Organic geochemical evidence for the origin of ancient anthropogenic soil deposits at Tofts Ness, Sanday, Orkney

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Abstract

Lipid biomarker components of soils constituting three Orcadian archaeological fossil soil profiles were analysed. The combined assessment of lipid distributional and compound specific stable carbon isotope data enabled the identification of grass turves as the most probable material used in the formation of the anthropogenic soil deposits. Appraisal of $5β$-stanol components indicated a faecal input to one of the soils which, on considering distributional evidence, was ascribed a human/porcine origin. Additional study of polar bile acids from this profile revealed a distribution exhibiting a predominance of deoxycholic acid indicating the primary faecal input to be mainly derived from humans although the minor occurrence of hyodeoxycholic acid, a characteristic component of pig faeces, attested to a limited porcine input. © 1999 Elsevier Science Ltd. All rights reserved.

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1. Introduction

Analyses of archaeological soils have largely paralleled those of more general soil formations in the preferential use of bulk variables such as TOC, total phosphate and other elements (Farswan and Nautiyal, 1997; Entwistle and Abrahams, 1997). Understandably, these types of analysis are attractive, the potentially high throughput of samples favouring their joint use with physical prospective methods in examining former settlement sites. In contrast, lipid analysis is more time-consuming but provides a highly diagnostic means of answering specific questions relating to subtle differences in soil organic matter engendered by various overlying vegetation changes or anthropogenic activity such as manuring (van Bergen et al., 1997; Bull et al., 1999 and references therein).

One prominent application of organic geochemical methods has been in the detection of ancient inputs of faecal matter to soils through the use of highly specific biological markers, i.e. $5β$-stanols and bile acids. The presence of characteristic $5β$-stanol signatures in soils has already enabled positive identifications of ancient latrines and cess-pits to be made (Knights et al., 1983; Bethell et al., 1994; Evershed and Bethell, 1996), whilst similar analyses of ancient Cretian agricultural terrace soils have established the existence of an off-site manuring regime in the Minoan period (Bull et al., in...
press). A further study of modern-day agricultural soils, where a 5β-stanol signal persisted for more than 120 years after the cessation of manuring, attests to the relative longevity of these compounds and supports their use in less exposed archaeological soils (Bull et al., 1998). Recent advances by this laboratory (Bristol) have shown bile acids to be diagnostic faecal markers with an even greater resistance to degradation than 5β-stanols (Elhmmali et al., 1997). Together both compound classes provide a reliable method by which ancient faecal deposition, and in many cases the actual source of the faecal matter, may be determined (Evershed and Bethell, 1996; Simpson et al., 1998; Simpson et al., in press).

Similar investigations to trace other components exogenous to the pedosphere are scarce. However, the prospect of determining further inputs is not unfeasible. It has already been demonstrated that a change in overlying vegetation has a discernible effect on the composition of low molecular weight soil components (van Bergen et al., 1997; van Bergen et al., 1998). Hence, the potential exists for such changes to be exploited and to provide information regarding differences in ancient vegetation cover or physical transfer of soils and vegetation from one environment to another. Furthermore, techniques such as stable carbon isotope analysis can provide additional information regarding the source of organic soil.

Fig. 1. A map depicting the location of settlement sites on the Tofts Ness peninsula of Sanday, Orkney. Insets: maps of anthropogenic soil depths associated with Mounds 4 and 11 as determined by auger. Dashed areas represent the extent of the settlement mound and middens, including post abandonment spreading out of settlement site material, they do not represent the extent of anthropogenic soils.
Fig. 2. Soil profile descriptions of the pits dug around Mounds 4, 8 and 11.
constituents. As with soil organic matter such isotope analyses have been largely restricted to bulk measurement of total organic carbon in studies of various ancient environments, such as Holocene habitat change (Ambrose and Sikes, 1991) and marine inputs to Orcadian soil formations (Simpson, 1985; Davidson et al., 1986). Advances enabling the measurement of compound specific stable carbon isotope ratios have further increased the level of information which may be derived from the analysis of low molecular weight components of soil (Hayes et al., 1990; Freeman et al., 1990; Simpson et al., 1998; Simpson et al., in press).

This study combines elemental, lipid, lipid biomarker and compound specific stable carbon isotope analyses to investigate the origin of three buried anthropogenic soil formations at Tofts Ness, on the Isle of Sanday, Orkney, UK.

2. Description of study site

2.1. Anthropogenic soils

A buried, dark coloured loam soil horizon of between 35 and 75 cm in thickness, embedded at various depths between calcareous wind blown sand deposits, can be identified in three areas of the Tofts Ness landscape. The close association with early settlement sites and enhanced total phosphate levels indicate that these fossil soils are anthropogenic in origin and formed by a process analogous to the plaggen manuring process found extensively in north-west Europe (Dockrill and Simpson, 1994; Simpson et al., 1998). Such soils are thought to have arisen within a mixed pastoral-arable economy as a result of efforts to maintain and enhance the fertility of arable areas. Heather or grass turves were stripped from podsolic soils and used as animal bedding; the composted turf and animal manure were then applied to the arable land where the mineral component attached to the turf gradually contributed to the creation of a thick, artificial, soil horizon (Pape, 1970; Simpson, 1997). Radiocarbon dating and stratigraphic relations between the fossil anthropogenic soils at Tofts Ness and settlement sites indicate that the horizon is associated with Bronze Age cultural landscape activity and may have commenced formation during the late Neolithic period (Simpson et al., 1998). Such a chronology places these anthropogenic soils amongst the earliest of their type.

Soils around three settlement sites, Mound 4 (M4), Mound 11 (M11) and Mound 8 (M8), in the Tofts Ness peninsula (Royal Commission on the Ancient and Historic Monuments of Scotland notation; Fig. 1) were surveyed to determine the extent and thicknesses of buried, fossil, anthropogenic soil horizons. Soils around Mound 4 and Mound 11 were surveyed by hand auger where Munsell colours of 10YR 3/2 or 10YR 3/3 (dark brown/black) and hand textures of sandy loam and sandy silt loam distinguished the horizons. At Mound 8, where the horizons of interest are generally buried deeper and thicknesses could not be fully ascertained by conventional hand auger, a fluxgate gradiometer was used to determine the spatial extent of the buried soil (Simpson et al., 1998). These observations indicated that the buried soils are slightly less than 1 ha in extent. Pits were dug in soil stratigraphies around each settlement mound (two from around Mound 8) and samples (ca 400 g) excised from the exposed anthropogenic layers in the soil profile (Mound 4: 0–5, 30–35, 39–44 and 49–54 cm; Mound 8: 35–40, 45–50, 55–60, 65–70, 75–80, 85–90, 95–100 and 105–110 cm; Mound 8/2: 60–65, 70–75 and 78–83 cm; Mound 11: 0–5, 33–36, 39–42, 45–48 and 51–54 cm; Fig. 2). Surficial soil from Mound 8 was not analysed since, unlike that of Mounds 4 and 11, it had been subjected to more recent ploughing. In addition, a number of contemporary materials of the type which may have been used in anthropogenic soil formation were collected (1–5 g of each). These included single samples of: cattle dung, midden (mixed turf and cattle dung), roofing turf, surface vegetation litter and seaweed (Fucus vesiculosus). A number of soil analyses have been made on archaeological sediments obtained from Mounds 4, 8 and 11 (these studies include thin section micromorphology, bulk δ13C analysis, total phosphate analysis, radiocarbon dating and a gradiometric analysis of Mound 8; Simpson et al., 1998).

3. Experimental

3.1. Sample preparation and solvent extraction

All soil and putative inputs were supplied air dried. Samples were partially crushed with a pestle and mortar and, subsequently, sieved using 2 mm and 75 μm sieves yielding a single <75 μm particle size fraction. Dried vegetation samples were processed by the same method but liquid nitrogen was added to facilitate the crushing process. All samples were Soxhlet extracted for 24 h using a dichloromethane/acetone (9:1 v/v) solvent system to obtain a total lipid extract (TLE).

3.2. Acid/neutral separation of TLEs

TLEs were initially separated into two fractions, ‘acid’ and ‘neutral’, using an extraction cartridge with a bonded amionopropyl solid-phase (Jones Chromatography). Extracts dissolved in DCM/isopropanol (2:1) were flushed through a cartridge pre-eluted with the same solvent system. After further elution
with DCM/isopropanol (2:1, 6 ml) the collected ‘neutral’ fraction was removed and the cartridge flushed with 2% acetic acid in diethylether (6 ml) thereby eluting the ‘acid’ fraction. Both fractions were evaporated under reduced pressure, transferred to vials and residual solvent removed by a stream of nitrogen.

### 3.3. Column chromatography of neutral lipids

Columns were packed with dried activated silica gel 60 (160°C, > 24 h; Fluka) and pre-eluted with hexane. Samples were applied to the column as a mixture of dissolved and finely suspended particulates in hexane. Gradient elution was performed under positive pressure supplied by a stream of nitrogen providing an elution rate of 15 ml min⁻¹. The eluents used comprised five separate solvent systems: hexane, hexane/DCM (9:1), DCM, DCM/methanol (1:1) and methanol, applied in elutropic order to give five fractions: ‘hydrocarbon’, ‘aromatic’, ‘ketone/wax-ester’, ‘alcohol’ and ‘polar’, respectively. The relative volumes of solvents applied were determined by the ratio 2:3:2:2, following the above elutropic series, and the size of the column being used for a particular separation. Column fractions were collected and solvent removed in an identical manner to fractions from the acid/neutral separation.

### 3.4. Urea adduction

Samples (~10 mg) dissolved in hexane/acetone (2:1, 1.5 ml), in a Pyrex test-tube, were agitated with a vortex mixer whilst a warm, saturated solution of urea in methanol (1 ml) was added dropwise forming a dense white precipitate of urea. The solvent was removed under a stream of nitrogen (ensuring the complete evaporation of methanol) and 3 ml of DCM added to resuspend the urea precipitate. After centrifugation (2500 rpm, 15 min) the non-adduct in DCM was removed by pipette and passed through a small plug of pre-extracted cotton wool (ensuring complete removal of urea crystals). The adduction procedure was then repeated and the second non-adduct solution combined with the first. The adduct was recovered by washing the urea crystals with DCM (10 ml) then dissolving them in double distilled water (10 ml) and extracting with DCM (2 ml). The urea saturated aqueous layer was removed and a further aliquot of double distilled water (10 ml) was mixed with the DCM and left to partition (ensuring complete removal of urea from the organic layer). The organic layer was then removed and passed through a small column of anhydrous sodium sulphate to remove any residual water. Solvent was removed from the eluates by evaporation under a gentle stream of nitrogen.

### 3.5. Derivatization

All fractions except ‘hydrocarbons’ and ‘n-alkanoic acids’ were derivatized by heating sample aliquots with 30 μl of N,O-bis(trimethylsilyl)triﬂuoroacetamide (BSTFA), containing 1% trimethylchlorosilane (TMCS), at 70°C for 1 h. Residual BSTFA was evaporated under nitrogen and the sample redissolved in hexane.

n-Alkanoic acids were converted to their corresponding methyl esters by reaction with a BF₃-methanol complex (14% w/v) at 70°C for 10 min. Methyl esters were isolated by adding 1 ml of double distilled water and extracting into hexane.

### 3.6. Elemental analysis

Elemental analyses were performed using a Perkin Elmer 240C elemental analyser to determine total carbon, hydrogen and nitrogen compositions of the soils. Inorganic carbon content was determined using a Strohlein Instruments Coulomat 702 carbon analyser adapted to analyse CO₂ liberated from H₃PO₄ digestion; the TOC value was then calculated as the difference between total carbon and total inorganic carbon. Each sample was analysed a total of four times and a mean TOC value calculated; values typically exhibited standard errors < ±0.1%dwt.

### 3.7. Gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS)

GC analyses were performed using a Hewlett-Packard 5890 series II gas chromatograph fitted with a fused silica capillary column (Chrompack CPSil-5 CB, 50 m × 0.32 mm) coated with a 100% dimethylpolysiloxane stationary phase (film thickness 0.12 μm). Derivatized samples were injected (1.0 μl) via an on-column injector as solutions in hexane. Hydrogen was used as carrier gas for all samples except the hydrocarbon fraction when helium was used as carrier gas in order to facilitate resolution of the internal standard. GC analyses of wax ester fractions were made using a column capable of performing at elevated temperature (J & W Scientific DB1, 15 m × 0.32 mm × 0.1 μm film thickness). The temperature was programmed from 40°C (1 min) to 200°C at a rate of 10°C min⁻¹ then to 300°C at a rate of 3°C min⁻¹ with a final time of 20 min when employing the former column and from 50°C (2 min) to 350°C at a rate of 10°C min⁻¹ with a final time of 10 min when using the latter. The majority of GC/MS chromatographic separations were performed using a Carlo Erba 5160 GC equipped with a fused silica capillary column (Chrompack CPSil-5 CB, 50 m × 0.32 mm) coated with a 100% dimethylpolysiloxane stationary phase (film thickness 0.12 μm, He
carrier gas) whilst 'wax-ester' fraction analyses employed a high temperature column (J & W Scientific DB1, 15 m x 0.32 mm x 0.1 µm film thickness; He carrier gas). The GC oven temperature was programmed from 40°C (1 min) to 200°C at a rate of 10°C min⁻¹ then to 300°C at a rate of 3°C min⁻¹ with a final time of 20 minutes when employing the former column and from 50°C (2 min) to 350°C at a rate of 10°C min⁻¹ with a final time of 10 minutes when using the latter column. Samples were introduced using on-column injection coupled, via a heated transfer line, to a Finnigan MAT 4500 quadrupole mass spectrometer scanning in the range of m/z 50–850 with a cycle time of 1.5 s. Electron energy was maintained at 300 eV with an ion source temperature of 190°C. The mass spectrometer was operated with an electron voltage of 70 eV.

3.8. Gas chromatography combustion/isotope ratio mass spectrometry (GCC/IRMS)

Analyses were made on 1 µl sample aliquots using a Varian 3400 gas chromatograph fitted with a fused silica capillary column (SGE BP-1, 50 m x 0.32 mm) coated with a 100% dimethylpolysiloxane stationary phase (film thickness 0.12 µm, He carrier gas), fitted with a septum equipped temperature programmable injector (SPI) coupled to a Finnigan MAT Delta S stable isotope mass spectrometer (electron ionisation, electron energy maintained at 300 eV with an ion source temperature of 190°C. The mass spectrometer was operated with an electron voltage of 70 eV.

Fig. 3. Levels of total organic carbon determined for each soil profile.

Fig. 4. Histograms depicting the distribution of n-alkane components exhibited by: (a) a typical mound excavated anthropogenic soil, and (b) the soil associated with a roofing turf.
100 eV electron voltage, 1mA electron current, 3 Faraday cup collectors masses 44, 45 and 46, CuO/Pt combustion reactor set to a temperature of 850°C).

4. Results

4.1. Total organic carbon (TOC) analysis

Fig. 3 depicts the changes in TOC levels, with depth, for each of the four soil profiles analysed. Values are observed to vary between 1.0 and 6.2% on a soil dry weight basis. Whilst the surficial samples of soils around Mounds 4 and 11 (0–5 cm) contain the highest quantities of TOC (4.9 and 6.2% respectively), the majority of anthropogenic soil samples [all except Mound 4 (0–5 cm), Mound 11 (0–5 cm) and Mound 11 (33–36 cm)] exhibit lower amounts (<2.7%). In the sub-40 cm region of each profile, TOC levels can be seen to both increase and decrease within a much narrower range (1.0–2.7%).

4.2. Analysis of n-alkyl lipids

Extracts of each soil sample show practically identical homologies of dominant, odd n-alkanes typified by a
Fig. 6. Histograms depicting the distribution of $n$-alkanol components exhibited by soils taken from the profiles adjacent Mounds 4, 8 and 11.
Fig. 7. Histograms depicting the distribution of \( n \)-alkanoic acid components exhibited by soils taken from the profiles adjacent to Mounds 4, 8, and 11; M8 (45–50, 65–70, 85–90 and 95–100 cm) not analysed.
monomodal distribution with a maximum at C₃₁ (Fig. 4); the immediate peripheral homologues (C₂₇, C₂₉, and C₃₃) are also abundant. n-Alkane distributions obtained from the turf soil, turf vegetation and cattle dung reveal almost identical homologous series of n-alkanes.

Fig. 5 summarises the wax ester distributions obtained from the majority of soil samples collected from the Tofts Ness peninsula. Homologous series are observed in the C₃₆–C₆₀ range with the C₄₄–C₅₂ homologues generally exhibiting a markedly higher abundance. Further inspection of this latter range reveals the C₅₀ wax ester, in each case, as the characteristically most minor component barring a few notable exceptions (M₄—30–35 cm, M₈/₂—78–83 cm and M₁₁—0–5 cm). Interestingly, soils obtained from the M₁₁ profile exhibit C₅₂ components which are significantly more abundant than peripheral homologues, especially in the uppermost soil layer. The surficial sample obtained from soils around the Mound 4 profile also exhibits a dominant C₅₂ homologue. Analysis of the moieties forming the observed wax esters reveals a range of components for each individual peak (n-alkanols, C₂₄–C₃₀; n-alkanoic acids, C₁₆–C₂₆). Of particular note is the observation that the most abundant acid fragment ions, for each peak, correspond with the loss of a C₂₆ n-alkanol moiety, this is particularly pronounced for the C₄₄ and C₄₈ wax ester components which exhibit dominant ions at m/z 285 (C₁₈ n-alkanoic acid) and m/z 341 (C₂₂ n-alkanoic acid) respectively. Additionally, Fig. 5 gives details of the distribution obtained from the roof turf vegetation which also exhibits a homologous series (C₃₆–C₆₀) dominated by components from C₄₂ to C₅₆, inclusive, with an unpronounced maximum at C₅₂.

The relative abundances of n-alkanol homologues...
observed for the soil samples are summarised in Fig. 6. Each homologous series lies within a C_{20}–C_{34} range with a central maximum at C_{26} and prominent but progressively less abundant C_{28} and C_{30} components. The overall abundance of n-alkanoid distributions fluctuates between different profiles and depths. Inspection of the n-alkanoid distribution obtained from the roof turf soil reveals a homologous series almost identical to those observed for the anthropogenic soils, albeit with a slightly more dominant C_{26} component.

n-Alkanoic acid distributions obtained for the majority of soil profile samples are shown in Fig. 7. Each sample exhibits a bimodal distribution of components with a lower maximum at C_{16} and a higher maximum at C_{24}, C_{26} or C_{28}. Only two anthropogenic soils actually exhibit a latter maximum at C_{24}, these being the two surficial samples from adjacent Mound 4 (0–5 cm) and Mound 11 (0–5 cm). Additionally, the distributions of both surficial samples have even/odd CPI values which are significantly higher (14.0 and 14.1 respectively) than those calculated for the remaining soil derived distributions (2.6–4.9). Inspection of the distribution derived from the roof turf vegetation reveals a similar relative abundance of latter homologues (≥C_{22}) with a maximum at C_{24}. In comparison, the homologous series obtained for the associated roof turf soil exhibits C_{26} and peripheral homologues at a significantly higher relative abundance compared with the C_{16} component. Whilst, the distribution observed for the mixed vegetation litter has a latter maximum at C_{24} albeit a lower maximum still occurs at C_{16}.

4.3. Stable carbon isotopic analysis of individual n-alkyl lipids

δ^{13}C values obtained for the three dominant n-alkanes (C_{29}, C_{31} and C_{33}) have been reported previously by Simpson et al. (1998). Values for homologues generally fell within a range of −32 to −35 % and exhibited a remarkable similarity between all four soil profiles.

Further stable carbon isotope analyses were performed on the n-alkanoic acid components of the anthropogenic soil profiles, the results of which are depicted graphically in Fig. 8. Initial inspection reveals all values to lie within a range of −27 to −37 % with homologues becoming progressively depleted in δ^{13}C in direct relation to chain length. The majority of δ^{13}C values obtained for any single homologue all lie within a very narrow range. Two notable exceptions to this pattern are the 0–5 cm surficial soil samples taken from soils around Mounds 4 and 11. For both samples, the majority of n-alkanoic acid homologues are depleted by ca 1.5 % relative to the isotopic composition of identical homologues obtained from samples at greater depth. Interestingly, the C_{16} and C_{18} components are consistently 3–6 % less depleted in δ^{13}C than the C_{20} homologue; a significantly larger difference than is observed between consecutive higher homologues.

4.4. Analysis of steroidal components—putative inputs

Table 1 summarises the relative abundance distributions of sterols and sterones in each of the potential inputs. Distributional data are presented as a percentage of the identified components in each sample. Inspection of the results obtained for the cattle dung and dung-based midden reveals very similar distributions. Each is dominated by stanols, with the single most abundant component in each sample being 24-ethyl-5β-cholestan-3β-ol (5β-stigmastanol; 1c) which occurs with its 3α-epimer 24-ethyl-5β-cholestan-3α-ol (2c). Other 5β(H)-stanols present are 5β-cholestan-3β-ol (coprosterol; 1a), 24-methyl-5β-cholestan-3β-ol (5β-campestanol; 1b), 24-

Table 1
A distributional summary of steroidal components in the putative inputs, % of identified components

<table>
<thead>
<tr>
<th>Input</th>
<th>1a</th>
<th>2a</th>
<th>3a</th>
<th>1b</th>
<th>2b</th>
<th>1c</th>
<th>2c</th>
<th>3c</th>
<th>4c</th>
<th>4d</th>
<th>5b</th>
<th>6b</th>
<th>7b</th>
<th>6c</th>
<th>7e</th>
<th>8e</th>
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<tbody>
<tr>
<td>Cattle Dung</td>
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<td>2</td>
<td>2</td>
<td>6</td>
<td>2</td>
<td>1</td>
<td>Trace</td>
<td>3</td>
<td>33</td>
<td>10</td>
<td>10</td>
<td>17</td>
<td>4</td>
<td>2</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Midden</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>4</td>
<td>1</td>
<td>3</td>
<td>Trace</td>
<td>2</td>
<td>21</td>
<td>10</td>
<td>10</td>
<td>11</td>
<td>4</td>
<td>3</td>
<td>23</td>
<td></td>
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<tr>
<td>Roof Turf (Soil)</td>
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<td>2</td>
<td>3</td>
<td>2</td>
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<td>5</td>
<td>12</td>
<td>11</td>
<td>3</td>
<td>1</td>
<td>9</td>
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<tr>
<td>Roof Turf (Vegetation)</td>
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<td>2</td>
<td>6</td>
<td>2</td>
<td>1</td>
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<td>6</td>
<td>2</td>
<td>18</td>
<td>14</td>
<td>5</td>
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<tr>
<td>Plant Litter</td>
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<td>1</td>
<td>3</td>
<td>5</td>
<td>6</td>
<td>1</td>
<td>Trace</td>
<td>8</td>
<td>7</td>
<td>5</td>
<td>4</td>
<td>3</td>
<td>23</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seaweed (Fucus vesiculosus)</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>0</td>
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* 5β-cholestan-3β-ol (1a); 5β-cholestan-3α-ol (2a); cholest-5-en-3β-ol (4a); 5α-cholestan-3β-ol (3a); 24-methyl-5β-cholestan-3β-ol (1b); 24-methyl-5β-cholestan-3α-ol (2b); 24-ethyl-5β-cholest-22-en-3β-ol (1e); 24-ethyl-5β-cholest-22-en-3α-ol (2e); 24-methylcholestan-5-en-3β-ol (4b); 24-methyl-5α-cholestan-3β-ol (3b); 24-ethyl-5β-cholest-3α-ol (2e); 24-ethylcholestan-5,22-dien-3β-ol (4c); 24-ethylcholesterol-5-en-3β-ol (4e); 24-ethylcholesterol-3β-ol (3β); 24-methyl-5β-cholestan-3-one (5b); 24-methyl-5α-cholestan-3-one (6b); 24-ethyl-5β-cholestan-3-one (5c); 24-methylcholesterol-4-en-3-one (7b); 24-ethyl-5α-cholestan-3-one (6c); 24-ethylcholesterol-4-en-3-one (7c).
ethyl-5β-cholest-22-en-3β-ol (1e) and their corresponding 3α-epimer analogues. Only two stanols with a 5α(H) configuration are observed to occur, i.e. 5α-cholestan-3β-ol (5α-cholestanol; 3a) and 24-ethyl-5α-cholestanol-3β-ol (5α-stigmastanol; 3c), although this latter compound is still the second most abundant com-

Fig. 9. Partial gas chromatograms showing the sterol distributions observed for the surficial (0–5 cm) and deeper (33–36 cm) soils taken from the soil profile adjacent Mound 11. For structures see appendix.
Fig. 10. Chromatograms m/z 129 and m/z 215 mass and reconstructed ion chromatograms obtained for the sterol fractions from the uppermost (35–40 cm) and deepest (105–110 cm) soils taken from the soil profile adjacent Mound 8. For structures see appendix A.
ponent of each distribution. Additionally, 24-ethyl-5β-cholestan-3-one (5c), 24-ethyl-5α-cholestan-3-one (6c) and 24-ethylcholest-4-en-3-one (7c) are observed, albeit in low abundance.

The soil associated with the roof turf exhibits a less complicated sterol distribution lacking a single dominant component. Inspection of the distribution reveals the presence of the unsaturated phytosterols 24-methylcholest-5-en-3β-ol (campesterol; 4b), 24-ethylcholest-5,22-dien-3β-ol (stigmasterol; 4e) and 24-ethylcholest-5-en-3β-ol (sitosterol, 4c) as well as the C27 congener cholest-5-en-3β-ol (cholesterol, 4a). Also present are the 5α(H)-stanol analogues 5α-cholestan-3β-ol (3a), 24-methyl-5α-cholestan-3β-ol (3b) and 24-ethyl-5α-cholestan-3β-ol (3c). Of note is the particularly high proportion of 24-ethyl-5α-cholestan-3-one (6c) and 24-ethylcholest-4-en-3-one (7c).

Vegetation associated with the roof turf yields a similarly uncomplicated distribution of components in which the only sterols 24-methyl-5α-cholestan-3β-ol (3b), 24-ethylcholest-5-en-3β-ol (4c) and 24-ethyl-5α-cholestan-3β-ol (3e) exist at low abundance. The dominant component at 50% is 24-ethylcholest-4-en-3-one (7c) whilst 24-methylcholest-4-en-3β-ol (7b) and 24-ethyl-5α-cholestan-3-one (6c) are present at a moderately high abundance and a 2% proportion of 24-methyl-5α-cholestan-3-one (6b) is also observable.

The distribution observed for the miscellaneous plant litter is very simple. The dominant compound is 24-ethylcholest-5-en-3β-ol (4c; 36%) with a correspondingly high proportion of 24-ethylcholest-4-en-3-one (7c). 24-methylcholest-5-en-3β-ol (4b), 24-ethylcholest-5,22-dien-3β-ol (4e) and the 5α(H)-stanol analogue 24-ethyl-5α-cholestan-3β-ol (3e) all reside at lower levels of abundance.

Seaweed (Fucus vesiculosus) represents the only putative input of marine origin. As expected, the ‘sterol’ fraction obtained is significantly different to those of previous samples. The distribution consists of a single component identified as 24-ethylcholest-5,24(24E)E-dien-3β-ol (fucosterol; 4g) recognised as the major sterol constituent of nearly all macroscopic brown algae (Phaeophyceae; Volkman, 1986).

4.5. Analysis of steroidal components in the anthropogenic soils

The majority of anthropogenic soils exhibit steroidal components at abundances amenable to GC analysis. However, the sterol fractions of the Mound 4 fossil
soils are extremely low in abundance, hampering any reliable analysis, although the surficial sample (0–5 cm) does exhibit a sterol profile dominated by 24-ethylcholest-5-en-3β-ol (4e) with lower, but still significant, quantities of 24-methylcholest-5-en-3β-ol (4b), 24-ethylcholest-5,22-dien-3β-ol (4e) and 24-ethylcholest-4-en-3-one (7e).

Anthropogenic soils around Mound 11 contain the highest quantity of sterols relative to aliphatic components. Fig. 9 depicts two representative sterol fractions from soils around Mound 11 as determined by GC. The surficial sample excised from 0–5 cm depth is overwhelmingly dominated by 24-ethylcholest-5-en-3β-ol (4c) and the relatively less abundant components 24-methylcholest-5-en-3β-ol (4b), 24-ethylcholest-5,22-dien-3β-ol (4e) and 24-ethylcholest-4-en-3-one (7e), largely paralleling the surficial soil from around Mound 4. Cholest-5-en-3β-ol (4a) is also present as are the 5x(H) reduced analogues of this component and the phytosterol congeners mentioned above. Soils taken from the profile at greater depth generally exhibit mostly the same steroidal components although the 5x(H)-stanols are all observed at higher relative abundances than their stenol analogues. Additionally, minor quantities of 24-methylcholest-5,22-dien-3β-ol (4d) and 5β-cholestan-3β-ol (1a) are also observed to occur at low concentrations. Mound 4. Cholest-5-en-3β-ol (4a) is also present as are the 5x(H) reduced analogues of this component and the phytosterol congeners mentioned above. Soils taken from the profile at greater depth generally exhibit mostly the same steroidal components although the 5x(H)-stanols are all observed at higher relative abundances than their stenol analogues. Additionally, minor quantities of 24-methylcholest-5,22-dien-3β-ol (4d) and 5β-cholestan-3β-ol (1a) are also observed to occur at low concentrations.

The sterol distributions for the anthropogenic soils from around Mound 8 (8 and 8/2) show many similarities to those of fossil anthropogenic soils from around Mound 11. However, a number of minor components observed in soils around Mound 11, e.g. 24-methylcholest-5,22-dien-3β-ol (4d), are not detected. Fig. 10 depicts total ion traces and mass chromatograms (m/z 129 and m/z 215) for the sterol fractions of the shallowest and deepest fossil soils taken from around Mound 8. Of particular significance is the increasing abundance of 5β-cholestan-3β-ol (1a), relative to 5x-cholestan-3β-ol (3a), observed at greater depth. The m/z 215 mass chromatogram is particularly useful since under EI conditions (70 eV) the 3β,5β-molecule is observed to fragment to produce a lower yield of ions of m/z 215 compared with the 3β,5x-epimer. Hence, the m/z 215 mass chromatogram unequivocally confirms the greater abundance of 5β-cholestan-3β-ol (1a) compared with 5x-cholestan-3β-ol (3a). Again paralleling soils around Mound 11, soils from around Mound 8 do not exhibit any detectable levels of 24-ethylcholest-5,24(24‘)-E-dien-3β-ol (4g).

4.6. Bile acids analysis of soils associated with Mound 8

Fig. 11 depicts the bile acid distribution of the soil excised at 105–110 cm depth from the Mound 8 profile. It may be readily observed that, disregarding the internal standard, the distribution is dominated by a large peak corresponding to the secondary bile acid deoxycholic acid (9). A number of related primary and secondary bile acids are present at a much lower abundance amongst which a small peak corresponding to hyodeoxycholic acid (12) is observed.

5. Discussion

5.1. General organic composition

Information regarding changes in the overall composition of material used to construct the mounds may be obtained by analysing soil TOC levels. The relatively large amount of organic carbon present in the surficial layers around Mound 4 (0–5 cm) and Mound 11 (0–5 cm) is most likely the result of contributions from modern-day flora and fauna. Whilst the narrower range of TOC levels exhibited by all samples deeper than 40 cm indicates a relatively uniform composition of material deposited over time, the low levels of organic matter being consistent with an input of mineral topsoils (Davidson et al., 1986; Simpson, 1993).

5.2. Identification of fossil anthropogenic soil constituents using n-alkyl components

The identification of material(s) used in the formation of the Tofts Ness anthropogenic soils is facilitated somewhat by environmental history data concerning the Orkneys. For example, due to increasingly adverse weather conditions and increased anthropogenic activity by 3000 BC the only remaining vegetation in the Orkneys consisted of scrub and open grassland. Additionally, the Isle of Sanday had few deposits of peat so seaweed, turf and animal dung were used as primary fuel sources, the burning of turf giving rise to iron rich orange/red soil deposits which may be observed at Tofts Ness (Ritchie, 1995). Such knowledge severely limits the number of putative inputs to fossil soils, the most probable of these being: cattle dung, dung-turf midden, grass turf, miscellaneous low lying vegetation and seaweed. Lipid analysis provides a highly diagnostic method which, as well as enabling differentiation between these various inputs,
can provide further information regarding the pedological fate of lipid components derived from them.

Further evidence for a majority input of unchanging composition is provided by the practically identical homologues of \( n \)-alkanes exhibited by all anthropogenic soils (Fig. 3). The large odd-over-even predominance observed for the components being indicative of an input from terrestrial vegetation (Peters and Moldowan, 1993). Additionally, the maximum at \( C_{31} \) supports the notion that grasses may have been a major organic input to each anthropogenic soil since the distribution observed is consistent with those of most temperate grasses (Maffei, 1996). The similarity between \( n \)-alkane distributions from fossil anthropogenic soils and those from turf soil, turf vegetation and cattle dung lend further support to a grass turf input, the cattle dung distribution having derived from the original food source, i.e. grass.

Wax esters commonly occur as components of epicuticular leaf waxes, usually exhibiting a suite of homologues (\( C_{30} \)–\( C_{56} \); Walton, 1990). Vegetation-derived esters commonly contain \( C_{26} \) and \( C_{28} \) \( n \)-alkanol moieties (depending upon the dominant free \( n \)-alkanol component) and a range of \( C_{18} \)–\( C_{32} \) of \( n \)-alkanoic acids (Tulloch and Hoffman, 1973a, b; Tulloch 1984, 1987). The larger ranges of constituent moieties exhibited by esters extracted from the fossil soils are most likely the result of transesterification processes operating within the soil (Amblès et al., 1994; Jambu et al., 1995). Despite this, the preferential occurrence of the \( C_{26} \) \( n \)-alkanol moiety parallels the occurrence of a dominant \( C_{28} \) \( n \)-alkanol component in the free \( n \)-alkanol distributions derived from turf soil and turf vegetation (see below). The exceptionally high abundance of the \( C_{52} \) wax ester homologue, in the two surficial samples (Mound 4: 0–5 cm and Mound 11: 0–5 cm), may be attributed to a more recent input of higher plant detritus, the predominant \( C_{26}, C_{28} \) \( n \)-alkanol-\( n \)-alkanoic acid composition of the ester lending support to this conclusion. The relative prominence of the \( C_{32} \) component in all samples from the Mound 11 profile is noteworthy and is possibly the consequence of enhanced preservation compared with the soils of other mound profiles. Whilst it may be compared favourably with distributions from the profiles associated with Mounds 4 and 8 the wax ester distribution obtained from the roof turf vegetation lacks the more dominant \( C_{32} \) component characteristic of samples from the Mound 11 profile. However, given the age and former environmentally exposed position of the roof turf, one would expect the sample to have suffered considerable degradation with no new input of lipid from vegetation. Under such a scenario the dominant \( C_{32} \) component would exhibit considerable degradation (Jambu et al., 1993) whilst the gradual increase in abundance, with chain length, observed may well arise from an inverse relationship between rate of loss and chain length as previously reported for aliphatic compounds (Amblès et al., 1994).

It is interesting to note the overall abundance of \( n \)-alkanol distributions fluctuating with both profile and depth. Jambu et al. (1993) reported an increase in free primary \( n \)-alkanols from L to F to A1 horizon in a soil profile beneath a cover of pine trees (\textit{Pinus maritima} ssp.). The soil samples in this study differ in that they have, within any profile, been largely excised from the same horizon which originates as a consequence of anthropogenic and not natural processes. Therefore, the fluctuations observed probably arise from increased input of vegetation and/or increased post-depositional environmental exposure, the latter leading to enhanced degradation. The similarities in distributions observed between fossil soils and the turf soil infer a probable majority input of turf soil and related materials. As mentioned above, enhanced degradation of the more abundant \( C_{26} \) component could yield an identical distribution; a phenomenon previously observed by both this laboratory (Bristol; Bull, 1997) and other authors (Disnar and Héroux, 1995). However, it is possible that the apparent increase in abundance of the peripheral homologues is also the result of other additional processes, e.g. enzymatic reduction of \( n \)-alkanoic acids (Kolattukudy, 1971, 1975). The same process may be used to explain the ‘increase’ of peripheral homologues between the roof turf vegetation and the roof turf soil.

Appraisal of \( n \)-alkanoic acid distributional data provides both further evidence concerning fossil anthropogenic soil formation materials and insights into soil lipid diagenesis. The high even/odd CPI values exhibited by distributions from the surficial soils (Mounds 4: 0–5 cm and Mound 11: 0–5 cm) reflect recent incorporation of plant detritus. The lower values exhibited by distributions from deeper soils indicate the action of pedogenic microbial processes (Peters and Moldowan, 1993). The similarity between the surficial soil and turf vegetation distributions implies, not surprisingly, that recently incorporated plant matter is of the same type associated with the ancient turf. Lehtonen and Ketola (1990) have suggested that the release of \( n \)-alkanoic acids from decaying plant detritus is the process responsible for observable increases in even long chain \( n \)-alkanoic acids. Whilst the hydrolytic release of long chain \( n \)-alkanoic acids from cerides undoubtedly contributes to the increase of higher homologues in the roof turf soil distribution, the most important contributory mechanism is the terminal oxidation of long-chain \( n \)-alkanols. Amblès et al. (1994) report significant correlation between the carbon number of even \( n \)-alkanoic acid components and the even \( n \)-alkanoic acid: \( n \)-alkanol ratio in a hydro-morphic forest podzol, it was furthermore noted that the extent of oxidation did not decrease with the
increase in chain length of even n-alkanols. This provides an explanation for the relative increase in C26, C30, and C32 n-alkanoic acids, observed in distributions derived from anthropogenic soils, since the respective n-alkanol analogues are the dominant components in each corresponding soil n-alkanol distribution. The n-alkanoic acid distribution obtained from the miscellaneous vegetation litter does not correlate well with any of the derived distributions including, most importantly, the relatively young surficial soils thereby affirming grassy turf as the most probable major component used in anthropogenic soil formation.

Stable carbon isotope analysis of the n-alkanoic acid components increased the evidence for a major terrestrial input to the Tofits Ness anthropogenic soils. The narrow range of δ13C values between soils, obtained for each homologue, is consistent with the idea of a fairly uniform composition of formation material thus agreeing with n-alkane stable carbon isotope data previously reported by Simpson et al. (1998). The ca 1.5% depletion exhibited by homologues from the two 0–5 cm surficial soils may be attributed to the assimilation of isotopically lighter, contemporary CO2 (Keeling et al., 1984; Friedli et al., 1986). This result holds particular importance regarding the question of mobility of n-alkanoic components in the soil environment. A number of studies (e.g. Jaffé et al., 1996) have highlighted the physical movement of lipids within soil/sediments. However, the relative immobility of n-alkanoic acids in the mounds studied is supported by these data since the n-alkanoic acids in the surficial soils are isotopically distinct from deeper homologues thereby inferring little, or no, detectable vertical migration. Furthermore, this result also supports the notion that lipids in the deeper fossil soil are indeed ancient in origin and not derived from modern-day sources although ideally 13C dating of lipids would provide better support for this proposition (Huang et al., 1996).

5.3. Identification of anthropogenic soil constituents based on steroidal components

Steroidal components remain one of the most useful suites of biomarkers for inter- and intra-environmental investigations having been the subject of much study (Mackenzie et al., 1982; Volkman, 1986). The specificity of certain sterols for particular sources of organics makes them ideal indicators for determining the nature of material used in anthropogenic soil formation. Additionally, the availability of modern-day inputs for comparative purposes not only enhances the reliability of source determinations but also facilitates further study of the chemical behaviour of sterol components in the natural environment. It should be noted that the fractions discussed in the following section, whilst designated as, and predominantly composed of sterols, do contain a limited number of sterenone components.

All of the putative inputs, except seaweed, exhibit distributions containing phytosterols, commonly observed in higher plants, and/or their diagenetic products both reductive (5α-stanols) and oxidative (stanes/stanones) (Mackenzie et al., 1982; Goad, 1991). If the original materials used to form the anthropogenic soils were significantly terrestrial in origin then the fossil soils would be expected to yield a similar suite of components and this is indeed the observed case. Whilst lower in abundance, soils around Mound 8, and to a greater extent around Mound 11, exhibit characteristically terrestrial steroidal distributions. The surficial (0–5 cm) and deeper (33–36 cm) soil sterol distributions depicted in Fig. 9 are of particular interest. When compared with the sterol distributions of the potential inputs, that of the 0–5 cm sample most resembles the plant litter whilst the deeper (33–36 cm) sample bears a strong resemblance to the distribution from the mineral soil of the roofing turf. The lower relative abundance of 24-ethylcholest-4-en-4-one (7c) is a product of oxidative degradation (Ren et al., 1996), in the fossil soil indicates a higher state of preservation compared with the roof turf. Significantly, 24-ethylcholest-5,24(24′)E-dien-3β-ol (4g) is not detected in any anthropogenic soils indicating little, or no, input of Fucus vesiculosus and/or any other related brown macroscopic algae. This agrees favourably with bulk δ13C values previously obtained for the anthropogenic soils which were strongly terrestrial (−26.0 to −26.1%; Simpson et al., 1998). Whilst, based on these data, seaweed cannot be considered a major component of anthropogenic soil formation there still remains the possibility that small amounts have remained undetected due to degradation of 24-ethylcholest-5,24(24′)E-dien-3β-ol (4g). The Δ24(24′) unsaturation is a relatively labile functionality and burial in the soil environment could well result in the complete conversion of this sterol to other steroidal and/or non-steroidal compounds. A search for these compounds would be a potentially useful future avenue of research.

The single most abundant component in both the cattle dung and the midden is 24-ethyl-5β-cholestane-3β-ol (1c). 5β-Stanols are widely known as intestinal reduction products of Δ4 unsaturated sterols in mammals (e.g. Knights et al., 1983; Laureillard and Saliot, 1993; Bethell et al., 1994). The presence of a dominant C29 5β-stanol homologue, resulting from reduction of the phytosterol 24-ethylcholest-5-en-3β-ol (4e), attests to the ruminant faecal source (Evershed and Bethell, 1996; Evershed et al., 1997). However, inspection of the fossil soil sterol distributions reveals the total absence of this component indicating that cattle manures were not a significant constituent of fossil soil for-
Moreover, the only detectable 5β-stanols in the fossil soils are 5β-cholestan-3β-ol (1a, Mounds 8 and 11) and 5β-cholestan-3α-ol (2a, Mound 8). 5β-cholestan-3β-ol (1a) is present as a minor component in the fossil soils from around Mound 11 and although there is a slight increase in abundance, with depth, its presence is most likely attributable to the multi-sourced background of 5β-stanols that is observed to occur in many soils (Bethell et al., 1994). Conversely, 5β-cholestan-3β-ol (1a) and 5β-cholestan-3α-ol (2a) are observed to occur at much higher abundances, relative to other sterol components, in soil profiles from around Mound 8. Whilst it can also derive from porcine manures, the potential use of 5β-cholestan-3β-ol (1a) as a biomarker of human faecal deposition has already been extensively investigated and reported (Hatcher and McGillivary, 1979; Knights et al., 1983; Bethell et al., 1994; Evershed and Bethell, 1996; Evershed et al., 1997). Previous work has made use of a stanol ratio, first proposed by Grimalt et al. (1990) and later modified, for archaeological soil studies, to include the epimer 5β-cholestan-3α-ol (2a) as a more reliable proxy for determining faecal deposition in antiquity, i.e.:

\[
\frac{(1a + 2a)}{(1a + 2a + 3a)}
\]

When applied to the C27 stanol components, determined for soils around Mound 8 and Mound 11, an increase in the ratio with depth is observed for all profiles (Fig. 12). Whilst the values generated for soils around Mound 11 remain ≤0.42 in line with the above conclusion of only a minor faecal background contribution to these soils, those from around Mound 8 increase to a much greater extent approaching the theoretical 0.7 threshold for faecal deposition (as determined by Grimalt et al., 1990) in the deepest sample, i.e. Mound 8 (105–110 cm; 0.63). Given the possibility of partial degradation of soil components with time it is not unreasonable to expect a decrease in this lower limit required to positively satisfy this parameter for faecal deposition. Given their inherently greater thermodynamic stability, 5α-stanols will be less susceptible to abiotic degradation than 5β-stanols (Mackenzie et al., 1982). Hence, we would interpret these results to indicate faecal material as a significant input during initial formation of anthropogenic soils around Mound 8 (> 78 cm) and a more minor component during later episodes of soil formation (45–78 cm). As mentioned above, since the C27 5β-stanols are the only detectable 5β-stanol components, the most likely faecal source is either human or porcine. Differentiation between these two sources requires additional analysis of bile acid components (Bethell et al., 1994).

![Fig. 12. A plot of values obtained by applying equation (i) to the C27 stanol components detected in anthropogenic soil adjacent Mounds 8 and 11.](image)
5.4. Appraisal of bile acid components to distinguish between pig and human inputs

Bile acids represent another group of metabolic products, structurally related to stanols, which have been used in the detection of faecal deposition (Evershed and Bethell, 1996; Elhmmali et al., 1997). Whilst the predominant secondary bile acid in human faeces is deoxycholic acid (9) the main secondary bile acid component of porcine faeces is the C-6 hydroxylated analogue hyodeoxycholic acid (12), thereby offering a potential method of faecal source discrimination in soils around Mound 8 (Bethell et al., 1994). Considering the predominance of deoxycholic acid in the deepest (105–110 cm) soil taken from around Mound 8 the faecal signal can be attributed to a majority human faecal input. However, it is important to note the occurrence of hyodeoxycholic acid (12) albeit at a low level. Since the compound is not produced by microflora in the human gut it is most likely derived from a minor input of porcine faecal material. Interestingly, other bile acid analyses concerning anthropogenic Orcadian top-soils, surrounding a medieval farm settlement, have recorded porcine faeces as the major component of a faecal input (Simpson et al., in press).

5.5. Archaeological implications

Evidence obtained from the lipid analysis of fossil soils from around Mounds 4, 8 and 11 indicates that these soils arose from the systematic deposition of grass turves. This agrees with both bulk $\delta^{13}$C analyses previously made on the same samples and observed morphological features such as the occurrence of phytoliths (Simpson et al., 1998). The additional human faecal input determined for soils around Mound 8 is consistent with an ancient input of household waste from the adjacent settlement and is further supported by the occurrence of small bone shards, another indicator of domestic waste application, in the soils. Radiocarbon measurements of soils from the profiles indicate a Bronze Age date for each mound with formation continuing into the Iron Age (Simpson et al., 1998). The soils can be considered to have been an integral component in the intensive manuring regime required in order to sustain any viable agricultural activity in the Bronze Age landscape of Tofts Ness, where the abundance of calcareous sand would have inhibited water retention during summer periods thus presenting a high risk of erosion when cultivated. One distinctive feature is the human origin of the faecal material input to soils around Mound 8. The apparent lack of high amounts of animal derived manures is significant and infers either the practice of direct application of this material to agricultural plots or its preferential use as a fuel source. Soils around Mound 8 appear to have been intimately associated with a human settlement although the relative proportion of faecal material applied to the mound decreased after initial formation.

6. Conclusions

This investigation has involved a synthesis of lipid distributions, specific lipid biomarkers and compound specific $\delta^{13}$C measurements in an attempt to identify the organic components that contribute to the formation of three Bronze Age anthropogenic fossil soil deposits located at Tofts Ness, Sanday, Orkney. The study has yielded a number of interesting points, namely:

- Analysis of aliphatic lipid components has led to the identification of grassy turves as a major component of anthropogenic soil formation,
- Analysis of sterol and n-alkanoic $\delta^{13}$C data failed to reveal any evidence supporting the use of seaweed as a major component of anthropogenic soil formation.
- Use of a stanol index ratio enabled the detection of a significant faecal input to soil around Mound 8 at initial stages of formation, dropping to a moderate input at later stages,
- Further analysis of bile acid components from soils around Mound 8 identified the faecal material as predominantly human in origin with possibly a very minor porcine contribution.

This study has shown that organic geochemical analysis is both an applicable and highly useful tool for investigations concerning archaeological soils and can reveal information unobtainable by more traditional forms of soil and archaeological analysis.

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Appendix A
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