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1 **Signatures of degraded body tissues and environmental conditions in grave soils from a**
2 **Roman and an Anglo-Scandinavian age burial from Hungate, York**

3 Matthew D. Pickering^a, Sabina Ghislandi^b, Maria Raimonda Usai^{b,ct}, Clare Wilson^d, Peter Connelley^e,
4 Don R. Brothwell^{b†} and Brendan J. Keely^{a*}

5 ^a Department of Chemistry, University of York, Heslington, York, YO10 5DD, UK

6 ^b Department of Archaeology, University of York, The King's Manor, York, YO1 7EP, UK

7 ^c Dipartimento di Architettura e Design, Wilson, Piazza Pau Salit, Alghero, Italy

8 ^d School of Biological and Environmental Science, University of Stirling, Stirling. FK9 4LA, UK

9 ^e York Archaeological Trust, 47 Aldwark, York YO1 7BX, UK

10 ORCHID ID

11 Keely: [0000-0002-8560-1862](https://orcid.org/0000-0002-8560-1862)

12 Pickering: [0000-0002-6234-2108](https://orcid.org/0000-0002-6234-2108)

13 Wilson: [0000-0002-0287-8576](https://orcid.org/0000-0002-0287-8576)

14 **Highlights**

- 15 • Signatures of degraded body tissues, gut contents and microbial activity from grave soils.
16 • Spatial variation in signatures with anatomical location and features of burial environment.
17 • *n*-Alkanals reveal highly localised regions of anoxia.
18 • Grave chemistry and preservation of the skeletal remains has been impacted by an adjacent
19 cess pit.
20 • Pedogenic spherulites record broad changes in redox conditions within the Roman grave.

21 **Keywords:** Human burials; scientific archaeology; triacylglycerols; *n*-alkanals; bone cholesterol; redox
22 conditions; Roman; Anglo-Scandinavian.

[†] Deceased on 26 September 2016 (DRB) and 13 May 2018 (MRU).

23 **Abstract**

24 Despite the importance of human burials in archaeological investigations of past peoples and their
25 lives, the soil matrix that accommodates the remains is rarely considered, attention being focused
26 mainly on visible features. The decomposition of a buried corpse and associated organic matter
27 influences both the organic composition and, directly or indirectly, the microstructure of the burial
28 matrix, producing signatures that could be preserved over archaeological timescales. If preserved,
29 such signatures have potential to reveal aspects of the individual's lifestyle and cultural practices as
30 well as providing insights into taphonomic processes. Using organic chemical analysis and soil
31 micromorphology we have identified organic signatures and physical characteristics relating to the
32 presence of the body, and its decomposition in grave soils associated with two human skeletons (one
33 Roman age and one Anglo-Scandinavian age) from Hungate, York, UK. The organic signatures,
34 including contributions from body tissues, gut contents, bone degradation and input from microbiota,
35 exhibit spatial variations with respect to anatomical location and features of the immediate burial
36 environment. In the Roman grave broad changes in redox conditions associated with the
37 decomposition of the corpse and disturbance from the excavation and use of an Anglo-Scandinavian
38 age cess pit that partially cuts the grave were evident. Leachate from the cess pit was shown to
39 exacerbate the degradation of the skeletal remains in the regions closest to it, also degrading and
40 depleting spherulites in the soil, through decalcification of the bone and liberation of bone-derived
41 cholesterol into the soil matrix. The findings from this work have implications for future archaeo- and
42 contemporary forensic investigations of buried human remains.

43 **1 Introduction**

44 The study of human burials in the archaeological record provides a unique glimpse into the lives (and
45 deaths) of our ancestors. Investigations typically focus on the recording, recovery and analysis of the
46 visible human remains, grave goods and burial structures (Brothwell, 1981). Although some well-
47 preserved burials have provided evidence of clothing (Barfield, 1994; Hadian et al., 2012), organic
48 grave goods (Barfield, 1994) and body tissues (O'Connor et al., 2011; Stead et al., 1986), the survival
49 of perishable materials in a condition that can be visually recognised is rare and generally limited to
50 particular burial environments (e.g. waterlogged, arid, frozen) and modes of preparation of the corpse
51 (e.g. embalming and mummification). While organic materials were undoubtedly significant,
52 widespread and varied components of human burials, in the vast majority of burials the extent of
53 decay is such that evidence of these components is invisible to traditional archaeological techniques.
54 Organic chemical analysis is now an established tool in the field of archaeology and has been applied
55 to many different types of archaeological remains (Evershed, 2008), including human remains. Bone

56 collagen can survive over archaeological timescales and is routinely isolated for stable isotope and
57 radiocarbon analysis. Exceptional examples of soft tissue preservation have provided rare
58 opportunities to study their protein and lipid compositions and thereby the extent and mechanisms
59 of preservation (Evershed and Connolly, 1988; Gulaçar et al., 1990; Mayer et al., 1997; O'Connor et
60 al., 2011). Likewise, chemical characterisation of organic residues associated with the wrappings of
61 Egyptian mummies has provided information on the preparations used by ancient embalmers (Buckley
62 and Evershed, 2001). By contrast, the potential for the soil in contact with buried human remains to
63 retain molecular information relating to decayed organic components of the burials has received little
64 attention. Such organic remains could relate to components from the body tissues of the interred,
65 from the stomach and gut contents, from formulations used in preparing the body for burial and from
66 substances associated with grave goods and burial structures, such as a coffin. Each of these sources
67 has potential to inform the archaeological interpretation: components established to derive from the
68 body tissues, whether or not the physical remains survive, could provide opportunities for isotopic
69 analysis and hence consideration of differences in diet among past populations (Stott et al., 1999);
70 stomach contents can reveal information regarding the balance of plant vs meat in the meals
71 immediately prior to death (Pickering et al., 2014); formulations used in burial rituals could reveal
72 commonalities and differences among individuals within localities and over time; signatures from
73 grave goods could reveal the nature of now decayed materials that were placed within the graves and
74 signatures of the burial structures (Burns et al., 2017) could also reveal commonalities and differences
75 over time and with geographical location. In order for organic signatures to be meaningfully employed
76 it is necessary to gain insights into the nature and extent of their alteration in the environments
77 particular to graves. Whilst much variation can be expected according to burial practice, soil
78 composition and chemistry, hydrology and age of burial, some generalities can be anticipated owing
79 to the presence of a substantial amount of organic matter in a somewhat defined space.

80 Grave soils from contemporary human burials have been shown to contain chemical signatures of
81 degraded adipose tissue (Bull et al., 2009; Forbes et al., 2002). Given that the recalcitrance of lipids
82 allows their survival over geological timescales (Eigenbrode et al., 2008), informative chemical
83 signatures from wide variety of sources have the potential to be preserved in archaeological grave
84 soils. We present results of the chemical and micromorphological analysis of grave soils and
85 sediments from two human burials from Hungate, York (UK), one of Roman age and the other of
86 Anglo-Scandinavian age.

87 **2 Experimental**

88 *2.1 Excavation*

89 The Roman age grave (C51364, 1st – 4th C AD) and Anglo-Scandinavian age grave (C53700, ¹⁴C date AD
90 870 - 980) were sampled for the InterArChive project in 2010 and 2011 during excavation of the
91 Hungate site. Both were undisturbed burials, containing articulated skeletons (Fig. 1). The graves
92 presented differences in the levels of preservation, both in terms of completeness of the skeletal
93 remains and in the physical condition of the bones. Whereas the bone in the Anglo-Scandinavian
94 burial was very well preserved, that in the Roman burial had lost much structural integrity and was
95 incomplete, particularly the upper left side of the remains (Fig. 1).



96 **Figure 1.** Left panel C53700: Anglo-Scandinavian age burial (AD 870-980) containing the skeletal
97 remains of an adult. During excavation, degraded wood was identified believed to have once been a
98 wooden lid covering the remains. Right panel C51364: poorly preserved skeletal remains of an adult
99 (Roman age ~ 3rd century AD) and a nearby Anglo-Scandinavian age cess pit (Context 2652) that cuts
100 the grave.

101 *2.2 Sampling*

102 Controls were collected from non-grave soil from the site (C1) and from grave fill above the level of
103 the skeletal remains, C2 and C3, as essential comparators of grave fill that has not been in contact
104 with the buried remains (Fig. 2).

105 Samples of the burial matrix were collected in line with the InterArChive sampling strategy (Usai et al.,
106 2014) from key anatomical locations on the skeleton (Table 1). The maximum number of prescribed
107 points around the skeletal remains were targeted for chemical analysis and 4 key areas; head (1),
108 pelvis (2) and feet (3/4) were targeted for micromorphological analysis (Table 1). Additional samples
109 (prefixed by the letter 'A') were collected in response to specific features of the individual graves and
110 from a cess pit adjacent to the Roman burial. Samples for micromorphology were collected from
111 undisturbed archaeological sediment or grave fills using Kubiena tins (83 × 50 × 32 mm, 83 × 55 × 42
112 mm, and 65 × 38 × 28 mm). On return to the laboratory, samples were refrigerated prior to thin
113 section preparation. Samples for chemical analysis were collected proximal to the skeletal remains or
114 feature using a spatula, wrapped in pre-cleaned foil, placed in geochemical sample bags and stored
115 cold. On return to the laboratory, samples were stored frozen until required for analysis.

116 *2.3 Manufacture of thin sections*

117 The samples were dried in the tins through acetone vapour exchange (Miedema et al., 1974) and
118 impregnated under vacuum with a slow curing polyester resin (Polylite 32320-00). The resulting
119 consolidated soil blocks were cut to produce the thin sections. The cut sections were back-polished,
120 mounted, cut and ground to 30 µm thickness, with final 3 µm and 1 µm polishes.

121 *2.4 Optical microscopy*

122 Micromorphological observations were performed using a petrographic microscope (Zeiss AxioLab A1)
123 equipped with a Zeiss AxioCamERc5s camera and AxioVision imaging software. The standard
124 terminologies proposed by Bullock et al. and by Stoops (Bullock et al., 1985; Stoops, 2003) were
125 adopted for the descriptions of the slides and semi-quantitative estimation of the features.

126 *2.5 Scanning electron microscopy-energy dispersive X-ray analysis (SEM-EDS)*

127 SEM-EDS analyses were conducted at the University of Stirling employing an EVO MA 15 Zeiss scanning
128 electron microscope with workflow automation and an Oxford Instruments INCA X-Max EDS to
129 provide micro-chemical data of fine material and inorganic pedofeatures of uncertain interpretation
130 (Courty et al., 1989; Fitzpatrick, 1993; Goldberg and Macphail, 2003). Standard operating conditions
131 were 8.5 mm working distance and 20 kV accelerating voltage. Calibration was achieved through the
132 analysis of standard cobalt every 2 h and standard dolomite at the beginning of each session. A
133 minimum of seven individual point analyses were carried out for each measured region. The
134 measurements of O and C were excluded owing to the presence of the resin in which the samples
135 were consolidated and weight percent data were normalized to 100%.

136

137 **Table 1.** Soil samples collected for the two graves from Hungate, York, UK.

| Anglo-Scandinavian burial C53700 | | Roman burial C51364 | |
|----------------------------------|----------------------------|----------------------------|------------------------------------------------------|
| Sample Number [†] | Position | Sample Number [†] | Position |
| C1 | Control (non-grave soil) | C2 | M Control (upper grave fill) |
| C2 | Control (lower grave fill) | C3 | M Control (lower grave fill level with top of skull) |
| C3 | Control (lower grave fill) | 1 | M Skull |
| 1 | M Skull | 2 | M Sacrum |
| 1b | Below skull | 3 | Left foot |
| 2 | M Sacrum | 4 | Right foot |
| 2b | Below sacrum | 3 & 4 | M Between feet |
| 3 | Both feet | 5 | Left shoulder |
| 5 | Below left shoulder | 6 | Right shoulder |
| 6 | Below right shoulder | 7 | Left elbow |
| 7 | Below left elbow | 8 | Right elbow |
| 8 | Below right elbow | 9 | Left hip |
| 9 | Below left hip | 10 | Right hip |
| 10 | Below right hip | 11 | Left knee |
| 11 | Below left knee | 12 | Right knee |
| 12 | Below right knee | 13 | Left iliac |
| 13 | Below left iliac | 14 | Right iliac |
| 14 | Below right iliac | 15 | Sternum |
| 15 | Below sternum | 16 | Right hand |
| A1 | Right scapula | A1 | Wall of cess pit (context 2652) |
| A2 | Dark soil right pelvis | A2 | Material excavated from cess pit (context 2652) |
| A3 | Coccyx | A3 | Intracranial sediment |
| | | A4 | Abdominal region |
| | | A5 | Dark soil within pelvis |

138 [†] Sample number corresponds to the standard sampling locations defined in Usai et al., (2014). Unless specified otherwise the soil adjacent
 139 to the bone was collected. Samples prefixed by the letter A represent samples additional to the standard sampling locations. M indicates
 140 that an undisturbed block sample for micromorphological analysis was collected in addition to a loose soil sample.

141 **2.6 Preparation of materials and extracts for chemical analysis**

142 All solvents used were analytical grade or higher. All glassware was baked (450°C, 6 h) prior to use
 143 using a Pyroclean Trace oven (Barnstead International, USA) to remove organic contaminants. Frozen
 144 soil samples were freeze dried using a HETO PowerDry PL3000 (Thermo, Hemel Hempstead, UK). Dry
 145 soils were disaggregated with a pestle and mortar, and sieved using 1 mm, 400 µm and 200 µm sieves.
 146 All subsequent work was performed on the sub 200 µm fraction. Extraction was performed using an
 147 accelerated solvent extraction system (ASE 350, Dionex, Hemel Hempstead, UK). Prior to loading soil
 148 samples, the empty extraction cells were subjected to extraction using the same solvents and
 149 conditions as for the samples. Soil (3-6 g) was loaded into 5 ml stainless steel cells and extracted three

150 times with dichloromethane:methanol (9:1 v/v; 5 min at 100°C and 1500 psi). A blank extraction was
151 also performed to assess whether any contamination was being introduced at the extraction step.
152 Solvent was removed using a rotary vacuum concentrator (RVC 2-25, Christ, Osterode am Harz, DE).
153 The total extract was divided into two portions, one of which was fractionated using a custom-made
154 glass chromatography column (90 mm x 10 mm i.d.) packed with silica gel (750 mg, height in column
155 = 20 mm). The column was conditioned by washing with three bed volumes each of
156 dichloromethane:methanol (1:1, v/v) and dichloromethane followed by equilibration with hexane.
157 The extract to be fractionated was dissolved in dichloromethane (200 µl), spiked onto silica gel (40 mg)
158 and the solvent removed in vacuo. The impregnated silica was loaded onto the top of the packed
159 chromatography column. The column was washed with three bed volumes each of i) hexane, ii)
160 hexane:toluene (1:1, v/v), iii) hexane:ethyl acetate (4:1, v/v) and iv) dichloromethane:methanol (1:1,
161 v/v) to generate four chromatographic fractions: i) aliphatic hydrocarbons, ii) aromatic hydrocarbons
162 and *n*-alkanals, iii) alcohols and esters and iv) acids, respectively. Solvent was removed using a rotary
163 vacuum concentrator.

164 2.7 Derivatisation

165 Total extracts and polar (iii and iv) fractions were reconstituted in dichloromethane:methanol (2:1,
166 v/v; 300 µl) followed by addition of trimethylsilyl diazomethane (20 µl) and allowed to react for 30
167 min, before drying under a gentle stream of nitrogen gas. Immediately prior to analysis, total extracts
168 and polar fractions were heated with *N,O*-bis(trimethylsilyl)trifluoroacetamide (100 µl, containing 1%
169 trimethylchlorosilane) and 5 drops of pyridine for 1.5 h at 60°C before removing excess derivatising
170 agent under a gentle stream of nitrogen gas.

171 2.8 Total organic carbon (TOC) analysis

172 Total organic carbon (TOC) analysis was performed using a Flash 2000 elemental analyser (Thermo,
173 Hemel Hempstead, UK) fitted with a MAS200R autosampler, chromatographic column and thermal
174 conductivity detector. Helium was used as a carrier gas at a flow rate of 140 ml min⁻¹. Soil samples
175 (10-15 mg) were weighed using a XS3DU microbalance (Mettler Toledo, Leicester, UK) into silver foil
176 capsules. Samples were treated with 2 drops of hydrochloric acid (6 M) to destroy carbonates before
177 heating to 80°C for 6 min to drive off excess acid solution. Foil capsules were folded to exclude air.
178 Samples were combusted in a quartz reactor tube packed with copper oxide granules and electrolytic
179 copper wires held at 900°C. Sample introduction coincided with a pulse of oxygen (250 ml min⁻¹, 5 s).
180 Instrument control, data acquisition and processing was by Eager Xperience V1.11 (Thermo, Hemel
181 Hempstead, UK).

182 *2.9 Gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS)*

183 Total lipid extracts were analysed using a Trace GC Ultra gas chromatograph (Thermo, Hemel
184 Hempstead, UK) equipped with a Triplus autosampler, a fused silica capillary column (J&W DB-5, 60
185 m x 0.32 mm i.d., 0.25 µm film thickness, Agilent, Wokingham, UK) and a flame ionisation detector
186 (FID). Samples were prepared in dichloromethane and volumes of 1 µl injected onto the column via
187 a split/splitless injection port operating in splitless mode (280°C, split flow 1:25, splitless time 0.8 min).
188 The oven temperature was programmed from an initial temperature of 70°C to 130°C at a rate of 20°C
189 min⁻¹ and then to 320°C at a rate of 4°C min⁻¹ where it was held for 40 min. Helium was used as a
190 carrier gas at a flow rate of 2 ml min⁻¹. The FID was held at 330°C. Instrument control, data acquisition
191 and analysis was by ChromQuest 5.0 V3.2.1 (Thermo, Hemel Hempstead, UK).

192 Chromatographic fractions (i-iv) were analysed using an identical gas chromatograph to that above
193 equipped with an ultrafast column module (UFC-5, 10 m x 0.1 mm i.d., 0.1 µm film thickness, Thermo,
194 Hemel Hempstead, UK). Samples were prepared in dichloromethane for analysis and volumes of 1 µl
195 injected onto the column via a split/splitless injection port operating in split mode (280°C, split flow
196 1:100) and the FID held at 330°C. The column oven was programmed from an initial temperature of
197 50°C (0.5 min isothermal) to 330°C at a rate of 90°C min⁻¹ where it was held for 4 min. Helium was
198 used as the carrier gas at a flow rate of 0.5 ml min⁻¹.

199 GC-MS analysis was performed on selected fractions using a 7860A gas chromatograph (Agilent,
200 Wokingham, UK) equipped with a 7683B Series autosampler coupled to a GCT Premier Micromass
201 time of flight mass spectrometer (Waters, Elstree, UK). Separation was achieved on a fused silica
202 capillary column (Zebron, ZB-5, 30 m x 0.25 mm i.d., 0.25 µm film thickness, Phenomenex,
203 Macclesfield, UK). Samples were prepared in dichloromethane for analysis and volumes of 1 µl
204 injected onto the column via a split/splitless injection port operating in split mode (280°C, split flow
205 1:5). The oven was programmed from an initial temperature of 70°C to 130°C at a rate of 20°C min⁻¹
206 and then to 320°C at a rate of 4°C min⁻¹ where it was held for 40 min. Helium was used as the carrier
207 gas at a flow of 1 ml min⁻¹ and the MS transfer line set to 300°C. Mass spectra were acquired over the
208 range *m/z* 50-750 (cycle time 0.2 s) using an electron ionisation energy of 70 eV. Instrument control,
209 data acquisition and processing was by MassLynx V4.1 (Waters, Elstree, UK). Compounds were
210 identified from interpretation of their mass spectra and comparison with library spectra (NIST 08)
211 where available.

212 *2.10 High performance liquid chromatography-multistage tandem mass spectrometry (HPLC-MSⁿ)*

213 HPLC-MSⁿ analysis of triacylglycerols was performed on selected total extracts according to Method J
214 of (Hasan, 2010) using a Dionex Ultimate 3000 rapid separation liquid chromatograph, controlled by

215 Chromeleon 6.8 (Dionex, Hemel Hempstead, UK), coupled to a HCTultra ETD II quadrupole ion trap
216 mass spectrometer (Bruker Daltonics; Coventry, UK) fitted with an atmospheric chemical ionisation
217 (APCI) source. Briefly, samples were prepared in hexane:propan-2-ol:acetonitrile (1:1:1, v/v/v). Lipid
218 separation was accomplished using two 3 μm Spherisorb ODS2 columns (150 x 4.6 mm i.d.) coupled
219 in series, using a ternary solvent gradient system comprising acetonitrile, dichloromethane and
220 ammonium acetate in methanol (0.01 M) at a flow rate of 1 ml min⁻¹. APCI was operated in positive
221 ion mode using the following settings: vaporiser temperature 450°C, nebuliser gas (N₂) 50 psi, drying
222 gas (N₂) flow rate 3 L min⁻¹, drying gas temperature 150°C, corona 4000 nA and capillary voltage -4000
223 V. Mass spectra were acquired in Ultra Scan mode over the range m/z 300-950 with a cycle time of
224 0.025 s. Online multistage tandem mass spectra (to MS³) were generated following selection of the
225 most abundant ion in the mass spectrum for collision induced dissociation. The isolation width was
226 set to 4 m/z units, maximum accumulation time was fixed at 200 ms and Smart Fragmentation set to
227 between 30-200% of a fragmentation amplitude of 1.0 V. MS control, data acquisition and processing
228 was by Compass EsquireControl 6.2 and Compass DataAnalysis 4.0 (Bruker Daltonics; Coventry, UK).

229 **3 Results and Discussion**

230 Chemical and micromorphological analyses of samples of burial soil, collected from numerous specific
231 locations (e.g. Fig. 2; full sample list in Table 1) around the skeletal remains of the Roman and Anglo-
232 Scandinavian burials, were compared with controls to identify features and signatures uniquely
233 associated with the burials. The soil particles were dominated by coarse grains of quartz with clays
234 dominating the fine material (Table 2). The soil in the Roman burial was apedal whereas that in the
235 Anglo-Scandinavian burial contained weakly developed granular peds and more voids. Given their
236 different positions within the stratigraphic profile it is not possible to determine if the difference
237 between the two graves relates to intrinsic differences during soil formation or to post depositional
238 disaggregation of peds in the Roman grave. The sediment from both graves contained fragments of
239 humified plant matter including gymnosperm wood in the Anglo-Scandinavian grave. Fungal hyphae
240 were also identified in the Anglo-Scandinavian grave in the area of the pelvis and sclerotia in the areas
241 of the skull and pelvis. The dominant pedofeatures in the Roman grave were spherulites, goethite
242 crystals, Fe/Mn nodules and clay coatings indicating variations in redox conditions and water
243 saturation. Amorphous phosphate was absent or very low around the torso in the Roman grave and
244 the concentrations at the feet and in the controls were anomalously low compared with most other
245 graves examined in Hungate (Ghislandi, 2016). The Anglo-Scandinavian grave contained higher levels
246 of amorphous phosphate throughout the thin sections and vivianite in association with the bone.
247 Hence, parts of the Roman grave may have been subjected to processes that caused leaching of

248 phosphorus. The presence of Fe/Mn nodules in the Anglo-Scandinavian grave indicate changes in
249 water saturation, though in a generally more oxidising environment than in the Roman grave.

250 The organic extracts of the soils were analysed by gas chromatography-mass spectrometry (GC-MS)
251 and high performance liquid chromatography-multistage tandem mass spectrometry (HPLC-MSⁿ) to
252 determine the distributions of lipid components in the soils. The extracts of all samples and controls
253 from both graves were dominated by autochthonous components of soil organic matter, mainly
254 homologous series of *n*-alkanoic acids (C₁₆-C₃₄), *n*-alkanols (C₁₈-C₃₄) and *n*-alkanes (C₂₀-C₃₅), the
255 distributions being characteristic of the leaf waxes of vascular plants (Eglinton and Hamilton, 1967).
256 In addition, phytosterols 24-ethylcholest-5-en-3 β -ol (sitosterol), 24-ethylcholest-5,22-dien-3 β -ol
257 (stigmasterol) and 24-methylcholest-5-en-3 β -ol (campesterol) were detected.

258 Despite the strong overprint from the natural soil organic matter, the samples associated with the
259 skeletal remains exhibited distinct differences to the controls, generally exhibiting elevated levels of
260 triacylglycerols (TAGs) (e.g. Figs. 2a and 3); di-C_{16:0} and C_{16:0}/C_{18:0} 1,2- and 1,3-diacylglycerols (DAGs);
261 C_{16:0} and C_{18:0} monoacylglycerols (MAGs); C_{16:0} and C_{18:0} fatty acids (Fig. 2b); C_{16:0} and C_{18:0} *n*-alkan-1-als
262 (Fig. 2b) and branched chain *iso* (*i*-) and *anteiso* (*ai*-) C_{15:0} and C_{17:0} fatty acids (Fig. 2c). The TAG
263 concentrations associated with the Anglo-Scandinavian remains were around an order of magnitude
264 greater than those for the Roman remains. Though TAGs have been identified from within the
265 protective environment of archaeological ceramics (Garnier et al., 2009; Kimpe et al., 2001; Mirabaud
266 et al., 2007; Šoberl et al., 2008) their occurrence in grave soils has not been reported previously. The
267 elevated levels of the TAGs, the primary constituents of animal fats and vegetable oils, associated with
268 the skeletal remains suggests they derive from adipose tissue. The low levels associated with the
269 controls (e.g. Fig. 2) may reflect minor contributions from plant sources or background levels from
270 previous burials within the cemetery. Notably, the TAG distributions in the soil samples are dominated
271 by saturated components (Fig. 3b) whereas fresh human adipose tissue comprises predominantly
272 (99%) unsaturated TAGs (Mayer et al., 1997). Generally, unsaturated components are more
273 susceptible to degradation (e.g. via oxidative cleavage) than their saturated counterparts. In addition,
274 human lipases have been shown to exhibit a preference for acyl moieties at the *sn*-1 and *sn*-3 positions
275 on the glycerol backbone as well as for unsaturated moieties (Jensen et al., 1994; Raclot and Groscolas,
276 1993). Preferential degradation of TAGs containing unsaturated acyl chains could explain the
277 observed predominance of saturated TAGs in the soil extracts. Comparison of the acyl chain
278 distributions of the saturated TAGs in the soil extracts from around the skeletal remains (Fig. 3c) with
279 the saturated TAG-derived fatty acid distributions of fresh human adipose tissue (Hodson et al., 2008)
280 reveals a strong similarity (A; Fig. 3c). By contrast, complete reduction of monounsaturated and of all
281 unsaturated fatty acids of human adipose TAGs would generate the predicted ratios shown for B and

282 C (Fig. 3c), respectively. Thus, extensive post-depositional alteration of the body fats is evident and
283 involves preferential loss of unsaturated TAG moieties. The TAG distributions in the soils around the
284 skeletal remains (Fig. 3b) exhibit a striking similarity to those reported for the adipocere of a 65 year
285 old ice mummy and the skin of a 5300 year old ice mummy (Mayer et al., 1997), supporting the genesis
286 of these signatures from human adipose tissue. In those cases, preservation of TAGs may be
287 attributed largely to the physiochemical barriers to degradation: encasement in ice and desiccation,
288 respectively. A study involving augmentation of three different soils with a model saturated TAG
289 (tristearin) showed that release of fatty acids within the first weeks of incubation at 20°C could be
290 attributed to the soil microbial community (Hita et al., 1996). It is remarkable, therefore, that TAG
291 signatures of adipose tissue can be recovered from grave soils after more than 1600 years since
292 interment. TAGs and cell membrane phospholipids are degraded in soils by stepwise hydrolysis to
293 fatty acids and glycerol (and phosphate in the case of phospholipids), which explains the elevated
294 levels of DAGs, MAGs and C_{16:0} and C_{18:0} fatty acids compared with the controls (e.g. Fig. 2b).

295 The C_{16:0} and C_{18:0} *n*-alkan-1-als associated with the skeletal remains have not been reported as
296 significant components of soils and were not detected in the controls (Fig. 2b). Short chain *n*-alkan-1-
297 als, principally C₁₆ and C₁₈ components, occur as both free and plasmalogen-bound components in
298 various animal tissues (Gilbertson et al., 1967; Rapport and Lerner, 1959; Wittenberg et al., 1956)
299 although their amounts are too low (relative to other lipid classes such as fatty acids) to represent a
300 significant source of *n*-alkan-1-als in the soil extracts, where they represent approximately 10%
301 abundance relative to summed C_{16:0} and C_{18:0} fatty acids (Fig. 2b). The most likely source that would
302 explain the concentrations of *n*-alkan-1-als in the soil extracts is reduction of fatty acids (e.g. mediated
303 by anaerobic bacteria). The concentrations of *n*-alkan-1-als in the soils are not, however, controlled
304 by fatty acid concentration alone: the differences in C_{16:0} and C_{18:0} fatty acids concentrations with
305 anatomical location do not correlate with those of the corresponding *n*-alkan-1-als (Fig. 2b), most
306 notably around the pelvis in the Anglo-Scandinavian grave. Features of bone morphology (for example
307 the skull or sacrum) could restrict water movement and contribute to highly localised regions of anoxia
308 that favour reduction of organic matter. Thus, the reductive transformation of fatty acids to *n*-alkan-
309 1-als, and the survival of the relatively labile aldehyde species themselves, is strongly indicative of
310 persistent oxygen-limited conditions within areas of the grave, with higher *n*-alkanal:fatty acid ratios
311 reflecting localised regions of more extensive anoxia.

312 Table 2a. Micromorphological descriptions of the sediments from the Roman age grave (C51364) and Scandinavian age grave (C53700) from Hungate, York,
 313 UK). PPL = plane polarised light, XPL = cross polarised light.

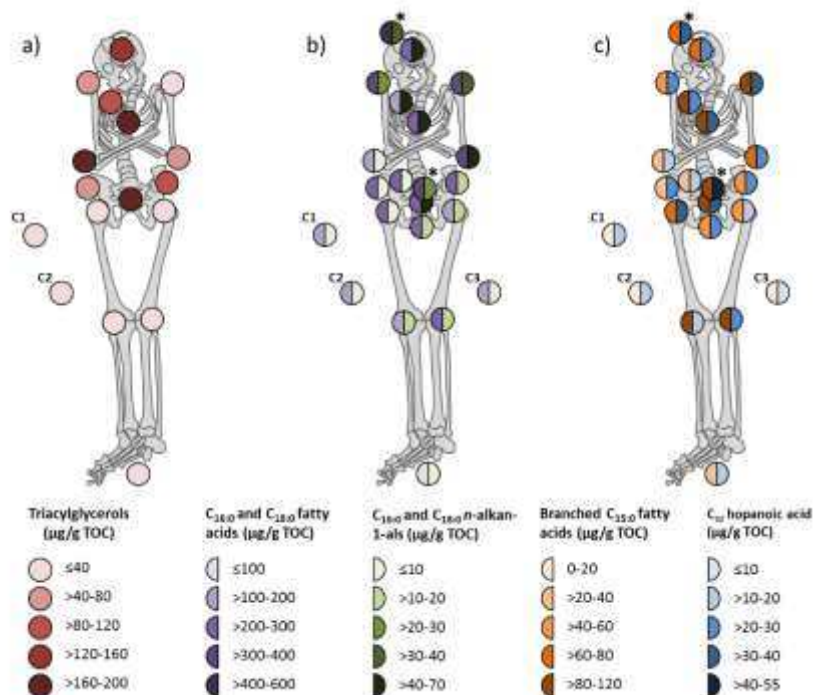
| Burial | Microstructure | Voids | Mineral components | Organic components | Pedofeatures |
|-------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| C51364 (Roman) | Two principal c/f related distributions either strongly separated or intermingled: porphyric and poorly/moderately sorted (10-30% by area) or chitonic and moderately/well sorted (70-90% by area). Several intermediate distributions. Porphyric areas were brown/light brown/yellowish brown in PPL and brown/yellow or isotropic in XPL, with dotted limpidity and speckled b-fabric. Chitonic areas were yellowish or reddish brown in PPL and orange/yellow or isotropic in XPL, with speckled or parallel striated b-fabric. Samples were apedal. | Chambers, 500-2000 µm with undulating surfaces, in the areas of the feet (8%), skull and C3 (5%) and pelvis (2%). Channels at all positions (5% except for skull: 8%). Modified complex voids only in the areas of skull and pelvis (5%) and control C2 (8%). Frequency of packing voids increased from the skull to the control C2, absent from the pelvis. Planar voids, thickness 100-1500 µm, in areas of skull (15%), pelvis and feet (5%) and C3 (8%). | Mineral components mostly sub-angular and not weathered quartz (45-55%). Occasional fragments of quartzite in the areas of the feet (2%) and C2 (5%). Rare grains of biotite were in the area of the skull (2%). | Fragments of weathered or partially weathered humified plant structures in all samples (2%) except control C3. Plant remains, 50-2000 µm, brown/black in PPL and isotropic in XPL. Some coarser fragments exhibit vessels, hence angiosperm-derived. | Sideritic spherulites in the areas of skull, pelvis, feet and C3 exhibit morphological differences: Group 1 (skull 5-10%, 5-12 µm; feet 10-20%, 2-5 µm; pelvis 2-5%, 5-12 µm): orange in PPL; Maltese cross extinction in XPL. Infillings in pores and within fine material in the areas of skull and pelvis. Sealed between well-preserved layered coatings of Fe rich fine material in area of feet. Group 2 (C3, skull and pelvis): 5-20 µm; yellow in PPL, amorphous in XPL; infillings in pores and among mineral grains. Some have coalesced to form dumbbell structures. Group 3 (feet): 5-15 µm; orange/yellow in PPL. Some present two distinct layers with high contrast at junction; only outer layer exhibits Maltese cross extinction; core amorphous in XPL. Infillings of goethite in voids in area of skull (8%): loose discontinuous or dense incomplete. Occurs as fan-like crystals, orange/red colour in PPL and reddish brown or isotropic in XPL and with parallel striated b-fabric. Sub-angular/sub-rounded Fe/Mn nodules (50-500 µm, 5%) in all samples except control C2. Thin dark brown coating around voids, probably organic in origin, in areas of feet (8%), skull and pelvis (5%). External quasicocoatings of clay around voids in area of pelvis (8%) and C2 (5%), clay textural impregnations in area of skull. Coatings of amorphous phosphate in control samples (5-10%). |

314

315 Table 2b. Micromorphological descriptions of the sediments from the Roman age grave (C51364) and Scandinavian age grave (C53700) from Hungate, York,
 316 UK).

| Burial | Microstructure | Voids | Mineral components | Organic components | Pedofeatures |
|------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| C53700 (Anglo- Scandinavian) | Two different fabrics: A (50-85%), B (20-50%) and C (10%) in varying proportions. Type A: close porphyric c/f related distribution, good sorting. Fine material, 10-40%, yellowish brown/brown in PPL and reddish brown or yellow/brown in XPL, with dotted limpidity and speckled b-fabric. Type B: open porphyric and well sorted. Fine material, 60-80%, reddish brown or yellowish brown in PPL and yellow/brown in XPL, dotted limpidity and speckled or parallel striated b-fabric. Type C (area of vertebrae): chitonic c/f related distribution, good sorting, yellow/orange in PPL, yellow or isotropic in XPL, limpud or dotted limpidity, parallel striated b-fabric. Samples from areas of skull and pelvis exhibit spongy appearing microstructures. Weakly developed granular peds (5-10%) in the area of vertebrae: fine in size and unaccommodated. | Chambers in areas of pelvis (8-15%) and skull (2%). Channels in areas of pelvis (8-15%) and vertebrae (10%). Modified complex voids in areas of skull (8-25%), vertebrae and pelvis (8%). Packing voids in the areas of the skull (8%), pelvis (5%) and vertebrae (15%). | Quartz dominant in all samples (30-50%), quartzite only in areas of pelvis (2%) and vertebrae (5%). Flint in area of pelvis (2%). | Amorphous organic matter in area of skull (2%). Humified plant structures (200-2000 µm) in all samples (2-5%). Occasional fragments characterized by vessels, hence angiosperm-derived. Gymnosperm wood indicated by single imprint of fragment in fine material in area of pelvis. Same sample exhibits humified plant (>2000 µm) comprising four fragments oriented parallel to the resting plane, circular/angular brown cells with infilling of fine material. Light brown in PPL and brown or isotropic in XPL. Fungal hyphae present in groundmass in area of pelvis. Sclerotia (20-200 µm) in areas of skull and pelvis (2%). Occasional roots in area of pelvis: orange in PPL and partially white in the internal section in XPL. | Amorphous phosphates in all samples, most frequent in area of pelvis (5-20%). Occur as coatings voids, nodules and strong impregnations in fine material; light yellow or yellow in PPL and isotropic in XPL; some exhibit crystallitic layers, occasionally weathered with granular aspect in pelvic area. Occasional amorphous phosphate coatings around spherical Mn nodules in areas of skull and pelvis. Single occurrence of vivianite (100-150 µm) in area of pelvis. Red redox impregnations in areas of pelvis and vertebrae (5%). Fe/Mn nodules in area of skull (2-8%) and occasional in the other samples. Clay coatings around mineral grains in areas of skull (2%) and pelvis (5%). |

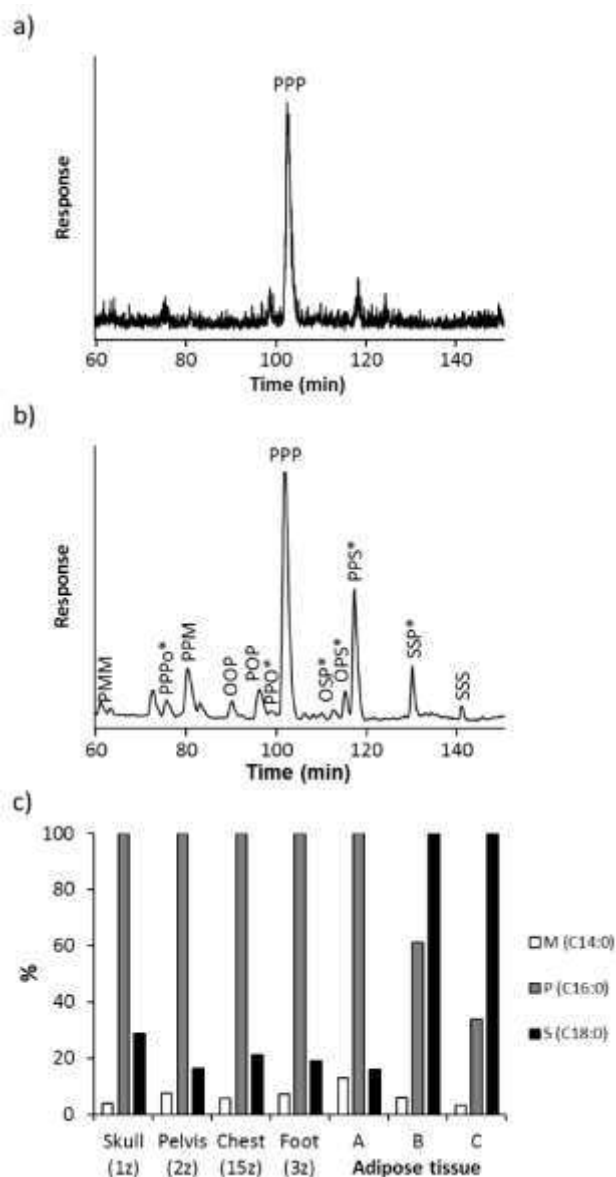
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318

319 **Figure 2.** Variation in concentrations of specific lipids in the Anglo-Scandinavian age burial C53700: **a)**
 320 combined triacylglycerols (TAGs; red shades), **b)** combined C_{16:0} and C_{18:0} fatty acids (purple shades)
 321 combined C_{16:0} and C_{18:0} *n*-alkan-1-als (green shades) and **c)** combined *i*- and *ai*-C_{15:0} fatty acids (orange
 322 shades) and bishomohopanoic acid (C₃₂; blue shades). All soil samples were collected from below the
 323 skeletal remains except for samples marked with an asterisk (*) which were collected from adjacent
 324 to the skeletal remains. C1 = control from outside the grave cut, C2 = control from fill at the edge of
 325 the grave above the level of the skeleton, C3 = control of the grave fill, level with the skeleton.

326 Branched chain fatty acids (*i*-C_{15:0}, *ai*-C_{15:0}, *i*-C_{17:0}, and *ai*-C_{17:0}) are significant components of bacterial
 327 lipids while being scarce in other sources. The spatial variation of these components in the Anglo-
 328 Scandinavian grave (Fig. 2c) was also mirrored by a series of C₃₀-C₃₃ hopanoic acids and hopanols (e.g.
 329 Fig. 2c), diagenetic products of the bacteriohopanetetrol and/or aminobacteriohopanetriol
 330 components of bacterial cell membranes (Quirk et al., 1984; Winkler et al., 2001). The variability in
 331 the bacterial markers across the remains (Fig. 2c) may be explained by the development and spread
 332 of populations evolved from the gut microbial fauna during decomposition (Can et al., 2014) together
 333 with soil microbe invasion in response to the corpse providing a plentiful supply of organic substrate.
 334 Anatomical locations where the highest proportions of body tissue are distributed appear to contain
 335 more abundant bacterial markers, indicating more intense/protracted microbial activity.

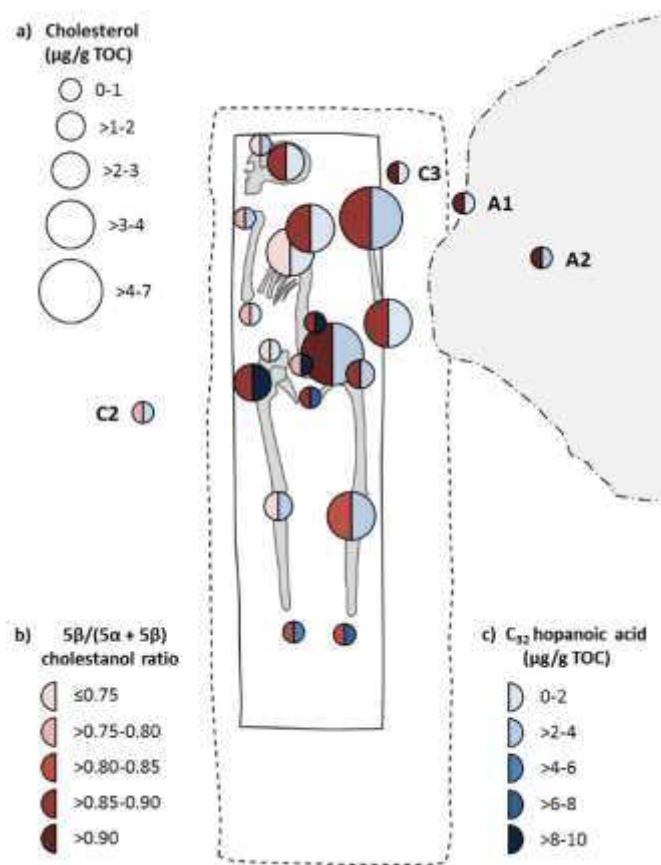


336

337 **Figure 3.** Partial HPLC-MS chromatograms (m/z 300-950) of the total lipid extracts for **a)** the control
 338 (C1) and **b)** pelvis (2) from C53700 acquired using Method J (Hasan, 2010). Triacylglycerols (TAGs) are
 339 labelled with letters to denote the acyl moieties attached to the glycerol backbone e.g. tripalmitin =
 340 PPP. M = myristyl ($C_{14:0}$), P = palmityl ($C_{16:0}$), Po = palmitoleyl ($C_{16:1}$), S = stearyl ($C_{18:0}$) and O = oleyl
 341 ($C_{18:1}$). *Indicates that only one of several possible positional isomers is shown. **c)** Comparison of the
 342 relative abundances of saturated TAG fatty acids (M = $C_{14:0}$, P = $C_{16:0}$ and S = $C_{18:0}$), normalised to the
 343 major component, in the soil samples from around the skeletal remains of C53700 to those of fresh
 344 human adipose tissue (Hodson et al., 2008). A = relative abundance of saturated fatty acids for human
 345 adipose tissue; B = relative abundance of saturated fatty acids for human adipose tissue assuming
 346 reduction of all monounsaturated fatty acids to saturated components; C = relative abundance of
 347 saturated fatty acids for human adipose tissue assuming reduction of all unsaturated fatty acids to
 348 saturated components.

349 In both graves the animal sterol cholest-5-en-3 β -ol (cholesterol) was generally present in greater
350 concentration in the samples associated with skeletal remains than in the controls (e.g. Fig. 4a) with
351 the Anglo-Scandinavian burial having the higher concentrations. The preservation state of the Roman
352 skeletal remains, C51364, was extremely poor compared with the Anglo-Scandinavian remains,
353 consistent with its greater age and wetter burial environment. Interestingly, cholesterol
354 concentrations were more varied, being particularly pronounced for soils associated with the more
355 degenerated regions of the skeleton, closest to an Anglo-Scandinavian age cess pit that was proximal
356 to the upper left hand side of the remains at a level slightly above the burial (Fig. 4a). Identification
357 of cholesterol as a significant lipid component of archaeological bone (Evershed et al., 1995; Jim et al.,
358 2004) together with the degenerated skeletal remains indicate it to have been released into the soil
359 as the bone degraded. Hence, persistent cholesterol signatures may have value in aiding the
360 identification of the original location of human remains in graves where the bones have not survived,
361 subject to appropriate caution being exercised to differentiate signatures from alternative sources
362 such as soil fauna. The cholesterol in the extracts was accompanied by its reduction products 5 α -
363 cholestan-3 β -ol and 5 β -cholestan-3 β -ol (coprostanol). 5 α -Cholestan-3 β -ol is the major product of
364 microbial reduction of cholesterol in soils and plant and mammalian tissues (Bethell et al., 1994). Its
365 epimer, 5 β -cholestan-3 β -ol, typically a minor product of cholesterol reduction in the environment, is
366 formed in significant amounts during microbial transformation of cholesterol in the guts of most
367 higher animals and is the major sterol in human faecal material (Leeming et al., 1996). Accordingly,
368 the ratio of 5 β /(5 α + 5 β) cholestanols has been used as a proxy to indicate faecal contamination of
369 sediments and water systems: values >0.7 indicate pollution (Grimalt et al., 1990). For the Anglo-
370 Scandinavian grave, 5 β /(5 α + 5 β) cholestanol index values suggestive of faecal contamination were
371 restricted to the samples from the pelvic region, signifying inputs from the gastrointestinal tract. For
372 the Roman grave, however, high ratios were observed throughout the burial (Fig. 4b), revealing clear
373 evidence for the ingress from the cess pit of material having a strong faecal signature. The high 5 β /(5 α
374 + 5 β) cholestanol index values of samples from the cess pit combined with their lower cholesterol
375 levels than the closest samples from within the grave fully support the interpretations discussed
376 above. Despite the influence of the cess pit, the organic signature from the pelvic region of C51364
377 can still be distinguished by abundant bacterially-derived C₃₀-C₃₃ hopanoic acids (Fig. 4c).
378 Furthermore, hopanoic acid distributions at the feet suggest migration of gut-derived organic matter
379 within the coffin. The evidence for organic signatures derived from the cess pit leachate affecting the
380 preservation of the bones comes from the generally worse preservation of the skeletal remains nearer
381 to the pit. Hence ingress of leachate from the Anglo-Scandinavian age cess pit into the grave
382 contributed a strong faecal signature to the soils of the upper left section of the remains. Such organic

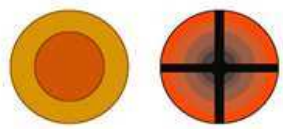

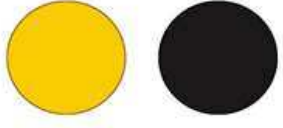

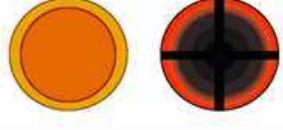

383 rich leachate would have been strongly anoxic and acidic, the latter accounting for the decalcification
 384 of the bone and consequent liberation of cholesterol within its area of influence. The acidic and
 385 reducing nature of the leachate means that it would have also carried ferrous iron in solution and is
 386 likely to have played a significant role in controlling phosphate levels in the Roman grave, mobilisation
 387 of phosphate being most prevalent in mildly acidic solutions (Nriagu, 1972). The absence/low levels
 388 of phosphorus in the burial soils of the Roman grave may be due either to low input to the original
 389 sediment or, given the greater age of the Roman burial, to the natural decrease in phosphorus levels
 390 with soil weathering (Cross and Schlesinger, 1995).



391 **Figure 4.** Variation in concentrations of specific lipids in Roman age burial C51364: **a)** cholesterol
 392 (circle sizes), **b)** 5β-cholestanol/(5α-cholestanol + 5β-cholestanol) ratio (red shades) and **c)**
 393 bishomohopanoic acid (C₃₂; blue shades). C2 = control from natural soil of grave cut above skeleton,
 394 C3 = control of grave fill level with top of skull, A1 = soil from natural soil of cess pit cut, A2 = soil
 395 excavated from the cess pit. The solid line outlines the soil stain from a coffin, the dashed line outlines
 396 the grave cut and the dashed and dotted line marks the boundary of an Anglo-Scandinavian age cess
 397 pit that cuts the grave.

398 Micromorphological analysis of undisturbed soil from control C3 and the head, pelvis and feet of the
 399 Roman burial revealed spherulites, features absent from control C2 and from all samples from the
 400 Anglo-Scandinavian burial. Morphological differences allowed the spherulites to be classified into

401 three distinct groups (Fig. 5, Table 2a). Group 1 spherulites: were mostly present near the skull and
 402 feet, and were less abundant near the pelvis. They occurred as infillings in the pores and within the
 403 fine material in the areas of the skull and pelvis with intermediate size range (Figs 5 and 6). Around
 404 the feet a smaller and narrower size range was present sealed between well preserved layered
 405 coatings of Fe rich fine material. Group 2 spherulites were present as infillings in pores and among
 406 mineral grains. They were most abundant in C3 and around the pelvis, with lower levels present in
 407 the skull area and only traces at the feet (Fig. 5). In some cases, the spherulites had coalesced to form
 408 dumbbell structures (Fig, 6a). Group 3 was identified only around the pelvis; some presented two
 409 distinct layers with high contrast at their junction with only the outer part of the spherule showing
 410 Maltese cross extinction and the core appearing amorphous in XPL.

| Designation | Morphology and optical characteristics | | Size / μm | Frequency / % | Location |
|-------------|-------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------|----------------------|---------------|----------|
| | PPL | XPL | | | |
| Group 1 |  |  | 5-12 | 5-10 | skull |
| | | | | 2-5 | pelvis |
| | | | 2-10 | 10-20 | feet |
| Group 2 |  |  | 5-20 | 10-20 | C3 |
| | | | | 2-5 | skull |
| | | | | 10-20 | pelvis |
| | | | | <2-5 | feet |
| Group 3 |  |  | 5-15 | 5-10 | pelvis |

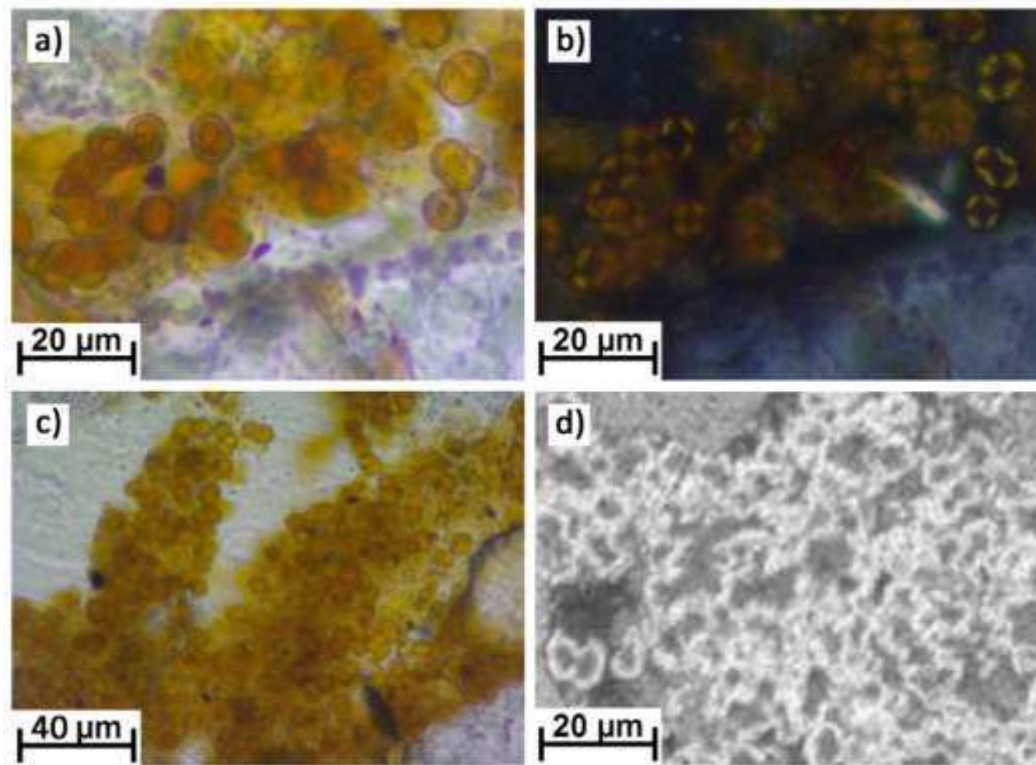
411 **Figure 5.** Key characteristics and occurrences of different forms of siderite present in the Roman
 412 grave from Hungate, York. Detailed of the different spherulite characteristics are given in Table 2a.

413 Scanning electron microscopy-energy dispersive X-ray spectroscopy (SEM-EDS) analysis revealed the
 414 spherulites to have appreciable Fe contents. Taking account of literature reports (Driese et al., 2010;
 415 Ludvigson et al., 2013; Pye et al., 1990), the composition is attributed to siderite (FeCO_3). Pedogenic
 416 siderite is characteristic of wet and persistent saturated soils (Browne and Kingston, 1993), often in
 417 association with vivianite and more rarely with goethite, where it occurs as small crystallites or
 418 spherulitic aggregates in the groundmass or as pore infillings (Stoops and Delvigne, 1990). It forms as
 419 a colourless mineral in reducing conditions, remaining stable at neutral to basic pH and can only
 420 precipitate from weakly acid solution if the concentration of dissolved iron is abnormally high (Lemos

421 et al., 2007). Siderite spherules have been observed in organic-rich archaeological floor deposits, in
422 areas inhabited by livestock where reducing conditions were inferred (Gebhardt and Langohr, 1999;
423 Milek, 2012). The Fe contents of the Group 1 spherulites were greater than those of Group 2, with
424 those of Group 1 from the area of the feet also having higher levels of Ca. Group 3 spherulites were
425 characterized by an Fe rich corona with significantly lower Fe levels in the core. This difference is
426 evident in the SEM-EDS images as a bright outer ring and a dull centre (Fig. 6d). The spherulites of the
427 first group showed the highest reflectivity. Sideritic spherulites were observed in several other graves
428 from Hungate, though exclusively in the sediments surrounding the skeletons and not in the upper
429 layers of the backfill, occurring in association with vivianite. The Roman grave, C51364, was unique
430 among those examined from Hungate in containing fan-like crystals of goethite and an absence of
431 vivianite. The location of the goethite crystals within soil pores around the skull indicate it to be a
432 secondary mineral (Fitzpatrick and Schwertmann, 1982) and the multi-layered fan-like coatings
433 indicate that the soil was very wet (Stoops and Delvigne, 1990). Goethite is formed under changing
434 redox conditions, being produced in intense oxidizing periods (Lindbo et al., 2010). Following oxidative
435 weathering, siderite is converted to goethite, assuming brownish colours (Stoops and Delvigne, 1990).

436 The siderite in the Roman grave formed in the sediments surrounding the skeleton during a phase of
437 reducing conditions, suggested to be associated with the decomposition of the organic matter of the
438 corpse and the coffin under water-logged conditions. The higher frequency of siderite associated with
439 the pelvis can be explained by the greater organic matter content and the consequently more intense
440 reducing sub-environment. Furthermore, the amorphous nature of the cores of the Group 2
441 spherulites may reflect inhibition of crystallisation by the higher amounts of organic matter associated
442 with the torso (cf. Fitzpatrick and Schwertmann, 1982). The orange colour of the siderite is indicative
443 of partial oxidation, presumably resulting as the decay of organic matter slowed reducing oxygen
444 sequestration and/or caused by the excavation, during Anglo-Scandinavian times, of the cesspit
445 located in the proximity of the skull and pelvis and partially cutting the backfill (Fig. 4). The excavation
446 of the cesspit most likely increased water flow through the grave, causing oxygenation. Further
447 evidence for higher fluid flow comes from the amorphous phosphatic coatings around the pore walls
448 in the C3 sample, located in the proximity of the cesspit. During the oxidative phase the sideritic
449 spherules from the sediments closest to the cess pit (C3) underwent the most extensive weathering
450 and goethite fan-like crystals were formed within C3 and on the pore walls in the area around the
451 skull. The presence of the Fe-rich corona on the Group 3 spherulites indicates a period of intense
452 anoxia during which the Fe-rich deposits accumulated, consistent with leaching and precipitation of
453 Fe derived from the cess pit following its extended use. The higher Ca contents of the spherulites
454 from the area of the feet can be attributed an origin from their proximity to bone and limited extent

455 of decalcification, the smaller size and higher Fe contents resulting from their rapid sealing and
456 preservation by fine material coatings. Notably, the results from the analysis of the spherulites are
457 entirely consistent with the interpretation from the profiles of the organic signatures and loss of bone
458 from the area of the grave closest to the cess pit.



459 **Figure 6.** Sideritic spherulites in the Roman age burial C51364: a) and b) from the area of the skull,
460 showing layered structure in PPL and characteristic Maltese cross extinction in XPL, c) accumulation
461 in packing voids in the area of the pelvis and d) showing elemental compositional variation with Fe-
462 rich outer rim in SEM-EDS image.

463 **4 Conclusions**

464 GC-MS and HPLC-MSⁿ analyses revealed distinct spatial variations in lipid profiles within the graves.
465 TAGs, derived from adipose tissue, were observed in the extracts from both graves, being most
466 abundant in samples from around the torso. The TAG acyl chain distributions, dominated by saturated
467 components, suggest loss of unsaturated components, either by oxidative cleavage of the unsaturated
468 bonds or by preferential hydrolysis of the unsaturated acyl moieties, rather than reductive
469 transformation into saturated components. The highest levels of DAGs, MAGs, fatty acids and *n*-alkan-
470 1-als also represent inputs from degraded body tissues and were observed in extracts from the upper
471 torso and pelvis. *n*-Alkan-1-als, by virtue of their formation from reductive transformation of fatty
472 acids and their survival in the soil, indicate regions of persistent anoxia within the grave. Their

473 preservation is highly localised to regions on the skeleton (e.g. skull, pelvis) where the bone
474 morphology provides a barrier to free movement of water thus can allow anoxic conditions to develop.
475 The higher abundances of microbial markers (branched chain fatty acids and hopanoids) in samples
476 from around the skeleton than in the controls reflects a legacy of increased microbial activity in
477 response to the remains, most likely through their involvement with the degradation of the corpse.
478 In the case of the Roman burial, abundant hopanoids clearly distinguish the gut region of the remains,
479 while their occurrence at the feet indicates migration of some material derived from the gut to the
480 foot of the coffin. The gut region of the Anglo-Scandinavian burial is characterised by elevated levels
481 of the faecal sterol, coprostanol. By contrast, coprostanol is present throughout the Roman grave and
482 instead records the ingress of material from an Anglo-Scandinavian age cess pit adjacent to the burial.
483 The acidic and anoxic nature of the leachate is indicated by the mobilisation of ferrous iron and the
484 formation and persistence of Fe-rich sideritic spherulites. It is clear that leachate from the cess pit has
485 affected the preservation of the skeletal remains, which is notably worse in areas closest to the pit.
486 Decalcification of the bone by acidic fluids from the cess pit has led to release of bone-cholesterol into
487 the soil in those regions, and depleted Ca contents recorded in spherulites from the skull and pelvis.
488 The spherulites record several changes in the redox conditions within the Roman grave, from their
489 initial formation, during a phase of intense reducing conditions associated with decomposition of the
490 corpse, to a phase of partial oxidation as the decay progressed and oxygen demand decreased. This
491 was followed by a second, more intensive phase of oxidation associated with the construction of the
492 cess pit and increased water flow through the grave, indicated by goethite formation and phosphatic
493 coatings on pore walls in the controls. An intense period of anoxia then followed with the extended
494 usage of the cess pit, marked by deposition on the spherulites of iron rich layers.

495 The results exemplified by analysis of the two graves demonstrate that a valuable unexploited archive
496 of information pertinent to the taphonomic interpretation of human burials is recorded in the burial
497 matrix. The results demonstrate the preservation of signatures relating to the body tissues and gut
498 contents which have the potential to inform archaeological investigation of diet (body tissues) and
499 last meals (gut contents) both within particular communities and between different communities.
500 Furthermore, the recognition that such organic signatures can and do survive should provide a
501 stimulus to further research. Notably, the potential for survival of signatures from grave offerings
502 provides scope for gaining further insights into their nature and use. Understanding of the factors
503 that influence the survival/destruction of organic signatures in graves could also reveal characteristics
504 that relate to particular styles of burial. The methods described here could also have potential in the
505 identification of temporary or disinterred graves including in determining the anatomical orientation
506 from the chemical signatures preserved in the soil, though further research is necessary.

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