REVISITING THE POTENTIAL OF CARBONISED GRAIN TO PRESERVE BIOGENIC $^{87}SR/^{86}SR$ SIGNATURES WITHIN THE BURIAL ENVIRONMENT

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Strontium isotope analysis of archaeological crops is a potential method of provenancing and identifying the movement of crops in the past but there remains uncertainty as to whether original $^{87}Sr/^{86}Sr$ values can be obtained from carbonised buried grains. We have determined that HCl leaching removes some, but not all, exogenous strontium from carbonised cereal grains buried in soil for up to one year. We conclude that while further work could refine the leaching method, strontium isotope analysis of archaeological cereal grains can distinguish crops sourced from outside a particular (e.g. local) area if it can be shown that leaching moves grain $^{87}Sr/^{86}Sr$ values significantly away from the expected strontium signature.

Keywords: strontium; cereal grains; carbonisation; leaching; contamination; burial experiment

INTRODUCTION

Mobility studies in archaeology routinely use the strontium (Sr) isotope composition (specifically the fractionation corrected ratio of $^{87}Sr/^{86}Sr$) of human and faunal tooth enamel to identify whether the Sr isotope composition is consistent with local sources, whether the individual moved during the period of tooth formation, and therefore whether they were local to the area in which their remains were deposited (e.g. Bentley et al., 2012; Bogaard et al., 2014). Such studies enable reconstruction of the interactions between people—and their animals—in the past and can contribute to wider understanding of how mobility related to, and impacted on, concepts of cultural identity that are inferred in other ways from the archaeological record.

Strontium in biological systems stems from a combination of *in situ* weathering of bedrock during soil formation and input of Sr from precipitation and air-borne particles (Bentley, 2006). Strontium-87 is the product of radioactive decay of rubidium-87 (^{87}Rb) and so the $^{87}Sr/^{86}Sr$ value of bedrock depends on the initial $^{87}Sr/^{86}Sr$ values, Rb/Sr ratios and age of the rocks (and the minerals within these rocks) that it is composed of. Old rocks with high Rb/Sr ratios, such as granites, tend to be enriched in ^{87}Sr and have high $^{87}Sr/^{86}Sr$ values, whereas younger rocks with lower Rb/Sr ratios, such as basalts, tend to have lower $^{87}Sr/^{86}Sr$ values (Faure and Powell, 1972). Plants take up Sr from the biologically available fraction of the soil in which they grow (mainly Sr dissolved in the soil pore water) and thus have $^{87}Sr/^{86}Sr$ values that reflect their geographical origin. When ingested, these values are preserved in the tissues of the consumer (Ericson, 1985). Tooth enamel is the most commonly sampled material in Sr isotope studies, since it has been shown to preserve its original $^{87}Sr/^{86}Sr$ value during burial (Budd et al., 2000).

Strontium in archaeological crop remains

The $^{87}Sr/^{86}Sr$ values of modern plants growing in the locality of an archaeological site are often determined in studies into past mobility to establish the variation in the $^{87}Sr/^{86}Sr$ values of the locally bioavailable Sr. But there are also good reasons for determining the $^{87}Sr/^{86}Sr$ values of *archaeological* crops. Strontium isotope analysis of archaeological crop remains presents a potential method of provenancing and identifying the movement of crops in the past as well as establishing the bioavailable Sr isotope composition of plant foods available to humans during their lifetime. This in turn may provide a means of reconstructing ancient trade networks and identifying the redistribution of crop surpluses from producer to consumer sites. Strontium isotope analysis of archaeological crop remains has not been routinely employed, however, due to concerns about contamination with Sr from the burial environment and uncertainty about whether biogenic $^{87}Sr/^{86}Sr$ values are retained in botanical remains that have been buried for long periods of time.

Strontium has been found—in order of decreasing concentration—in the straw, bran and endosperm of cereal plants (Runia, 1987). While little work has focussed on Sr specifically, its similarity in chemical behaviour to calcium (Ca) means that inferences can be made about Sr based on studies into Ca in plants (Isermann, 1981). Chemical imaging by laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) has shown that Ca and other metals tend to be concentrated in the seed coat and aleurone layers of mature wheat grains (Wu et al., 2013), an observation that is consistent with the theory that metal cations (like Sr^{2+}) are readily bound by chelation to phytic acid (Reddy et al., 1982), which is concentrated in aleurone cells

(Regvar 2011). In general, cereal grains contain a relatively low amount of Sr (c. 2 ppm; Laursen et al., 2011) which means that any contamination with Sr from the burial environment is likely to swamp the biogenic $^{87}Sr/^{86}Sr$ values of cereal grains.

Moreover, cereal grains have an amorphous and fairly open structure once carbonised (Charles et al., 2015) that could permit ready sorption of exogenous Sr. Carbonised cereal grains are essentially a type of biochar, whose high internal surface area and negative charge means that it has a high sorption capacity for heavy metals. As a result, the use of biochars—formed from a range of organic materials—in the remediation of contaminated soils has been the subject of much research (reviewed by Beesley et al., 2011). The surface of biochars produced by pyrolysis at temperatures between 200 and 400˚C (similar to archaeological preserved cereal grains; Charles et al., 2015) are rich in oxygen-containing functional groups, which enable the creation of surface complexes between cations (like Sr^{2+}) and the biochar surface (Uchimiya et al., 2011). Biochars formed at higher temperatures tend to have higher carbon/oxygen ratios and a more electronegative surface. As a result, metal sorption occurs via electrostatic interaction between the metal cations and the negative charge associated with delocalised electrons in aromatic structures (Harvey et al., 2011). Given the high aromaticity of archaeological carbonised grains compared to their modern carbonised counterparts (Styring et al., 2013), it is possible that the relative importance of these two sorption mechanisms (complex formation versus electrostatic interaction) changes during burial of carbonised cereal grains. What remains unclear is whether the high affinity of biochars for metal cations is an advantage for the use of archaeological crop remains as geographical tracers, since biogenic Sr will also be

strongly bound, or whether it is a disadvantage, since exogenous Sr that has been adsorbed from the burial environment will be difficult to remove.

Few studies have investigated the potential of using archaeological crop remains as geographical tracers. Larry V. Benson and colleagues have investigated the reliability of maize kernel $^{87}Sr/^{86}Sr$ values (as well as trace metal concentrations and lead isotope values) in determining the location of agricultural fields in the Americas (reviewed in Benson, 2012). They used a procedure employed by palynologists to remove minerals from sediment samples in order to clean carbonised and uncarbonised maize cobs. This involved leaching in c. 3 M hydrochloric acid (HCl) to dissolve carbonate minerals, followed by leaching in hydrofluoric acid (HF) to dissolve silicate minerals (Benson et al., 2010). They suggested that low aluminium and Sr concentrations ($\leq$ 4.2 ppm Sr) could indicate the removal of soil mineral contamination (Benson et al., 2010; Benson, 2012). They also found, however, that this leaching removed much of the biogenic Sr (and other metals) from uncarbonised maize cobs, raising the question of whether any biogenic Sr would be retained after leaching (Benson et al., 2010). They did not test this on carbonised cobs, however, which might have a better retention capacity for metals.

Heier et al. (2009) investigated: i) the effect of charring on the concentration and $^{87}Sr/^{86}Sr$ value of hulled barley grains; ii) the adsorption of Sr by carbonised and uncarbonised barley grains soaked in a chalk solution; and iii) the effectiveness of several leaching methods in removing the adsorbed Sr. They found that charring decreased the $^{87}Sr/^{86}Sr$ value of cereal grains by 0.0001 and they posited that this could be due to inherent variability in the $^{87}Sr/^{86}Sr$ values of different grains, or

contamination during the charring process. They also found that both carbonised and uncarbonised grains absorbed Sr from the chalk solution in which they were soaked, but that leaching in 6M HCl for 24 h removed > 95% of the exogenous Sr from carbonised grains, but not from uncarbonised grains. Leaching of uncontaminated carbonised grains in 6 M HCl for 24 h was found to reduce the biogenic Sr concentration by 60%, and reduce the $^{87}Sr/^{86}Sr$ value by 0.0001 (Heier et al. 2009). This demonstrated the risk of using harsh leaching methods to remove exogenous Sr.

While the Heier et al. (2009) study has demonstrated the potential for biogenic $^{87}Sr/^{86}Sr$ values to be preserved and recovered in carbonised cereal grains found on archaeological sites, since this study was published only one study by Bogaard et al. (2014) has employed Sr isotope analysis of archaeological crop remains to identify the location of ancient crop fields. Moreover, there remained some uncertainty in the study's conclusions as to where the archaeological plants were grown. While the behaviour of archaeological charred plant $^{87}Sr/^{86}Sr$ values under leaching in 6 M HCl for 24 h suggested that their cultivation in one particular landscape zone could be ruled out, it remained uncertain whether or not all of the exogenous Sr had been removed. It is clear, then, that further work is required to address these outstanding concerns.

Given that both Benson (2012) and Heier et al. (2009) found that carbonised plant material retained biogenic Sr better than uncarbonised, and since the majority of archaeological crop material is found in a carbonised state, we focussed our investigations on carbonised cereal grains only. We also investigated the potential of using cereal rachis (the 'stem' within the cereal ear, constituting lignin-rich 'chaff')

that is often preserved on archaeological sites as a geographical tracer. The relatively high Sr content of wheat straw compared to grains (Runia 1987) suggests that rachis may be a good material for Sr isotope analysis.

The aim of this study is to address some of the outstanding questions from the Heier et al. (2009) study, in the hope of establishing whether or not archaeological crop remains can be used as reliable geographical tracers. Do carbonised cereal grains adsorb Sr to the same extent when they are buried in soil compared to being soaked in a chalk solution? Can this exogenous Sr be removed by leaching to yield the biogenic Sr isotope signature? And does leaching of uncontaminated grains change the Sr isotope signature?

METHODS AND SAMPLES

Burial experiment

Bread wheat (*T. aestivum* L.) grains and rachis grown without manure were sampled in 2004 from the long-term agricultural experiment at Rothamsted, UK (51.81 N, -0.37 E). Grains and rachis were placed in porcelain crucibles, which were covered with a lid and buried in individual beakers of sand to exclude oxygen, before heating in a Gallenkamp Plus II electric oven. The oven was preheated, and when the oven reached 230˚C the grains were placed in the oven and removed after 24 hours. These conditions have been found to produce carbonised grains that are morphologically similar to well preserved, undistorted grains found on archaeological sites (Charles et

al. 2015). The carbonised cereal grains and rachis were buried in soil collected from between 0–15 cm depth in Achnasheen, northwest Scotland (57.58 N, -5.07 E). Sub-samples of 50 carbonised grains or rachis were placed in open-gauze fabric bags, soil was added and mixed with the carbonised crop material and then the bags were tied up and buried 10 cm deep in a large plastic pot. High-purity deionised (18.2 MΩ) water (Millipore; hereafter referred to as ultrapure deionised water) was added throughout the duration of burial to keep the soil damp. Crop material remained buried for 3, 9 and 12 months. On retrieval, crop material was recovered by dry-sieving, washed in ultrapure deionised water to remove adhering soil and freeze-dried.

These cereal grains and rachis and soil were selected because the biosphere $^{87}Sr/^{86}Sr$ map created by Evans et al. (2010) allowed us to predict that the crop material and soil would have significantly different $^{87}Sr/^{86}Sr$ values, so that even low levels of Sr contamination could be detected. Based on the biosphere $^{87}Sr/^{86}Sr$ map in Evans et al. (2010), the $^{87}Sr/^{86}Sr$ values of the grains from Rothamsted were expected to be 0.708–0.709 and the $^{87}Sr/^{86}Sr$ value of the soil from Achnasheen was expected to be 0.713–0.720 (Evans et al. 2010). The $^{87}Sr/^{86}Sr$ values of the grains from Rothamsted after burial in the soil from Achnasheen were therefore expected to have $^{87}Sr/^{86}Sr$ values between these two extremes. The $^{87}Sr/^{86}Sr$ values of an ultrapure deionised water leach of the soil and of the unburied carbonised cereal grains and rachis from the same plot as the crop material used in the burial experiment are given in Table 1.

Long-term burial

The Sr concentrations and $^{87}Sr/^{86}Sr$ values of carbonised naked barley (*Hordeum vulgare* var. *nudum* L.) grains retrieved from the archaeological site of Çatalhöyük, Turkey (37.67 N, 32.83 E), were also determined in this study. While three samples were comprised of grains carbonised *in situ* in a clay bin, preventing the grains from mixing directly with the soil matrix, another three samples were comprised of grains that had spilled from the bin and mixed with the surrounding soil. The grains carbonised *in situ* were expected to have adsorbed a lower concentration of exogenous Sr than the grains that had direct contact with the soil.

Leaching experiments

Three samples of the carbonised grains and rachis from the unmanured plot at Rothamsted, UK that had been buried for 12 months, each comprising 15 grains or 20 rachis internode segments, were selected for leaching experiments. Each sample was crushed using an agate mortar and pestle and then divided into three sub-samples, which were subjected to one of the following protocols:

(1) No leaching

(2) Leaching in 10 mL 6 M HCl for 24 h at room temperature

(3) Leaching in a mixture of 10 mL 6 M HCl and 2 mL 29 M HF for 24 h at room temperature

A fourth sample of carbonised grains (n = 25) from the unmanured plot at Rothamsted, UK that had been buried for 12 months was selected for additional leaching experiments. This sample was also crushed using an agate mortar and pestle

and then divided into five sub-samples, which were subjected to one of the following protocols:

(1) No leaching

(2) Leaching in 10 mL 1 M ammonium acetate (NH_4OAc) for 24 h at room temperature

(3) Leaching in 10 mL 6 M HCl for 36 h at room temperature

(4) Leaching in 10 mL 6 M HCl for 48 h at room temperature

(5) Leaching in 10 mL 6 M HCl for 72 h at room temperature

In order to test whether the leaching procedures remove biogenic Sr, we took a sample of 30 bread wheat grains also harvested from the long-term agricultural experiment at Rothamsted in 2004, but from a different plot that had received 35 t/ha/yr cattle manure since 1852. There was insufficient crop material remaining from the unmanured field at Rothamsted to use in these experiments. These grains were carbonised under the same conditions as grains used in the burial experiment and were crushed using an agate mortar and pestle and then divided into six sub-samples, which were subjected to one of the following protocols:

(1) No leaching

(2) Leaching in 10 mL 1 M ammonium acetate (NH_4OAc) for 24 h at room temperature

(3) Leaching in 10 mL 6 M HCl for 24 h at room temperature

(4) Leaching in 10 mL 6 M HCl for 36 h at room temperature

(5) Leaching in 10 mL 6 M HCl for 48 h at room temperature

(6) Leaching in 10 mL 6 M HCl for 72 h at room temperature

Crushing ensured that the samples were homogenised and that the leaching treatment was therefore the cause of any differences in the $^{87}Sr/^{86}Sr$ values of sub-samples, rather than natural differences in $^{87}Sr/^{86}Sr$ values among grains/rachis.

After leaching, the sub-samples of leached crop material were centrifuged, the supernatant leachates collected in clean 7 mL Savillex™ perfluoroalkoxy (PFA) vials and the leached crop material rinsed in ultrapure deionised water three times. The ultrapure deionised water from each rinse was added to the leachate. Sr isotope analysis of both leached crop material and the leachates was carried out in order to fully characterise the $^{87}Sr/^{86}Sr$ values of the Sr that was removed during leaching. The HCl used in the leaching process was double distilled in-house and the HNO_3 was Teflon distilled in-house. The HF used was a Super Purity Acid (ROMIL-SpA™). All acids were diluted with ultrapure deionised water.

Digestion and determination of $^{87}Sr/^{86}Sr$ values

Prior to digestion, all crop samples were crushed using an agate mortar and pestle. Samples were digested and their $^{87}Sr/^{86}Sr$ values determined at the NERC Isotope Geoscience Laboratory (NIGL), Keyworth, UK. Crop samples were weighed into 7 mL Savillex™ PFA vials and ^{84}Sr-enriched tracer solution was added (this allows determination of Sr concentration at the same time as $^{87}Sr/^{86}Sr$ values). Crop samples were digested with 8 M HNO_3 and 30% H_2O_2 on a hot plate at c. 100 °C, until solutions were pale orange-yellow. Digests were dried and converted into their chloride form by addition of 6 M HCl. This solution was then dried down and the residues dissolved in 1 mL 2.5 M HCl. Strontium was separated using Eichrom® AG

cation exchange resin, 50W-X8 (200–400 mesh). The column chemistry is described in Appendix 1. The column eluate was dried down at c. 100 °C, a few drops of 8 M HNO_3 were added to oxidise any residual organics and the sample was dried down at c. 100 °C ready for Sr isotope analysis. Sr isotope composition and concentration were determined by thermal ionization mass spectroscopy (TIMS), using a Thermo Triton multi-collector mass spectrometer. Parafilm was melted onto the edges of a single rhenium filament and Sr was taken up in 1 μL of tantalum fluoride and placed between the parafilm boundaries on the filament. The sample was gently dried down by raising the filament current and then increased until it glowed for a few seconds before switching off the current (adapted from Birck 1986). All samples were run using a peak jumping routine of 100 * 8 second integrations. The average total Sr beam size was 2.9 V. Sr ratios were normalized for instrumental mass discrimination using a fixed $^{86}Sr/^{88}Sr$ value of 0.1194. The $^{87}Sr/^{86}Sr$ ratios were measured to an internal precision of ± 0.00006 (1SE, n = 71). The samples were run at a time when the international standard for $^{87}Sr/^{86}Sr$, NIST 987, gave a value of 0.710255 ± 0.00001 (2σ, n = 34). Procedural Sr blanks were ~80 pg. Procedural reproducibility of plant dissolutions were monitored using NIST 1515 apple leaf standard which gave a $^{87}Sr/^{86}Sr$ value of 0.713950 ± 0.00004 (2σ, n = 18) and a Sr concentration of 25.5 ± 3.9 ppm (2σ, n = 18). The $^{87}Sr/^{86}Sr$ value and Sr concentration of each of the samples are given in Appendix 2.

RESULTS

Adsorption of Sr from the burial environment – short-term burial

Table 1 and Figure 1 show the Sr concentrations and $^{87}Sr/^{86}Sr$ values of carbonised bread wheat grains grown in the same field at Rothamsted agricultural station, UK, and buried for different durations in soil with a labile $^{87}Sr/^{86}Sr$ value of 0.71478 (determined from a water leach of the soil). Unburied grains have a Sr concentration of 2.2 ± 0.1 ppm and $^{87}Sr/^{86}Sr$ value of 0.70805 ± 0.00003 (1σ, n = 3). Grains buried for 3 months have a Sr concentration of 5.4 ± 0.5 ppm and $^{87}Sr/^{86}Sr$ value of 0.71281 ± 0.00002 (1σ, n = 2). Grains buried for 9 months have a higher Sr concentration of 8.7 ± 0.6 ppm and $^{87}Sr/^{86}Sr$ value of 0.71343 ± 0.00002 (1σ, n = 2). Grains buried for 12 months have a Sr concentration of 8.3 ± 0.4 ppm and $^{87}Sr/^{86}Sr$ value of 0.71352 ± 0.00007 (1σ, n = 4).

Table 1 and Figure 1 also show the Sr concentrations and $^{87}Sr/^{86}Sr$ values of carbonised bread wheat rachis grown in the same field at Rothamsted agricultural station, UK, and buried for different durations in soil with a labile $^{87}Sr/^{86}Sr$ value of 0.71478. The unburied rachis sample has a Sr concentration of 4.6 ppm and $^{87}Sr/^{86}Sr$ value of 0.70890 (n = 1). Rachis samples buried for 3 months have a markedly higher Sr concentration of 95.3 ± 0.6 ppm and $^{87}Sr/^{86}Sr$ value of 0.71453 ± 0.00002 (1σ, n = 2). Rachis samples buried for 9 months have a Sr concentration of 102.1 ± 1.6 ppm and $^{87}Sr/^{86}Sr$ value of 0.71455 ± 0.00001 (1σ, n = 2). Rachis samples buried for 12 months have a Sr concentration of 92.5 ± 11.6 ppm and $^{87}Sr/^{86}Sr$ value of 0.71451 ± 0.00010 (1σ, n = 3).

Adsorption of Sr from the burial environment – long-term burial

Table 1 and Figure 2 show the Sr concentrations and $^{87}Sr/^{86}Sr$ values of carbonised naked barley grains retrieved from the archaeological site of Çatalhöyük, Turkey. The soil that surrounded the clay bin from which they were recovered has a labile $^{87}Sr/^{86}Sr$ value of 0.70800 (determined from a water leach of the soil). The carbonised naked barley grains have a Sr concentration of 292.2 ± 12.3 ppm and $^{87}Sr/^{86}Sr$ value of 0.70800 ± 0.00008 (1σ, n = 6). A Welch two sample t-test shows that there is no significant difference between the Sr concentrations or $^{87}Sr/^{86}Sr$ values of naked barley grains that were recovered from within a clay bin—with no contact with soil—and those that had not ($t(2.89) = 0.09$, $p = 0.933$ and $t(3.13) = 0.90$, $p = 0.431$, respectively).

Leaching experiments – removing exogenous Sr from buried grains

Figure 3a shows that the Sr concentrations of carbonised bread wheat grains from the manured plot at Rothamsted, UK that had been buried for 12 months decreased from 8.3 ± 0.4 ppm (1σ, n = 4) to 0.7 ± 0.1 ppm (1σ, n = 5) after leaching in 6 M HCl for up to 72 h at room temperature. The buried grains leached in 6 M HCl for 48 h failed to yield a Sr concentration or $^{87}Sr/^{86}Sr$ value and are therefore not included in our analysis. The $^{87}Sr/^{86}Sr$ values of the leached grains also decreased (in the direction of the $^{87}Sr/^{86}Sr$ value of unburied grains from the same field) from 0.71352 ± 0.00007 (1σ, n = 4) to 0.71089 ± 0.00036 (1σ, n = 5). There is no decrease in Sr concentration or $^{87}Sr/^{86}Sr$ value with increased leaching time in 6 M HCl—all leachable Sr seems to have been removed after 24 h. Leaching in 6 M HCl and HF or 1 M NH_4OAc for 24 h at room temperature also decreased the Sr concentrations and $^{87}Sr/^{86}Sr$ values of the buried carbonised bread wheat grain samples, but not to the same extent as leaching in

6 M HCl only. Leaching in 6 M HCl and HF decreased the Sr concentrations and $^{87}Sr/^{86}Sr$ values to 1.8 ± 0.3 ppm and 0.71173 ± 0.00025 (1σ, n = 3), respectively and leaching in 1 M NH_4OAc decreased the Sr concentration and $^{87}Sr/^{86}Sr$ value to 4.3 ppm and 0.71279 (n = 1), respectively (Figure 3a).

The $^{87}Sr/^{86}Sr$ values of the material removed from buried grains leached in 6 M HCl and HF are similar to that of the soil (0.71445 ± 0.00012; 1σ, n = 3; Figure 3a and Table 1), but the $^{87}Sr/^{86}Sr$ values of the material removed from buried grains leached in 6 M HCl for 24 h and over are lower (0.71373 ± 0.00008; 1σ, n = 5; Figure 3a and Table 1). This could indicate removal of biogenic Sr from the grain themselves and/or that HF has taken residual soil material, with a more radiogenic $^{87}Sr/^{86}Sr$ value, into solution whereas the HCl alone has not. The $^{87}Sr/^{86}Sr$ value of the material removed from buried grains leached in 1 M NH_4OAc for 24 h (0.71406, n = 1; Figure 3a and Table 1) falls between the $^{87}Sr/^{86}Sr$ values of the material removed during leaching in 6 M HCl and in 6 M HCl and HF.

Figure 3b shows that the mean Sr concentration of carbonised bread wheat rachis that had been buried for 12 months decreased from 92.5 ± 11.6 ppm (1σ, n = 3) to 1.6 ± 0.2 ppm (1σ, n = 3) after leaching in 6 M HCl for 24 h at room temperature. Their mean $^{87}Sr/^{86}Sr$ value did not decrease, but actually increased slightly from 0.71451 ± 0.00010 to 0.71501 ± 0.00067. Figure 3b also shows that leaching in 6 M HCl and HF for 24 h at room temperature did not markedly decrease the $^{87}Sr/^{86}Sr$ value of these carbonised bread wheat rachis samples (0.71359; n = 1). The $^{87}Sr/^{86}Sr$ values of the material removed from buried rachis leached in 6 M HCl are similar to that of the soil (0.71451 ± 0.00009; 1σ, n = 3; Figure 3b and Table 1), as are the $^{87}Sr/^{86}Sr$ values of

the material removed from buried grains leached in 6 M HCl and HF (0.71493 ± 0.00014; 1σ, n = 3; Figure 3b and Table 1).

Leaching experiments – quantifying the removal of biogenic Sr

Figure 4 shows that after leaching in 6 M HCl for between 24 and 72 h at room temperature, the Sr concentrations of carbonised bread wheat grains from the manured plot at Rothamsted, UK that had not been buried decreased from 6.5 ppm (n = 1) to 1.2 ± 0.2 ppm (1σ, n = 4). There is no correlation between leaching duration and Sr concentration; 5.3 ppm or 82% biogenic Sr was removed during leaching in 6 M HCl for 24 h or longer. The $^{87}Sr/^{86}Sr$ values of the leached grains (excluding the grains leached for 24 h in 6 M HCl due to their anomalously low $^{87}Sr/^{86}Sr$ value) are slightly higher than the un-leached grain sample (0.71037 ± 0.000009; 1σ, n = 3 compared to 0.71031; Figure 4), whereas the $^{87}Sr/^{86}Sr$ values of material leached out of the leached grains are slightly lower than the $^{87}Sr/^{86}Sr$ value of the un-leached grains (0.71027 ± 0.00001; 1σ, n = 4 compared to 0.71031; Figure 4). The difference in $^{87}Sr/^{86}Sr$ values are at the fifth decimal place, however, which suggests that the biogenic Sr that is removed during leaching is not from a distinct isotopic pool. The carbonised grains leached in 1 M NH_4OAc for 24 h failed to yield a Sr concentration or $^{87}Sr/^{86}Sr$ value and are therefore not included in our analysis.

DISCUSSION

It is clear from the burial experiments that cereal grains and rachis adsorb exogenous Sr and that this adsorption of Sr is significant after less than 3 months of burial in soil.

Rachis shows a much greater susceptibility to adsorb exogenous Sr than grain and seemed to reach saturation after 3 months of burial. This is likely to be due to the greater surface area of rachis fragments compared to grains, permitting faster and greater adsorption of Sr.

The high Sr concentration and consistent $^{87}Sr/^{86}Sr$ values (close to the labile $^{87}Sr/^{86}Sr$ value of the soil) of the cereal grain samples from the archaeological site of Çatalhöyük demonstrates that all of the cereal grains had adsorbed exogenous Sr. In addition, the cereal grains that had remained physically separate from the surrounding soil matrix (recovered *in situ* from a clay storage bin) contain as much Sr as those that were recovered from the soil itself. It therefore seems that adsorption of exogenous Sr occurs through movement of water soluble Sr (similar to uranium uptake by buried bone; Hedges and Millard, 1995), rather than physical contamination of cereal grains with soil *per se*. The $^{87}Sr/^{86}Sr$ values of these cereal grain samples were very close to those determined in 24 h 6 M HCl leaches of other plant material (almonds, naked barley grain, clubrush nutlets, wild mustard seeds, pea seeds; mean $^{87}Sr/^{86}Sr$ value = 0.70802) recovered from Çatalhöyük, presumed to reflect the $^{87}Sr/^{86}Sr$ value of the soil in which they were buried (Bogaard et al., 2014).

Leaching in 6 M hydrochloric acid, 6 M hydrochloric acid and hydrofluoric acid, or 1 M ammonium acetate for 24 h or longer succeeded in removing some of the exogenous Sr from carbonised cereal grains, but none of these leaching protocols resulted in buried grains yielding a $^{87}Sr/^{86}Sr$ value similar to unburied grains. It should be noted, however, that the short-term burial experiment deliberately used soil and carbonised cereal grains with very different $^{87}Sr/^{86}Sr$ values, so even a very small

contribution of exogenous Sr changes the $^{87}Sr/^{86}Sr$ value of the buried cereal grains significantly. Since leaching of carbonised grains in 6 M HCl for 24 h or longer removes biogenic as well as exogenous Sr, it is not possible to use the concentration of Sr in leached buried grains as an indicator of the amount of exogenous Sr remaining after leaching.

Leaching in 6M HCl + HF was less successful at yielding cereal grains with $^{87}Sr/^{86}Sr$ values close to those of their unburied counterparts than leaching in 6 M HCl only. This might be because the HF dissolved some recalcitrant mineral particles still adhering to the buried grains, which contributed additional Sr compared to grains that were leaching in HCl only. Benson et al. (2010) also observed that cleaning in HF resulted in higher concentrations of aluminium in carbonised archaeological corn cobs. They suggest that HF was dissolving residual aluminosilicate minerals that originated from the burial environment. Similarly, leaching in 1 M ammonium acetate was less effective at removing exogenous Sr than leaching in 6 M HCl. Ammonium acetate is commonly used to extract exchangeable cations from soil (Ure et al., 1993), making it a gentler treatment compared to HCl, which also extracts Sr bound to Fe-Mn oxides, adsorbed to clays, and bound in carbonates (Négrel et al., 2000). The $^{87}Sr/^{86}Sr$ values of buried carbonised rachis leached in 6 M HCl and 6 M HCl and HF did not change compared to unleached rachis, suggesting that the leaching procedures failed to remove exogenous Sr. This is despite the reduction in Sr concentration of leached rachis and indicates that biogenic Sr was preferentially removed during leaching.

CONCLUSIONS

This study has shown that carbonised cereal grains adsorb exogenous Sr from the soil in which they are buried and that though leaching in 6 M HCl for 24 h was found to be the most successful method, it does not remove all of this exogenous Sr. Furthermore, leaching of carbonised cereal grains removes biogenic Sr as well as exogenous Sr, and so it is not possible to use the Sr concentration of leached cereal grains as a means of determining when all exogenous Sr has been removed. However, if the $^{87}Sr/^{86}Sr$ value of a cereal grain sample is determined before and after leaching in 6 M HCl, and its $^{87}Sr/^{86}Sr$ value becomes more different from—and does not overlap with—the range of $^{87}Sr/^{86}Sr$ values determined within a defined region (e.g. within a 2 km radius of the site from which it was recovered), it is possible to say that the cereal grains were not grown in that region. In the case of the short-term burial experiment, for example, if the cereal grains grown at Rothamsted were recovered in northwest Scotland, it would not be possible to identify their growing location as southeast England (based on their post-burial, post-leaching $^{87}Sr/^{86}Sr$ value of 0.711 and the expected $^{87}Sr/^{86}Sr$ value of the chalklands in southeast England of 0.708–0.709). It would be clear, however, that the grains did not grow in soil with a similar $^{87}Sr/^{86}Sr$ value to that in which they were buried, or indeed in soil with a $^{87}Sr/^{86}Sr$ value of greater than 0.711, because after leaching their $^{87}Sr/^{86}Sr$ value becomes less radiogenic. The incomplete removal of exogenous Sr contamination by leaching therefore only results in an *underestimation* of the likelihood that cereals were grown outside the local area. Unfortunately rachis is not a suitable material for Sr isotope analysis, despite its higher initial concentration of Sr compared to grain.

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REFERENCES

Beesley, L., Moreno-Jiménez, E., Gomez-Eyles, J.L., Harris, E., Robinson, B., Sizmur, T., 2011. A review of biochars' potential role in the remediation, revegetation and restoration of contaminated soils. *Environmental Pollution* 159, 3269–3282.

Benson, L.V., 2012. Development and application of methods used to source prehistoric southwestern maize: a review. *Journal of Archaeological Science* **39**, 791–807.

Benson, L.V., Taylor, H.E., Plowman, T.I., Roth, D.A., Antweiler, R.C., 2010. The cleaning of burned and contaminated archaeological maize prior to 87Sr/86Sr analysis. *Journal of Archaeological Science* **37**, 84–91.

Bentley, R.A., 2006. Strontium Isotopes from the Earth to the Archaeological Skeleton: A Review. *Journal of Archaeological Method and Theory* **13**, 135–187.

Bentley, R.A., Bickle, P., Fibiger, L., Nowell, G.M., Dale, C.W., Hedges, R.E.M., Hamilton, J., Wahl, J., Francken, M., Grupe, G., Lenneis, E., Teschler-Nicola, M., Arbogast, R.-M., Hofmann, D., Whittle, A., 2012. Community differentiation and kinship among Europe's first farmers. *Proceedings of the National Academy of Sciences* **109**, 9326–9330.

Bogaard, A., Henton, E., Evans, J.A., Twiss, K.C., Charles, M.P., Vaiglova, P., Russell, N., 2014. Locating land use at Neolithic Çatalhöyük, Turkey: the implications of 87Sr/86Sr signatures in plants and sheep tooth sequences. *Archaeometry* **56**, 860–877.

Budd, P., Montgomery, J., Barreiro, B., Thomas, R.G., 2000. Differential diagenesis of strontium in archaeological human dental tissues. *Applied Geochemistry* **15**, 687–694.

Charles, M., Forster, E., Wallace, M., Jones, G., 2015. "Nor ever lightning char thy grain": establishing archaeologically relevant charring conditions and their effect on glume wheat grain morphology. *STAR: Science & Technology of Archaeological Research* **1**, 1-6.

Ericson, J.E., 1985. Strontium isotope characterization in the study of prehistoric human ecology. *Journal of Human Evolution* **14**, 503–514.

Faure, G., Powell, T., 1972. *Strontium Isotope Geology*. Springer, New York.

Harvey, O.R., Herbert, B.E., Rhue, R.D., Kuo, L.-J., 2011. Metal interactions at the biochar-water interface: energetics and structure-sorption relationships elucidated by flow adsorption microcalorimetry. *Environmental Science & Technology* **45**, 5550–5556

Hedges, R.E.M., Millard, A.R., 1995. Bones and Groundwater: Towards the Modelling of Diagenetic Processes. *Journal of Archaeological Science* **22**, 155–164.

Heier, A., Evans, J.A., Montgomery, J., 2009. The potential of carbonized grain to preserve biogenic 87Sr/86Sr signatures within the burial environment*. *Archaeometry* **51**, 277–291.

Isermann, K., 1981. Uptake of stable strontium by plants and effects of plant growth, in: Skoryna, S. (Ed.), *Handbook of Stable Strontium*. Springer, New York, pp.65-86.

Laursen, K.H., Schjoerring, J.K., Olesen, J.E., Askegaard, M., Halekoh, U., Husted, S., 2011. Multielemental fingerprinting as a tool for authentication of organic wheat, barley, faba bean, and potato. *Journal of Agricultural and Food Chemistry* **59**, 4385–4396.

Négrel, P., Grosbois, C., Kloppmann, W., 2000. The labile fraction of suspended matter in the Loire River (France): multi-element chemistry and isotopic (Rb–Sr and C–O) systematics. *Chemical Geology* **166**, 271–285.

Reddy, N.R., Sathe, S.K., Salunkhe, D.K., 1982. Phytates in legumes and cereals, in: C.O. Chichester, E.M.M. and G.F.S. (Ed.), *Advances in Food Research*. Academic Press, pp. 1–92.

Runia, L.T., 1987. Strontium and calcium distribution in plants: effect on palaeodietary studies. *Journal of Archaeological Science* **14**, 599–608.

Styring, A.K., Manning, H., Fraser, R.A., Wallace, M., Jones, G., Charles, M., Heaton, T.H.E., Bogaard, A., Evershed, R.P., 2013. The effect of charring and burial on the biochemical composition of cereal grains: investigating the integrity of archaeological plant material. *Journal of Archaeological Science* **40**, 4767–4779.

Uchimiya, M., Chang, S., Klasson, K.T., 2011. Screening biochars for heavy metal retention in soil: role of oxygen functional groups. *Journal of Hazardous Materials* **190**, 432–441.

Ure, M., Thomas, R., Littlejohn, D., 1993. Ammonium acetate extracts and their analysis for the speciation of metal ions in soils and sediments. International *Journal of Environmental Analytical Chemistry* **51**, 65–84.

Wu, B., Andersch, F., Weschke, W., Weber, H., Becker, J.S., 2013. Diverse accumulation and distribution of nutrient elements in developing wheat grain studied by laser ablation inductively coupled plasma mass spectrometry imaging. *Metallomics* **5**, 1276.

FIGURE CAPTIONS

Figure 1. The change in Sr concentration (note the logarithmic scale) and $^{87}Sr/^{86}Sr$ values of carbonised cereal grain and rachis samples after burial in soil (whose $^{87}Sr/^{86}Sr$ value is marked with a dashed line) for 3, 9 and 12 months.

Figure 2. The: Sr concentration and $^{87}Sr/^{86}Sr$ values of carbonised cereal grain samples recovered from the archaeological site of Çatalhöyük (dated to c. 6000 cal BC). The $^{87}Sr/^{86}Sr$ value of the soil in which the grain samples were buried is marked with a dashed line.

Figure 3. The effect of leaching in 6 M HCl, 6 M HCl + HF, or 1 M NH_4OAc for between 24 and 72 hours on the Sr concentration and $^{87}Sr/^{86}Sr$ values of: a) carbonised cereal grain and b) carbonised rachis samples that had been buried in soil (whose $^{87}Sr/^{86}Sr$ value is marked with a dashed line) for 12 months. Circular symbols correspond to the first leaching experiment on buried grains and rachis, diamond symbols correspond to the second leaching experiment on buried grains. All of these grains and rachis were grown on an unmanured plot at Rothamsted, UK, carbonised and buried in soil for 12 months.

Figure 4. The effect of leaching in 6 M HCl for between 24 and 72 hours on the Sr concentration and $^{87}Sr/^{86}Sr$ values of unburied carbonised cereal grain samples.

Table 1. Summary of crop sample and soil Sr concentrations and $^{87}Sr/^{86}Sr$ values determined in this study. In cases where the $^{87}Sr/^{86}Sr$ values of multiple samples were

determined, the $^{87}Sr/^{86}Sr$ value and Sr concentration are the average of the replicates ± 1σ. The Sr concentration of leachates are calculated by subtracting the Sr concentration of the leached sample from the Sr concentration of the unleached sample.