



# Using portable X-ray fluorescence (pXRF) spectrometry to discriminate burned skeletal fragments

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## Abstract

Identifying the individuals who make up burned and commingled skeletal assemblages represents a labour-intensive challenge. Portable X-ray fluorescence (pXRF) is a potential tool for reconciling fragmented and mixed individuals using the unique elemental content of bone. While the method's usefulness has been demonstrated with unburned bone, further work is needed to identify if the elemental signatures embedded in bone remain consistent enough, regardless of exposure temperature, to allow the discrimination of burned individuals. We test whether pXRF can discriminate between individuals with variable degrees of burning and further, whether the elemental profiles reliably reflect burning temperatures. Tibiae and femora from five fresh lambs (*Ovis aries*) were sectioned and experimentally burned for 30 min at 200 °C, 400 °C, 600 °C, 800 °C and 900 °C. Elemental profiles from the unburned and burned fragments were analysed using discriminant function analysis. Whether burned, unburned or variably exposed to heat, fragments from the five individuals were successfully distinguished using aggregate elements (more than 80% of fragments correctly classified). The elemental profiles did vary by degree of burning allowing the distinction of fragments burned at < 200 °C, 400 °C, 600–800 °C and 900 °C (> 90% correctly classified). Collectively, these results show the promise of pXRF in the analysis of burned and commingled assemblages if the elements used are carefully considered and aggregated. However, further work considering diagenetic effects needs to be undertaken.

**Keywords** Burned bone · Trace element analysis · Portable X-ray fluorescence · Commingled

## Introduction

The discrimination of individuals from burned, fragmented remains is labour-intensive. Consequently, burned commingled assemblages are underrepresented in mortuary analyses (Naji et al. 2014; Knüsel and Robb 2016; Williams et al. 2017; Osterholtz 2019). Methods allowing fast and non-destructive analysis while maintaining high reliability are key to addressing this problem. In this paper, we investigate whether portable X-ray fluorescence (pXRF) spectrometry can discriminate among unburned and burned fragmented remains of individuals using trace elemental composition of skeletal tissue. We further explore whether the same technique is of value in identifying the temperature of burning.

This research builds upon recent work demonstrating the utility of XRF when applied to human remains in archaeological and forensic contexts (Gonzalez-Rodriguez and Fowler 2013; Perrone et al. 2014; Winburn et al. 2017; Finlayson et al. 2017). Such studies have either targeted a select number of chemical elements for investigation (Gonzalez-Rodriguez and Fowler 2013) or compared individual chemical elements from individuals on a case-by-case basis (Perrone et al. 2014; Winburn et al. 2017; Finlayson et al. 2017). Importantly, the latter approach requires the prior assessment of the minimum number of individuals (MNI) and respective elemental profiles of elements. Typically, when working with burned remains, hundreds to thousands of fragments are present, rendering a case-by-case approach impractical. Further work is needed in controlled experimental settings prior to applying pXRF to burned and commingled skeletal remains in archaeological or forensic contexts.

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In this study, we assess whether an aggregate elemental approach to fragmented bone is able to discriminate among individuals effectively even when variably burned. We also examine the impact of burning on elemental profiles and whether pXRF might assist in characterising combustion temperature. Results indicate pXRF provides a useful tool for investigating burned and commingled contexts and their varied permutations.

## Background: pXRF and bone

XRF spectrometry is a fast, versatile and relatively inexpensive tool which allows the reliable detection of elements between Z19 and Z41 (K to Nb) (Shackley 2011). It is increasingly used to investigate the elemental profiles of biological specimens to address physiological, environmental and legal issues (Fleming and Gherase 2007; Nie et al. 2011; Gilpin and Christensen 2015; Towett et al. 2016; Buddhachat et al. 2017). Because the method is non-destructive and the pXRF units are portable, allowing the analysis of large quantities of material in the field, this method is increasingly used in archaeological and anthropological sciences (Little et al. 2014; Emmitt et al. 2018).

With regard to human remains, pXRF presents an opportunity to analyse the complex databank of elemental signatures embedded in teeth and bone to answer archaeological and forensic questions. Skeletal tissue is a heterogeneous material comprised of both organic and inorganic components (Zimmerman et al. 2015). The inorganic components of bone and teeth act as a biological reservoir and primarily consist of calcium phosphate crystals (Bronner 2008). A number of other elements (such as Zn, Fe, Cu and Sr) are found in the mineralised tissue (Smrčka 2005; Gilpin and Christensen 2015). The concentration in which these elements occur is unique to individuals and is mediated by multiple factors. These include when during development tissues form, bone type, bone location, as well as diet, environmental exposures and individual variability in metabolic function (Pemmer et al. 2013; Zimmerman et al. 2015; Zaichick and Zaichick 2016). Together, these pathways produce an elemental fingerprint which forms the basis of established bioarchaeological toolkits, such as stable isotope analysis.

pXRF has been explored as a supporting method for parsing out commingled assemblages, first by Gonzalez-Rodriguez and Fowler (2013), followed by Perrone et al. (2014), Winburn et al. (2017) and Finlayson et al. (2017). Perrone et al. (2014) demonstrate that a bone with unknown origins can be excluded from an individual of interest if no overlap in element concentration is present between the bone and the skeletal remains already established as belonging to a single individual. The initial establishment of which elements belong to an individual is essential to this exclusionary

method and is conducted using traditional sorting methods, such as pair-matching. Winburn et al. (2017) and Finlayson et al. (2017) support this finding using a similar 95% confidence interval exclusionary approach. However, this element-by-element overlap approach ignores the value of using multiple chemical elements in unison. Furthermore, the element-by-element approach is less useful when the commingled human remains under investigation are fragmentary since it requires that elemental concentrations and the related confidence intervals for an individual are created from multiple bone elements belonging to a consolidated individual. Only then can other bones be comparatively assessed against the calculated confidence intervals. However, in fragmentary commingled deposits, the necessary preliminary reassociations of individuals may not be possible, preventing the calculation of confidence intervals. This is especially true for burned human remains, where shrinking, warping and fragmentation at high temperatures obscure morphological variation between individuals (Gonçalves et al. 2011; Carroll and Squires 2020).

In this paper, we explore whether a multi-element approach that does not work from known elemental profiles is a useful alternative for analysing a collection of commingled fragments. However, expanding this approach to burned bone necessitates consideration of how skeletal tissue and elemental signatures in bone change with heat exposure since the elemental signatures in bone burned at 900 °C, for example, may not match the chemical signatures obtained from an unburned bone from the same individual (Grupe and Hummel 1991). Variability in heat exposure, both between and within individuals, is expected in burning contexts since the duration of heat exposure, oxygen supply, fire management, local weather conditions, insulation effects and the condition of the remains all contribute to variation in exposure temperature (McKinley 1997; Symes et al. 2015). This variability needs to be understood when attempting to use elemental profiles within bone fragments to identify individuals in burned and fragmented contexts.

Experimental studies exploring how bone responds to heating have demonstrated that substantial structural and elemental bone changes correspond with increasing heat exposure (Grupe and Hummel 1991; Person et al. 1996; Harbeck et al. 2011; Thompson et al. 2011; Piga et al. 2016). Burning is a diagenetic process, the same processes which lead to the shifting bone dimensions through changes in the crystalline structure of the bone matrix impact bone chemistry, particularly above 500 °C (Greenwood et al. 2013; Piga et al. 2013; Mamede et al. 2017; Schmahl et al. 2017; Carroll and Squires 2020). While the chemical components of bone are known to shift and new crystalline phases are introduced (see Greenwood et al. 2013 and Iriarte et al. 2020 for review), previous studies have demonstrated that heat-induced impact to trace elements is variable (Grupe

and Hummel 1991; Subira and Malgosa 1993; Iriarte et al. 2020).

Despite the experimental work to date, the precise mechanisms underpinning proportional changes in bone element content with heat exposure are not completely understood, remaining “the least well studied aspect of heat-induced change in bone” (Thompson et al. 2017: 328). The elemental changes in bone may be due to a number of reactive processes, such as (1) the dynamic elemental uptake and substitution in bone during the heating and cooling of the inorganic phase, (2) changes in the relative proportions of elements with the loss of water and organic phase as well as (3) heat-induced responses inherent to the elements present in bone (Trueman et al. 2011; Greenwood et al. 2013; Thompson et al. 2017; Mamede et al. 2017).

Elemental analyses under controlled conditions are needed to identify which elements change and under what conditions, as this information may introduce further interpretive opportunities. If heavier elements, such as Sr, remain stable despite being exposed to high temperatures (Grupe and Hummel 1991; Harbeck et al. 2011), is this element enough to identify and re-associate commingled and burned remains? Additionally, are the results from pXRF able to assist in parsing out the variable and complex processes involved in the preparation and burning of bodies? For example, if systematic changes are observed in light elemental concentrations with increasing temperatures, may elemental concentrations be used along with macroscopic bone changes to estimate burning temperature, potentially providing insight into uniformities in burning practices?

Given that pXRF can characterise the elements within samples quickly, its use may present an opportunity for the analysis of burned bone. This paper investigates how well the method reassociates variably burned commingled individuals. In order to assess the application of pXRF analysis on burned skeletal material, the following questions are addressed: (1) do bones of individuals within a species exhibit unique elemental values resulting from differences in diet, physiology, environment and metabolism that allow them to be reliably distinguished and (2) do observed differences in elemental values that distinguish among individuals hold across a range of different burning temperatures?

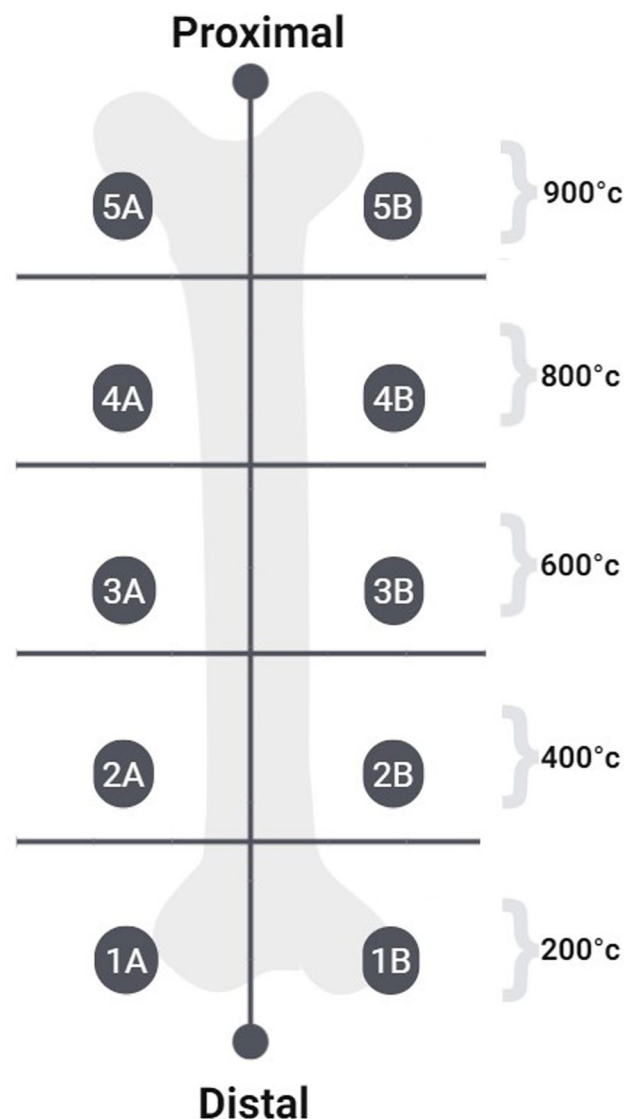
## Materials and methods

### Sample preparation

Due to the sensitivities around the use of human material and in line with previous experimental work (for example, Thompson 2005; Thompson et al. 2011; Ellingham et al. 2016), faunal osteological material is used in this study. Two articulating long bones, a femur and a tibia, were obtained from

five fresh lambs aged between 5 and 12 months (*Ovis aries*, labelled A to E) sourced from local butchers to ensure that the bone elements used in this study belong to discrete individuals. Each bone was defleshed and sectioned into five similar-sized bone sections (32 mm in length, on average) large enough to cover the pXRF analytical window and associated with one of five temperature regimes (as outlined in Fig. 1). To simulate the fragmentation and admixture of burned and unburned commingled contexts, each of the five bone sections was cut vertically so that every bone element was represented by ten fragments (Fig. 1). This produced 20 fragments per individuals for a total of 100 fragments.

Fragments were placed in the centre of a Carbolite-Gero rapid wire chamber furnace (RWF12/5) and exposed to set temperatures for 30 min which is sufficient for key bone



**Fig. 1** Illustration of how each bone element per lamb was sectioned into ten fragments, labelled and subsequently burned

changes to occur: 200 °C, 400 °C, 600 °C, 800 °C and 900 °C. The stepped exposure temperatures used in this study capture four identified stages of heat-induced bone alteration: dehydration (100 °C–600 °C, loss of water bound to the bone matrix), decomposition (300 °C–800 °C, pyrolysis of the organic components of bone such as collagen and proteins), inversion (500 °C–1100 °C, loss of carbonate) and fusion (above 700 °C, melting of the crystal matrix) (Mayne Correia 1997; Thompson 2004; Etok et al. 2007; Mamede et al. 2017; Marques et al. 2018). To ensure consistency, exposure temperature was determined by the fragment number, as demonstrated in Fig. 1. This schedule of burning resulted in 20 fragments per temperature.

## pXRF

Samples were analysed using a portable XRF Bruker Tracer III-SD with a Rh target and silicon drift detector. The device was operated with a live time of 30 s, at 40keV and 10 µA. All assays were analysed without a filter to ensure that lighter elements were better captured. Industry geological standards were analysed before and after each new analysis period, allowing assessment of instrument consistency across the study.

All fragments, bar one (a femur fragment belonging to lamb A, see Table 1 and Supplementary Table S1), were measured three times using the pXRF device before and after burning. This produced a net of 297 assays for unburned bone fragments and 297 burned assays distributed evenly across temperature categories (see Table 1 for details). Scanning was focused on the flattest area of dense cortical bone which was able to cover the lens in its entirety to minimise non-uniform distances between the instrument's aperture and the bone. While every effort was made to diminish the distance the X-rays travelled and therefore the amount of air attenuation, fragments had varied surface morphology which means some attenuation was unavoidable. The net peak areas were calculated in the Artax 7.4 software where manual Bayesian deconvolution was undertaken. Once exported, the element readings were ratioed to the Rh peak (18.5–22 keV) produced by the Rhodium X-ray tube, which assists in compensating for differences in the shape and density of the analysed samples (Shackley 2011; Conrey et al. 2014). The three ratioed elemental assessments per fragment were

then averaged (Table 1, see Supplementary Table S1 for the averaged values obtained for the individuals analysed).

## Evaluating the consistency of pXRF element detection

Portable XRF is focused on the “mid-Z X-ray region” and is therefore best suited to the reliable detection of elements between Z19 and Z41 (Shackley 2011). The device's detection limits, in combination with air attenuation and mass absorption effects, necessitate a critical look at the elements detected by pXRF (Shackley 2011).

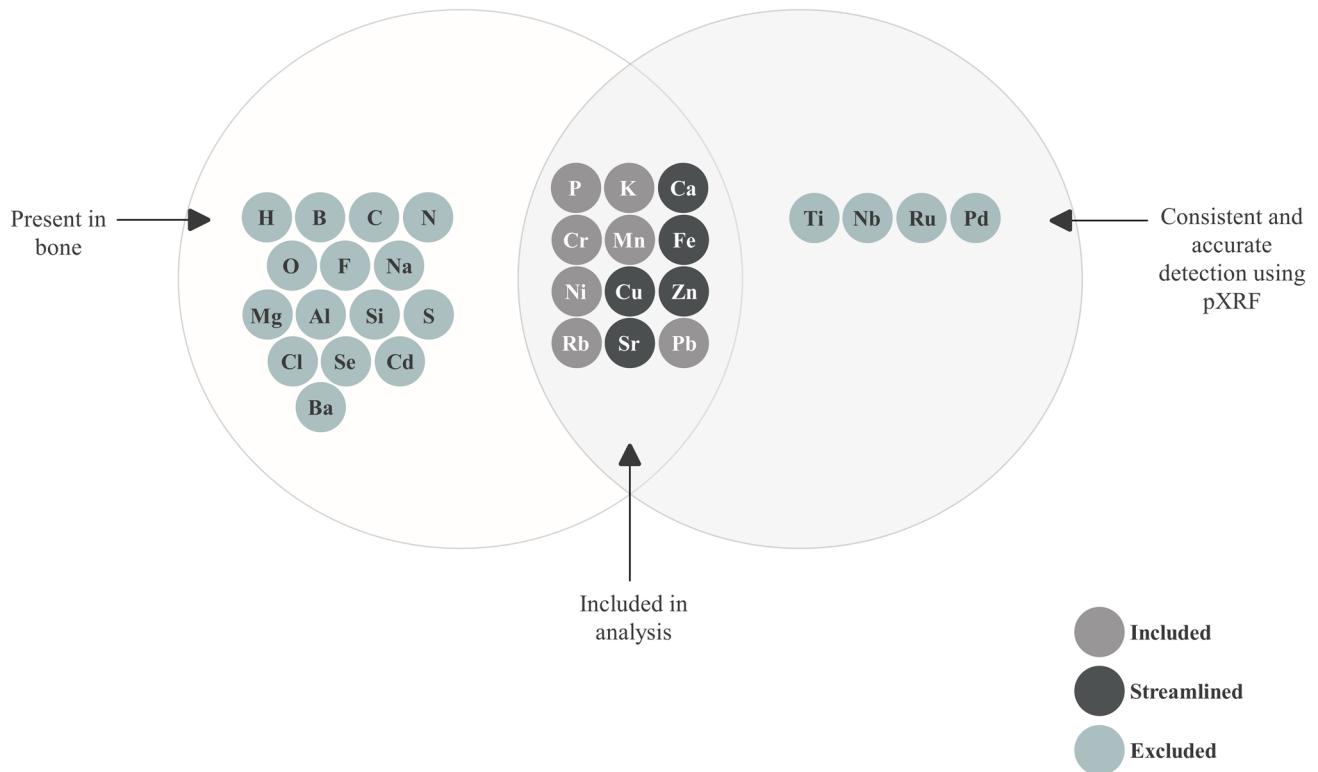
The given values for the elements represented in the Geological Standards (in weight percent) were compared to the pXRF detected values for those standards. The following standards were used: GSJ (Geological Survey of Japan: JA-2, JB-2, JB-3, JG-1A, JG-2, JSI-2), NIST (National Institute of Standards and Technology: NIST278, NIST1400, NIST1486, NIST2710, NIST2711), USGS (United States Geological Survey: COQ-1) and MINTEK (Mineral Research Organization, South Africa: SARM-2, SARM3, SARM6). Overall, the elements present in bone performed as expected: the lighter elements (such as Mg, Al and Si) are poorly captured ( $r^2 < 0.5$ ) by the pXRF device. In contrast, with some exceptions (such as Ba), the Mid-Z elements (P to Pb) perform well ( $r^2 > 0.8$ ). Together with an evaluation of the standard deviation and coefficient of variation ( $CV = \sigma/\bar{x}$ ) for each element measured in both the geological and bone fragments, the examination of expected and measured values confirms the need to be cautious of the Mg, Al, Si, S and Ba values when interpreting results. These elements are therefore excluded from this study. The remaining elements present in bone show a strong congruence between known and measured element values in the Geological Standards and are therefore included in this study (Fig. 2).

Examining the spectra visually demonstrated that matrix effects impacted a number of elements. For example, on average, the Sr peak appeared to be free of interfering elements. However, while Ni did exhibit a peak, closer inspection indicated that this peak was the result of Sr  $K\beta$  and not Ni  $K\alpha$ . Similarly, the escape peak of Ca impacted K, while S, P, Si and Al all variably impacted each other. In addition, several papers have highlighted that elements lighter than Fe are influenced by the surface

**Table 1** Summary of spectra obtained

	Unburned	200 °C	400 °C	600 °C	800 °C	900 °C
Total (3 assays per fragment)	297 <sup>a</sup>	57 <sup>a</sup>	60	60	60	60
Averaged	99 <sup>a</sup>	19 <sup>a</sup>	20	20	20	20

<sup>a</sup>Mould present on fragment LAFF1B. This fragment was therefore excluded from pXRF analysis at both unburned and burned states. The absence of LAFF1B is reflected in the total number of spectra obtained from unburned fragments and fragments burned at 200 °C



**Fig. 2** Venn diagram displaying chemical elements present in bone (Iyengar and Tandon 1999; Smrčka 2005) and elements that fall within the device's detection limits and provide accurate and consistent results using pXRF

morphology of an item of interest (for example, see Forster et al. 2011). These nuances need to be accounted for when investigating the variance between burning condition and individual lambs. Therefore, a “streamlined” analysis which removed elements impacted by matrix effects was conducted to assess whether reducing the number of elements used decreases the effectiveness of pXRF. This streamlined analysis was performed using Cu, Sr, Zn, Ca and Fe (Fig. 2).

### Statistical analysis

The spectral data from the bone fragments were imported into R, version 4.0.2 (R Core Team 2020). The relationships between the groups making up the pilot study were explored using discriminant function analysis. This classification method uses characteristics to construct a series of linear functions that maximise differences between classes. Multiple discriminant analyses were conducted to explore how well pXRF spectra collectively are able to accurately classify fragments by (1) lamb or (2) burn temperature. Given that the unburned XRF data contained a larger number of mean XRF readings ( $n = 99$ , Table 1), when the unburned bone data are included in analyses alongside burned bone

spectra, a random subset of 19 bone fragment spectra from each category is used. Each discriminant function model was developed using a 70% random training subset of the data, and the resulting models were tested on the remaining test subset (30%).

## Results

### Discriminating by individual

We first examined how well the 12 selected elements discriminated a random selection ( $n = 70$ ) of unburned fragments. Fragments in the 70% training set were discriminated with 98.6% accuracy. The equation developed using the training set was applied to the remaining 30% of the fragments ( $n = 29$ ), where discrimination fell modestly but remained high (86.2% accuracy rate) (Table 2, Fig. 3A). This demonstrates that the unburned individuals used in this study can be reliably discriminated using pXRF.

This approach was then applied to both the variably burned fragments and a combination of burned and unburned fragments to assess whether burning temperatures attenuate the discrimination of individuals. Models generated using the 70% training sets for the burned ( $n = 70$ ) and combined group ( $n$

**Table 2** Summary of discriminant function analysis classification results for the individuation and identification of burning temperature

Grouping variable	Exposure condition	Elements included <sup>a</sup>	Contribution of LD1 to variance	Accuracy of the model (training set, 70%)	Accuracy of the model (test set, 30%)
<b>Individuals</b>					
Lamb ID	Unburned	12	64.86%	98.6%	86.2%
Lamb ID	Burned	12	91.13%	91.4%	86.2%
Lamb ID	Unburned and burned <sup>b</sup>	12	89.76%	92.8%	83.9%
Lamb ID	Unburned and burned <sup>b</sup>	5	94.76%	78.3%	61.3%
<b>Burn temperature</b>					
Temperature	Unburned and burned <sup>b</sup>	12	87.28%	83.3%	56.7%
Temperature, adjusted <sup>c</sup>	Unburned and burned <sup>b</sup>	12	90.53%	96.3%	90.6%

<sup>a</sup>See Fig. 2 for a list of elements included and excluded from analysis

<sup>b</sup>Random subset of unburned spectra included to ensure equal sample sizes across burn categories

<sup>c</sup>Temperature adjusted refers to modifications of how burning temperatures are grouped following the first analysis. Unburned fragments and those burned at 200 degrees are combined, as are those burned at 600 °C and 800 °C, reducing the initial six categories (0 °C, 200 °C, 400 °C, 600 °C, 800 °C, 900 °C) to four ( $\leq 200$  °C, 400 °C, 600–800 °C and 900 °C); see text for details

= 83) also resulted in the strong separation of the lamb individuals, with 91.4% and 92.8% accuracy, respectively (Table 2, Fig. 3A, C). When the resulting equations derived from the training sets were applied to the remaining 30% of fragments from the burned ( $n = 29$ ) and variably burned and unburned ( $n = 31$ ) subsets, the accuracy fell modestly but remained high (86.2% and 83.9% respectively). Therefore, while heat exposure does attenuate the discrimination of individuals, changes in bone condition related to burning do not prevent a high rate of discrimination using bone elemental content.

The discriminant function analysis was further tested exclusively on elements demonstrating the least amount of matrix interference: Ca, Cu, Fe, Sr and Zn. Both training and test sets examined using the subset of five elements provided lower but still good discrimination, 79.8% and 71.4% respectively (Table 2, Fig. 3D). While the discrimination accuracy remains high, there is a much greater degree of overlap between results indicating that using all 12 elements is a better approach, at least for the individuals used in this study. This conclusion is supported by examining element performance across the heat exposure conditions, which demonstrates that no single element alone can discriminate individuals.

Among the elements which contribute the most to discrimination, Cr is the only element which consistently contributes heavily (a relatively high absolute value) towards the separation of individuals regardless of whether the bone has been burned or not (Table 3). Elements heavily loaded within the developed discriminant equations for burned bone, such as Cu, Pb and Fe, are found to contribute less to the separation of unburned fragments. The inverse is true for Mn and Rb, which are strongly loaded (positively or negatively) in the separation of unburned fragments but less in burned fragments. The contributing weighting of Cu and Fe in the combined discriminant equation is continued in the streamlined analysis, suggesting that Fe and Cu, along with Cr and Rb, may be able to parse out

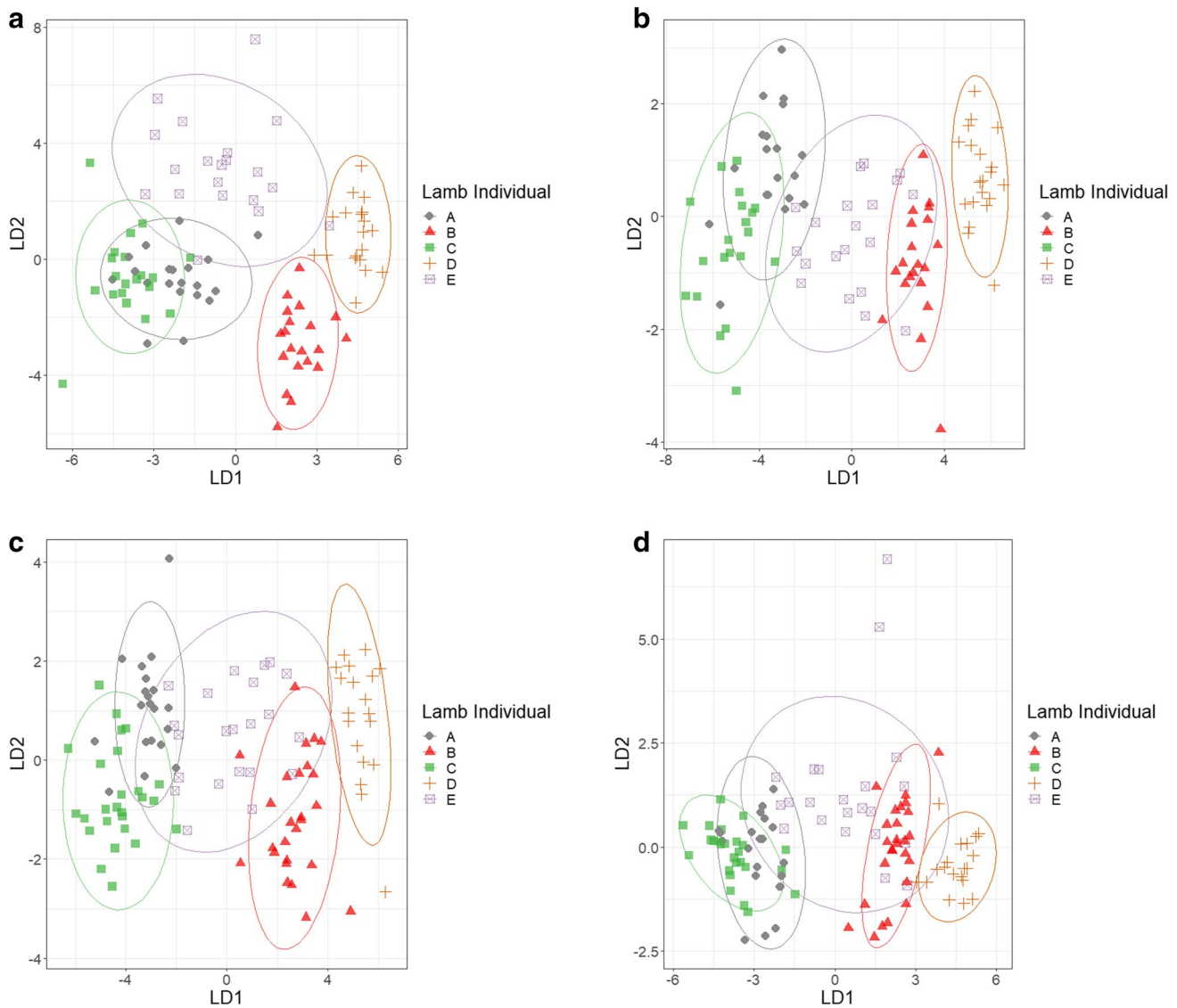
individuals who have been variably exposed to heat. Overall, the results show that while a number of elements contribute relatively little to the discrimination of individuals when considering LD1, the first linear discriminant function (Ca, K, P and Zn), a multi-element approach is preferable to ensure that maximum variation between the individuals is captured.

## Discriminating by temperature

Discriminant analysis was also employed to investigate how well pXRF spectra can distinguish the burning temperature. The unburned and burned spectra were analysed together while holding the exposure temperature as the known grouping variable. While a high discriminating accuracy was achieved using the training set (83.3%), applying the resulting equation to the test sample demonstrated a marked reduction in success (56.7%) (Table 2). Bone surface colour followed the expected sequential changes reported elsewhere (Mamede et al. 2017; Krap et al. 2019; Egeland and Pickering 2021). Bone colour progressed as follows: ivory at 0 °C, slightly darkened ivory at 200 °C, black at 400 °C, mottled grey and white at 600 °C and then white at both 800 °C and 900 °C.

The discriminant function analysis shows two non-overlapping groupings: 0 °C and 200 °C and then 600 °C to 900 °C (Fig. 4). The 400 °C group appears to be an intermediary stage between the two groups, and the overlapping clusters of 600 °C, 800 °C and 900 °C are reflected in the assignment errors. The difficulty in discriminating between the higher temperatures is unfortunate as these temperatures typically result in uniformly calcined bone, making a macroscopic distinction between these narrow temperature ranges difficult.

The relative position of the burn temperatures evident in Fig. 4 broadly reflects the heat-induced bone transformation stages identified to date, and the overlapping



**Fig. 3** Plot demonstrating the operation of LD1 and LD 2 on the lamb individuals using **a** unburned fragments, **b** burned fragments, **c** unburned and burned fragments and **d** streamlined analysis of

unburned and burned fragments. Both the 70% training set and the 30% test set are included in each plot

temperatures at which these varied changes are taking place (Mayne Correia 1997; Thompson 2004; Etok et al. 2007). While not examined in the present study, the loss of carbon represents a significant heat-induced transformation, and the effects of this loss are likely reflected in the results obtained here. For example, 400 °C represents a transitional phase where the organic components of bone, such as carbon and collagen, are still undergoing pyrolysis (Mamede et al. 2017). Around 600 °C, however, a second CO<sub>2</sub> release has taken place with the loss of structural carbonate (Mamede et al. 2017). At higher temperatures where the carbon has burned off, the pXRF

spectra are likely picking up the primary signals for the remaining inorganic bone structure. With the loss of the organic bone components, the inorganic components are no longer buffered by tissue shielding and significant changes to crystallite size and organisation are typically observed by investigators using alternative methodologies such as X-ray diffraction and spectroscopy (Etok et al. 2007; Thompson 2015). The changes to bone crystallite size and organisation above 500 °C are observed in other studies (for example, see Thompson 2015) result in bone apatite being more susceptible to elemental substitutions (Marques et al. 2016).

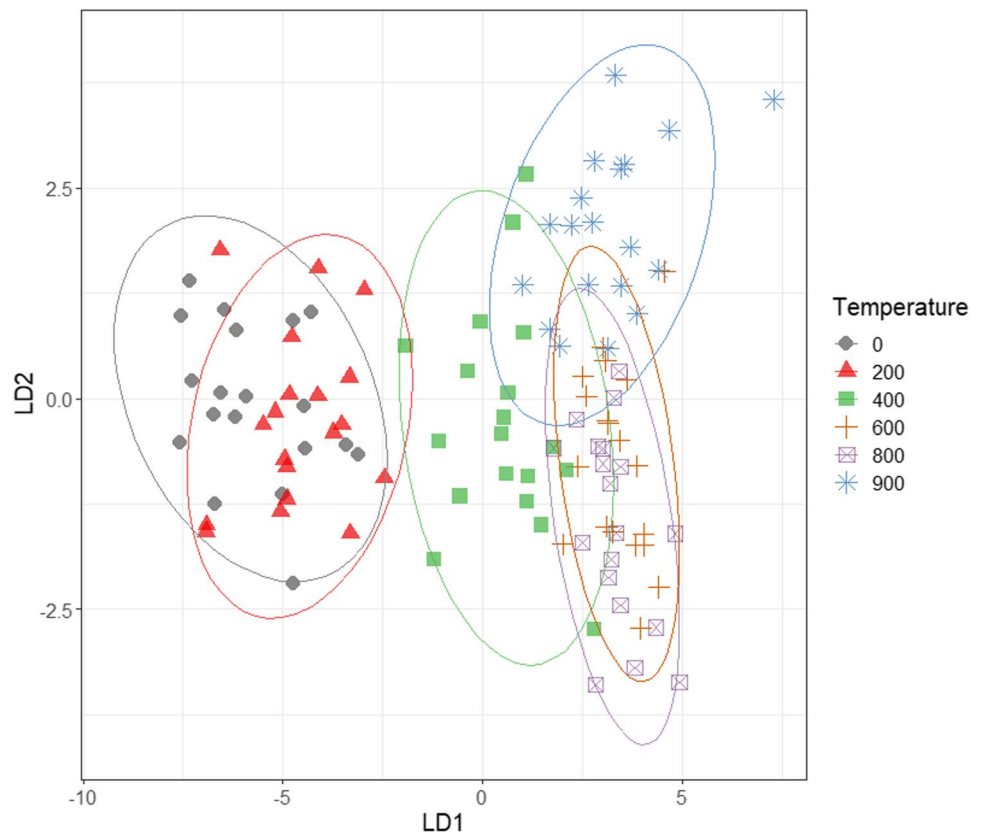
**Table 3** Coefficients of first linear discriminant functions (LD1) obtained from the discrimination of study individuals and exposure temperature, ordered from most negatively weighted to the most positively weighted

Discriminating by individual				Discriminating by temperature			
Unburned	Burned	Combined	Streamlined	Combined (0 °C, 200 °C, 400 °C, 600 °C, 800 °C, 900 °C)		Combined, adjusted (≤ 200 °C, 400 °C, 600–800 °C and 900 °C)	
Rb -17.337	Cu -35.487	Cu -27.026	Cu -22.139	Mn -31.691	Mn -44.026		
Sr -12.216	Cr -21.063	Cr -25.615	Sr -9.695	Rb -29.762	Rb -22.318		
Fe -5.826	Sr -12.316	Sr -11.116	Zn -0.003	Cu -8.684	Cu -5.383		
Ni -3.429	Ni -9.380	Ni -3.957	Ca 0.072	Zn -0.881	Zn -3.131		
Cu -1.295	Rb -6.994	Zn -2.546	Fe 10.035	Ca -0.082	Cr -0.720		
P -0.848	Zn -1.051	Ca 0.007		Sr 0.276	Ca -0.096		
Ca 0.119	Ca 0.000	K 0.350		K 1.080	Sr 0.166		
K 2.449	K 0.849	P 0.911		P 3.356	K 0.962		
Zn 3.219	P 0.932	Pb 7.545		Pb 6.062	Ni 1.681		
Pb 13.637	Mn 4.610	Mn 7.940		Cr 7.972	P 3.433		
Cr 35.994	Fe 10.953	Fe 10.113		Fe 8.025	Pb 5.619		
Mn 37.565	Pb 13.010	Rb 20.699		Ni 11.263	Fe 9.532		

For the sample considered here and plausibly for others, using broader temperature ranges produces more certain discrimination. The six exposure temperatures were regrouped into four exposure ranges which represent the key landmarks of bone mineral change with heat, as identified by Etok et al. (2007): ≤ 200 °C (0 °C and 200 °C consolidated), 400 °C,

600–800 °C (600 °C and 800 °C consolidated) and 900 °C. These broader ranges produced a marked improvement in discrimination accuracy, particularly in the 30% (n = 32) test set (Table 2). The results indicate that sufficient elemental difference exists to assist in parsing out bones burned within the consolidated temperature categories as defined.

**Fig. 4** Discriminating by exposure temperature. Both the 70% training set and the 30% test set are included in the plotted data





## Discussion

### Identifying individuals

The results demonstrate that the five lambs have elemental values that are sufficiently distinct to allow the individuals to be successfully differentiated much of the time. This holds true regardless of whether the assemblage is unburned, burned at varying temperatures or represents a mix of burned and unburned fragments. However, persistent overlap, such as between Lamb A and C as seen in Fig. 3, indicates that while pXRF will assist in differentiating individuals, it cannot necessarily identify a unique fingerprint for every individual. Increasing the number of individuals in an analysis will likely decrease this tool's effectiveness, as suggested by Perrone et al. (2014), enabling identification of a minimum but not necessarily the total number of individuals.

This work demonstrates that a multi-element approach is better able to capture the elemental content of bone. Prior work on pXRF in commingled contexts has primarily focused on selected elements and element ratios (Gonzalez-Rodriguez and Fowler 2013; Perrone et al. 2014). In contrast, this study employed a maximum target approach where elements which are reliably detected by pXRF are used collectively. The utility in a multi-element approach is highlighted by the assignment accuracy seen in the streamlined analysis: using a smaller number of elements which see limited matrix effects significantly reduced the discriminatory power of the analysis (by 14.5%). The decrease is expected given that elements such as Cr and Rb were excluded in the streamlined analysis, both of which played large contributing roles in discriminating individuals.

Overall, elements consistently found to contribute towards the discrimination of individuals are Cr, Sr, Rb, Pb, Cu and Fe (Table 3). These elements, with the exception of Cr, have variably been identified as elements of interest in the discrimination of commingled remains (for example, Castro et al. 2010). The utility of Sr in elemental investigations is well established in anthropology and has been successfully used to investigate cremated remains given its stability at high temperatures (Grupe and Hummel 1991; Harbeck et al. 2011; Harvig et al. 2014; Snoeck et al. 2015; Marsteller et al. 2017). It was therefore expected that Sr would be weighted heavily in the discrimination of individuals regardless of temperature exposure and marginally weighted in the discrimination of temperature. Results were largely consistent with these expectations, though Sr was not the strongest discriminator of elements used (see Table 3).

The contributing impact of Pb, Cu and Fe in discriminating individuals is expected given the varied and

complex factors behind the accumulation and storage of elements in bone. Pb stores within the body are primarily held within the bone and are reflective of individual exposure toxicity, as is Rb (for example, from lead water pipes) (Castro et al. 2010; Pemmer et al. 2013). In contrast, Cu and Fe are elements necessary to biological processes, and while we have uncertainties as to the precise biological function of these elements, deficiencies in Fe and Cu result in anaemia, growth arrest and skeletal abnormalities (Smrčka 2005). As the biological need for elemental stores differs across individuals, the relative proportions of elements such as Cu and Fe are likely to differ from individual to individual (Bronner 2008; Pemmer et al. 2013).

The repeated presence of Cu as one of the most effective discriminating elements may also be linked to the individuals chosen for this study. The five individuals that make up this study are immature sheep, without fully fused long bones. Cu is linked to bone mineralisation, cartilage maintenance and the ossification of growth centres so whether the results obtained here would be reproduced in adult individuals cannot be ascertained with the current data. The lambs were all between 5 and 12 months old so the individual differences in elements associated with bone growth and development, such as Cu, are likely to be the result of a combination of diet (feed enrichment protocols), exogenous mineral uptake from their particular native environments, metabolism and development.

Cr, an element implicated in metabolising glucose (Smrčka 2005), was found to be a major contributor to the discrimination of the individuals across all three analyses. This element has not featured largely in examinations of burned bone (though see Brooks et al. 2006) and requires further consideration. It will be useful to examine in future studies how stable the contributions of various elements are to discriminant function analyses of fragmentary skeletal material using the approach proposed here. As part of this, it will also be important to consider how the diets and life histories of other species influence the success of this analytical approach.

### Temperature indicators

Studies of elemental change with temperature have primarily focused on light elements such as H, C and O, which are the first to indicate bone content restructure due to dehydration and the combustion of organic matter (Greenwood et al. 2013; Snoeck et al. 2014). Given their known correlation with heat, it is anticipated that an analysis which includes these elements may be better able to discriminate between unburned bones and those exposed to low-temperature burning. These elements were not examined as they fell beyond the reliable detection limits of pXRF in this study (Fig. 2).

While the precise mechanisms underpinning thermal-induced physiochemical modification, and therefore the function of elements in differentiating burning conditions, is beyond the scope of this paper, some tentative conclusions can be made. Elements associated with the organic components of bone that are burned off completely at temperatures exceeding 500 °C represent one driver for elemental change. The presence of organic matter may suppress the fluorescence of elements embedded in the mineral structure. The study design meant that fragments from the same bone locations were burned at 200 °C, 400 °C, 600 °C, 800 °C and 900 °C. Fragments from sections 1 and 5 cover growth plates with potentially different micro-nutrients (Grupe and Hummel 1991; Rasmussen et al. 2019). While the fragments were manually stripped of marrow and soft tissue, the network of trabeculae at the epiphyses was not completely cleared of adhering organics. Fragments 1 (both burned and unburned) and 5 (unburned) may therefore be returning spectra reflecting both bone content and residual marrow. The results obtained for Fe and to a lesser extent, Cu, may reflect both the accumulation of elements at the epiphysis as well as the presence of marrow as both elements have been known to collect at the bone metaphysis (Grupe and Hummel 1991).

Mn, Rb and Fe account for a large amount of variance in the discrimination of temperature. However, there is diminished certainty around the higher exposure temperatures (Fig. 4). The model indicates that the unburned and incompletely burned fragments (0 °C and 200 °C), the charred fragments (400 °C) and the fragments exposed to high temperatures (600 °C, 800 °C and 900 °C) form four mostly distinct groups. This elemental analysis tracks onto established macroscopic groupings using bone surface colour (Thompson 2015; Wärmländer et al. 2019). This is expected, as the macroscopic changes in bone result from changes in bone structure and chemical content driven by heat exposure (Snoeck et al. 2014; Mamede et al. 2017; Thompson et al. 2017). While it is well established that discriminating by temperature can loosely be done via macroscopic analysis, further work is needed to bridge our understanding of macroscopic and microscopic changes with heat exposure. The volume of factors that collectively contribute to individual burning contexts (such as temperature, duration, fuel source, humidity and oxygenation and the position and condition of the body) necessitate a considered and multi-method approach. The rapid detection of elements using pXRF can provide a powerful complementary tool to the wide array of analytic techniques used by investigators globally, particularly those that allow the investigation of bone crystallinity (for example, vibrational spectroscopy, Fourier-transform infrared spectroscopy and X-ray diffraction. see Mamede et al. 2017 for a review).

Several elements, such as Ca, K, P and Zn, showed little discriminatory strength, whether by individual or by

temperature exposure. This result is expected for Ca and P given their ubiquitous nature in the bone matrix (Dermience et al. 2015). Though individuals may have varying Ca and P uptake, these differences are expected to be very slight unless an individual has had long-term deficiencies in one of these elements.

The stable nature of some elements at higher temperatures, such as Sr and Cr, is likely behind their negligible performance in discriminating burn temperatures. In contrast, Grupe and Hummel (1991) identified that Zn has a fluctuating relationship with temperature—though unstable across temperature ranges, the authors could not identify a consistent pattern. In this study, Zn contributed negligibly towards temperature discrimination (Table 2), supporting Grupe and Hummel's finding.

### Future work

While the results of this study support the use of elemental analysis using pXRF to discriminate individuals and explore exposure temperature, further work is needed to identify whether the identified elements are able to routinely distinguish individuals (and are therefore applicable to different species) and what elements are reflective of the degree of burning. There is a paucity of background information on the expected elemental content within the bone. While sheep have been used as a representative analogue in this study, humans have different life histories, a more diverse diet, are more mobile and likely have greater environmental exposure to heavy metal toxins. In the absence of human remains, expanding this study to include additional species which are penned, fed and managed in different ways, such as *Sus domesticus*, may provide greater insight into the role the identified elements play across different contexts.

Precisely how heat affects individual element caches within the bone matrix requires further exploration and represents a lacuna within our current understanding of burned bone. Furthermore, heat-induced transformation closely mimics diagenesis; therefore, identifying heat-induced decomposition signatures specific to exposure temperatures would be valuable in discriminating between diagenetically altered unburned bone and bone burned at low temperatures. Teasing out these nuances with experimental studies using pXRF and methods that allow the examination of bone crystallinity (such as Fourier-transform infrared spectroscopy and X-ray diffraction) will contribute to understanding how bone structures respond to different processes (see Paba et al. 2021).

We used freshly butchered individuals so the elements contained within the bone are highly likely to represent in vivo accumulation. Given how often Pb, and to a lesser extent Fe, Mn and Sr, was weighted in the linear discriminants, it is expected that bone diagenesis will negatively

impact the power of pXRF to discriminate buried individuals and their exposure temperatures. It is unlikely that the pXRF will be able to identify biogenic versus diagenetic element accumulation, aside from allowing an observation of increased levels of elements such as Sr, Pb, Mn and Fe. Future studies in this vein need to include bones exposed to known diagenetic processes alongside known variation in the burning conditions (see also Snoeck et al. 2014). However, though diagenesis is an ever-present concern in archaeology, a number of experimental studies have demonstrated that bones burned at high temperatures are better buffered from further diagenesis (see Lanting et al. 2001), although we cannot always be certain that burning immediately follows death.

The study design saw bones burned for a set duration of 30 min. It is not yet clear if the identification of burn temperature using the identified coefficients could be applied to fragments burned for longer or shorter durations. Indications from experimental work, for example Greenwood et al. (2013) and Ellingham et al. (2016), suggest that microstructural changes take place swiftly and over a short temperature range. However, the issue of variability in bone condition (for example, dry, green and fleshed), burn temperature and burn duration is a problem that needs to be addressed within experimental work more broadly. The wide range of controlled experimental conditions across studies makes inter-study comparison difficult, and using uniform experimental approaches is a necessary step prior to uncontrolled experimental work (such as open-air fires).

## Conclusion

This paper has demonstrated that when a multi-element approach is taken, pXRF can discriminate bone fragments belonging to five individuals with a high degree of success. While diminished success is seen in the discrimination of the narrow burn temperatures used in this study, pXRF can discriminate broad temperature categories, providing an additional avenue to mortuary and taphonomic investigations of burned remains. Furthermore, the non-destructive and fast analysis reduces the labour-intensive nature of burned and commingled assemblage analysis. The careful and critical application of this method can assist in piecing together the fragmented remains of the individuals who make up these assemblages and the practices of the individuals who engaged with their bodies. In re-associating these remains, we may begin to unlock the data potential of these otherwise underrepresented assemblages.

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## Declarations

**Conflict of interest** The authors declare no competing interests.

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