

Turning up the heat: Assessing the impact of charring regime on the morphology and stable isotopic values of cereal grains

Elizabeth Stroud^{*}, Michael Charles, Amy Bogaard, Helena Hamerow

School of Archaeology, University of Oxford, UK

ARTICLE INFO

Keywords:

Stable isotope analysis
Grain morphology
Charring experiment
Archaeobotany
Carbon isotope analysis
Nitrogen isotope analysis
Cereals

ABSTRACT

The stable isotopic values of charred crops are now frequently analysed in archaeology. While previous research has highlighted how grain morphology and stable carbon and nitrogen isotope values change with grain charring temperature, such research has been limited to temperature ranges under 260 °C and using predominately Mediterranean cereals and pulses. For the first time, this study provides experimental data on the impact of charring on two northern European cereals, rye and oat, both morphologically and isotopically. New experimental charring of rye, oat, bread wheat and hulled barley extends the charring window to 300 °C, providing an insight into the morphological changes to the grains as well as the difference between charred and uncharred isotopic values. This range of cereals and conditions opens up potential for stable isotopic investigation of medieval agricultural growing conditions and practices in Britain. The results indicate that isotopically, a 0.16‰ and a 0.32‰ offset should be applied to $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, respectively, of grains charred between 230 and 300 °C. Morphological and internal structural changes, as well as external distortion, are key attributes which vary with charring temperature and duration. Guidelines are provided to enable assessment of whether archaeological grains of bread wheat, hulled barley, rye and oat fall within the acceptable charring window for isotopic analysis.

1. Introduction

Use of stable isotopic values from archaeological crops provides an increasingly valuable tool, in conjunction with crop weed analysis, for the understanding of past agricultural systems. The interpretation of isotopic data from plant remains preserved at archaeological sites by charring requires knowledge of the effect of the heating regime on their isotopic composition. The charring process, partially involving the Maillard reaction, which converts sugars and amino acids to more stable compounds, also influences the isotopic composition of the grains (Nitsch et al., 2015). Two papers published in 2015 investigated the effect of a range of heating regimes on crop seed morphology and isotopic values and have shown that heating grains at 220–240 °C produces grains which resemble well-preserved archaeobotanical material (Charles et al., 2015; Nitsch et al., 2015). Furthermore, research indicates that grains charred within a temperature window of 215–260 °C have altered isotopic ratios and consequently, if isotopic ratios of charred grain are compared to uncharred grains, or if the isotopic results of charred grains are used within palaeodietary reconstructions, an

offset is needed. Research has addressed this issue by producing modelled offsets to “correct” the charred isotopic value back to an “uncharred” value (Aguilera et al., 2008; Fraser et al., 2013; Nitsch et al., 2015; Styring et al., 2019).

Previous research by Nitsch et al. (2015) has investigated the effect of charring on the stable carbon and nitrogen isotope values of a suite of crop taxa typically found at Mediterranean/South West Asian archaeological sites (see Table 1): bread wheat, emmer, einkorn, barley, lentil and pea. However, the applicability of their offset to taxa outside this crop suite requires testing. This paper builds on such research by investigating the effect of heating regimes on the morphology and stable carbon and nitrogen isotope values of rye, oat, bread wheat and hulled barley, the four species common to Northern European sites, and in particular, Medieval contexts (Hamerow et al., 2020). Bread wheat and hulled barley have been previously studied (see. Nitsch et al., 2015); however, to understand the effect of higher temperatures on grain morphology and isotopic values, this paper extends their heating temperature range to 300 °C.

This paper reports on the impact that increasing the charring range

^{*} Corresponding author.

E-mail address: elizabeth.stroud@arch.ox.ac.uk (E. Stroud).

<https://doi.org/10.1016/j.jas.2023.105754>

Received 2 September 2022; Received in revised form 16 February 2023; Accepted 20 February 2023

Available online 10 March 2023

0305-4403/© 2023 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

Table 1

Various research conducted on the effect of charring, including the range of species examined, the temperatures and durations used.

Study	Species	Temperatures (°C)	Time (hrs)	Effect		Isotopic offset	
				$\delta^{15}\text{N}$	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$
Poole et al., (2002)	Pea	130 to 700	1				
		190, 250, 340	2				
Nitsch et al., (2015)	Bread wheat, hulled barley, einkorn, emmer, pea, lentil	215, 230, 245, 260	4, 8, 24	Increase by 0.04‰ for every 4 h and 0.12‰ for every 15 °C	Increase by 0.016‰ for every 4 h	0.31	0.11
Hartman et al., (2020)	Lentil	100 to 400	2	Constant till 200 °C, +0.8‰ at 300 °C, +2.2‰ at 400 °C	Significant change above 400 °C		
Fraser et al., (2013)	Barley, bread wheat, einkorn, emmer, broomcorn millet, pea, lentil, broad bean	230	2,4,8,24	gradual increase resulting in a +0.8‰ difference at 24 h s		1	
Aguilera et al., (2008)	Wheat, barley	250				0.68	
Styring et al., (2019)	Pearl millet	215, 230, 245, 260	4, 8, 24	Maximum difference of 0.34		0.34	
Hart and Feranec (2020)	Maize	180, 220, 260	2	Increase with temperature to 0.96 ± 0.2 at 260 °C	Increase with temperature, +0.56 ± 0.38 by 260 °C	0.54	
		180, 220	24				

to 300 °C has on the morphology of bread wheat, hulled barley, rye and oat and the isotopic consequence of a higher charring temperature. The 215–260 °C charring window advocated by Nitsch et al. (2015) as the optimal range from which to select isotopic samples is based on temperatures which experimentally produce well preserved and identifiable grains of their studied species; some species are difficult to separate visually when charred above 260 °C (Charles et al., 2015). The relevance of this cut-off will be explored for the expanded range of taxa as there may be no isotopic reason for discounting grains charred above this threshold.

This study aimed to investigate three questions. Firstly, what are the morphological indicators of charring for bread wheat, hulled barley, rye and oat, at a range of temperatures and durations (215–300 °C, 4–24 h) and how can these be used to help select samples suitable for isotopic analysis? Secondly, what is the effect of charring on the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of these crops under these conditions? And thirdly, can the changes in isotopic ratios be compensated for by using charring offsets for the four species at different temperature range combinations?

2. Methods

2.1. Material and sampling

This experiment followed the methodology set out by Nitsch et al. (2015), allowing comparability and the use of their bread wheat and hulled barley samples charred at 215 °C, 230 °C, 245 °C and 260 °C for 4, 8 and 24 h.

Rye (*Secale cereale* L.) and oat (*Avena sativa* L.) grains were obtained from organic farms. The rye grains were obtained from Whitehall Farm, Peterborough, UK and the oat grains from Tamarisk Farm, Dorset, UK. The hulled barley grains (*Hordeum vulgare* var. *distichum* L.) came from the same batch of material that Nitsch et al. (2015) used in their experiment, a single field in the Sault region of Provence, France, harvested in 2013. The bread wheat (*Triticum aestivum* L.) came from plot 18 of the Bad Lauchstädt long term static fertilization experiment in Germany, harvested 2004. A third new species, spelt, was also charred but not included in the statistical calculations below as these were tailored for northern European medieval assemblages; full isotopic details for spelt are included in Stroud et al. (submitted).

A total of 800 grains each of rye and oat were required to cover the 16 different combinations of temperature and time (15 charred batches + 1 uncharred). 50 grains per taxon were selected for each of the different conditions, providing three replicates of 10 grains per condition and a spare 20 grains for photography and morphological

assessment. The batches were weighed before and after charring to understand mass loss for the different charring conditions (see Stroud et al. (submitted) for data). For the barley and wheat batches, only 200 grains were required, as they were only charred at 300 °C for 4hr, 8hrs or 24hrs, as well as a batch of 50 grains used as a control uncharred sample.

2.2. Charring

Oat and rye were charred at five different temperatures for 4, 8 or 24 h, and an additional batch of bread wheat and hulled barley from the Nitsch et al. (2015) experiment was charred at 300 °C for 4, 8 or 24 h. The grains to be charred were wrapped in foil envelopes and buried in sand for the allotted duration (see Fraser et al., 2013; Nitsch et al., 2015). A Gallenkamp Plus II oven was used for charring, with the oven preheated to the required temperature before the samples were placed inside. Thermocouples were buried inside beakers of sand at three points in the oven, while a fourth thermocouple monitored the oven temperature outside the sand. A datalogger recorded the temperature over the duration of charring and it indicated that average temperature variability once the oven reached the set temperature was less than 3%. The grains were removed from the oven at the end of the heating period and left to cool to room temperature within their beakers of sand.

2.3. Sectioning and photography

A subset of grains not used for isotopic analysis was examined under a microscope to understand the impact of charring on the external and internal morphology of the grains at each temperature and duration combination. The grains were sectioned in half, at right angles to the ventral groove, allowing for the internal structure to be examined. Photographs were taken of the internal and external morphology of the grains using a Lecia stereo microscope with a Lumenera infinity 3–6 UR digital camera.

2.4. Isotopic analysis

Three batches containing 10 grains each, for each temperature/duration combination, were analysed isotopically at the University of Oxford's Research Laboratory for Archaeology and the History of Art. Batches of ten grains of the charred material were homogenised using an agate mortar and pestle. The uncharred materials, due to their harder, less brittle nature, were homogenised using a Spex 2760 Freezer/Mill. The resultant homogenised powders were weighed into tins and

Table 2

The charring matrix displaying scores for colour, distortion and internal structure of the four species, rye, bread wheat, hulled barley and oat, at the three durations and five temperature combinations. A brief summary of scoring criteria is shown below with full details in supplementary materials.

		Colour					Distortion					Internal Structure				
		215°C	230°C	245°C	260°C	300°C	215°C	230°C	245°C	260°C	300°C	215°C	230°C	245°C	260°C	300°C
Rye	4hrs	2	3	4	4	4	1	1	2	2	3	0	2	2	4	3
	8hrs	3	4	4	4	4	1	1	3	3	3	1	2	2	3	3
	24hrs	4	4	4	4	4	3	3	3	4	4	2	2	3	4	4
Wheat	4hrs	3	4	4	4	4	1	2	3	3	4	1	3	3	3	4
	8hrs	4	4	4	4	4	2	2	3	3	4	2	3	3	3	4
	24hrs	4	4	4	4	4	2	2	3	3	4	2	3	3	3	4
Barley	4hrs	3	4	4	4	4	2	3	4	4	4	1	2	4	4	4
	8hrs	4	4	4	4	4	2	3	4	4	4	1	3	4	4	4
	24hrs	4	4	4	4	4	3	4	4	4	4	1	3	4	4	4
Oat	4hrs	3	4	4	4	4	2	2	2	3	4	0	2	2	3	4
	8hrs	4	4	4	4	4	2	3	2	4	4	1	2	2	3	3
	24hrs	4	4	4	4	4	2	3	3	4	4	1	3	3	4	4
Scoring criteria																
Score	Colour					Distortion					Internal structure					
0	unchanged					unchanged					unchanged					
1	pale					slight					dense, no voids					
2	light brown					slight to moderate					dense, no voids (but possible expansion cracks)					
3	dark brown					moderate to major					less dense, no voids but possible expansion cracks					
4	black					major					less dense, voids					

analysed on a Sercon EA-GSL mass spectrometer. An internal alanine was used to obtain raw and drift-corrected delta values, while carbon and nitrogen were measured in separate runs.

A two-point calibration was conducted using IAEA- N1 and IAEA-N2 for nitrogen, and IAEA-C7 and IAEA-C6 for carbon for the majority of

samples, with a small number calibrated using the internal standards of SEAL and EMA-P2. Check standards of Alanine, and EMA-P2 or Leucine, as well as the duplication of every tenth sample, were used in conjunction with the calibration standards to understand accuracy and precision (as per Szpak et al., 2017) (see Stroud et al. (submitted) for

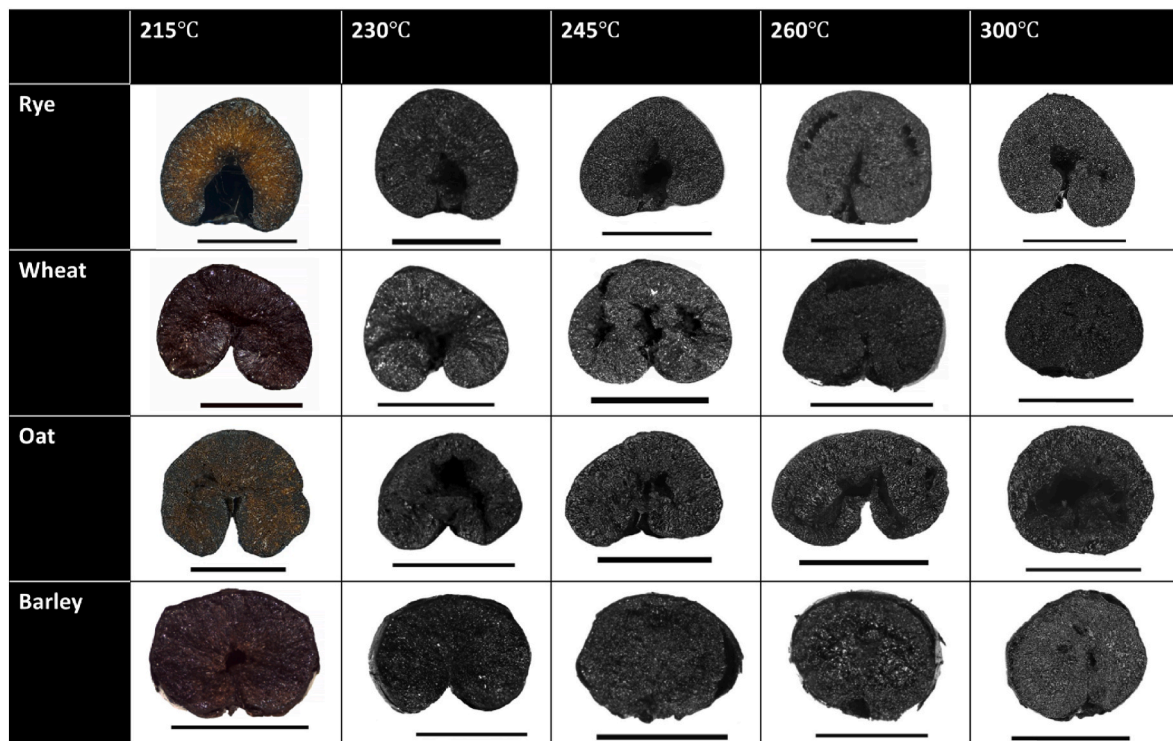


Fig. 1. The cross-section photographs of rye, bread wheat, oat and hulled barley for 4hr duration at temperatures of 215, 230, 245, 260 and 300 °C. Scale bar = 2 mm.

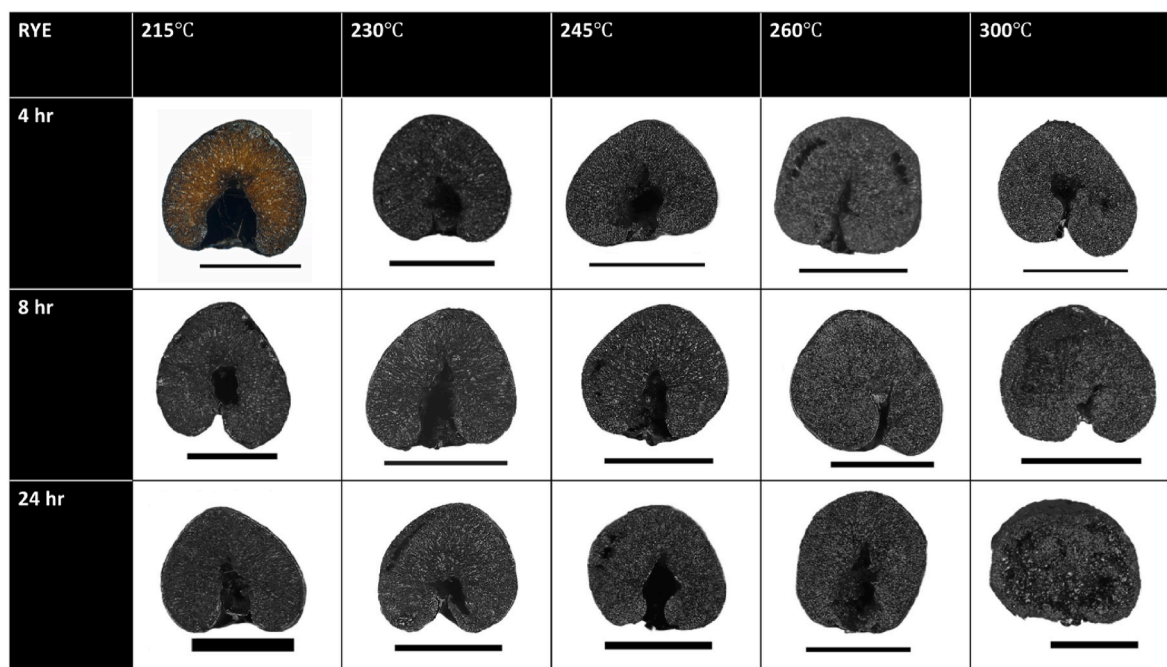


Fig. 2. The cross-section photographs of rye at the 15 different time and temperature combinations (high resolution photographs of all species can be found in [Stroud et al. \(submitted\)](#)). Scale bar = 2 mm.

analytical details). The accuracy (u (bias)) of the carbon runs was $\pm 0.16\%$, while the precision (u (Rw)) was $\pm 0.08\%$. Overall total analytical uncertainty for $\delta^{13}\text{C}$ values was $\pm 0.18\%$. For nitrogen the accuracy was $\pm 0.52\%$, while precision is $\pm 0.27\%$ and overall analytical uncertainty for $\delta^{15}\text{N}$ values is $\pm 0.58\%$.

Previous isotopic analysis by [Nitsch et al. \(2015\)](#) of bread wheat and hulled barley was used in the statistical calculations below. This study used slightly different calibration standards; USGS40 was used instead of IAEA-N2. Their measurement uncertainty was calculated using Kragten approximation methods ([Kragten 1994](#)) and no samples were duplicated. To compare the two sets of data, the Kragten approximation method was also applied to the new isotopic samples' results. The average measurement uncertainty for all the isotopic values used below, new and old (as per [Kragten 1994](#)), was 0.08% for $\delta^{13}\text{C}$ values and 0.31% for $\delta^{15}\text{N}$ values.

All statistical analysis and graphing were conducted using R-Studio and R version 4.1. For ease of comparison, graphs have been designed to replicate those published in [Nitsch et al. \(2015\)](#).

3. Results

3.1. 1 Physical changes

The experimental results show that the colour, distortion and internal structure of the grain closely reflects the heating regime. The morphological changes (distortion) seen in the grains for each heating regime cannot be explored fully in this paper. Instead, a summary of the major changes in the three main categories for each species is shown in [Table 2](#), which classifies such changes into scores ranging from 0 (unchanged/undistorted) to 4 (major distortion/significant internal changes). These changes are summarised below, while [Fig. 1](#) shows photographs of each species from 4hrs for each temperature. Full photographs are shown for Rye ([Fig. 2](#)), while the other species are available in [Stroud et al. \(submitted\)](#).

The experimental charring showed that the colour of a grain section is consistently black at temperatures of 230°C and higher, even after the shortest time period tested (4 h). The results resemble those for einkorn and emmer wheat ([Charles et al., 2015](#)), suggesting that the grain is

sufficiently altered to allow archaeological preservation by charring. At 215°C grains of all taxa are fully blackened after 8 h except for rye where the change only occurs after 24 h (see [Table 2](#) for details).

The second category, distortion, refers to both internal and external evidence of change to the size and shape of the grain. Cross sections of all the taxa revealed the tendency of the grains to become rounder at higher temperatures. This was also evident in changes to the morphology of the grain's ventral groove. Changes to the ventral groove are species dependant due to differences in ventral groove morphology. Typically, the ventral groove becomes shallower as the grain swells and in most cases the ventral groove acts like an expansion pleat, allowing the grain to swell without splitting. All the grains in the study were substantially distorted at 300°C . However, unlike colour, distortion is significantly variable across the different taxa, presumably due to their different chemical composition, which cause the taxa to react differently at varying temperature levels/durations. Significant distortion (score of 4) was recorded in barley at 230°C at 24 h, oat at 260°C for 8 h, bread wheat at 300°C for 4 h and rye for 24 h in at 260°C .

The final category relates to internal structure and covers two main attributes of the grain's cross-section: cell/matrix arrangement, and the appearance of cracks and voids. Cracks occurred at lower temperatures, hypothesised as a consequence of rapid grain dehydration ([Charles et al., 2015](#)), while voids occurred at a higher temperature and tended to be rounded in appearance and more commonly found in the centre of the matrix. Changes to the internal structure of grains occurs from 215°C onwards ([Table 2](#)). Again, there are differences between the four species similar to and associated with those observed in grain distortion. Barley grains show voids at the lowest temperature (245°C) followed by rye and oat (260°C) and lastly bread wheat (300°C) and barley tending to score higher at the lower temperatures than oat and rye.

Overall morphology, in particular grain colour and distortion, suggests that grains charred under 215°C will not commonly be recovered at archaeological sites. The incomplete blackening of the grain at 215°C is seen in all species and suggests that at lower temperatures these grains would only be partially charred, and the starches and proteins in the grains may not have been converted to microbially unavailable Maillard reaction products.

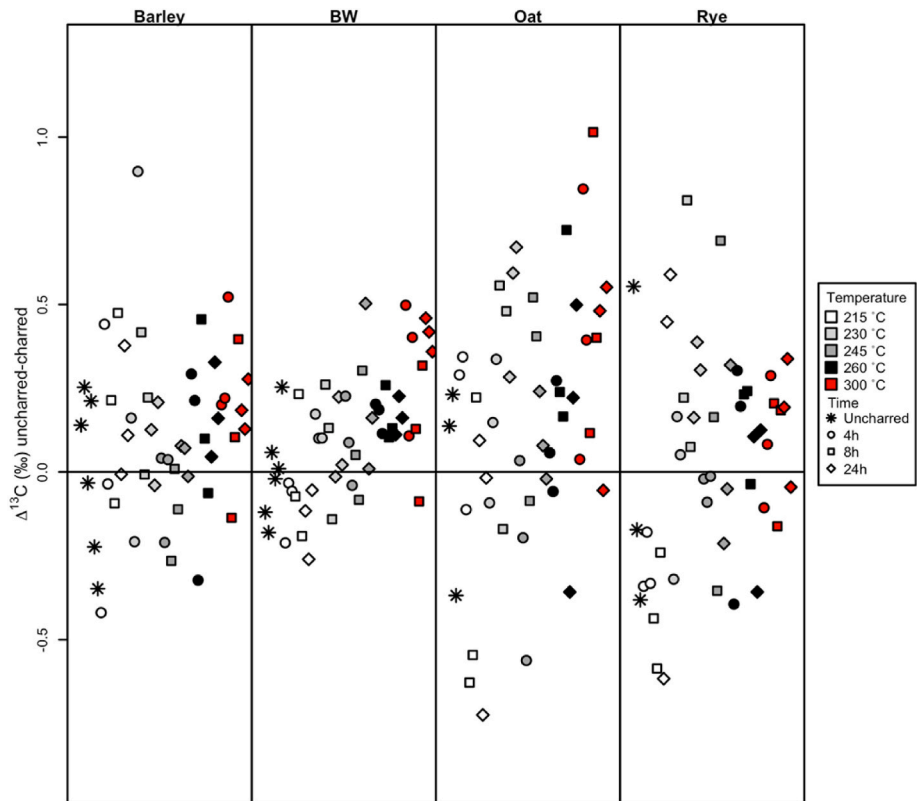


Fig. 3. The deviation $\delta^{13}\text{C}$ values of barley, bread wheat (BW), oat and rye for the different times and temperature combinations from the mean $\delta^{13}\text{C}$ value of uncharred replicates. Horizontal line represents the mean for the uncharred replicates.

Table 3
The results of a multiple linear regression with coefficients of time and temperature on $\delta^{13}\text{C}$ values, showing the p-value and the beta value rounded to 2 decimal places.

		215–260 °C	215–300 °C	230–260 °C	230–300 °C
Temperature	p-value	0.04	<0.01	0.27	0.06
	Beta	0.01	0.01	−0.01	0.01
Time	p-value	0.28	0.3	0.33	0.36
	Beta	0.01	0.01	0.01	0.01

3.2. Carbon isotope results

The four taxa have $\delta^{13}\text{C}$ values ranging from −27.9‰ to −24.9‰ for the uncharred material, while the charred material is slightly more variable with a range of −28.3‰ to −24.6‰. Comparing the deviation of charred grains’ $\delta^{13}\text{C}$ values from the uncharred replicates average $\delta^{13}\text{C}$ values shows this variability especially in the rye and oat (Fig. 3). Bread wheat has an upwards trend in the $\delta^{13}\text{C}$ value with temperature, as noted by Nitsch et al. (2015); the added 300 °C batches are consistent with this trend. Oat and rye $\delta^{13}\text{C}$ values are significantly more variable than the $\delta^{13}\text{C}$ values of bread wheat, in some cases deviating from the mean uncharred value by 1‰.

The charred material was examined to ascertain the impact of heating regime on $\delta^{13}\text{C}$ values. A multiple linear regression with coefficients for temperature, time and species was used, following Nitsch et al. (2015), and Table 3 details the results using the differing combinations of temperature ranges. For $\delta^{13}\text{C}$ values of the four species, temperature is only significant ($p < 0.05$) if the 215 °C batches are included in the analysis – regardless of whether the highest temperature is 260 °C or 300 °C. Time is never significant in any of the permutations (Table 3). The effect that temperature has on the $\delta^{13}\text{C}$ value is limited, with the greatest impact in the 215–260 °C and 215–300 °C analysis. Using the beta coefficient (the mean change between the outcome variable – $\delta^{13}\text{C}$ – for every unit of change of the predictor variable – time or

temperature) it can be calculated that there is a 0.05‰ change for every 15 °C, resulting in a 0.14‰ difference between 215 °C and 260 °C, and a difference of 0.26‰ between 215 °C and 300 °C.

3.3. Nitrogen isotope results

The four taxa have $\delta^{15}\text{N}$ values ranging from 0.17 to 4.1‰ for the uncharred material while the charred material is more variable (−0.1 to 6.2‰). Rye is the most variable of the four taxa, with the $\delta^{15}\text{N}$ values of its 215 °C material particularly so. Wheat’s $\delta^{15}\text{N}$ values are variable at lower charring temperature batches, compared to the 260 °C and 300 °C samples. The variability is higher for $\delta^{15}\text{N}$ values compared to $\delta^{13}\text{C}$ values, something also noted by Nitsch et al. (2015).

There are notable trends detected when comparing the charred samples to the averaged uncharred value (Fig. 4). Rye’s 215 °C 4- and 8-h samples have a mean similar to that of the uncharred material, while two of the subsequent 215 °C 24-h samples have some of the highest deviation from the uncharred mean. From 230 °C onwards $\delta^{15}\text{N}$ values decrease as temperature increases till the 300 °C samples, which have similar values to the uncharred material. Oat samples show an initial increase in $\delta^{15}\text{N}$ value from uncharred to 215 °C, the largest difference from the uncharred mean, while the 230 °C–245 °C temperature batches subsequently decrease from that high, with the mean $\delta^{13}\text{C}$ values plateauing for the 260 °C–300 °C batches. Hulled Barley and bread wheat

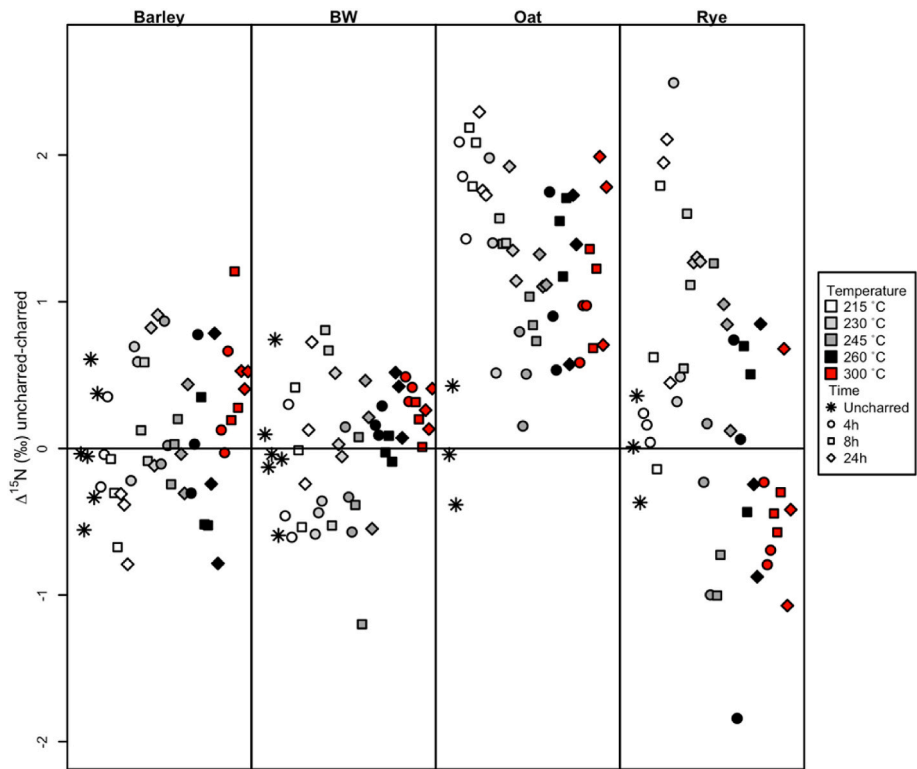


Fig. 4. The $\delta^{15}\text{N}$ values of barley, bread wheat (BW), oat and rye for the different times and temperature compared to the mean $\delta^{15}\text{N}$ value of uncharred replicates.

Table 4

The results of a multiple linear regression with coefficients for time and temperature on the $\delta^{15}\text{N}$ values showing the p-value and the beta value rounded to 2 decimal places.

		215–260 °C	215–300 °C	230–260 °C	230–300 °C
Temperature	p-value	<0.01	0.02	<0.01	0.1
	Beta	−0.01	−0.01	−0.02	−0.01
Time	p-value	0.02	<0.01	0.05	0.03
	Beta	0.01	0.01	0.01	0.01

$\delta^{15}\text{N}$ value variabilities differ, being less variable than rye and oat. Bread wheat shows a trend of increasing $\delta^{15}\text{N}$ values as temperature increases, resulting in the higher temperatures having the largest difference from the uncharred material, corroborating a similar observation by Nitsch et al. (2015).

Statistical analysis of the isotopic values of charred material was conducted using a multiple linear regression with coefficients for temperature, time and species; the same method as used for the carbon isotope analysis. When the 215 °C batches are included as the lowest temperature, both time and temperature are significant in the regression model – regardless of whether the highest temperature is 260 °C or 300 °C (Table 4). The results suggest that there is a 0.05–0.14‰ decrease in $\delta^{15}\text{N}$ value for every 15 °C when the 215 °C batches are included. This is a negative relationship with the highest $\delta^{15}\text{N}$ value at 215 °C; the value decreases by 0.41‰ by 260 °C (0.31‰ between 215 °C and 300 °C using the 215–300 °C model). When the temperature range is restricted to just 230–260 °C there is a significant difference between the batches for temperature, and for time, though not significant at the 0.05 level, it is very close. Analyses of the 230 °C–300 °C range finds that just time is significant at the 0.05 level changing by 0.01‰ for every hour (beta coefficient), resulting in a 0.20‰ difference between the 4hr and 24hr batches.

3.4. Difference between charred and uncharred: calculating a charring offset

Previous research has noted that variations in the biochemical compositional of grains could potentially result in different species or even landraces reacting to charring conditions in different ways (Nitsch et al., 2015). Moreover, modern charring experiments only recreate a subset of the potential combinations of temperature and duration that are relevant to preservation by charring. As in Nitsch et al.’s (2015) work, the approach taken in the present study was not to attempt calculation of individual species’ charring offsets, for which a larger number of observations per species would be needed. Rather, the present study aimed to capture the range of variations observed across the four species examined, providing an indicative offset applicable to all of the species. Full data are published in Stroud et al. (submitted), including all data from Nitsch et al. (2015). This dataset provides a basis for charring offsets that could be in future tailored to particular crops suites, or to explore single species differences, but these further steps are outside the scope of this paper.

A charring offset was calculated using the same method as Nitsch et al. (2015). Nitsch et al. (2015) compared the isotopic ratios of all the charred samples to uncharred samples, advocating this method due to the difficulty in distinguishing between the different temperatures and times of archaeological seeds. The charring morphology experiment conducted for this paper highlights the difficulty in distinguishing

Table 5

The results from the first (LM1) and second (LM2) linear models based on the $\delta^{13}\text{C}$ values, showing the R^2 value, p-value of the model, p-value of the charred-fresh coefficient, the Beta value and the confidence intervals, for the four different temperature ranges rounded to 2 decimal places.

	215–300 °C		215–260 °C		230–300 °C		230–260 °C	
	LM1	LM2	LM1	LM2	LM1	LM2	LM1	LM2
Adjusted R^2	0.87	0.87	0.87	0.87	0.89	0.89	0.89	0.89
Model P value	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Charred-fresh P value		0.11		0.26		0.02*		0.06
Beta		−0.12		−0.08		−0.16		−0.13
CI 2.5%		−0.26		−0.23		−0.29		−0.3
CI 97.5%		0.03		0.06		−0.03		0.01

between grains charred between 230 °C and 260 °C, while duration of charring appears very difficult to distinguish morphologically. This is compounded by the fact that archaeological specimens may have undergone a range of different temperatures for different durations during charring.

3.4.1. Carbon offset

The charred materials' $\delta^{13}\text{C}$ values were compared to the uncharred material using two different linear models as per Nitsch et al. (2015). The first linear model examined the relationship between $\delta^{13}\text{C}$ values and species; the second included an extra coefficient for charring (i.e., charred vs non-charred values with no regard for charring time or temperature). Table 5 summarises the results for the different temperature batches used.

The first model (LM1), using all temperatures (215–300 °C and the uncharred batches) for $\delta^{13}\text{C}$ values produces a good fit ($R^2 = 0.87$) with all species' coefficients significant (see Table 5). The addition of the charring coefficient to the model (LM2) results in a negligible increase in the fit of the model (see Table 5) and the p-value for charred-fresh is not significant. Table 5 also shows the results of the second linear model when using different temperature range combinations. The 215–260 °C range results suggests that there is no need for a charring offset as the difference between the charred and uncharred values are not significant. This does not change if the charring range is increased to 215–300 °C as detailed above. This is because the $\delta^{13}\text{C}$ values of the 215 °C batches have very similar $\delta^{13}\text{C}$ values to the uncharred batches. When the 215 °C batches are removed, there is a significant difference between the charred and uncharred material ($p = 0.02$). A 0.16‰ offset is recommended if the temperature range is restricted to 230–300 °C. The 230–300 °C model predicts that there is a 0.16‰ difference between the $\delta^{13}\text{C}$ value of the charred material compared to the uncharred material shown as the beta coefficient, falling within a 95% confidence interval (CI) of −0.03 to −0.29‰ (Table 5). Restricting the temperature range to 230–260 °C results in p-value between charred and uncharred of 0.06, a similar result to Nitsch et al. (2015) model's p-value of 0.057 and while neither of those values are significant at the 0.05 level, both are very close to it.

The offset of this paper's model for a 230–260 °C temperature range is 0.13‰, very similar to Nitsch et al. (2015), who proposed an offset of 0.11‰ for the temperature range of 215–260 °C. However, the confidence intervals for the 230–260 °C model does include zero indicating

that there is a possibility that this offset could also be zero. The charring offset for the four species within this study, bread wheat, hulled barley, rye and oat differs only slightly from Nitsch et al. (2015) and the need for an offset is dependent on the range of charring temperatures chosen for the model.

The similarity of the $\delta^{13}\text{C}$ values of charred grains to the uncharred values (see Fig. 3) at 215 °C is evident in all four species. As reported above some of the grains, when charred at 215 °C for some time durations (i.e., 4 h and 8 h), are still brown internally, raising the question as to whether grains charred at this temperature would survive within the archaeological record. Chemical research into whether grains at the lower temperatures have undergone the necessary chemical changes for survival is still required. If the 215 °C batches are removed, the results suggest that inclusion of grains charred between 230 and 300 °C would need an offset of 0.16‰ to be subtracted from the $\delta^{13}\text{C}$ values of charred material in order to convert the values back to something comparable with uncharred grains.

3.4.2. Nitrogen offset

The first linear model constructed with the $\delta^{15}\text{N}$ data from all batches (215–300 °C) (LM1) shows a good fit of the model with the data ($R^2 = 0.84$), with all species' coefficients significant (see Table 6). The addition of the charring coefficient (charred-fresh, LM2) only slightly increases the fit of the model ($R^2 = 0.84$); however, the charring coefficient is significant ($p = 0.04$). For the four different temperature permutations the charred-fresh coefficients are significant within the models, indicating that a charring offset is required, regardless of the inclusion of the 300 °C samples. When all temperature batches are included (215–300 °C), the difference between charred and uncharred (beta value) is 0.33‰ with a 95% CI between −0.64 and −0.02‰ (see Table 6).

There is always a significant difference between the charred and uncharred groups' $\delta^{15}\text{N}$ values if the 300 °C temperature batches are included in the models, but without their inclusions p-values of the models sit around 0.05 (Table 6). The offsets (beta values in Table 6) for the four temperature combinations differ only slightly to that recommended by Nitsch et al. (2015); Nitsch et al. (2015) proposed a $\delta^{15}\text{N}$ value charring offset of 0.31‰, while this study's offsets range from 0.31 to 0.33‰. Unlike the carbon results, the inclusion of the 215 °C batches does not significantly change the results. The 215 °C samples from some of the taxa have $\delta^{15}\text{N}$ values which differ greatly from the uncharred

Table 6

The results from the first (LM1) and second (LM2) linear models based on the $\delta^{15}\text{N}$ values, showing the R^2 value, p-value of the model, p-value of the charred-fresh coefficient, the Beta value and the confidence intervals, for the four different temperature ranges rounded to 2 decimal places.

	215–300 °C		215–260 °C		230–300 °C		230–260 °C	
	LM1	LM2	LM1	LM2	LM1	LM2	LM1	LM2
R^2 (adjusted)	0.84	0.84	0.85	0.85	0.83	0.83	0.83	0.84
P-model	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Charred-fresh P value		0.04		0.04		0.04		0.05
Beta		−0.33		−0.33		−0.32		−0.31
CI 2.5%		−0.64		−0.64		−0.62		−0.62
CI 97.5%		−0.02		−0.01		−0.02		0.01

Table 7

The residual standard error of a multiple regression model accounting for the effects of time and temperature (as per Nitsch et al., 2015).

Taxon	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)
Bread Wheat	0.19	0.43
Hulled barley	0.25	0.48
Rye	0.33	0.93
Oat	0.38	0.53

material, while the 300 °C samples of some samples tend to have lower deviation in charred grains $\delta^{15}\text{N}$ values compared to uncharred material's values (see Fig. 4). The different temperature combinations indicate that the charring window for these four species (bread wheat, hulled barley, rye and oat) can range from 215 to 300 °C with limited impact on the overall $\delta^{15}\text{N}$ charring offset. However, the analysis of charred material only also indicates a -0.31‰ difference between specimens charred at 215 °C and those charred at 300 °C highlighting how differences in charring temperature could also affect the $\delta^{15}\text{N}$ values. As also reported above, when the 215 °C batches are removed, the within charred model finds only time is significant and the different between the lowest temperature and highest temperature is lower at 0.20‰.

As explained above for the $\delta^{13}\text{C}$ value offset, the inclusion of the 215 °C batches could be problematic since there is uncertainty as to whether they have completed the chemical processes necessary for resistance to microbial decay. Furthermore, the differences noted with the rye and oat isotopic values highlight the need to conduct charring experiments of species potentially chemically different to other crop species which have been charred experimentally.

3.5. Variability and issues of comparability

Combining the Nitsch et al. (2015) isotopic values with the new values was carried out to reduce comparability issues. The hulled barley and bread wheat grains used in this study are from the same field as those examined in the Nitsch et al. (2015) study; the hulled barley the same material as used by Nitsch et al. (2015) and the bread wheat also from the same field as Nitsch et al. (2015) but a from different year. To confirm there was no significant difference between the old and new grains, batches of uncharred barley and wheat were isotopically analysed and their values compared to those of the uncharred grain from the Nitsch et al. (2015) study. There is no statistically significant difference between the uncharred Nitsch et al. (2015) samples and the uncharred samples from this study (Welch two sample *t*-test, hulled barley $\delta^{13}\text{C}$ *p*-value = 0.3, $\delta^{15}\text{N}$ *p*-value = 0.3, bread wheat $\delta^{13}\text{C}$ *p*-value = 0.2, $\delta^{15}\text{N}$ *p*-value = 0.9; see Stroud et al. (submitted) for more detail).

There is a high amount of variability in the rye and oat batches compared to the bread wheat and to a lesser extent the barley. The higher variability seen in the rye and oat isotopic values is most likely due to the grains used in the experiment: the material was sourced from modern farms, the grains coming from a larger cultivated area than the bread wheat which was grown under experimental field conditions in a small plot. The oat came from a single field of ~5 ha, while the rye was also from a single field although its size is unknown. The bread wheat and barley, cultivated or collected from experiments, had some degree of uniform topography and soil conditions across the small plot/collection areas. Oat and rye grain, however, would have higher isotopic variability due to the wider range of cultivation conditions within large fields affecting $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values.

Nitsch et al. (2015) attempted to calculate the likely range of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of samples from a single growing condition. They used the residual standard error (SE) of a multiple regression model which accounted for the effect of time and temperature ($y = \text{temp}(x) + \text{time}(x)$, where $y = \delta^{13}\text{C}$ or $\delta^{15}\text{N}$). Their calculations showed that 0.25‰ for $\delta^{13}\text{C}$ and 0.75‰ for $\delta^{15}\text{N}$ could provide a rough estimate of the expected

population standard error of a given growing condition. Extrapolating from that (1.96 x SE) they calculated that a 95% CI of $\pm 0.5\text{‰}$ for $\delta^{13}\text{C}$ and $\pm 1\text{‰}$ for $\delta^{15}\text{N}$ would account for the variability within a single growing condition (Nitsch et al., 2015).

The standard error of the species examined in this paper are within a similar range to those of Nitsch et al.'s (2015) (Table 7). Rye has the most variable isotopic values as noted above, and its standard error is high for $\delta^{15}\text{N}$ but still below 1‰: similar to the high $\delta^{15}\text{N}$ standard error that pea produced in the Nitsch et al. study. The variability of rye as mentioned above is mostly a consequence of the large area from which from the material derived (a large modern field).

The $\pm 0.5\text{‰}$ and $\pm 1.0\text{‰}$ 95% CI for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, respectively, suggested by Nitsch et al. (2015), would still account for variability within a single growing condition when using bulk samples of ten grains for the species examined within this study. Studies using bulk samples of multiple grains of wheat, rye, oat or barley should interpret results with the understanding that the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values are only 95% likely to represent the true population mean using a confidence range of $\pm 0.5\text{‰}$ and $\pm 1.0\text{‰}$ respectively. Thus, as Nitsch et al. (2015) pointed out, any difference in isotopic means of less than 0.5‰ ($\delta^{13}\text{C}$) and 1.0‰ ($\delta^{15}\text{N}$) should not be interpreted as significant.

4. Discussion

This study has shown that while the effect of heat on crop grain morphology and $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values follow broadly similar patterns to previous studies, there is some variation between taxa and that this needs to be accounted for in isotopic analysis. At 230 °C after 4 h, the grain of the four species become 'charred', i.e. pre-dominantly blackened across the cut section, level of grain distortion is low and identification to species level is relatively straightforward, and the internal cell structure has undergone a transformation (manifest as a more open/less dense appearance). Hulled barley is the most sensitive to heating temperature, with substantial morphological distortion occurring above 230 °C. Increasing temperatures cause marked distortions in grain morphology and at 300 °C, grain morphology is substantially altered, both internally and externally but identification to species is still possible and critically the effect on $\delta^{15}\text{N}$ or $\delta^{13}\text{C}$ values and the associated charring offsets is relatively limited. Consequently, such grain can be included in isotopic analysis.

At the other end of the heating range (215 °C) grain is not undergoing the transformations necessary for archaeological preservation and there is considerable deviation in isotopic values from the uncharred samples, especially in the case of oat and rye. For these reasons we propose that grains charred at this low temperature should not be included in isotopic sampling. The uncertainty about the survival of grains charred below 230 °C in the archaeological record, and the very different isotopic values of oat and rye grains charred at 215 °C compared to the other temperatures, suggest that these grains should not be considered for isotopic analysis.

The results of this study, in conjunction with those of Nitsch et al. (2015), and other recent charring offset experiments on other species such as pearl millet (Styring et al., 2019), indicate that the application of a "charring offset" is necessary if either comparing the isotopic values of charred and uncharred archaeological material or using the isotopic value of charred grains in palaeodietary reconstruction. As seen in this study and others (Styring et al., 2019; Nitsch et al., 2015), the nitrogen offset appears to be around 0.3‰, with the four species in this study requiring a 0.32‰ offset. The use of a $\delta^{13}\text{C}$ value offset appears to be dependent on the species and temperature range of the seeds analysed. For the four species examined here, bread wheat, rye, hulled barley and oat, a $\delta^{13}\text{C}$ value offset is only necessary if the lower temperature is removed (215 °C) and the higher temperature of 300 °C is included. This suggests that assemblages of extremely well-preserved material, charred below 260 °C, may not require a $\delta^{13}\text{C}$ value charring offset. However, if grains charred to 300 °C are included, a charring offset, while small, is

Table 8

The differences in colour, distortion and internal structure of archaeological wheat, barley, rye and oat grains which are good, borderline or badly suited for isotopic analysis () indicate the charring scores used in Table 2.

	Good (230 to +260 °C)	Borderline (+260 - +300 °C)	Bad (+300 °C)
Colour	black (4), matt appearance	black (4), matt appearance	black (4), matt or glassy appearance
Distortion	slight to moderate (1–3)	moderate to major (2–4)	moderate to major (3–5+)
Internal structure	no to minimal voids, matrix dense (1–2)	moderate voids, matrix dense to moderately dense (2–4)	major voids, less than half dense matrix surviving, matrix can look bubbly (5+)

recommended.

4.1. Selection of grains suitable for isotopic analysis

Through experimental charring, this paper has found that three categories of internal and external traits change depending on charring temperature and duration. The next step requires these traits to be translated into useable criteria to identify grains suitable for isotopic analysis. This paper advocates shifting the “charring window” set by Nitsch et al. (2015), from 215–260 °C to 230–300 °C for the four species studied. The dissection of the grain to understand any internal changes is key to assessing the suitability of the grains for isotopic analysis. The authors’ experience with grains from northern Europe has found that while many grains fulfilled the external morphological attributes of grain charred at an isotopically suitable temperature range (230 °C–300 °C), internal changes indicated that they were more likely charred above 300 °C. These grains were therefore not suitable for isotopic analysis because of uncertainty regarding their isotopic offset (Hamerow et al. in prep).

A set of criteria was developed to help select archaeological grains of wheat, barley, rye and oat which fall within the 230 °C–300 °C temperature range for the new isotopic offsets presented in this paper. Table 8 details the proposed criteria required to select suitable grains for isotopic analysis classifying them as either *good*, *borderline* or *bad*. It is advised that *bad* grains should not be isotopically analysed, while *borderline* grains can be. However, any interpretation of the resultant isotopic values should consider their possibly high charring temperature and different offset.

In addition to having a completely blackened grain matrix, the

matrix of a grain suitable for isotopic analysis should be matte. Archaeological grains can, however, present with a matrix which appears glassy or vitrified (Fig. 5). We hypothesise that this glassiness is the result of high temperatures which have vitrified the grain’s matrix; such glassiness has not yet been replicated experimentally in either grains or wood and needs further investigation (see Courty et al., 2020 cf. with McParland et al., 2010 for debate within anthracology as to whether high temperatures cause vitrification in charcoal). The lack of such glassiness in any experimental studies suggests that the conditions required to vitrify cells are not the same as those used thus far to char modern grains to determine isotopic offsets. Consequently, glassiness should currently be used as an attribute to rule out grains for isotopic analysis.

Grains should be selected that have limited distortion, i.e., changes to size and shape of grain (Table 8). However, it has been observed that some archaeological grains have limited external distortion but internally have large areas of glassy matrix or large voids (see Fig. 5). This highlights the need for grains to be dissected in half to ascertain their suitability for isotopic analysis, and furthermore, that all attributes in Table 8 must be used when selecting grains for analysis.

The charring experiment above (and others, such as Charles et al., 2015) shows that as charring temperature and duration increases, the matrix of a grain loses density, potentially a consequence of the cells losing internal material, and at higher temperatures cells can merge. The density of the matrix is especially important in separating archaeological grains suitable for isotopic analysis from those which are not: grains with limited amounts of dense matrix or large merged voids are hypothesised to be indicative of higher temperatures. The low-density matrix, coupled with large voids, are sound attributes for ruling out archaeological grains from isotopic analysis.

Species specific differences do occur; the experimental charring showed differences between wheat/barley compared with oat and rye. Hulled barley tended to show higher amounts of distortion and internal changes at lower temperatures compared with rye, bread wheat and oat. The differences between species does highlight the importance of charring experiments to understand how different species change because of different charring conditions. Consequently, the above criteria, while they may be suitable for wheat, rye, barley and oat (and glume wheats such as spelt – see Stroud et al. (submitted)), other crop species such as pulses, millets or sorghums may present with different changes due to charring and warrant further investigation.

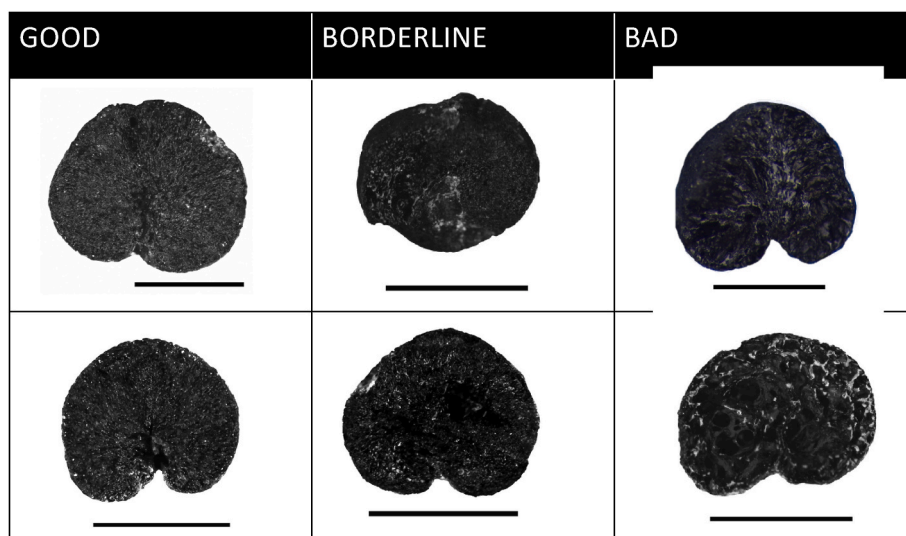


Fig. 5. Examples of archaeological grains which show characteristic internal structures of the good, borderline and bad categories. Scale bar = 2 mm.

5. Conclusion

This paper investigated the impact of charring, both isotopically and morphologically, on bread wheat, hulled barley, rye and oat. The construction of a model to predict a charring offset indicates that, within the range of charring between 230 °C and 300 °C for the four species, small offsets are required for both $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values. At the lower temperature of 215 °C grains are only partially charred (different isotopic values, only partially colour change), and so this material is excluded from the prediction of offsets.

Between 230 °C and 300 °C, the isotopic offsets for wheat, barley, rye and oat are 0.32‰ ($\delta^{15}\text{N}$, 95% with a 95% confidence interval of −0.62 to −0.02) and 0.16‰ ($\delta^{13}\text{C}$, 95% with a confidence interval of −0.29 to −0.03). The research also confirms the findings of Nitsch et al. (2015) that variability of $\pm 0.5\%$ for $\delta^{13}\text{C}$ values and $\pm 1\%$ for $\delta^{15}\text{N}$ values should be expected in a single growing condition. This reiterates the point that smaller isotopic differences should not be considered significant.

This research shows that colour, distortion and internal structure change depending on the charring temperature and time range for the four species. Furthermore, examination of the internal structure of the grain is extremely important for selecting grains suitable for isotopic analysis, given findings that external shape may not always reflect high distortion in archaeological grains. The criteria presented here provide archaeologists who wish to conduct isotopic analysis of wheat, barley, rye or oat with guidelines to follow when selecting samples.

Funding

This work was funded by the European Research Council under the European Union's Horizon 2020 research and innovation programme (ERC-2016-ADG-741751).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

Thanks are extended to the ERC, as well as Samantha Neil, Peter Ditchfield, and Erika Nitsch for assistance during the data collection phase, and Amy Styring for comments on a draft manuscript.

References

- Aguilera, M., Araus, J.L., Voltas, J., Rodríguez-Ariza, M.O., Molina, F., Rovira, N., Buxo, R., Ferrio, J.P., 2008. Stable carbon and nitrogen isotopes and quality traits of fossil cereal grains provide clues on sustainability at the beginnings of Mediterranean agriculture. *Rapid Commun. Mass Spectrom.* 22 (11), 1653–1663.
- Charles, M., Forster, E., Wallace, M., Jones, G., 2015. Nor ever lighting char they grain": establishing archaeological relevant charring conditions and their effect on glume wheat and grain morphology. *Science & Technology of Archaeological Research* 1 (1), 1–6. <https://doi.org/10.1179/2054892315Y.0000000008>.
- Courty, M.-A., Allue, E., Henry, A., 2020. Forming mechanisms of vitrified charcoals in archaeological firing assemblages. *J. Archaeol. Sci.: Report* 30, 102215. <https://doi.org/10.1016/j.jasrep.2020.102215>.
- Fraser, R.A., Bogaard, A., Charles, M., Styring, A.K., Wallace, M., Jones, G., Ditchfield, P., E Heaton, T.H., 2013. 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. *J. Archaeol. Sci.* 40 (12), 4754–4766.
- Hamerow, H., Bogaard, A., Charles, M., Forster, E., Holmes, M., McKerracher, M., Neil, S., Ramsey, C.B., Stroud, E., Thomas, R., 2020. An integrated bioarchaeological approach to the medieval 'agricultural revolution': a case study from Stafford, England, c. AD 800–1200. *Eur. J. Archaeol.* 23 (4), 585–609. <https://doi.org/10.1017/eea.2020.6>.
- Hamerow, H., Bogaard, A., Charles, M., Forster, E., Holmes, M., McKerracher, M., Stroud, E., and R. Thomas. (in prep) Feeding Medieval England: the Bioarchaeology of a Long Agricultural Revolution. Oxford University Press.
- Hart, J.P., Feranec, R.S., 2020. Using Masze $\delta^{15}\text{N}$ values to assess soil fertility in fifteenth- and sixteenth-century ad Iroquoian agricultural fields. *PLoS One* 15 (4), e0230952. <https://doi.org/10.1371/journal.pone.0230952>.
- Hartman, G., Brittingham, A., Gilboa, A., Hren, M., Maas, K., Pilver, J., Weiss, E., 2020. Post-charring diagenetic alterations of archaeological lentils by bacterial degradation. *J. Archaeol. Sci.* 117, 105119. <https://doi.org/10.1016/j.jas.2020.105119>.
- Kragten, J., 1994. Calculating standard deviations and confidence intervals with a universally applicable spreadsheet technique. *Analyst* 119 (10), 2161–2165.
- McParland, L.C., Collinson, M.E., Scott, A.C., Campbell, G., Veal, R., 2010. Is vitrification in charcoal a result of high temperature burning of wood? *J. Archaeol. Sci.* 37, 2679–2687.
- Nitsch, E.K., Charles, M., Bogaard, A., 2015. Calculating a statistically robust $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ offset for charred cereal and pulse seeds. *Science & Technology of Archaeological Research* 1 (1). <https://doi.org/10.1179/2054892315Y.0000000001>.
- Poole, I., Braadaart, F., Boon, J.J., van Bergen, P.F., 2002. Stable carbon isotope changes during artificial charring of propagules. *Org. Geochem.* 33 (12), 1675–1681. [https://doi.org/10.1016/S0146-6380\(02\)00173-0](https://doi.org/10.1016/S0146-6380(02)00173-0).
- Stroud, E.A., Charles, M., Bogaard, A., Nitsch, E., Hamerow, H., submitted. The experimental heating of rye, oat, spelt, wheat and barley between 215 and 300°C: the stable carbon and nitrogen isotope data and the photographic evidence of changes to the morphology of the grains. Data in Brief.
- Styring, A.K., Diop, A.M., Bogaard, A., Champion, L., Fuller, D.Q., Gestrich, N., MacDonald, K.C., K. Neumann, K., 2019. Nitrogen isotope values of *Pennisetum glaucum* (pearl millet) grains: towards a reconstruction of past cultivation conditions in the Sahel, West Africa. *Veg. Hist. Archaeobotany* 28, 663–678.
- Szpak, P., Metcalfe, J.Z., Macdonald, R.A., 2017. Best practices for calibrating and reporting stable isotope measurements in archaeology. *J. Archaeol. Sci.: Report* 13, 609–616.