ of Aleppo pine wood from tree rings of the same period. $\Delta^{13}C_{pine}$= 4.63 + 1.91×ln (precipitation). (Modified from Ferrio et al. 2003)

botanical remains represents an appealing way to obtain local information on the climate of the past, despite the lower temporal resolution of this approach and uncertainties about the use of charcoal $\delta^{13}C$ associated with carbonisation events [21,23].

3.1 Methodological Aspects

Information on past climates can be recovered from archaeobotanical remains provided that a number of prerequisites are considered: (i) the need to account for past changes in the $\delta^{13}C$ of atmospheric CO_2, (ii) the removal of soil contaminants and (iii) the quantification of $\delta^{13}C$ shifts during carbonisation.

The source of carbon for most terrestrial plants is atmospheric CO_2 and, accordingly, changes in $\delta^{13}C$ of atmospheric CO_2 ($\delta^{13}Ca$) are reflected in plant tissues (see Eq. 2). As $\delta^{13}Ca$ is not constant over time (e.g. is currently increasing due to fossil fuel emissions), past $\delta^{13}Ca$ values need to be estimated before modern and archaeological data or fossil samples from different ages can be compared. Antarctic ice cores provide a direct and extensive record of global atmospheric changes, although they cannot account for local variations. Nonetheless, ice core data are still the most suitable reference values for $\delta^{13}Ca$ in archaeological material. A $\delta^{13}Ca$ curve covering the entire Holocene, fitted by the authors and obtained by interpolating data from Antarctic ice cores together with modern data obtained from two Antarctic stations of the CU-INSTAAR/NOAA-CMDL network for atmospheric CO2, can be found at http://web.udl.es/usuaris/x3845331/AIRCO2_LOESS.xls [20].

Plant material recovered from archaeological sediments often carries soil substances that may alter $\delta^{13}C$. The most common contaminants are carbonate precipitates and humic/fulvic acids, although their amounts strongly depend on soil conditions. DeNiro and Hastorf [15] provided a simple protocol that allows for contaminant removal without altering the charred fragments.

Most archaeobotanical remains are found in the carbonised state. Carbonisation prevents microbial degradation, yet the process can involve significant shifts in the original $\delta^{13}C$. However, De Niro and co-workers [15,39] studied carbonisation in grains and plant fragments but found little change in $\delta^{13}C$. We have compared the ^{13}C signal of cereal grains grown under contrasting environmental conditions with their corresponding values after being charred at 250ºC (Ferrio, Voltas, Araus, unpublished results). The slope and intercept of the relationship between intact and charred material did not differ significantly from unity and zero, respectively, further suggesting that $\delta^{13}C$ in cereal grains remains unaffected after charring. In contrast to

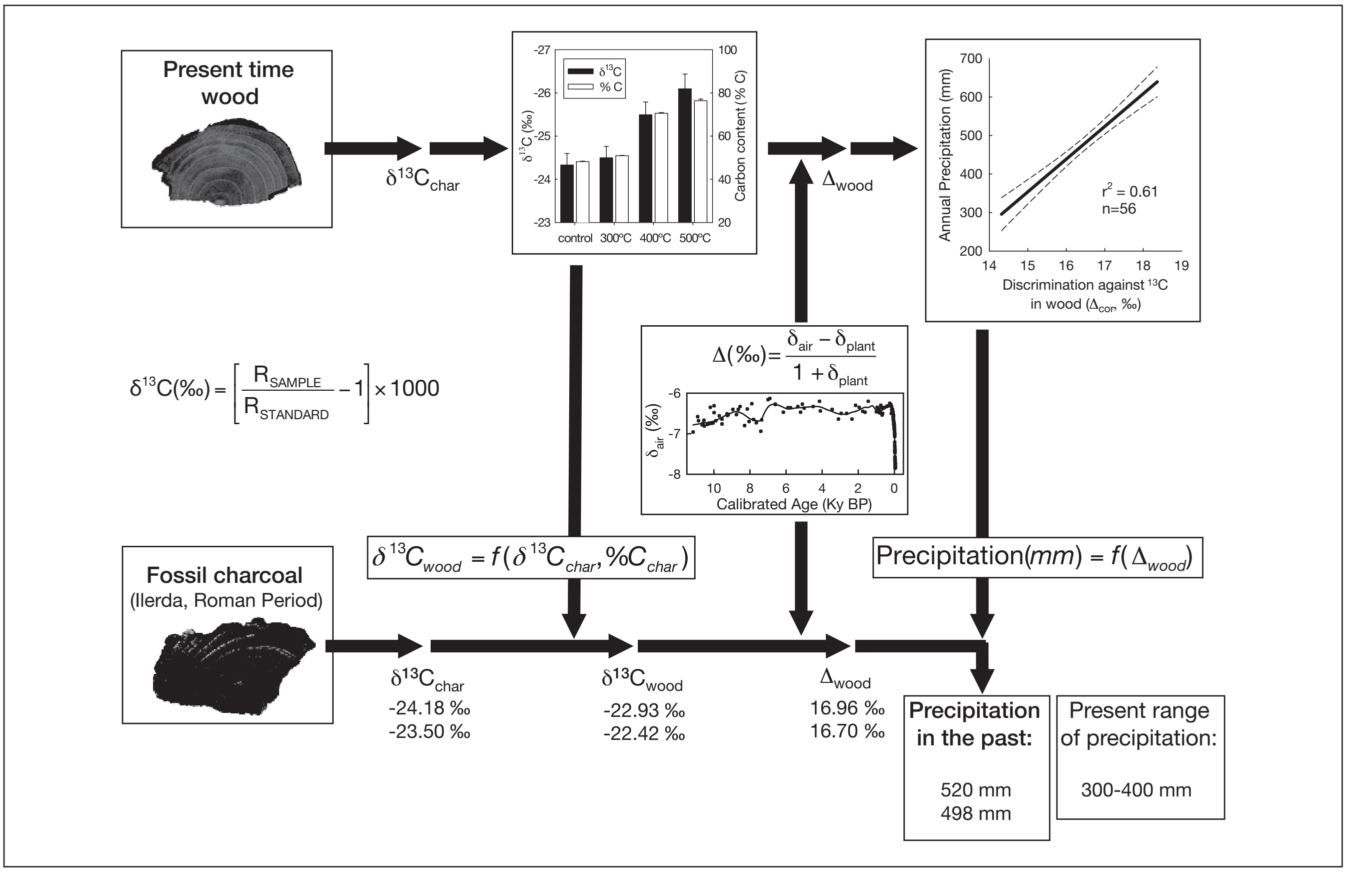

Figure 2. Methodological steps for the inference of annual precipitation from fossil charcoal of Aleppo pine. The charcoal carbon isotope composition ($\delta^{13}C_{char}$) is determined by mass spectrometry whereas the effect of charring on $\delta^{13}C_{char}$ can be evaluated by means of experimental carbonisation using present-time wood samples. Next, $\delta^{13}C_{char}$ values are corrected in order to obtain $\delta^{13}C$ estimates of intact wood ($\delta^{13}C_{wood}$) using %C as an indicator of the level of carbonisation ($\delta^{13}C_{wood}$= -0.706 × $\delta^{13}C_{char}$ +0.031 × $\%C_{char}$ -8.07). Carbon isotope discrimination ($\Delta^{13}C$) is then calculated to account for variations in air $\delta^{13}C$ ($\delta^{13}Ca$). The relationship between wood $\Delta^{13}C$ and annual precipitation was established from extant samples (Ferrio et al. 2003, see Fig. 1). The resulting equation is then used to infer past precipitation from charcoal $\Delta^{13}C$.

the effect on cereals, the effect of carbonisation on wood may be considerable owing to its greater chemical complexity. Based on a variety of morphophysical features [16,21,24,32], the usual range of temperatures for charcoal formation as determined from the fossil record appears to have been rather narrow (around 350–450ºC), indicating a compromise between increasing resistance to chemical/biological degradation and decreasing toughness. Earlier studies [13,21,32] have shown that wood charred at low temperatures (150–300ºC) becomes slightly enriched in $\delta^{13}C$, but that $\delta^{13}C$ values decrease at higher temperatures (300–600ºC). Fortunately, the $\delta^{13}C$ of charcoal is still strongly related to the original wood $\delta^{13}C$ in conifers such as *Pinus halepensis*, and changes due to carbonisation can be successfully corrected by taking the charcoal %C as an indicator of the degree of carbonisation [21]. This remains to be more widely tested in hardwoods, which have different chemical and physical wood properties. In this regard, we have found contrasting results for the effect of carbonisation on the wood $\delta^{13}C$ of Mediterranean oaks sampled across a rainfall gradient [23]. Although the carbonisation levels of oak wood were comparable to those of conifer wood, there were few changes in its $\delta^{13}C$ values. In short, we can conclude that the environmental signal recorded in wood is at least partly preserved in charcoal, but further work is needed to understand the fate of isotope fractionation during carbonisation.

With these prerequisites as a background, the relationships shown in Fig. 1 may be applied to archaeobotanical samples in order to infer past trends in water availability. An outline of the methodology is presented in Fig. 2 for the fossil wood of Aleppo pine. The procedure becomes simpler in charred cereal grains since a correction for the impact of carbonisation in $\delta^{13}C$ is unnecessary.

Table 1. Main geographic and environmental variables (average of present values from the closest meteorological station), dating and cultural period of the archaeological sites together with the number of individual grains (N) analysed for each species

Archaeological Site	*Lat.*	*Long.*	*Alt.* (m)	P_{an} (mm)	T_{an} (ºC)	P/E_{an}	*Calendar year (BCE/CE)* Mean	Interval	*Cultural Period*	*N* Barley	Wheat	Aleppo pine
1. Minferri	41º30'N	0º45'E	240	389	13.5	0.51	1890 cal. BCE	2131-1692 cal. BCE	Bronze Age	2	2	1
							1673 cal. BCE	1879-1517 cal. BCE		2	2	4
2. Masada de Ratón	41º29'N	0º22'E	130	350	14.6	0.43	1262 cal. BCE	1524-999 cal. BCE	Late Bronze Age	-	-	11
							1110 cal. BCE	1185-982 cal. BCE		-	-	8
							1062 cal. BCE	1048-936 cal. BCE		2	1	5
3. Vincamet	41º30'N	0º21'E	110	350	14.6	0.43	988 cal. BCE	1288-928 cal. BCE	Late Bronze Age	-	-	8
4. El Vilot de Montagut	41º38'N	0º30'E	250	355	14.7	0.45	1412 cal. BCE	1412 cal. BCE	Late Bronze Age	2	2	-
							888 cal. BCE	1125-795 cal. BCE	First Iron Age	2	2	8
							773 cal. BCE	801-396 cal. BCE		2	2	9
5. Tozal de los Regallos	41º30'N	0º01'E	317	359	14.9	0.44	896 cal. BCE	896 cal. BCE	First Iron Age	2	-	28
6. Els Vilars	41º34'N	0º56'E	300	403	14.2	0.51	793 ca. BCE	793 cal. BCE	Iron Age (Early Iberian)	2	2	-
							781 cal. BCE	781 cal. BCE		2	1	-
							488 BCE	550-425 BCE		2	2	8
7. Roques del Sarró	41º38'N	0º38'E	195	361	14.8	0.46	225 BCE	275-175 BCE	Iron Age (Iberian)	-	2	11
8. Margalef	41º35'N	0º49'E	232	370	13.7	0.49	200 BCE	200 BCE	Iron Age (Iberian)	12	2	-
9. Tossal de les Tenalles	41º39'N	0º52'E	230	369	14.9	0.46	200 BCE	200 BCE	Iron Age (Iberian)	2	12	-
10. Ciutat de Lleida	41º37'N	0º38'E	220	361	14.8	0.46	26 BCE	50-1 BCE Roman		4	2	9
							67 CE	20-100 CE		-	-	6
							200 CE	175-225 CE		7	2	2
							300 CE	8250-350 CE		5	5	10
							925 CE	900-950 CE	Middle Age	-	-	9
							990 CE	950-1030 CE		2	2	6
							1010 CE	990-1030 CE		-	-	10
							1025 CE	950-1100 CE		4	3	6
							1065 CE	1030-1099 CE		-	-	2
							1298 CE	1298 CE		2	1	-
							1450 CE	1450 CE		2	3	-
							1600 CE	1550-1650 CE	Modern Age	-	-	3
							1750 CE	1700-1800 CE		-	-	5
Total										60	50	169

4 Changes in Aridity During the last 4000 Years in the NW Mediterranean Basin

The approach developed thus far was applied to a series of archaeobotanical samples from the Mid-Ebro Basin (NE Spain). The samples consisted of charred grains of naked wheat and barley and of charcoal from Aleppo pine, all recovered from ten archaeological sites in the Segre and Cinca valleys (Fig. 3, Table 1). These sites display similar climatic and soil conditions and are characterised by a semi-arid Mediterranean climate. The samples were collected from various archaeological contexts, such as domestic fires, cooking ovens, storage jars, the floors of dwellings, and levels of rubble from housing structures and pits (for further details, see [1,3]).

The investigations described herein were aimed at obtaining evidence supporting increasing aridity throughout the Late Holocene in the Western Mediterranean Basin. In particular, we attempted to quantitatively reconstruct the evolution of seasonal (spring) and annual precipitation during the last 4000 years based on $\delta^{13}C$ evidence. To this end, information previously published by our team was reviewed, and data on cereal crops [22] and Aleppo pine [21] were combined. Fossil cereal grains were used as a palaeoenvironmental proxy for ancient moisture conditions during the mid-spring (second half of April and May period), while Aleppo pine remains provided a reliable record of changes in annual water availability (i.e. precipitation). Together, both types of data allow valuable information on changes in rainfall seasonality and dynamics through time to be retrieved.

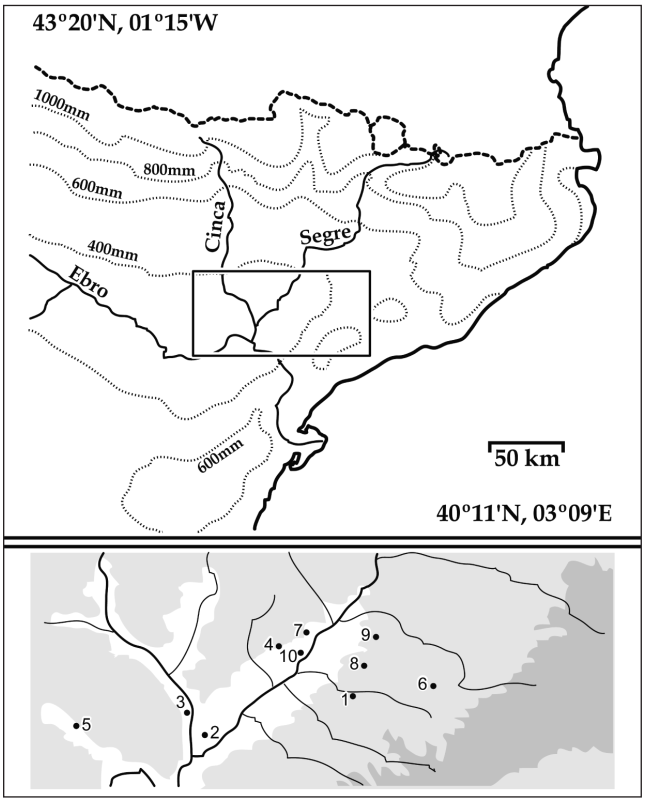

Figure 3. Geographic location of the archaeological sites. *Top*: isohyets of mean annual precipitation; *bottom*: altitude (metres above mean sea level).

4.1 Past Growing Conditions and the ^{13}C Signal in Ancient Crops

The $\delta^{13}C$ values of cereal grains varied considerably across sites/periods, ranging from -24.8 to -19.6‰ in barley, and from -24.4 to -20.4‰ in wheat. In both cases, the estimated range in $\Delta^{13}C$ (Eq. 2) fell within the calibration range of the models used to predict total water inputs (TWI) during grain filling [20], confirming that these $\Delta^{13}C$ values provide reliable TWI approximations. As an example, changes in $\Delta^{13}C$ values and estimated TWI over time are shown in Fig. 4 for barley. The comparison between estimated and current TWI during grain filling revealed a main phase of greater water availability in spring (500 BCE–500 CE) alternating with two drier periods (2000–500 BCE; 1000 CE to the present). Although less pronounced, a similar pattern of evolution through time was observed for $\Delta^{13}C$ ([22]; Fig. 5A) and estimated TWI values (Fig. 5B) in wheat. This consistency in the changes over time for both species further supports the validity of this palaeoenvironmental proxy. However, $\delta^{13}C$ values in cereal grains may be affected not only by climatic fluctuations but also by changes in cultural practices, like irrigation or planting in alluvial soils [5]. To assess anthropogenic-derived environmental changes, $\delta^{13}C$ data from different crop species, such as wheat and barley, grown in a common

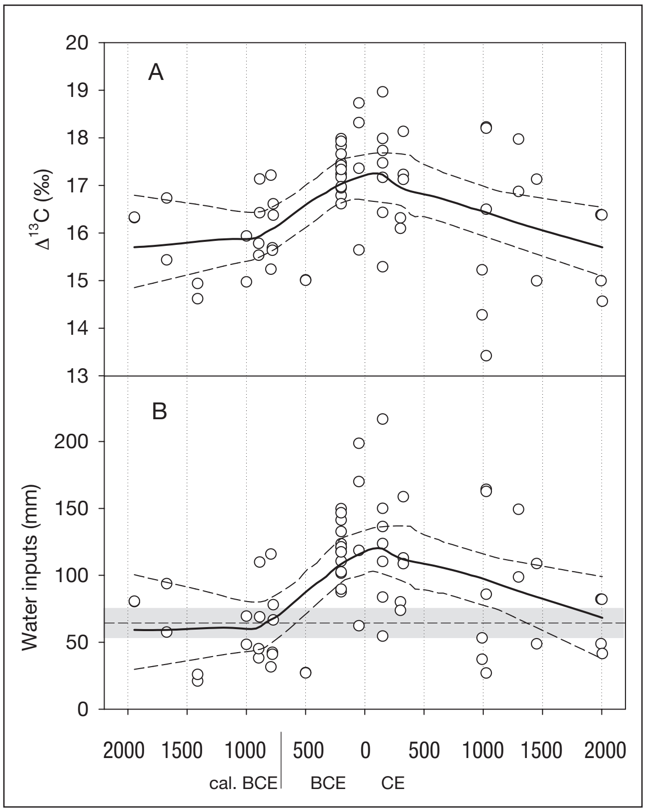

Figure 4. Evolution of (**A**) $\Delta^{13}C$ in charred grains and (**B**) estimated water inputs during grain filling of barley recovered in archaeological sites from the Mid-Ebro Basin (NE Spain) over the last four millennia. Modern material is also included for reference. Trend lines depict locally weighted least squares regression curves (LOESS, Cleveland 1979), together with their confidence intervals (95%, dashed lines), fitted to the data using a smoothing parameter (span=0.7) that minimised a bias-corrected Akaike statistic. The horizontal dashed *line* and darkened area in (**B**) indicate the current average precipitation value for the city of Lleida ±2SE. Drawn from original data in Ferrio et al. 2006.

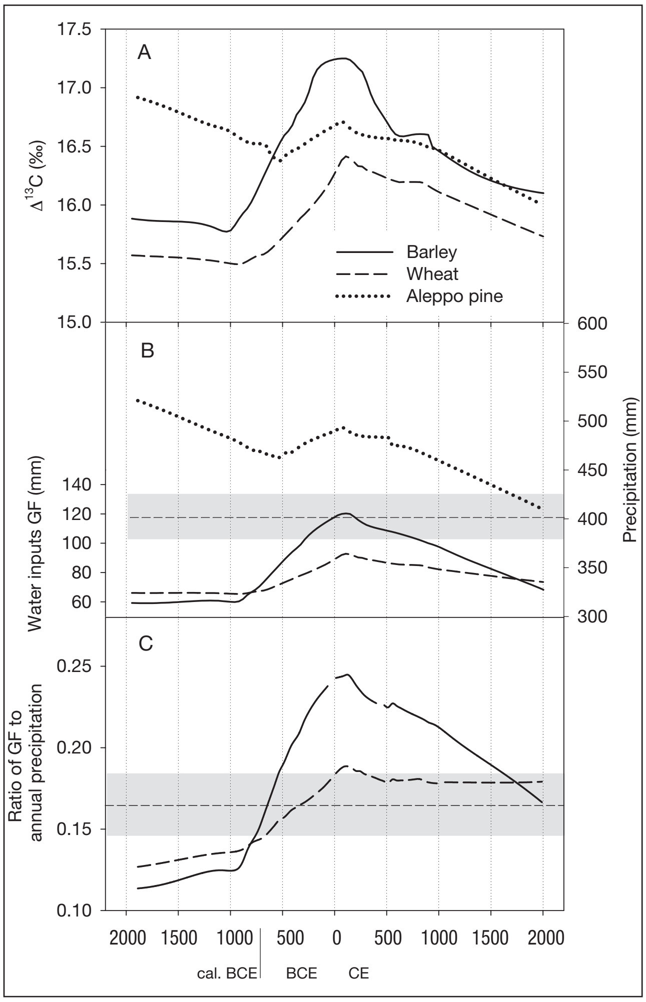

Figure 5. (**A**) Evolution of $\Delta^{13}C$ in charred grains of barley (solid line) and wheat (dashed line), and in wood charcoal of Aleppo pine (dotted line) recovered in archaeological sites from the Mid-Ebro Basin (NE Spain) over the last four millennia. (**B**) Estimated water inputs during grain filling of barley (solid line) and wheat (dashed line) (left y-axis), and estimated annual rainfall from Aleppo pine (dotted line) (right y-axis). The horizontal dashed line and darkened area indicate the current average annual rainfall for the city of Lleida city ±2SE. Drawn from original data in Ferrio et al. 2006ab. (**C**) Estimated ratio of rainfall during grain filling to annual rainfall using barley (solid line) or wheat (dashed line) as indicators of grain filling rainfall. The horizontal dashed line and darkened area indicate the current average ratio for Lleida ±2SE. Trend lines depict locally weighted least squares regression curves (LOESS, Cleveland 1979) fitted to the data using a smoothing parameter (span=0.7 for wheat and barley, span=0.5 for Aleppo pine) that minimised a bias-corrected Akaike statistic.

area can be combined (this approach is outlined in [20]). Alternatively, the $\delta^{13}C$ values of cultivated plants can be compared with those from wild species, for example forest trees, which are not directly affected by agricultural practices. This specific issue is discussed below.

4.2 Past Climate and ^{13}C Signal in Charcoal Wood Remains

Based on $\delta^{13}C$ data from wood charcoal of Aleppo pine (Fig. 2), annual precipitation estimates ranging from 257 to 698 mm (mean of 478 mm) were determined. These values are, on average, higher than those reported in recent meteorological records for the city of Lleida (mean value of 404 mm) during the period 1941–1986. Thus, throughout the study period (from ca. 2000 BCE to the 18th century), annual precipitation was consistently higher than is presently the case [21]. The present-day semi-arid conditions prevailing in this region therefore seem to be the result of a shift toward a harsher climate, most likely enhanced by recent anthropogenic disturbances (i.e. agriculture and other land-use practices). For example, a considerable decrease in precipitation in the NW Mediterranean after 1850 was reported, as shown by tree-ring analyses [12,33], lake-level records [43], and documentary studies [9].

The fitted trends over time are shown in Fig. 5A ($\Delta^{13}C$ values) and Fig. 5B (precipitation estimates). A gradual increase in aridity, starting about 4200 BP (the so-called 4.2 ka drought event), has been identified in numerous studies and seems to have taken place on a large scale in the Mediterranean, including the Near East [42], North Africa [34], and the Iberian Peninsula [36,14]. Our data suggest, however, that this increase did not develop uniformly. Indeed, two important phases of greater precipitation (1800–900 BCE; 300 BCE–300 CE) alternated with drier periods (900–300 BCE; 900 CE–present). Overall, these findings are supported by other palaeoenvironmental proxies, mostly derived from pollen and geomorphologic studies [14,43,25,31]. For example, the first humid phase coincided with the interphase between two aridification periods (2350–1450 BCE and 900 BCE–220 CE) as defined in [31] for the NW Mediterranean on the basis of pollen transects. Likewise, the humid phase between 300 BCE and 300 CE was also confirmed by pollen data from the city of Lleida (0–200 CE), which indicated a relatively wet climate (S. Riera, unpublished results). The most consistent arid phase within the timeframe of this study corresponded to the period 900–300 BCE (Fig. 5A, B). Nevertheless, even then, estimated water availability was significantly higher than in present-time records (about 24%) [21]. In this regard, pollen analyses performed at the archaeological site of El Vilot de Montagut [2] showed an increase in aridity at the beginning of the third millennium BP, which is in agreement with the low $\Delta^{13}C$ values found for this site. The evolution of $\Delta^{13}C$ in charcoal remains from other co-occurring species in the archaeological record, such as the Mastic tree (*Pistacia lentiscus* L.) and evergreen and deciduous oaks (*Quercus* spp.), is also consistent with the results obtained for Aleppo pine [23]. Interestingly, even the $\delta^{13}C$ values reported in the study of Vernet et al. [52] for archaeological sites from SE France showed a comparable trend [23]. Similar to the outcome of cereal remains and $\delta^{13}C$, these results substantiate the use of stable isotopes of carbon as a valuable tool for inferring the palaeoclimate.

4.3 Climate Seasonality and ^{13}C Signal in Different Archaeobotanical Remains

Earlier studies have shown that a direct comparison of $\delta^{13}C$ data from different plant remains is a fruitful approach to discriminate between climatic and anthropogenic causes of environmental fluctuations [20] and to investigate changes in climate seasonality through time [23]. The common trend in $\Delta^{13}C$ changes for wheat and barley, as outlined in Fig. 5A, seems to refute the adoption of irrigation technology for wheat cultivation, since barley is a more drought-adapted species and wheat is preferred for human consumption. Thus, the observed changes in cereal water inputs during mid-spring (Fig. 5B) can be attributed to climate. Combining this information with data on the dynamics of annual precipitation, as inferred from charcoal $\delta^{13}C$ (Fig. 5AB), should provide clues as to possible changes in rainfall seasonality, especially in relation to the variability in the spring rainfall peak typical of the Western Mediterranean Basin [35]. In particular, the Mid-Ebro Basin is presently characterised by a bimodal regime of rainfall seasonality, with peaks in autumn and in spring.

The ratio of mid-spring precipitation (second half of April and May), as inferred from wheat or barley, to annual precipitation, estimated for the last 4000 years, is shown in Fig. 5C. Compared to recent times (mean ratio of 0.165), the relative significance of spring rainfall in the past was variable. In particular, our results suggest that, from approximately 300 BCE until recent time, spring rainfall accounted for either a slightly higher (according to wheat) or clearly higher (according to barley) proportion of annual rainfall than is the case nowadays. Precipitation trends for cereals and the Aleppo pine during this period have followed essentially the same pattern (Fig. 5B), suggesting that fluctuations in annual rainfall have been mainly caused by concomitant changes in the contribution of spring rainfall. By contrast, during the period 1800–800 BCE the contribution of spring rainfall to annual rainfall was consistently less than is the case today (Fig. 5C). While mid-spring water inputs in ancient times were comparable to those measured in recent times, annual precipitation in the past was initially higher but then decreased (Fig. 5B). During the transition phase from about 800 to 300 BCE, there was still a decline in annual precipitation, but also a sudden recovery of mid-spring rainfall. In this regard, the gradual decrease in annual precipitation beginning in 1800 BCE and the associated changes in rainfall seasonality seem to mark the onset of the modern dry summer regime in the Mediterranean [46,36]. Indeed, it has been suggested that, before 2200 BCE, the climate was characterised by wet summers—very different than the present-day typical Mediterranean rainfall regime [30]. A gradual reduction in the amount of summer rainfall was most likely the main reason for the steady decrease in total precipitation from 1800 to 800 BCE. Henceforth, and according to our results, fluctuations in annual rainfall were probably driven mainly by changes in the contribution of the spring rainfall.

Meaningfully, the transition phase shown in Fig. 5C coincides with the 'Cold Iron Age Epoch' (ca. 900–300 BCE), which was characterised by an abruptly cooling climate in Europe [49]. According to charcoal $\delta^{13}C$ analyses, this period appears to have been an important arid phase in the Mid-Ebro Basin. The increase in aridity is confirmed by pollen data from archaeological sites in the area [2], as well as by hydrological studies from the NW Mediterranean [37]. Other palaeoenvironmental proxies, however, provide (apparently) contradictory evidence for this period. This evidence has been interpreted as supporting an increase in vegetation cover and a colder/wetter climate [25,14]. Are these findings compatible with current $\delta^{13}C$ evidence? The answer may come from examining the role of seasonality. According to Fig. 5C, the divergence between Aleppo pine and cereals can be regarded as an increase in spring precipitation along with a continuous decrease in summer rainfall starting about a millennium earlier. In this regard, a decline in summer precipitation may also explain the relative prevalence of evergreen oaks previously reported [31] for this period, as deciduous oaks are less adapted than evergreen oaks to summer drought. Similarly, a reduction in soil erosion might have arisen from the absence of summer convective storms [25], together with an increase in deep-rooted cold-resistant plant species. After this transition phase, the synchrony of $\delta^{13}C$ changes in charred grains and charcoal points to the installation of the current Mediterranean climate of seasonal rainfall and summer drought during the second half of the third millennium.

5 Concluding Remarks

In recent years, information derived from the analysis of carbon isotope signatures in different types of fossil plant remains has been extensively employed to reconstruct past climate and environmental conditions. However, studies using charred material are still scarce. Nowadays, archaeobotanists are becoming increasingly interested in these methods, and our review has attempted to show that this approach can provide reliable estimations of past precipitation regimes. A further step would consist of combining multiple isotopic approaches to solve specific archaeological uncertainties. For instance, we have shown that proper matching of the information on carbon isotopes from different species provides additional insight into the phenomenon under study. Oxygen isotopes can also be employed to determine the origin of source water in cultivated and wild plants [53], and nitrogen isotopes serve as valuable tools to assess nutrient management practices in ancient agriculture [10]. We are currently applying a multi-isotopic approach to characterise the climatic conditions of the Mid-Holocene by using sub-fossil (i.e. waterlogged) wood and using different crop remains to determine the sustainability of early Mediterranean agricultural systems.

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