1   Assessing natural variation and the effects of charring, burial and pre-treatment on the stable

2   carbon and nitrogen isotope values of archaeobotanical cereals and pulses

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

5   and T.H.E. Heaton[4]

6   [1] School of Archaeology, 36 Beaumont Street, University of Oxford, Oxford, UK
7   [2] Organic Geochemistry Unit, Biogeochemistry Research Centre, School of Chemistry, University of Bristol,
8   Bristol, UK
9   [3] Department of Archaeology, University of Sheffield, Sheffield, UK
10   [4] NERC Isotope Geosciences Laboratory, British Geological Survey, Keyworth, UK

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

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17

18   **Abstract**

19   The aim of this study is to assess the potential of charred archaeobotanical cereal grain and
20   pulse seed δ^{13}C and δ^{15}N values to provide evidence of crop growing conditions and as a
21   potential component of palaeodietary studies. In order to reliably interpret archaeobotanical
22   δ^{13}C and δ^{15}N values it is necessary to take into account the impact of charring, burial and
23   laboratory pre-treatment procedures. We examine the effects of charring and burial on bulk
24   δ^{13}C, δ^{15}N, %C, %N and C:N ratios in modern cereal and pulse material, and of cleaning by
25   acid-base-acid (ABA) pre-treatment on modern and archaeobotanical charred material. Our
26   study utilised bulk grain and seed samples to help account for within-ear/pod and between-
27   plant variability in δ^{13}C and δ^{15}N values. Heating at relatively low temperatures and for
28   prolonged times (230°C for up to 24 hours) is conducive to the formation of well preserved,
29   undistorted charred cereal grain and pulse seed. Heating for 24 hours has a systematic and
30   predictable effect on δ^{15}N values, with increases of around 1‰ on average in cereal grains
31   and pulse seeds, and no consistent impact on δ^{13}C values. Increases in δ^{15}N are likely due to
32   the loss of lighter ^{14}N via N-containing volatiles. Burial (for up to 2 years) and ABA pre-
33   treatment have no significant effects on δ^{13}C or δ^{15}N values. After pre-treatment, however,
34   the %C and %N contents of the archaeobotanical material more closely resembles that of the
35   modern charred grains and seeds, suggesting that archaeobotanical remains accumulate non-
36   structural material during burial but retain their original carbon and nitrogen content.
37   Therefore %C, %N contents and C:N ratios can provide useful criteria for assessing
38   archaeobotanical preservation.
39

40   **1.   Introduction**

41   The charred remains of seed crops, especially cereals and pulses, form a large component of

42   the Eurasian archaeobotanical record from the later prehistoric period onwards and provide

43   evidence of plant use and consumption (e.g. Dennell 1976; Jacomet and Kreuz 1999).

44   Associated weed evidence offers further valuable information about farming practices and

45   land management (e.g. Jones 2000). More recently, the stable carbon and nitrogen isotope

46 analysis of crops has been explored as a means of obtaining *direct* and complementary
47 evidence of crop growing conditions. Carbon isotope values (δ^{13}C) of cereal crop remains
48 have mainly been used to explore water management regimes, and to a lesser extent changes
49 in local precipitation/moisture and climate conditions (Ferrio et al. 2005; Ferrio et al. 2006;
50 Ferrio et al. 2007; Fiorentino et al. 2008; Riehl 2008; Riehl et al. 2008; Voltas et al. 2008;
51 Heaton et al. 2009; Roberts et al. 2011; Wallace et al. in prep). Nitrogen isotope values (δ^{15}N)
52 have been shown to be a useful tool to identify land-use practices such as manuring and
53 associated changes in soil productivity as the nitrogen cycle changes with human intervention
54 (Bogaard et al. 2007; Aguilera. et al. 2008; Fraser et al. 2011). In palaeodiet studies the stable
55 isotope values of plant-diet components are usually the 'unknown factors' and their values
56 are estimated by subtracting trophic level diet to bone collagen fractionation values (usually
57 +3 to +5‰) from the collagen isotope values of herbivores. The addition of actual plant-diet
58 *food* isotope values to dietary models can potentially refine palaeodietary reconstructions.

59

60 In order to reliably interpret archaeobotanical crop stable isotope values it is necessary to take
61 the impact of preservation processes and burial conditions into account. The depositional
62 contexts of crop remains can vary from concentrated storage deposits to middens where
63 sweepings from cooking hearths and crop processing areas accumulated (van der Veen,
64 2007). Buried grain and seeds are thus variously exposed to potential contaminants and
65 diagenesis in soil, where original molecules can be removed or replaced by microbes or
66 infiltrating groundwater. The crop remains retrieved from archaeological sites are often
67 preserved by charring or carbonisation, a condition that involves heating but not combustion.
68 The charring processes are assumed responsible for preserving many of the original
69 biomolecules in ancient plant remains and rendering them less susceptible to degradation and
70 microbial attack (Knicker et al. 1996; Almendros and Dorado 1999). It is widely recognised
71 that charring can affect the morphology (e.g. size and shape) of crop remains (Boardman and
72 Jones 1990; Braadbaart and van Bergen 2005; Braadbaart 2008; Märkle and Rösch 2008), but
73 the effects of charring on cereal and pulse crop bulk δ^{13}C and δ^{15}N values have not been
74 explored in detail. In addition, the isotope effects of burial in soil have rarely been tested
75 under experimental conditions.

76

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

80 Base-Acid (ABA, see section 2.5) pre-treatment technique is routinely applied to charred

81 plant remains undergoing radiocarbon dating (Higham et al. 2009) and is considered essential

82 to remove non-structural and exogenous younger radiogenic ^{14}C because even a small amount

83 of contamination can result in incorrect dates (Taylor 1987). However, effects on δ^{13}C values

84 are of less concern for radiocarbon dating and little is known about the effects of the ABA

85 pre-treatment on the nitrogen that remains in ancient charred plant materials.

86

87 In this study we consider three main areas of stable isotope investigation. First, we examine

88 the effects of charring, under a set of conditions conducive to the optimal preservation of

89 morphologically intact, undistorted cereal grains and pulse seeds, in a set of Eurasian crops.

90 Secondly, we examine post-depositional effects through burial of charred crop remains in

91 garden soil and submersion in humic acid solution. Thirdly, we examine the effects of the

92 ABA pre-treatment as a cleaning method. Overall, we seek to identify if there are systematic

93 and significant changes in bulk δ^{13}C and δ^{15}N values, as well as percentage carbon and

94 nitrogen content and resulting C:N ratios, arising from any of these factors. It is also

95 necessary to take natural variation in the stable isotope values of crops grown under

96 standardized conditions into account. This assessment can help with determining adequate

97 sample sizes and identifying meaningful differences in isotope values among samples. We

98 report on the magnitude of stable isotope variations (from two associated studies - Bogaard et

99 al., 2007; Fraser et al., 2011) observed in grains and seeds from a single plant and in different

100 plants grown on the same experimental field/plot and discuss how these variations may

101 influence our interpretations. In this paper we focus principally on the effects that are

102 detectable at the bulk stable isotope level because these analyses are commonly used and

103 interpreted in archaeological science. Solid state ^{13}C NMR and FT-IR techniques examining

104 the molecular composition of modern and archaeological charred crop remains to assess

105 molecular changes due to charring, post-depositional preservation and ABA pre-treatment are

106 investigated by Styring et al (this volume) in conjunction with our studies.

107

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

109 DeNiro and Hastorf (1985) drew attention to the 'effects of charring' as a necessary precursor

110 for preserving the isotopic signatures of archaeobotanical remains; this was because the δ^{13}C

111 and δ^{15}N values of uncharred (desiccated) plants (many of which were edible legumes, seeds

112 and fruits), in contrast to charred plants, were highly variable and differed considerably from

113 their modern counterparts (e.g. 8-21‰ for δ^{15}N). Another early study (Marino and Deniro

114 1987) observed that the effects of various cooking procedures, one of which included
115 carbonization, indicated negligible effects on $\delta^{13}C$, $\delta^{18}O$ and δD values of plant cellulose; on
116 this basis, the authors suggested that there is potential for obtaining 'palaeoclimatic'
117 information from either cooked or uncooked plant remains. A substantial amount of research
118 relevant to our study has been undertaken on the isotopic effects of heating and charring of
119 wood, grasses and leaves (Turekian et al. 1998; Czimczik et al. 2002; Turney et al. 2006;
120 Bird and Ascough 2012). These studies focus mainly on the changes in $\delta^{13}C$ values rather
121 than $\delta^{15}N$ values. While contradictory and highly variable effects have been observed, the
122 magnitude of change in plant tissue $\delta^{13}C$ values due to charring have mostly been in the range
123 of 1 to 3‰ (Turekian et al. 1998; Czimczik et al. 2002; Turney et al. 2006; Ascough et al.
124 2008).

125

126 The preservation potential and $\delta^{13}C$ isotope composition of charred wood are recognised as
127 being the result of a complex set of parameters, including charring temperatures and times,
128 aerobic or anaerobic conditions, soil pH and microbial or fungal attack, and also the initial
129 composition (e.g., proportions of cellulose and lignin) (Czimczik et al. 2002; Ascough et al.
130 2008; Ascough et al. 2010b). Wood charcoal studies provide highly relevant background to
131 the charring of crop remains studied here, but the biochemical composition and physical
132 structures of cereal grains and pulse seeds vary significantly from wood. Wood is comprised
133 predominantly of ~65-75% carbohydrates (~35 to 40% cellulose and ~25 to 35%
134 hemicelluloses), ~18- 28% lignin and the remainder are ~4 to 10% organic extractives, which
135 can include compounds such as lipids, waxes, alkaloids, proteins, phenolics, simple sugars,
136 starches and essential oils (Pettersen 1984). In contrast, cereal grains and pulse seeds are
137 composed of negligible lignin or cellulose (some in the outer epidermis), many
138 polysaccharides (with ~60% starch), smaller amounts of proteins (~3 to 8%) and very low
139 amounts of lipids (~2%) (Braadbaart et al. 2004a). For this reason, the effects of charring and
140 preservation specific to crop remains warrant more investigation.

141

142 The changes in plant $\delta^{13}C$ values observed due to heating can be influenced by the different
143 stable isotope compositions of individual biochemical components, such as lipids, lignins,
144 carbohydrates and proteins. Differences in the $\delta^{13}C$ values of these components can depend
145 on the plant species and the tissues analysed. For example, lignin can be ~1‰ lower than and
146 cellulose ~2‰ higher than bulk wood $\delta^{13}C$ values (O'Leary 1981; Benner et al. 1987; Tieszen
147 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*

180 A number of studies have sought to replicate the conditions under which charred
181 archaeobotanical crop seeds/grains are preserved, including wheats (Boardman and Jones
182 1990; Braadbaart et al. 2004a, 2005; Braadbaart 2008), pea (Braadbaart et al. 2004b), millet
183 (Yang et al. 2011), sunflower (Braadbaart and Wright 2007) and maize (Goette et al. 1994).
184 Different authors have defined the point at which 'charring' occurs in different ways: as
185 colour change from brown to black (Boardman and Jones 1990), or as the complete
186 conversion of starch and proteins into larger and more stable polymorphic compounds (e.g.
187 Maillard reaction products) relatively resistant to microbial attack (Bland et al. 1998;
188 Braadbaart et al. 2004b; Silván et al. 2006).

189

190 Through experimental charring of emmer wheat grain, Braadbaart et al. (2004) concluded
191 that charred archaeobotanical grain must have been heated to temperatures in excess of
192 310℃, since it is only above this threshold after two hours' heating (the maximum period
193 considered) that sufficient molecular changes are considered to occur to prevent microbial
194 degradation after burial. Charring above ~250℃, however, causes marked swelling,
195 protrusions and other distortions in both emmer and free-threshing wheat grain (Braadbaart
196 2008). Other work with millet suggests that these grains may be reduced to ash above 300℃
197 (Yang et al. 2011). In peas, cracking of the cotyledons is common at temperatures in excess
198 of 310℃ (Braadbaart et al. 2004b). Experimental charring of einkorn grain by Charles and
199 colleagues (in prep) shows that, even when the temperature is raised very gradually over an
200 extended period, characteristic distortions consistently occur when grains are heated above
201 250℃. The important implication is that very well preserved and undistorted
202 archaeobotanical grain was heated to lower temperatures.

203

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

213

214 For the purposes of this study, therefore, most experimental charring was conducted in

215 reducing conditions at 230℃, with variable but lengthy charring times, from 2 to 24 hours.

216 Charring for up to 24 hours can cause very slight swelling but little other distortion, thus

217 replicating well preserved assemblages of cereal grain with fully intact morphology (e.g.,

218 'stores' of cereal grain protected from direct heat – e.g. Jones et al. 1986). Our reasoning is

219 that such archaeobotanical material provides the best conditions for interpreting stable

220 isotope values, both because the grain is highly identifiable (often to species) and because it

221 tends to derive from primary deposits that are well defined stratigraphically.

222

223 *2.2.   Burial in soil*

224 While charring processes can be simulated experimentally, the diagenetic processes that

225 occur in the burial environment, perhaps over many thousands of years, remain the most

226 unpredictable factors. We sought to replicate a likely depositional environment by burying

227 charred wheat grains and peas in common garden soil. We recognise that diagenetic

228 alterations may occur at any time during burial and that experimental burial is constrained by

229 project research time. Nevertheless, we consider that, if there are original molecules less

230 resistant to attack remaining in the charred materials, they will most likely change, degrade or

231 be removed in the initial years of burial and hence these early changes may be detectable in a

232 relatively short-term experiment.

233

234 *2.3.   Humic acid contamination*

235 Humic acids are considered a potential contaminant in buried organic matter. However,

236 humic acids and their structures and effects are highly variable and the longer-term isotope

237 effects on buried plant matter remain largely undetermined (Head and Zhou 2000; Ascough

238 et al. 2010a). Our research includes a two-year long experiment to intentionally contaminate

239 charred modern and archaeological millet seeds by soaking the seeds in a humic acid solution

240 mixed with garden soil. This experiment used millet as a cereal because it is a C_4 plant and its

241 grains have $\delta^{13}C$ values of approximately 9 to 13‰ in accordance with this photosynthetic

242 group (O'Leary 1988). The modern charred millet used here had a $\delta^{13}C$ value of -12.4‰ and

243 the archaeological charred millet was -10.8‰. The humic acid used here has a $\delta^{13}C$ value of

244 -26.7‰, which is within the range typical for C_3 plants; therefore contamination with carbon

245 from the humic acid solution should cause the $\delta^{13}C$ values of the millet seeds to become more

246 negative. Seeds from archaeological bitter vetch were also included in the experiment to

247 monitor changes in C$_3$ crop plants; however, contamination of the bitter vetch carbon will be
248 harder to detect than in the millet because the δ^{13}C values of the bitter vetch, ~ -25.2‰, are
249 similar to the δ^{13}C values of the humic acid solution. The humic acid solution has a very low
250 %N content of ~0.6% and a δ^{15}N value of 1.4‰, which is similar to the original δ^{15}N values
251 of the un-soaked charred millet (0.9‰) and bitter vetch (1.2‰) seeds; therefore, significant
252 changes in the soaked samples are unlikely to be detected within machine error (see section
253 3.5). Changes in the %C and %N contents of the soaked seeds are also examined.
254
255 *2.4. ABA pretreatment*
256 The ABA pre-treatment consists of three main steps, 1) the application of a strong acid, such
257 as hydrochloric acid (HCl), to remove non-structural carbon, 2) application of a base, such as
258 sodium hydroxide (NaOH), to remove humic acid contaminants, 3) final application of acid
259 to remove CO$_2$ absorbed from the air during step 2. For our studies we wish to examine the
260 effects of the ABA pre-treatment method on the bulk δ^{13}C and δ^{15}N values of modern and
261 archaeological charred crop remains. We are particularly concerned with the fate of nitrogen
262 because the N contents in cereal grains and pulse seeds are low (~0.5% to ~4%) compared to
263 carbon (~30% to 45%); we seek to examine the effects on %N and δ^{15}N values to see if
264 original N is retained and/or detrimentally altered by the ABA pre-treatment methods. The
265 work of Styring et al. (this volume) considers the biochemical compositions of a subset of
266 these same samples.
267
268 2.5. *Natural stable isotope variability in modern cereal and pulse crops*
269 Our investigation of crop sample stable isotope variability identified different levels of
270 isotope variation that need to be considered. Variations (1 SD) in δ^{13}C values within and
271 between wheat plants may range up to 0.7‰ (Heaton et al., 2009). A mean variation of 1.8 ±
272 1‰ in the δ^{15}N values of individual grains within single ears of wheat (N = 4 ears, each
273 containing ~17 to 21 individual grains) was observed by Bogaard et al. (2007), and our study
274 measured a 0.7‰ range in δ^{15}N values between 6 individual broad beans within a single pod
275 (Supplementary Table 5). In addition, there were mean ranges of 1.8 ± 1.2‰ for δ^{15}N values
276 and 0.4 ± 0.2‰ for δ^{13}C values observed in 38 sets of bulk samples of wheats (each
277 comprising ~50 well-homogenised powdered grains) taken from three-four replicate field
278 plots at the same site, studied by Fraser et al (2011) (Supplementary Table 6). One important
279 implication here is that, even though sub-samples used for experiments are taken from the

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

285

286

287 **3.   Experimental methods and materials**

288 *3.1.   Charring cereal grains and pulse seeds*

289 Cereal and pulse samples were obtained from a collection of crops grown on experimental
290 field sites in the United Kingdom, central Europe and Syria; site descriptions and field
291 collection methods are published previously in Bogaard et al. (2007) and Fraser et al. (2011).
292 Bulk crop samples from different field plots were randomly split into sub-samples containing
293 25 to 50 cereal grains, or 10 pulse seeds. One sub-sample from each plot was retained as the
294 uncharred control. Crop sub-samples were subject to one of the following charring
295 conditions: at 230℃ for 2, 4, 8 or 24 hours; at 250℃ for 6 hours, at 270℃ for 6 hours, at
296 300℃ for 2 or 6 hours, at 400℃ for 2 or 6 hours. Although charred modern crop seeds and
297 pulses are not an exact biochemical replicate for the archaeological grains, we consider that
298 they are the closest available analogue to use as controls for our experiments.

299

300 Crop sub-samples were charred in a Heraeus electric oven. To achieve low oxygen
301 conditions, sub-samples were wrapped in aluminum foil and placed in a 250 ml beaker filled
302 with sand and then sealed with more foil. This set-up excludes oxygen sufficiently enough to
303 produce reducing conditions, and can be considered comparable to the conditions amongst
304 embers and ash in the bottom of a hearth or building fire. The burial of sub-samples in sand
305 also served to reduce the heating rate to which the material was exposed. The temperature
306 inside the beakers (measured using K-type thermocouple probes) increased at a rate of 1.5-
307 2℃/min. After reaching 230°C the samples were held at this temperature for 2 to 24 hours
308 and then allowed to cool within the sand in the closed oven. Sample weight loss due to
309 charring at 230℃ for 24 hours was calculated from pre- and post-charring weights

310

311 *3.2.   Burial in soil experiment*

312   A set of bread wheat, pea and broad bean sub-samples, previously charred at 230℃ for 24

313   hours, were placed in bags of agricultural soil and then buried under common garden soil in

314   Birmingham, United Kingdom (climate: temperate maritime, mean precipitation of 660

315   mm/year, mean high and low temperatures ~13 and 6℃, respectively), for up to 24 months

316   from May 2008 until May 2010. Sub-samples were placed in 15 cm^2 open-gauze fabric bags,

317   soil was added and mixed with the charred crop remains and then the bags were secured with

318   string and buried 30 cm deep. The agricultural soils surrounding the charred crop remains had

319   a δ^{13}C value of -27.6‰ and a δ^{15}N value of 15.6‰. Bags remained buried for 6, 12, 18 or 24

320   months. On retrieval, crop samples remains were recovered by wet-sieving, washed in

321   distilled water to remove adhering soil and air dried at ~40℃.

322

323   *3.3.   Humic acid contamination experiment*

324   Charred modern and archaeobotanical millet and archaeological bitter vetch seeds were

325   soaked in sealed vials of a humic acid solution (Fluka Analytical®) for periods of 6, 12 and

326   24 months. The humic acid soaking solution was a 10% solution made up with distilled water

327   and an additional 50 grams of garden soil; the pH was 5.8. The modern millet was charred at

328   230℃ for 24 hours prior to soaking. The charred archaeological material came from a bulk-

329   storage room in a burned-down building at the tell site of Late Bronze Age Assiros Toumba,

330   Greece (see Heaton and Jones, 2009). Each sub-sample consisted of approximately 50 to 100

331   seeds; after soaking, seeds were washed in distilled water and air- dried at 30℃, then ground

332   to a fine homogeneous powder using a mortar and pestle.

333

334   *3.4.   Acid-Base-Acid (ABA) pre-treatment*

335   Archaeobotanical cereal grain and pulse samples from six Neolithic or Bronze Age

336   archaeological sites (one in Jordan, three in Bulgaria, one in Greece and one in Germany)

337   were used to assess the effect of the ABA pre-treatment on isotope values, %C and %N

338   content. The material was recovered by a range of techniques including wet sieving and

339   direct sampling from primary concentrations. All of the archaeobotanical cereal grain and

340   pulse seeds derived from deposits that were very well preserved, with virtually no distortion

341   and fully intact morphology – that resembles modern material charred under optimal

342   conditions (see above). Two random sub-samples of around 10-20 grains/pulse seeds each

343   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.

377

3.6.   C:N ratio comparison

379   Our previous research on the $\delta^{13}C$ and $\delta^{15}N$ values of modern cereals and pulses (Bogaard et

380   al. 2007; Fraser et al. 2011, and additional unpublished data) enables us to measure the %C

381   and %N contents of 208 cereal and 93 pulse samples. The C:N ratios of cereals ranged from

382   20.9 to 33.3 and the ratios for pulses from 11.8 to 14.6 (Supplementary Table 4). The C:N

383   ratios of these uncharred modern cereals are compared to our experimental samples that

384   undergo charring, burial, contamination and ABA pre-treatments. In a similar way to the use

385   of C:N ratios in modern bone collagen to define collagen 'quality' (DeNiro 1985), we seek to

386   determine whether or not a specific range(s) of C:N values can be developed for assessing the

387   preservation of archaeobotanical crop stable isotope values.

388

389

390   **4.   Results**

391   *4.1.   The effects of heating on grain weight, morphology and %C and %N contents*

392   Table 1 summarises grain weight losses due to heating at 230℃ for 24 hours across a range of

393   cereals and pulses. Losses range from ~48 to 56% of fresh weight. The %C and %N contents

394   of cereal grain and pulses all increase with heating. Figure 1b and Table 3 show that the %C

395   content of cereal grains, heated at a constant 230℃ over a time series of 2, 4, 6 or 8, and 24

396   hours, increased steadily from ~39% to 65%; most of the increases occur within the first 4

397   hours of heating. Under higher temperatures, up to 400℃ for 2 to 6 hours, %C increases only

398   slightly more (to ~73 to 78%, Tables 2 and 3). The millet seeds show a markedly smaller

399   increase, starting at ~ 40%C (i.e. similar to other cereal grains), but rising to only 54%C with

400   charring at 230ºC. Figure 1d and Table 2, show that the %N contents of grains, heated at

401   230℃ over the same time series (0 to 24 hrs) also increased steadily.

402   For all samples heated at 230℃ for 24 hours (Supplementary Tables 1 and 2), the %C

403   contents of cereal grains (n=20) increased approximately 22% and pulse seeds (n=15)

404   increased approximately 20%. Heating increased the %N of cereal grains approximately

405   1.5% and pulse seeds approximately 2.9%. The decreases in weight and the increases in %C

406   and %N contents during heating largely reflect dehydration through loss of free water

407   (Styring et al. this volume). Although the overall relative increase in %N was slightly larger

408   than that of %C, changes in C:N ratios were small and still resulted in the cereal and pulse

409   groups remaining distinct (Figure 2).

410

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).

443

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).

452

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.

469

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

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477 the presence and removal of humic acids. The modern samples rinsed clear and to a neutral

478 pH after approximately two to three rinses in distilled water; in contrast the majority of the

479 archaeological samples required additional rinses for the solutions to become clear and reach

480 neutrality. The second and final addition of acid caused mild effervescence in only some of

481 the archaeological samples.

482

483 The responses of the charred material to the ABA pre-treatment are shown in Figure 5 and

484 Supplementary Table 3. There were small changes in the %C and %N of the modern charred

485 cereal and pulse samples, ranging from 9.9 to 0.5% (mean 1.2% ± 5.1) and 2.2 to 0.0% (mean

486 0.3% ±0.6, respectively (Figure 5b). The largest extremes in these two ranges occurred in the

487 samples charred for only 2 hours; we consider these short charring times results in a less inert

488 internal matrix that is more reactive to removal by the ABA pre-treatment. The

489 archaeological grains and pulses show larger and more systematic changes in the %C and %N

490 contents in response to the ABA treatment than the modern samples (Figure 5b). The %C and

491 %N always increased, approximately 19% ±7 and 1.4% ±0.8 respectively. These data

492 indicate material, either original/structural or exogenous, is being removed from the

493 archaeological samples during the ABA pretreatment that is acting to concentrate the more

494 recalcitrant carbon and nitrogen in charred matrix of the samples. Importantly, the percent

495 C:N ranges of cereals and pulses remain distinct (Figure 7, Supplementary table 3) and are

496 very similar to their modern charred counterparts (above, Figure 2).

497

498 The ABA pretreatment shows no consistent isotope effects on the $\delta^{13}C$ or $\delta^{15}N$ values of the

499 modern charred grains and pulses (Figure 5a, upper portion). The majority of the differences

500 between untreated and ABA treated $\delta^{13}C$ and $\delta^{15}N$ values are within the standard deviation of

501 machine error of their untreated counterparts. Only three $\delta^{13}C$ and two $\delta^{15}N$ values have

502 differences greater than the standard deviation of the machine error. There were no

503 particularly different effects on the samples charred for 2 and 4 hours; the differences were

504 0.0‰ and +0.6‰, respectively.

505

506 The effects of the ABA pretreatment on the isotope values of the archaeological samples

507 were similarly small and often within error (Figure 5a, lower portion). There were no

508 relationships between the changes in the %C and N contents and the changes in stable isotope

509 values. This suggests that, although substances were removed (even if selectively removed),

510 resulting in increases in %C and N contents, these components did not have stable isotope

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

513

514

5.   Discussion

516   In comparison to previous studies of experimentally charred cereal and pulse remains, the
517   temperatures used here were lower (230°C) and the heat exposure times longer (up to 24
518   hours). These conditions result in cereal and pulse grains that are not distorted with
519   protrusions or cracks, or partially reduced to ash. Morphologically intact grains can be more
520   easily assigned to species and be identified as likely dietary components. The support for
521   charring at higher temperatures (>300°C) in other studies is largely based on it being
522   considered necessary to render crop remains inert and enabling preservation in the burial
523   environment (Braadbaart et al. 2004b). Charring processes cause major chemical and
524   structural changes in plant materials that can result from losses by volatisation of gases, the
525   degradation of molecules and/or rearrangement of existing molecules into larger polymorphic
526   compounds. In particular, the formation of melanoidins during Maillard reactions between
527   free amino acid groups and sugars is a common reaction observed when plant materials are
528   heated (Bland et al. 1998; Silván et al. 2006). Melanoidins and the amino acids within them,
529   as well as other high molecular weight aromatic compounds formed during charring, are
530   considered very stable to oxidation and resistant to microbial attack (Knicker et al. 1996;
531   Almendros and Dorado 1999). The biochemical analyses of Styring et al (this volume)
532   indicate that Maillard reactions already occur in cereal grain experimentally charred for over
533   4 hours at 230°C, likely forming polymeric melanoidins. Importantly, our burial and humic
534   acid experiments, indicated no significant physical changes or isotopic effects on charred
535   crop remains, which suggests crop remains charred at lower temperatures for longer times are
536   sufficiently inert and resistant to microbial attack and soil contamination.

537

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

16

544 previous work of Kanstrup et al. (2012) concluded no significant isotope effects of charring
545 on the δ^{15}N values of single emmer, spelt and barley samples at temperatures ranging from
546 100 to 400℃ and times of up to 6 hours; the δ^{15}N values in the heavily distorted and partially
547 ashed wheat samples exposed up to 550℃ increased, but these were considered not
548 archaeologically relevant. Our study extended the variety of crop types and sample sizes
549 previously studied (including pilot data from Bogaard et al. 2007) and increased the charring
550 time up to 24 hours. Our new data clearly indicate a significant and systematic trend where
551 δ^{15}N values increase due to charring; all charring offsets are positive with a mean difference
552 of +1.0 ±0.4‰ (see Figure 3). The contrasting result with Kanstrup et al. (2012) could
553 represent the effects of prolonged charring times on increasing crop δ^{15}N values, which can
554 occur under a different set of charring conditions that are also archaeologically relevant.

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

571 The ABA pre-treatment appeared to have no significant influence on stable isotope values or
572 the %C and %N contents of the charred modern grains and pulses. Attention has been called
573 to the potential changes in original carbon and nitrogen contents in organic plant materials
574 analysed for palaeoecological studies caused by different acidification methods during
575 sample cleaning by Brodie et al. (2011) and Fernandes and Krull (2008). However, our
576 results indicate that there were no significant isotopic biases imposed by the acid or base
577 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 δ^{13}C and δ^{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 δ^{13}C or δ^{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

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

623

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

639

640

641 **6.   Conclusion**
642 We conclude that well preserved and undistorted charred cereal grain and pulse seed,
643 indicative of extended heating at low temperature, offer an important source of information
644 on past growing conditions and potential dietary contributions. While δ^{13}C values remain

645 largely unaffected by charring, there is a systematic and predictable increase in $\delta^{15}N$ values in
646 both cereal grains and pulse seeds of around 1‰ on average. Percentage C and N and C:N
647 data provide useful criteria for assessing archaeobotanical preservation and their
648 comparability to modern charred analogue data. Our work on ABA pre-treatment suggests
649 that it is a safe means of removing contaminants and appears to have a beneficial effect on
650 archaeobotanical C:N composition. Our burial and contamination studies indicate that
651 charred cereal grains and pulse seeds are remarkably inert and resistant to microbial action.
652 Any sampling strategy needs to take account of natural variation in plant $\delta^{13}C$ and $\delta^{15}N$
653 values, and the bulk sampling strategy used here (i.e., homogenisation of *c.* 20-30 grains)
654 targeting archaeological contexts with primary concentrations of stored material should be
655 particularly useful for assessing spatial or chronological differences. More work is needed to
656 establish relevant charring conditions for other crop and other plant types, and their isotopic
657 effects. A wider range of burial conditions and durations would also help to broaden the
658 conclusions reached here. Further bulk isotope work also needs to be underpinned by
659 complementary biochemical analyses to better understand changes in molecular structure.
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859
860 Table 5. The stable carbon and nitrogen isotope values, percentage carbon and nitrogen
861 contents and C:N ratios of charred bread wheat grains and peas buried in garden soil for up to
862 24 months.
863
864 Table 6. The stable carbon and nitrogen isotope values and percentage carbon and nitrogen
865 contents of charred modern and archaeological broomcorn millet and archaeological bitter
866 vetch soaked in humic acid solution for periods of 6, 12 and 24 months (1SD in parentheses).
867
868 **List of supplementary tables**
869 Supplementary Table 1. Stable carbon isotope values and percentage carbon content of
870 uncharred and charred cereals and pulses. Charring conditions were at 230°C for 24 hours.
871
872 Supplementary Table 2. Stable nitrogen isotope values and percentage nitrogen content of
873 uncharred and charred cereals and pulses. Charring conditions were at 230°C for 24 hours.
874
875 Supplementary Table 3. The stable carbon and nitrogen isotope values, percentage carbon
876 and nitrogen contents and C:N ratios of untreated and ABA pre-treated modern and
877 archaeological cereals and pulses (1SD in parentheses).
878
879 Supplementary Table 4. The mean percentage carbon and nitrogen contents and C:N ratios of
880 modern experimentally cereal and pulse crops (1SD in parentheses) (data from Fraser et al.
881 2011).
882
883 Supplementary Table 5. Stable carbon and nitrogen isotope variability in pulse seeds
884 observed: 1) within a single pod containing six beans, and 2) from bulk samples taken from
885 replicate experimental field plots at the same site (lentil data are from Fraser et al. 2011).
886
887 Supplementary Table 6. The stable carbon and nitrogen isotope variability of cereal grains
888 observed: 1) within individual grains in single ears of wheat, and 2) bulk samples taken from
889 replicate experimental field plots at the same location (data from Fraser et al. 2011).
890

Table 1

Table 1

The weights of uncharred and charred cereal grains and pulses seeds heated at 230°C 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

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 δ^{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	
δ^{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
δ^{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

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

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°C 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

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

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

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℃
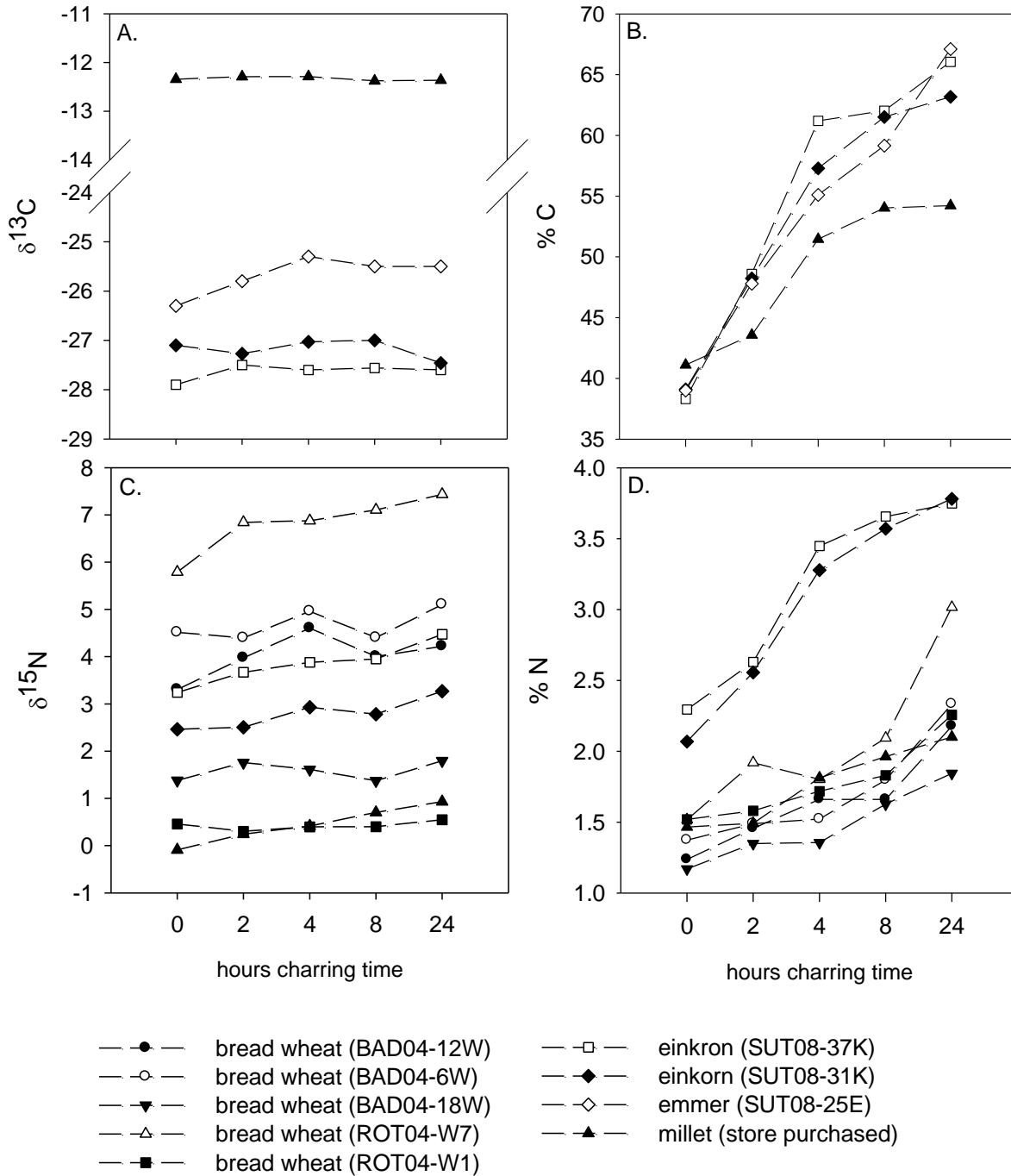
Figure 2

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)
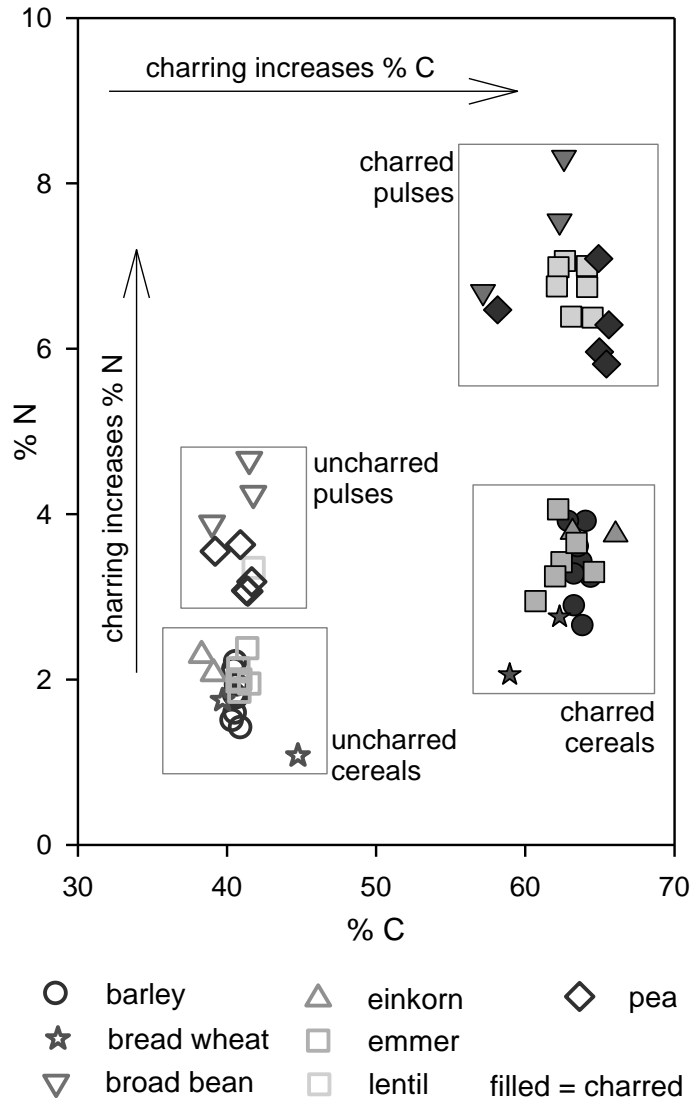
Figure 3

Figure 3

The effects of heating, at 230°C 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)

‰ change in δ values

●   uncharred δ values normalised to zero

○   offset in charred $\delta^{15}N$ values

▽   offset in charred $\delta^{13}C$ values
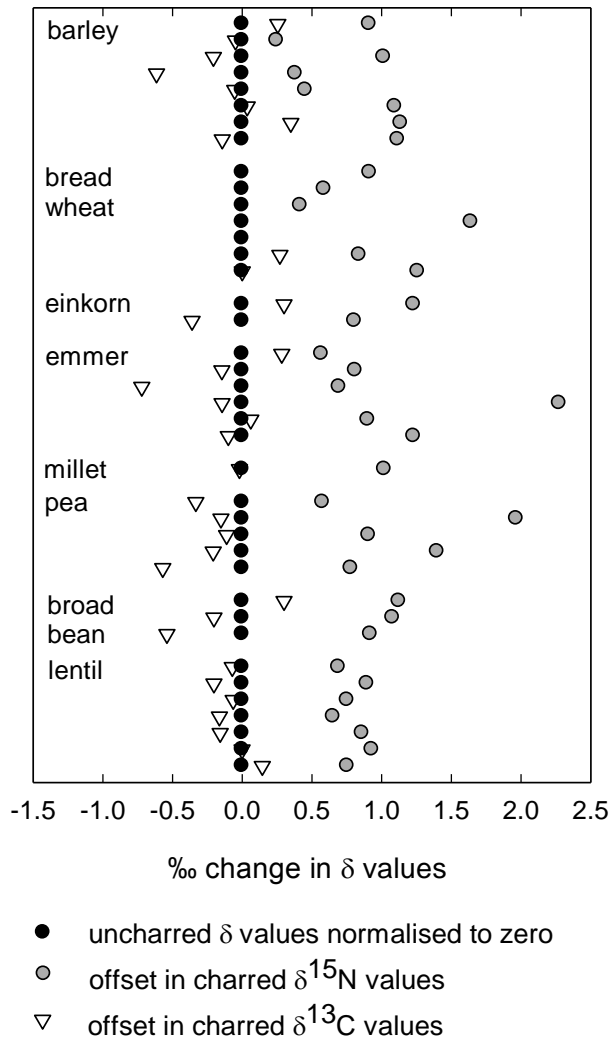
Figure 4

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
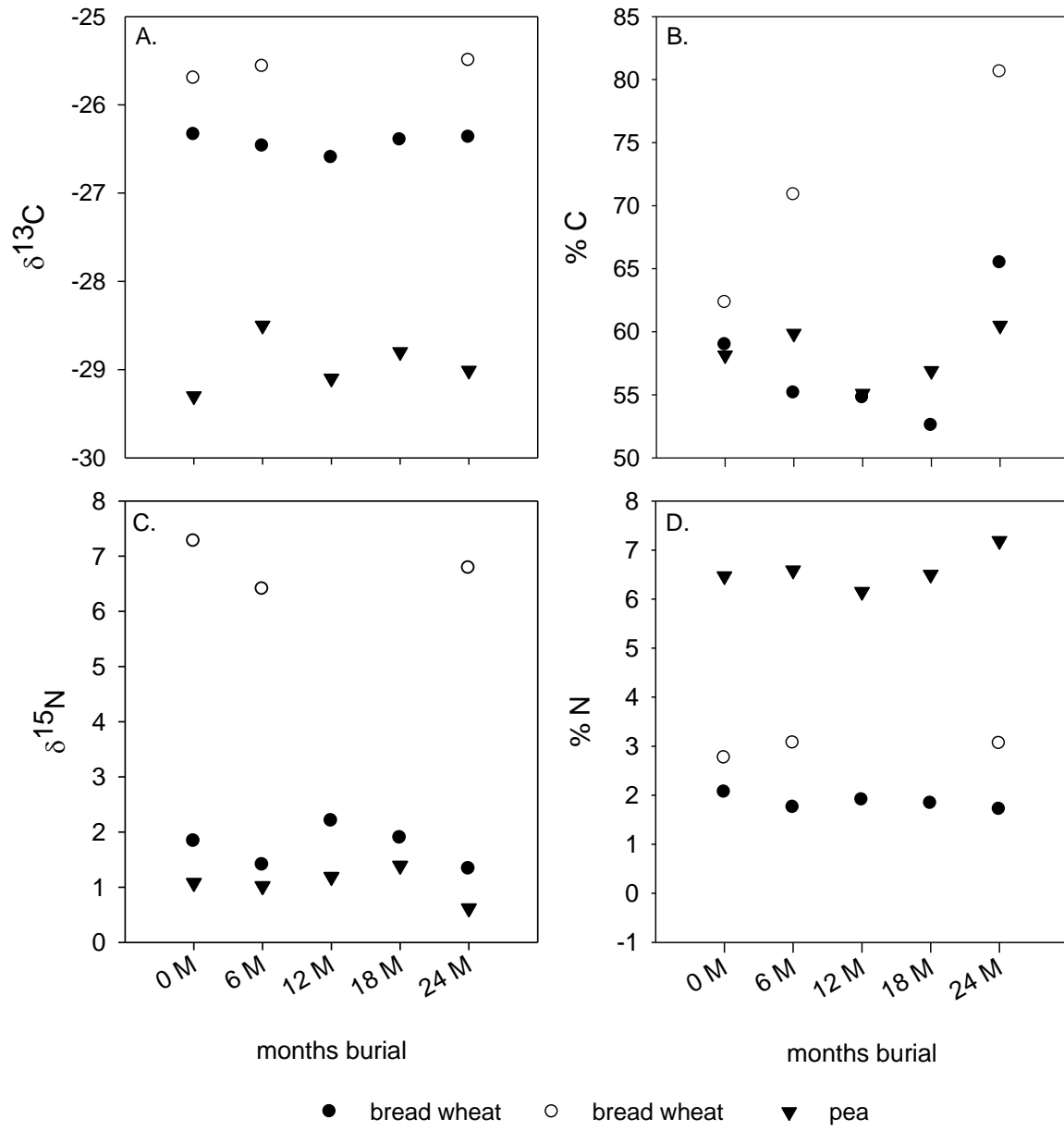
Figure 5

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.
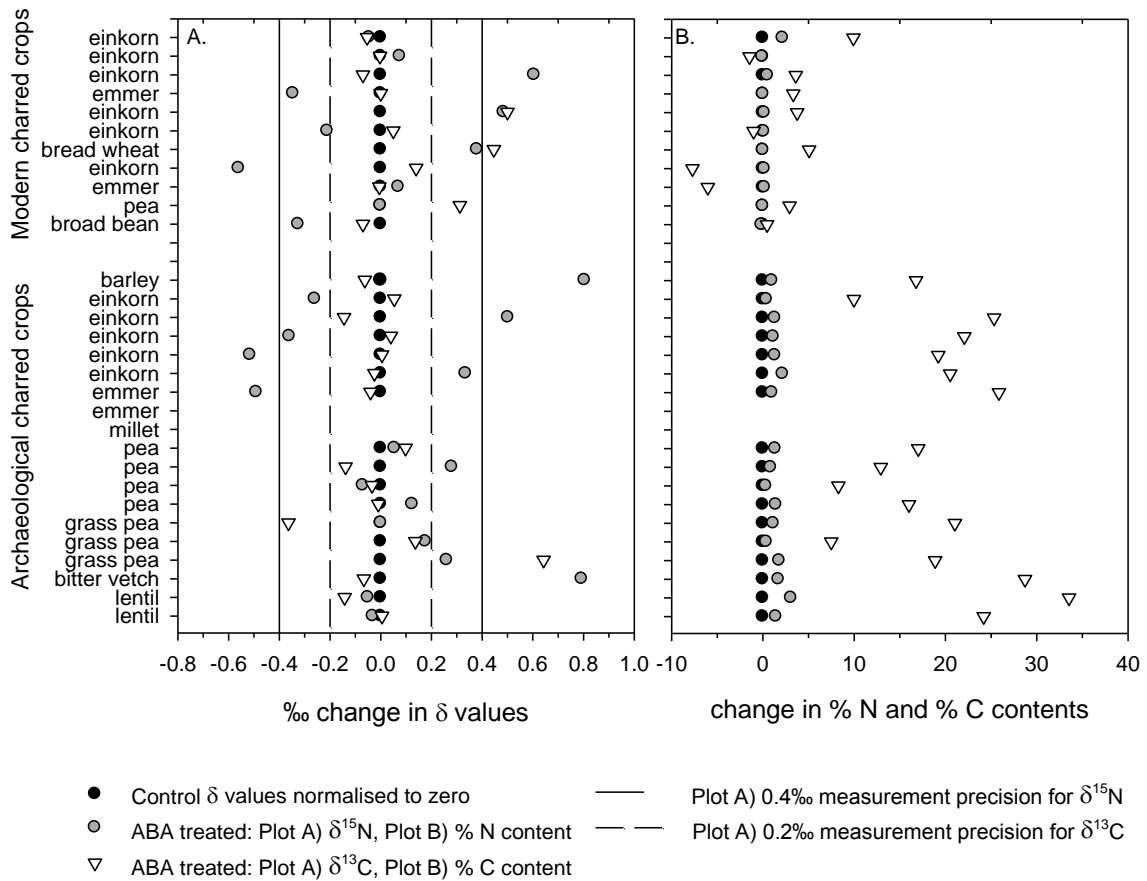
Figure 6

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.
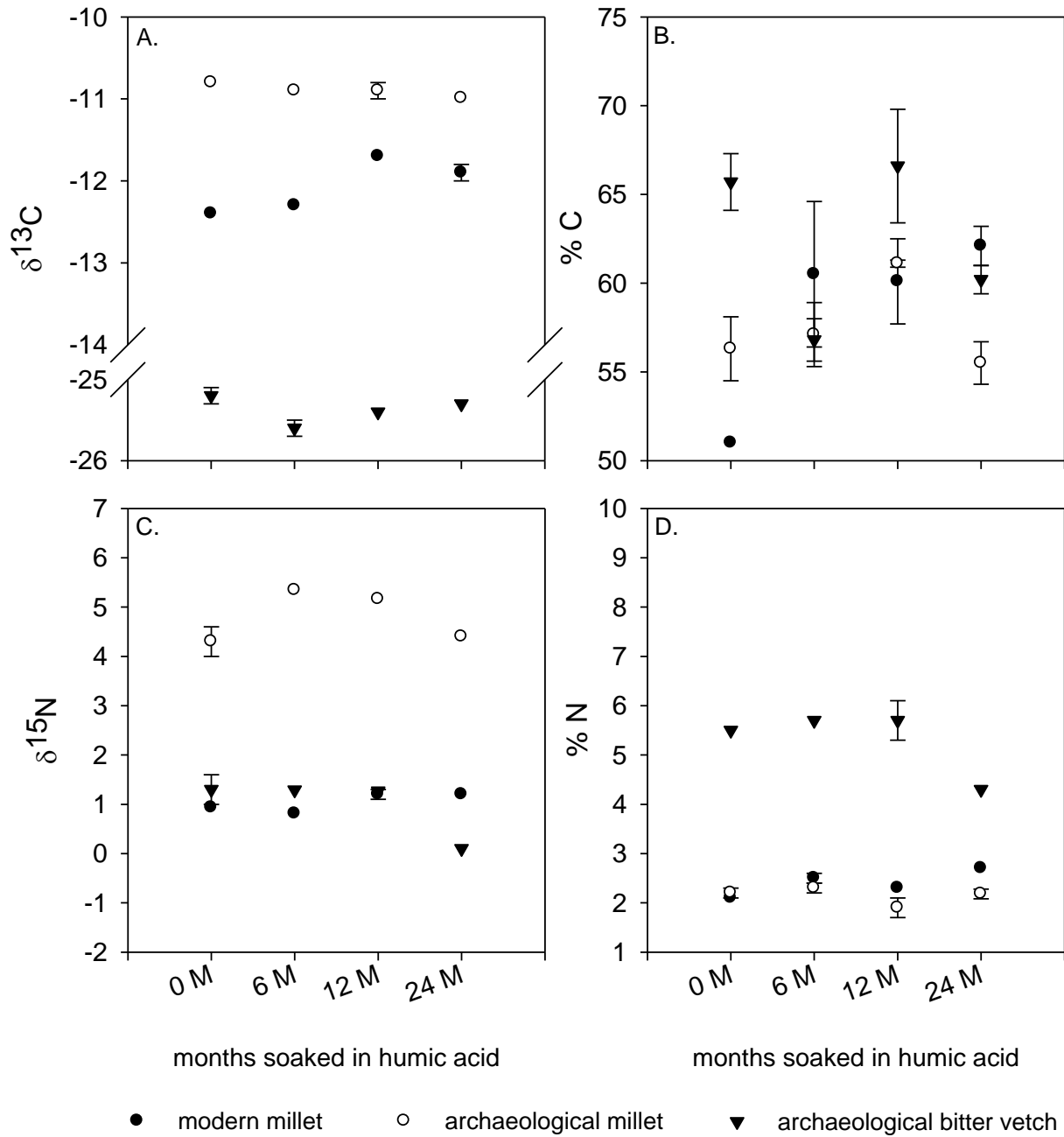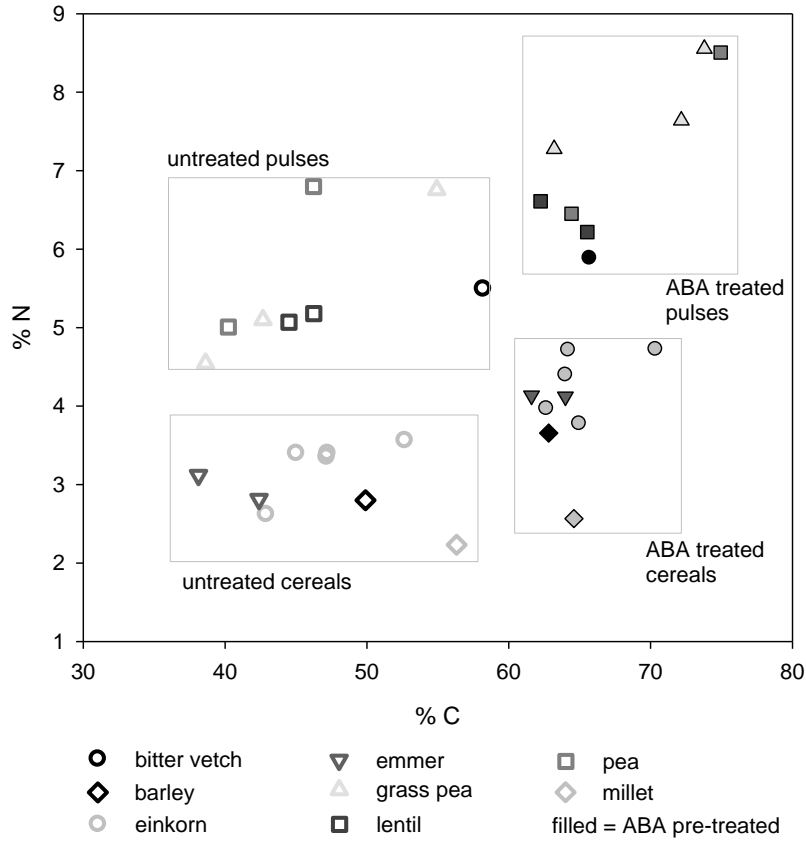
Figure 7

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

Supplementary Material_Table 2

Supplementary Material_Table 3

Supplementary Material_Table 4

Supplementary Material_Table 5

Supplementary Material_Table 6