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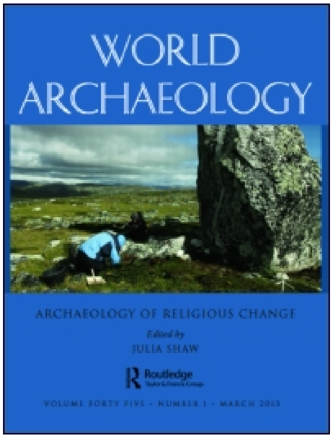

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Stable carbon isotope analysis as a direct means of inferring crop water status and water management practices

M. Wallace [a] , G. Jones [a] , M. Charles [b] , R. Fraser [b] , P. Halstead [a] , T.H.E. Heaton [c] & A. Bogaard [b]

[a] University of Sheffield

[b] University of Oxford

[c] NERC Isotope Geosciences Laboratory, British Geological Survey

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Stable carbon isotope analysis as a direct means of inferring crop water status and water management practices

M. Wallace, G. Jones, M. Charles, R. Fraser, P. Halstead, T.H.E. Heaton and A. Bogaard

Abstract

Stable carbon isotope analysis of plant remains is a promising tool for researchers studying palaeoclimate and past agricultural systems. The potential of the technique is clear: it offers a direct measure of the water conditions in which plants grew. In this paper, we assess how reliably stable carbon isotope discrimination can be used to infer water conditions, through the analysis of present-day crop plants grown at multiple locations across the Mediterranean and south-west Asia. The key findings are that: (1) $\Delta^{13}C$, as expected, provides an indication of water conditions, (2) even for plants grown in similar conditions there is variation in $\Delta^{13}C$ and (3) $\Delta^{13}C$ may reflect crop water status for a period beginning well before the grain filling period. A new framework is presented which increases the robustness with which $\Delta^{13}C$ values of plant remains can be interpreted in terms of the water conditions in which ancient crops grew.

Keywords

Carbon isotopes; Archaeobotany; Experimental archaeology; Cereals; Pulses; Water; Rainfall; Irrigation.

Introduction

The Fertile Crescent was the setting for the emergence of agriculture in south-west Asia, yet much of the region exists within arid or semi-arid climate zones and experiences sparse and unreliable rainfall. Although past climatic conditions differed from those of today, these areas are likely to have been water-limited environments throughout the Holocene. Consequently, the management

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of surface water resources has been a focus of archaeologists studying the ancient agriculture of the region (Butzer 1996; Charles 1988; Davies 2009; Mithen 2010). Traditionally, information on ancient crop watering has been derived from *indirect* lines of evidence, such as irrigation infrastructure (Bruins 1990; Clement and Moseley 1991; Farrington 1980; Kirchner 2009) and the ecology of weed species found in unprocessed harvests or processing by-products (Charles and Hoppé 2003; Charles et al. 2003; Jones et al. 1995). Such evidence is useful, but often does not enable water conditions to be inferred for specific crops, preventing investigation of, for example, the differential treatment of crop species or the importation of crops grown elsewhere.

In recent years, greater emphasis has been placed on *direct* means of inferring water conditions from archaeological crop remains themselves, through phytolith (Jenkins, Jamjoun and Al Nuimat 2011; Madella et al. 2009; Mithen et al. 2008; Piperno 2006; Rosen and Weiner 1994) and stable carbon isotope analysis (Araus and Buxó 1993; Araus et al., 'Changes' 1997a; Araus et al., 'Identification' 1997b; Araus, Febrero et al. 1999; Araus, Ferrio et al. 2007; Ferrio et al. 2005; Fiorentino, Caracuta, Calcagnile et al. 2008; Fiorentino, Caracuta, Casiello et al. 2012; Flohr, Mülder and Jenkins 2011; Heaton et al. 2009; Riehl 2008; Riehl, Bryson and Pustovoytov 2008; Stokes, Mülder and Jenkins 2011; Voltas, Ferrio et al. 2008). Isotopic investigations have tended to interpret results in relative terms, for example using stable carbon isotopes to determine whether certain time periods were wetter or drier than others. The level of moisture availability in absolute terms has rarely been considered. In addition, it is only through the study of present-day crops, grown under known conditions, that the relationship between plant carbon isotopes, specific water conditions and water management practices can be explored. To date, such present-day studies have been conducted for only a handful of crop species, grown in few locations, under highly controlled experimental conditions (Araus et al., 'Identification' 1997b; Araus, Febrero et al. 1999; Araus, Villegas et al. 2003; Fiorentino, Caracuta, Casiello et al. 2012; Flohr, Mülder and Jenkins 2011; Stokes, Mülder and Jenkins 2011).

While controlled experimental conditions allow the precise relationship between water availability and stable carbon isotopes to be established, they do not necessarily reflect the effect of water *management practices* (e.g. irrigation) on stable carbon isotopes, which is an issue of particular interest in archaeology. In order to cover the relationship of both water input and water management practices with crop isotopes, we have undertaken two types of investigation. First, controlled experiments were conducted to explore the relationship between crop stable isotopes and water availability for two crops, complementing work published by others and allowing us to address specific questions of archaeological relevance such as the period of growth during which isotopic values are determined. Second, crop samples were collected from working farms where crops were grown under both irrigation and dry-farming regimes, to establish how reliably water management practices can be inferred from $\Delta^{13}C$, given the environmental and biological background 'noise' inherent in the cultivated field and the inevitable variations encountered in 'real-life' farming conditions. This article presents the findings of these investigations, and evaluates the utility and reliability of the stable carbon isotope technique in archaeological research relating to crop water status and water management practices. It forms part of a broader project investigating the use of crop stable isotope analysis to provide a better understanding of past cultivation practices (Bogaard et al. 2007; Fraser, Bogaard et al. 2011; Fraser, Styring et al. in press; Styring et al. in press; Styring, Sealy and Evershed 2010).

Carbon stable isotopes in plants

Plants assimilate carbon from atmospheric carbon dioxide (CO_2) where the lighter carbon isotope (^{12}C) predominates over the heavier isotope (^{13}C). During photosynthesis the heavier ^{13}C isotope is discriminated against, and so plant structural carbon is ^{13}C-depleted relative to atmospheric carbon dioxide (Ehleringer, Hall and Farquhar 1993; Farquhar and Richards 1984). The ratio of ^{13}C to ^{12}C is usually expressed as $\delta^{13}C$ (Farquhar, O'Leary and Berry 1982). The $\delta^{13}C$ value of plant structural carbon reflects ^{13}C discrimination during carbon fixation (primarily determined by photosynthetic pathway of a species: C_3, C_4 or CAM) and the $\delta^{13}C$ value of CO_2 assimilated by the plant. As the $\delta^{13}C$ of atmospheric CO_2 ($\delta^{13}C_{air}$) has changed over time, this needs to be taken into account when comparing the $^{13}C/^{12}C$ ratio for plants grown in different time periods. Discrimination during photosynthesis, independent of source CO_2, is usually denoted by $\Delta^{13}C$ and calculated as follows (Farquhar, O'Leary and Berry 1982):

$$\Delta^{13}C = \frac{\delta^{13}C_{air} - \delta^{13}C_{plant}}{1 + \delta^{13}C_{plant}}$$

The data presented in this paper are for modern crops only, and so calculations utilize the global average $\delta^{13}C_{air}$ of −8‰ (Tans 2011). Results are presented as $\Delta^{13}C$, however, to enable direct comparison with archaeological data.

Within the C_3 photosynthetic pathway, the degree of ^{13}C discrimination during photosynthesis is closely linked to the opening and closing of stomata. Open stomata, which permit the escape of water (cooling the plant and facilitating the movement of nutrients), ensure free assimilation of CO_2, such that discrimination against ^{13}C is at its maximum. Closed stomata, which conserve water, restrict the availability of CO_2 and so ^{13}C discrimination is at its minimum. Since one of the major determinants of stomatal conductance is water availability, $\Delta^{13}C$ serves as a proxy for water availability (Ehleringer, Hall and Farquhar 1993; Farquhar, Ehleringer and Hubick 1989; Farquhar, O'Leary and Berry 1982; Farquhar and Richards 1984; Stewart et al. 1995).

The degree of discrimnation against ^{13}C varies between plant species, and so a model for interpreting $\Delta^{13}C$ in one species may not apply to another. $\Delta^{13}C$ has also been shown to vary between species genotypes, although variation is often small, especially between landraces (Araus, Ferrio et al. 2007; Condon, Farquhar and Richards 1990). Different plant parts have inherently different $\Delta^{13}C$ values owing to their different biochemical compositions, with more complex compounds having higher $\Delta^{13}C$ values (Condon, Richards and Farquhar 1992; Hillaire-Marcel 1986). Grains, for example, which are mainly comprised of simple biochemical compounds (e.g. starch), usually have a lower $\Delta^{13}C$ than other plant parts (Araus et al. 1992; Merah, Deléens and Monneveux 2002).

Photosynthesis and carbon fixation are complex biological processes, and the degree of ^{13}C discrimination is influenced by a myriad of other environmental and biological factors as well as water availability. Water availability itself is determined by various forms of water input (rainfall, ground-water flow, anthropogenic watering, etc.) and also by water losses (evaporation and evapotranspiration, in turn affected by temperature, humidity, wind speed, etc.). The water-holding properties of soil also influence the amount of available water. Temperature (Heaton 1999; Hillaire-Marcel 1986; Tieszen 1991; Troughton and Card 1975) and light intensity (Broadmeadow and Griffiths 1993; Heaton 1999; Hidy et al. 2009; Smith, Oliver and McMillan 1976) influence $\delta^{13}C$, either directly or through their effect on evaporation rate. Soil nutrients and the alteration of soil fertility through the application of chemical fertilizer can also result in changes in isotopic discrimination (Condon, Richards and Farquhar 1992; Heaton 1999; Tieszen 1991). In dry regions of the world, however, where water is the main limiting factor on photosynthesis, water availability is the primary determinant of stomatal activity during periods of carbon fixation, and therefore the primary determinant of $\Delta^{13}C$.

Materials and methods

Experimental crop trials were undertaken at Tal Jebbin, 17km north of Aleppo, Syria. Bread wheat (*Triticum aestivum*) and lentil (*Lens culinaris*) were grown in separate 2m by 5m plots. Wheat was sown in November and harvested in early June. Lentils were sown in December and harvested in mid-May.

Three levels of irrigation were applied to both manured (20t/ha of manure from penned sheep) and unmanured plots of each crop (Table 1), with three replicates per combination of irrigation and manuring (a total of eighteen plots per crop). Plots were irrigated by flood irrigation. Three levels of irrigation were defined by the number of irrigations between sowing and crop maturity (Table 1). The timing of irrigations within each level was based on weather conditions, to avoid over-watering. The experiment was first carried out in 2007/8 and replicated in 2008/9.

The controlled Syrian experiments were complemented by collections of crop material from present-day farms, where both flood-irrigated and rain-fed cultivation were practised. Three study areas were chosen (all of which have been the subject of research on the relationship between crop-growing conditions and weed ecology): the Borja area of northern Spain (Charles, Jones and Hodgson 1997; Jones et al. 1995), the area around the village of Tharounia on the Greek island of Evvia (Jones 2005; Jones et al. 1999), and the area in and around Wadi Ibn Hammâd in Jordan (Charles and Hoppé 2003; Charles et al. 2003). Records of crop treatment were compiled through interviews with local cultivators and irrigation supervisors to enable a comparison between water availability and $\Delta^{13}C$ values (Table 1), but variability in water conditions is expected, given that multiple farmers were involved, and fields were spread over large areas.

Free threshing wheat (*Triticum aestivum* and *T. durum*) and two-row barley (*Hordeum vulgare* var. *distichum*) at Borja were sown in November and harvested in late June. Some chemical fertilizers were applied at Borja, with unirrigated fields tending to receive less nitrogen-rich fertilizers. On Evvia, broad beans (*Vicia faba*) were sown in small fields or garden plots in October or November and harvested green in May or dry in June. Some of the garden plots received stall manure and most received dung from livestock penned on previous stubble or sown pasture. At Wadi Ibn Hammâd the wheat (*Triticum aestivum* and *T. durum*) growing period was December to early June and organic farming was practised.

At Borja, unirrigated fields (usually under biennial 'bare' fallow) were located just above moderately irrigated fields on the valley side. Fully irrigated fields (usually under continuous cultivation) were located on the lower slopes and valley floor. Moderately irrigated fields received water subject to availability, and fully irrigated fields as a matter of course. On Evvia, garden plots were hand watered, as deemed appropriate by individual cultivators, and fields were unwatered. At Wadi Ibn Hammâd, wheat crops were grown either along the wadi (irrigated and unirrigated) or on the plateau above (unirrigated), the former receiving some additional water from surface flow after heavy rain. Along the wadi, fields benefited from

Table 1 Irrigation regimes practised at the study sites

Regime	*Irrigations*	*Irrigation amount (and method)*
Aleppo, Syria, 2007/8, wheat		
No irrigation	–	–
Moderate irrigation	1x in November, March and May	110–160mm (flooded from pump)
Full irrigation	1x in November, January, March and April, 2x in May	110–160mm (flooded from pump)
Aleppo, Syria, 2008/9, wheat		
No irrigation	–	–
Moderate irrigation	1x in November, April and May	110–160mm (flooded from pump)
Full irrigation	1x November and April, 3x in May	110–160mm (flooded from pump)
Aleppo, Syria, 2007/8, lentil		
No irrigation	–	–
Moderate irrigation	1x in March	90–95mm (flooded from pump)
Full irrigation	1x in March, April and May	90–95mm (flooded from pump)
Aleppo, Syria, 2087/9, lentil		
No irrigation	–	–
Moderate irrigation	1x in April	90–95mm (flooded from pump)
Full irrigation	1x in April, 2x in May	90–95mm (flooded from pump)
Borja, Spain, 2006/7 and 2007/8, wheat and barley		
No irrigation	–	–
Moderate irrigation	Up to 1x in March and May	Subject to availability (flooded from earthen channels)
Full irrigation	At least 1x in March and May	To field saturation (flooded from earthen channels)
Evvia, Greece, 2006/7, broad bean		
Unwatered	–	–
Watered	Throughout growth period as decided by cultivators	Varied (hand watering)
Wadi Ibn Hammâd, Jordan, 2006/7, wheat		
No irrigation (plateau)	–	–
No irrigation (wadi)	None but surface flow through wadi	–
Moderate irrigation (wadi)	None during wheat growth but prior vegetables irrigation	–
Full irrigation (wadi)	As above, plus throughout growth period as decided by cultivators	Varied (flooded from earthen channels)

intensive watering of vegetables grown there immediately prior to sowing of wheat. Irrigated fields were flooded during the cereal growing season, between December and early June.

Rainfall, temperature and, where available, humidity and wind speed data were collected for each site from the closest weather station(s) of comparable elevation and with good data (see

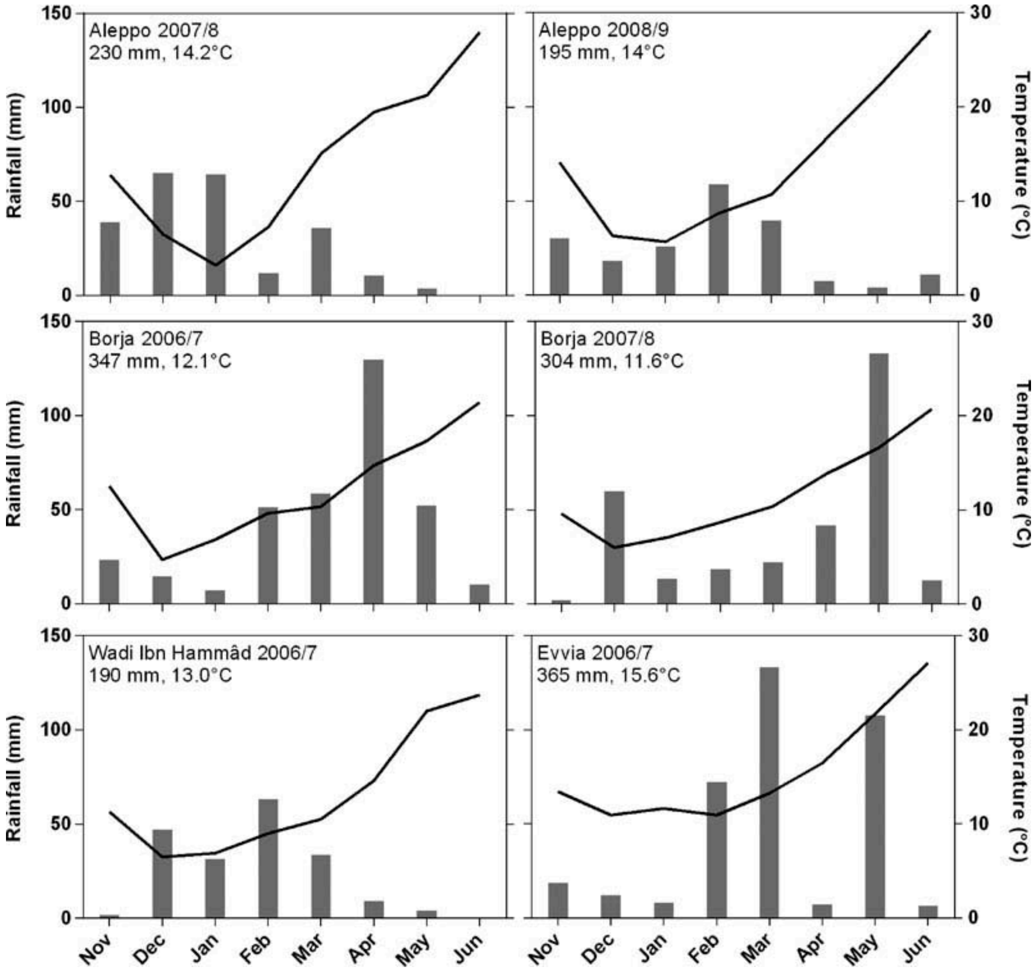

Figure 1 Monthly total rainfall (grey bars) and mean temperature (black line) for each of the study sites for the crop-growing season, with the total rainfall and mean temperature stated for the period shown. For sources, see the Appendix.

Appendix). Rainfall and temperature records are depicted in Figure 1. In the case of the Aleppo experiments, potential monthly evapotranspiration (based on the Penman-Monteith equation:, Allen, Pereira and Smith 1998) and an aridity index (rainfall amount + irrigation amount / potential evapotranspiration, modified after Middleton and Thomas 1997) were calculated.

For both experimental trials and farm collections, crops were sampled immediately prior to harvest. Crop material from each field in the farm studies was collected at six approximately evenly spaced points along a diagonal transect. Collected crop plants were dehusked or shelled, and twenty-five to fifty grains randomly selected from each plot or field were combined, as were five to ten complete rachises for the cereals. Fresh material was homogenized under liquid nitrogen using a Spex 6850 freezer mill. Between 0.75 and 1.50 mg of the resulting powder was then weighed into tin capsules. Isotopic analyses were performed at the NERC Isotope Geosciences Laboratory at Keyworth (UK) by combustion in a Costech 4010 elemental analyser on-line to a VG TripleTrap and Optima dual-inlet mass spectrometer. $\delta^{13}C_{plant}$ values were calculated to the VPDB scale by comparison to a within-run laboratory standard plant material calibrated against NBS-19 and NBS-22, with analytical precision better than <±0.1‰ (1 SD). Differences in $\Delta^{13}C$ between irrigation regimes (and manuring levels) were tested for their significance using one-way ANOVA with Bonferroni *post hoc* analysis to compare pairs of regimes.

Results

Wheat and lentil grown in experimental trials

The results of the two experimental trials in consecutive years at Aleppo, Syria, are summarized in Tables 2 and 3. In both years, higher $\Delta^{13}C$ values are usually associated with greater water input from irrigation, though the ranges of $\Delta^{13}C$ values for different irrigation regimes overlap.

Though not statistically significant at the 0.05 level, grain from wheat grown in 2007/8 in fully irrigated plots has a mean $\Delta^{13}C$ value around 1‰ higher than that of moderately irrigated and unirrigated wheat grown in the same year. The difference in $\Delta^{13}C$ between grain of moderately irrigated and unirrigated wheat is negligible. Among the wheat grain grown in the following year (2008/9), there are no significant differences between the mean $\Delta^{13}C$ for each regime. Overall, the wheat grain $\Delta^{13}C$ values for 2008/9 are similar to those for grain from the fully irrigated plots of 2007/8, when there was less rainfall and greater potential evapotranspiration from January onwards (Fig. 1). Also, the winter peaks in rainfall occurred in December/January in 2007/8 and in February in 2008/9, which would have resulted in a greater quantity of soil moisture being carried into the later periods of the 2008/9 growth period, when rainfall was low.

Lentil seeds from the fully irrigated plots of 2007/8 have a mean $\Delta^{13}C$ value around 1.4‰ higher than that of moderately irrigated and unirrigated lentils, and the differences are significant. As is the case for wheat grain from the same year, the difference in $\Delta^{13}C$ between moderately irrigated and unirrigated lentils is not significant. The lentil results for 2008/9 follow a similar pattern to those for 2007/8, but at higher $\Delta^{13}C$ values, and with a greater difference in mean $\Delta^{13}C$ between each regime. Fully irrigated lentils have $\Delta^{13}C$ values around 2‰ greater than those that were moderately irrigated, and around 2.6‰ greater than those that were unirrigated. Both of these differences are significant but that between the two lower levels is not. So, whereas the moister conditions in 2008/9 appear to have brought wheat grain $\Delta^{13}C$ to a maximum (with irrigation resulting in no further $\Delta^{13}C$ increase), irrigation of lentils in 2008/9 continued to result in $\Delta^{13}C$ increases.

By plotting the $\Delta^{13}C$ results against the aridity index, we can take account of differences in weather to compare lentil and wheat results purely in terms of water availability. Aridity indices were calculated, for both wheat and lentil, for a series of periods incrementally decreasing by one month, the first beginning in November, when wheat was sown, but all ending in May, just before harvest (Table 4). For each period and crop, Pearson correlation coefficients were calculated for the relationship of $\Delta^{13}C$ with aridity index. Correlations were stronger and more significant for periods starting from January onwards than for those starting earlier (Table 4). The strongest correlations were for February to May and April to May. These relationships are plotted in Figure 2, showing that, for both periods, the effect of aridity on $\Delta^{13}C$ is greater for lentil than for wheat.

Table 2 Δ¹³C results for present-day crops

	Grain, Δ¹³C			*Rachis, Δ¹³C*		
	Number of fields/ plots	*Mean (‰)*	*S.D. (‰)*	*Number of fields/ plots*	*Mean (‰)*	*S.D. (‰)*
Aleppo, Syria – wheat						
2007/8						
No irrigation	6	16.72	0.48	6	18.85	0.31
Moderate irrigation	6	16.66	0.69	6	19.02	0.62
Full irrigation	6	17.50	0.43	6	19.36	0.38
2008/9						
No irrigation	6	17.20	0.34	6	18.90	0.27
Moderate irrigation	6	17.56	0.41	6	18.80	0.48
Full irrigation	6	17.40	0.35	6	18.97	0.65
Aleppo, Syria – lentil						
2007/8						
No irrigation	6	14.79	0.79	0		
Moderate irrigation	6	14.88	0.43	0		
Full irrigation	6	16.28	0.25	0		
2008/9						
No irrigation	6	16.04	0.33	0		
Moderate irrigation	6	16.62	0.57	0		
Full irrigation	6	18.63	0.48	0		
Borja, Spain – wheat						
2006/7						
No irrigation	18	16.99	0.62	13	19.13	0.35
Moderate irrigation	18	16.36	0.54	10	18.28	0.43
Full irrigation	6	17.81	0.81	5	19.12	0.55
2007/8						
No irrigation	5	16.80	0.79	5	18.56	0.70
Moderate irrigation	3	16.13	0.27	3	17.91	0.29
Full irrigation	3	18.14	0.56	3	19.85	0.63
Borja, Spain – barley						
2006/7						
Moderate irrigation	1	18.43		1	19.93	
Full irrigation	6	18.80	0.56	6	20.61	0.25
2007/8						
No irrigation	2	18.88	0.20	2	20.34	0.47
Moderate irrigation	5	18.65	0.45	5	18.87	0.94
Full irrigation	3	19.57	1.01	3	19.64	0.80
Wadi Ibn Hammâd, Jordan – wheat						
2006/7						
No irrigation (plateau)	2	14.94	0.03	2	17.21	0.84
No irrigation (wadi)	2	15.30	0.54	2	18.01	0.58
Moderate irrigation (wadi)	3	15.53	1.09	3	18.21	1.24
Full irrigation (wadi)	3	16.33	0.85	3	18.54	0.37
Evvia, Greece – broad bean						
2006/7						
Unwatered	10	16.08	1.08	0		
Watered	6	17.70	1.50	0		

Table 3 One-way analysis of variance (ANOVA) of the differences between Δ^{13}C for different irrigation regimes, with Bonferroni *post hoc* analysis of paired irrigation regimes

				Bonferroni post hoc analysis		
		One-way ANOVA		*Unirrigated vs. partially irrigated*	*Partially vs. fully irrigated*	*Unirrigated vs. fully irrigated*
Taxon	*Year*	*F*	*p*	*p*	*p*	*p*
Aleppo, Syria						
Wheat	2007/8	4.608	0.015*	>0.999	0.068	0.103
Wheat	2008/9	1.713	0.202	0.706	>0.999	>0.999
Lentil	2007/8	8.934	<0.001*	>0.999	0.012*	0.005*
Lentil	2008/9	36.426	<0.001*	0.292	<0.001*	<0.001*
Borja, Spain						
Wheat	2006/7	13.566	<0.001*	0.011*	<0.001*	0.022*
Wheat	2007/8	7.666	0.014*	0.575	0.015*	0.065
Barley	2007/8	1.738	0.244	>0.999	0.320	0.899
Evvia, Greece						
Broad bean	2006/7	6.776	0.020*	0.020*	n/a	n/a

* = significant at the 0.05 level.

Table 4 Pearson's correlation coefficients of Δ^{13}C with aridity index calculated for a range of time periods

	Wheat grain		*Lentil seed*	
Month range	*r*	*Non-zero slope, p*	*r*	*Non-zero slope, p*
Nov. – May	0.29	0.084	0.20	0.249
Dec. – May	0.26	0.133	0.24	0.169
Jan. – May	0.42	0.011	0.75	<0.001
Feb. – May	0.50	0.002	0.86	<0.001
Mar. – May	0.38	0.021	0.63	<0.001
Apr. – May	0.46	0.005	0.87	<0.001
May – May	0.34	0.043	0.79	<0.001

Although Δ^{13}C is unlikely to be useful for the detection of manuring, this experiment provided the opportunity to assess the effect of manure on Δ^{13}C. For both crops, the effect of manuring on Δ^{13}C is inconsistent in both direction and magnitude, such that the difference in mean Δ^{13}C between grain from manured and unmanured plots under the same irrigation regime is 0.01‰ (see Appendix). In some cases, however, the difference in Δ^{13}C between manuring regimes can be around ±0.6‰. Manuring should thus be considered a potential source of noise in the archaeobotanical Δ^{13}C record.

The Δ^{13}C values of wheat rachis are always higher than those of wheat grain from the same plot (Table 2). This grain-rachis difference is slightly greater for 2007/8 (mean = 2.1‰, standard deviation = 0.4‰) than for 2008/9 (mean = 1.5‰, standard deviation = 0.5‰). The reason for the slightly smaller difference in 2008/9 is not clear, but it seems that, when grain reaches high Δ^{13}C values (above c.17.5‰), the difference between rachis and grain Δ^{13}C values is less.

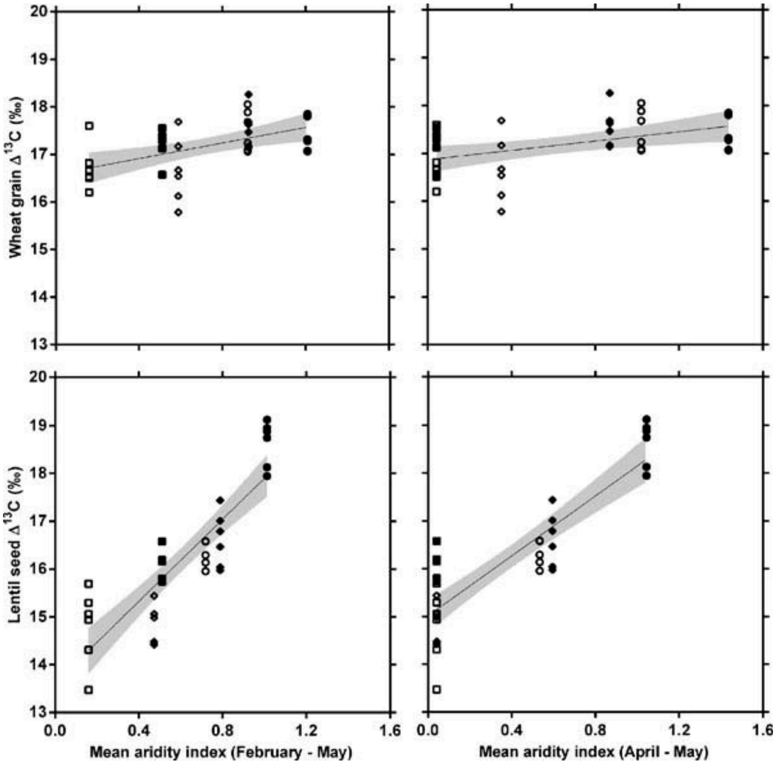

Figure 2 Plots of $\Delta^{13}C$ for wheat grain and lentil seed against mean aridity index, with linear regression lines; grey area denotes 95% confidence band. Open symbols = 2007/8, filled symbols = 2008/9; □■ = no irrigation, ◊♦ = moderate irrigation, ○●= full irrigation.

Wheat, barley and broad beans from farm collections

The $\Delta^{13}C$ values of crops collected from present-day farms are summarized in Tables 2 and 3, and Figure 3. As might be expected, the variation in $\Delta^{13}C$ within irrigation regimes is often greater in the farm collections than in the controlled experiments, which probably reflects variation in both natural water availability and crop husbandry practices.

The majority of samples derive from farms in the Borja region of Spain, where cereals were sampled in 2007 and 2008. In both years, wheat grain from fully irrigated fields has a mean $\Delta^{13}C$ value significantly higher than that of grain from moderately irrigated fields, but grain from unirrigated wheat also has a higher mean $\Delta^{13}C$ than that of moderately irrigated wheat. The $\Delta^{13}C$ values of rachis are on average 1.9‰ (standard deviation = 0.6‰) higher than those of grain from the same field. The relatively high mean $\Delta^{13}C$ value for unirrigated wheat was unexpected, especially as the unirrigated and moderately irrigated fields were within 2km of one another, and therefore subject to similar weather conditions. At Borja, however, unirrigated crops received less nitrogen-rich fertilizer than irrigated plots. Nitrogen stress can increase $\Delta^{13}C$ but usually by less than 0.5‰ (Clay et al. 2001; Condon, Richards and Farquhar 1992; Livingston et al. 1999; Serret et al. 1992), and so is insufficient to account for the lower values for moderately irrigated cereals. Other contributory factors may be the variable terrain (and

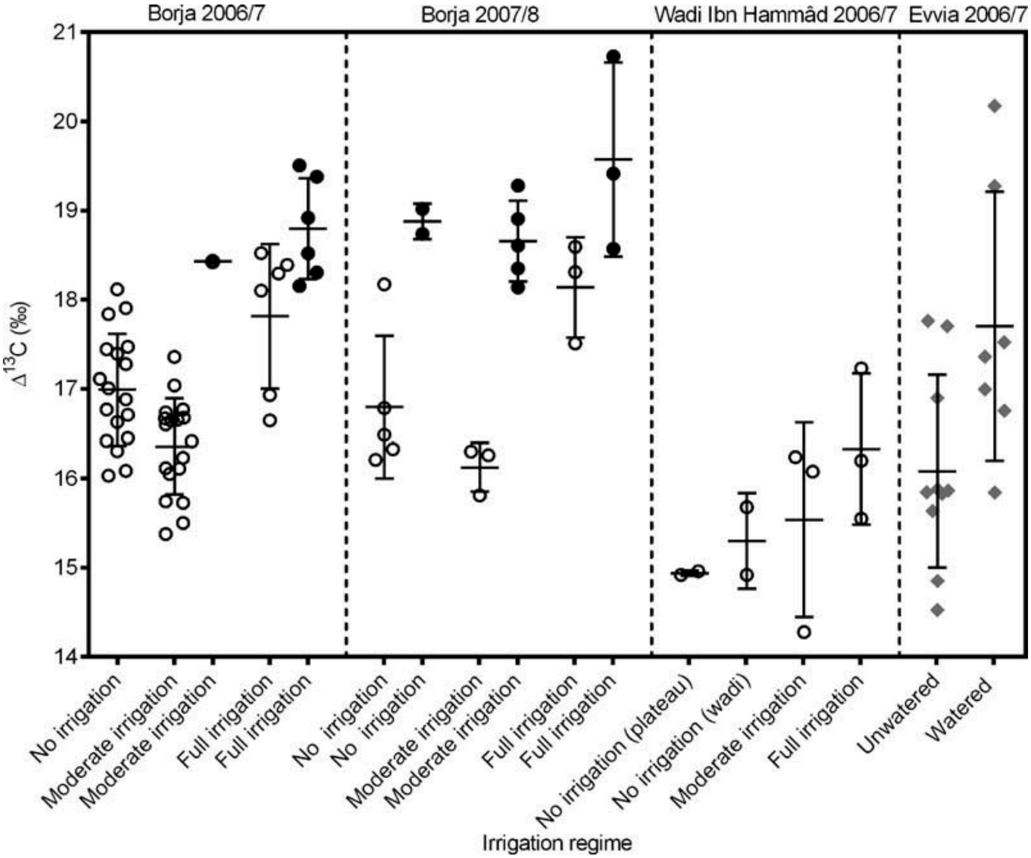

Figure 3 Plot of Δ^{13}C values of wheat grain (○), barley grain (●) and broad beans (♦) from the farm collections; bars and whiskers denote means and standard deviations.

hence drainage) or management practices (e.g. biennial bare fallow enhancing infiltration of rainfall of unirrigated fields).

Barley grain from Borja has Δ^{13}C values typically 1–2‰ higher than those of wheat grain grown under the same irrigation regime (Table 2 and Fig. 3). This difference in Δ^{13}C between wheat and barley has been reported elsewhere, and is usually explained by barley ceasing photosynthesis earlier than wheat, thus avoiding the driest parts of summer (Araus et al., ‘Identification’ 1997b, 730). With the exception of irrigated barley from 2007/8, for which grain and rachis Δ^{13}C values are very similar, the Δ^{13}C of barley rachis is on average 1.7% (standard deviation = 0.4‰) higher than that of grain from the same field. Rainfall in the 2006/7 growing season was 100mm higher than in 2007/8 (Fig. 1) but this is not reflected in the Δ^{13}C results. This difference occurred very early in the growth cycle (September–October), however, which may have been too early to affect the Δ^{13}C results, particularly as the rainfall in both years was high, and probably enough on its own to account for the high Δ^{13}C values.

At Wadi Ibn Hammâd the greater the water availability the greater the mean Δ^{13}C: fully irrigated wheat exhibits higher values than moderately irrigated wheat, while unirrigated wheat in the wadi, that benefits from run-off, has higher values than that on the plateau. It is notable, however, that the Δ^{13}C values for all wheat grain, even that grown with irrigation, are considerably lower than those for Aleppo and Borja. The Wadi Ibn Hammâd region experienced widespread drought-induced crop failure in the year these samples were taken (2006/7), and so they can be considered representative of crops grown under very poor water conditions. The Δ^{13}C offset between grain and rachis from the same fields is, on average, 2.5‰ (standard deviation = 0.6‰). This offset is larger than that at other sites, perhaps indicating that the offset between rachis and grain tends to be greater when water availability is low.

On Evvia the Δ^{13}C values of the seeds from watered plots are on average higher than those from plots receiving no additional water, with mean values comparable to the Borja fully irrigated and partially irrigated wheat, respectively. The Δ^{13}C values are particularly variable for both regimes, with standard deviations greater than those for crops in the other farm collections. The small-scale arable practices used on Evvia were less standardized, particularly for the intensively cultivated gardens, than for the large-scale agriculture practised at Borja (or the controlled conditions at Aleppo). This variation in the practices of individual farmers may well explain the high degree of Δ^{13}C variation in the Evvia broad beans, and highlights the levels of variation that might be expected in a traditional farming system.

Discussion

Our experimental and agricultural findings show that Δ^{13}C is positively correlated with water availability, in line with theoretical expectations that stable carbon isotope ratios are a reflection of stomatal activity. These results are consistent with previous work (e.g. Araus, Amaro et al. 1997; Araus et al., 'Changes' 1997a; Araus, Febrero et al. 2003; Craufurd et al. 1991; Flohr, Mülder and Jenkins 2011; Merah, Deléens and Monneveux 1999; Monneveux et al. 2006; Stokes, Mülder and Jenkins 2011; Voltas, Romagosa et al. 1999) summarized in Table 5 (and the Appendix). Several key points emerge: (a) Δ^{13}C values for wheat grain grown in conditions where water is not a limiting factor are typically above 17‰; (b) wheat grown under very dry conditions has grain Δ^{13}C values of 14–16‰; (c) the Δ^{13}C values for barley grain are at least 1‰ higher than those for wheat grown under comparable water conditions, though this offset may be another *c*. 1‰ higher for the more water-demanding six-row variety due to greater use of reserve carbohydrates (Anyia et al. 2007; Jiang, Roche and Hole et al. 2006; Voltas, Romagosa et al. 1999); and (d) Δ^{13}C values for rachis are *c*. 2‰ higher those for grain, at least for wheat for which we have sufficient data (Fig. 4).

In the farm studies, however, irrigation regimes that are recognized as distinct by farmers cannot always be differentiated using Δ^{13}C and, even in the controlled conditions of the Aleppo experiments, crops under the same water management regime exhibit a range of Δ^{13}C values, which can be as much as 1‰. The reasons for this are two-fold. First, as Δ^{13}C reflects all aspects of water availability, the effects of irrigation on Δ^{13}C can be masked by those of natural water sources. Second, there is considerable variation in the Δ^{13}C of plants grown in similar conditions, and this variation reduces our ability to detect distinct levels of water availability. Such variation in Δ^{13}C values may be, at least in part, due to differences in soil water availability at individual fields. This may be due to terrain and soil properties. The inference of water management practices can also be made more difficult by year-to-year climatic variation, such as that seen in the Aleppo experiments, or variation in the agricultural practices, such as those on Evvia. Undoubtedly other environmental

Table 5 Summary of mean Δ^{13}C values and mean standard deviation (or range, denoted by *) for present-day crops grown in outdoor experiments reported by other researchers

Location	*Rainfall*	*Irrigation*	*Mean $\Delta^{13}C$ (‰)*	*Mean $\Delta^{13}C$ S.D. (‰)*
Triticum durum (expected $\Delta^{13}C$: poorly watered <16‰; well-watered >17‰)				
Stokes et al. (2011)				
E Jordan	Low	Unirrigated/partially irrigated	13.6	0.6
E Jordan	Low	Irrigated	15.0	0.2
E Jordan	Medium	Unirrigated/partially irrigated	17.9	0.5
E Jordan	Medium	Irrigated	19.7	0.3
NE Jordan	Medium	Unirrigated/partially irrigated	15.7	1.1
NE Jordan	Medium	Irrigated	17.7	0.9
Araus, Amaro et al. (1997)				
N Syria	Low	Unirrigated	14.0	0.4
NW Syria	Medium	Unirrigated	15.3	0.4
NW Syria	Medium	Irrigated	16.7	0.5
Araus, Villegas et al. (2003)				
SE Spain	Low	Unirrigated	15.3	
NE Spain	Low–Medium	Unirrigated	15.4	
SE Spain	Low–Medium	Irrigated	16.9	
NE Spain	Medium	Irrigated	17.9	
Merah et al. (1999)				
S France	Medium	Unirrigated	15.4	0.7
S France	High	Unirrigated	16.6	0.7
S France	Very high	Unirrigated	18.0	0.7
Monneveux et al. (2006)				
S France	Medium	Unirrigated	15.4	
S France	Medium	Irrigated	16.9	
Hordeum vulgare (expected $\Delta^{13}C$: poorly watered <17.5‰; well-watered >18.5‰)				
Flohr et al. (2011)				
E Jordan	Low	Unirrigated/partially irrigated	15.1	
E Jordan	Low	Irrigated	17.7	
E Jordan	Medium	Unirrigated/partially irrigated	17.5	
E Jordan	Medium	Irrigated	19.3	
NE Jordan	Medium	Unirrigated/partially irrigated	17.0	
		Irrigated		

(*continued*)

Table 5 (*Continued*)

Location	*Rainfall*	*Irrigation*	*Mean* $\Delta^{13}C$ *(‰)*	*Mean* $\Delta^{13}C$ *S.D. (‰)*
NE Jordan	Medium		19.2	
Craufurd et al. (1991)				
N Syria	Low	Unirrigated	14.6	0.6
N Syria	Medium	Unirrigated	17.1	0.9
Araus et al., ‘Changes’ (1997a)				
NE Spain	Low	Unirrigated	16.0	1.8*
NE Spain	Medium	Unirrigated	17.0	1.9*
NE Spain	High	Unirrigated	17.7	1.6*
NE Spain	Unknown	Irrigated	17.3	2.7*
Voltas et al. (1999)				
NE Spain	Low	Unirrigated	14.6	
NE Spain	Medium	Unirrigated	16.2	
NE Spain	High	Unirrigated	16.6	

Notes

Depending on the quantification method reported, water conditions are grouped according to the following criteria. Rainfall during the growth period: low = less than 250mm, medium = 250 to 499mm, high = 500 to 750mm, very high = more than 750mm. Ratio of evaporation to rainfall for the growth period: low = less than 0.25, medium = 0.25 to 0.5, high = more than 0.5. Rainfall during the grain filling period: low = less than 50mm, medium = 50–100mm, high = more than 100mm. Partially irrigated = <50% optimum water requirements. A more detailed version of this table is provided in the Appendix.

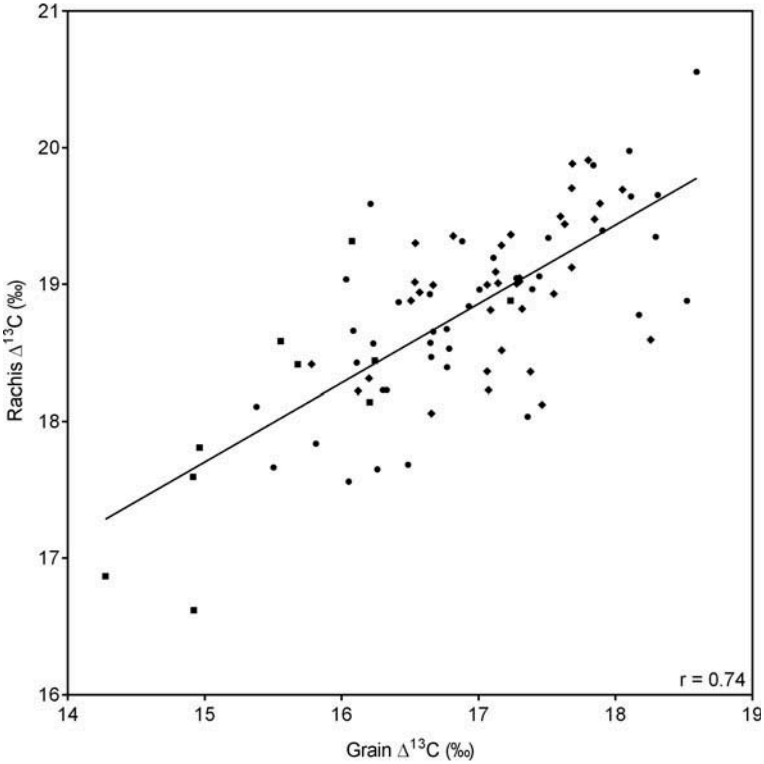

Figure 4 Plot of Δ^{13}C values of wheat grain and rachis for each sample from Aleppo (♦), Borja (●) and Wadi Ibn Hammâd (■); line denotes linear regression.

variables, such as manuring, cause a small degree of Δ^{13}C noise. However, our results clearly show that variation in Δ^{13}C is normal, and so this variation must be taken into account when interpreting Δ^{13}C results archaeologically. Taking our results and those presented in Table 5, this normal variation is in the range of ±0.5–1.0‰ for crops grown in the same conditions, which leaves scope for the inference of water conditions from Δ^{13}C.

The period during a plant's growth cycle in which the Δ^{13}C of, for example, grain is determined is also crucial to the interpretation of Δ^{13}C values in terms of water availability. If determined only during grain filling at the end of the growth cycle, as is usually assumed (Araus et al., 'Identification' 1997b, 730), Δ^{13}C may inform on early summer precipitation or the extension of the growing season through irrigation. On the other hand, if determined over a longer period, Δ^{13}C may reflect water conditions throughout growth. Grain filling occurs over six to eight weeks before ripening in the case of cereals, but staggered ripening complicates the issue for lentils. Our results for both wheat and lentils show a strong correlation between Δ^{13}C and water availability for the main grain filling period (April to May), but there are equally strong correlations for longer periods (e.g. February to May).

There are at least two possible reasons why water input prior to grain filling might determine Δ^{13}C. First, water added to the soil earlier in the growth period (e.g. in spring rainfall) may remain available to plants when grain filling commences. Second, reserve carbohydrates, that can make up as much as 40 ‰ of grain mass (Gebbing and Schnyder 1999; Tahir and Nakata 2005;

Takahashi, Tsuchihashi and Nakaseko 1993), form over several weeks prior to grain filling and so reflect water availability earlier in the growth period. Archaeological interpretations of $\Delta^{13}C$ values, therefore, should not assume that they represent the grain filling period only.

Taking account of our own and previously published results, we propose an interpretative framework for the inference of water status from the $\Delta^{13}C$ of archaeological crop remains. This framework is based on the following premises: (a) due to the influence of other environmental and biological factors, $\Delta^{13}C$ values provide only broad approximations of water status during growth; (b) individual crop samples may exhibit anomalous $\Delta^{13}C$ values due to variable localised conditions; (c) $\Delta^{13}C$ reflects all aspects of water availability, including water input and losses due to weather, anthropogenic activity and soil conditions; (d) $\Delta^{13}C$ values of cereal grain may reflect a substantial part of the growth period, rather than just the grain filling period; (e) the relationship between water availability and $\Delta^{13}C$ is likely to be strongest in dry conditions where water is the main limiting factor on photosynthesis. The first premise implies that small differences in $\Delta^{13}C$ (e.g. 0.5‰) should not be accorded great significance, while the second premise means that interpretation of $\Delta^{13}C$ values is most reliable when applied to groups of samples. In addition, while $\Delta^{13}C$ informs directly on soil moisture levels, a consequence of the third premise is that the natural and human factors contributing to water availability must be inferred from environmental and archaeological context.

Our proposed framework (Fig. 5) distinguishes three broad levels of crop water status: *poorly watered* crops for which water availability imposes major limitations on growth and for which $\Delta^{13}C$ values are typically below 16‰ for wheat grain; *well watered* crops for which water is not a major limitation on growth and for which $\Delta^{13}C$ values are typically in excess of 17‰ for wheat grain; *moderately watered* crops with intermediary $\Delta^{13}C$ values. The upper band, representing well-watered wheat, equates closely to the 17‰ and 17.5‰ thresholds used by Araus et al. (‘Identification’ 1997b, 734, 1999, 206) to indicate irrigated (and so presumably well-watered) wheat crops. For barley grain, these thresholds are 1–2‰ higher than for wheat (Fig. 5). For lentil, the thresholds seem comparable with those for wheat grain (Fig. 5), although lentil $\Delta^{13}C$ seems more sensitive to water input. The fact that the $\Delta^{13}C$ values for wheat rachis are consistently c. 2‰ higher than those of grain enables archaeobotanists to estimate or corroborate grain $\Delta^{13}C$ on sites where only cereal chaff is abundant. In addition, comparison of rachis and grain $\Delta^{13}C$ *may* help to differentiate between water availability early and late in the growing season.

This framework provides interpretatively useful information without exaggerating the reliability with which water conditions can be inferred from $\Delta^{13}C$ under farming conditions where local environmental factors and management practices other than irrigation are variable. Given that the latter also affect crop productivity, particular caution is needed in using $\Delta^{13}C$ as a measure of past crop yields (cf. Araus, Slafer et al. 2003).

Conclusion

Archaeobotanical stable carbon isotope analysis is a promising research tool for the inference of water conditions directly from ancient crop remains. As is often the case with new approaches, however, this potential must be tempered by the realities and limitations of the technique. Our

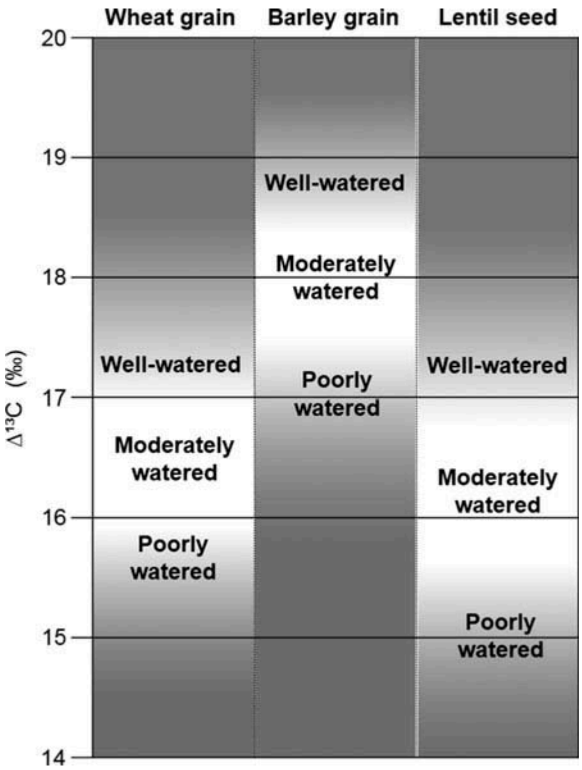

Figure 5 Diagrammatic representation of the framework for the interpretation of archaeobotanical $\Delta^{13}C$ values in terms of water availability.

research has shown that precise inferences of water input, for specific parts of the year, may be misleading, and that interpretations of crop water status should be made in broader terms. The interpretative framework presented here thus provides a reliable means of inferring water conditions from the $\Delta^{13}C$ values of crop remains, particularly in water-limited environments such as the Mediterranean region and south-west Asia. This in turn improves the confidence with which past climate, agricultural systems and land-use patterns can be explored through crop isotope analysis.

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M. Wallace, G. Jones and P. Halstead
University of Sheffield
m.p.wallace@sheffield.ac.uk

M. Charles, R. Fraser and A. Bogaard
University of Oxford

T.H.E. Heaton
NERC Isotope Geosciences Laboratory, British Geological Survey

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Michael Wallace is a post-doctoral research assistant at the University of Sheffield, Department of Archaeology. His doctoral research on the assessment and application of stable carbon isotope analysis in archaeobotany was completed at the same institution in 2011.

Glynis Jones is Professor of Archaeological Science at the University of Sheffield. Her research interests lie in the area of past agriculture and land use, particularly in the archaeobotanical investigation of crops and weeds, the development and application of ethnographic and ecological approaches, and the use of ancient biomolecules.

Mike Charles is a senior research fellow in environmental archaeology at the University of Oxford, Institute of Archaeology. He has published widely on the archaeobotany of south-west Asia, the identification of crop husbandry practices through weed ecology and several other topics.

Rebecca Fraser is a post-doctoral researcher at the University of Oxford. Her recent publications cover the use of stable nitrogen isotope analysis to infer land management practices and address taphonomic issues regarding isotopic analysis of archaeological remains.

Paul Halstead is a Professor of Archaeology at the University of Sheffield. His main research interests are the later prehistory of Greece, zooarchaeology, and traditional and ancient farming in the Mediterranean.

Tim Heaton is an isotope geochemist at the British Geological Survey. He has many publications covering a variety of topics, including the nitrogen cycle and plant isotopes.

Amy Bogaard is a lecturer in Neolithic and Bronze Age archaeology at the University of Oxford, Institute of Archaeology. She has research interests in early farming in Europe and western Asia, and in the development and refinement of techniques for inferring farming practices.