Assessing natural variation and the effects of charring, burial and pre-treatment on the stable carbon and nitrogen isotope values of archaeobotanical cereals and pulses

R.A. Fraser[1], A. Bogaard[1], M. Charles[1], A.K. Styring[2], M. Wallace[3], G. Jones[3], P. Ditchfield[1] and T.H.E. Heaton[4]

[1] School of Archaeology, 36 Beaumont Street, University of Oxford, Oxford, UK
[2] Organic Geochemistry Unit, Biogeochemistry Research Centre, School of Chemistry, University of Bristol, Bristol, UK
[3] Department of Archaeology, University of Sheffield, Sheffield, UK
[4] NERC Isotope Geosciences Laboratory, British Geological Survey, Keyworth, UK

* Correspondence to: R.A. Fraser, School of Archaeology, 36 Beaumont Street, University of Oxford, Oxford OX1 2PG, UK
E-mail: Rebecca.fraser@earth.ox.ac.uk

Keywords: archaeobotany, carbon isotopes, nitrogen isotopes, preservation, pre-treatment

Abstract

The aim of this study is to assess the potential of charred archaeobotanical cereal grain and pulse seed $\delta^{13}C$ and $\delta^{15}N$ values to provide evidence of crop growing conditions and as a potential component of palaeodietary studies. In order to reliably interpret archaeobotanical $\delta^{13}C$ and $\delta^{15}N$ values it is necessary to take into account the impact of charring, burial and laboratory pre-treatment procedures. We examine the effects of charring and burial on bulk $\delta^{13}C$, $\delta^{15}N$, %C, %N and C:N ratios in modern cereal and pulse material, and of cleaning by acid-base-acid (ABA) pre-treatment on modern and archaeobotanical charred material. Our study utilised bulk grain and seed samples to help account for within-ear/pod and between-plant variability in $\delta^{13}C$ and $\delta^{15}N$ values. Heating at relatively low temperatures and for prolonged times (230°C for up to 24 hours) is conducive to the formation of well preserved, undistorted charred cereal grain and pulse seed. Heating for 24 hours has a systematic and predictable effect on $\delta^{15}N$ values, with increases of around 1‰ on average in cereal grains and pulse seeds, and no consistent impact on $\delta^{13}C$ values. Increases in $\delta^{15}N$ are likely due to the loss of lighter ^{14}N via N-containing volatiles. Burial (for up to 2 years) and ABA pre-treatment have no significant effects on $\delta^{13}C$ or $\delta^{15}N$ values. After pre-treatment, however, the %C and %N contents of the archaeobotanical material more closely resembles that of the modern charred grains and seeds, suggesting that archaeobotanical remains accumulate non-structural material during burial but retain their original carbon and nitrogen content. Therefore %C, %N contents and C:N ratios can provide useful criteria for assessing archaeobotanical preservation.

1. Introduction

The charred remains of seed crops, especially cereals and pulses, form a large component of the Eurasian archaeobotanical record from the later prehistoric period onwards and provide evidence of plant use and consumption (e.g. Dennell 1976; Jacomet and Kreuz 1999). Associated weed evidence offers further valuable information about farming practices and land management (e.g. Jones 2000). More recently, the stable carbon and nitrogen isotope

analysis of crops has been explored as a means of obtaining *direct* and complementary evidence of crop growing conditions. Carbon isotope values ($\delta^{13}C$) of cereal crop remains have mainly been used to explore water management regimes, and to a lesser extent changes in local precipitation/moisture and climate conditions (Ferrio et al. 2005; Ferrio et al. 2006; Ferrio et al. 2007; Fiorentino et al. 2008; Riehl 2008; Riehl et al. 2008; Voltas et al. 2008; Heaton et al. 2009; Roberts et al. 2011; Wallace et al. in prep). Nitrogen isotope values ($\delta^{15}N$) have been shown to be a useful tool to identify land-use practices such as manuring and associated changes in soil productivity as the nitrogen cycle changes with human intervention (Bogaard et al. 2007; Aguilera. et al. 2008; Fraser et al. 2011). In palaeodiet studies the stable isotope values of plant-diet components are usually the 'unknown factors' and their values are estimated by subtracting trophic level diet to bone collagen fractionation values (usually +3 to +5‰) from the collagen isotope values of herbivores. The addition of actual plant-diet *food* isotope values to dietary models can potentially refine palaeodietary reconstructions.

In order to reliably interpret archaeobotanical crop stable isotope values it is necessary to take the impact of preservation processes and burial conditions into account. The depositional contexts of crop remains can vary from concentrated storage deposits to middens where sweepings from cooking hearths and crop processing areas accumulated (van der Veen, 2007). Buried grain and seeds are thus variously exposed to potential contaminants and diagenesis in soil, where original molecules can be removed or replaced by microbes or infiltrating groundwater. The crop remains retrieved from archaeological sites are often preserved by charring or carbonisation, a condition that involves heating but not combustion. The charring processes are assumed responsible for preserving many of the original biomolecules in ancient plant remains and rendering them less susceptible to degradation and microbial attack (Knicker et al. 1996; Almendros and Dorado 1999). It is widely recognised that charring can affect the morphology (e.g. size and shape) of crop remains (Boardman and Jones 1990; Braadbaart and van Bergen 2005; Braadbaart 2008; Märkle and Rösch 2008), but the effects of charring on cereal and pulse crop bulk $\delta^{13}C$ and $\delta^{15}N$ values have not been explored in detail. In addition, the isotope effects of burial in soil have rarely been tested under experimental conditions.

Chemical pre-treatment of ancient plant material and charcoal prior to $\delta^{13}C$ and $\delta^{15}N$ analysis is considered necessary to remove exogenous carbonates deposited from groundwaters and humic acids (Kelly et al. 1998; Fernandes and Krull 2008; Ascough et al. 2010a). The Acid-

Base-Acid (ABA, see section 2.5) pre-treatment technique is routinely applied to charred plant remains undergoing radiocarbon dating (Higham et al. 2009) and is considered essential to remove non-structural and exogenous younger radiogenic ^{14}C because even a small amount of contamination can result in incorrect dates (Taylor 1987). However, effects on $\delta^{13}C$ values are of less concern for radiocarbon dating and little is known about the effects of the ABA pre-treatment on the nitrogen that remains in ancient charred plant materials.

In this study we consider three main areas of stable isotope investigation. First, we examine the effects of charring, under a set of conditions conducive to the optimal preservation of morphologically intact, undistorted cereal grains and pulse seeds, in a set of Eurasian crops. Secondly, we examine post-depositional effects through burial of charred crop remains in garden soil and submersion in humic acid solution. Thirdly, we examine the effects of the ABA pre-treatment as a cleaning method. Overall, we seek to identify if there are systematic and significant changes in bulk $\delta^{13}C$ and $\delta^{15}N$ values, as well as percentage carbon and nitrogen content and resulting C:N ratios, arising from any of these factors. It is also necessary to take natural variation in the stable isotope values of crops grown under standardized conditions into account. This assessment can help with determining adequate sample sizes and identifying meaningful differences in isotope values among samples. We report on the magnitude of stable isotope variations (from two associated studies - Bogaard et al., 2007; Fraser et al., 2011) observed in grains and seeds from a single plant and in different plants grown on the same experimental field/plot and discuss how these variations may influence our interpretations. In this paper we focus principally on the effects that are detectable at the bulk stable isotope level because these analyses are commonly used and interpreted in archaeological science. Solid state ^{13}C NMR and FT-IR techniques examining the molecular composition of modern and archaeological charred crop remains to assess molecular changes due to charring, post-depositional preservation and ABA pre-treatment are investigated by Styring et al (this volume) in conjunction with our studies.

2. Background: stable isotopes and preservation of charred plant remains

DeNiro and Hastorf (1985) drew attention to the 'effects of charring' as a necessary precursor for preserving the isotopic signatures of archaeobotanical remains; this was because the $\delta^{13}C$ and $\delta^{15}N$ values of uncharred (desiccated) plants (many of which were edible legumes, seeds and fruits), in contrast to charred plants, were highly variable and differed considerably from their modern counterparts (e.g. 8-21‰ for $\delta^{15}N$). Another early study (Marino and Deniro

1987) observed that the effects of various cooking procedures, one of which included carbonization, indicated negligible effects on $\delta^{13}C$, $\delta^{18}O$ and δD values of plant cellulose; on this basis, the authors suggested that there is potential for obtaining 'palaeoclimatic' information from either cooked or uncooked plant remains. A substantial amount of research relevant to our study has been undertaken on the isotopic effects of heating and charring of wood, grasses and leaves (Turekian et al. 1998; Czimczik et al. 2002; Turney et al. 2006; Bird and Ascough 2012). These studies focus mainly on the changes in $\delta^{13}C$ values rather than $\delta^{15}N$ values. While contradictory and highly variable effects have been observed, the magnitude of change in plant tissue $\delta^{13}C$ values due to charring have mostly been in the range of 1 to 3‰ (Turekian et al. 1998; Czimczik et al. 2002; Turney et al. 2006; Ascough et al. 2008).

The preservation potential and $\delta^{13}C$ isotope composition of charred wood are recognised as being the result of a complex set of parameters, including charring temperatures and times, aerobic or anaerobic conditions, soil pH and microbial or fungal attack, and also the initial composition (e.g., proportions of cellulose and lignin) (Czimczik et al. 2002; Ascough et al. 2008; Ascough et al. 2010b). Wood charcoal studies provide highly relevant background to the charring of crop remains studied here, but the biochemical composition and physical structures of cereal grains and pulse seeds vary significantly from wood. Wood is comprised predominantly of ~65-75% carbohydrates (~35 to 40% cellulose and ~25 to 35% hemicelluloses), ~18- 28% lignin and the remainder are ~4 to 10% organic extractives, which can include compounds such as lipids, waxes, alkaloids, proteins, phenolics, simple sugars, starches and essential oils (Pettersen 1984). In contrast, cereal grains and pulse seeds are composed of negligible lignin or cellulose (some in the outer epidermis), many polysaccharides (with ~60% starch), smaller amounts of proteins (~3 to 8%) and very low amounts of lipids (~2%) (Braadbaart et al. 2004a). For this reason, the effects of charring and preservation specific to crop remains warrant more investigation.

The changes in plant $\delta^{13}C$ values observed due to heating can be influenced by the different stable isotope compositions of individual biochemical components, such as lipids, lignins, carbohydrates and proteins. Differences in the $\delta^{13}C$ values of these components can depend on the plant species and the tissues analysed. For example, lignin can be ~1‰ lower than and cellulose ~2‰ higher than bulk wood $\delta^{13}C$ values (O'Leary 1981; Benner et al. 1987; Tieszen 1991; Loader et al. 2003). The lipid components show the greatest differences; $\delta^{13}C$ values

148 can be ~3 to 8‰ lower than bulk plant $\delta^{13}C$ values (Park and Epstein 1961). It follows that
149 preferential losses or changes in a biochemical component over another during charring may
150 significantly change the final bulk isotope value of the material.
151
152 Archaeological studies that have concentrated on the isotope effects of cooking or charring of
153 plants have focused mainly on pot residues (Hart et al. 2007; Boyd et al. 2008; Hart et al.
154 2009), and it is acknowledged that little experimental stable isotope research has been done
155 on actual crop/plant-food remains themselves (Dürrwächter et al. 2006; Bogaard et al. 2007).
156 The work of Araus et al. (1997) included a study of relationships between %C and $\delta^{13}C$
157 values in charred cereal grains (none found) and also observed changes in $\delta^{13}C$ of between ~-
158 0.5 and +1.2‰ over a range of charring times from 15 to 120 minutes at temperatures up to
159 400℃ (Araus et al. 1997, Figure 2, p734). Poole et al (2002) charred peas for 2 hours over a
160 range of temperatures, 100 to 700℃ and observed increases in $\delta^{13}C$ values of up to 1.5‰.
161 Yang et al. (2011) charred millets for 2 or 3 hours over temperatures from 50 to 300℃ and
162 observed a less than 1‰ change in $\delta^{13}C$ values (both directions). Increases in $\delta^{13}C$ values of
163 crop remains (and other plant materials) are usually attributed to the preferential loss of ^{13}C-
164 depleted lipids during charring. However, the lipid $\delta^{13}C$ values in peas and millets have rarely
165 been analysed; therefore, accounting for the selective loss of the ~2 to 3.6% average lipid
166 contents can only be estimated in broad terms. For example, assuming a plausible lipid $\delta^{13}C$
167 value that is ~5‰ less than other biochemical components and represents only ~2% of the
168 total content, in mass balance terms, this may not be enough to account for all of the 1.5‰
169 increase in $\delta^{13}C$ values observed in the peas charred by Poole et al. (2002). For nitrogen,
170 Kanstrup et al. (2012) charred wheat grains for 0.5, 2 and 6 hours at 250℃ and inferred little
171 change in $\delta^{15}N$ values under these conditions.
172
173 Past charring conditions at any one site may have been highly variable. However in order to
174 undertake our experimental investigations, we identified a set of standardised charring
175 conditions that replicate well preserved, undistorted archaeobotanical charred crop remains as
176 closely as possible and allows for comparison of the other effects of burial, contamination
177 and ABA pre-treatment methods.
178
179 *2.1. Establishing relevant experimental charring conditions*

A number of studies have sought to replicate the conditions under which charred archaeobotanical crop seeds/grains are preserved, including wheats (Boardman and Jones 1990; Braadbaart et al. 2004a, 2005; Braadbaart 2008), pea (Braadbaart et al. 2004b), millet (Yang et al. 2011), sunflower (Braadbaart and Wright 2007) and maize (Goette et al. 1994). Different authors have defined the point at which 'charring' occurs in different ways: as colour change from brown to black (Boardman and Jones 1990), or as the complete conversion of starch and proteins into larger and more stable polymorphic compounds (e.g. Maillard reaction products) relatively resistant to microbial attack (Bland et al. 1998; Braadbaart et al. 2004b; Silván et al. 2006).

Through experimental charring of emmer wheat grain, Braadbaart et al. (2004) concluded that charred archaeobotanical grain must have been heated to temperatures in excess of 310℃, since it is only above this threshold after two hours' heating (the maximum period considered) that sufficient molecular changes are considered to occur to prevent microbial degradation after burial. Charring above ~250℃, however, causes marked swelling, protrusions and other distortions in both emmer and free-threshing wheat grain (Braadbaart 2008). Other work with millet suggests that these grains may be reduced to ash above 300℃ (Yang et al. 2011). In peas, cracking of the cotyledons is common at temperatures in excess of 310℃ (Braadbaart et al. 2004b). Experimental charring of einkorn grain by Charles and colleagues (in prep) shows that, even when the temperature is raised very gradually over an extended period, characteristic distortions consistently occur when grains are heated above 250℃. The important implication is that very well preserved and undistorted archaeobotanical grain was heated to lower temperatures.

These observations suggest that charring conditions that create undistorted archaeobotanical cereal grain involve extended exposure to relatively low temperatures, around 230℃, for periods of 6 hours or more, in reducing conditions. Such a scenario is not implausible in collapsed, smouldering buildings partly or completely destroyed by fire, and also in association with domestic hearths/ovens kept burning for extended periods; while open fires are too hot/oxidised to produce undistorted charred grain, experimental work suggests that 'buried' material protected from direct exposure becomes charred through heating at relatively low temperatures (*c.* 150-300℃) for extended periods (Goette et al. 1994; Sievers and Wadley 2008).

For the purposes of this study, therefore, most experimental charring was conducted in reducing conditions at 230℃, with variable but lengthy charring times, from 2 to 24 hours. Charring for up to 24 hours can cause very slight swelling but little other distortion, thus replicating well preserved assemblages of cereal grain with fully intact morphology (e.g., 'stores' of cereal grain protected from direct heat – e.g. Jones et al. 1986). Our reasoning is that such archaeobotanical material provides the best conditions for interpreting stable isotope values, both because the grain is highly identifiable (often to species) and because it tends to derive from primary deposits that are well defined stratigraphically.

2.2. Burial in soil

While charring processes can be simulated experimentally, the diagenetic processes that occur in the burial environment, perhaps over many thousands of years, remain the most unpredictable factors. We sought to replicate a likely depositional environment by burying charred wheat grains and peas in common garden soil. We recognise that diagenetic alterations may occur at any time during burial and that experimental burial is constrained by project research time. Nevertheless, we consider that, if there are original molecules less resistant to attack remaining in the charred materials, they will most likely change, degrade or be removed in the initial years of burial and hence these early changes may be detectable in a relatively short-term experiment.

2.3. Humic acid contamination

Humic acids are considered a potential contaminant in buried organic matter. However, humic acids and their structures and effects are highly variable and the longer-term isotope effects on buried plant matter remain largely undetermined (Head and Zhou 2000; Ascough et al. 2010a). Our research includes a two-year long experiment to intentionally contaminate charred modern and archaeological millet seeds by soaking the seeds in a humic acid solution mixed with garden soil. This experiment used millet as a cereal because it is a C_4 plant and its grains have $\delta^{13}C$ values of approximately 9 to 13‰ in accordance with this photosynthetic group (O'Leary 1988). The modern charred millet used here had a $\delta^{13}C$ value of -12.4‰ and the archaeological charred millet was -10.8‰. The humic acid used here has a $\delta^{13}C$ value of -26.7‰, which is within the range typical for C_3 plants; therefore contamination with carbon from the humic acid solution should cause the $\delta^{13}C$ values of the millet seeds to become more negative. Seeds from archaeological bitter vetch were also included in the experiment to

monitor changes in C_3 crop plants; however, contamination of the bitter vetch carbon will be harder to detect than in the millet because the $\delta^{13}C$ values of the bitter vetch, ~ -25.2‰, are similar to the $\delta^{13}C$ values of the humic acid solution. The humic acid solution has a very low %N content of ~0.6% and a $\delta^{15}N$ value of 1.4‰, which is similar to the original $\delta^{15}N$ values of the un-soaked charred millet (0.9‰) and bitter vetch (1.2‰) seeds; therefore, significant changes in the soaked samples are unlikely to be detected within machine error (see section 3.5). Changes in the %C and %N contents of the soaked seeds are also examined.

2.4. ABA pretreatment

The ABA pre-treatment consists of three main steps, 1) the application of a strong acid, such as hydrochloric acid (HCl), to remove non-structural carbon, 2) application of a base, such as sodium hydroxide (NaOH), to remove humic acid contaminants, 3) final application of acid to remove CO_2 absorbed from the air during step 2. For our studies we wish to examine the effects of the ABA pre-treatment method on the bulk $\delta^{13}C$ and $\delta^{15}N$ values of modern and archaeological charred crop remains. We are particularly concerned with the fate of nitrogen because the N contents in cereal grains and pulse seeds are low (~0.5% to ~4%) compared to carbon (~30% to 45%); we seek to examine the effects on %N and $\delta^{15}N$ values to see if original N is retained and/or detrimentally altered by the ABA pre-treatment methods. The work of Styring et al. (this volume) considers the biochemical compositions of a subset of these same samples.

2.5. *Natural stable isotope variability in modern cereal and pulse crops*

Our investigation of crop sample stable isotope variability identified different levels of isotope variation that need to be considered. Variations (1 SD) in $\delta^{13}C$ values within and between wheat plants may range up to 0.7‰ (Heaton et al., 2009). A mean variation of 1.8 ± 1‰ in the $\delta^{15}N$ values of individual grains within single ears of wheat (N = 4 ears, each containing ~17 to 21 individual grains) was observed by Bogaard et al. (2007), and our study measured a 0.7‰ range in $\delta^{15}N$ values between 6 individual broad beans within a single pod (Supplementary Table 5). In addition, there were mean ranges of 1.8 ± 1.2‰ for $\delta^{15}N$ values and 0.4 ± 0.2‰ for $\delta^{13}C$ values observed in 38 sets of bulk samples of wheats (each comprising ~50 well-homogenised powdered grains) taken from three-four replicate field plots at the same site, studied by Fraser et al (2011) (Supplementary Table 6). One important implication here is that, even though sub-samples used for experiments are taken from the

same ‘parent’ bulk sample, a certain amount of variation among sub-samples is to be expected. Therefore, our studies undertook experimental repeats to identify whether or not a treatment introduces a systematic and quantifiable bias. We seek to identify if the isotope effects of our experiments are greater than the inherent natural isotopic variability and also that of instrument measurement precision (section 3.5).

3. Experimental methods and materials

3.1. Charring cereal grains and pulse seeds

Cereal and pulse samples were obtained from a collection of crops grown on experimental field sites in the United Kingdom, central Europe and Syria; site descriptions and field collection methods are published previously in Bogaard et al. (2007) and Fraser et al. (2011). Bulk crop samples from different field plots were randomly split into sub-samples containing 25 to 50 cereal grains, or 10 pulse seeds. One sub-sample from each plot was retained as the uncharred control. Crop sub-samples were subject to one of the following charring conditions: at 230℃ for 2, 4, 8 or 24 hours; at 250℃ for 6 hours, at 270℃ for 6 hours, at 300℃ for 2 or 6 hours, at 400℃ for 2 or 6 hours. Although charred modern crop seeds and pulses are not an exact biochemical replicate for the archaeological grains, we consider that they are the closest available analogue to use as controls for our experiments.

Crop sub-samples were charred in a Heraeus electric oven. To achieve low oxygen conditions, sub-samples were wrapped in aluminum foil and placed in a 250 ml beaker filled with sand and then sealed with more foil. This set-up excludes oxygen sufficiently enough to produce reducing conditions, and can be considered comparable to the conditions amongst embers and ash in the bottom of a hearth or building fire. The burial of sub-samples in sand also served to reduce the heating rate to which the material was exposed. The temperature inside the beakers (measured using K-type thermocouple probes) increased at a rate of 1.5-2℃/min. After reaching 230^{o}C the samples were held at this temperature for 2 to 24 hours and then allowed to cool within the sand in the closed oven. Sample weight loss due to charring at 230℃ for 24 hours was calculated from pre- and post-charring weights

3.2. Burial in soil experiment

A set of bread wheat, pea and broad bean sub-samples, previously charred at 230℃ for 24 hours, were placed in bags of agricultural soil and then buried under common garden soil in Birmingham, United Kingdom (climate: temperate maritime, mean precipitation of 660 mm/year, mean high and low temperatures ~13 and 6℃, respectively), for up to 24 months from May 2008 until May 2010. Sub-samples were placed in 15 cm^2 open-gauze fabric bags, soil was added and mixed with the charred crop remains and then the bags were secured with string and buried 30 cm deep. The agricultural soils surrounding the charred crop remains had a $\delta^{13}C$ value of -27.6‰ and a $\delta^{15}N$ value of 15.6‰. Bags remained buried for 6, 12, 18 or 24 months. On retrieval, crop samples remains were recovered by wet-sieving, washed in distilled water to remove adhering soil and air dried at ~40℃.

3.3. Humic acid contamination experiment

Charred modern and archaeobotanical millet and archaeological bitter vetch seeds were soaked in sealed vials of a humic acid solution (Fluka Analytical®) for periods of 6, 12 and 24 months. The humic acid soaking solution was a 10% solution made up with distilled water and an additional 50 grams of garden soil; the pH was 5.8. The modern millet was charred at 230℃ for 24 hours prior to soaking. The charred archaeological material came from a bulk-storage room in a burned-down building at the tell site of Late Bronze Age Assiros Toumba, Greece (see Heaton and Jones, 2009). Each sub-sample consisted of approximately 50 to 100 seeds; after soaking, seeds were washed in distilled water and air- dried at 30℃, then ground to a fine homogeneous powder using a mortar and pestle.

3.4. Acid-Base-Acid (ABA) pre-treatment

Archaeobotanical cereal grain and pulse samples from six Neolithic or Bronze Age archaeological sites (one in Jordan, three in Bulgaria, one in Greece and one in Germany) were used to assess the effect of the ABA pre-treatment on isotope values, %C and %N content. The material was recovered by a range of techniques including wet sieving and direct sampling from primary concentrations. All of the archaeobotanical cereal grain and pulse seeds derived from deposits that were very well preserved, with virtually no distortion and fully intact morphology – that resembles modern material charred under optimal conditions (see above). Two random sub-samples of around 10-20 grains/pulse seeds each was extracted from each archaeological sample, one for pre-treatment prior to isotopic

344 analysis and the other for isotopic analysis without pre-treatment. The archaeobotanical
345 results from individual sites will be presented elsewhere.
346
347 Sub-samples of 25 modern and archaeological charred grains and seeds were treated first
348 with 10 ml of 0.5M HCl at 70℃ for 30 to 60 minutes, or until any effervescing ceased, and
349 then rinsed in distilled water three times. The second treatment was 10 ml of 0.1M NaOH at
350 70℃ for 60 minutes, and rinsed in distilled water until the solution was clear and the pH
351 neutral, with a minimum of three rinses. The final treatment was a repeat of the 0.5M HCl
352 step one followed by freeze drying. Samples were ground to fine homogeneous powder using
353 a mortar and pestle ready for stable isotope analysis.
354
355 *3.5. Elemental and stable isotope analyses*
356 Crop samples for elemental and stable isotope analysis were ground to a fine homogeneous
357 powder under liquid nitrogen using a Spex 6850 freezer mill. Samples were weighed into tin
358 capsules and analysed using two systems:
359 1) %C, %N and $^{13}C/^{12}C$ analyses were performed by combustion in a Costech 4010 on-line
360 to a VG TripleTrap and Optima dual-inlet mass spectrometer. The %C and %N
361 calculation, and $^{13}C/^{12}C$ ratio calculation as $\delta^{13}C$ values on the VPDB scale, were
362 undertaken using a within-run laboratory standard plant material calibrated against
363 acetanilide and NBS-19 and NBS-22.
364 2) $^{15}N/^{14}N$ analyses were performed on a ThermoFinnigan system comprising an elemental
365 analyser linked under continuous flow with a Delta+XL mass spectrometer. $^{15}N/^{14}N$ ratios
366 were calculated as $\delta^{15}N$ versus atmospheric N_2 by comparison with a laboratory standard
367 plant material calibrated against IAEA-N-1 and N-2.
368
369 The precision (1σ) among replicates of a homogenized barley sample was 0.2 for %N and
370 0.4‰ for $\delta^{15}N$ analysed in 29 separate runs. The precision (1σ) among replicates of a
371 homogenised wheat grain sample was 3.5 for %C and 0.1‰ for $\delta^{13}C$, analysed in 21 separate
372 runs. While much of the natural variability in stable isotope ratios and %C and %N contents
373 of crop materials is difficult to fully quantify for each site, these levels of measurement
374 precision provide initial baseline minimum quantities for assessing whether significant and
375 meaningful isotopic differences exist between untreated and treated crop materials in this
376 study.

3.6. C:N ratio comparison

Our previous research on the $\delta^{13}C$ and $\delta^{15}N$ values of modern cereals and pulses (Bogaard et al. 2007; Fraser et al. 2011, and additional unpublished data) enables us to measure the %C and %N contents of 208 cereal and 93 pulse samples. The C:N ratios of cereals ranged from 20.9 to 33.3 and the ratios for pulses from 11.8 to 14.6 (Supplementary Table 4). The C:N ratios of these uncharred modern cereals are compared to our experimental samples that undergo charring, burial, contamination and ABA pre-treatments. In a similar way to the use of C:N ratios in modern bone collagen to define collagen 'quality' (DeNiro 1985), we seek to determine whether or not a specific range(s) of C:N values can be developed for assessing the preservation of archaeobotanical crop stable isotope values.

4. Results

4.1. The effects of heating on grain weight, morphology and %C and %N contents

Table 1 summarises grain weight losses due to heating at 230℃ for 24 hours across a range of cereals and pulses. Losses range from ~48 to 56% of fresh weight. The %C and %N contents of cereal grain and pulses all increase with heating. Figure 1b and Table 3 show that the %C content of cereal grains, heated at a constant 230℃ over a time series of 2, 4, 6 or 8, and 24 hours, increased steadily from ~39% to 65%; most of the increases occur within the first 4 hours of heating. Under higher temperatures, up to 400℃ for 2 to 6 hours, %C increases only slightly more (to ~73 to 78%, Tables 2 and 3). The millet seeds show a markedly smaller increase, starting at ~ 40%C (i.e. similar to other cereal grains), but rising to only 54%C with charring at 230^{o}C. Figure 1d and Table 2, show that the %N contents of grains, heated at 230℃ over the same time series (0 to 24 hrs) also increased steadily.

For all samples heated at 230℃ for 24 hours (Supplementary Tables 1 and 2), the %C contents of cereal grains (n=20) increased approximately 22% and pulse seeds (n=15) increased approximately 20%. Heating increased the %N of cereal grains approximately 1.5% and pulse seeds approximately 2.9%. The decreases in weight and the increases in %C and %N contents during heating largely reflect dehydration through loss of free water (Styring et al. this volume). Although the overall relative increase in %N was slightly larger than that of %C, changes in C:N ratios were small and still resulted in the cereal and pulse groups remaining distinct (Figure 2).

An important observation is that at 230℃ for up to 24 hours, the wheat grains remained morphologically intact with little distortion in shape. Noticeable changes were that free threshing wheat grain became slightly plumper (decrease in length, increase in breadth and/or thickness), and that the outer testa of the pulses flaked off. The duration of charring had only a limited impact on the level of distortion, whereas increasing the charring temperature to 300℃ and 400℃ resulted in greater distortion (cf. Boardman and Jones 1990; Braadbaart 2008).

4.2. Effect of charring on $\delta^{13}C$ values

Heating of cereal grains at 230℃ over a time-series of 2, 4, 8 and 24 hours had a small (mean 0.2‰ ±0.5) yet variable impact on $\delta^{13}C$ values (Figures 1a, Tables 2 and 3). The largest change in $\delta^{13}C$ is +0.8‰ for sample SUT08-25E. Figure 3 (triangular symbols) and Table 4 summarise the carbon isotope differences between all uncharred and charred sub-samples of cereal grains and pulse seeds heated at 230℃ for 24 hours (n = 35); 72% percent of the offsets are within measurement error of 0.2‰, 83% are within 0.3‰, and the trend appears non-directional. The mean ‰ difference between uncharred and charred $\delta^{13}C$ values is 0.0 ±0.3‰ for cereal grains and peas, -0.1 ±0.4‰ for broad beans and -0.1 ±0.1‰ for lentils. Moreover, heating cereal grains at a range of higher temperatures, from 250℃ to 400℃, for periods of 2 to 6 hours also showed little further impact on $\delta^{13}C$ values; the offsets remained within the range of 0.1‰ to 0.4‰ (Table 3).

4.3. Effects of charring on $\delta^{15}N$ values

Heating of cereal grains at 230℃ over a time-series from 2, 4, 8 and 24 hours showed a gradual increase in $\delta^{15}N$ values (Figure 1c, Table 2); we observed a mean increase of ~0.3‰ after 2 hours and up to 0.8‰ after 24 hours; the final offsets ranged between 0.4‰ and 1.6‰. Figure 3 and Table 4 summarise the nitrogen isotope differences between all uncharred and charred subsamples of cereal grains and pulse seeds heated at 230℃ for 24 hours (n = 38); 92% of the offsets are larger than the standard deviation of measurement error (here, 0.4‰). In contrast to the offsets in $\delta^{13}C$ values, the charred $\delta^{15}N$ values are directional, all being more positive than the uncharred values; this suggests a systematic bias with a mean value of +1.0 ±0.4‰ for grains and pulses examined here. These increases in $\delta^{15}N$ values are shown in Figure 3 (circular symbols).

4.4. Effects of burial in soil

The post-charring burial within common garden soil for up to 24 months has no consistent or significant effects on either the $\delta^{13}C$ and $\delta^{15}N$ values or the %C and %N contents of the grains and pulses (Figure 4 and Table 5). The bread wheat sample BOR07-44W had a %C content of 80% after 24 months' burial; this single analysis could be an outlier and remains unexplained at this stage. The small-scale variability amongst buried sub-samples is predominantly within the standard deviation of measurement error and the levels of natural variability observed between samples (see section 3.5).

4.5. Effects of humic acid contamination

The results of the humic acid contamination study are shown in Figure 6 and Table 6. The humic acid soak over the 6- to 24-month period has no significant effect on the $\delta^{13}C$, $\delta^{15}N$ or %N values of the archaeological millet and bitter vetch samples. The effects on archaeological %C contents were more variable during the 24 months; however, the differences are similar to the natural levels of variation we have observed in archaeological and modern grain and pulse %C contents (Bogaard et al. 2007; Fraser et al. 2011; and unpublished data). There was a 0.5‰ shift in the $\delta^{13}C$ values of the modern charred millet from -12.4 to -11.9‰, and an approximate 20% increase in the %C content (51% to 62%); the latter was recognizable and maintained after the first 6 months. This result is considered significant and could indicate that exogenous carbon inclusion can occur quite rapidly. Although the change in $\delta^{13}C$ values is above that of the standard deviation of machine error (here, 0.2‰), and therefore significant, it was in the opposite direction to what would be expected if it were contaminated with a C_3 humic substance with a $\delta^{13}C$ value of -26.7‰. More detailed molecular analyses may help explain the unexpected positive change in the modern millet $\delta^{13}C$ values, at least beyond that of natural sample variation.

4.6. Effects of the ABA pre-treatment

When the acid (0.5M HCl) was added to the samples mild effervescence occurred in a small number of archaeological samples, which may indicate the removal of exogenous carbonate via CO_2. The addition of the base (0.1M NaOH) caused most of the solutions containing the modern samples to become a transparent mild tan colour (some solutions remained colourless) and the archaeological solutions mostly became an opaque dark brown to black colour. The dark colour obtained after the application of the base solution is associated with

the presence and removal of humic acids. The modern samples rinsed clear and to a neutral pH after approximately two to three rinses in distilled water; in contrast the majority of the archaeological samples required additional rinses for the solutions to become clear and reach neutrality. The second and final addition of acid caused mild effervescence in only some of the archaeological samples.

The responses of the charred material to the ABA pre-treatment are shown in Figure 5 and Supplementary Table 3. There were small changes in the %C and %N of the modern charred cereal and pulse samples, ranging from 9.9 to 0.5% (mean 1.2% ± 5.1) and 2.2 to 0.0% (mean 0.3% ±0.6, respectively (Figure 5b). The largest extremes in these two ranges occurred in the samples charred for only 2 hours; we consider these short charring times results in a less inert internal matrix that is more reactive to removal by the ABA pre-treatment. The archaeological grains and pulses show larger and more systematic changes in the %C and %N contents in response to the ABA treatment than the modern samples (Figure 5b). The %C and %N always increased, approximately 19% ±7 and 1.4% ±0.8 respectively. These data indicate material, either original/structural or exogenous, is being removed from the archaeological samples during the ABA pretreatment that is acting to concentrate the more recalcitrant carbon and nitrogen in charred matrix of the samples. Importantly, the percent C:N ranges of cereals and pulses remain distinct (Figure 7, Supplementary table 3) and are very similar to their modern charred counterparts (above, Figure 2).

The ABA pretreatment shows no consistent isotope effects on the $\delta^{13}C$ or $\delta^{15}N$ values of the modern charred grains and pulses (Figure 5a, upper portion). The majority of the differences between untreated and ABA treated $\delta^{13}C$ and $\delta^{15}N$ values are within the standard deviation of machine error of their untreated counterparts. Only three $\delta^{13}C$ and two $\delta^{15}N$ values have differences greater than the standard deviation of the machine error. There were no particularly different effects on the samples charred for 2 and 4 hours; the differences were 0.0‰ and +0.6‰, respectively.

The effects of the ABA pretreatment on the isotope values of the archaeological samples were similarly small and often within error (Figure 5a, lower portion). There were no relationships between the changes in the %C and N contents and the changes in stable isotope values. This suggests that, although substances were removed (even if selectively removed), resulting in increases in %C and N contents, these components did not have stable isotope

values significantly different to the remaining material, or the magnitude of difference is undetectable at either the machine precision or the bulk isotope level.

5. Discussion

In comparison to previous studies of experimentally charred cereal and pulse remains, the temperatures used here were lower (230°C) and the heat exposure times longer (up to 24 hours). These conditions result in cereal and pulse grains that are not distorted with protrusions or cracks, or partially reduced to ash. Morphologically intact grains can be more easily assigned to species and be identified as likely dietary components. The support for charring at higher temperatures (>300°C) in other studies is largely based on it being considered necessary to render crop remains inert and enabling preservation in the burial environment (Braadbaart et al. 2004b). Charring processes cause major chemical and structural changes in plant materials that can result from losses by volatisation of gases, the degradation of molecules and/or rearrangement of existing molecules into larger polymorphic compounds. In particular, the formation of melanoidins during Maillard reactions between free amino acid groups and sugars is a common reaction observed when plant materials are heated (Bland et al. 1998; Silván et al. 2006). Melanoidins and the amino acids within them, as well as other high molecular weight aromatic compounds formed during charring, are considered very stable to oxidation and resistant to microbial attack (Knicker et al. 1996; Almendros and Dorado 1999). The biochemical analyses of Styring et al (this volume) indicate that Maillard reactions already occur in cereal grain experimentally charred for over 4 hours at 230°C, likely forming polymeric melanoidins. Importantly, our burial and humic acid experiments, indicated no significant physical changes or isotopic effects on charred crop remains, which suggests crop remains charred at lower temperatures for longer times are sufficiently inert and resistant to microbial attack and soil contamination.

Changes in $\delta^{13}C$ values due to charring, at all temperatures and time periods considered here, were small and the majority were within measurement error (here 0.2‰). For crop remains charred at 230°C for 24 hours the mean change was less than 0.1‰. Overall, our results indicate that the archaeologically relevant charring conditions applied here have no significant isotope effects on the $\delta^{13}C$ values of crop remains. The changes in $\delta^{15}N$ values due to charring were larger and more significant than those observed for $\delta^{13}C$ in this study. The

previous work of Kanstrup et al. (2012) concluded no significant isotope effects of charring on the $\delta^{15}N$ values of single emmer, spelt and barley samples at temperatures ranging from 100 to 400℃ and times of up to 6 hours; the $\delta^{15}N$ values in the heavily distorted and partially ashed wheat samples exposed up to 550℃ increased, but these were considered not archaeologically relevant. Our study extended the variety of crop types and sample sizes previously studied (including pilot data from Bogaard et al. 2007) and increased the charring time up to 24 hours. Our new data clearly indicate a significant and systematic trend where $\delta^{15}N$ values increase due to charring; all charring offsets are positive with a mean difference of +1.0 ±0.4‰ (see Figure 3). The contrasting result with Kanstrup et al. (2012) could represent the effects of prolonged charring times on increasing crop $\delta^{15}N$ values, which can occur under a different set of charring conditions that are also archaeologically relevant.

Increases in %N and %C contents of experimentally charred crop remains, especially after the initial 2 hours to 4 hours of heating, are attributed to the loss of free and chemically bound water and as charring time increases more complex molecular changes occur along with the loss of volatiles (Styring et al, this volume; Braadbaart et al. 2004b). Increases in crop %N were associated with increases in $\delta^{15}N$ values. The molecular analyses of the amino acids in these charred grains by Styring et al. (this volume) suggests that retention and increase in N is evidence for the conversion of original amino acids into other N-containing compounds during Maillard reactions, and/or preferential losses of non N-containing molecules after the original amino acid structures are lost; the increase in $\delta^{15}N$ values is most likely due to the loss of lighter ^{14}N via N-containing volatiles (such as alkyl pyrazines, although the $\delta^{15}N$ values of N-volatiles were not measured). The lack of change in $\delta^{13}C$ values with increasing %C indicates little systematic or significant isotope effects associated with the molecular changes and loss of C containing volatiles from crop remains during charring.

The ABA pre-treatment appeared to have no significant influence on stable isotope values or the %C and %N contents of the charred modern grains and pulses. Attention has been called to the potential changes in original carbon and nitrogen contents in organic plant materials analysed for palaeoecological studies caused by different acidification methods during sample cleaning by Brodie et al. (2011) and Fernandes and Krull (2008). However, our results indicate that there were no significant isotopic biases imposed by the acid or base treatments applied here.

578 The effects of the ABA pre-treatment on the isotope values of the archaeological samples in
579 this study were generally minimal, yet variable, which is expected for samples with different
580 and long burial histories. The chemical pre-treatment of archaeological charred crop remains
581 prior to $\delta^{13}C$ and $\delta^{15}N$ analysis in previous studies has received mixed consideration.
582 Archaeological grain/seed of barley (*Hordeum vulgare*), wheat (*Triticum aestivum*) and faba
583 bean (*Vicia faba*) from Los Castillejos, SE Spain were treated with 6M HCl by Aguilera et al.
584 (2008), whereas the ancient wheat from the Middle East analysed by Araus et al. (2007) and
585 the wheat, emmer (*T. dicoccum*), einkorn (*T. monococcum*) and spelt (*T. spelta*) from Bronze
586 Age Assiros in Greece analysed by Heaton and Jones (2009) underwent no chemical pre-
587 treatments. Chemical pre-treatment may not appear necessary for crop samples preserved in
588 sealed bulk storage deposits (e.g. the Assiros samples mentioned above), but many crop
589 remains are dispersed within a sediment matrix and/or also soaked in potentially
590 contaminating water during retrieval by flotation, and so may require chemical pretreatment
591 as a precautionary measure.
592
593 The untreated archaeological samples were more variable in %C than the treated or the
594 modern uncharred and charred crops (Figures 2 and 7). After ABA pre-treatment the
595 archaeobotanical material more closely resembles the modern charred %C and %N values.
596 The implication is that during burial the archaeological material accumulates non-structural
597 material but retains its original carbon and nitrogen content. More biochemical work is
598 needed to address the nature of the potential contaminants affecting %C and %N in
599 (untreated) archaeobotanical remains.
600
601 Clearly, the experimental burial times investigated here cannot be compared directly with
602 millennia of archaeological time. Nevertheless, our results demonstrated that contamination
603 during short-term burial does not affect $\delta^{13}C$ or $\delta^{15}N$ values. Furthermore, the fact that with
604 pre-treatment we can recover what appears to be the original charred %C and %N suggests
605 that any exogenous material taken into the grain does not alter these aspects of composition.
606 This conclusion is supported by the work of Styring et al. (this volume), who show that, after
607 pre-treatment the biochemical composition of the archaeological grains closely resembles that
608 of modern charred grain. Overall, this work suggests that archaeological grains, once charred,
609 retain their original C and N content.
610

In this study we observed considerable natural variability in $\delta^{13}C$ and especially $\delta^{15}N$ values at varying scales, from the individual cereal ear or pulse pod to replicate samples across the same experimental treatments. These results have two major implications for assessing both modern and archaeobotanical crop isotope values. First, individual grain analyses only make sense if they form part of broader sampling strategies that can capture the mean and spread of values in a given set of growing conditions. Here we have opted to analyse bulk samples per cultivation plot and where possible replicate plots of the same treatment so that we can average out within-ear and between-plant variability. Secondly, in order to assess levels of variation within an archaeobotanical assemblage, as many such bulk samples as possible should be analysed in order to assess the central tendency and range of variation. It is only with a thorough bulk sampling strategy that we can begin to understand isotopic differences between, for example, contexts chronological phases or archaeological sites.

Our study was constructed to assess the potential and reliability of archaeobotanical crop $\delta^{13}C$ and $\delta^{15}N$ values as evidence of crop growing conditions and as a component of palaeodietary studies. The results confirm that, with adequate allowance for natural variability and charring effects, well preserved archaeobotanical cereal grains and pulse seeds do provide valuable archives of isotopic information of direct relevance to land use and dietary reconstruction. The observation that prolonged heating systematically increases $\delta^{15}N$ values, by *c.* 1‰ on average, in morphologically intact cereal grains and pulse seeds suggests that adjustment should be made to estimate pre-charring values from archaeobotanical determination (i.e., by deducting 1‰). With regard to land use reconstruction, elevation of $\delta^{15}N$ on the order of 1‰ may affect overall interpretation, especially where values fall near thresholds identified through modern work on manuring regimes (Fraser et al. 2011). In a palaeodietary context, increases in $\delta^{15}N$ values of 1‰ could have a significant effect on modelling of ancient human diet (Hedges and Reynard 2006). Finally, we suggest that archaeobotanical %C and %N data be used to help assess the preservation of the original organic molecules and the reliability of the isotope data.

6. Conclusion

We conclude that well preserved and undistorted charred cereal grain and pulse seed, indicative of extended heating at low temperature, offer an important source of information on past growing conditions and potential dietary contributions. While $\delta^{13}C$ values remain

largely unaffected by charring, there is a systematic and predictable increase in $\delta^{15}N$ values in both cereal grains and pulse seeds of around 1‰ on average. Percentage C and N and C:N data provide useful criteria for assessing archaeobotanical preservation and their comparability to modern charred analogue data. Our work on ABA pre-treatment suggests that it is a safe means of removing contaminants and appears to have a beneficial effect on archaeobotanical C:N composition. Our burial and contamination studies indicate that charred cereal grains and pulse seeds are remarkably inert and resistant to microbial action. Any sampling strategy needs to take account of natural variation in plant $\delta^{13}C$ and $\delta^{15}N$ values, and the bulk sampling strategy used here (i.e., homogenisation of *c.* 20-30 grains) targeting archaeological contexts with primary concentrations of stored material should be particularly useful for assessing spatial or chronological differences. More work is needed to establish relevant charring conditions for other crop and other plant types, and their isotopic effects. A wider range of burial conditions and durations would also help to broaden the conclusions reached here. Further bulk isotope work also needs to be underpinned by complementary biochemical analyses to better understand changes in molecular structure.

Acknowledgements

The work reported here was funded by the Natural Environment Research Council (NERC standard grant NE/E003761/1, PI Bogaard).

REFERENCES

Aguilera., M., J. L. Araus, et al. (2008). "Stable carbon and nitrogen isotopes and quality traits of fossil cereal grains provide clues on sustainability at the beginnings of Mediterranean agriculture." Rapid Communications in Mass Spectrometry 22(11): 1653-1663.

Almendros, G. and J. Dorado (1999). "Molecular characteristics related to the biodegradability of humic acid preparations." European Journal of Soil Science 50(2): 227-236.

Araus, J. L., A. Febrero, et al. (1997). "Identification of Ancient Irrigation Practices based on the Carbon Isotope Discrimination of Plant Seeds: a Case Study from the South-East Iberian Peninsula." Journal of Archaeological Science 24(8): 729-740.

Ascough, P. L., M. I. Bird, et al. (2010a). "Alkali extraction of archaeological and geological charcoal: evidence for diagenetic degradation and formation of humic acids." Journal of Archaeological Science 38(1): 69-78.

Ascough, P. L., M. I. Bird, et al. (2010b). "Charcoal reflectance measurements: Implications for structural characterization and assessment of diagenetic alteration." Journal of Archaeological Science 37(7): 1590-1599.

684 Ascough, P. L., M. I. Bird, et al. (2008). "Influence of production variables and starting
685 material on charcoal stable isotopic and molecular characteristics." Geochimica et
686 Cosmochimica Acta 72(24): 6090-6102.
687 Benner, R., M. L. Fogel, et al. (1987). "Depletion of C in lignin and its implications for stable
688 carbon isotope studies." Nature 329(6141): 708-710.
689 Bird, M. I. and P. L. Ascough (2012). "Isotopes in pyrogenic carbon: A review." Organic
690 Geochemistry 42(12): 1529-1539.
691 Bird, M. I. and D. R. Grocke (1997). "Determination of the abundance and carbon isotope
692 composition of elemental carbon in sediments." Geochimica et Cosmochimica Acta
693 61(16): 3413-3423.
694 Bland Helen, A., F. van Bergen Pim, et al. (1998). Early Diagenetic Transformations of
695 Proteins and Polysaccharides in Archaeological Plant Remains. Nitrogen-Containing
696 Macromolecules in the Bio- and Geosphere, American Chemical Society. 707: 113-
697 131.
698 Boardman, S. and G. Jones (1990). "Experiments on the effects of charring on cereal plant
699 components." Journal of Archaeological Science 17(1): 1-11.
700 Bogaard, A., T. H. E. Heaton, et al. (2007). "The impact of manuring on nitrogen isotope
701 ratios in cereals: archaeological implications for reconstruction of diet and crop
702 management practices." Journal of Archaeological Science 34(3): 335-343.
703 Boyd, M., T. Varney, et al. (2008). "Reassessing the northern limit of maize consumption in
704 North America: stable isotope, plant microfossil, and trace element content of
705 carbonized food residue." Journal of Archaeological Science 35(9): 2545-2556.
706 Braadbaart, F. (2008). "Carbonisation and morphological changes in modern dehusked and
707 husked *Triticum dicoccum* and *Triticum aestivum* grains." Vegetation History and
708 Archaeobotany 17(1): 155-166.
709 Braadbaart, F. and P. F. van Bergen (2005). "Digital imaging analysis of size and shape of
710 wheat and pea upon heating under anoxic conditions as a function of the
711 temperature." Vegetation History and Archaeobotany 14(1): 67-75.
712 Braadbaart, F., J. van der Horst, et al. (2004). "Laboratory simulations of the transformation
713 of emmer wheat as a result of heating." Journal of Thermal Analysis and Calorimetry
714 77(3): 957-973.
715 Braadbaart, F., J. J. Boon, et al. (2004b). "Laboratory simulations of the transformation of
716 peas as a result of heat treatment: changes of the physical and chemical properties."
717 Journal of Archaeological Science 31(6): 821-833.
718 Czimczik, C. I., C. M. Preston, et al. (2002). "Effects of charring on mass, organic carbon,
719 and stable carbon isotope composition of wood." Organic Geochemistry 33(11):
720 1207-1223.
721 DeNiro, M. J. (1985). "Postmortem preservation and alteration of in vivo bone collagen
722 isotope ratios in relation to palaeodietary reconstruction." Nature 317: 806-809.
723 Dürrwächter, C., O. E. Craig, et al. (2006). "Beyond the grave: variability in Neolithic diets
724 in Southern Germany?" Journal of Archaeological Science 33(1): 39-48.
725 Fernandes, M. and E. Krull (2008). "How does acid treatment to remove carbonates affect the
726 isotopic and elemental composition of soils and sediments?" Environmental
727 Chemistry 5(1): 33-39.
728 Ferrio, J. P., N. Alonso, et al. (2006). "Carbon isotope composition of fossil charcoal reveals
729 aridity changes in the NW Mediterranean Basin." Global Change Biology 12(7):
730 1253-1266.
731 Ferrio, J. P., J. L. Araus, et al. (2005). "Water management practices and climate in ancient
732 agriculture: Inferences from the stable isotope composition of archaeobotanical
733 remains." Vegetation History and Archaeobotany 14(4): 510-517.

734 Ferrio, J. P., J. Voltas, et al. (2007). Reconstruction of Climate and Crop Conditions in the
735 Past Based on the Carbon Isotope Signature of Archaeobotanical Remains. Terrestrial
736 Ecology. 1: 319-332.
737 Fiorentino, G., V. Caracuta, et al. (2008). "Third millennium B.C. climate change in Syria
738 highlighted by Carbon stable isotope analysis of 14C-AMS dated plant remains from
739 Ebla." Palaeogeography, Palaeoclimatology, Palaeoecology 266(1-2): 51-58.
740 Fraser, R. A., A. Bogaard, et al. (2011). "Manuring and stable nitrogen isotope ratios in
741 cereals and pulses: towards a new archaeobotanical approach to the inference of land
742 use and dietary practices." Journal of Archaeological Science 38(10): 2790-2804.
743 Goette, S., M. Williams, et al. (1994). "Toward reconstructing ancient maize: Experiments in
744 processing and charring." Journal of Ethnobiology 14(1): 1-21.
745 Hart, J. P., W. A. Lovis, et al. (2007). "Paleodietary implications from stable carbon isotope
746 analysis of experimental cooking residues." Journal of Archaeological Science 34(5):
747 804-813.
748 Hart, J. P., G. R. Urquhart, et al. (2009). "Non-linear relationship between bulk $\delta^{13}C$ and
749 percent maize in carbonized cooking residues and the potential of false-negatives in
750 detecting maize." Journal of Archaeological Science 36(10): 2206-2212.
751 Head, M. J. and W. J. Zhou (2000). "Evaluation of NaOH leaching techniques to extract
752 humic acids from palaeosols." Nuclear Instruments and Methods in Physics Research
753 Section B: Beam Interactions with Materials and Atoms 172(1-4): 434-439.
754 Heaton, T. H. E., G. Jones, et al. (2009). "Variations in the $^{13}C/^{12}C$ ratios of modern wheat
755 grain, and implications for interpreting data from Bronze Age Assiros Toumba,
756 Greece." Journal of Archaeological Science 36(10): 2224-2233.
757 Higham, T. F. G., H. Barton, et al. (2009). "Radiocarbon dating of charcoal from tropical
758 sequences: results from the Niah Great Cave, Sarawak, and their broader
759 implications." Journal of Quaternary Science 24(2): 189-197.
760 Kelly, E. F., S. W. Blecker, et al. (1998). "Stable isotope composition of soil organic matter
761 and phytoliths as paleoenvironmental indicators." Geoderma 82(1-3): 59-81.
762 Knicker, H., G. Almendros, et al. (1996). "13C- and 15N-NMR spectroscopic examination of
763 the transformation of organic nitrogen in plant biomass during thermal treatment."
764 Soil Biology and Biochemistry 28(8): 1053-1060.
765 Loader, N. J., I. Robertson, et al. (2003). "Comparison of stable carbon isotope ratios in the
766 whole wood, cellulose and lignin of oak tree-rings." Palaeogeography,
767 Palaeoclimatology, Palaeoecology 196(3–4): 395-407.
768 Marino, B. D. and M. J. Deniro (1987). "Isotopic analysis of archaeobotanicals to reconstruct
769 past climates: Effects of activities associated with food preparation on carbon,
770 hydrogen and oxygen isotope ratios of plant cellulose." Journal of Archaeological
771 Science 14(5): 537-548.
772 Märkle, T. and M. Rösch (2008). "Experiments on the effects of carbonization on some
773 cultivated plant seeds." Vegetation History and Archaeobotany 17(0): 257-263.
774 O'Leary, M. H. (1981). "Carbon isotope fractionation in plants." Phytochemistry 20: 553-567.
775 O'Leary, M. H. (1988). "Carbon Isotopes in Photosynthesis." BioScience 38(5): 328-336.
776 Park, R. and S. Epstein (1961). "Metabolic fractionatin of C^{13} and C^{12} in plants." Plant
777 Physiology 36(2).
778 Pettersen, R. C. (1984). The chemical composition of wood. Chemistry of Solid Wood. R. M.
779 Rowe. Washington, DC, American Chemical Society: 74.
780 Riehl, S. (2008). "Climate and agriculture in the ancient Near East: a synthesis of the
781 archaeobotanical and stable carbon isotope evidence." Vegetation History and
782 Archaeobotany 17(0): 43-51.

783 Riehl, S., R. Bryson, et al. (2008). "Changing growing conditions for crops during the Near
784 Eastern Bronze Age (3000-1200 BC): the stable carbon isotope evidence." Journal of
785 Archaeological Science 35(4): 1011-1022.
786 Roberts, N., W. J. Eastwood, et al. (2011). "Climatic, vegetation and cultural change in the
787 eastern Mediterranean during the mid-Holocene environmental transition." The
788 Holocene 21(1): 147-162.
789 Silván, J. M., J. van de Lagemaat, et al. (2006). "Analysis and biological properties of amino
790 acid derivates formed by Maillard reaction in foods." Journal of Pharmaceutical and
791 Biomedical Analysis 41(5): 1543-1551.
792 Taylor, R. E. (1987). Radiocarbon Dating. An archaeological perspective. Orlando, USA,
793 Academic Press.
794 Tieszen, L. L. (1991). "Natural variations in the carbon isotope values of plants: Implications
795 for Archaeology, Ecology and Palaeoecology." Journal of Archaeological Science 18:
796 227-248.
797 Turekian, V. C., S. Macko, et al. (1998). "Causes of bulk carbon and nitrogen isotopic
798 fractionations in the products of vegetation burns: laboratory studies." Chemical
799 Geology 152(1-2): 181-192.
800 Turney, C. S. M., D. Wheeler, et al. (2006). "Carbon isotope fractionation in wood during
801 carbonization." Geochimica et Cosmochimica Acta 70(4): 960-964.
802 van der Veen, M. (2007). "Formation processes of desiccated and carbonized plant remains -
803 the identification of routine practice." Journal of Archaeological Science 34(6): 968-
804 990.
805 Voltas, J., J. P. Ferrio, et al. (2008). "Stable carbon isotopes in archaeobotanical remains and
806 palaeoclimate." Contributions to Science 4(1): 21-31.
807
808

List of figures

List of tables

Table 5. The stable carbon and nitrogen isotope values, percentage carbon and nitrogen contents and C:N ratios of charred bread wheat grains and peas buried in garden soil for up to 24 months.

Table 6. The stable carbon and nitrogen isotope values and percentage carbon and nitrogen contents of charred modern and archaeological broomcorn millet and archaeological bitter vetch soaked in humic acid solution for periods of 6, 12 and 24 months (1SD in parentheses).

List of supplementary tables

Supplementary Table 1. Stable carbon isotope values and percentage carbon content of uncharred and charred cereals and pulses. Charring conditions were at 230°C for 24 hours.

Supplementary Table 2. Stable nitrogen isotope values and percentage nitrogen content of uncharred and charred cereals and pulses. Charring conditions were at 230°C for 24 hours.

Supplementary Table 3. The stable carbon and nitrogen isotope values, percentage carbon and nitrogen contents and C:N ratios of untreated and ABA pre-treated modern and archaeological cereals and pulses (1SD in parentheses).

Supplementary Table 4. The mean percentage carbon and nitrogen contents and C:N ratios of modern experimentally cereal and pulse crops (1SD in parentheses) (data from Fraser et al. 2011).

Supplementary Table 5. Stable carbon and nitrogen isotope variability in pulse seeds observed: 1) within a single pod containing six beans, and 2) from bulk samples taken from replicate experimental field plots at the same site (lentil data are from Fraser et al. 2011).

Supplementary Table 6. The stable carbon and nitrogen isotope variability of cereal grains observed: 1) within individual grains in single ears of wheat, and 2) bulk samples taken from replicate experimental field plots at the same location (data from Fraser et al. 2011).

Table 1

The weights of uncharred and charred cereal grains and pulses seeds heated at 230℃ for 24 hours.

Cereal taxon	N of grains	Weight in grams				% weight loss	Morphological observations
		uncharred		charred			
		total sample	mean per seed	total sample	mean per seed		
bread wheat	25	1.45	0.06	0.72	0.03	50.39	intact
emmer	25	0.94	0.04	0.52	0.02	44.70	intact
einkorn	25	0.84	0.03	0.47	0.02	44.00	intact
naked barley	25	1.40	0.06	0.73	0.03	47.55	intact
pea	25	7.67	0.31	3.75	0.15	51.14	much testa flaked off
lentil	25	0.97	0.04	0.50	0.02	48.40	much testa flaked off
millet	25	0.14	0.01	0.07	0.00	50.70	many loose flakes
broad bean	10	5.31	0.53	2.55	0.26	51.90	much testa flaked off

Table 2

The stable carbon and nitrogen isotope values and percentage nitrogen content of uncharred and charred cereal grains and broomcorn millet seeds heated for a time-series from 2, 4, 8 and 24 hours at 230°C. The data marked with an asterisk are from Bogaard et al. (2007) and these data were not analysed for $\delta^{13}C$ values or % C contents.

Sample ID.	Cereal taxon	Charring time (at 230°C)					Difference 0 to 24 hrs charred
		0 hrs	2 hrs	4 hrs	8 hrs	24 hrs	
$\delta^{15}N$							
BAD04-12W	bread wheat	3.3	4.0	4.6	4.0	4.2	0.9
BADD04-6W	bread wheat	4.5	4.4	5.0	4.4	5.1	0.6
BAD04-18W	bread wheat	1.4	1.8	1.6	1.4	1.8	0.4
ROT04-W7	bread wheat	5.8	6.8	6.9	7.1	7.4	1.6
ROT04-W1	bread wheat	0.5	0.3	0.4	0.4	0.5	0.1
SUT08-37K	einkorn	3.2	3.7	3.9	4.0	4.5	1.2
SUT08-31K	einkorn	2.5	2.5	2.9	2.8	3.3	0.8
RC_millet	millet	-0.1	0.2	0.4	0.7	0.9	1.0
%N							
BAD04-12W	bread wheat	1.2	1.5	1.7	1.7	2.2	0.9
BADD04-6W	bread wheat	1.4	1.5	1.5	1.8	2.3	1.0
BAD04-18W	bread wheat	1.2	1.3	1.4	1.6	1.8	0.7
ROT04-W7	bread wheat	1.5	1.9	1.8	2.1	3.0	1.5
ROT04-W1	bread wheat	1.5	1.6	1.7	1.8	2.3	0.7
SUT08-37K	einkorn	2.3	2.6	3.4	3.7	3.7	1.5
SUT08-31K	einkorn	2.1	2.6	3.3	3.6	3.8	1.7
RC_millet	millet	1.5	1.5	1.8	2.0	2.1	0.6
$\delta^{13}C$							
SUT08-37K	einkorn	-27.9	-27.5	-27.6	-27.6	-27.6	0.3
SUT08-31K	einkorn	-27.1	-27.3	-27.0	-27.0	-27.5	-0.4
SUT08-25E	emmer	-26.3	-25.8	-25.3	-25.5	-25.5	0.8
RC_millet	millet	-12.3	-12.3	-12.3	-12.4	-12.4	0.0
%C							
SUT08-37K	einkorn	38.3	48.6	61.2	62.0	66.1	27.8
SUT08-31K	einkorn	39.1	48.2	57.3	61.5	63.2	24.1
SUT08-25E	emmer	39.0	47.8	55.1	59.2	67.1	28.1
RC_millet	millet	41.1	43.6	51.4	54.0	54.2	13.1
C:N ratio							
SUT08-37K	einkorn	19.5	21.6	20.7	19.8	20.6	1.1
SUT08-31K	einkorn	22.1	22.0	20.4	20.1	19.5	-2.6
RC_millet	millet	32.7	34.1	33.1	32.1	30.1	-2.6

Table 3

Stable carbon isotope values and percentage carbon contents of uncharred and charred wheat grains and broomcorn millet seeds heated at 230°C from 2 to 24 hours and between 250-400°C for either 2 or 6 hours.

Cereal taxon		Charring temperatures								
		230°C			250°C	270°C	300°C		400°C	
	0 hrs	2 hrs	6 hrs	8 hrs	6 hrs	6 hrs	2 hrs	6 hrs	2 hrs	6 hrs
$\delta^{13}C$										
bread wheat	-23.8	-23.9	-23.8	nd	nd	nd	-23.7	-23.9	-23.9	-24.0
durum wheat	-25.9	-25.9	-25.6	nd	nd	nd	-25.6	-25.5	-25.5	-25.6
durum wheat	-23.3	-23.3	-23.2	-22.9	-22.9	-23.1	nd	nd	nd	nd
%C										
bread wheat	44.0	49.1	63.9	nd	nd	nd	64.8	72.9	66.6	73.6
durum wheat	41.1	48.4	73.9	nd	nd	nd	73.1	74.5	67.7	78.5
durum wheat	42.1	51.1	57.6	nd	64.3	64.1	nd	nd	nd	nd

Table 4

Summary of the mean changes in stable carbon and nitrogen values, percentage carbon and nitrogen and C:N ratios of cereal grains and pulses heated at 230℃ for 24 hours (1SD in parentheses).

Crop taxon	N of bulk samples*	Mean changes after heating at 230°C for 24 hours		
		$\delta^{15}N$		%N
grain	25	0.9 (0.5)		1.3 (0.4)
pea	5	1.1 (0.6)		3.0 (0.3)
broad bean	3	1.0 (0.1)		3.2 (0.7)
lentil	7	0.8 (0.1)		3.0 (0.1)
		$\delta^{13}C$		%C
grain	20	0.0 (0.3)		22.0 (4.1)
pea	5	0.0 (0.4)		22.9 (2.2)
broad bean	3	-0.1 (0.4)		19.9 (1.5)
lentil	7	-0.1 (0.1)		21.3 (1.2)
		C:N ratio		
grain	20	-3.3 (4.0)		
pea	5	-2.7 (0.3)		
broad bean	3	-1.7 (1.0)		
lentil	7	-2.0 (0.5)		
* each bulk sample consists of a minimum of either 20 to 30 individual grains for wheat, or 10 individual seeds for peas, lentils or broad beans				

Table 5

The stable carbon and nitrogen isotope values, percentage carbon and nitrogen contents and C:N ratios of charred bread wheat grains and peas buried in garden soil for up to 24 months

Sample ID.	Cereal taxon	Plant part	Uncharred	Charred 24 hrs at 230°C	Burial time (months)			
					6	12	18	24
$\delta^{15}N$								
BAD04-18W	bread wheat	grain	1.0	1.8	1.4	2.2	1.9	1.3
BOR07-44W	bread wheat	grain	6.0	7.3	6.4	nd	nd	6.8
BAD07-18P	pea	seed	0.6	1.1	1.0	1.2	1.4	0.6
% N								
BAD04-18W	bread wheat	grain	1.4	2.1	1.8	1.9	1.8	1.8
BOR07-44W	bread wheat	grain	1.8	2.8	3.1	nd	nd	3.1
BAD07-18P	pea	seed	3.6	6.5	6.6	6.2	6.5	7.2
$\delta^{13}C$								
BAD04-18W	bread wheat	grain	-26.6	-26.3	-26.5	-26.6	-26.4	-26.4
BOR07-44W	bread wheat	grain	-25.7	-25.7	-25.6	na	na	-25.5
BAD07-18P	pea	seed	-28.5	-29.3	-28.5	-29.1	-28.8	-29.0
% C								
BAD04-18W	bread wheat	grain	44.8	59.0	55.1	54.8	52.6	65.5
BOR07-44W	bread wheat	grain	39.7	62.3	70.9	na	na	80.6
BAD07-18P	pea	seed	39.2	58.1	59.9	55.1	56.9	60.5
C:N ratio								
BAD04-18W	bread wheat	grain	37.3	33.4	36.8	33.6	33.5	42.4
BOR07-44W	bread wheat	grain	26.5	26.3	27.0	na	na	30.8
BAD07-18P	pea	seed	12.9	10.5	10.6	10.5	10.2	9.8

Table 6

The stable carbon and nitrogen isotope values and percentage carbon and nitrogen contents of charred modern and archaeological broomcorn millet and archaeological bitter vetch soaked in humic acid solution for periods of 6, 12 and 24 months (1SD in parentheses)

Charred cereal taxon	unsoaked	Months of humic acid exposure			unsoaked	Months of humic acid exposure		
		6	12	24		6	12	24
	$\delta^{13}C$				% C			
modern millet	-12.4 (0.0)	-12.3 (0.0)	-11.7 (0.0)	-11.9 (0.01)	51	60.5 (4.1)	60.1 (2.4)	62.1 (1.1)
arch. millet	-10.8 (0.0)	-10.9 (0.0)	-10.9 (0.1)	-10.9 (0.0)	56.3 (1.8)	57.1 (1.8)	61.1 (0.2)	55.5 (1.2)
arch. bitter vetch	-25.2 (0.1)	-25.6 (0.1)	-25.4 (0.0)	-25.3 (0.0)	65.7 (1.6)	56.8 (1.2)	66.6 (3.2)	60.2 (0.8)
	$\delta^{15}N$				% N			
modern millet	0.9	0.8	1.2 (0.1)	1.2	2.10	2.5 (0.1)	2.3 (0.0)	2.7
arch. millet	4.3 (0.3)	5.3	6.2	4.4	2.2 (0.1)	2.3 (0.1)	1.9 (0.2)	2.2 (0.1)
arch. bitter vetch	1.3 (0.3)	1.3	1.3	0.1	5.5 (0.0)	5.7 (0.0)	5.7 (0.4)	4.3 (0.0)

Figure 1

A time-series of stable carbon and nitrogen isotope values and the percentage carbon and nitrogen contents of cereal grain and broomcorn millet, from uncharred (0) to heated for 2, 4, 8 and 24 hours at 230℃

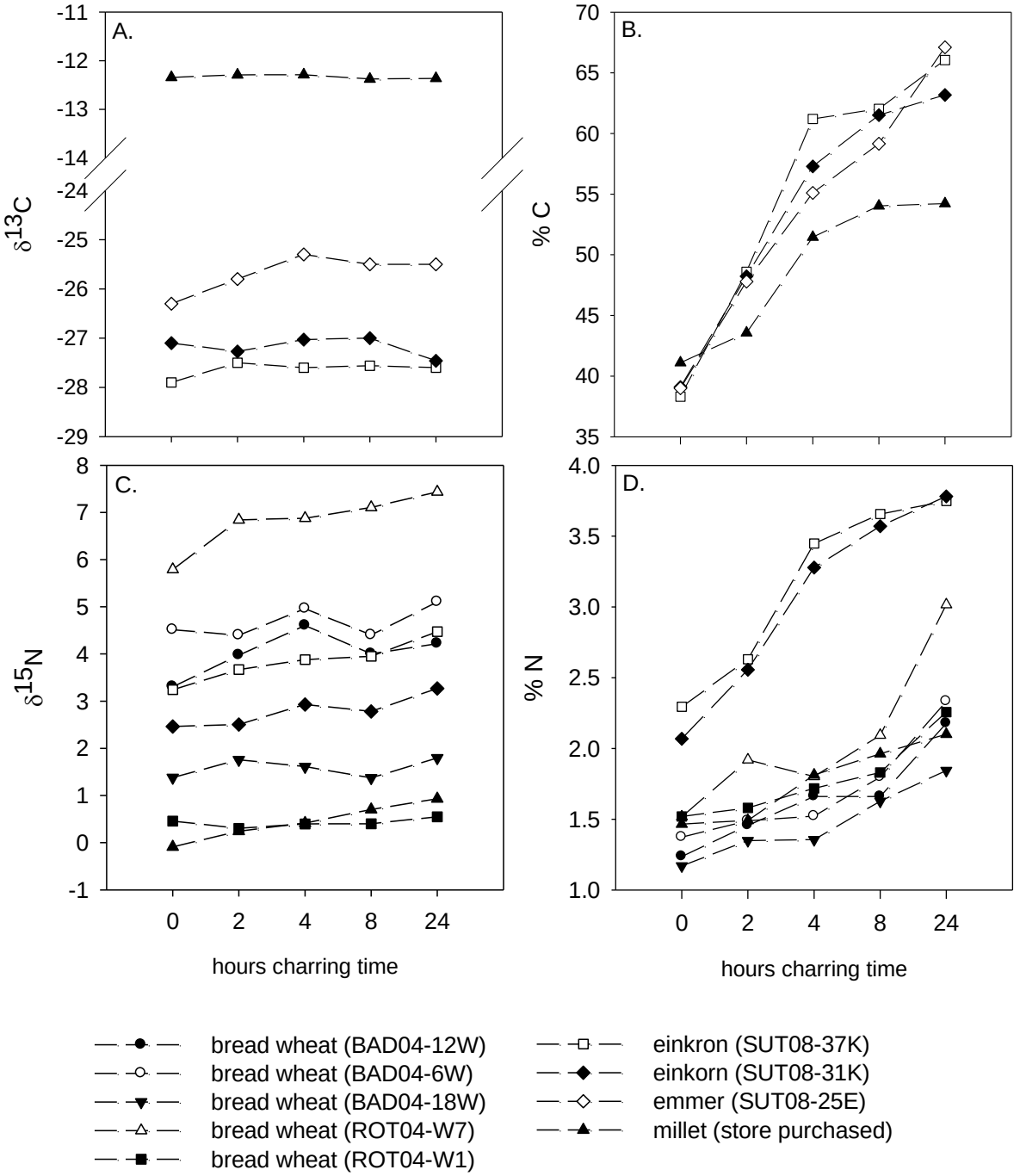


Figure 2

The effects of heating at 230℃ for 24 hours on the percentage carbon and nitrogen contents of cereal grains and pulse seeds (open symbols = uncharred crops and closed symbols = charred crops)

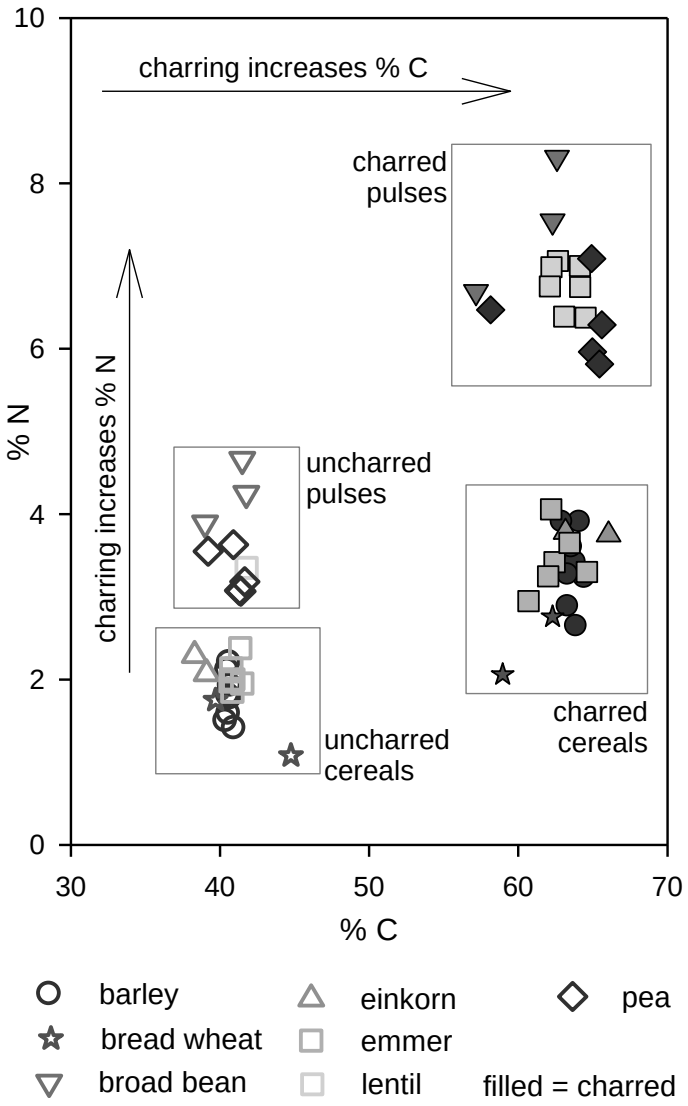


Figure 3

The effects of heating, at 230℃ for 24 hours, on the $\delta^{13}C$ and $\delta^{15}N$ values of cereal grains and pulse seeds, the uncharred δ values are normalised to zero to show individual isotopic differences due to charring (triangle = ‰ change in $\delta^{13}C$ values, circle = ‰ change in $\delta^{15}N$ values)

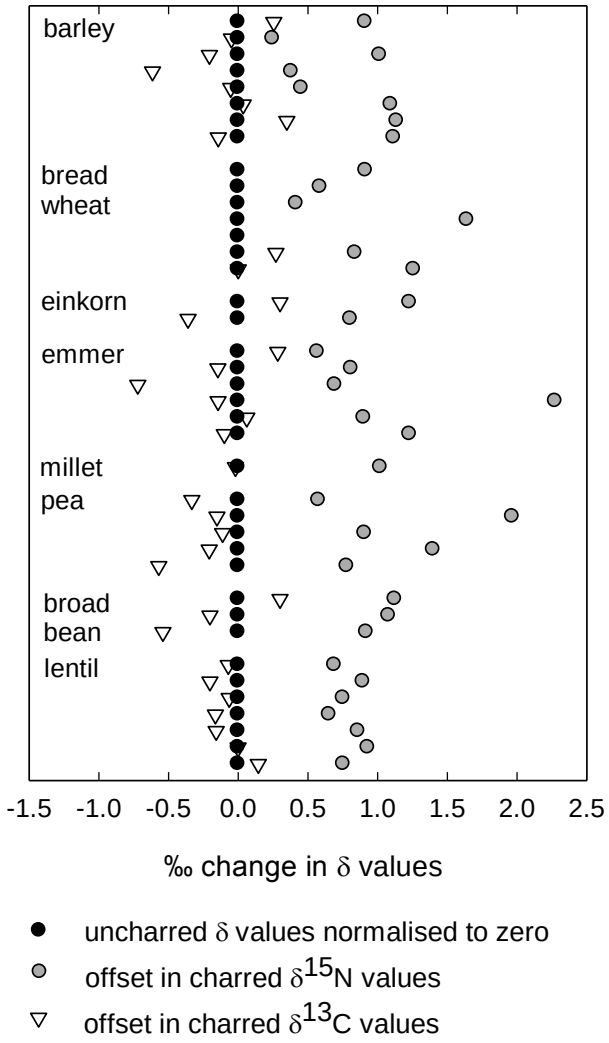


Figure 4

The stable carbon and nitrogen isotope values and percentage carbon and nitrogen contents of modern charred bread wheat grain and peas buried in garden soil for 6 to 24 months

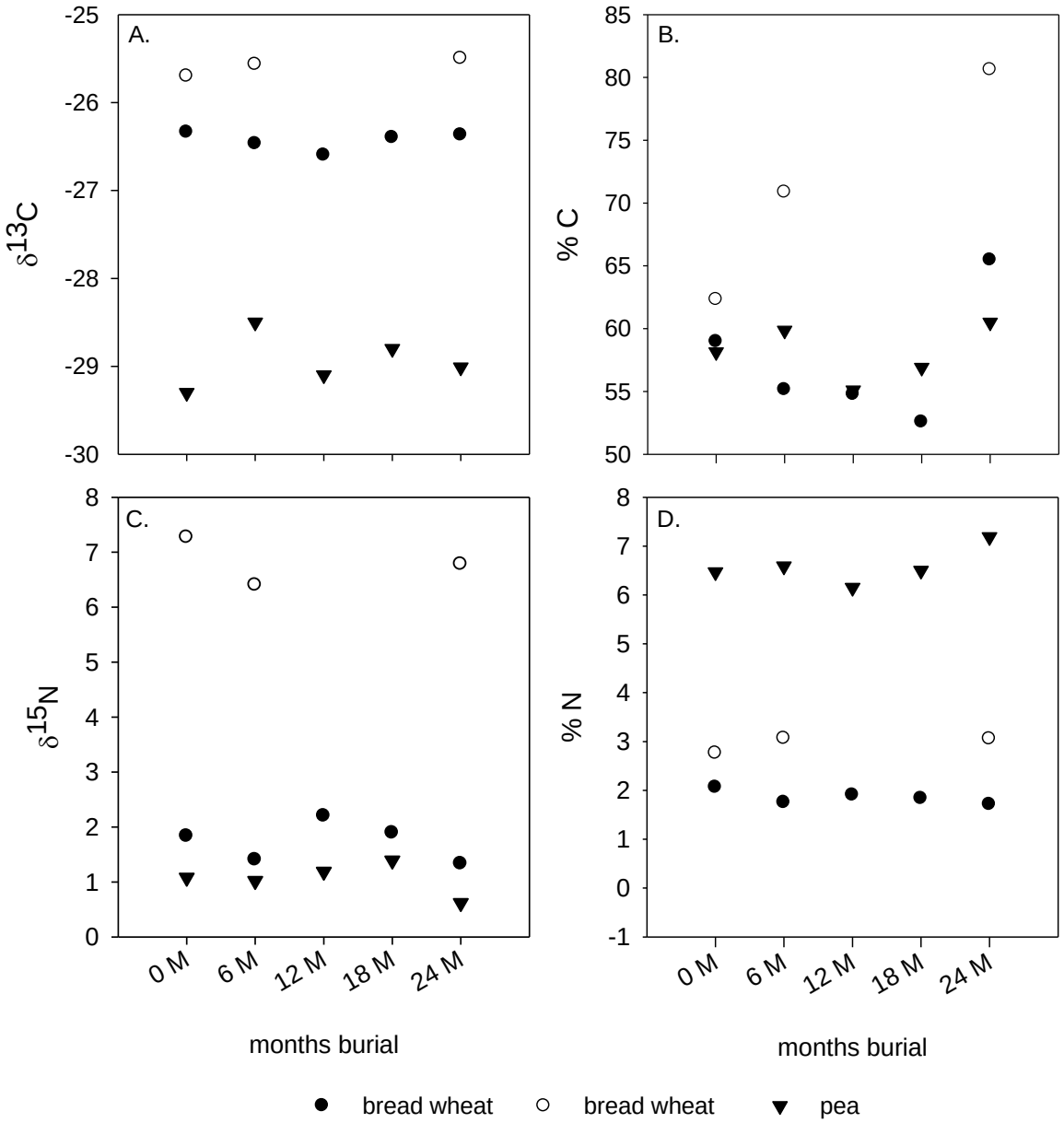


Figure 5

The differences in stable carbon and nitrogen isotope values and percentage carbon and nitrogen contents between control (untreated) and ABA pre-treated modern and archaeological cereal grains and pulses. The control values are normalised to zero in both graphs to show the quantity (difference) and direction of the effect pre-treatment had on each sample. Lines on Plot A) show the standard deviation (1SD) of the measurement errors for $\delta^{13}C$ and $\delta^{15}N$ values in this study.

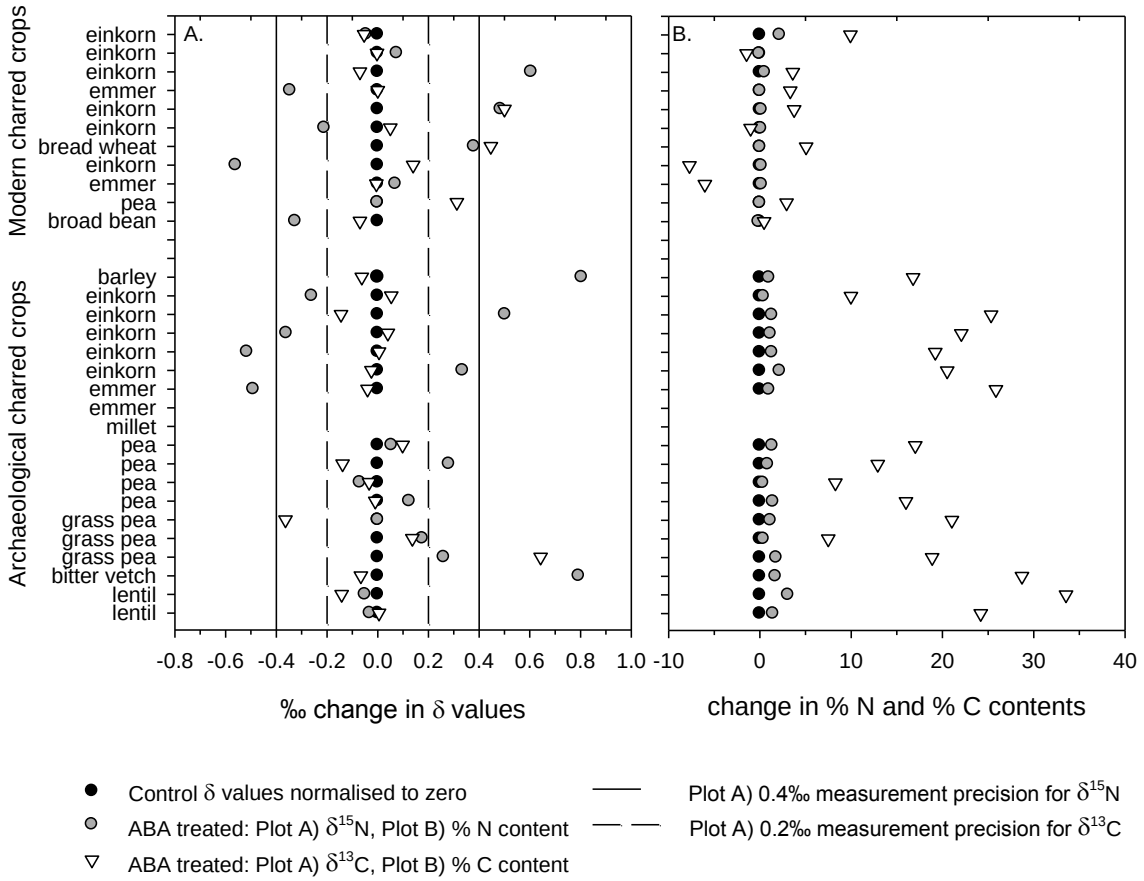


Figure 6

Stable carbon and nitrogen isotope values and percentage carbon and nitrogen contents of charred millet and bitter vetch seeds soaked in humic acid solution for up to 24 months.

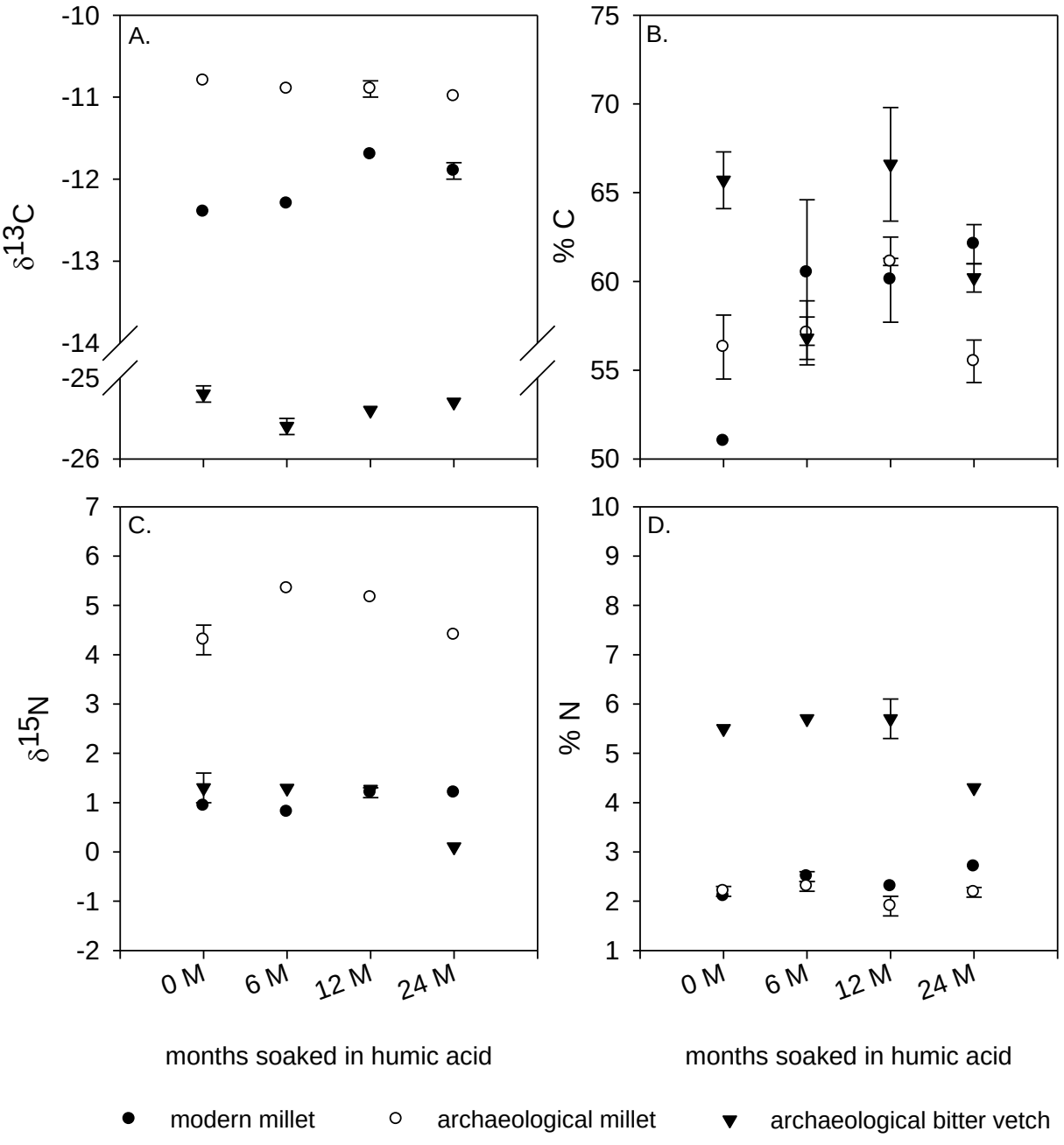
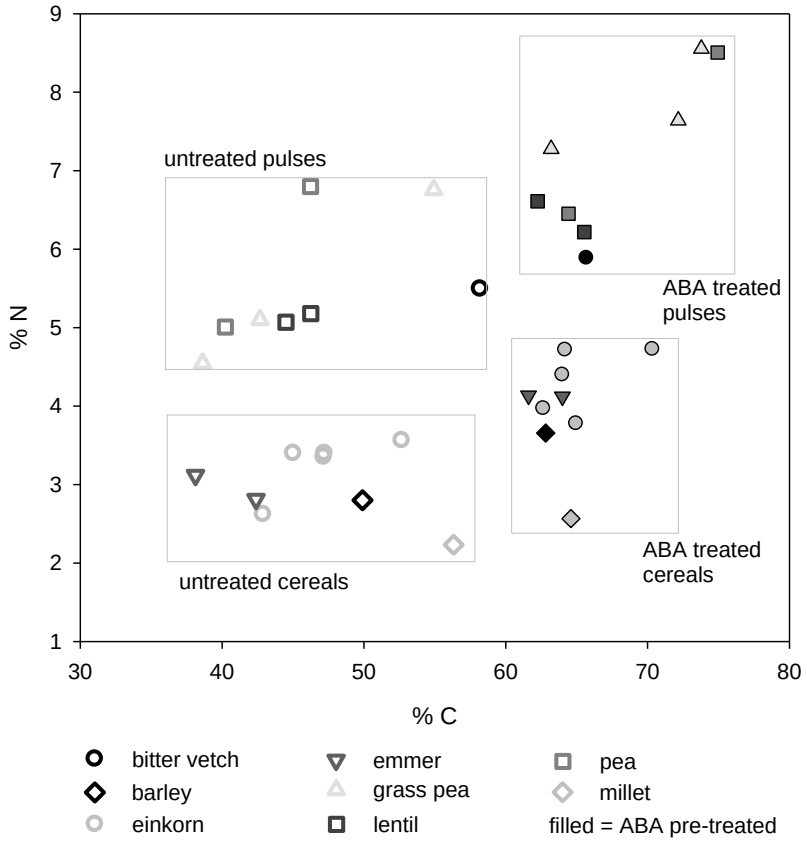


Figure 7

Figure 7

The percent carbon and nitrogen contents of untreated (control) and ABA pre-treated archaeological grains and pulses (open symbols = untreated, closed symbols = ABA pre-treated samples)



Supplementary Material_Table 1

Click here to download Supplementary Material: Sup Table 1.doc

Supplementary Material_Table 2

Click here to download Supplementary Material: Sup Table 2.doc

Supplementary Material_Table 3

Click here to download Supplementary Material: Sup Table 3.doc

Supplementary Material_Table 4

Click here to download Supplementary Material: Sup Table 4.doc

Supplementary Material_Table 5

Click here to download Supplementary Material: Sup Table 5.doc

Supplementary Material_Table 6

Click here to download Supplementary Material: Sup Table 6.doc