Appendix 1 : Organic Residue Analysis of Soils

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INTRODUCTION

A suite of analyses was carried out on samples of soil collected from several key locations around the skeletal remains within the mass grave to test for signatures of the bodies and of any materials buried with them. The analyses consisted of the following:

- Micromorphological analysis to identify microscopic remains, characterise the soil structures and elucidate the soil forming processes within the grave.
- Measurement of elements (carbon, hydrogen, nitrogen and sulfur) and of total organic carbon (TOC) content to indicate the abundance and type (eg protein, carbohydrate, lipid) of organic matter within the soil.
- Gas chromatography (GC) and gas chromatography mass spectrometry (GC-MS) to separate and identify extractable organic residues, which can provide important indicators of the origins and preservation status of organic matter in the soil, including signatures from the body, material added to the body and materials placed in the grave.
- High performance liquid chromatography mass spectrometry (LC-MS) and UV/vis spectroscopy to identify different extractable organic residues than those determined by GC-MS.
- Pyrolysis gas chromatography to assess the nature of the non-extractable organic matter in the soil.

METHODS

Sampling

Soil samples for chemical analysis were collected from skulls 3571 and 3758 and from around the infra-cranial skeletons of individuals 3753, 3754 and 3755 (samples are listed in Table A1.1 and their locations shown in Figs. A1.1-1.6). In addition, two samples were collected from the centre of the mass grave (grave 1 and 2) at a level slightly below that of the skeletons. Two control samples of soil were collected; one from the spoil heap of material removed from the mass grave (C2) and the other from an undisturbed section beyond the western edge of the grave, above the level of the skeletons (C3). Samples were stored at -20°C pending analysis. Samples of undisturbed soil for micromorphological analysis were collected in Kubiena tins adjacent to skulls 3751 and 3758, the pelvis of skeleton 3756 and the pelvis and heel of skeleton 3755 (Table A1.1, Figs. A1.1 and A1.6). Unfortunately, the nature of the site and the closeness of the skeletal remains to each other in the grave precluded the collection of undisturbed replicates for many of the samples collected for chemical analysis. Samples were stored at +4°C pending preparation for micromorphological analysis.

Table A1.1 List of samples collected for chemical (C) and micromorphological (MM) analysis

Sample	*Sample type and location*	
	Chemical analysis (C)	*Micromorphological analysis (MM)*
C2	From spoil heap	
C3	From beyond W edge of pit	
Skull 3751	Beneath skull	
	Orange brown stain behind skull	
	Inside skull	
		Skull A
		Skull A
		Skull B
Skeleton 3753	L foot	
	L hand	
	Pelvis	
	Pelvis base	
Skeleton 3754	R foot	
	L hand	
	Pelvis	
Skeleton 3755	R hand	
	R Foot	Heel
	Pelvic region next to MM	Pelvis
	Pelvis base	
Skeleton 3756		Pelvis
Skull 3758	Beneath skull	Skull
Centre of grave	1	
	2	

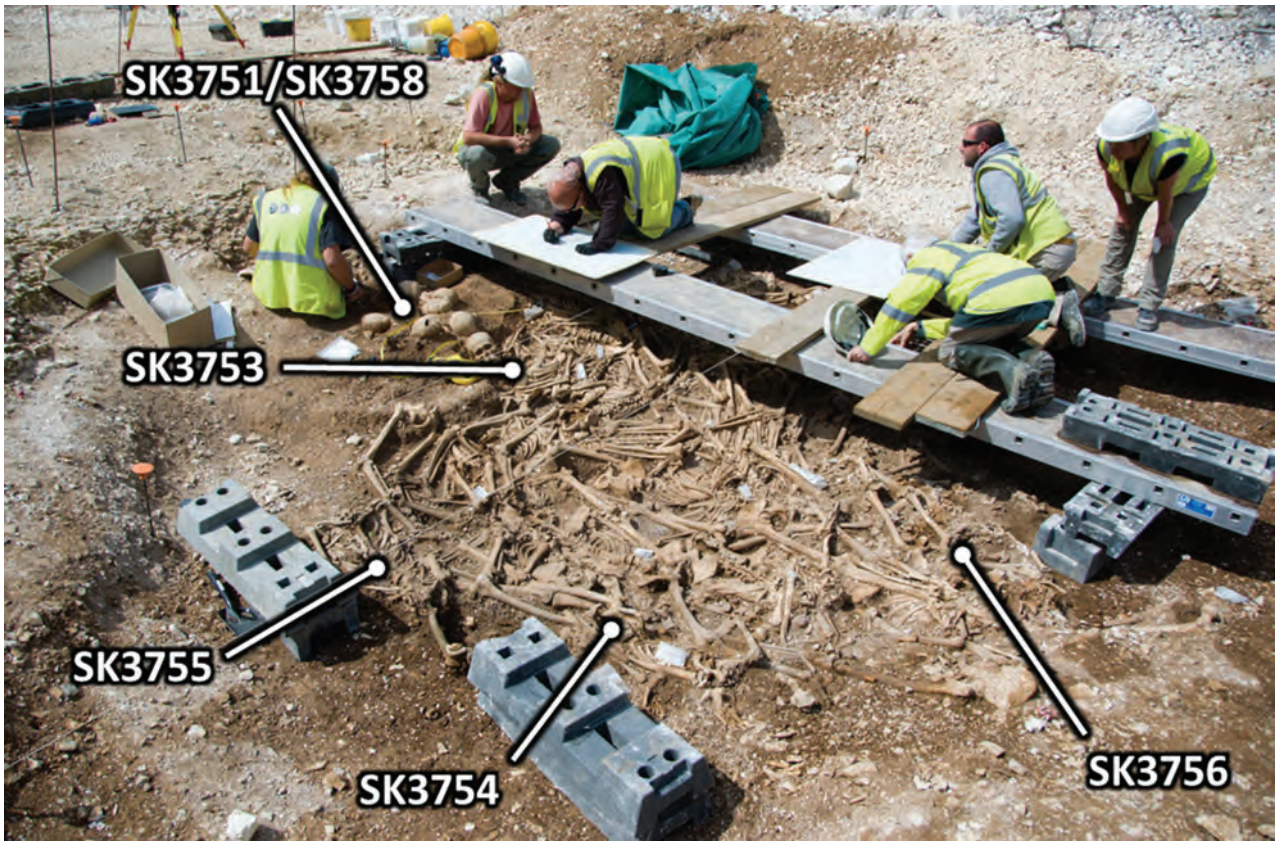

Fig. A1.1 Burial pit and sample (SK) locations

Micromorphology

Moisture was removed from the samples of undisturbed soil collected in Kubiena tins by exchange with acetone vapour and impregnation with crystic resin. The impregnated blocks were cut into thin sections, mounted on glass slides and ground to a thickness of 30μm (Murphy 1986). Micro-morphological analysis was carried out using a Zeiss Axio Scope.A1 binocular microscope with x/y motorised stage and a Zeiss Axio Lab.A1 microscope with rotary stage.

Chemical analysis

Frozen soil samples were freeze dried, disaggregated with a pestle and mortar, and sieved to ≤ 200μm.

Elemental Analysis (EA)

Carbon, hydrogen, nitrogen, and sulfur (CHNS) and total organic carbon (TOC) elemental analysis was performed on the soils using a Thermo Flash 2000 Elemental Analyser fitted with MAS200R autosampler, chromatographic column and thermal

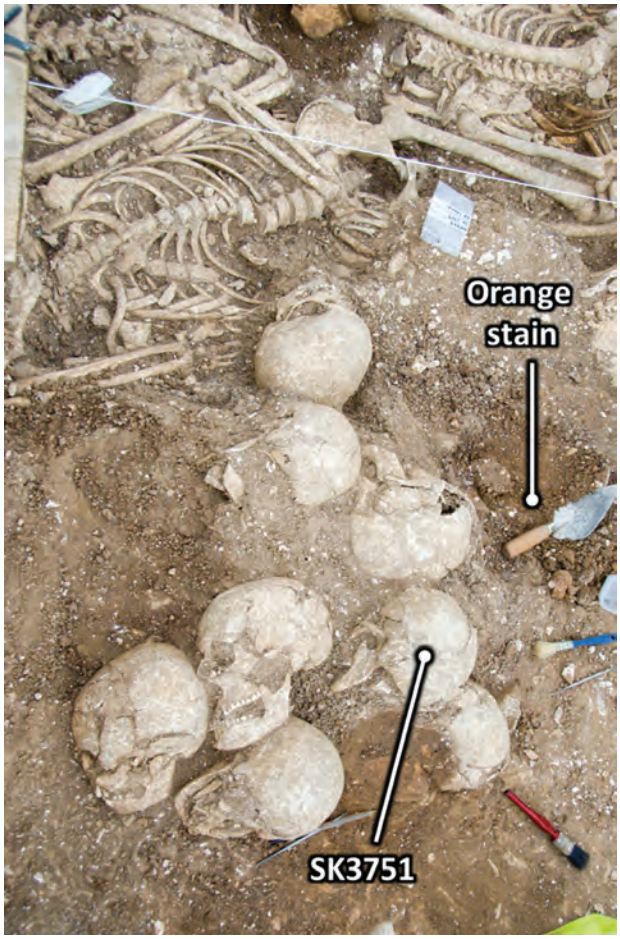

Fig. A1.2 (right) Sampling skulls

Fig. A1.3 Sampling the interior sediment of SK3751

conductivity detector. For TOC analysis, samples were treated with aqueous hydrochloric acid to remove carbonates.

Py-GC was performed on the soils using a CDS Pyroprobe 5150 coupled to a Thermo Trace GC Ultra gas chromatograph fitted with a fused silica capillary column (J&W DB-5, 60m x 0.32mm i.d., 0.25μm film thickness) and a flame ionisation detector (FID). Assignments were made on the basis of comparisons with authentic reference materials and literature data.

Extraction and fractionation of soils for chemical analysis

Soils were extracted by pressurised liquid extraction with dichloromethane and methanol at 100°C using a Dionex ASE 350. Solvent was removed using a rotary vacuum concentrator (RVC 2-25, Christ) and the total extract was divided into two portions. One half of the total extract was fractionated using a small scale flash column packed with silica to separate the complex mixture of compounds into groups sharing similar chemical properties. The total extract was loaded onto the silica and the column washed with i) hexane, ii) hexane:toluene, iii) hexane: ethyl acetate, and iv) dichloromethane:methanol to generate the hydrocarbon (HC), aromatic hydrocarbon (ArHC), medium polar (MP), highly polar (HP) chromatographic fractions, respectively. Solvent was removed using a rotary vacuum concentrator. Total extracts and polar (MP and HP) fractions were methylated and silylated prior to GC-MS analysis.

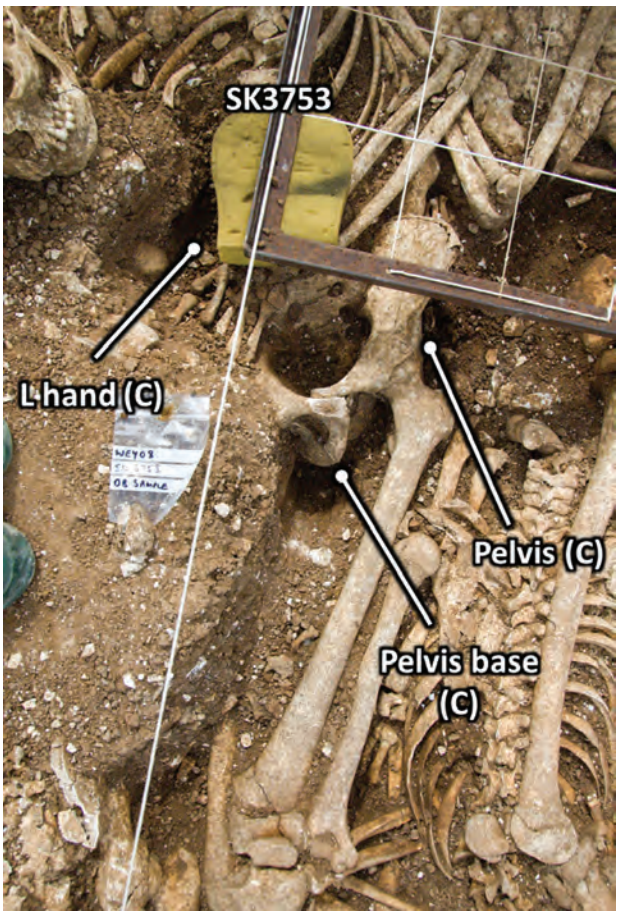

Fig. A1.4 SK3753 sampling positions. Sampling position for the left foot not shown. C = sample for chemical analysis

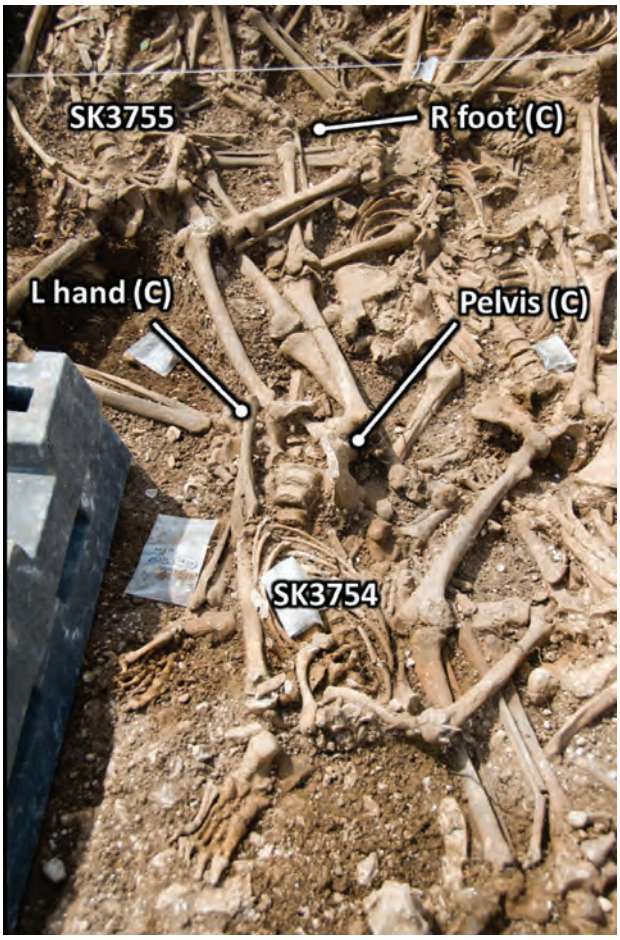

Fig. A1.5 SK3754 sampling locations and proximity to SK3755. C = sample for chemical analysis

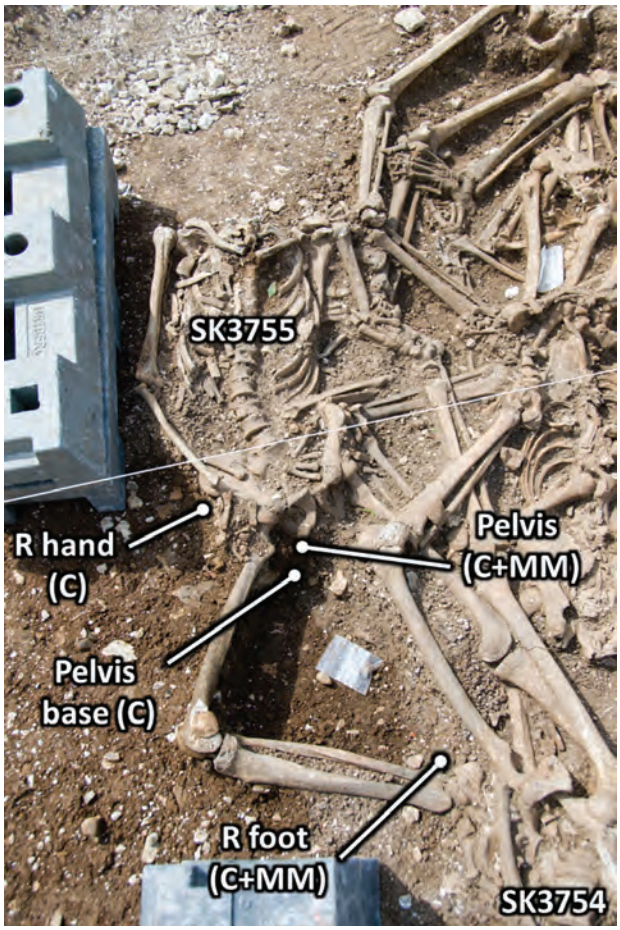

Fig. A1.6 SK3755 sampling locations and proximity to SK3754. C = sample for chemical analysis, MM = sample for micromorphological analysis

A selection of extracted soil samples (namely, C2, grave 1, grave 2 and skeleton 3755 pelvis base) were subjected to a further extraction with methanolic sulfuric acid according to Pickering (2009).

Gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS)

Total lipid extracts were analysed using a Thermo Trace GC Ultra gas chromatograph equipped with a Triplus autosampler, a fused silica capillary column (J&W DB-5, 60m x 0.32mm i.d., 0.25μm film thickness) and a flame ionisation detector (FID). Chromatographic fractions were analysed using an identical gas chromatograph to that above equipped with an ultrafast column module (Thermo UFC-5, 10m x 0.1mm i.d. 0.1μm film thickness).

GC-MS was performed on selected fractions using an Agilent 7860A gas chromatograph equipped with a 7683B Series autosampler coupled to a Waters GCT Premier Micromass time of flight mass spectrometer. Separation was achieved on a fused silica capillary column (Zebron, ZB-5, 30m x 0.25mm i.d., 0.25μm film thickness). Compounds were identified from their chromatographic retention times and interpretation of their mass spectra by comparison with library spectra (NIST 08) where available.

UV/vis spectroscopy

Acid extracts were dissolved in acetone and UV/vis spectroscopy carried out using a Shimadzu UV-1800 spectrophotometer. Spectra were acquired over the λ range 350-800μm using a slit width of 1nm, scan speed of 3000μm min-1 and a cell path length of 1cm.

High performance liquid chromatography-mass spectrometry (LC-MS)

Acid extracts were analysed using a Dionex Ultimate 3000 RSLC system with online diode array detector, coupled to a Bruker HCTultra ETD II quadrupole ion trap mass spectrometer fitted with an atmospheric chemical ionisation (APCI) source. Analysis was performed according to Method B of Airs *et al.* (2001).

RESULTS AND DISCUSSION

Micromorphological analysis

Slides prepared from undisturbed blocks of soil were examined by microscopy to look for microscale remains, soil features and soil forming processes within the grave.

Coarse fraction

The coarse material primarily comprises partially weathered sub-angular quartz (accounting for *c* 20% of the area of the slide), and weathered iron (Fe) stained calcite (*c* 10%), exhibiting first order cream/brown/red colours under cross polarised light (XPL). This is consistent with the local chalk geology. A high incidence of shell (mollusc) fragments was observed in all samples apart from the right foot of skeleton 3755. Fragments of bone were also prevalent throughout the thin sections with the exception of the pelvic sample of skeleton 3756 which did not contain any. When present, the fragments of bone exhibit a range of low birefringence levels under XPL. The birefringence arises from the collagen present within the bone. Thus, the range of birefringence exhibited by the bone fragments indicates that they are in varying states of degradation, the most weathered fragments exhibiting no birefringence suggesting absence/low levels of organic matter (Babel 1975). The presence of bone fragments mixed in with the soil fabric indicates disturbance of the soil (eg via root or

faunal activity). Evidence of pedoturbation by roots is apparent in the pelvic samples from skeleton 3755 and skeleton 3756. The roots (accounting for *c* 2% of the area of the slide) had become partially ferruginised and a significant amount of lignin still remained, as indicated by its high birefringence under XPL (Babel 1975).

Fine fraction

The samples contain a greater proportion of fine material than coarse material (fine:coarse ratio ≈ 7:3) with a speckled b-fabric present throughout, exhibiting a yellow/red/black (1st order) interference colours under XPL. The formation of the b-fabric is supported by a dotted limpidity observed under plane polarised light (PPL). Both characteristics indicate a poorly developed fine material where the organic matter present has limited the formation of distinct clay domains (Kovda and Mermut 2010).

Voids and peds

Moderate to strongly developed sub-angular blocky ped structures were observed throughout the majority of samples. These were separated by partly accommodated inter-pedal channels and displayed intra-pedal voids throughout. The exception is the skull sample (A) which exhibits a strong granular pedality separated by well-developed complex packing voids (accounting for 50% of the area of the slide). Second generation planar voids were observed in both pelvic samples, as they transect initial inter-pedal channel voids, though these are most likely to have arisen post sampling as a consequence of clay shrinkage at points of weakness, during the drying of the samples prior to processing (Courty *et al*. 1989; Oades 1993).

Pedofeatures

Well-developed textural pedofeatures occur throughout the samples, possibly indicating disturbance or a lack of cohesion in the upper layers of the soil with attendant translocation of particles through the soil profile (Usai 2001). The pedofeatures are predominant in the sample from the heel of skeleton 3755 with some displaying high levels of Fe staining, as determined by SEM-EDS inorganic elemental analysis. Fe staining is also prevalent on fragments of degraded calcite in the pelvic samples. Calcitic/clay intercalations are observed throughout the samples. Furthermore, typic and aggregated Fe/Mn redoximorphic nodules, as determined by SEM-EDS analysis, are present in the granular peds of the skull sample from skull 3751 (skull A), indicating redoximorphic conditions have occurred at some point (Lindbo *et al*. 2010). Excremental pedofeatures, indicating bioturbation by soil fauna, were observed in all samples, with the highest concentration in the pelvic sample of skeleton 3755.

Elemental analysis

Elemental carbon, hydrogen, nitrogen and sulfur measurements were performed on the soils in order to provide an indication of the nature and quantity of organic matter present. The total organic carbon (TOC) measurements (after acid digestion) for all samples are extremely similar and range between 1.76 and 2.15% (Table A1.2). The total inorganic carbon (TIC) measurements range between 0.74 and 3.06%, indicating that inorganic carbon (eg carbonate) contributes a significant portion of the total carbon (TC) present in the soil (Table A1.2). This is consistent with the local chalk geology and high levels of calcite identified by micromorphological analysis of the soils. In order to visualise the variation in bulk elemental composition across the soil samples a cross plot was constructed of the atomic ratios of nitrogen to TOC (N/C) and hydrogen to TOC (H/C) (Fig. A1.7). Clustering of certain groups of data points is observed. In some cases the clusters correspond to samples associated with the same set of skeletal remains (eg skeleton 3755), in other cases they correspond to samples collected from the same region (eg samples from the pelvic regions of skeletons 3753 and 3755). The similar ratios observed at the left hand of skeleton 3754 and right foot of skeleton 3755 are consistent with the close proximity of these two sampling points within the grave. The soil samples taken from the centre of the pit (Grave 1 and 2) both plot in the centre of the distribution, perhaps reflecting the mixed nature of the inputs to these samples.

The N/C ratio was examined to assess whether the nitrogen contents of any of the samples were elevated above the level of the soil background (represented by the controls) which might suggest inputs of proteinaceous organic matter deriving from the interred remains. While control C3 exhibits a lower N/C ratio than the other soils, resulting from a lower nitrogen value (Table A1.2), the C2 control plots close to the samples from the pit (Fig. A1.7). The similarity of the elemental composition of C2 to the soils from the pit could be due to it having been taken from the spoil heap. As such, it may comprise material excavated from a significant depth within the pit and consequently may not be a suitable comparator to assess the soil background. The N/C ratios of the soil samples from within the pit are greater than that of C3 and indicate a greater nitrogen content, suggestive of an input of protein-derived organic matter. The H/C ratio was

Table A1.2 Bulk elemental carbon hydrogen nitrogen sulfur (CHNS) and total organic carbon (TOC) contents of the soils. Total inorganic carbon (TIC) was calculated from the difference between the measured total carbon (TC) and measured total organic carbon (TOC)

Sample	Elemental content (% by weight)					
	Carbon (C)			Nitrogen (N)	Hydrogen (H)	Sulfur (S)
	TC	TOC	TIC			
C2 control	4.08	1.87	2.22	0.29	0.69	0.00
C3 replacement	4.91	1.86	3.06	0.23	0.62	0.00
SK3751 beneath skull	3.94	1.85	2.08	0.29	0.80	0.00
SK3751 orange brown stain behind skull	4.36	2.03	2.33	0.30	0.77	0.00
SK3751 inside skull	4.28	1.86	2.42	0.30	0.76	0.00
SK3753 L foot	3.17	1.91	1.25	0.30	0.89	0.00
SK3753 L hand	3.88	2.02	1.86	0.30	0.84	0.00
SK3753 pelvis	3.50	2.11	1.39	0.30	0.82	0.00
SK3753 pelvis base	3.44	2.00	1.43	0.29	0.80	0.00
SK3754 R foot	3.40	1.76	1.63	0.29	0.84	0.00
SK3754 L hand	3.76	1.83	1.93	0.28	0.78	0.00
SK3754 pelvis	3.00	1.93	1.07	0.29	0.83	0.00
SK3755 R hand	3.15	2.13	1.02	0.32	0.83	0.00
SK3755 R foot	2.99	2.15	0.84	0.32	0.88	0.00
SK3755 pelvic region next to MM	3.11	2.04	1.06	0.31	0.82	0.00
SK3755 pelvis base	2.81	2.07	0.74	0.31	0.83	0.00
SK3758 beneath skull	4.03	2.05	1.98	0.30	0.81	0.00
Centre of pit 1	3.22	1.99	1.23	0.30	0.82	0.00
Centre of pit 2	3.13	1.97	1.16	0.29	0.77	0.00

examined to provide an indication of the contribution from aliphatic organic compounds. With the exception of C3 (H/C ratio = 3.97) the H/C ratios for all samples are greater than 4, the maximum possible for organic matter (eg methane, CH4). Given that the samples were all rigorously dried prior to elemental analysis, these values indicate a contribution from inorganic hydrogen, from the dehydroxylation and dewatering of clay minerals (cf. Gualtieri and Ferrari 2006). Thus, comparisons between H/C ratios can be made, assuming the mineral background contributes a constant amount for each sample. Notably, within the samples for each skeleton, the foot samples consistently plot with higher H/C ratios, with those from skeletons 3753 and 3754 exhibiting the highest H/C values of all samples studied. Notably, while skeletons 3753 and 3754 were situated at the margins of the grave, the feet of these individuals were located further into the pit. Thus, the greater H/C values could indicate a higher proportion of lipid material derived from the more densely packed remains towards the centre of the grave.

Trace organic chemical analysis

Analysis of the extracts by GC and GC-MS reveal all of the extracts to exhibit distributions of n-alkanes, n-alkanols and n-alkanoic acids consistent with those of the leaf waxes of vascular plants (Eglinton and Hamilton 1967). Similar distributions have been observed in extracts from a range of soils (Jambu *et al*. 1991; Jambu *et al*. 1993; Ambles *et al*. 1994; Bull *et al*. 2000a; Bull *et al*. 2000b; Jansen *et al*. 2008). C16 and C18 alkanoic acids, present in the extracts, have numerous sources and occur in animals, plants, fungi and bacteria (Brockerhoff 1965; Zelles 1997; Ruess *et al*. 2002). In addition, the HP fractions contain a narrow envelope of C22-C28 ω-hydroxyalkanoic acids dominated by the C22 and C24 components. Notably, C22 and C24 moieties occur as polyesters in the biopolymer suberin which is associated with the exterior surfaces of woody plants eg bark, roots (Kogel-Knabner 2002). Accordingly the presence ω-hydroxyalkanoic acids in soil extracts have been suggested to arise via microbial hydrolysis of plant root material (Van Bergen *et al*. 1998; Bull *et al*. 2000a). Notably, roots were identified as abundant features in the soils subjected to micromorphological analysis. The ubiquity of the signatures pertaining to higher plant waxes and suberin in the samples associated with the controls and skeletal remains indicates that they reflect background plant material naturally present within the soil.

A number of steroidal components were identified in the extracts including, cholest-5-en-3β-ol (cholesterol), 24-ethylcholest-5-en-3β-ol (sitosterol), 24-ethylcholest-5, 22-dien-3β-ol (stigmasterol), 24-methylcholest-5-en-3β-ol (campesterol) accompanied

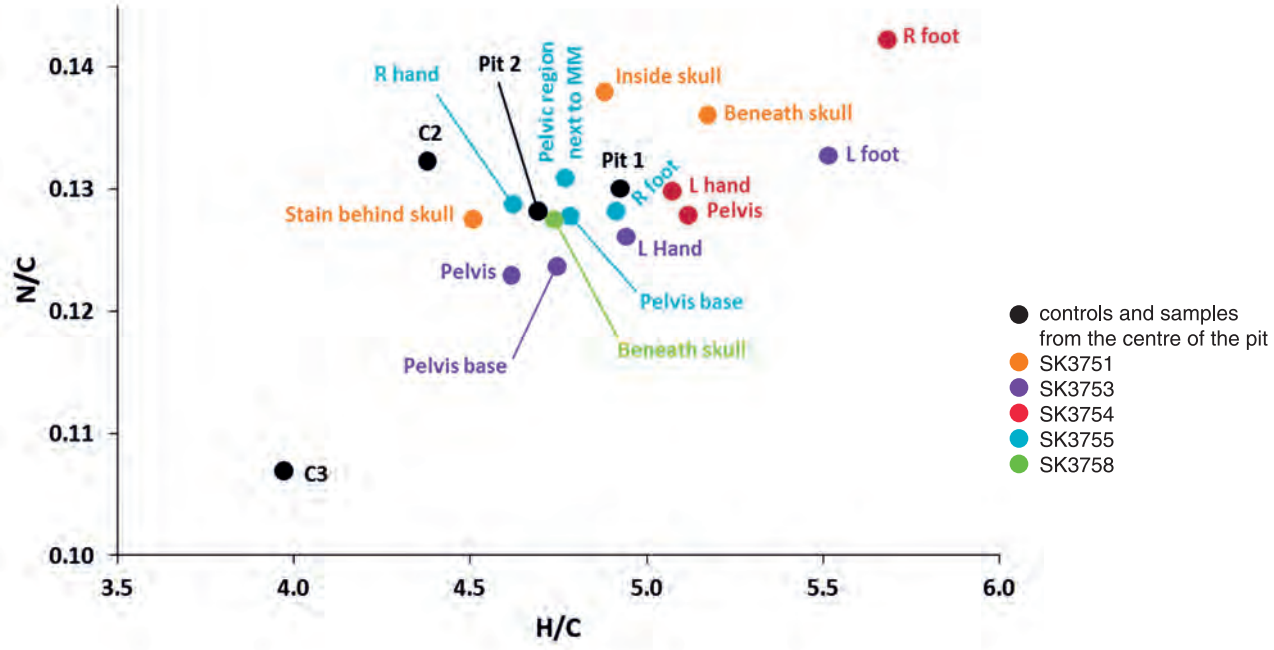

Fig. A1.7 Cross plot of nitrogen/organic carbon and hydrogen/organic carbon atomic ratios for the soils.

by some of the corresponding stanols (chemically reduced products), 24-ethyl-5α-cholestan-3β-ol (5α-stigmastanol), 24-ethyl-5α-cholestan-3β-ol (24-ethylcoprostanol), 5α-cholestan-3β-ol (5α-cholestanol), and 5α-cholestan-3β-ol (coprostanol).

Sitosterol, stigmasterol and campesterol are major plant sterols (Puglisi *et al*. 2003). Their occurrence throughout all of the samples studied, including the controls C2 and C3, is indicative of plant material present within the soil and consistent with the observations of plant waxes and root-derived material in the samples. Cholesterol is primarily an animal sterol, although it also occurs to a lesser extent in plants (Puglisi *et al*. 2003). Its occurrence in the controls suggests a background input from soil fauna. Notably, cholesterol and its stanol derivatives are generally elevated, relative to the controls, in the samples from around the skeletal remains indicating additional inputs (eg 71.7μg/gTOC for skeleton 3754 pelvis cf. 11.6μg/gTOC for control C3). Exceptions to this are the orange stain adjacent to skull 3751 inside skull 3751 and skeleton 3755 right foot which contain sterol profiles similar to the controls. Cholesterol occurs in all animal tissues. It is also a persistent lipid component of bone (Evershed *et al*. 1995; Jim *et al*. 2004). Thus, the elevated levels of cholesterol derivatives in the samples associated with the remains are most likely to arise from that released during degradation of the body tissues. The elevated levels of cholesterol derivatives in the sample from beneath the skull of 3751 suggest that it contains organic matter derived from the head. By contrast, the similarity of the sterol profiles of the sample from inside the cranial cavity of skull 3751 to those of the controls indicate that these sediments may have formed from ingress of material after extensive degradation of the organic tissues had taken place. In the majority of samples cholesterol is accompanied by smaller amounts of the corresponding stanols 5α-cholestanol and 5β-cholestanol (coprostanol), the latter typically being present in lower abundance. In a number of samples, however, 5β-cholestanol is present in greater abundance than 5α-cholestanol and sometimes cholesterol; namely skeleton 3753 pelvis and pelvis base, skeleton 3754 pelvis, skeleton 3755 pelvis base and pelvis next to MM and the centre of grave samples 1 and 2. An enhancement in the proportion of 24-ethylcoprostanol is also observed for these samples. 5α-stanols are the major products of the microbial reduction of sterols in soils and plant and mammalian tissues (Bethell *et al*. 1994). The diastereomeric forms, 5β-stanols, are typically minor products of sterol reduction in the environment (Nishimura 1982). They are, however, formed in significant amounts during microbial transformation of cholesterol in the guts of most higher animals (Bethell *et al*. 1994; Leeming *et al*., 1996). Accordingly, 24-ethylcoprostanol has been used as a manuring indicator (Bull *et al*. 1998; Bull *et al*. 2000b; Hjulstrom and Isaksson 2009) and coprostanol has been used as a marker for the input of human and animal faecal matter to soils, sediments and water systems (Grimalt *et al*. 1990; Bethell *et al*. 1994; Leeming *et al*.1996; Hjulstrom and Isaksson 2009). The elevated levels of 5β-stanols in the pelvic samples studied (from skeletons 3753, 3754 and 3755) and the two

samples from the centre of the grave indicate the presence of faecal material. The signal is strongest for the samples from the skeleton 3753 pelvis and skeleton 3755 pelvis next to MM. In the case of the samples collected from the centre of the pit, the high levels of coprostanol could indicate transport of faecal material from the gut (for example pooling of the products of body decomposition).

The steroid profiles contained in coprolites (fossilised faecal matter) greater than 2000 years old have been used to infer dietary information (Lin *et al*. 1978; Lin and Connor 2001). Faecal steroids derive from both dietary and endogenous sources (Lin and Connor 2001). Thus, an overprint of endogenous cholesterol and its derivatives is observed, even in the case of predominantly vegetarian diet. The situation is further complicated in the case of the pelvic soil samples studied here as there are contributions arising from sterols naturally present in the soil and from cholesterol deriving from the body tissues, some of which may have also have been converted to coprostanol by gut-derived microflora. The former contribution can be corrected for by subtracting the steroid profile of the C3 control, representing the background sterol signature, from those of the pelvic samples. The ratio of cholesterol to plant sterols (after correction for the background contribution) varies considerably between samples.

Notably, several of the samples collected in close proximity from the same pelvis exhibit different cholesterol to plant sterol ratios and different proportions of coprostanol; in the case of skeleton 3753, the sample from the pelvis exhibits a greater cholesterol:plant sterol ratio and a greater proportion of coprostanol than the pelvis base. Similarly, in the case of skeleton 3755, the sample from the pelvis collected next to MM exhibits a greater cholesterol:plant sterol ratio and a greater proportion of coprostanol than the other sample from the same pelvis. These samples, collected close together, would be expected to receive similar inputs of endogenous cholesterol derived from the degradation of body tissues. Thus, it appears that the sterol variations observed result from differences in concentration caused either by better preservation or by sampling in closer proximity to the original source of the sterol. As the proportion of coprostanol is greatest in samples from the skeleton 3753 pelvis and skeleton 3755 pelvis next to MM, these samples appear to represent the most productive locations with regards to recovering faecal signatures. The cholesterol to plant sterol ratios for the two samples are greater than those observed for the stools of modern day Tarahumara Indians and Americans consuming high cholesterol diets (Lin and Connor 2001) but not as great as that observed for the coprolite of a Greenland Eskimo whose diet was believed to consist almost exclusively of meat (Lin *et al*. 1978). This suggests that that both individuals (skeletons 3753 and 3755) had consumed food derived from both plant and animal sources, with significant contribution from the latter, shortly before death.

Notably, this interpretation should be considered with caution; a soil sample is a more open system than that of a coprolite and, therefore, subject to greater external influence. Sterols have been shown to persist in soils over archaeological timescales (Knights *et al*. 1983; Bull *et al*. 2003). The recalcitrance of this class of compounds stems from their hydrophobicity, acting as a barrier to their being leached from the original site of deposition by percolating groundwater, and their polycyclic structure which offers inherent resistance to biological and chemical weathering (Atlas *et al*. 1981; Hornick *et al*. 1983). Nevertheless, inputs of cholesterol from the decomposition of body tissues or activity of soil fauna could alter the distributions of "dietary" sterols in the samples. Given that the sterol ratios in the samples are observed to vary over short distances, however, it appears that the variations reflect significant changes relating to the anatomy.

Acid methanolytic extraction was performed on a selection of soil samples (C2, grave 1, grave 2 and skeleton 3755 pelvis base) to look for heme (a component of hemoglobin, the oxygen transport protein in red blood cells) which would indicate the remains of blood in the pit. Acid extracts were analysed by UV/vis spectroscopy and high performance liquid chromatography with online photodiode array and mass spectrometric detection (LC-MS). No trace of an absorption band at ≈ 400 nm, characteristic of heme derivatives (Calzavara-Pinton *et al*. 2007), could be observed in the UV/vis spectra. Similarly, no trace of heme derivatives could be detected by LC-MS. The proportion of tetrapyrroles (such as heme) that survive in oxic terrestrial environments is small (Hendry *et al*. 1987). In the case of the grave, however, the amount of organic matter present as a result of the inhumations would surely lead to the development of anoxic conditions in the pit. Conditions during this period would be favourable to the preservation of heme. In addition, the structure of heme possesses two propionic acid acidic moieties which have been proposed to bind, via ester linkages, to mineral surfaces in sediments (Huseby and Ocampo 1997), leading to an enhanced potential for preservation of these compounds. The ester linkages are broken during acid methanolytic extraction, thus, the lack of detection of any heme derivatives in the extracts suggests that the soils from the pit do not contain significant mineral-bound heme. The acid moieties of heme also render it partially soluble in water

(0.169 mg ml-1 at 25°C) (Yalkowsky and Dannenfelser 1992). Thus, solubilisation in interstitial waters would make heme more readily available for degradation by microorganisms and could lead to it being leached away from the site of deposition by percolating groundwater.

The soil samples were examined by pyrolysis-gas chromatography to look for any traces of complex polymeric organic matter, such as protein, carbohydrates or lignin, which could relate to clothing or other organic materials that might have been present within the grave. No differences were detected between the pyrograms of the samples associated with the skeletal remains and the controls. Similarly, no compounds indicative of clothing (eg lanosterol from sheep's wool) could be detected in the sample extracts and no evidence for textiles was identified in the samples studied by micromorphology.

SUMMARY

Micromorphological analysis revealed the soil to comprise calcite with clay inclusions. The development of sub-angular peds (indicative of soil aggregation), and speckled birefringence exhibited by the fine material, points to the presence of high levels of organic matter. The occurrence of textural pedofeatures throughout all of the thin sections indicates surface disturbance or poor cohesion in the upper soil layers. Evidence of pedoturbation was observed in all thin sections studied, with the highest frequency of root fragments occurring in the pelvic samples.

The majority of the organic matter in the sample extracts derives from plant waxes and root material naturally present within the soil. Significant variations were observed in the sterol distributions of the soils. The samples associated with the skeletal remains generally exhibited a greater proportion of cholesterol, probably derived from the degradation of body tissues, than the controls. In addition, samples collected from the pelvic regions and from the centre of the pit contained coprostanol, a marker for human faecal material. In the case of the samples from the centre of the pit this is most likely to indicate a mixed signal deriving from the pooling of organic matter from several individuals. The occurrence of coprostanol in the pelvic samples indicates the presence of faecal material deriving from the digestive tract. Examination of the sterol distributions in the pelvic samples with the strongest faecal signals suggests that these individuals may have consumed a diet of plant and animal origin, comprising significant quantities of the latter, within 48 hours prior to death. This interpretation should be taken with caution because, while dietary information has been obtained from coprolites, soils are much more dynamic systems susceptible to external influence.

The inability to detect blood-derived heme derivatives in the samples could be the result of them having been leached away or degraded. An alternative explanation is that the bodies were exsanguinated before they were placed into the pit. The absence of signatures for clothing in the soil samples does not necessarily mean that it was not present in the burial and could equally result from a lack of preservation.