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153 **Supplementary Table 1. Summary of results, all samples.**

Sample number	Sample name	Details	Location	Age	Results
1	Isbister	DL42 M40-50 Sk8 SC1 ST2	Orkney, Scotland	Middle to Late Neolithic 3200–2800 BC	Seaweed, meat/fish/dairy exposure to fire/cooking
2	Isbister	DL2104 M17-23 Sk11 BC8/31 SC3 IS1976	Orkney, Scotland	Middle to Late Neolithic 3200–2800 BC	Seaweed, meat/fish/dairy exposure to fire/cooking
3	Isbister	DL52.14 Sk78 BC5 IS113	Orkney, Scotland	Middle to Late Neolithic 3200–2800 BC	Highly oxidised organic material (trace)
4	Isbister	DL86 Sk78 BC4 IS115	Orkney, Scotland	Middle to Late Neolithic 3200–2800 BC	Seaweed, meat/fish/dairy
5	Isbister	DL143 1-2 Sk78 SC3	Orkney, Scotland	Middle to Late Neolithic 3200–2800 BC	Non-specific protein and lipid biomarkers
6	Isbister	DL168 Sk78 SC1 ST2 mandible IS1958	Orkney, Scotland	Middle to Late Neolithic 3200–2800 BC	Non-specific protein and lipid biomarkers
7	Isbister	DL170 Sk78 SC1 ST2	Orkney, Scotland	Middle to Late Neolithic 3200–2800 BC	Seaweed, meat/fish/dairy
8	Isbister	DL180 Sk78 BC2 (a+b) SC1 ST2 mandible IS1958	Orkney, Scotland	Middle to Late Neolithic 3200–2800 BC	Meat/fish/dairy
9	Isbister	DL188 1-3 Sk78 BC7 jaw fragment IS1976	Orkney, Scotland	Middle to Late Neolithic 3200–2800	Non-specific protein and lipid biomarkers

				BC	
10	Isbister	DL196 5 BC4 ST4 ISB incisor IS1976	Orkney, Scotland	Middle to Late Neolithic 3200–2800 BC	Non-specific protein, lipid and carbohydrate biomarkers
11	Isbister	DL715 24 Sk77 BC4 mandible IS1976	Orkney, Scotland	Middle to Late Neolithic 3200–2800 BC	Seaweed (red), meat/fish/dairy
12	Isbister	DL5083 x3 Sk77 BC2c IS128 mandible IS1976	Orkney, Scotland	Middle to Late Neolithic 3200–2800 BC	Seaweed, meat/fish/dairy
13	Isbister	DL5117 x3 Sk77 BC6(3) ST5 mandible 5 IS1976	Orkney, Scotland	Middle to Late Neolithic 3200–2800 BC	Highly oxidised organic material (trace)
14	Isbister	DL5119 x4 Sk77 BC6(3) ST5 mandible 2 IS1976	Orkney, Scotland	Middle to Late Neolithic 3200–2800 BC	Seaweed, meat/fish/dairy
15	Isbister	DL5838 Sk77 BC5 IS113 incisor IS1976	Orkney, Scotland	Middle to Late Neolithic 3200–2800 BC	Seaweed (red), meat/fish/dairy, higher plant wax (e.g. from leafy greens) exposure to fire/cooking
16	Quanterness	Bag 10/Box 4 Bag 209 (bag 4) 1139- 01: M1 right lingual	Orkney, Scotland	Middle to Late Neolithic c. 3200–2800 BC	Highly oxidised organic material (trace)
17	Quanterness	Box 10/Box L/Bag 216 (bag 2) 1190- A?1: M2 left labial	Orkney, Scotland	Middle to Late Neolithic c. 3200–2800 BC	Highly oxidised organic material (trace)
18	Quanterness	Box 10/Box K/bag 203 (Bag 3) 249- 01: M2 right lingual	Orkney, Scotland	Middle to Late Neolithic c. 3200–2800 BC	Seaweed (red)
19	Quanterness	Box 20 (Box A Bag 666 Bag 1 or 4) 2730-2734 (mandible split):	Orkney, Scotland	Neolithic Middle to Late Neolithic c.	Highly oxidised organic material

		M1 left lingual		3200–2800 BC	
20	Quanterness	Box 20/Bag 675 (Box A: Bag 2) 2552-02: M2 right lingual	Orkney, Scotland	Neolithic Middle to Late Neolithic c. 3200–2800 BC	Seaweed (red)
21	Quanterness	Box 19/Box F/Bag 663 from skull 1408: Loose maxillary molar Lingual	Orkney, Scotland	Neolithic Middle to Late Neolithic c. 3200–2800 BC	Highly oxidised organic material
22	Distillery Cave	Box 6, adult male '1': Canine right lingual	Oban, West Scotland	Early Neolithic c. 3700 BC	Seaweed (red)
23	Distillery Cave	Box 6, prob male '3': PM2 right labial	Oban, West Scotland	Early Neolithic c. 3700 BC	Seaweed
24	Raschoille Cave	ORC III 24.1: M1 right lingual	Oban, West Scotland	Neolithic 4000-2900 cal BC	Highly oxidised organic material
25	Boatbridge Quarry	Cist 2, Thankerton, S Lanarkshire, PM2 left labial S1	Central Scotland	Chalcolithic /Early Bronze Age 2460–2140 cal BC	Submerged freshwater aquatic plant (<i>Nymphaea spp.</i>) exposure to fire/cooking
26	Boatbridge Quarry	Cist 2, Thankerton, S Lanarkshire, M2 right labial S2	Central Scotland	Chalcolithic /Early Bronze Age 2460–2140 cal BC	Submerged freshwater aquatic plant (<i>Nymphaea spp.</i>) exposure to fire/cooking
27	Juniper Green	(NMS X.ET 33): M3 right back of tooth	Central Scotland	Chalcolithic /Early Bronze Age 2335–2135 cal BC	Submerged freshwater aquatic plant; (<i>Nymphaea spp.</i>)
28	Ruchlaw Mains	PM1,2 right lingual	East Scotland	Chalcolithic /Early Bronze Age 2340–2140 cal BC	Bacterially-derived biomarkers exposure to fire/cooking
29	Skateraw	(Roy Richie 1958 cist),		2340–2195 cal BC	Highly oxidised organic material
30	Lesmurdie	cist B, Banff (NMS X.ET 14): R mandibular 2 nd	East Scotland	2295–2040 cal BC	Highly oxidised organic material

		molar, labial			
31	Kretuonas	SA1	Lithuania	Neolithic	Highly oxidised organic material (trace)
32	Donkalnis	SA4	Lithuania	Neolithic	Highly oxidised organic material
33	Spiginas	SA5	Lithuania	Mesolithic	Submerged freshwater aquatic plant (<i>Nymphaea spp.</i>)
34	Spiginas	SA6	Lithuania	Neolithic	Non-specific lipid (bacterial?) biomarkers
35	Gyvakarai	SA7	Lithuania	Neolithic/Br onze Age	Highly oxidised organic material
36	Obeliai	Sk1128 male 50-55 yrs T32 labial, 1A5	Lithuania	AD 5 th -6 th century	Submerged freshwater aquatic plant (<i>Nymphaea spp.</i>) exposure to fire/cooking
37	Obeliai	Sk1132 female 30-35 yrs T41 lingual, 1A6	Lithuania	AD 5 th -6 th century	Non-specific (bacterial?) lipid biomarkers
38	Obeliai	Sk1289 male 30-35 yrs T32 labial, 1A8	Lithuania	AD 5 th -6 th century	Freshwater micro/macroalgae
39	Obeliai	Sk1294 male 50-55 yrs T43 lingual, 1A9	Lithuania	AD 5 th -6 th century	Freshwater micro/macroalgae, meat/fish/dairy
40	Obeliai	Sk0984 female >55 yrs T33 lingual, 1A10	Lithuania	AD 5 th -6 th century	Submerged freshwater aquatic plant (<i>Nymphaea spp.</i>)
41	Pagrybis	Sk1410A female 40-45 yrs T43 labial, 1A17	Lithuania	AD 5 th -6 th century	No detectable organic compounds
42	Pagrybis	, Sk1409C male 30-35 yrs T31 labial, 1A18	Lithuania	AD 5 th -6 th century	Highly oxidised organic material
43	Pagrybis	Sk1402 female 45-50 yrs T42 labial, 1A19	Lithuania	AD 5 th -6 th century	Non-specific (bacterial?) lipid biomarkers
44	Pagrybis	Sk1398 male >55 yrs T27 bucal, 1A20	Lithuania	AD 5 th -6 th century	Non-specific (bacterial?) lipid biomarkers
45	Pagrybis	Sk1058 male 30-35 yrs T41 labial, 1A21	Lithuania	AD 5 th -6 th century	Non-specific (bacterial?) lipid biomarkers
46	Vėluikiai	Sk1421 female 25-30 yrs T46 lingual, 1A28	Lithuania	AD 5 th -6 th century	Highly oxidised organic material
47	Pašatrijis	Sk7224 male 40-45 yrs T27 bucal, 1A29	Lithuania	AD 1 st -3 th century)	Non-specific (bacterial?) lipid biomarkers
48	Pašatrijis	Sk7227 male 20-25	Lithuania	AD 1 st -3 th	Highly oxidised organic

		yrs T32 labial, 1A30		century	material
49	Jakštaičiai-Meškia	Sk1388B male 20-25 yrs T36 lingual, 1A31	Lithuania	AD 7 th -12 th century	Non-specific (bacterial?) lipid biomarkers
50	Jakštaičiai-Meškia	Sk1386 female 20-25 yrs T46 lingual, 1A32	Lithuania	AD 7 th -12 th century	Freshwater micro/macroalgae
51	Jakštaičiai-Meškia	Sk1382 male 35-40 yrs T36 lingual, 1A33	Lithuania	AD 7 th -12 th century	Freshwater micro/macroalgae
52	Kanaljorden	122	Sweden	Mesolithic 5880–5620 cal BC	Meat/fish
53	Kanaljorden	126	Sweden	Mesolithic 5710–5570 cal BC	Meat/fish exposure to fire/cooking
54	La Corona	25	Spain	Mesolithic	Seaweed, meat/fish
55	La Corona	2 M1 mfieq (lingual)	Spain	Mesolithic	Exposure to fire/cooking
56	La Corona	La Corona 4 M3 mfdch (lingual)	Spain	Mesolithic	Exposure to fire/cooking
57	Bòbila Madurell,	MF18 male	Catalonia, Sain	Neolithic	Highly oxidised organic material (trace)
58	Moita do Sebastião, Muge	MSD male	Portugal	Mesolithic	Highly oxidised organic material (trace)
59	Moita do Sebastião, Muge	MSY female	Portugal	Mesolithic	Highly oxidised organic material (trace)
60	Moita do Sebastião, Muge	MS7.3 male	Portugal	Mesolithic	Highly oxidised organic material (trace)
61	Moita do Sebastião, Muge	MS29 child F.1-6 (female)	Portugal	Mesolithic	No detectable organic compounds
62	Moita do Sebastião, Muge	MS32 male	Portugal	Mesolithic	Highly oxidised organic material (trace)
63	Moita do Sebastião, Muge	MS56 male	Portugal	Mesolithic	Highly oxidised organic material (trace)
64	Moita do Sebastião, Muge	MS60 female	Portugal	Mesolithic	No detectable organic compounds
65	Cabeço da Arruda, Muge, Portugal:	ARR XIII, female	Portugal	Mesolithic	Beeswax

	ARR XIII				
66	Cabeço do Pez, Sado Valley	B251-b female	Portugal	Mesolithic	Submerged freshwater aquatic plant (<i>Potamogeton</i> spp. pondweed)
67	Cabeço do Pez, Sado Valley		Portugal	Mesolithic	Submerged freshwater aquatic plant (<i>Potamogeton</i> spp. pondweed)
68	Cabeço do Pez, Sado Valley	6250-D Esquina A- B Jordi male	Portugal	Mesolithic	Submerged freshwater aquatic plant (<i>Potamogeton</i> spp. pondweed)
69	Schipluiden	Grave 1 Individual 1 TMS0081: maxilla labial molar	Netherlands	Neolithic	Non-specific (bacterial?) lipid biomarkers exposure to fire/cooking
70	Schipluiden	Grave 1 Individual 2 TMS0077: mandible labial right PM1	Netherlands	Neolithic	Non-specific protein and lipid biomarkers
71	Schipluiden	Grave 1 Individual 2 TMS0077: maxilla/mandible? lingual left incisor 1-2	Netherlands	Neolithic	Non-specific protein and lipid biomarkers
72	Schipluiden	Grave 1 Individual 2 TMS0077: maxilla/mandible? lingual right M2-3	Netherlands	Neolithic	Non-specific protein and lipid biomarkers exposure to fire/cooking
73	Schipluiden	Grave 3 TMS0058: lower jaw- mandible labial molar	Netherlands	Neolithic	Non-specific protein and lipid biomarkers exposure to fire/cooking
74	Schipluiden	Grave 3 TMS0058: upper jaw-maxilla labial molar	Netherlands	Neolithic	Non-specific protein and lipid biomarkers exposure to fire/cooking
75	Schipluiden	Grave 5 child TMS0051: maxilla labial PM1	Netherlands	Neolithic	Highly oxidised organic material
76	Hardinxveld - Giessendam De Bruin	Grave 2 ('Elvis'), S22-1004, find nr. 11.014 p.22 VI.35: (male)	Netherlands	Late Mesolithic c. 5200- 4970 cal BC	Non-specific protein and lipid biomarkers exposure to fire/cooking
77	Hardinxveld - Giessendam De Bruin	Grave 1 ('Henk'), S21-1021, find nr. 15.165 mandible lingual right PM1(male)	Netherlands	Late Mesolithic (5500-4900 cal. BC)	No detectable organic compounds
78	Hardinxveld Giessendam Polderweg	Grave 1 (('Trijntje'), S25- 1004) mandible incisor lingual	Netherlands	Late Mesolithic (c. 5500- 4900 cal.	Highly oxidised organic material (trace)

		(female)		BC)	
79	Kruszyn	Site 13, Grave G93, male 35-40/45 years old, Linear Band Pottery culture (LBK)	Poland	Neolithic	Highly oxidised organic material
80	Rønstenen	AS 15/06 tandsten	Denmark	Mesolithic	Highly oxidised organic material

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Detailed Results

Scotland

Isbister chambered cairn, Orkney Islands

Isbister is a chambered cairn on the island of South Ronaldsay, Orkney, North Scotland containing the disarticulated remains of around 85 individuals^{1,2}. It was built during the late fourth millennium and remained in funerary use into the third millennium³. It is located in one of Europe's richest Neolithic landscapes combining numerous chambered cairns, two stone circles, the stone-built Late Neolithic village of Skara Brae and the huge ceremonial settlement of Ness of Brodgar, whose *floruit* was c. 3200–2800 BC⁴. Wild resources including bones from inshore and deep-water fish, birds and wild plants were found at some of these sites, in addition to bones of domesticated animals.

Fifteen samples of dental calculus taken from fifteen individuals from Isbister were analysed by sequential thermal desorption-gas chromatography-mass spectrometry (TD-GC-MS) and pyrolysis-gas chromatography-mass spectrometry (Py-GC-MS). This technique facilitates the identification of both free/unbound and bound/polymeric organic components. All fifteen calculus samples produced very little free organic material, with only three samples containing free compounds if the essentially ubiquitous and thermally generated carbon dioxide is excluded. In contrast, thirteen of the fifteen samples revealed a minor to significant amount of a bound/polymeric organic constituent.

1. Isbister DL42 M40-50 Sk8 SC1 ST2 (calculus: 2.57 mg)

The thermal desorption total ion chromatogram (TIC) revealed carbon dioxide and sulphur dioxide as the only detectable components, indicating the virtual absence of free, thermally extractable organic components in this sample. It should be noted, however, that highly polar material could have been present, which would not have successfully eluted from the column, or indeed volatilised sufficiently in the probe. It is not possible to determine the significance of the carbon dioxide, beyond it being indicative of oxidised organic material. The origin of the sulphur dioxide is also difficult to determine and its absence in other samples prevents any meaningful interpretation here.

In contrast to the thermal desorption profile, the pyrolysis-GC-MS TIC (Fig. 1) is dominated by a complex suite of organic compounds. These include a series of C₈ to C₁₈ *n*-1-alkenes and

187 *n*-alkanes, typical of unsaturated and saturated fat/oil-derived acyl lipids^{5,6}. A bimodal
188 distribution with maxima at C₁₂ and C₁₄ suggests the original acyl lipids may have been high
189 in polyunsaturated fatty acids. The absence of these compounds in the TD profile provides
190 molecular evidence for a polymeric/bound acyl lipid source. However, in addition to *n*-1-
191 alkenes and *n*-alkanes, typical of fat/oil derived lipids, the pyrogram also displayed a series of
192 C₁₀ to C₁₆ iso-methyl-1-alkenes (isoalkenes) and 2-methylalkanes (isoalkanes), presumably
193 deriving from the branched fatty acids which are significant components of bacterial
194 triglycerides.

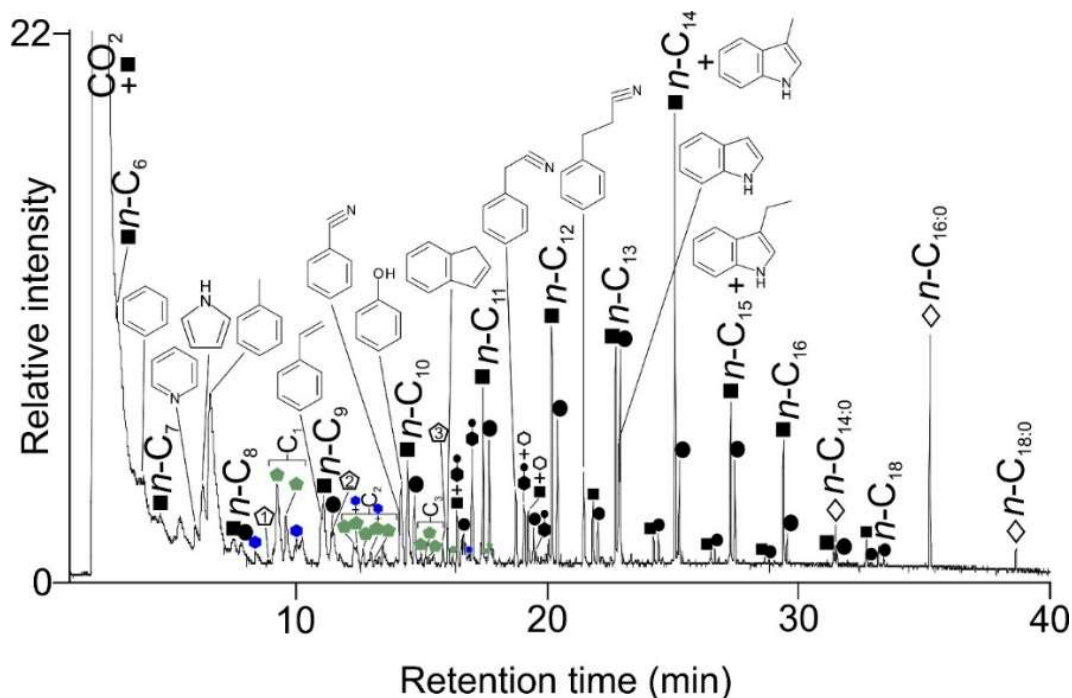
195 There were components identified in the pyrolysate TIC which can be indicative of 'black
196 carbon', i.e. charcoal or soot⁷⁻¹⁰. However, although this included the presence of
197 naphthalenes and biphenyls, the dominance of toluene, rather than the normally dominant
198 aromatic hydrocarbon benzene, is notably atypical of chars⁷⁻¹⁰. The markers identified here
199 can also be indicative of proteins^{6,11} and combined with the abundant related protein markers
200 pyrrole, benzonitrile, benzyl nitrile (phenylacetone nitrile), benzenepropanenitrile and indoles^{6,11}
201 identified this is likely to be the main origin of these compounds in this sample pointing to a
202 substantial protein-derived component^{6,11}. This is also corroborated by the presence of
203 aliphatic nitriles (carbon numbers C_{14:0}, C_{16:0} (max) and C_{18:0}) suggesting a lipid (fat/oil) in
204 this sample which has combined with protein-derived nitrogen to produce these moieties⁵.
205 Notably however, the naphthalenes and biphenyls, combined with the polynuclear aromatic
206 hydrocarbon (PAH) phenanthrene (a combustion marker), provides tentative molecular
207 evidence for exposure to fire/cooking. The proteinaceous origin for the aromatic markers is
208 further corroborated by the significant abundance of phenol, 2-methylphenol (*o*-cresol), 4-
209 methylphenol (*p*-cresol) (with the 4-methyl isomer being markedly predominant), and lesser
210 amounts of 2,4-dimethylphenol and 4-ethylphenol, all of which, collectively, are indicative of
211 the amino acid tyrosine¹²⁻¹⁴. With an absence of guaiacol itself (all isomers) and other
212 guaiacol moieties characteristic of lignin^{15,16}, the possibility that the phenols may originate
213 from wood or woody plant material can be excluded. Alkyl phenols have also been observed
214 in wood smoke and smoked foods^{17,18}, however the absence of methoxyphenols, diagnostic of
215 lignin-derived smoke, means this possibility can also be discounted. Taken together, the
216 presence of toluene, styrene and phenylacetone nitrile (benzyl nitrile) can indicate the amino
217 acid phenylalanine^{12,13}. In addition, the major constituents indole and 3-methylindole
218 (skatole), with a lesser but significant amount of a C₂ indole (3-ethylindole?), were also
219 identified, which are consistent with a tryptophan amino acid source^{12,13,19,20}. Proteins rich in

the amino acids tyrosine, phenylalanine and tryptophan include meat, fish and dairy products, although the presence of bacterial markers means the possibility that at least a partial origin could be microbial cannot be excluded.

However, highly notable in this context is the additional presence of appreciable amounts of the more unusual 2- and 3-methylpyrroles, 2- and 3-ethylpyrroles, 2,4-, 2,3- and 3,4-dimethyl-pyrroles, 2-ethyl-3(?)-methylpyrrole, 4-ethyl-2-methylpyrrole, 3-ethyl-4-methylpyrrole (trace), 2,3,5-trimethylpyrrole, a propylmethylpyrrole, an ethyldimethylpyrrole and 4-ethyl-2,3-dimethylpyrrole (trace). Collectively, these specific C₁, C₂, C₃ and C₄ alkyl pyrroles suggest a significant tetrapyrrole/porphyrin-derived origin and are indicative of an algal source observed in fossils and expected in similarly diagenetically altered archaeological material^{11,21,22}. Notable also in this context were the presence of the moderately abundant nitrogen containing aromatic compounds 1-H-pyrazole-4-carbonitrile, ethylcyanobenzene, 2-pyridinecarbonitrile, tolyl isocyanide (x2), a C₄ pyrimidine, along with pyridine and its methyl-, dimethyl- and trimethyl derivatives (these pyridines can result from the pyrolysis of polypeptides containing the amino acid alanine²³). The presence here of the abundant protein markers and specifically the C₁ to C₄ alkyl pyrroles, combined with the particular lipid profile and specific carbohydrate markers (see below) identified, suggests the presence of a macroalgae, i.e. seaweed, in this sample^{21,23,24} (see discussion).

The thermally-derived carbohydrate markers 2-methylfuran, 2-methyl-2-cyclopenten-1-one and 2,3-dimethyl-2-cyclopenten-1-one were identified as moderately significant components, along with an unidentified carbohydrate marker. That they were absent in the TD profile points to their originating from the original polymeric carbohydrate source (e.g. starch)²⁵. Although levoglucosan (1,6-anhydro- β -D-glucopyranose) is usually the major component in the thermally-derived products of carbohydrates analysed by TD/Py-GC-MS, its absence from the TD profile here is consistent with the removal of hydroxyl groups in the original carbohydrate polymer, so preventing its formation in the probe. The additional absence of the normally significant carbohydrate markers 2-hydroxy-3-methyl-2-cyclopenten-1-one and 3-hydroxy-2-methyl-2-cyclopenten-1-one observed in pure standard material (glycogen, starch and cellulose) would also support this interpretation. This shows a strong matrix effect likely to be due to the inorganic component of the calculus and the microflora in the mouth. This dehydration/dehydroxylation has also been observed in dog calculus and 19th century human calculus⁶, where other markers, e.g. a prevalence of nitriles, with amides very minor, deriving from dehydrating conditions in the mouth of dogs and humans (though not sheep⁶) supporting

this view⁶. The presence of carbon dioxide is also consistent with a lipid, amino acid/protein and carbohydrate component in this sample. Notably, these findings did not reveal key biomarkers typically observed in soils^{20,23}. It should also be noted that highly polar material could have been present, which would not have successfully eluted from the column, or indeed volatilised sufficiently in the probe.



Supplementary Figure 1 Reconstructed total ion chromatogram of the pyrogram (pyrolysis profile) (610 °C for 10s) of sample DL42, after thermal desorption (310 °C for 10 s). Key to Fig. 1: Peak identities (x indicates carbon chain length): filled squares, Cx indicates alkenes; filled circles, Cx indicates alkanes; open diamonds, Cx:y indicates acyclic nitriles; blue filled hexagons indicates alkyl pyridines; green filled pentagons indicates alkyl pyrroles (with alkyl chain length above, Cx); filled hexagons with filled circles attached indicates alkyl phenols; open hexagons indicates alkyl indenenes; open pentagons with numerals inside indicates carbohydrate pyrolysis markers, 1 is 2-methylfuran, 2 is 2-methyl-2-cyclopenten-1-one and 3 is 2,3-dimethyl-2-cyclopentene-1-one. Also shown are the structures of thirteen aromatic compounds identified: benzene, pyridine, pyrrole, toluene, styrene, benzonitrile, phenol, indene, benzyl nitrile, benzenepropanenitrile, indole, 3-methylindole (skatole) and 3-ethylindole. CO₂ indicates carbon dioxide.

2. Isbister DL2104 M17-23 Sk11 BC8/31 SC3 IS1976 (calculus: 3.19 mg)

The thermal desorption total ion chromatogram (TIC) (Fig. 2, inset) revealed two 2,5-diketopiperazine derivatives (DKPs). These were identified as the DKP derivative of proline-glycine (pro-gly), which was the major component, and the DKP of proline-alanine (pro-ala), which was present only as a relatively minor constituent. These are thermally generated in the pyroprobe from the amino acids proline and glycine, and proline and alanine respectively, and is indicative of proteinaceous material containing these three protein constituents^{5,12,13}. Carbon dioxide was the only other compound observed indicating very little free, thermally extractable, organic material in this sample. It should be noted, however, that highly polar material could have been present, which would not have successfully eluted from the column, or indeed volatilised sufficiently in the probe. It is not possible to determine the significance of the carbon dioxide, beyond it being indicative of oxidised organic material.

In contrast to the thermal desorption profile, the pyrolysis-GC-MS TIC (Fig. 2) is dominated by a complex suite of organic compounds. These include a series of C₈ to C₁₈ *n*-1-alkenes and *n*-alkanes, typical of unsaturated and saturated fat/oil-derived acyl lipids^{5,6}. A bimodal distribution with maxima at C₁₂ and C₁₄ suggests the original acyl lipids may have been high in polyunsaturated fatty acids. The absence of these compounds in the TD profile provides molecular evidence for a polymeric/bound acyl lipid source. However, in addition to *n*-1-alkenes and *n*-alkanes, typical of fat/oil derived lipids, the pyrogram also displayed a series of C₁₀ to C₁₈ iso-methyl-1-alkenes (isoalkenes) and 2-methylalkanes (isoalkanes), presumably deriving from the branched fatty acids which are significant components of bacterial triglycerides.

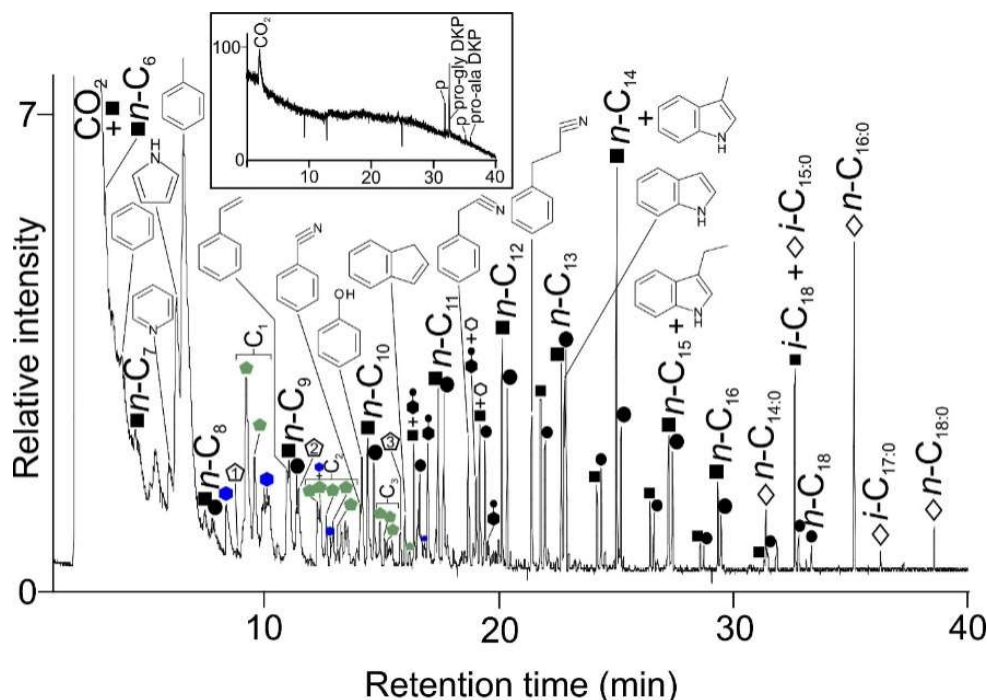
There were components identified in the pyrolysate TIC which can be indicative of 'black carbon', i.e. charcoal or soot⁷⁻¹⁰. However, although this included the presence of naphthalenes, the dominance of toluene, rather than the normally dominant aromatic hydrocarbon benzene, is notably atypical of chars⁷⁻¹⁰. The markers identified here can also be indicative of proteins^{6,11} and combined with the abundant related protein markers pyrrole, benzonitrile, benzyl nitrile (phenylacetone nitrile), benzenepropanenitrile and indoles^{6,11} identified this is likely to be the main origin of these compounds in this sample pointing to a substantial protein-derived component^{6,11}. This is also corroborated by the presence of aliphatic nitriles (carbon numbers C_{14:0}, *i*-C_{15:0}, C_{16:0} (max) *i*-C_{17:0} and C_{18:0}) suggesting a lipid (fat/oil) in this sample which has combined with protein-derived nitrogen to produce

304 these moieties⁵. However, the naphthalenes and biphenyl (trace), combined with the
305 polynuclear aromatic hydrocarbon (PAH) phenanthrene (trace; a combustion marker),
306 provides very tentative molecular evidence for exposure to fire/cooking. The proteinaceous
307 origin for the aromatic markers is further corroborated by the significant abundance of
308 phenol, 2-methylphenol (*o*-cresol), 4-methylphenol (*p*-cresol) (with the 4-methyl isomer
309 being markedly predominant), and lesser amounts of 2,4-dimethylphenol and 4-ethylphenol,
310 all of which, collectively, are indicative of the amino acid tyrosine¹²⁻¹⁴. With an absence of
311 guaiacol itself (all isomers) and other guaiacol moieties characteristic of lignin^{15,16}, the
312 possibility that the phenols may originate from wood or woody plant material can be
313 excluded. Alkyl phenols have also been observed in wood smoke and smoked foods^{17,18},
314 however the absence of methoxyphenols, diagnostic of lignin-derived smoke, means this
315 possibility can also be discounted. Taken together, the presence of toluene, styrene and
316 phenylacetonitrile (benzyl nitrile) can indicate the amino acid phenylalanine^{12,13}. In addition,
317 the major constituents indole and 3-methylindole (skatole), with a lesser but significant
318 amount of a C₂ indole (3-ethylindole?), were also identified, which are consistent with a
319 tryptophan amino acid source^{12,13,19,20}. Proteins rich in the amino acids tyrosine,
320 phenylalanine and tryptophan include meat, fish and dairy products, although the presence of
321 bacterial markers means the possibility that at least a partial origin could be microbial cannot
322 be excluded.

323 However, highly notable in this context is the additional presence of appreciable amounts of
324 the more unusual 2- and 3-methylpyrroles, 2- and 3-ethylpyrroles, 2,4-, 2,3- and 3,4-
325 dimethyl-pyrroles, 2-ethyl-3(?)-methylpyrrole, 4-ethyl-2-methylpyrrole, 3-ethyl-4-
326 methylpyrrole (trace), 2,3,5-trimethylpyrrole, a propylmethylpyrrole, an ethyldimethylpyrrole
327 (trace) and 4-ethyl-2,3-dimethylpyrrole (minor trace). Collectively, these specific C₁, C₂, C₃
328 and C₄ alkyl pyrroles suggest a significant tetrapyrrole/porphyrin-derived origin and are
329 indicative of an algal source observed in fossils and expected in similarly diagenetically
330 altered archaeological material^{11,21,22}. Notable also in this context were the presence of the
331 moderately abundant nitrogen containing aromatic compounds 1-H-pyrazole-4-carbonitrile,
332 ethylcyanobenzene, 2-pyridinecarbonitrile, tolyl isocyanide (x2), a C₃ and C₄ pyrimidine,
333 along with pyridine and its methyl-, dimethyl- and trimethyl derivatives (these pyridines can
334 result from the pyrolysis of polypeptides containing the amino acid alanine²³). The presence
335 here of the abundant protein markers and specifically the C₁ to C₄ alkyl pyrroles, combined

with the particular lipid profile and specific carbohydrate markers (see below) identified, suggests the presence of a macroalgae, i.e. seaweed, in this sample^{21,22,24} (see discussion).

The thermally-derived carbohydrate markers 2-methylfuran, 2-methyl-2-cyclopenten-1-one and 2,3-dimethyl-2-cyclopenten-1-one were identified as moderately significant components, along with an unidentified carbohydrate marker. That they were absent in the TD profile points to their originating from the original polymeric carbohydrate source (e.g. starch)²⁵. Although levoglucosan (1,6-anhydro- β -D-glucopyranose) is usually the major component in the thermally-derived products of carbohydrates analysed by TD/Py-GC-MS, its absence from the TD profile here is consistent with the removal of hydroxyl groups in the original carbohydrate polymer, so preventing its formation in the probe. The additional absence of the normally significant carbohydrate markers 2-hydroxy-3-methyl-2-cyclopenten-1-one and 3-hydroxy-2-methyl-2-cyclopenten-1-one observed in pure standard material (glycogen, starch and cellulose) would also support this interpretation. This shows a strong matrix effect likely to be due to the inorganic component of the calculus and the microflora in the mouth. This dehydration/dehydroxylation has also been observed in dog calculus and 19th century human calculus⁶, where other markers, e.g. a prevalence of nitriles, with amides very minor, deriving from dehydrating conditions in the mouth of dogs and humans (though not sheep⁶) supporting this view⁶. The presence of carbon dioxide is also consistent with a lipid, amino acid/protein and carbohydrate component in this sample. Notably, these findings did not reveal key biomarkers typically observed in soils^{20,23}. It should also be noted that highly polar material could have been present, which would not have successfully eluted from the column, or indeed volatilised sufficiently in the probe.



Supplementary Figure 2 Reconstructed total ion chromatogram of the pyrogram (pyrolysis profile) (610 °C for 10s) of sample DL2104, after thermal desorption (310 °C for 10 s). Key to Fig. 2: Peak identities (x indicates carbon chain length): filled squares, Cx indicates alkenes; filled circles, Cx indicates alkanes; open diamonds, Cx:y indicates acyclic nitriles; blue filled hexagons indicates alkyl pyridines; green filled pentagons indicates alkyl pyrroles (with alkyl chain length above, Cx); filled hexagons with filled circles attached indicates alkyl phenols; open hexagons indicates alkyl indenenes; open pentagons with numerals inside indicates carbohydrate pyrolysis markers, 1 is 2-methylfuran, 2 is 2-methyl-2-cyclopenten-1-one and 3 is 2,3-dimethyl-2-cyclopentene-1-one. Also shown are the structures of thirteen aromatic compounds identified: benzene, pyridine, pyrrole, toluene, styrene, benzonitrile, phenol, indene, benzyl nitrile, benzenepropanenitrile, indole, 3-methylindole (skatole) and 3-ethylindole. CO₂ indicates carbon dioxide. Inset displays a reconstructed total ion chromatogram of the thermal desorption profile (310 °C for 10 s) of this sample. Peak identities: pro-gly DKP indicates the 2,5-diketopiperazine derivative of the amino acids proline and glycine and pro-ala indicates the 2,5-diketopiperazine derivative of the amino acids proline and alanine. CO₂ indicates carbon dioxide and p indicates contaminants from the pyroprobe.

3. Isbister DL52.14 Sk78 BC5 IS113 mandible IS1976 (calculus: 1.40 mg)

The thermal desorption total ion chromatogram (TIC) revealed carbon dioxide as the only detectable (trace) component, indicating the essential absence of free, thermally extractable organic components in this sample. It should be noted, however, that highly polar material could have been present, which would not have successfully eluted from the column, or indeed volatilised sufficiently in the probe. It is not possible to determine the significance of the carbon dioxide, beyond it being indicative of oxidised organic material.

The pyrolysate TIC revealed carbon dioxide and toluene as the only detectable (trace) components, indicating the essential absence of organic material in this sample. It should be noted, however, that highly polar material could have been present, which would not have successfully eluted from the column, or indeed volatilised sufficiently in the probe. It is not possible to determine the significance of the carbon dioxide, beyond it being indicative of oxidised organic material. The toluene is in too low an abundance to provide any meaningful interpretation of its presence, beyond it being indicative of an organic origin.

4. Isbister DL86 Sk78 BC4 IS115 mandible IS1976 (calculus: 4.89 mg)

The thermal desorption total ion chromatogram (TIC) revealed carbon dioxide and acetonitrile as the only detectable components, indicating the virtual absence of free, thermally extractable organic components in this sample. It should be noted, however, that highly polar material could have been present, which would not have successfully eluted from the column, or indeed volatilised sufficiently in the probe. It is not possible to determine the significance of the carbon dioxide, beyond it being indicative of oxidised organic material. The origin of the acetonitrile is also difficult to determine, beyond it being indicative of a relatively labile nitrogen-containing polymeric/bound biomolecule (hence the generation of acetonitrile at only ~300°C), although it has been correlated with the pyrolysis of proteins high in phenylalanine and tyrosine, in particular²⁶.

In contrast to the thermal desorption profile, the pyrolysis-GC-MS TIC (Fig. 3) is dominated by a relatively complex suite of organic compounds. These include a series of C₈ to C₁₆ *n*-1-alkenes and *n*-alkanes, typical of unsaturated and saturated fat/oil-derived acyl lipids^{5,6}. A bimodal distribution with maxima at C₁₁/C₁₂ and C₁₄ suggests the original acyl lipids may have been high in polyunsaturated fatty acids. The absence of these compounds in the TD profile provides molecular evidence for a polymeric/bound acyl lipid source. However, in

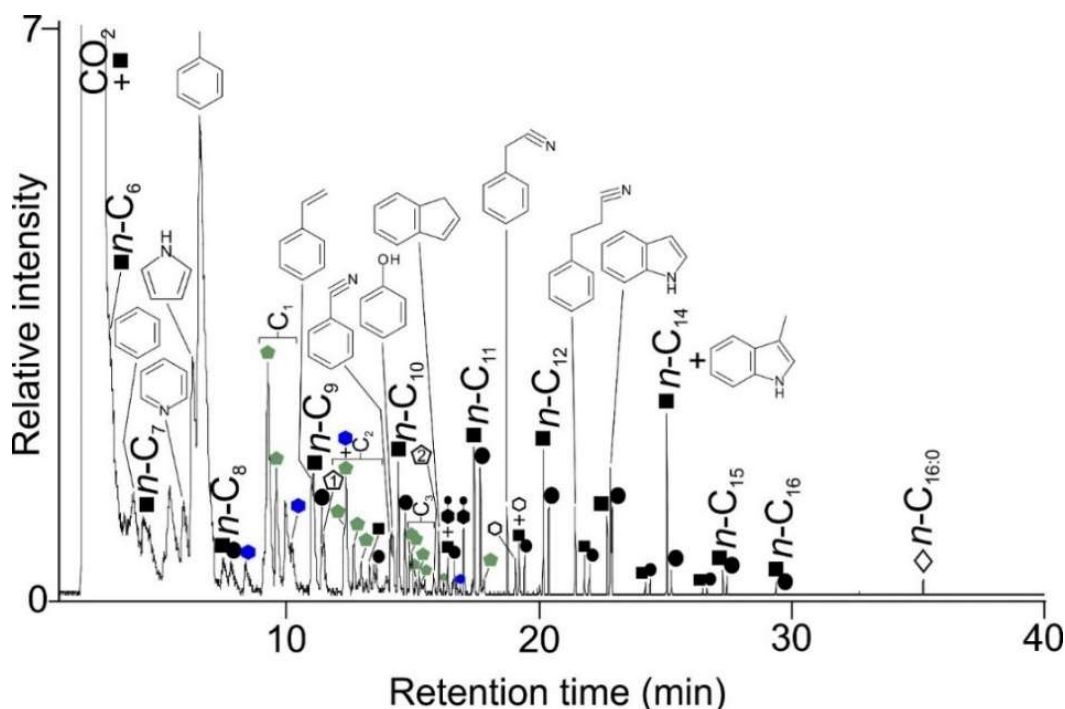
410 addition to *n*-1-alkenes and *n*-alkanes, typical of fat/oil derived lipids, the pyrogram also
411 displayed a series of C₁₀ to C₁₅ iso-methyl-1-alkenes (isoalkenes) and 2-methylalkanes
412 (isoalkanes), presumably deriving from the branched fatty acids which are significant
413 components of bacterial triglycerides.

414 There were components identified in the pyrolysate TIC which can be indicative of ‘black
415 carbon’, i.e. charcoal or soot⁷⁻¹⁰. However, lack of alkyl naphthalenes and biphenyls and the
416 dominance of toluene, rather than the normally dominant aromatic hydrocarbon benzene, is
417 notably atypical of chars⁷⁻¹⁰. The markers identified here can also be indicative of proteins^{6,11}
418 and combined with the abundant related protein markers pyrrole, benzonitrile, benzyl nitrile
419 (phenylacetone nitrile), benzenepropanenitrile and indoles^{6,11} identified this is likely to be the
420 main origin of these compounds in this sample pointing to a substantial protein-derived
421 component^{6,11}. This is also corroborated by the presence of the C_{16:0} aliphatic nitrile,
422 suggesting a lipid (fat/oil) in this sample which has combined with protein-derived nitrogen
423 to produce these moieties⁵. The proteinaceous origin for the aromatic markers is further
424 corroborated by the significant abundance of phenol, 2-methylphenol (*o*-cresol) and 4-
425 methylphenol (*p*-cresol) (with the 4-methyl isomer being markedly predominant), which
426 collectively are indicative of the amino acid tyrosine¹²⁻¹⁴. With an absence of guaiacol itself
427 (all isomers) and other guaiacol moieties characteristic of lignin^{15,16}, the possibility that the
428 phenols may originate from wood or woody plant material can be excluded. Alkyl phenols
429 have also been observed in wood smoke and smoked foods^{17,18}, however the absence of
430 methoxyphenols, diagnostic of lignin-derived smoke, means this possibility can also be
431 discounted. Taken together, the presence of toluene, styrene and phenylacetone nitrile (benzyl
432 nitrile) can indicate the amino acid phenylalanine^{12,13}. In addition, the major constituents
433 indole and 3-methylindole (skatole) are consistent with a tryptophan amino acid
434 source^{12,13,19,20}. Proteins rich in the amino acids tyrosine, phenylalanine and tryptophan
435 include meat, fish and dairy products, although the presence of bacterial markers means the
436 possibility that at least a partial origin could be microbial cannot be excluded.

437 However, highly notable in this context is the additional presence of appreciable amounts of
438 the more unusual 2- and 3-methylpyrroles, 2- and 3-ethylpyrroles, 2,3-dimethyl-pyrrole, 2-
439 ethyl-3(?)-methylpyrrole, 4-ethyl-2-methylpyrrole, 3-ethyl-4-methylpyrrole, 2,3,5-
440 trimethylpyrrole and a propylmethylpyrrole. Collectively, these specific C₁, C₂, C₃ and C₄
441 alkyl pyrroles suggest a tetrapyrrole/porphyrin-derived origin and are indicative of an algal
442 source observed in fossils and expected in similarly diagenetically altered archaeological

material^{11,21,22}. Notable also in this context were the presence of the relatively moderately abundant nitrogen containing aromatic compounds 1-H-pyrazole-4-carbonitrile, ethylcyanobenzene, 2-pyridinecarbonitrile and tolyl isocyanide (x2), along with pyridine and its methyl-, dimethyl- and trimethyl derivatives (these pyridines can result from the pyrolysis of polypeptides containing the amino acid alanine²³). The presence here of the abundant protein markers and specifically the C₁ to C₄ alkyl pyrroles, combined with the particular lipid profile and specific carbohydrate markers (see below) identified, suggests the presence of a macroalgae, i.e. seaweed, in this sample^{21,22,24} (see discussion).

The thermally-derived carbohydrate markers 2-methyl-2-cyclopenten-1-one and 2,3-dimethyl-2-cyclopenten-1-one were identified as significant components, along with an unidentified carbohydrate marker. That they were absent in the TD profile points to their originating from the original polymeric carbohydrate source (e.g. starch)²⁵. Although levoglucosan (1,6-anhydro- β -D-glucopyranose) is usually the major component in the thermally-derived products of carbohydrates analysed by TD/Py-GC-MS, its absence from the TD profile here is consistent with the removal of hydroxyl groups in the original carbohydrate polymer, so preventing its formation in the probe. The additional absence of the normally significant carbohydrate markers 2-hydroxy-3-methyl-2-cyclopenten-1-one and 3-hydroxy-2-methyl-2-cyclopenten-1-one observed in pure standard material (glycogen, starch and cellulose) would also support this interpretation. This shows a strong matrix effect likely to be due to the inorganic component of the calculus and the microflora in the mouth. This dehydration/dehydroxylation has also been observed in dog calculus and 19th century human calculus⁶, where other markers, e.g. a prevalence of nitriles, with amides very minor, deriving from dehydrating conditions in the mouth of dogs and humans (though not sheep⁶) supporting this view⁶. The presence of carbon dioxide is also consistent with a lipid, amino acid/protein and carbohydrate component in this sample. Notably, these findings did not reveal key biomarkers typically observed in soils^{20,23}. It should also be noted that highly polar material could have been present, which would not have successfully eluted from the column, or indeed volatilised sufficiently in the probe.



Supplementary Figure 3 Reconstructed total ion chromatogram of the pyrogram (pyrolysis profile) (610 °C for 10s) of sample DL86, after thermal desorption (310 °C for 10 s). Key to Fig. 3: Peak identities (x indicates carbon chain length): filled squares, Cx indicates alkenes; filled circles, Cx indicates alkanes; open diamonds, Cx:y indicates acyclic nitriles; blue filled hexagons indicates alkyl pyridines; green filled pentagons indicates alkyl pyrroles (with alkyl chain length above, Cx); filled hexagons with filled circles attached indicates alkyl phenols; open hexagons indicates alkyl indenenes; open pentagons with numerals inside indicates carbohydrate pyrolysis markers, 1 is 2-methylfuran, 2 is 2-methyl-2-cyclopenten-1-one and 3 is 2,3-dimethyl-2-cyclopentene-1-one. Also shown are the structures of thirteen aromatic compounds identified: benzene, pyridine, pyrrole, toluene, styrene, benzonitrile, phenol, indene, benzyl nitrile, benzenepropanenitrile, indole, 3-methylindole (skatole) and 3-ethylindole. CO₂ indicates carbon dioxide.

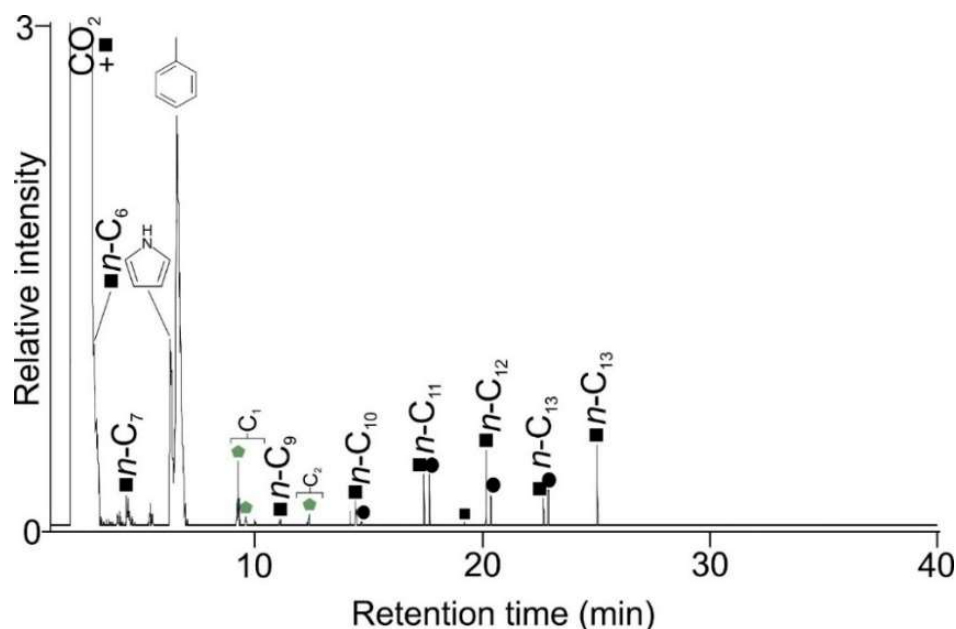
5. Isbister DL143 1-2 Sk78 SC3 mandible IS1976 (calculus: 4.92 mg)

The thermal desorption total ion chromatogram (TIC) revealed carbon dioxide as the only detectable (trace) component, indicating the essential absence of free, thermally extractable organic components in this sample. It should be noted, however, that highly polar material could have been present, which would not have successfully eluted from the column, or

indeed volatilised sufficiently in the probe. It is not possible to determine the significance of the carbon dioxide, beyond it being indicative of oxidised organic material.

In contrast to the thermal desorption profile, the pyrolysis-GC-MS TIC (Fig. 4) revealed a number of organic compounds, albeit in relatively low abundance. These include a series of C₉ to C₁₄ *n*-1-alkenes and *n*-alkanes, typical of unsaturated and saturated fat/oil-derived acyl lipids^{5,6}. A bimodal distribution with maxima at C₁₂ and C₁₄ suggests the original acyl lipids may have been high in polyunsaturated fatty acids. The absence of these compounds in the TD profile provides molecular evidence for a polymeric/bound acyl lipid source. Although the series of ~C₁₀ to ~C₁₅ iso-methyl-1-alkenes (isoalkenes) and 2-methylalkanes (isoalkanes), indicative of a bacterial lipid input and identified in previous samples from Isbister (see above), were not observed, the detection of the *i*-C₁₂ 1-alkene as a very minor component suggests their overall absence in this sample is due to the relatively small amount of organic material present.

There were a number of chemical markers identified in the pyrolysate TIC which can be indicative of proteins, i.e. toluene, pyrrole, 2-methylpyrrole, 3-methylpyrrole and 2-ethylpyrrole^{6,11}. The complex suite of nitrogen-containing protein markers observed in previous samples (see above) were not detected, which given the notably similar pyrolysis profile of this sample when compared to those with appreciable amounts of organic material above, suggests this is again due to the relatively small amount of organic material present. This may also explain the absence of nitriles, phenols, pyridines, C₃ and C₄ alkyl pyrroles and carbohydrate markers in the pyrolysate of this sample. The presence of carbon dioxide is also consistent with a lipid and amino acid/protein component in this sample. It should also be noted that highly polar material could have been present, which would not have successfully eluted from the column, or indeed volatilised sufficiently in the probe. The low abundance of organic material in this sample does not allow any further inferences to be made (but see discussion).



Supplementary Figure 4. Reconstructed total ion chromatogram of the pyrogram (pyrolysis profile) (610 °C for 10s) of sample DL143, after thermal desorption (310 °C for 10 s). Key to Fig. 4: Peak identities (x indicates carbon chain length): filled squares, Cx indicates alkenes; filled circles, Cx indicates alkanes; green filled pentagons indicate alkyl pyrroles (with alkyl chain length above, Cx). Also shown are the structures of two aromatic compounds identified: pyrrole and toluene. CO₂ indicates carbon dioxide.

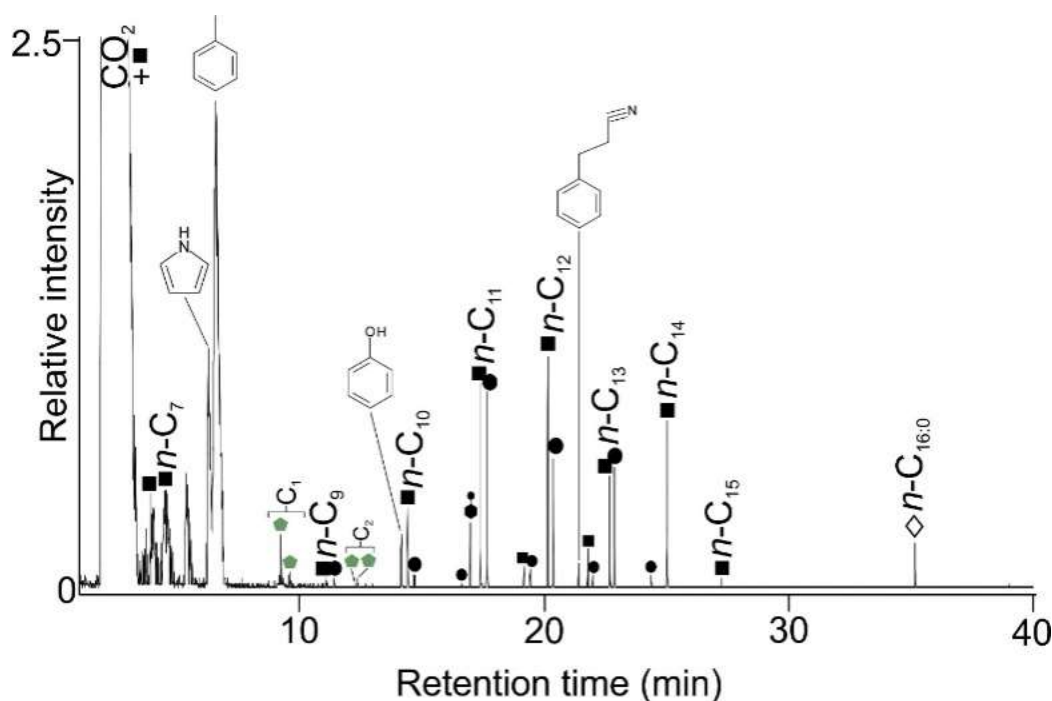
6. Isbister DL168 Sk78 SC1 ST2 mandible IS1958 (calculus: 1.88 mg)

The thermal desorption total ion chromatogram (TIC) revealed carbon dioxide and acetonitrile as the only detectable (trace) components, indicating the virtual absence of free, thermally extractable organic components in this sample. It should be noted, however, that highly polar material could have been present, which would not have successfully eluted from the column, or indeed volatilised sufficiently in the probe. It is not possible to determine the significance of the carbon dioxide, beyond it being indicative of oxidised organic material. The origin of the acetonitrile is also difficult to determine, beyond it being indicative of a relatively labile nitrogen-containing polymeric/bound biomolecule (hence the generation of acetonitrile at only ~300°C), although it has been correlated with the pyrolysis of proteins high in phenylalanine and tyrosine, in particular²⁶.

In contrast to the thermal desorption profile, the pyrolysis-GC-MS TIC (Fig. 5) revealed a number of organic compounds, albeit in relatively low abundance. These include a series of

C₉ to C₁₅ *n*-1-alkenes and *n*-alkanes, typical of unsaturated and saturated fat/oil-derived acyl lipids^{5,6}. A bimodal distribution with maxima at C₁₁/C₁₂ and C₁₄ suggests the original acyl lipids may have been high in polyunsaturated fatty acids. The absence of these compounds in the TD profile provides molecular evidence for a polymeric/bound acyl lipid source. However, in addition to *n*-1-alkenes and *n*-alkanes, typical of fat/oil derived lipids, the pyrogram also displayed a series of C₁₁ to C₁₄ iso-methyl-1-alkenes (isoalkenes) and 2-methylalkanes (isoalkanes), albeit in minor abundance due to the relatively small amount of organic material present; these are again presumed to derive from the branched fatty acids which are significant components of bacterial triglycerides.

There were a number of chemical markers identified in the pyrolysate TIC which can be indicative of proteins, i.e. toluene, pyrrole, 2-methylpyrrole, 3-methylpyrrole, 2-ethylpyrrole, a dimethylpyridine and benzenepropanenitrile^{6,11}. The complex suite of nitrogen-containing protein markers observed in previous samples (see above) were not detected, which given the notably similar pyrolysis profile of this sample when compared to those with appreciable amounts of organic material above, suggests this is yet again due to the relatively small amount of organic material present. Despite this, the C_{16:0} nitrile was observed as a significant component, as was both phenol and 4-methylphenol, although the C₃ and C₄ alkyl pyrroles and carbohydrate markers were not detected in the pyrolysate. The presence of carbon dioxide is also consistent with a lipid and amino acid/protein component in this sample. It should also be noted that highly polar material could have been present, which would not have successfully eluted from the column, or indeed volatilised sufficiently in the probe. The low abundance of organic material in this sample does not allow any further inferences to be made in isolation, but see discussion below.



Supplementary Figure 5. Reconstructed total ion chromatogram of the pyrogram (pyrolysis profile) (610 °C for 10s) of sample DL168, after thermal desorption (310 °C for 10 s). Key to Fig. 5: Peak identities (x indicates carbon chain length): filled squares, Cx indicates alkenes; filled circles, Cx indicates alkanes; open diamond, Cx:y indicates acyclic nitrile; green filled pentagons indicates alkyl pyrroles (with alkyl chain length above, Cx); filled hexagon with filled circle attached indicates alkyl phenol. Also shown are the structures of four aromatic compounds identified: pyrrole, toluene, phenol and benzenepropanenitrile. CO₂ indicates carbon dioxide.

7. Isbister DL170 Sk78 SC1 ST2 mandible IS1958 (calculus: 3.74 mg)

The thermal desorption total ion chromatogram (TIC) revealed carbon dioxide as the only detectable (trace) component, indicating the essential absence of free, thermally extractable organic components in this sample. It should be noted, however, that highly polar material could have been present, which would not have successfully eluted from the column, or indeed volatilised sufficiently in the probe. It is not possible to determine the significance of the carbon dioxide, beyond it being indicative of oxidised organic material.

In contrast to the thermal desorption profile, the pyrolysis-GC-MS TIC (Fig. 6) revealed a number of organic compounds, albeit in low to moderate abundance. These include a series

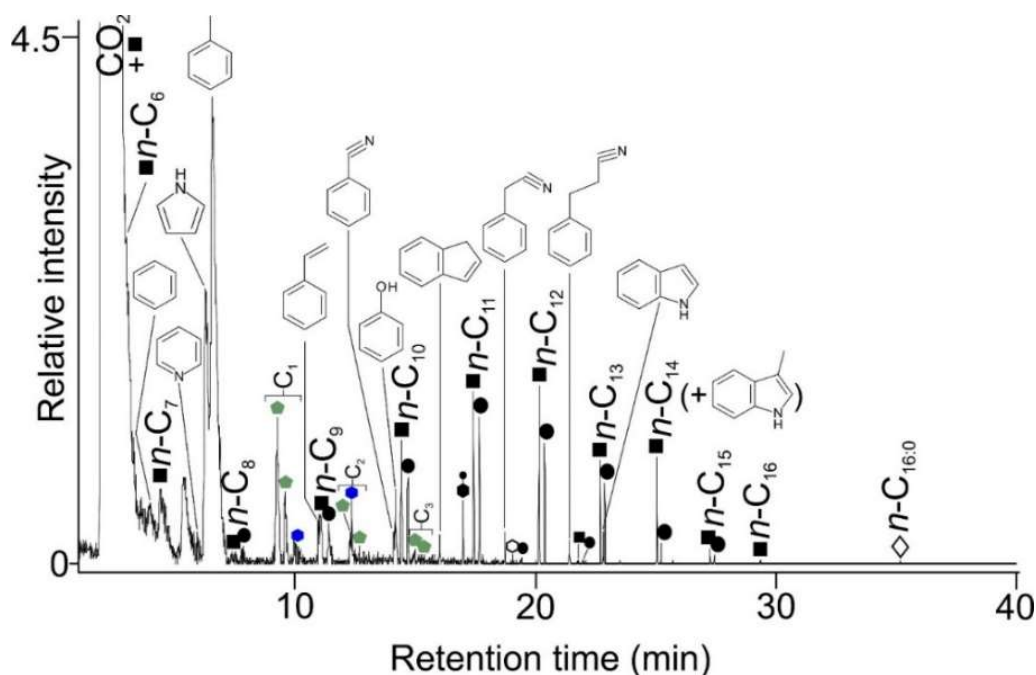
of C₈ to C₁₆ *n*-1-alkenes and *n*-alkanes, typical of unsaturated and saturated fat/oil-derived acyl lipids^{5,6}. A bimodal distribution with maxima at C₁₁/C₁₂ and C₁₄ suggests the original acyl lipids may have been high in polyunsaturated fatty acids. The absence of these compounds in the TD profile provides molecular evidence for a polymeric/bound acyl lipid source. Although the series of ~C₁₀ to ~C₁₅ iso-methyl-1-alkenes (isoalkenes) and 2-methylalkanes (isoalkanes), indicative of a bacterial lipid input and identified in previous samples from Isbister (see above), were not observed, the detection of the *i*-C₁₂ and *i*-C₁₃ 1-alkenes as a very minor components suggests their overall absence in this sample is due to the relatively small amount of organic material present.

There were components identified in the pyrolysate TIC which can be indicative of 'black carbon', i.e. charcoal or soot⁷⁻¹⁰. However, lack of alkyl naphthalenes and biphenyls and the dominance of toluene, rather than the normally dominant aromatic hydrocarbon benzene, is notably atypical of chars⁷⁻¹⁰. The markers identified here can also be indicative of proteins^{6,11} and combined with the relatively abundant related protein markers pyrrole, benzonitrile, benzyl nitrile (phenylacetone nitrile), benzenepropanenitrile and indoles^{6,11} identified this is likely to be the origin of these compounds in this sample, pointing to a substantial protein-derived component^{6,11}. This is also corroborated by the presence of the C_{16:0} aliphatic nitrile, suggesting a lipid (fat/oil) in this sample which has combined with protein-derived nitrogen to produce these moieties⁵. The proteinaceous origin for the aromatic markers is further corroborated by the relatively significant abundance of phenol and 4-methylphenol (*p*-cresol), which collectively are indicative of the amino acid tyrosine¹²⁻¹⁴. With an absence of guaiacol itself (all isomers) and other guaiacol moieties characteristic of lignin^{15,16}, the possibility that the phenols may originate from wood or woody plant material can be excluded. Alkyl phenols have also been observed in wood smoke and smoked foods^{17,18}, however the absence of methoxyphenols, diagnostic of lignin-derived smoke, means this possibility can also be discounted. Taken together, the presence of toluene, styrene and phenylacetone nitrile (benzyl nitrile) can indicate the amino acid phenylalanine^{12,13}. In addition, the significant constituents indole and 3-methylindole (skatole) are consistent with a tryptophan amino acid source^{12,13,19,20}. Proteins rich in the amino acids tyrosine, phenylalanine and tryptophan include meat, fish and dairy products, although the presence of bacterial markers means the possibility that at least a partial origin could be microbial cannot be excluded.

However, notable in this context is the additional presence of appreciable amounts of the more unusual 2- and 3-methylpyrroles, 2-ethylpyrrole and 2,3-dimethyl-pyrrole, with minor

but detectable amounts of 2-ethyl-3(?)-methylpyrrole, 4-ethyl-2-methylpyrrole and 2,3,5-trimethylpyrrole. Collectively, these specific C₁, C₂, and C₃ (C₄ not detected due to the low abundance of organic material present) alkyl pyrroles suggest a tetrapyrrole/porphyrin-derived origin and are indicative of an algal source observed in fossils and expected in similarly diagenetically altered archaeological material^{11,21,22}. Notable also in this context were pyridine and its methyl-, and dimethyl derivatives (these pyridines can result from the pyrolysis of polypeptides containing the amino acid alanine²³). The presence here of the protein markers and specifically the C₁ to C₄ alkyl pyrroles, combined with the particular lipid profile, tentatively suggests the presence of a macroalgae, i.e. seaweed, in this sample^{21,22,24} (see discussion).

The carbohydrate markers 2-methyl-2-cyclopenten-1-one and 2,3-dimethyl-2-cyclopenten-1-one were not detected in the pyrolysate, which given the notably similar pyrolysis profile of this sample when compared to those above, suggests this is due to the relatively small amount of organic material present. The presence of carbon dioxide is also consistent with a lipid and amino acid/protein component in this sample. It should also be noted that highly polar material could have been present, which would not have successfully eluted from the column, or indeed volatilised sufficiently in the probe. The low abundance of organic material in this sample does not allow any further inferences to be made (but see discussion).



Supplementary Figure 6. Reconstructed total ion chromatogram of the pyrogram (pyrolysis profile) (610 °C for 10s) of sample DL170, after thermal desorption (310 °C for 10 s). Key to Fig. 6: Peak identities (x indicates carbon chain length): filled squares, C_x indicates alkenes; filled circles, C_x indicates alkanes; open diamond, C_x:y indicates acyclic nitrile; blue filled hexagons indicates alkyl pyridines; green filled pentagons indicates alkyl pyrroles (with alkyl chain length above, C_x); filled hexagon with filled circle attached indicates alkyl phenol; open hexagon indicates alkyl indene. Also shown are the structures of twelve aromatic compounds identified: benzene, pyridine, pyrrole, toluene, styrene, benzonitrile, phenol, indene, benzyl nitrile, benzenepropanenitrile, indole and 3-methylindole (skatole) (minor). CO₂ indicates carbon dioxide.

8. Isbister DL180 Sk78 BC2 (a+b) SC1 ST2 mandible IS1958 (calculus: 2.18 mg)

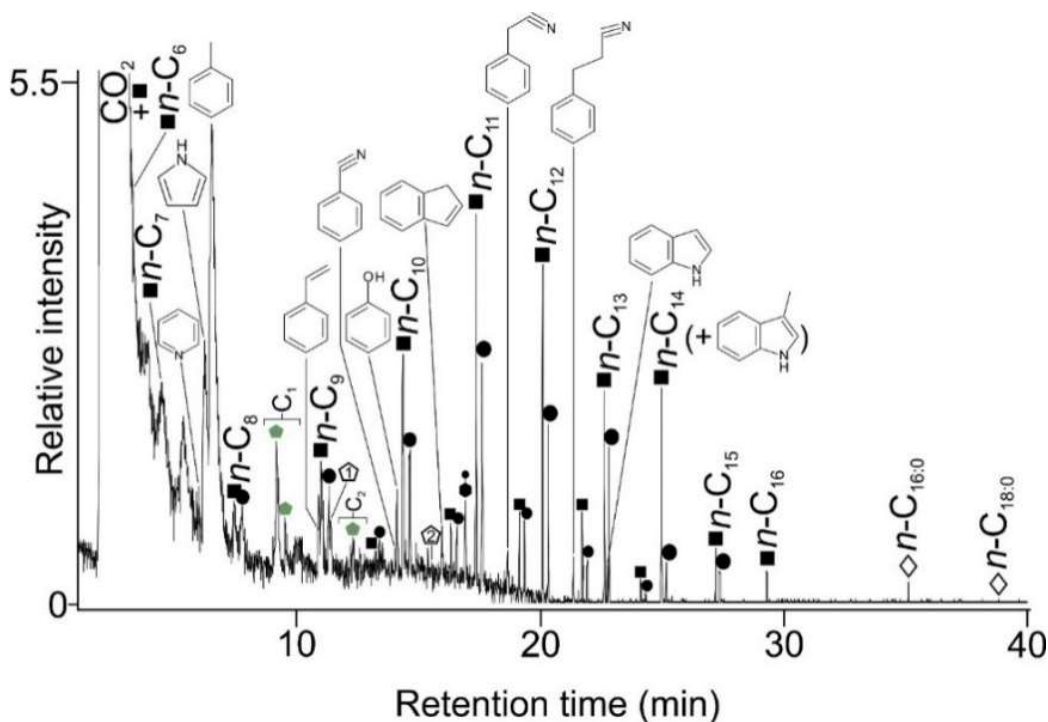
The thermal desorption total ion chromatogram (TIC) revealed carbon dioxide and acetonitrile as the only detectable (trace) components, indicating the virtual absence of free, thermally extractable organic components in this sample. It should be noted, however, that highly polar material could have been present, which would not have successfully eluted from the column, or indeed volatilised sufficiently in the probe. It is not possible to determine the significance of the carbon dioxide, beyond it being indicative of oxidised organic material. The origin of the acetonitrile is also difficult to determine, beyond it being indicative of a relatively labile nitrogen-containing polymeric/bound biomolecule (hence the generation of acetonitrile at only ~300°C), although it has been correlated with the pyrolysis of proteins high in phenylalanine and tyrosine, in particular²⁶.

In contrast to the thermal desorption profile, the pyrolysis-GC-MS TIC (Fig. 7) revealed a number of organic compounds, albeit in relatively low abundance. These include a series of C₈ to C₁₆ *n*-1-alkenes and *n*-alkanes, typical of unsaturated and saturated fat/oil-derived acyl lipids^{5,6}. A bimodal distribution with maxima at C₁₁/C₁₂ and C₁₃/C₁₄ suggests the original acyl lipids may have been high in polyunsaturated fatty acids. The absence of these compounds in the TD profile provides molecular evidence for a polymeric/bound acyl lipid source. However, in addition to *n*-1-alkenes and *n*-alkanes, typical of fat/oil derived lipids, the pyrogram also displayed a series of C₁₀ to C₁₄ iso-methyl-1-alkenes (isoalkenes) and 2-methylalkanes (isoalkanes), presumably deriving from the branched fatty acids which are significant components of bacterial triglycerides.

666 There were components identified in the pyrolysate TIC which can be indicative of ‘black
667 carbon’, i.e. charcoal or soot⁷⁻¹⁰. However, lack of alkyl naphthalenes and biphenyls and the
668 dominance of toluene, rather than the normally dominant aromatic hydrocarbon benzene, is
669 notably atypical of chars⁷⁻¹⁰. The markers identified here can also be indicative of proteins^{6,11}
670 and combined with the relatively abundant related protein markers pyrrole, benzonitrile,
671 benzyl nitrile (phenylacetone nitrile), benzenepropanenitrile and indoles^{6,11} identified this is
672 likely to be the origin of these compounds in this sample, pointing to a substantial protein-
673 derived component^{6,11}. This is also corroborated by the presence of the C_{16:0} and C_{18:0}
674 aliphatic nitriles, suggesting a lipid (fat/oil) in this sample which has combined with protein-
675 derived nitrogen to produce these moieties⁵. The proteinaceous origin for the aromatic
676 markers is further corroborated by the relatively significant abundance of phenol and 4-
677 methylphenol (*p*-cresol), which collectively are indicative of the amino acid tyrosine¹²⁻¹⁴.
678 With an absence of guaiacol itself (all isomers) and other guaiacol moieties characteristic of
679 lignin^{15,16}, the possibility that the phenols may originate from wood or woody plant material
680 can be excluded. Alkyl phenols have also been observed in wood smoke and smoked
681 foods^{17,18}, however the absence of methoxyphenols, diagnostic of lignin-derived smoke,
682 means this possibility can also be discounted. Taken together, the presence of toluene, styrene
683 and phenylacetone nitrile (benzyl nitrile) can indicate the amino acid phenylalanine^{12,13}. In
684 addition, the significant constituents indole and 3-methylindole (skatole) are consistent with a
685 tryptophan amino acid source^{12,13,19,20}. Proteins rich in the amino acids tyrosine,
686 phenylalanine and tryptophan include meat, fish and dairy products, although the presence of
687 bacterial markers means the possibility that at least a partial origin could be microbial cannot
688 be excluded.

689 However, notable in this context is the additional presence of appreciable amounts of the
690 more unusual 2- and 3-methylpyrroles and 2-ethylpyrrole, although C₃ and C₄ alkyl pyrroles
691 were not detected, quite possibly due to the low abundance of organic material present.
692 Consequently, this does not allow any further inferences to be made in isolation, but see
693 discussion below. The carbohydrate markers 2-methyl-2-cyclopenten-1-one and 2,3-
694 dimethyl-2-cyclopenten-1-one were detected in the pyrolysate, albeit only as trace
695 components (for their likely significance here see discussion below). The presence of carbon
696 dioxide is also consistent with a lipid and amino acid/protein component in this sample. It
697 should also be noted that highly polar material could have been present, which would not
698 have successfully eluted from the column, or indeed volatilised sufficiently in the probe. The

low abundance of organic material in this sample does not allow any further inferences to be made here (but see discussion).



Supplementary Figure 7. Reconstructed total ion chromatogram of the pyrogram (pyrolysis profile) (610 °C for 10s) of sample DL180, after thermal desorption (310 °C for 10 s). Key to Fig. 7: Peak identities (x indicates carbon chain length): filled squares, Cx indicates alkenes; filled circles, Cx indicates alkanes; open diamonds, Cx:y indicates acyclic nitriles; green filled pentagons indicates alkyl pyrroles (with alkyl chain length above, Cx); filled hexagon with filled circle attached indicates alkyl phenol; open pentagons with numerals inside indicates carbohydrate pyrolysis markers, 1 is 2-methyl-2-cyclopenten-1-one and 2 is 2,3-dimethyl-2-cyclopentene-1-one (trace). Also shown are the structures of eleven aromatic compounds identified: pyridine, pyrrole, toluene, styrene, benzonitrile, phenol, indene, benzyl nitrile, benzenepropanenitrile, indole and 3-methylindole (skatole) (minor). CO₂ indicates carbon dioxide.

713

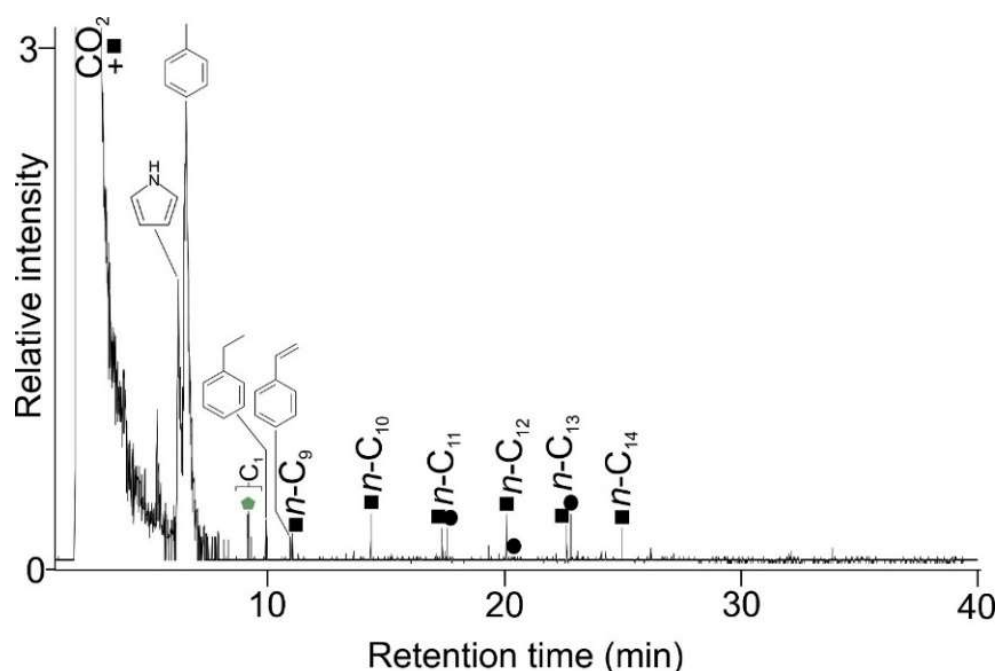
9. Isbister DL188 1-3 Sk78 BC7 jaw fragment IS1976 (calculus: 4.05 mg)

The thermal desorption total ion chromatogram (TIC) revealed carbon dioxide as the only detectable (trace) component, indicating the essential absence of free, thermally extractable organic components in this sample. It should be noted, however, that highly polar material

could have been present, which would not have successfully eluted from the column, or indeed volatilised sufficiently in the probe. It is not possible to determine the significance of the carbon dioxide, beyond it being indicative of oxidised organic material.

In contrast to the thermal desorption profile, the pyrolysis-GC-MS TIC (Fig. 8) revealed a number of organic compounds, albeit in very low abundance. These include a series of C₉ to C₁₄ *n*-1-alkenes and *n*-alkanes, typical of unsaturated and saturated fat/oil-derived acyl lipids^{5,6}. The absence of these compounds in the TD profile provides molecular evidence for a polymeric/bound acyl lipid source. Although the series of ~C₁₀ to ~C₁₅ iso-methyl-1-alkenes (isoalkenes) and 2-methylalkanes (isoalkanes), indicative of a bacterial lipid input and identified in previous samples from Isbister (see above), were not observed in this sample this is likely to be due to the very small amount of organic material present.

There were a small number of chemical markers identified in the pyrolysate TIC which can be indicative of proteins, i.e. toluene, styrene, pyrrole, and 2-methylpyrrole^{6,11}. The complex suite of nitrogen-containing protein markers observed in previous samples (see above) were not detected, which again is likely to be due to the very small amount of organic material present. This may also explain the absence of nitriles, phenols, pyridines, C₂, C₃ and C₄ alkyl pyrroles, and carbohydrate markers in the pyrolysate of this sample. The presence of carbon dioxide is also consistent with a lipid and amino acid/protein component in this sample. It should also be noted that highly polar material could have been present, which would not have successfully eluted from the column, or indeed volatilised sufficiently in the probe. The low abundance of organic material in this sample does not allow any further inferences to be made (but see discussion).



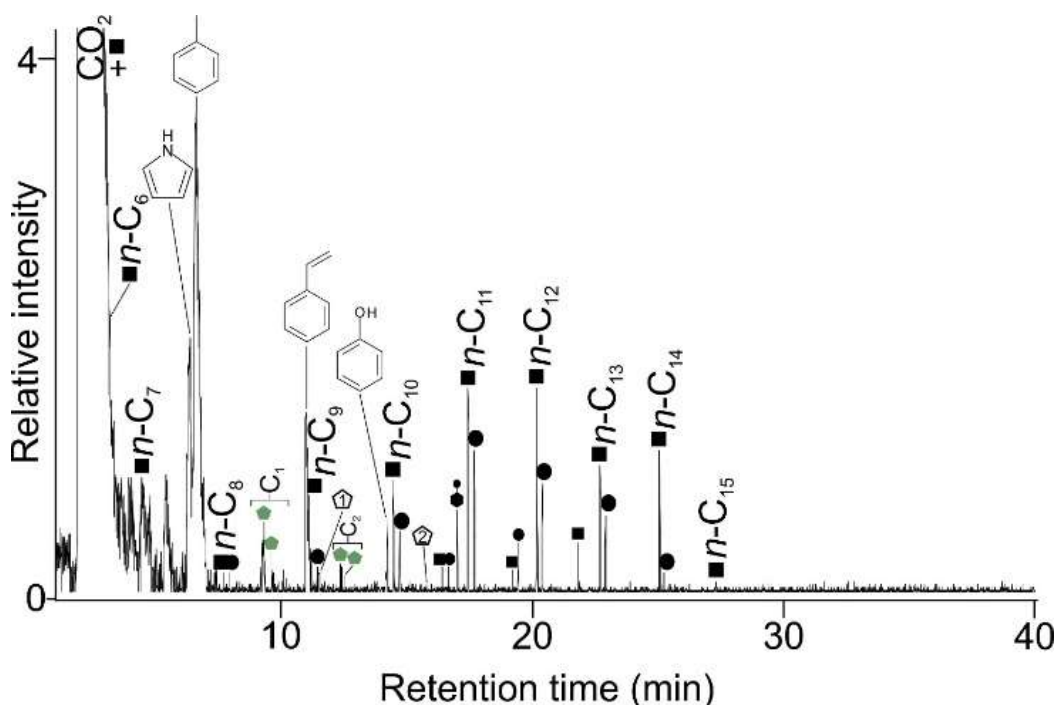
Supplementary Figure 8. Reconstructed total ion chromatogram of the pyrogram (pyrolysis profile) (610 °C for 10s) of sample DL188, after thermal desorption (310 °C for 10 s). Key to Fig. 8: Peak identities (x indicates carbon chain length): filled squares, Cx indicates alkenes; filled circles, Cx indicates alkanes; green filled pentagon indicates alkyl pyrrole (with alkyl chain length above, Cx); filled. Also shown are the structures of four aromatic compounds identified: pyrrole, toluene, ethyl benzene and styrene. CO₂ indicates carbon dioxide.

10. Isbister DL196 5 BC4 ST4 ISB incisor IS1976 (calculus: 2.58 mg)

The thermal desorption total ion chromatogram (TIC) revealed carbon dioxide and acetonitrile as the only detectable components, indicating the virtual absence of free, thermally extractable organic components in this sample. It should be noted, however, that highly polar material could have been present, which would not have successfully eluted from the column, or indeed volatilised sufficiently in the probe. It is not possible to determine the significance of the carbon dioxide, beyond it being indicative of oxidised organic material. The origin of the acetonitrile is also difficult to determine, beyond it being indicative of a relatively labile nitrogen-containing polymeric/bound biomolecule (hence the generation of acetonitrile at only ~300°C), although it has been correlated with the pyrolysis of proteins high in phenylalanine and tyrosine, in particular²⁶.

In contrast to the thermal desorption profile, the pyrolysis-GC-MS TIC (Fig. 9) revealed a suite of organic compounds, albeit in relatively low abundance. These include a series of C₉ to C₁₅ *n*-1-alkenes and *n*-alkanes, typical of unsaturated and saturated fat/oil-derived acyl lipids^{5,6}. A bimodal distribution with maxima at C₁₁/C₁₂ and C₁₄ suggests the original acyl lipids may have been high in polyunsaturated fatty acids. The absence of these compounds in the TD profile provides molecular evidence for a polymeric/bound acyl lipid source. However, in addition to *n*-1-alkenes and *n*-alkanes, typical of fat/oil derived lipids, the pyrogram also displayed a series of C₁₁ to C₁₃ iso-methyl-1-alkenes (isoalkenes) and 2-methylalkanes (isoalkanes), albeit in relatively moderate abundance due to the relatively small amount of organic material present; these are again presumed to derive from the branched fatty acids which are significant components of bacterial triglycerides.

There were a number of chemical markers identified in the pyrolysate TIC which can be indicative of proteins, i.e. toluene, styrene, pyrrole, 2-methylpyrrole, 3-methylpyrrole, 2-ethylpyrrole and a dimethylpyridine^{6,11}. The complex suite of nitrogen-containing protein markers observed in previous samples (see above) were not detected, which given the notably similar pyrolysis profile of this sample when compared to those with appreciable amounts of organic material above, suggests this is again due to the relatively small amount of organic material present. Despite this, the proteinaceous origin for the non-nitrogenous aromatic markers is further corroborated by the relatively significant abundance of both phenol and 4-methylphenol (*p*-cresol), which collectively are indicative of the amino acid tyrosine¹²⁻¹⁴. However, alkyl nitriles and the C₃ and C₄ alkyl pyrroles were not detected in the pyrolysate, possibly because of the low abundance of organic material present. Interesting, despite the small amount of macromolecular observed, the carbohydrate markers 2-methyl-2-cyclopenten-1-one and 2,3-dimethyl-2-cyclopenten-1-one were detected in the pyrolysate, albeit as relatively minor components (for their likely significance here see discussion below). The presence of carbon dioxide is also consistent with a lipid, amino acid/protein and carbohydrate component in this sample. It should also be noted that highly polar material could have been present, which would not have successfully eluted from the column, or indeed volatilised sufficiently in the probe. The low abundance of organic material in this sample does not allow any further inferences to be made in isolation, but see discussion below.



Supplementary Figure 9. Reconstructed total ion chromatogram of the pyrogram (pyrolysis profile) (610 °C for 10s) of sample DL196, after thermal desorption (310 °C for 10 s). Key to Fig. 9: Peak identities (x indicates carbon chain length): filled squares, Cx indicates alkenes; filled circles, Cx indicates alkanes; green filled pentagons indicates alkyl pyrroles (with alkyl chain length above, Cx); filled hexagon with filled circle attached indicates alkyl phenol; open pentagons with numerals inside indicates carbohydrate pyrolysis markers, 1 is 2-methyl-2-cyclopenten-1-one and 2 is 2,3-dimethyl-2-cyclopentene-1-one (trace). Also shown are the structures of four aromatic compounds identified: pyrrole, toluene, styrene and phenol. CO₂ indicates carbon dioxide.

11. Isbister DL715 24 Sk77 BC4 mandible IS1976 (calculus: 12.15 mg)

The thermal desorption total ion chromatogram (TIC) revealed carbon dioxide and acetonitrile as the only detectable components, indicating the virtual absence of free, thermally extractable organic components in this sample. It should be noted, however, that highly polar material could have been present, which would not have successfully eluted from the column, or indeed volatilised sufficiently in the probe. It is not possible to determine the significance of the carbon dioxide, beyond it being indicative of oxidised organic material. The origin of the acetonitrile is also difficult to determine, beyond it being

indicative of a relatively labile nitrogen-containing polymeric/bound biomolecule (hence the generation of acetonitrile at only ~300°C), although it has been correlated with the pyrolysis of proteins high in phenylalanine and tyrosine, in particular²⁶.

In contrast to the thermal desorption profile, the pyrolysis-GC-MS TIC (Fig. 10) is dominated by a complex suite of organic compounds. These include a series of C₈ to C₁₇ *n*-1-alkenes and *n*-alkanes, typical of unsaturated and saturated fat/oil-derived acyl lipids^{5,6}. A bimodal distribution with maxima at C₁₁/C₁₂ and C₁₄ suggests the original acyl lipids may have been high in polyunsaturated fatty acids. The absence of these compounds in the TD profile provides molecular evidence for a polymeric/bound acyl lipid source. However, in addition to *n*-1-alkenes and *n*-alkanes, typical of fat/oil derived lipids, the pyrogram also displayed a series of C₁₀ to C₁₆ iso-methyl-1-alkenes (isoalkenes) and 2-methylalkanes (isoalkanes), presumably deriving from the branched fatty acids which are significant components of bacterial triglycerides.

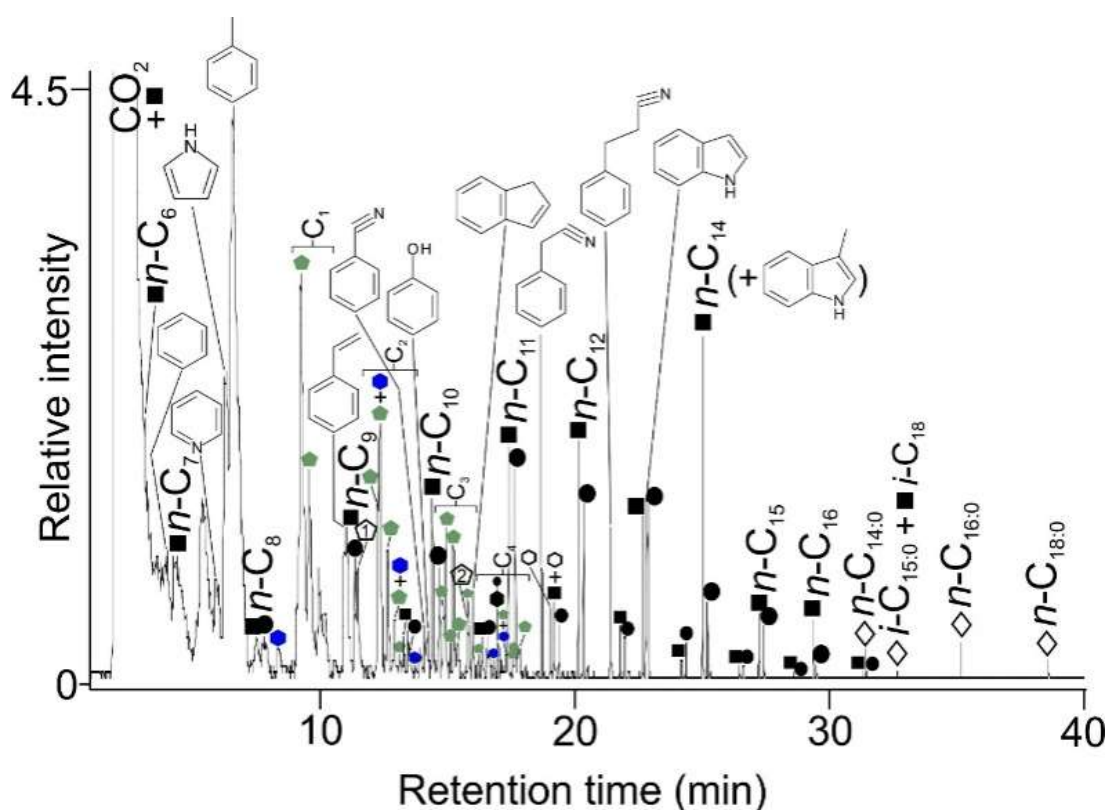
There were components identified in the pyrolysate TIC which can be indicative of 'black carbon', i.e. charcoal or soot⁷⁻¹⁰. However, lack of alkyl naphthalenes and biphenyls and the dominance of toluene, rather than the normally dominant aromatic hydrocarbon benzene, is notably atypical of chars⁷⁻¹⁰. The markers identified here can also be indicative of proteins^{6,11} and combined with the abundant related protein markers pyrrole, benzonitrile, benzyl nitrile (phenylacetone nitrile), benzenepropanenitrile and indoles^{6,11} identified this is likely to be the main origin of these compounds in this sample, pointing to a substantial protein-derived component^{6,11}. This is also corroborated by the presence of aliphatic nitriles (carbon numbers C_{14:0}, *i*-C_{15:0}, C_{16:0} (max) and C_{18:0}) suggesting a lipid (fat/oil) in this sample which has combined with protein-derived nitrogen to produce these moieties⁵. The proteinaceous origin for the aromatic markers is further corroborated by the significant abundance of phenol, 3-methylphenol (*m*-cresol), 4-methylphenol (*p*-cresol) (with the 4-methyl isomer being predominant), and lesser amounts of an unidentified C₂ phenol, all of which, collectively, are indicative of the amino acid tyrosine¹²⁻¹⁴. With an absence of guaiacol itself (all isomers) and other guaiacol moieties characteristic of lignin^{15,16}, the possibility that the phenols may originate from wood or woody plant material can be excluded. Alkyl phenols have also been observed in wood smoke and smoked foods^{17,18}, however the absence of methoxyphenols, diagnostic of lignin-derived smoke, means this possibility can also be discounted. Taken together, the presence of toluene, styrene and phenylacetone nitrile (benzyl nitrile) can indicate the amino acid phenylalanine^{12,13}. In addition, the major constituents indole and 3-

methyldindole (skatole) were also identified, which are consistent with a tryptophan amino acid source^{12,13,19,20}. Proteins rich in the amino acids tyrosine, phenylalanine and tryptophan include meat, fish and dairy products, although the presence of bacterial markers means the possibility that at least a partial origin could be microbial cannot be excluded.

However, highly notable in this context is the additional presence of appreciable amounts of the more unusual 2- and 3-methylpyrroles, 2- and 3-ethylpyrroles, 2,4-, 2,3- and 3,4-dimethyl-pyrroles, 2-ethyl-4(?)-methylpyrrole, 2-ethyl-3(?)-methylpyrrole, 4-ethyl-2-methylpyrrole, 3-ethyl-4-methylpyrrole (trace), 2,3,5-trimethylpyrrole, 2,3,4-trimethylpyrrole, a propylmethylpyrrole, 2,5(?)-diethylpyrrole, 2,3(?)-diethylpyrrole, an ethyldimethylpyrrole, 4-ethyl-2,3-dimethylpyrrole, 3-ethyl-2,4-dimethylpyrrole, 2,3(?)-diethyl-5?-methylpyrrole, 2,3-dimethyl-4-n-propylpyrrole and 3-methyl-4-neopentylpyrrole (trace). Collectively, these specific C₁, C₂, C₃, C₄, C₅ and C₆ alkyl pyrroles suggest a significant tetrapyrrole/porphyrin-derived origin and are indicative of an algal source observed in fossils and expected in similarly diagenetically altered archaeological material^{11,21,22}. Notable also in this context were the presence of the moderately abundant nitrogen containing aromatic compounds 1-H-pyrazole-4-carbonitrile, ethylcyanobenzene, 2-pyridinecarbonitrile and tolyl isocyanide, along with pyridine and its methyl-, dimethyl- and trimethyl derivatives (these pyridines can result from the pyrolysis of polypeptides containing the amino acid alanine²³). The presence here of the abundant protein markers and specifically the C₁ to C₆ alkyl pyrroles, combined with the particular lipid profile and specific carbohydrate markers (see below) identified, suggests the presence of a macroalgae, i.e. seaweed, in this sample^{21,22,24} (see discussion).

The thermally-derived carbohydrate markers 2-methyl-2-cyclopenten-1-one and 2,3-dimethyl-2-cyclopenten-1-one were identified as significant components, along with an unidentified carbohydrate marker. That they were absent in the TD profile points to their originating from the original polymeric carbohydrate source (e.g. starch)²⁵. Although levoglucosan (1,6-anhydro- β -D-glucopyranose) is usually the major component in the thermally-derived products of carbohydrates analysed by TD/Py-GC-MS, its absence from the TD profile here is consistent with the removal of hydroxyl groups in the original carbohydrate polymer, so preventing its formation in the probe. The additional absence of the normally significant carbohydrate markers 2-hydroxy-3-methyl-2-cyclopenten-1-one and 3-hydroxy-2-methyl-2-cyclopenten-1-one observed in pure standard material (glycogen, starch and cellulose) would also support this interpretation. This shows a strong matrix effect likely

to be due to the inorganic component of the calculus and the microflora in the mouth. This dehydration/dehydroxylation has also been observed in dog calculus and 19th century human calculus⁶, where other markers, e.g. a prevalence of nitriles, with amides very minor, deriving from dehydrating conditions in the mouth of dogs and humans (though not sheep⁶) supporting this view⁶. The presence of carbon dioxide is also consistent with a lipid, amino acid/protein and carbohydrate component in this sample. Notably, these findings did not reveal key biomarkers typically observed in soils^{20,23}. It should also be noted that highly polar material could have been present, which would not have successfully eluted from the column, or indeed volatilised sufficiently in the probe.



Supplementary Figure 10. Reconstructed total ion chromatogram of the pyrogram (pyrolysis profile) (610 °C for 10s) of sample DL715, after thermal desorption (310 °C for 10 s). Key to Fig. 10: Peak identities (x indicates carbon chain length): filled squares, C_x indicates alkenes; filled circles, C_x indicates alkanes; open diamonds, C_x:y indicates acyclic nitriles; blue filled hexagons indicates alkyl pyridines; green filled pentagons indicates alkyl pyrroles (with alkyl chain length above, C_x); filled hexagons with filled circles attached indicates alkyl phenols; open hexagons indicates alkyl indenenes; open pentagons with

numerals inside indicates carbohydrate pyrolysis markers, 1 is 2-methyl-2-cyclopenten-1-one and 2 is 2,3-dimethyl-2-cyclopentene-1-one. Also shown are the structures of twelve aromatic compounds identified: benzene, pyridine, pyrrole, toluene, styrene, benzonitrile, phenol, indene, benzyl nitrile, benzenepropanenitrile, indole and 3-methylindole (skatole) (minor). CO₂ indicates carbon dioxide.

12. Isbister DL5083 x3 Sk77 BC2c IS128 mandible IS1976 (calculus: 10.39 mg)

The thermal desorption total ion chromatogram (TIC) revealed carbon dioxide and acetonitrile as the only detectable components, indicating the virtual absence of free, thermally extractable organic components in this sample. It should be noted, however, that highly polar material could have been present, which would not have successfully eluted from the column, or indeed volatilised sufficiently in the probe. It is not possible to determine the significance of the carbon dioxide, beyond it being indicative of oxidised organic material. The origin of the acetonitrile is also difficult to determine, beyond it being indicative of a relatively labile nitrogen-containing polymeric/bound biomolecule (hence the generation of acetonitrile at only ~300°C), although it has been correlated with the pyrolysis of proteins high in phenylalanine and tyrosine, in particular²⁶.

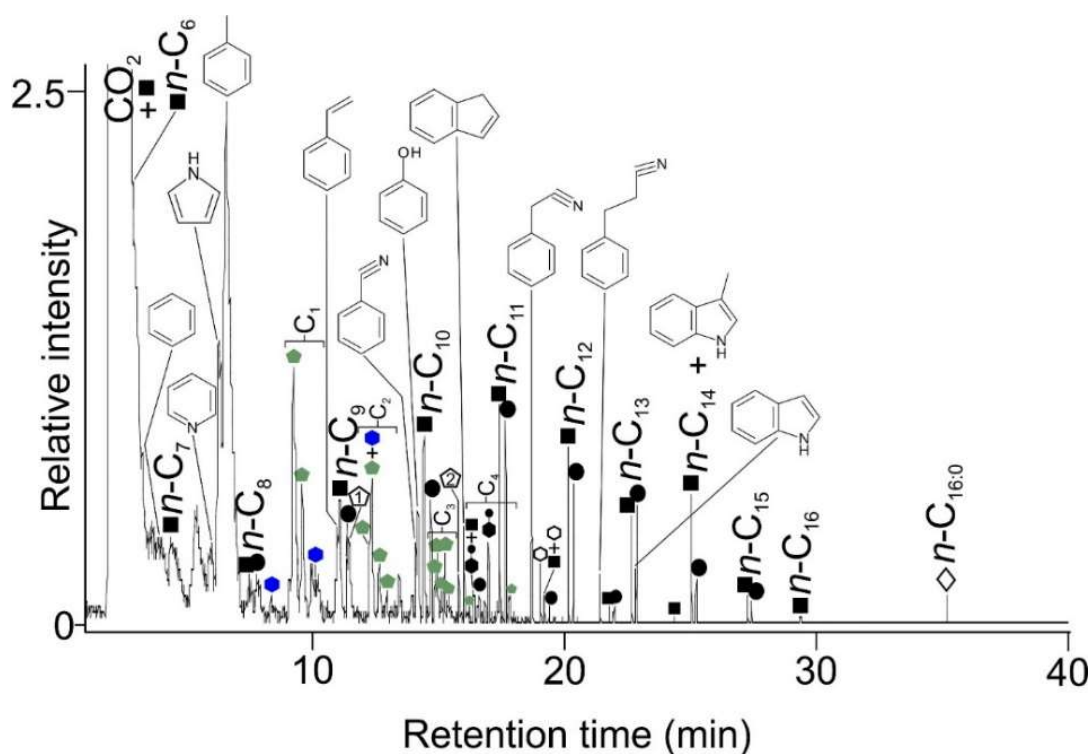
In contrast to the thermal desorption profile, the pyrolysis-GC-MS TIC (Fig. 11) is dominated by a relatively complex suite of organic compounds. These include a series of C₈ to C₁₆ *n*-1-alkenes and *n*-alkanes, typical of unsaturated and saturated fat/oil-derived acyl lipids^{5,6}. A bimodal distribution with maxima at C₁₁/C₁₂ and C₁₄ suggests the original acyl lipids may have been high in polyunsaturated fatty acids. The absence of these compounds in the TD profile provides molecular evidence for a polymeric/bound acyl lipid source. However, in addition to *n*-1-alkenes and *n*-alkanes, typical of fat/oil derived lipids, the pyrogram also displayed a series of C₁₁ to C₁₄ iso-methyl-1-alkenes (isoalkenes) and 2-methylalkanes (isoalkanes), presumably deriving from the branched fatty acids which are significant components of bacterial triglycerides.

There were components identified in the pyrolysate TIC which can be indicative of 'black carbon', i.e. charcoal or soot⁷⁻¹⁰. However, lack of alkyl naphthalenes and biphenyls and the dominance of toluene, rather than the normally dominant aromatic hydrocarbon benzene, is notably atypical of chars⁷⁻¹⁰. The markers identified here can also be indicative of proteins^{6,11} and combined with the abundant related protein markers pyrrole, benzonitrile, benzyl nitrile

(phenylacetonitrile), benzenepropanenitrile and indoles^{6,11} identified this is likely to be the main origin of these compounds in this sample pointing to a substantial protein-derived component^{6,11}. This is also corroborated by the presence of the C_{16:0} aliphatic nitrile, suggesting a lipid (fat/oil) in this sample which has combined with protein-derived nitrogen to produce these moieties⁵. The proteinaceous origin for the aromatic markers is further corroborated by the significant abundance of phenol, 2-methylphenol (*o*-cresol) and 4-methylphenol (*p*-cresol) (with the 4-methyl isomer being predominant), which collectively are indicative of the amino acid tyrosine¹²⁻¹⁴. With an absence of guaiacol itself (all isomers) and other guaiacol moieties characteristic of lignin^{15,16}, the possibility that the phenols may originate from wood or woody plant material can be excluded. Alkyl phenols have also been observed in wood smoke and smoked foods^{17,18}, however the absence of methoxyphenols, diagnostic of lignin-derived smoke, means this possibility can also be discounted. Taken together, the presence of toluene, styrene and phenylacetonitrile (benzyl nitrile) can indicate the amino acid phenylalanine^{12,13}. In addition, the major constituents indole and 3-methylindole (skatole) are consistent with a tryptophan amino acid source^{12,13,19,20}. Proteins rich in the amino acids tyrosine, phenylalanine and tryptophan include meat, fish and dairy products, although the presence of bacterial markers means the possibility that at least a partial origin could be microbial cannot be excluded.

However, highly notable in this context is the additional presence of appreciable amounts of the more unusual 2- and 3-methylpyrroles, 2- and 3-ethylpyrroles, 2,3-dimethyl-pyrrole, 2-ethyl-4(?) -methylpyrrole, 2-ethyl-3(?) -methylpyrrole, 4-ethyl-2-methylpyrrole, 3-ethyl-4-methylpyrrole, 2,3,5-trimethylpyrrole and a propylmethylpyrrole. Collectively, these specific C₁, C₂, C₃ and C₄ alkyl pyrroles suggest a tetrapyrrole/porphyrin-derived origin and are indicative of an algal source observed in fossils and expected in similarly diagenetically altered archaeological material^{11,21,22}. Notable also in this context were the presence of the relatively moderately abundant nitrogen containing aromatic compounds 1-H-pyrazole-4-carbonitrile, 2-pyridinecarbonitrile and tolyl isocyanide (x2), along with pyridine and its methyl-, dimethyl- and trimethyl derivatives (these pyridines can result from the pyrolysis of polypeptides containing the amino acid alanine²³). The presence here of the abundant protein markers and specifically the C₁ to C₄ alkyl pyrroles, combined with the particular lipid profile and specific carbohydrate markers (see below) identified, suggests the presence of a macroalgae, i.e. seaweed, in this sample^{21,22,24} (see discussion).

The thermally-derived carbohydrate markers 2-methyl-2-cyclopenten-1-one and 2,3-dimethyl-2-cyclopenten-1-one were identified as significant components, along with an unidentified carbohydrate marker. That they were absent in the TD profile points to their originating from the original polymeric carbohydrate source (e.g. starch)²⁵. Although levoglucosan (1,6-anhydro- β -D-glucopyranose) is usually the major component in the thermally-derived products of carbohydrates analysed by TD/Py-GC-MS, its absence from the TD profile here is consistent with the removal of hydroxyl groups in the original carbohydrate polymer, so preventing its formation in the probe. The additional absence of the normally significant carbohydrate markers 2-hydroxy-3-methyl-2-cyclopenten-1-one and 3-hydroxy-2-methyl-2-cyclopenten-1-one observed in pure standard material (glycogen, starch and cellulose) would also support this interpretation. This shows a strong matrix effect likely to be due to the inorganic component of the calculus and the microflora in the mouth. This dehydration/dehydroxylation has also been observed in dog calculus and 19th century human calculus⁶, where other markers, e.g. a prevalence of nitriles, with amides very minor, deriving from dehydrating conditions in the mouth of dogs and humans (though not sheep⁶) supporting this view⁶. The presence of carbon dioxide is also consistent with a lipid, amino acid/protein and carbohydrate component in this sample. Notably, these findings did not reveal key biomarkers typically observed in soils^{20,23}. It should also be noted that highly polar material could have been present, which would not have successfully eluted from the column, or indeed volatilised sufficiently in the probe.



Supplementary Figure 11. Reconstructed total ion chromatogram of the pyrogram (pyrolysis profile) (610 °C for 10s) of sample DL5083, after thermal desorption (310 °C for 10 s). Key to Fig. 11: Peak identities (x indicates carbon chain length): filled squares, Cx indicates alkenes; filled circles, Cx indicates alkanes; open diamond, Cx:y indicates acyclic nitrile; blue filled hexagons indicates alkyl pyridines; green filled pentagons indicates alkyl pyrroles (with alkyl chain length above, Cx); filled hexagons with filled circles attached indicates alkyl phenols; open hexagons indicates alkyl indenenes; open pentagons with numerals inside indicates carbohydrate pyrolysis markers, 1 is 2-methyl-2-cyclopenten-1-one and 2 is 2,3-dimethyl-2-cyclopentene-1-one. Also shown are the structures of twelve aromatic compounds identified: benzene, pyridine, pyrrole, toluene, styrene, benzonitrile, phenol, indene, benzyl nitrile, benzenepropanenitrile, indole and 3-methylindole (skatole). CO₂ indicates carbon dioxide.

13. Isbister DL5117 x3 Sk77 BC6(3) ST5 mandible 5 IS1976 (calculus: 5.69 mg)

The thermal desorption total ion chromatogram (TIC) revealed carbon dioxide as the only detectable (trace) component, indicating the essential absence of free, thermally extractable organic components in this sample. It should be noted, however, that highly polar material could have been present, which would not have successfully eluted from the column, or

indeed volatilised sufficiently in the probe. It is not possible to determine the significance of the carbon dioxide, beyond it being indicative of oxidised organic material.

The pyrolysate TIC revealed carbon dioxide, 1-butene, 1-heptene, a heptadiene, pyrrole, toluene and 2-methylpyrrole as the only detectable (trace) components, indicating the essential absence of organic material in this sample. It should be noted, however, that highly polar material could have been present, which would not have successfully eluted from the column, or indeed volatilised sufficiently in the probe. It is not possible to determine the significance of the carbon dioxide, beyond it being indicative of oxidised organic material. The short-chain alkenes, toluene and pyrroles are in too low an abundance to provide any meaningful interpretation of their presence, beyond being indicative of an organic origin.

14. Isbister DL5119 x4 Sk77 BC6(3) ST5 mandible 2 IS1976 (calculus: 6.03 mg)

The thermal desorption total ion chromatogram (TIC) revealed carbon dioxide and acetonitrile as the only detectable components, indicating the virtual absence of free, thermally extractable organic components in this sample. It should be noted, however, that highly polar material could have been present, which would not have successfully eluted from the column, or indeed volatilised sufficiently in the probe. It is not possible to determine the significance of the carbon dioxide, beyond it being indicative of oxidised organic material. The origin of the acetonitrile is also difficult to determine, beyond it being indicative of a relatively labile nitrogen-containing polymeric/bound biomolecule (hence the generation of acetonitrile at only ~300°C), although it has been correlated with the pyrolysis of proteins high in phenylalanine and tyrosine, in particular²⁶.

In contrast to the thermal desorption profile, the pyrolysis-GC-MS TIC (Fig. 12) is dominated by a relatively complex suite of organic compounds. These include a series of C₈ to C₁₆ *n*-1-alkenes and *n*-alkanes, typical of unsaturated and saturated fat/oil-derived acyl lipids^{5,6}. The absence of these compounds in the TD profile provides molecular evidence for a polymeric/bound acyl lipid source. However, in addition to *n*-1-alkenes and *n*-alkanes, typical of fat/oil derived lipids, the pyrogram also displayed a series of C₁₁ to C₁₄ iso-methyl-1-alkenes (isoalkenes) and 2-methylalkanes (isoalkanes), presumably deriving from the branched fatty acids which are significant components of bacterial triglycerides.

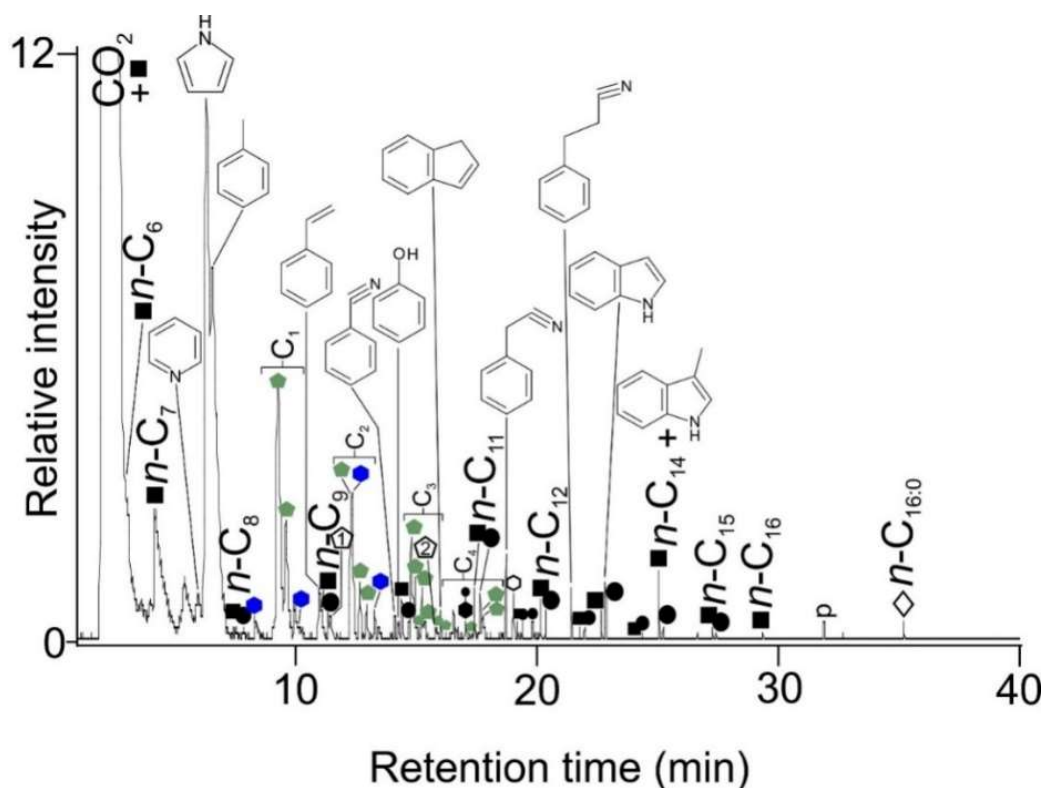
There were components identified in the pyrolysate TIC which can be indicative of 'black carbon', i.e. charcoal or soot⁷⁻¹⁰. However, lack of alkyl naphthalenes and biphenyls and the

dominance of toluene, rather than the normally dominant aromatic hydrocarbon benzene, is notably atypical of chars⁷⁻¹⁰. The markers identified here can also be indicative of proteins^{6,11} and combined with the abundant related protein markers pyrrole, benzonitrile, benzyl nitrile (phenylacetonitrile), benzenepropanenitrile and indoles^{6,11} identified this is likely to be the main origin of these compounds in this sample pointing to a substantial protein-derived component^{6,11}. This is also corroborated by the presence of the C_{16:0} aliphatic nitrile, suggesting a lipid (fat/oil) in this sample which has combined with protein-derived nitrogen to produce these moieties⁵. The proteinaceous origin for the aromatic markers is further corroborated by the moderate abundance of phenol and 4-methylphenol (*p*-cresol), which collectively are indicative of the amino acid tyrosine¹²⁻¹⁴. With an absence of guaiacol itself (all isomers) and other guaiacol moieties characteristic of lignin^{15,16}, the possibility that the phenols may originate from wood or woody plant material can be excluded. Alkyl phenols have also been observed in wood smoke and smoked foods^{17,18}, however the absence of methoxyphenols, diagnostic of lignin-derived smoke, means this possibility can also be discounted. Taken together, the presence of toluene, styrene and phenylacetonitrile (benzyl nitrile) can indicate the amino acid phenylalanine^{12,13}. In addition, the major constituents indole and 3-methylindole (skatole) are consistent with a tryptophan amino acid source^{12,13,19,20}. Proteins rich in the amino acids tyrosine, phenylalanine and tryptophan include meat, fish and dairy products, although the presence of bacterial markers means the possibility that at least a partial origin could be microbial cannot be excluded.

However, highly notable in this context is the additional presence of appreciable amounts of the more unusual 2- and 3-methylpyrroles, 2- and 3-ethylpyrroles, 2,4-, 2,3- and 3,4-dimethyl-pyrroles, 2-ethyl-4(?) -methylpyrrole, 2-ethyl-3(?) -methylpyrrole, 4-ethyl-2-methylpyrrole, 3-ethyl-4-methylpyrrole, 2,3,5-trimethylpyrrole, 2,3,4-trimethylpyrrole, two propylmethylpyrroles, 2,5(?) -diethylpyrrole, 2,3(?) -diethylpyrrole, 4-ethyl-2,3-dimethylpyrrole, 2,3(?) -diethyl-5?-methylpyrrole and 3-ethyl-2,4,5-trimethylpyrrole. Collectively, these specific C₁, C₂, C₃, C₄ and C₅ alkyl pyrroles suggest a tetrapyrrole/porphyrin-derived origin and are indicative of an algal source observed in fossils and expected in similarly diagenetically altered archaeological material^{11,21,22}. Notable also in this context were the presence of the relatively moderately abundant nitrogen containing aromatic compounds 1-H-pyrazole-4-carbonitrile, 2-pyridinecarbonitrile and tolyl isocyanide (x2), 1-H-pyrrole-2-carbonitrile(?), along with pyridine and its methyl-, dimethyl- and trimethyl derivatives (these pyridines can result from the pyrolysis of polypeptides containing

the amino acid alanine²³). The presence here of the abundant protein markers and specifically the C₁ to C₄ alkyl pyrroles, combined with the particular lipid profile and specific carbohydrate markers (see below) identified, suggests the presence of a macroalgae, i.e. seaweed, in this sample^{21,22,24} (see discussion).

The thermally-derived carbohydrate markers 2-methyl-2-cyclopenten-1-one and 2,3-dimethyl-2-cyclopenten-1-one were identified as moderately significant components, along with an unidentified carbohydrate marker. That they were absent in the TD profile points to their originating from the original polymeric carbohydrate source (e.g. starch)²⁵. Although levoglucosan (1,6-anhydro- β -D-glucopyranose) is usually the major component in the thermally-derived products of carbohydrates analysed by TD/Py-GC-MS, its absence from the TD profile here is consistent with the removal of hydroxyl groups in the original carbohydrate polymer, so preventing its formation in the probe. The additional absence of the normally significant carbohydrate markers 2-hydroxy-3-methyl-2-cyclopenten-1-one and 3-hydroxy-2-methyl-2-cyclopenten-1-one observed in pure standard material (glycogen, starch and cellulose) would also support this interpretation. This shows a strong matrix effect likely to be due to the inorganic component of the calculus and the microflora in the mouth. This dehydration/dehydroxylation has also been observed in dog calculus and 19th century human calculus⁶, where other markers, e.g. a prevalence of nitriles, with amides very minor, deriving from dehydrating conditions in the mouth of dogs and humans (though not sheep⁶) supporting this view⁶. The presence of carbon dioxide is also consistent with a lipid, amino acid/protein and carbohydrate component in this sample. Notably, these findings did not reveal key biomarkers typically observed in soils^{20,23}. It should also be noted that highly polar material could have been present, which would not have successfully eluted from the column, or indeed volatilised sufficiently in the probe.



Supplementary Figure 12. Reconstructed total ion chromatogram of the pyrogram (pyrolysis profile) (610 °C for 10s) of sample DL5119, after thermal desorption (310 °C for 10 s). Key to Fig. 12: Peak identities (x indicates carbon chain length): filled squares, Cx indicates alkenes; filled circles, Cx indicates alkanes; open diamond, Cx:y indicates acyclic nitrile; blue filled hexagons indicates alkyl pyridines; green filled pentagons indicates alkyl pyrroles (with alkyl chain length above, Cx); filled hexagons with filled circle attached indicates alkyl phenol; open hexagon indicates alkyl indene; open pentagons with numerals inside indicates carbohydrate pyrolysis markers, 1 is 2-methyl-2-cyclopenten-1-one and 2 is 2,3-dimethyl-2-cyclopentene-1-one. Also shown are the structures of eleven aromatic compounds identified: pyridine, pyrrole, toluene, styrene, benzonitrile, phenol, indene, benzyl nitrile, benzenepropanenitrile, indole and 3-methylindole (skatole). CO₂ indicates carbon dioxide and p indicates contaminant from the pyroprobe.

15. Isbister DL5838 Sk77 BC5 IS113 incisor IS1976 (calculus: 10.95 mg)

The thermal desorption total ion chromatogram (TIC) (Fig. 13, inset) revealed a moderate amount of free, thermally extractable organic components in this sample, although it was dominated by carbon dioxide. Other free biomolecules identified included the nitrogen-

1108 containing compounds pyridine, pyrrole, benzenepropanenitrile, indole, an unidentified
1109 derivative of benzenepropanenitrile and the C_{16:0} nitrile. Also identified were a furan
1110 derivative and minor amounts of the C₂₇, C₂₉ and C₃₁ (trace) *n*-alkanes, with C₂₉
1111 predominating. It should be also noted, however, that highly polar material could have been
1112 present, which would not have successfully eluted from the column, or indeed volatilised
1113 sufficiently in the probe. It is not possible to determine the significance of the carbon dioxide,
1114 beyond it being indicative of a significant amount of highly oxidised organic material. The
1115 nitrogen-containing compounds may be indicative of thermally-labile proteinaceous material,
1116 with the aliphatic nitrile also indicating a lipid component. In isolation, the furan derivative is
1117 tentatively indicative of a carbohydrate constituent (but see Py-GC-MS below). Although
1118 minor, the particular *n*-alkanes, i.e. C₂₇, C₂₉ and C₃₁ (trace), with C₂₉ predominant, suggest a
1119 higher plant wax origin⁶ (see discussion).

1120 In contrast to the thermal desorption profile, the pyrolysis-GC-MS TIC (Fig. 13) is dominated
1121 by a complex suite of organic compounds. These include a series of C₈ to C₂₀ *n*-1-alkenes and
1122 *n*-alkanes, typical of unsaturated and saturated fat/oil-derived acyl lipids^{5,6}. A distribution
1123 with maxima at C₁₁/C₁₂ and C₁₃/C₁₄ suggests the original acyl lipids may have been high in
1124 polyunsaturated fatty acids. The absence of these compounds in the TD profile provides
1125 molecular evidence for a polymeric/bound acyl lipid source. However, in addition to *n*-1-
1126 alkenes and *n*-alkanes, typical of fat/oil derived lipids, the pyrogram also displayed a series of
1127 C₁₀ to C₁₈ iso-methyl-1-alkenes (isoalkenes) and 2-methylalkanes (isoalkanes), presumably
1128 deriving from the branched fatty acids which are significant components of bacterial
1129 triglycerides.

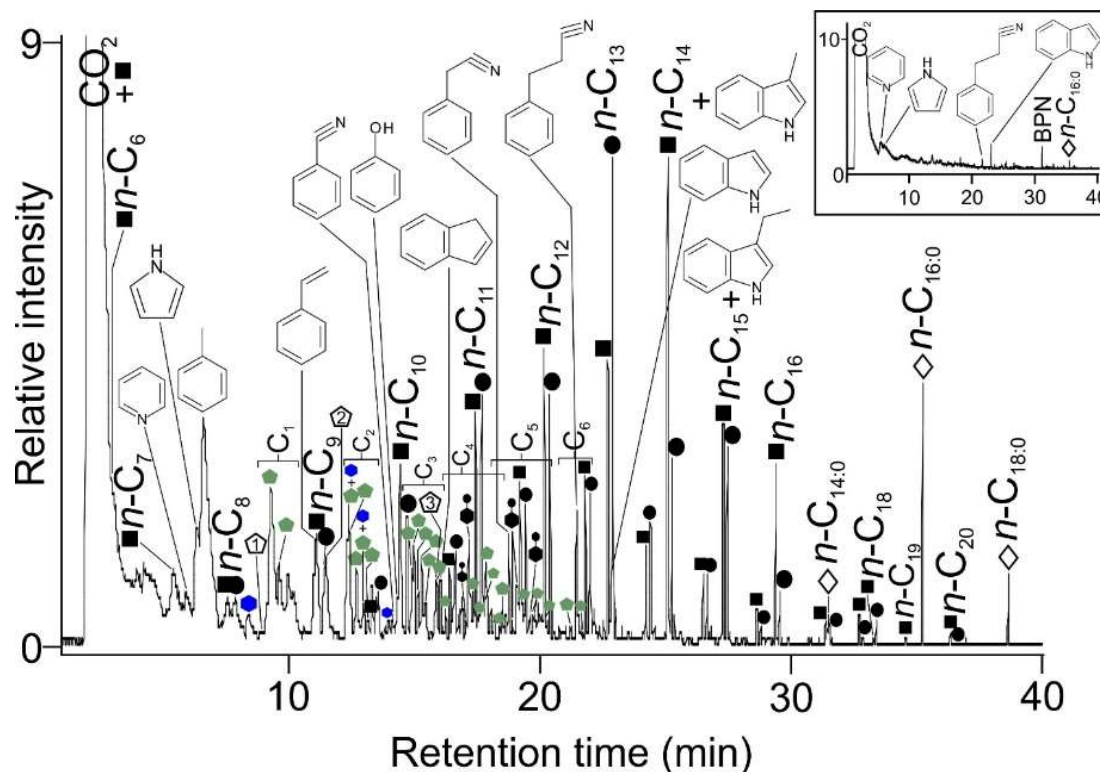
1130 There were components identified in the pyrolysate TIC which can be indicative of 'black
1131 carbon', i.e. charcoal or soot⁷⁻¹⁰. However, although this included the presence of
1132 naphthalenes, the dominance of toluene, rather than the normally dominant aromatic
1133 hydrocarbon benzene, is notably atypical of chars⁷⁻¹⁰. The markers identified here can also be
1134 indicative of proteins^{6,11} and combined with the abundant related protein markers pyrrole,
1135 benzonitrile, benzyl nitrile (phenylacetone nitrile), benzenepropanenitrile and indoles^{6,11}
1136 identified this is likely to be the main origin of these compounds in this sample, pointing to a
1137 substantial protein-derived component^{6,11}. This is also corroborated by the presence of
1138 aliphatic nitriles (carbon numbers C_{14:0}, C_{16:0} (max) and C_{18:0}) suggesting a lipid (fat/oil) in
1139 this sample which has combined with protein-derived nitrogen to produce these moieties⁵.
1140 The proteinaceous origin for the aromatic markers is further corroborated by the significant

1141 abundance of phenol, 2-methylphenol (*o*-cresol), 3-methylphenol (*m*-cresol), 4-methylphenol
 1142 (*p*-cresol) (interestingly, with the 3-methyl isomer being markedly predominant [*cf.* DL715]),
 1143 and similar amounts of 2,4-dimethylphenol and 3,4-dimethylphenol, all of which,
 1144 collectively, are indicative of the amino acid tyrosine¹²⁻¹⁴. With an absence of guaiacol itself
 1145 (all isomers) and other guaiacol moieties characteristic of lignin^{15,16}, the possibility that the
 1146 phenols may originate from wood or woody plant material can be excluded. Alkyl phenols
 1147 have also been observed in wood smoke and smoked foods^{17,18}, however the absence of
 1148 methoxyphenols, diagnostic of lignin-derived smoke, means this possibility can also be
 1149 discounted. Taken together, the presence of toluene, styrene and phenylacetonitrile (benzyl
 1150 nitrile) can indicate the amino acid phenylalanine^{12,13}. In addition, the major constituents
 1151 indole and 3-methylindole (skatole), with a lesser but significant amount of a C₂ indole (3-
 1152 ethylindole?), were also identified, which are consistent with a tryptophan amino acid
 1153 source^{12,13,19,20}. Proteins rich in the amino acids tyrosine, phenylalanine and tryptophan
 1154 include meat, fish and dairy products, although the presence of bacterial markers means the
 1155 possibility that at least a partial origin could be microbial cannot be excluded.

1156 However, highly notable in this context is the additional presence of notably significant
 1157 amounts of the more unusual alkyl pyrroles. C₁ alkyl pyrroles: 2- and 3-methylpyrroles; C₂
 1158 alkyl pyrroles: 2- and 3-ethylpyrroles, 2,4-, 2,3- and 3,4-dimethyl-pyrroles; C₃ alkyl pyrroles:
 1159 2-ethyl-4(?) -methylpyrrole, 2-ethyl-3(?) -methylpyrrole, 4-ethyl-2-methylpyrrole, 3-ethyl-4-
 1160 methylpyrrole, 2,3,5-trimethylpyrrole, 2,3,4-trimethylpyrrole; C₄ alkyl pyrroles: three
 1161 unidentified propylmethylpyrroles, five unidentified ethyldimethylpyrroles, 2,5(?) -
 1162 diethylpyrrole, 2,4(?) -diethylpyrrole, 2,3(?) -diethylpyrrole, 3,4(?) -diethylpyrrole 4-ethyl-2,3-
 1163 dimethylpyrrole, 3-ethyl-2,4-dimethylpyrrole and 2,3,4,5-tetramethylpyrrole; C₅ alkyl
 1164 pyrroles: 3-methyl-4-isobutylpyrrole, 2,3(?) -diethyl-5?-methylpyrrole, 2,3-diethyl-4-
 1165 methylpyrrole, 2,3-dimethyl-4-n-propylpyrrole and 3-ethyl-2,4,5-trimethylpyrrole; C₆ alkyl
 1166 pyrroles: 3-ethyl-4-isobutylpyrrole, 3-methyl-4-neopentylpyrrole and 2-methyl-3-ethyl-4-n-
 1167 propylpyrrole. Collectively, these specific C₁, C₂, C₃, C₄, C₅ and C₆ alkyl pyrroles are
 1168 characteristic of a significant tetrapyrrole/porphyrin-derived origin and are indicative of an
 1169 algal source observed in fossils and expected in similarly diagenetically altered
 1170 archaeological material^{11,21,22}. Notable also in this context were the presence of the
 1171 moderately abundant nitrogen containing aromatic compounds 1-H-pyrazole-4-carbonitrile,
 1172 ethylcyanobenzene, 2-pyridinecarbonitrile, tolyl isocyanide, a C₂ and C₄ pyrimidine, along
 1173 with pyridine and its methyl-, dimethyl- and trimethyl derivatives (these pyridines can result

from the pyrolysis of polypeptides containing the amino acid alanine²³). The presence here of the abundant protein markers and specifically the C₁ to C₆ alkyl pyrroles, combined with the particular lipid profile and specific carbohydrate markers (see below) identified, indicate the presence of a macroalgae, i.e. seaweed, in this sample^{21,22,24} (see discussion).

The thermally-derived carbohydrate markers 2-methylfuran, 2-methyl-2-cyclopenten-1-one and 2,3-dimethyl-2-cyclopenten-1-one were identified as significant components, along with an unidentified carbohydrate marker. That they were absent in the TD profile points to their originating from the original polymeric carbohydrate source (e.g. starch)²⁵. Although levoglucosan (1,6-anhydro-β-D-glucopyranose) is usually the major component in the thermally-derived products of carbohydrates analysed by TD/Py-GC-MS, its absence from the TD profile here is consistent with the removal of hydroxyl groups in the original carbohydrate polymer, so preventing its formation in the probe. The additional absence of the normally significant carbohydrate markers 2-hydroxy-3-methyl-2-cyclopenten-1-one and 3-hydroxy-2-methyl-2-cyclopenten-1-one observed in pure standard material (glycogen, starch and cellulose) would also support this interpretation. This shows a strong matrix effect likely to be due to the inorganic component of the calculus and the microflora in the mouth. This dehydration/dehydroxylation has also been observed in dog calculus and 19th century human calculus⁶, where other markers, e.g. a prevalence of nitriles, with amides very minor, deriving from dehydrating conditions in the mouth of dogs and humans (though not sheep⁶) supporting this view⁶. The presence of carbon dioxide is also consistent with a lipid, amino acid/protein and carbohydrate component in this sample. Notably, these findings did not reveal key biomarkers typically observed in soils^{20,23}. It should also be noted that highly polar material could have been present, which would not have successfully eluted from the column, or indeed volatilised sufficiently in the probe.



1199

1200 **Supplementary Figure 13.** Reconstructed total ion chromatogram of the pyrogram
 1201 (pyrolysis profile) (610 °C for 10s) of sample DL5838, after thermal desorption (310 °C for
 1202 10 s). Key to Fig. 13: Peak identities (x indicates carbon chain length): filled squares, Cx
 1203 indicates alkenes; filled circles, Cx indicates alkanes; open diamonds, Cx:y indicates acyclic
 1204 nitriles; blue filled hexagons indicates alkyl pyridines; green filled pentagons indicates alkyl
 1205 pyrroles (with alkyl chain length above, Cx); filled hexagons with filled circles attached
 1206 indicates alkyl phenols; open pentagons with numerals inside indicates carbohydrate
 1207 pyrolysis markers, 1 is 2-methylfuran, 2 is 2-methyl-2-cyclopenten-1-one and 3 is 2,3-
 1208 dimethyl-2-cyclopentene-1-one. Also shown are the structures of twelve aromatic compounds
 1209 identified: pyridine, pyrrole, toluene, styrene, benzonitrile, phenol, indene, benzyl nitrile,
 1210 benzenepropanenitrile, indole, 3-methylindole (skatole) and 3-ethylindole. CO₂ indicates
 1211 carbon dioxide. Inset displays a reconstructed total ion chromatogram of the thermal
 1212 desorption profile (310 °C for 10 s) of this sample. Peak identities: BPN indicates a
 1213 benzenepropanenitrile derivative; open diamond, Cx:y indicates an acyclic nitrile. Also
 1214 shown are four aromatic compounds: pyridine, pyrrole, benzenepropanenitrile and indole.
 1215 CO₂ indicates carbon dioxide.

1216

1217 **Discussion Isbister**

1218 Fifteen samples of dental calculus from fifteen individuals from Isbister, Orkney were
1219 analysed by sequential thermal desorption-gas chromatography-mass spectrometry (TD-GC-
1220 MS) and pyrolysis-gas chromatography-mass spectrometry (Py-GC-MS).

1221 The TD-GC-MS analyses revealed very little organic material in thirteen of the fifteen
1222 samples. Of the two samples in which ‘free’ organic compounds were detected, sample
1223 DL2104 revealed the 2,5-diketopiperazine derivative (DKP) of proline-glycine (pro-gly),
1224 along with a far lesser amount of the DKP of proline-alanine (pro-ala). These two
1225 biomarkers, along with carbon dioxide, dominated the thermal desorption profile as the only
1226 ‘free’ organic compounds detected, indicating little free, thermally extractable, organic
1227 components in this sample. These thermally generated DKPs suggest the amino acids proline,
1228 glycine, and alanine were constituents in a protein^{5,12,13}, presumably from food consumed by
1229 this individual (see below). Eight of the samples produced acetonitrile at the chosen thermal
1230 desorption temperature, which may suggest a proteinaceous component high in phenylalanine
1231 and/or tyrosine (see below). The only other calculus sample containing ‘free’ organic
1232 constituents was DL5838; in addition to the ubiquitous carbon dioxide, nitrogen-containing
1233 compounds dominated the profile; these included pyridine, pyrrole, benzenepropanenitrile,
1234 indole, an unidentified derivative of benzenepropanenitrile and the C_{16:0} nitrile. These
1235 nitrogen-containing compounds may be indicative of thermally-labile proteinaceous
1236 constituent, with the aliphatic nitrile also indicating a lipid component which has combined
1237 with the protein⁵. A furan derivative was also identified, which is tentatively indicative of a
1238 carbohydrate constituent (but see Py-GC-MS below).

1239 Albeit in minor amounts, the C₂₇, C₂₉ and C₃₁ (trace) *n*-alkanes, with the C₂₉ *n*-alkane
1240 predominating, were also detected. This is indicative of a higher plant wax origin (e.g. from
1241 leafy greens)⁶. Moreover, although the interpretation has to be somewhat tentative due to the
1242 relatively low abundance of these compounds, the C₂₉ *n*-alkane is notably dominant in
1243 Brassicaceae epicuticular leaf waxes such as *Brassica oleracea* (cabbage)²⁷⁻²⁹ and *Coincya*
1244 spp.³⁰ and this has also been found in archaeological studies where *Brassica* leaves (cabbage)
1245 have been identified^{29,31}. In modern and archaeological studies the C₂₉ *n*-alkane accounted for
1246 over 90% of the hydrocarbon fraction, with the C₂₉ *n*-alkane/total *n*-alkanes ratios being:
1247 ~95:5 and ~96:4 for *Brassica* and *Coincya* respectively²⁷⁻³⁰. The C₂₉ *n*-alkane/total *n*-alkanes
1248 ratio for DL5838 is 96:4 and is consistent with a Brassicaceae source. However, it should be
1249 noted that *Brassica* spp. vegetables such as cabbages and turnips would not have been

available in Neolithic Orkney, since they are not native³². Those Brassicaceae plants which are native to Orkney and possible sources of leafy greens include *Cakile maritima* (sea rocket), *Crambe maritima* (sea kale), *Cochlearia officinalis* (scurvy grass) and *Nasturtium officinale* (watercress)³². However, the very high C₂₉ *n*-alkane predominance has been linked to glaucousness in Brassicaceae leaf wax³⁰. This is notable since sea kale (*Crambe maritima*) has a distinct blue-grey-green waxy (glaucous) appearance in contrast to the other Brassicaceae native to Orkney³³⁻³⁵, reflecting the significant amount of wax on its leaves, with the dominance of the C₂₉ *n*-alkane making the wax monolayers/sheets more uniform and so providing a more protective and less permeable barrier³⁰. This protects against light, temperature and wind, but the amount of epicuticular leaf wax has also been linked to protection against salt stress³⁶, with an increase in wax production linked to significant salt exposure³⁶, which is consistent with *Crambe maritima*'s habitat of shingle beaches. Moreover, it's interesting to note that the distribution of *Crambe maritima* is limited in northern Scotland and Orkney, to South Ronaldsay's east coast shingle beaches, only a few miles from Isbister itself³⁷. Sea kale is mentioned by Pliny the Elder as a sailor's anti-scurvy remedy³⁸ and by Mrs Beeton as a 'type of asparagus'³⁹, following botanist William Curtis's recommendation of its use as a vegetable in Britain in 1799³⁴ and its use as a vitamin C-rich vegetable is well recognised, despite its use dramatically falling in recent times due to over-exploitation³³⁻³⁵.

In contrast to the TD-GC-MS analyses, the Py-GC-MS results revealed appreciable amounts of organic material in thirteen of the fifteen calculus samples, with nine containing moderate to major abundances of organic compounds. This indicates that although the cool environment on Orkney has allowed the survival of a wide range of biomolecules relating to fats/oil, proteins and carbohydrates (see below), diagenesis and biodegradation have resulted in the loss of most, if not all, free biomarkers. In contrast, bound/polymeric biomacromolecules (some clearly resulting from pre- and/or post-depositional biochemical reactions between the constituents of the original foods consumed) have been appreciably well preserved, albeit the more resilient moieties and compound classes are likely to have been preferentially preserved.

The pyrolysis-GC-MS analyses were dominated by a complex suite of organic compounds indicative of lipid (fat/oil), protein and carbohydrate components. These include a series of C₈/C₉ to C₁₆ (up to C₂₀ where more organic material was present) *n*-1-alkenes and *n*-alkanes, typical of unsaturated and saturated fat/oil-derived acyl lipids^{5,6}. A bimodal distribution with

maxima at C₁₁/C₁₂ and C₁₄ combined with abundant short chain (C₄-C₇) *n*-1-alkenes in eleven of the fifteen samples analysed suggests the original acyl lipids in these samples are likely to have been high in polyunsaturated fatty acids. The absence of these compounds in the TD profile provides molecular evidence for a polymeric/bound acyl lipid source. However, in addition to *n*-1-alkenes and *n*-alkanes, typical of fat/oil derived lipids, the pyrograms also displayed a series of C₁₀ to C₁₆ (up to C₂₀ where more organic material was present) iso-methyl-1-alkenes (isoalkenes) and 2-methylalkanes (isoalkanes), presumably deriving from the branched fatty acids which are significant components of bacterial triglycerides.

In thirteen of the samples analysed there were components in the pyrolysate which can be indicative of 'black carbon', i.e. charcoal or soot⁷⁻¹⁰. However, although this included the presence of naphthalenes, the dominance of toluene, rather than the normally dominant aromatic hydrocarbon benzene, is notably atypical of chars⁷⁻¹⁰. The markers identified here can also be indicative of proteins^{6,11} and combined with the abundant related protein markers pyrrole, benzonitrile, benzyl nitrile (phenylacetone nitrile), benzenepropanenitrile and indoles^{6,11} identified in six of the samples this is likely to be the main origin of these compounds in these samples, pointing to a substantial protein-derived component^{6,11}. This is also corroborated by the presence of aliphatic nitriles (carbon numbers C_{14:0}, C_{16:0} (max) and C_{18:0}) suggesting a lipid (fat/oil) in these same six samples which has combined with protein-derived nitrogen to produce these moieties¹. The proteinaceous origin for the aromatic markers is further corroborated by the significant abundance of phenol, methylphenols, and dimethylphenols, all of which, collectively, are indicative of the amino acid tyrosine¹²⁻¹⁴ (in turn, also consistent with the acetonitrile in the TD-GC-MS analyses). With an absence of guaiacol itself (all isomers) and other guaiacol moieties characteristic of lignin^{15,16}, the possibility that the phenols may originate from wood or woody plant material can be excluded. Alkyl phenols have also been observed in wood smoke and smoked foods^{17,18}, however the absence of methoxyphenols, diagnostic of lignin-derived smoke, means this possibility can also be discounted. Taken together, the presence of toluene, styrene and phenylacetone nitrile (benzyl nitrile) can indicate the amino acid phenylalanine^{12,13,40}, which is also consistent with the acetonitrile observed in the thermal desorption profiles of a number of the Isbister calculus samples. In addition, the major constituents indole and 3-methylindole (skatole), with a lesser amount of a C₂ indole (3-ethylindole?) in the two most organically-rich samples, were also identified, which are consistent with a tryptophan amino acid source^{12,13,19,20}. Proteins rich in the amino acids tyrosine, phenylalanine and tryptophan include meat, fish and dairy products,

although without further biomolecular corroboration the presence of bacterial markers means the possibility that at least a partial origin could be microbial cannot be excluded.

Far more diagnostic of a particular dietary source are the particular suite of alkyl pyrroles observed in the pyrogram of six of the samples (DL42, DL2104, DL86, DL715, DL5119 and DL5838), with an alkyl chain length of up to C₆ (see Table 1). The presence of C₅ alkyl pyrroles in three of these six samples and C₆ alkyl pyrroles in two of them, in addition to the C₁ to C₄ moieties in all six, is highly notable. In sample DL5838, which contained the highest abundance of observed biomacromolecularly-derived compounds in the analyses, and therefore provided the most detailed chemical information on this source of organic material, it revealed very significant amounts of these biomarkers. This included the 2- and 3-dimethylpyrroles, 2- and 3-ethylpyrroles, 2,4-, 2,3- and 3,4-dimethylpyrroles, four ethylmethylpyrroles, two trimethylpyrroles, two propylmethylpyrroles, seven ethyldimethylpyrroles, four diethylpyrroles, 2,3,4,5-tetramethylpyrrole, a methylisobutylpyrrole, two diethylmethylpyrroles, a dimethyl-*n*-propylpyrrole, 3-ethyl-2,4,5-trimethylpyrrole, and three C₆ alkyl pyrroles, tentatively identified as 3-ethyl-4-isobutylpyrrole, 3-methyl-4-neopentylpyrrole and 2-methyl-3-ethyl-4-*n*-propylpyrrole. These specific C₁ to C₆ alkyl pyrroles are highly diagnostic of the presence of a significant tetrapyrrole component. Their porphyrin-derived origin, and the relative abundance of some of the key biomarkers, is indicative of an algal source observed in fossils and expected in similarly diagenetically altered archaeological material^{11,21,22}, (see below). Although C₁ and C₂ alkyl pyrroles can also derive from protein-derived amino acids such as glycine, proline, hydroxyproline, serine and glutamic acid^{12-14,20,40,41}, the C₃+ alkyl pyrroles identified in these six samples point to a tetrapyrrole source^{21,22,40,41}. Moreover, the same specific C₁ to C₅ alkylpyrroles identified in the Py-GC-MS of an algal kerogen^{21,22} were observed in DL5838 (see Table 2).

As a ‘chemical fossil’, algae can be expected to reveal a distinctive suite of alkylpyrroles when analysed by pyrolysis-GC-MS^{21,22}. Key research in this area studied the composition of kerogen, i.e. macromolecular fossil organic matter, from the Miocene Monterey Formation, California, USA. The Monterey Formation is an area of marine deposits, known for its many fossils, most notably a significant number of large soft-bodied seaweeds, which are rarely found as fossils elsewhere⁴². These largely occur in the siliceous shales (diatomite), which are

formed from diatoms and other silica-producing microalgae⁴³ and a sediment sample was taken from the Upper Miocene Monterey Formation siliceous-carbonate rock at Santa Barbara County for organic geochemical investigation^{21,22} where, notably, fossil seaweed from the Upper Miocene has also been observed. Although microalgae are reasonably understood to be the primary source of organic matter in the Monterey kerogen, based on geological and organic geochemical data to date⁴³, macroalgae as a source of kerogen has usually been overlooked^{44,45}, with several studies showing macroalgae (Rhodophyta; red seaweeds) to be a major source of kerogen in some sedimentary environments,⁴⁴⁻⁴⁶. The organic geochemical investigation of the Monterey kerogen using Py-GC-MS revealed a highly distinctive suite of alkylpyrroles, which can be related chemically to the monomer building blocks constituting the tetrapyrrole pigments of chlorophylls, protoporphyrin IX and bilirubin^{21,22}. The study concluded that the abundant tetrapyrrole pigments were likely to derive from the major algal contribution to the sediments of the Monterey kerogen, preservation characteristics or a combination of the major algal input and the particular preservation conditions, with the same alkylpyrrole distributions also observed in similar kerogens from immature upwelling sedimentary environments such as the Namibian Shelf^{21,22}. This is consistent with the geological and organic geochemical evidence for abundant marine algae within the Monterey Formation, where ‘Organic matter is overwhelmingly amorphous marine algal debris’⁴³.

More specifically, the highly unusual alkyl pyrroles, 3-methyl-4-isobutylpyrrole, 2,3-dimethyl-4-*n*-propylpyrrole, 3-ethyl-4-isobutylpyrrole, 3-methyl-4-neopentylpyrrole and 2-methyl-3-ethyl-4-*n*-propylpyrrole (see Table 1) are known to derive from the C₈ side chain of bacteriochlorophylls c and d⁴⁷ and are biomarkers for the relatively rare bacteriochlorophylls c and d which are found in green bacteria such as *Chloroflexus* sp.⁴⁷ and are known to naturally occur in association with intertidal red seaweeds such as *Porphyra umbilicalis*⁴⁸⁻⁴⁹. Overall the six samples display a remarkably similar pyrolysis profile to algal-derived kerogen previously studied via very similar organic geochemical techniques^{21,22}.

1374

1375 **Supplementary Table 2. C₁ to C₆ alkyl pyrrole compounds¹ present in the Py-GC-MS of**
 1376 **the Isbister dental calculus samples**

Compound (alkyl pyrrole)	DL42	DL2104	DL86	DL715	DL511 9	DL583 8
2-methylpyrrole	Major	major	major	major	Major	major
3-methylpyrrole	√√√√√√	√√√√√√	√√√√√	√√√	√√√√	√√√
2-ethylpyrrole	√√√	√√√√	√√	√√√	√√√√	√√√
3-ethylpyrrole	√√	√√	√√	√	√√	√
2,5-dimethylpyrrole	-	-	-	-	-	-
2,4-dimethylpyrrole	√√√	√√√	√√√	√√√	√√	√√√
2,3-dimethylpyrrole	√√√	√√√	√√	√√	√√√	√√
3,4-dimethylpyrrole	√	√	√	√	√	√
ethylmethylpyrrole (2-Et-4(?)-Me)	-	-	-	√√	√√√	√√
ethylmethylpyrrole (2-Et-3(?)-Me)	√√	√√√	√√	√√	√√	√√√
4-ethyl-2-methylpyrrole	√√	√	√	√	√	√
2,3,5-trimethylpyrrole	√	√	√	√√	√√	√√
3-ethyl-4-methylpyrrole	√	√	√	√	√	√√
2,3,4-trimethylpyrrole	-	-	-	√	√	√
Propylmethylpyrrole	√	√	√	√	√	√
Ethyldimethylpyrrole	-	-	-	-	-	√
2,5(?)-diethylpyrrole	-	-	-	√	√	√

Ethyldimethylpyrrole	-	-	-	-	√	√
Propylmethylpyrrole	-	-	-	-	√	√
2,4(?)-diethylpyrrole	-	-	-	-	-	√
Ethyldimethylpyrrole	-	-	-	-	-	√
Ethyldimethylpyrrole	√	√	√	√	-	√
4-ethyl-2,3-dimethylpyrrole	√	√	-	√	√	√
3-ethyl-2,4-dimethylpyrrole	-	-	-	√	-	√
2,3(?)-diethylpyrrole	-	-	-	√	√	√
Propylmethylpyrrole	-	-	-	-	-	√
3,4(?)-diethylpyrrole	-	-	-	-	-	√
Ethyldimethylpyrrole	-	-	-	-	-	√
2,3,4,5-tetramethylpyrrole	-	-	-	-	-	√
3-methyl-4-isobutylpyrrole	-	-	-	-	-	√
2,3?-diethyl-5-methylpyrrole	-	-	-	√	√	√
2,3-diethyl-4-methylpyrrole	-	-	-	-	-	√
2,3-diethyl-4- <i>n</i> -propylpyrrole	-	-	-	√	-	√
3-ethyl-2,4,5-trimethylpyrrole	-	-	-	-	√	√
3-ethyl-4-isobutylpyrrole	-	-	-	-	-	√
2,3-diethyl-4,5-dimethylpyrrole	-	-	-	-	-	-
2,4-diethyl-3,5-dimethylpyrrole	-	-	-	-	-	-
3-methyl-4-neopentylpyrrole	-	-	-	√	-	√
2-methyl-3-ethyl-4- <i>n</i> -propylpyrrole	-	-	-	-	-	√

¹After Sinninghe Damste *et al.* 1992²²

Supplementary Table 3. C₁ to C₅ alkyl pyrrole compounds present in the Py-GC-MS of natural sources of tetrapyrroles¹ and Isbister dental calculus sample DL5838

Compound (alkyl pyrrole)	DL5838	Fossil algae (M. K. [†])	Bilirubi n	Chlorophyll- a	Protoporphyrin- IX
2-methylpyrrole	(√√√√√/*)	√	√	√	√
3-methylpyrrole	(√√√*)	√√	√	√√	√√
2,5-dimethylpyrrole	-	√	√	-	√
2,4-dimethylpyrrole	√√√	√√	√	√	√√
2,3-dimethylpyrrole	√√	√√	√	√	√√
3,4-dimethylpyrrole	√	√√	√	√	√√
ethylmethylpyrrole	√√	√	√	√	√
ethylmethylpyrrole	√√√	√	√	√	√
4-ethyl-2- methylpyrrole	√	√	√	√	√
2,3,5-trimethylpyrrole	√√	√√	√√	√	√√
3-ethyl-4- methylpyrrole	√√	√√√	√√	√√√	√√
2,3,4-trimethylpyrrole	√	√√	√√	√	√√√
ethyldimethylpyrrole	√	√	√	√	√
ethyldimethylpyrrole	√	√	√	√	√
4-ethyl-2,3- dimethylpyrrole	√	√√	√√	√√	√√

3-ethyl-2,4-dimethylpyrrole	√	√√√	√√√	√√√	√√√
2,3-diethyl-4-methylpyrrole	√	√	√	√	√
3-ethyl-2,4,5-trimethylpyrrole	√	√√	√√	√√	√√

¹After Sinninghe Damste *et al.* 1992²²

[†]Monterey kerogen (Sinninghe Damste *et al* 1992)²²

*High due to elevated levels of nitrate in environment (see text)

Having identified specific algal biomarkers in six of the fifteen samples, with a further six producing fewer organic compounds, but tentatively indicative of the same biological source, it is appropriate to consider the other compound classes (proteins, lipids and carbohydrates) in this context. Although diagenetic changes may have altered the original protein composition, protein markers observed in the Py-GC-MS of the six ‘algal’-containing samples suggest those high in the aromatic amino acids phenylalanine, tyrosine and tryptophan, in addition to significant levels of glycine, alanine, cysteine, proline (and possibly hydroxyproline), serine, arginine, glutamic acid and aspartic acid (see Table 3), were significant in the original food consumed. The TD-GC-MS was also indicative of a proteinaceous component relatively high in phenylalanine and glycine, proline and alanine. This is highly significant when combined with the presence of the abundant algal markers; seaweeds (macroalgae), especially red types, can be high in protein (up to 30-50% dry weight)⁵⁰⁻⁵⁴ and green, brown and red types contain significant amounts of these particular amino acids⁵¹⁻⁵⁵, with some species being higher in phenylalanine, tyrosine and tryptophan than egg proteins⁵³. Seaweed is known to have been exploited for food in antiquity⁵⁶, although its consumption by humans on Orkney seems only to have been tentatively considered to date^{2,57}.

1405 **Supplementary Table 4. Protein biomarkers¹ present in the Py-GC-MS of the Isbister**
1406 **dental calculus samples (relative %) and the protein-based amino acid sources from**
1407 **which they derive**

Compound (protein pyrolysis biomarkers)	Amino acid source	DL42	DL21 04	DL86	DL71 5	DL51 19	DL58 38
Pyrrole	pro	9.1	13.4	13.4	13.2	41.0	8.7
2-methylpyrrole	gly, pro, hypro, ser, glu, tetrapyr	6.8	6.9	9.7	15.7	13.9	13.2
3-methylpyrrole	gly, pro, hypro, ser, glu, tetrapyr	3.2	3.2	3.8	4.7	4.1	4.5
2-ethylpyrrole	gly, pro, hypro, ser, glu, tetrapyr	0.9	1.4	1.4	3.0	6.6	4.7
3-ethylpyrrole	gly, pro, hypro, ser, glu, tetrapyr	0.4	0.6	0.4	0.9	0.6	1.0
Pyridine	ala (asp?)	4.2	3.2	4.1	2.4	2.1	2.1
4-methylpyridine	ala, cys (asp?)	1.3	2.6	1.6	0.6	1.0	2.1
2-methylpyridine	ala (asp?)	1.1	1.6	0.5	0	0.2	0
2,4-dimethylpyridine	ala (asp?)	0.8	1.4	1.8	0.6	0	0.5
2,6?-dimethylpyridine	ala (asp?)	0.1	0.4	0	0	0	0.1
a C3 pyridine	ala (asp?)	0.4	0.5	0.1	0.3	0	1.6
1H-pyrazole-4- carbonitrile	arg(?)	0.5	0.4	0.4	0.4	0.05	0.1
2-pyridinecarbonitrile	arg(?)	0.8	0.8	0.2	0.2	0.4	0.7

Pyrimidines	arg	0.2	0.3	0	0.2	0	1.6
Imidazoles	arg, glu?	0	0	0	0	0	0.5
Toluene	phe	34.1	35.6	39.4	40.4	22.6	31.4
Ethylbenzene	phe	0.6	0.8	4.1	2.3	0.9	3.5
o+m-xylene	phe	1.1	0.9	1.4	1.4	0.2	1.4
p-xylene	phe	0	0	0	0	0	0
Styrene	phe	3.8	2.6	3.6	3.1	1.3	2.1
Benzonitrile	phe	1.5	0.9	1.1	0.6	0.6	0.7
ethylcyanobenzene	phe	0.4	0.4	0.2	0.2	0	0.3
benzyl nitrile	phe	2.5	2.2	1.2	1.1	1.3	2.4
benzenepropanenitrile	phe	3.0	2.6	1.2	1.7	0.8	3.1
Phenol	tyr	4.5	3.7	1.4	0.6	0.5	0.7
2-methylphenol	tyr	1.1	0.9	0.04	0	0	0.3
4-methylphenol	tyr	4.2	2.1	0.9	0.4	0.3	0.3
3-methylphenol	tyr	0	0	0	0.1	0	2.8
3,4?-dimethylphenol	tyr	0	0	0	0	0	0.3
2,4-dimethylphenol	tyr	0.8	0.9	0	0	0	0.2
2,6-dimethylphenol	tyr	0.4	0.3	0	0	0	0
4-ethylphenol	tyr	0	0	0	0.1	0	0.5
o-tolyl isocyanide(?)	trp	0.6	0.8	0.2	0.1	0.05	0.9

Indole	trp	3.8	2.9	1.8	1.9	0.6	3.5
3-methylindole	trp	3.0	1.8	0.6	0.7	0.2	1.9
3-ethylindole	trp	0.4	0.3	0	0	0	0.5
Non/less-specific protein markers							
Benzene	-	1.1	1.1	3.8	1.4	0	0
Indene	-	1.3	0.9	1.1	1.0	0.4	1.6
a methylindene	-	1.1	0.8	0.6	0.7	0.4	0
a methylindene	-	0.6	0.3	0.04	0.1	0	0
a C ₂ indene	-	0.4	0.3	0	0	0	0
Protein markers – Total Peak Area		100.1	99.8	100.0 8	100.1	100.1	99.8

¹After Stankiewicz *et al* 1996¹²; Stankiewicz, Hutchins *et al* 1997¹³; Stankiewicz, Briggs *et al* 1997¹⁴; Poinar and Stankiewicz 1999⁵⁸.

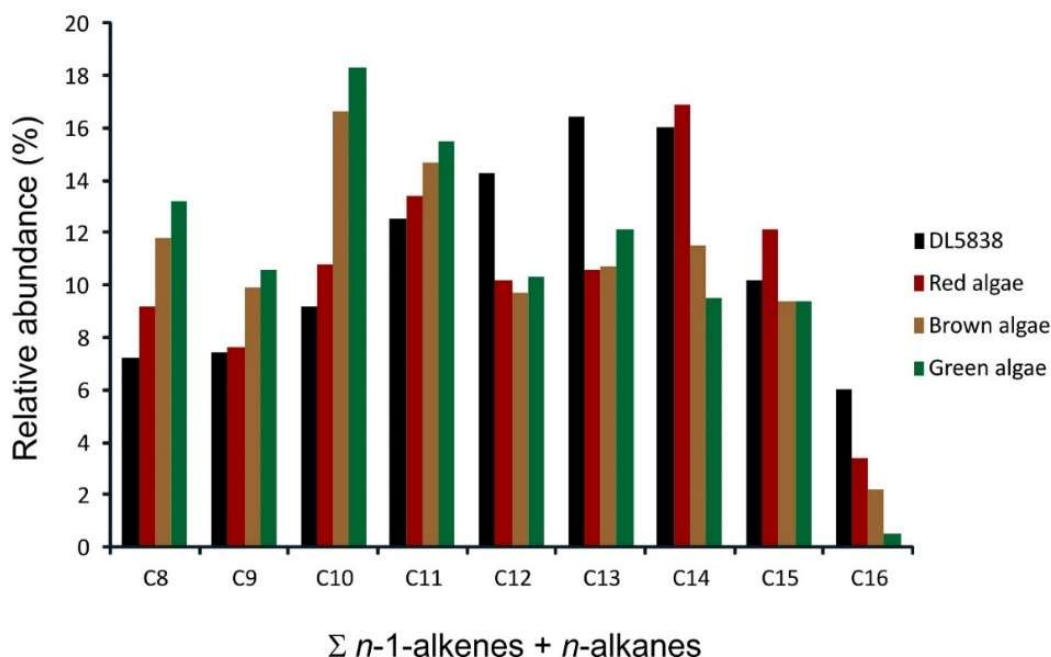
gly = glycine; pro = proline; hypro = hydroxylproline; ser = serine; glu = glutamic acid; ala = alanine; asp = aspartic acid; cys = cysteine; arg = arginine; phe = phenylalanine; tyr = tyrosine; trp = tryptophan; tetrapyr = tetrapyrrole

Although biomarkers for aspartic and glutamic acids are relatively low given their usual high abundance in fresh seaweeds, they would be expected to be preferentially degraded when compared to the aromatic amino acids such as phenylalanine and tyrosine. The high relative abundance of the C₁ and C₂ alkyl pyrroles may be explained by high nitrate levels in the environment from anthropogenic activity (agricultural and waste products entering the coastal waters close to the areas of seaweed exploitation), which has been shown to produce high levels of serine in macroalgae (seaweeds)⁵⁹ and terrestrial plants⁶⁰⁻⁶². Serine in the

macroalgae exposed to high nitrate levels was shown to be ~10% compared with the reference site of ~7% (the amino acid alanine, which does not produce alkyl pyrroles in Py-GC-MS, is essentially unaffected)⁵⁹. This nitrate can also raise the $\delta^{15}\text{N}$ values; *Catenellanipae* being raised by ~3.5 to 16.5‰ where appreciably elevated levels of NO_3^- was present⁵⁹. This translates to significantly increased levels of methyl and ethyl pyrroles in the Py-GC-MS analyses, deriving from the high serine content (their formation from serine during pyrolysis studied in some detail mechanistically⁶³).

As discussed above, the bimodal distribution of *n*-1-alkenes/*n*-alkanes with maxima at $\text{C}_{11}/\text{C}_{12}$ and C_{14} , combined with abundant short chain ($\text{C}_4\text{-C}_7$) *n*-1-alkenes, suggests the original acyl lipids are likely to have been high in polyunsaturated fatty acids, which is consistent with a marine – seaweed – source. Research on seaweeds has shown that green, brown and red seaweeds differ in the relative abundances of the particular unsaturated fatty acids present in their lipid component^{52,64-69}. Green seaweeds are high in C_{16} and C_{18} polyunsaturated fatty acids, with C_{20} acids in minor abundance and C_{22} acids very minor or absent; brown seaweeds have less abundant C_{16} acids, with C_{18} acids being major and C_{20} acids more significant than green; red seaweeds have C_{20} polyunsaturated acids dominating, with significant amounts of C_{22} acids^{52,64-69}. Although the polyunsaturated mid and long chain moieties characteristic of a marine macroalgal input were not detected, these compounds are particularly labile and susceptible to degradation. Perhaps notably, the expected biopolymeric products generated during pyrolysis if these biomolecules were incorporated into a bound organic fraction would be expected to maximise at $\sim\text{C}_{11}/\text{C}_{12}$ and $\sim\text{C}_{14}$ *n*-1-alkene/*n*-alkanes, i.e. promote a bimodal distribution of alkene/alkanes, as is observed in these samples. Moreover, previous studies specifically on the Py-GC-MS analysis of seaweed at similar pyrolysis temperatures (500/600°C) have revealed the C_{13} *n*-1-alkene and *n*-alkane in equal abundance, yet the C_{14} *n*-1-alkene in significantly higher quantity than both of these lipid markers, while the C_{14} *n*-alkane is not reported as a constituent of the pyrolysate, suggesting it is minor or absent⁷⁰; this is mirrored in the six samples containing macroalgal biomarkers. In addition, the same study revealed significant amounts of the protein markers phenol, 3-methyl phenol, benzenepropanenitrile, indole, 3-methylindole, and hexadecanenitrile, as also observed in the six Isbister samples. The microbial biomarkers (e.g. branched hydrocarbons) observed are consistent with the iso-and anteiso-branched chain fatty acids normally present in marine macroalgae (seaweeds)⁶. Although other possible food sources high in polyunsaturated fatty acids could also explain this molecular profile, the combination of

tetrapyrrole, protein and lipid biomarkers identify a seaweed component in these samples. Looking in more detail, the pyrolysis lipid profiles observed can be generated in appreciable amounts by the C_{20:4} and C_{20:5} fatty acids, but can also be indicative of significant abundances of C_{18:3} and C_{18:4} fatty acids, as are present in seaweeds^{52,64-69}; differences can, albeit somewhat tentatively, be related to typical expected biomarkers profiles from green, brown and red seaweeds. In this context, it is interesting to note that when relative abundance of the sum of *n*-1-alkenes/*n*-alkanes for carbon chain numbers C₈ to C₁₆ is plotted for green, brown, red and sample DL5838 (the sample with the highest abundance of organic material and macroalgal biomarkers), the distributions suggest the Isbister sample is most similar to red seaweeds (Fig 14).



Supplementary Figure 14. Histogram showing a comparison of the sum of the *n*-1-alkene + *n*-alkane distributions (Σ *n*-1-alkene + *n*-alkane) (C₈ to C₁₆ carbon numbers) of calculus sample DL5838 from Isbister (highest organic content and most abundant macroalgal biomarkers) and modelled relative abundances predicted for red, brown and green macroalgae (seaweed). These are based on their fatty acid composition and subsequent oxidative polymerisation and defunctionalisation, as observed in archaeological and ancient biomacromolecules – ‘chemical fossils’ – in previous studies^{5,71,72}. Source data are provided as a Source Data file.

Although the C_{12} and C_{13} moieties are somewhat high, this may be explained either by a small input from marine fish where those with a high oil content, in particular, contain high levels of docosahexaenoic acid ($C_{22:6}$; DHA)⁷³⁻⁷⁷, which is normally low in macroalgae, or a red seaweed high in both $C_{20:4}$ and $C_{22:6}$, such as *Palmaria palmata* (Dulse)⁶⁴. This would be expected to increase the relative abundance of the C_{12} and C_{13} moieties.

Previous research on human remains from Isbister has suggested that these individuals had a largely terrestrial diet, although it has been conceded that females may have exploited seaweed or shellfish as part of their diet². This same research has observed: ‘The values (–19.9 to –21.2‰) suggest little or no use of marine protein’, this despite the location of the monument directly on the shore, the abundant remains of marine fish found in the tomb and the historic importance of fishing in Orkney (Colley 1983a, 1983b)⁵⁷. However, this appears to ignore the fact that macroalgae (seaweeds) can have a very wide range of stable isotopic values for $\delta^{13}C$ (–12 to –36), despite this - and the possibility of some seaweed consumption by females - being noted in connection with this research^{2,78}. In fact, the isotopic data could also be consistent with a significant seaweed diet (a largely seaweed human diet at Isbister could produce the values previously reported for the site^{2,57}), with a small input from marine fish⁷⁹.

Importantly, the findings presented here show seaweed (with specific biomarkers consistent with red seaweeds), and therefore marine protein, formed a significant part of the diet for both males and females at Isbister. Although diagenesis means the biomolecular information is not necessarily reflecting the original diet consumed, it nevertheless shows the organic material entrapped within the dental calculus is providing profound insights into palaeodiets not evident by other means. Moreover, it shows the exploitation of a nutritious and readily available food resource, which has been surprisingly underplayed, if not dismissed, as a valued staple in Neolithic Orkney.

National Museums Scotland human remains collection

Fifteen samples of dental calculus taken from fourteen individuals from eight sites in Scotland, curated by National Museums Scotland, were analysed by sequential thermal desorption-gas chromatography-mass spectrometry (TD-GC-MS) and pyrolysis-gas chromatography-mass spectrometry (Py-GC-MS). This technique facilitates the identification of both free/unbound and bound/polymeric organic components. All fifteen calculus samples

1506 produced very little free organic material. The fifteen samples revealed highly variable
1507 amounts of a bound/polymeric organic constituent.

1508
1509 **Quanterness passage tomb, Orkney**

1510 Quanterness is a large, Maeshowe-type passage tomb on the mainland island of Orkney. It
1511 was excavated by Colin Renfrew in 1972–74⁸⁰. It is located in one of Europe's richest
1512 Neolithic landscapes containing numerous chambered tombs, two stone circles, the stone-
1513 built Neolithic village of Skara Brae and the huge ceremonial settlement of Ness of Brodgar
1514 whose *floruit* was c. 3200–2800 BC⁸¹. Wild resources including large numbers of bones from
1515 inshore and deep-sea fish, birds and wild plants were found at some of these sites, in addition
1516 to agricultural produce.

1517
1518 **16. Quanterness passage tomb, Orkney (NMS unreg): Bag 10/Box 4 Bag 209 (bag**
1519 **4) 1139-01: M1 right lingual (calculus: 1.55 mg)**

1520 The thermal desorption total ion chromatogram (TIC) revealed carbon dioxide as the only
1521 detectable component, indicating the essential absence of free, thermally extractable organic
1522 components in this sample. It should be noted, however, that highly polar material could have
1523 been present, which would not have successfully eluted from the column, or indeed
1524 volatilised sufficiently in the probe. It is not possible to determine the significance of the
1525 carbon dioxide, beyond it being indicative of oxidised organic material.

1526 The pyrolysate TIC revealed carbon dioxide, C₄ and C₅ 1-alkenes, cyclopentadiene and
1527 benzene as the only detectable components. The dominance of carbon dioxide, combined
1528 with the relatively minor abundance of the few small organic compounds present, suggests a
1529 highly oxidised sample. It is not possible to determine the significance of the carbon dioxide,
1530 beyond it being indicative of oxidised organic material and the other compounds are too low
1531 an abundance and too ubiquitous to provide any meaningful interpretation of their presence,
1532 beyond being indicative of an organic origin. It should be noted, however, that highly polar
1533 material could have been present, which would not have successfully eluted from the column,
1534 or indeed volatilised sufficiently in the probe.

17. Quanterness passage tomb, Orkney (NMS unreg): Box 10/Box L/Bag 216 (bag 2) 1190-A?1: M2 left labial (calculus: 2.76 mg)

The thermal desorption total ion chromatogram (TIC) revealed carbon dioxide as the only detectable component, indicating the essential absence of free, thermally extractable organic components in this sample. It should be noted, however, that highly polar material could have been present, which would not have successfully eluted from the column, or indeed volatilised sufficiently in the probe. It is not possible to determine the significance of the carbon dioxide, beyond it being indicative of oxidised organic material.

The pyrolysate TIC revealed carbon dioxide, C₄ and C₅ 1-alkenes, cyclopentadiene and benzene as the only detectable components. The dominance of carbon dioxide, combined with the relatively minor abundance of the few small organic compounds present, suggests a highly oxidised sample. It is not possible to determine the significance of the carbon dioxide, beyond it being indicative of oxidised organic material and the other compounds are too low an abundance and too ubiquitous to provide any meaningful interpretation of their presence, beyond being indicative of an organic origin. It should be noted, however, that highly polar material could have been present, which would not have successfully eluted from the column, or indeed volatilised sufficiently in the probe.

18. Quanterness passage tomb, Orkney (NMS unreg): Box 10/Box K/bag 203 (Bag 3) 249-01: M2 right lingual (calculus: 5.38 mg)

The thermal desorption total ion chromatogram (TIC) revealed carbon dioxide as the only detectable component, indicating the essential absence of free, thermally extractable organic components in this sample. It should be noted, however, that highly polar material could have been present, which would not have successfully eluted from the column, or indeed volatilised sufficiently in the probe. It is not possible to determine the significance of the carbon dioxide, beyond it being indicative of oxidised organic material.

In contrast to the thermal desorption profile, the pyrolysis-GC-MS TIC (Fig. 15) is dominated by a complex suite of organic compounds. These include a series of C₇ to C₁₈ *n*-1-alkenes and *n*-alkanes, typical of unsaturated and saturated fat/oil-derived acyl lipids^{5,6}. The absence of these compounds in the TD profile provides molecular evidence for a polymeric/bound acyl lipid source. However, in addition to *n*-1-alkenes and *n*-alkanes, typical of fat/oil derived lipids, the pyrogram also displayed a series of C₉ to C₁₆ iso-methyl-1-alkenes (isoalkenes)

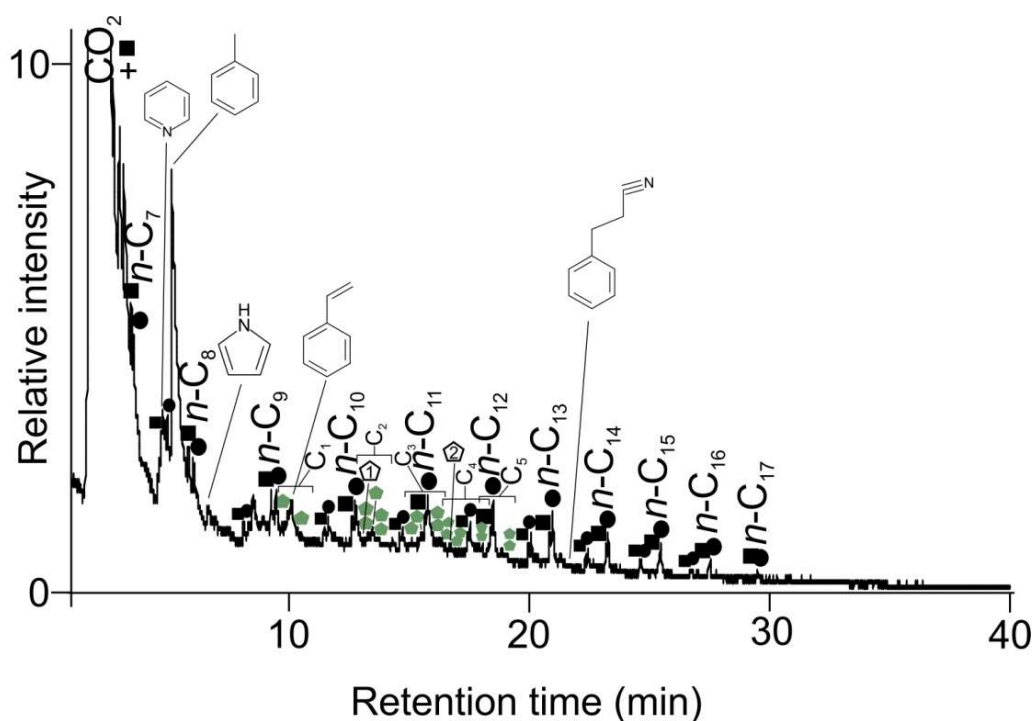
and 2-methylalkanes (isoalkanes), presumably deriving from the branched fatty acids which are significant components of bacterial triglycerides.

There were components identified in the pyrolysate TIC which can be indicative of ‘black carbon’, i.e. charcoal or soot⁷⁻¹⁰. However, the dominance of toluene, rather than the normally dominant aromatic hydrocarbon benzene, is notably atypical of chars⁷⁻¹⁰. The markers identified here can also be indicative of proteins^{6,11} and combined with the abundant related protein markers pyrrole and benzenepropanenitrile^{6,9} identified this is likely to be the main origin of these compounds in this sample, pointing to an appreciable protein-derived component^{6,9}. The proteinaceous origin for the aromatic markers is further corroborated by the moderate abundance of 4-methylphenol (*p*-cresol), which can be indicative of the amino acid tyrosine¹²⁻¹⁴. However, the presence of bacterial markers means the possibility that at least a partial origin could be microbial cannot be excluded.

However, highly notable in this context is the additional presence of notably significant amounts of the more unusual alkyl pyrroles. C₁ alkyl pyrroles: 2- and 3-methylpyrroles; C₂ alkyl pyrroles: 2- and 3-ethylpyrroles, 2,4-, 2,3- and 3,4-dimethyl-pyrroles; C₃ alkyl pyrroles: 2-ethyl-4(?) -methylpyrrole, 2-ethyl-3(?) -methylpyrrole, 4-ethyl-2-methylpyrrole, 2,3,5-trimethylpyrrole; C₄ alkyl pyrroles: an unidentified propylmethylpyrrole, 2,5(?) -diethylpyrrole, 4-ethyl-2,3-dimethylpyrrole, 3-ethyl-2,4-dimethylpyrrole and 2,3,4,5-tetramethylpyrrole; two C₅ alkyl pyrroles: 3-methyl-4-isobutylpyrrole, 2,3(?) -diethyl-5?-methylpyrrole. Collectively, these specific C₁, C₂, C₃, C₄ and C₅ alkyl pyrroles are characteristic of a significant tetrapyrrole/porphyrin-derived origin and are indicative of an algal source observed in fossils and expected in similarly diagenetically altered archaeological material^{11,21,22}. The presence here of the abundant protein markers and specifically the C₁ to C₅ alkyl pyrroles, combined with the particular lipid profile and specific carbohydrate markers (see below) identified, indicate the presence of a macroalgae, i.e. seaweed, in this sample^{21,22,24} (see discussion below).

The thermally-derived carbohydrate markers 2-methyl-2-cyclopenten-1-one and 2,3-dimethyl-2-cyclopenten-1-one were identified as moderately abundant components. That they were absent in the TD profile points to their originating from the original polymeric carbohydrate source (e.g. starch)²⁵. Although levoglucosan (1,6-anhydro-β-D-glucopyranose) is usually the major component in the thermally-derived products of carbohydrates analysed by TD/Py-GC-MS, its absence from the TD profile here is consistent with the removal of

hydroxyl groups in the original carbohydrate polymer, so preventing its formation in the probe. The additional absence of the normally significant carbohydrate markers 2-hydroxy-3-methyl-2-cyclopenten-1-one and 3-hydroxy-2-methyl-2-cyclopenten-1-one observed in pure standard material (glycogen, starch and cellulose) would also support this interpretation. This shows a strong matrix effect likely to be due to the inorganic component of the calculus and the microflora in the mouth. This dehydration/dehydroxylation has also been observed in dog calculus and 19th century human calculus⁶, where other markers, e.g. a prevalence of nitriles, with amides very minor, deriving from dehydrating conditions in the mouth of dogs and humans (though not sheep⁶) supporting this view⁶. The presence of carbon dioxide is also consistent with a lipid, amino acid/protein and carbohydrate component in this sample. Notably, these findings did not reveal key biomarkers typically observed in soils^{20,23}. It should also be noted that highly polar material could have been present, which would not have successfully eluted from the column, or indeed volatilised sufficiently in the probe.



Supplementary Figure 15. Reconstructed total ion chromatogram of the pyrogram (pyrolysis profile) (610 °C for 10s) of sample 249-01, Quanterness, after thermal desorption (310 °C for 10 s). Key to Fig. 15: Peak identities (x indicates carbon chain length): filled

squares, Cx indicates alkenes; filled circles, Cx indicates alkanes; green filled pentagons indicates alkyl pyrroles (with alkyl chain length above, Cx); open pentagons with numerals inside indicates carbohydrate pyrolysis markers, 1 is 2-methyl-2-cyclopenten-1-one and 2 is 2,3-dimethyl-2-cyclopentene-1-one. Also shown are the structures of five aromatic compounds identified: pyridine, pyrrole, toluene, styrene, and benzenepropanenitrile. CO₂ indicates carbon dioxide.

19. Quanterness passage tomb, Orkney (NMS unreg): Box 20 (Box A Bag 666 Bag 1 or 4) 2730-2734 (mandible split): M1 left lingual (calculus: 7.62 mg)

The thermal desorption total ion chromatogram (TIC) revealed carbon dioxide as the only detectable (trace) component, indicating the essential absence of free, thermally extractable organic components in this sample. It should be noted, however, that highly polar material could have been present, which would not have successfully eluted from the column, or indeed volatilised sufficiently in the probe. It is not possible to determine the significance of the carbon dioxide, beyond it being indicative of oxidised organic material.

The pyrolysate TIC revealed carbon dioxide, C₄ and C₅ 1-alkenes, cyclopentadiene and benzene as the only detectable components. The dominance of carbon dioxide, combined with the relatively minor abundance of the few small organic compounds present, suggests a highly oxidised sample. It is not possible to determine the significance of the carbon dioxide, beyond it being indicative of oxidised organic material and the other compounds are too low an abundance and too ubiquitous to provide any meaningful interpretation of their presence, beyond being indicative of an organic origin. It should be noted, however, that highly polar material could have been present, which would not have successfully eluted from the column, or indeed volatilised sufficiently in the probe.

20. Quanterness passage tomb, Orkney (NMS unreg): Box 20/Bag 675 (Box A: Bag 2) 2552-02: M2 right lingual (calculus: 9.31 mg)

The thermal desorption total ion chromatogram (TIC) revealed carbon dioxide as the only detectable component, indicating the essential absence of free, thermally extractable organic components in this sample. It should be noted, however, that highly polar material could have been present, which would not have successfully eluted from the column, or indeed

1651 volatilised sufficiently in the probe. It is not possible to determine the significance of the
1652 carbon dioxide, beyond it being indicative of oxidised organic material.

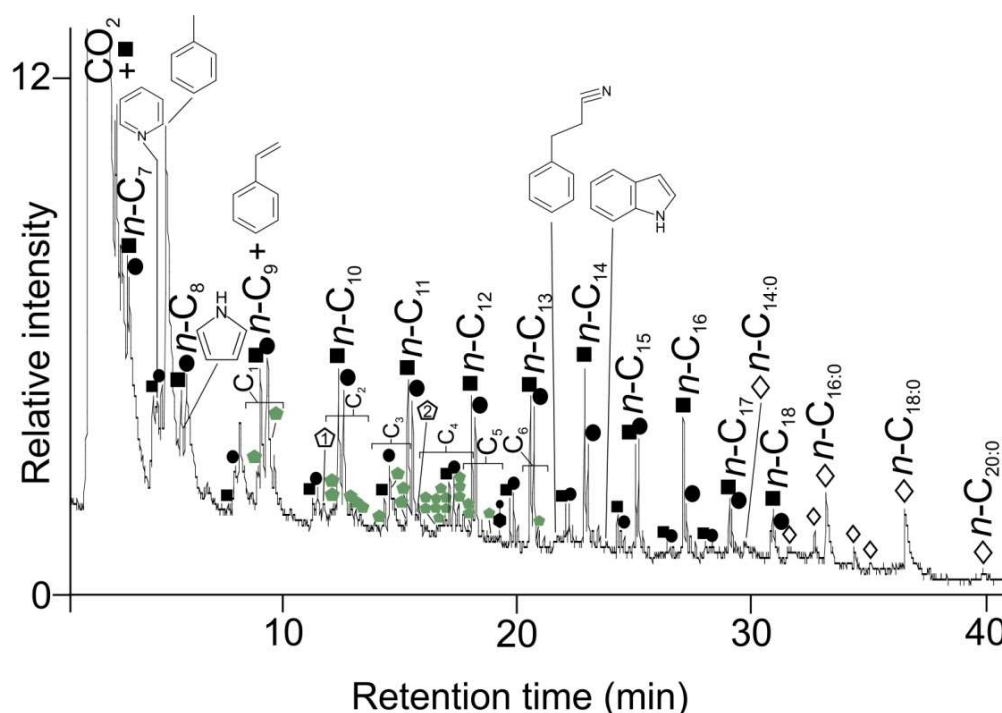
1653 In contrast to the thermal desorption profile, the pyrolysis-GC-MS TIC (Fig. 16) is dominated
1654 by a complex suite of organic compounds. These include a series of C₇ to C₁₈ *n*-1-alkenes and
1655 *n*-alkanes, typical of unsaturated and saturated fat/oil-derived acyl lipids^{5,6}. A distribution
1656 with maxima at C₁₀/C₁₁ and C₁₄ suggests the original acyl lipids may have been high in
1657 polyunsaturated fatty acids. The absence of these compounds in the TD profile provides
1658 molecular evidence for a polymeric/bound acyl lipid source. However, in addition to *n*-1-
1659 alkenes and *n*-alkanes, typical of fat/oil derived lipids, the pyrogram also displayed a series of
1660 C₉ to C₁₇ iso-methyl-1-alkenes (isoalkenes) and 2-methylalkanes (isoalkanes), presumably
1661 deriving from the branched fatty acids which are significant components of bacterial
1662 triglycerides.

1663 There were components identified in the pyrolysate TIC which can be indicative of 'black
1664 carbon', i.e. charcoal or soot^{7,10}. However, the dominance of toluene, rather than the normally
1665 dominant aromatic hydrocarbon benzene, is notably atypical of chars⁷⁻¹⁰. The markers
1666 identified here can also be indicative of proteins^{6,11} and combined with the abundant related
1667 protein markers pyrrole, benzenepropanenitrile and indole^{6,11} identified this is likely to be the
1668 main origin of these compounds in this sample, pointing to a substantial protein-derived
1669 component^{6,11}. This is also corroborated by the presence of aliphatic nitriles (carbon numbers
1670 C_{14:0}, , C_{15:0}, C_{16:0} (max), C_{17:0} and C_{18:0}) suggesting a lipid (fat/oil) in this sample which has
1671 combined with protein-derived nitrogen to produce these moieties⁸. The proteinaceous origin
1672 for the aromatic markers is further corroborated by the moderate abundance of 4-
1673 methylphenol (*p*-cresol), which can be indicative of the amino acid tyrosine¹²⁻¹⁴, and the
1674 moderately abundant constituent indole which is consistent with a tryptophan amino acid
1675 source^{12-14,19}. However, the presence of bacterial markers means the possibility that at least a
1676 partial origin could be microbial cannot be excluded.

1677 However, highly notable in this context is the additional presence of notably significant
1678 amounts of the more unusual alkyl pyrroles. C₁ alkyl pyrroles: 2- and 3-methylpyrroles; C₂
1679 alkyl pyrroles: 2- and 3-ethylpyrroles, 2,4-, 2,3- and 3,4-dimethyl-pyrroles; C₃ alkyl pyrroles:
1680 2-ethyl-4(?) -methylpyrrole, 2-ethyl-3(?) -methylpyrrole, 4-ethyl-2-methylpyrrole, 2,3,5-
1681 trimethylpyrrole; C₄ alkyl pyrroles: two unidentified propylmethylpyrroles, four unidentified
1682 ethyldimethylpyrroles, 2,5(?) -diethylpyrrole, 2,4(?) -diethylpyrrole, 2,3(?) -diethylpyrrole,

3,4(?)-diethylpyrrole 4-ethyl-2,3-dimethylpyrrole, 3-ethyl-2,4-dimethylpyrrole and 2,3,4,5-tetramethylpyrrole; two C₅ alkyl pyrroles: 3-methyl-4-isobutylpyrrole and 2,3(?)-diethyl-5?-methylpyrrole; a C₆ alkyl pyrrole: 3-ethyl-4-isobutylpyrrole. Collectively, these specific C₁, C₂, C₃, C₄, C₅ and C₆ alkyl pyrroles are characteristic of a significant tetrapyrrole/porphyrin-derived origin and are indicative of an algal source observed in fossils and expected in similarly diagenetically altered archaeological material^{11,21,22}. The presence here of the abundant protein markers and specifically the C₁ to C₆ alkyl pyrroles, combined with the particular lipid profile and specific carbohydrate markers identified, indicate the presence of a macroalgae, i.e. seaweed, in this sample^{21,22,24} (see discussion).

The thermally-derived carbohydrate markers 2-methyl-2-cyclopenten-1-one and 2,3-dimethyl-2-cyclopenten-1-one were identified as moderately abundant components. That they were absent in the TD profile points to their originating from the original polymeric carbohydrate source (e.g. starch)²⁵. Although levoglucosan (1,6-anhydro-β-D-glucopyranose) is usually the major component in the thermally-derived products of carbohydrates analysed by TD/Py-GC-MS, its absence from the TD profile here is consistent with the removal of hydroxyl groups in the original carbohydrate polymer, so preventing its formation in the probe. The additional absence of the normally significant carbohydrate markers 2-hydroxy-3-methyl-2-cyclopenten-1-one and 3-hydroxy-2-methyl-2-cyclopenten-1-one observed in pure standard material (glycogen, starch and cellulose) would also support this interpretation. This shows a strong matrix effect likely to be due to the inorganic component of the calculus and the microflora in the mouth. This dehydration/dehydroxylation has also been observed in dog calculus and 19th century human calculus⁶, where other markers, e.g. a prevalence of nitriles, with amides very minor, deriving from dehydrating conditions in the mouth of dogs and humans (though not sheep⁶) supporting this view⁶. The presence of carbon dioxide is also consistent with a lipid, amino acid/protein and carbohydrate component in this sample. Notably, these findings did not reveal key biomarkers typically observed in soils^{20,23}. It should also be noted that highly polar material could have been present, which would not have successfully eluted from the column, or indeed volatilised sufficiently in the probe.



Supplementary Figure 16. Reconstructed total ion chromatogram of the pyrogram (pyrolysis profile) (610 °C for 10s) of sample 2552-02, Quanterness, after thermal desorption (310 °C for 10 s). Key to Fig. 16: Peak identities (x indicates carbon chain length): filled squares, Cx indicates alkenes; filled circles, Cx indicates alkanes; open diamonds, Cx:y indicates acyclic nitriles; green filled pentagons indicates alkyl pyrroles (with alkyl chain length above, Cx); filled hexagon with filled circle attached indicates alkyl phenol; open pentagons with numerals inside indicates carbohydrate pyrolysis markers, 1 is 2-methyl-2-cyclopenten-1-one and 2 is 2,3-dimethyl-2-cyclopentene-1-one. Also shown are the structures of four aromatic compounds identified: pyridine, pyrrole, toluene, styrene, benzenepropanenitrile and indole. CO₂ indicates carbon dioxide.

21. Quanterness passage tomb, Orkney (NMS unreg): Box 19/Box F/Bag 663 from skull 1408: Loose maxillary molar Lingual (calculus: 0.80 mg)

The thermal desorption total ion chromatogram (TIC) revealed carbon dioxide and 1-butene as the only detectable components, indicating the essential absence of free, thermally extractable organic components in this sample. It should be noted, however, that highly polar material could have been present, which would not have successfully eluted from the column, or indeed volatilised sufficiently in the probe. The 1-butene possibly derives from lipid or

proteinaceous material in the sample¹¹, and perhaps produced at this relatively low temperature due to matrix effects. It is not possible to determine the significance of the carbon dioxide, beyond it being indicative of oxidised organic material.

The pyrolysate TIC revealed carbon dioxide and the C₄ and C₅ 1-alkenes as the only detectable components. The dominance of carbon dioxide suggests a highly oxidised sample. It is not possible to determine the significance of the carbon dioxide, beyond it being indicative of oxidised organic material. It should be noted, however, that highly polar material could have been present, which would not have successfully eluted from the column, or indeed volatilised sufficiently in the probe.

Discussion, Quanterness passage tomb, Orkney (NMS unreg)

The TD-GC-MS analyses revealed very little organic material in all of the six samples. Carbon dioxide was the only compound observed in the thermal desorption profile of all six samples (though even this was very minor in four of the six samples) indicating highly oxidised organic material with an essential absence of free, thermally extractable, organic constituents in these samples.

In contrast to the TD-GC-MS analyses, the Py-GC-MS results revealed appreciable amounts of organic material in all six calculus samples, although only two contained moderate to major abundances of organic compounds >C₅. This indicates that although the cool environment on Orkney has allowed the survival of a wide range of biomolecules relating to fats/oil, proteins and carbohydrates (see below), diagenesis and biodegradation have resulted in the loss of most, if not all, free biomarkers. In contrast, bound/polymeric biomacromolecules (some clearly resulting from pre- and/or post-depositional biochemical reactions between the constituents of the original foods consumed) have been appreciably well preserved in two of the samples, albeit the more resilient moieties and compound classes are likely to have been preferentially preserved. These two samples most rich in thermally extractable biomolecules (only) will be discussed below.

The pyrolysis-GC-MS analyses of the two samples containing significant amounts of organic material were dominated by a complex suite of organic compounds indicative of lipid (fat/oil), protein and carbohydrate components. These include a series of C₇ to C₁₈ *n*-1-alkenes and *n*-alkanes, typical of unsaturated and saturated fat/oil-derived acyl lipids^{5,6}. A bimodal distribution with maxima at C₁₀/C₁₁ and C₁₄ combined with abundant short chain

1763 (C₄-C₇) *n*-1-alkenes suggests the original acyl lipids in these samples are likely to have been
1764 high in polyunsaturated fatty acids. The absence of these compounds in the TD profile
1765 provides molecular evidence for a polymeric/bound acyl lipid source. However, in addition to
1766 *n*-1-alkenes and *n*-alkanes, typical of fat/oil derived lipids, the pyrograms also displayed a
1767 series of C₈ to C₁₇ iso-methyl-1-alkenes (isoalkenes) and 2-methylalkanes (isoalkanes),
1768 presumably deriving from the branched fatty acids which are significant components of
1769 bacterial triglycerides.

1770 There were components in the pyrolysate which can be indicative of 'black carbon', i.e.
1771 charcoal or soot⁷⁻¹⁰. However, the dominance of toluene, rather than the normally dominant
1772 aromatic hydrocarbon benzene, is notably atypical of chars⁷⁻¹⁰. The markers identified here
1773 can also be indicative of proteins^{6,11} and combined with the related protein markers pyrrole
1774 and benzenepropanenitrile^{6,11} identified this is most likely to be the main origin of these
1775 compounds in this sample, pointing to an appreciable protein-derived component^{6,11}. This is
1776 also corroborated by the presence of aliphatic nitriles (carbon numbers C_{14:0}, C_{15:0}, C_{16:0}
1777 (max), C_{17:0} and C_{18:0}) suggesting a lipid (fat/oil) in this sample which has combined with
1778 protein-derived nitrogen to produce these moieties⁵, although the presence of bacterial
1779 markers means the possibility that at least a partial origin could be microbial cannot be
1780 excluded.

1781 Far more diagnostic of a particular dietary source are the particular suite of alkyl pyrroles
1782 observed in the pyrograms of these samples, with an alkyl chain length of up to C₆. Both these
1783 samples revealed significant amounts of these biomarkers. This included the 2- and 3-
1784 dimethylpyrroles, 2- and 3-ethylpyrroles, 2,4-, 2,3- and 3,4-dimethylpyrroles, three
1785 ethylmethylpyrroles, a trimethylpyrrole, two propylmethylpyrroles, five
1786 ethyldimethylpyrroles, three diethylpyrroles, 2,3,4,5-tetramethylpyrrole, 3-methyl-4-
1787 isobutylpyrrole, a diethylmethylpyrrole and 3-ethyl-4-isobutylpyrrole (trace). These specific
1788 C₁ to C₆ alkyl pyrroles are highly diagnostic of the presence of a significant tetrapyrrole
1789 component. Their porphyrin-derived origin, and the relative abundance of some of the key
1790 biomarkers, is indicative of an algal source observed in fossils and expected in similarly
1791 diagenetically altered archaeological material^{11,21,22}. Although C₁ and C₂ alkyl pyrroles can
1792 also derive from protein-derived amino acids such as glycine, proline, hydroxyproline, serine
1793 and glutamic acid^{12-14,20,40,41}, the C₃+ alkyl pyrroles identified in these six samples point to a
1794 tetrapyrrole source^{21,22,40,41}. Moreover, the same specific C₁ to C₅ alkylpyrroles identified in
1795 the Py-GC-MS of an algal kerogen^{21,22} were observed in these samples. More specifically, the

highly unusual alkyl pyrroles, 3-methyl-4-isobutylpyrrole and 3-ethyl-4-isobutylpyrrole are known to derive from the C₈ side chain of bacteriochlorophylls c and d⁴⁷ and are biomarkers for the relatively rare bacteriochlorophylls c and d which are found in green bacteria such as *Chloroflexus* sp.⁴⁷ and are known to naturally occur in association with intertidal red seaweeds such as *Porphyra umbilicalis*⁴⁷⁻⁴⁹. Overall the two samples display a remarkably similar pyrolysis profile to algal-derived kerogen previously studied via very similar organic geochemical techniques^{21,22}.

As discussed above, the bimodal distribution of *n*-1-alkenes/*n*-alkanes with maxima at C₁₀/C₁₁ and C₁₄, combined with abundant short chain (C₄-C₇) *n*-1-alkenes, suggests the original acyl lipids are likely to have been high in polyunsaturated fatty acids, which is consistent with a marine source. Moreover, the presence here of the specific C₁ to C₆ alkyl pyrroles, combined with the particular lipid profile identified, indicate the presence of a macroalgae, i.e. seaweed, in these samples^{21,22,24}. Seaweed is known to have been exploited for food in antiquity⁵⁶, although its consumption by humans in Orkney seems only to have been tentatively considered to date^{2,57}.

Distillery Cave

Distillery Cave was accidentally discovered in 1890⁸². The archaeological levels were disturbed, and the site was eventually destroyed. However, the remains of four adults and four children were discovered. Three of those individuals have produced radiocarbon dates ranging from 3762–3644 cal BC to 3514–3353 cal BC⁸³. The Cave lay at around 12m above modern sea level.

22. Distillery Cave, Oban, Highland (NMS unreg) Box 6, adult male ‘1’: Canine right lingual (calculus: 5.62 mg)²⁸

The thermal desorption total ion chromatogram (TIC) revealed carbon dioxide and 1-butene as the only detectable components, indicating the essential absence of free, thermally extractable organic components in this sample. It should be noted, however, that highly polar material could have been present, which would not have successfully eluted from the column, or indeed volatilised sufficiently in the probe. The 1-butene possibly derives from lipid or proteinaceous material in the sample¹¹, and perhaps produced at this relatively low

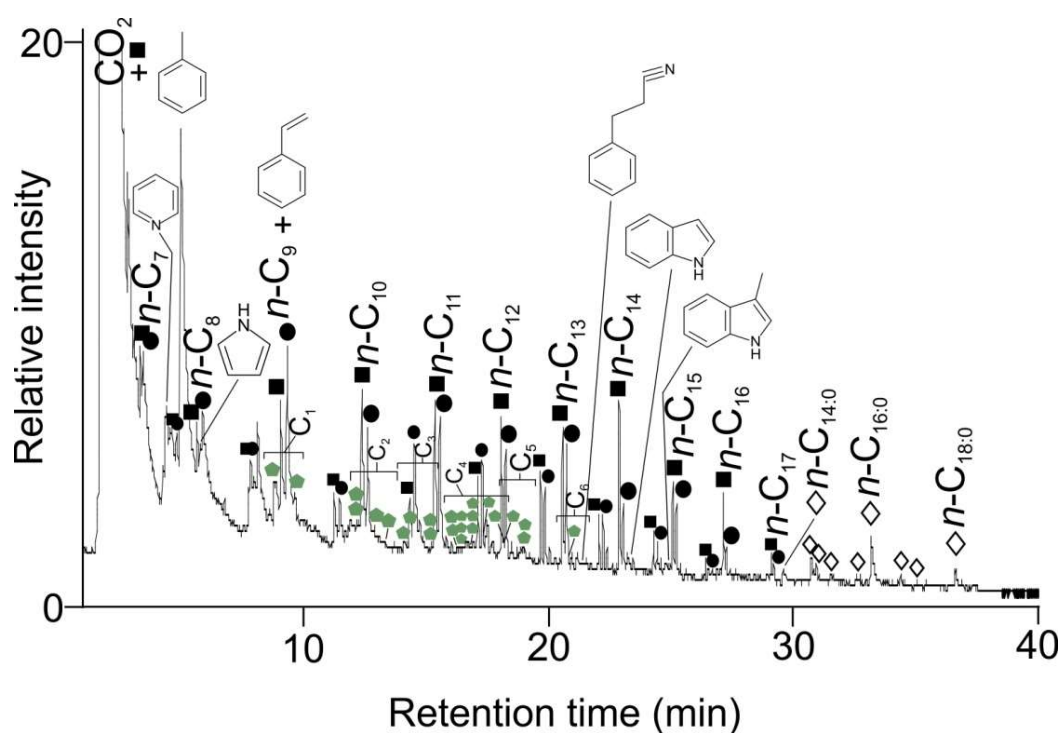
temperature due to matrix effects. It is not possible to determine the significance of the carbon dioxide, beyond it being indicative of oxidised organic material.

In contrast to the thermal desorption profile, the pyrolysis-GC-MS TIC (Fig. 17) is dominated by a complex suite of organic compounds. These include a series of C₇ to C₁₇ *n*-1-alkenes and *n*-alkanes, typical of unsaturated and saturated fat/oil-derived acyl lipids^{5,6}. A distribution with maxima at C₁₀/C₁₁ and C₁₄ suggests the original acyl lipids may have been high in polyunsaturated fatty acids. The absence of these compounds in the TD profile provides molecular evidence for a polymeric/bound acyl lipid source. However, in addition to *n*-1-alkenes and *n*-alkanes, typical of fat/oil derived lipids, the pyrogram also displayed a series of C₉ to C₁₆ iso-methyl-1-alkenes (isoalkenes) and 2-methylalkanes (isoalkanes), presumably deriving from the branched fatty acids which are significant components of bacterial triglycerides.

There were components identified in the pyrolysate TIC which can be indicative of 'black carbon', i.e. charcoal or soot⁷⁻¹⁰. However, the dominance of toluene, rather than the normally dominant aromatic hydrocarbon benzene, is notably atypical of chars⁷⁻¹⁰. The markers identified here can also be indicative of proteins^{6,11} and combined with the abundant related protein markers pyrrole, benzenepropanenitrile and indoles^{6,11} identified this is likely to be the main origin of these compounds in this sample, pointing to a substantial protein-derived component^{6,11}. This is also corroborated by the presence of aliphatic nitriles (carbon numbers C_{14:0}, C_{16:0} (max) and C_{18:0}) suggesting a lipid (fat/oil) in this sample which has combined with protein-derived nitrogen to produce these moieties⁵. In addition, the moderately abundant constituents indole and 3-methylindole (skatole) were also identified, which are consistent with a tryptophan amino acid source^{12,13,19,20}, although the presence of bacterial markers means the possibility that at least a partial origin could be microbial cannot be excluded.

However, highly notable in this context is the additional presence of notably significant amounts of the more unusual alkyl pyrroles. C₁ alkyl pyrroles: 2- and 3-methylpyrroles; C₂ alkyl pyrroles: 2- and 3-ethylpyrroles, 2,4-, and 2,3-dimethyl-pyrroles; C₃ alkyl pyrroles: 2-ethyl-4(?) -methylpyrrole, 2-ethyl-3(?) -methylpyrrole and 4-ethyl-2-methylpyrrole, 2,3,5-trimethylpyrrole; C₄ alkyl pyrroles: two unidentified propylmethylpyrroles, three unidentified ethyldimethylpyrroles, 2,5(?) -diethylpyrrole, 2,3(?) -diethylpyrrole, 3,4(?) -diethylpyrrole, 4-ethyl-2,3-dimethylpyrrole, 3-ethyl-2,4-dimethylpyrrole and 2,3,4,5-tetramethylpyrrole; C₅

alkyl pyrroles: 3-methyl-4-isobutylpyrrole, 3-ethyl-2,4,5-trimethylpyrrole and an unidentified diethylmethylpyrrole; C₆ alkyl pyrroles: 3-ethyl-4-isobutylpyrrole (trace). Collectively, these specific C₁, C₂, C₃, C₄, C₅ and C₆ alkyl pyrroles are characteristic of a significant tetrapyrrole/porphyrin-derived origin and are indicative of an algal source observed in fossils and expected in similarly diagenetically altered archaeological material^{11,21,22}. The presence here of the abundant protein markers and specifically the C₁ to C₆ alkyl pyrroles, combined with the particular lipid profile (see below) identified, indicate the presence of a macroalgae, i.e. seaweed, in this sample^{21,22,24} (see discussion below). The presence of carbon dioxide is also consistent with a lipid and amino acid/protein component in this sample. Notably, these findings did not reveal key biomarkers typically observed in soils^{20,23}. It should also be noted that highly polar material could have been present, which would not have successfully eluted from the column, or indeed volatilised sufficiently in the probe.



Supplementary Figure 17. Reconstructed total ion chromatogram of the pyrogram (pyrolysis profile) (610 °C for 10s) of sample adult male ‘1’, Distillery Cave, after thermal desorption (310 °C for 10 s). Key to Fig. 17: Peak identities (x indicates carbon chain length): filled squares, C_x indicates alkenes; filled circles, C_x indicates alkanes; open diamonds, C_x:y indicates acyclic nitriles; green filled pentagons indicates alkyl pyrroles (with alkyl chain

length above, Cx). Also shown are the structures of seven aromatic compounds identified: pyridine, pyrrole, toluene, styrene, benzenepropanenitrile, indole and 3-methylindole (skatole). CO₂ indicates carbon dioxide.

23. Distillery Cave, Oban, Highland (NMS unreg) Box 6, prob male '3': PM2 right labial (calculus: 4.58 mg)²⁸

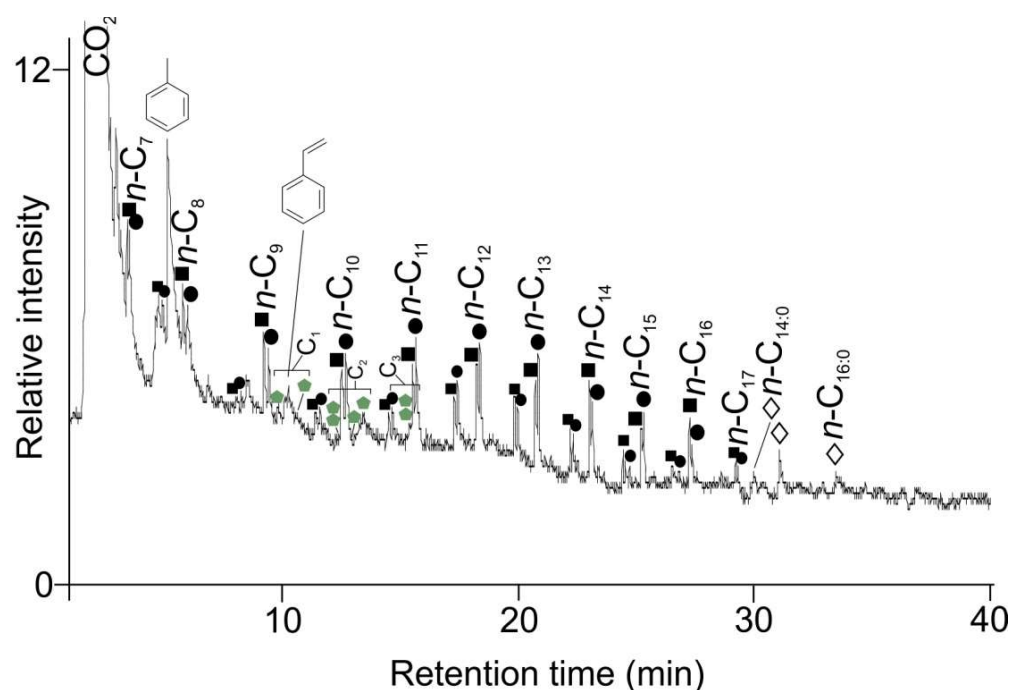
The thermal desorption total ion chromatogram (TIC) revealed carbon dioxide as the only detectable component, indicating the essential absence of free, thermally extractable organic components in this sample. It should be noted, however, that highly polar material could have been present, which would not have successfully eluted from the column, or indeed volatilised sufficiently in the probe. It is not possible to determine the significance of the carbon dioxide, beyond it being indicative of oxidised organic material.

In contrast to the thermal desorption profile, the pyrolysis-GC-MS TIC (Fig. 18) is dominated by a complex suite of organic compounds. These include a series of C₇ to C₁₇ *n*-1-alkenes and *n*-alkanes, typical of unsaturated and saturated fat/oil-derived acyl lipids^{5,6}. The absence of these compounds in the TD profile provides molecular evidence for a polymeric/bound acyl lipid source. However, in addition to *n*-1-alkenes and *n*-alkanes, typical of fat/oil derived lipids, the pyrogram also displayed a series of C₈ to C₁₆ iso-methyl-1-alkenes (isoalkenes) and 2-methylalkanes (isoalkanes), presumably deriving from the branched fatty acids which are significant components of bacterial triglycerides.

The components identified in the pyrolysate TIC of the previous sample from the same site and which can be indicative of 'black carbon' (i.e. charcoal or soot) and/or proteins were not detected, although this may reflect the lower abundance of the organic compounds in the pyrogram of this sample. Corroborating this is the presence of aliphatic nitriles (carbon numbers C_{14:0}, *i*-C_{15:0} (max) and C_{16:0}) suggesting a lipid (fat/oil) in this sample which has combined with protein-derived nitrogen to produce these moieties⁵, although the dominance of the *i*-C_{15:0} bacterial marker means at least a partial microbial origin for them is likely.

However, highly notable in this context is the additional presence of notably significant amounts of the more unusual alkyl pyrroles. C₁ alkyl pyrroles: 2- and 3-methylpyrroles; C₂ alkyl pyrroles: 2- and 3-ethylpyrroles, 2,4-, and 2,3-dimethyl-pyrroles; C₃ alkyl pyrroles: 4-ethyl-2-methylpyrrole and 2,3,5-trimethylpyrrole. Collectively, these specific C₁, C₂ and C₃ alkyl pyrroles are indicative of a significant tetrapyrrole/porphyrin-derived origin and suggest

an algal source observed in fossils and expected in similarly diagenetically altered archaeological material^{11,21,22}. The similarity to the previous sample from this site, albeit the biomarkers are in appreciably lower abundance, indicates the presence of a macroalgae, i.e. seaweed, in this sample^{21,22,24} (see discussion below). The presence of carbon dioxide is also consistent with a lipid and amino acid/protein component in this sample. Notably, these findings did not reveal key biomarkers typically observed in soils^{20,23}. It should also be noted that highly polar material could have been present, which would not have successfully eluted from the column, or indeed volatilised sufficiently in the probe.



Supplementary Figure 18. Reconstructed total ion chromatogram of the pyrogram (pyrolysis profile) (610 °C for 10s) of sample prob male ‘3’, Distillery Cave, after thermal desorption (310 °C for 10 s). Key to Fig. 18: Peak identities (x indicates carbon chain length): filled squares, Cx indicates alkenes; filled circles, Cx indicates alkanes; open diamonds, Cx:y indicates acyclic nitriles; green filled pentagons indicates alkyl pyrroles (with alkyl chain length above, Cx). Also shown are the structures of two aromatic compounds identified: toluene and styrene. CO₂ indicates carbon dioxide.

1927 **Discussion, Distillery Cave, Oban, Highland (NMS unreg)**

1928 Two samples of dental calculus from Distillery Cave were analysed by sequential thermal
1929 desorption-gas chromatography-mass spectrometry (TD-GC-MS) and pyrolysis-gas
1930 chromatography-mass spectrometry (Py-GC-MS). The samples contained both free and
1931 bound/polymeric organic material.

1932 Carbon dioxide dominated the thermal desorption profile, in addition to appreciable amounts
1933 of C₄ to C₆ 1-alkenes, as the only ‘free’ organic compounds detected, indicating highly
1934 oxidised organic material with little free, thermally extractable, organic constituents in these
1935 samples.

1936 In contrast to the TD-GC-MS analyses, the Py-GC-MS results revealed appreciable amounts
1937 of organic material in both calculus samples. This indicates that although the relatively cool
1938 environment has allowed the survival of a wide range of biomolecules relating to fats/oil and
1939 proteins, diagenesis and biodegradation have resulted in the loss of most, if not all, free
1940 biomarkers. In contrast, bound/polymeric biomacromolecules (some clearly resulting from
1941 pre- and/or post-depositional biochemical reactions between the constituents of the original
1942 foods consumed) have been appreciably well preserved, albeit the more resilient moieties and
1943 compound classes are likely to have been preferentially preserved.

1944 The pyrolysis-GC-MS analyses were dominated by a complex suite of organic compounds
1945 indicative of lipid (fat/oil) and protein components. These include a series of C₇ to C₁₇ *n*-1-
1946 alkenes and *n*-alkanes, typical of unsaturated and saturated fat/oil-derived acyl lipids^{5,6}. A
1947 bimodal distribution with maxima at C₁₀/C₁₁ and C₁₄ combined with abundant short chain
1948 (C₄-C₇) *n*-1-alkenes suggests the original acyl lipids in these samples are likely to have been
1949 high in polyunsaturated fatty acids. The absence of these compounds in the TD profile
1950 provides molecular evidence for a polymeric/bound acyl lipid source. However, in addition to
1951 *n*-1-alkenes and *n*-alkanes, typical of fat/oil derived lipids, the pyrograms also displayed a
1952 series of C₉ to C₁₆ iso-methyl-1-alkenes (isoalkenes) and 2-methylalkanes (isoalkanes),
1953 presumably deriving from the branched fatty acids which are significant components of
1954 bacterial triglycerides.

1955 There were components identified in the pyrolysate TIC which can be indicative of ‘black
1956 carbon’, i.e. charcoal or soot⁷⁻¹⁰. However, the dominance of toluene, rather than the normally
1957 dominant aromatic hydrocarbon benzene, is notably atypical of chars⁷⁻¹⁰. The markers
1958 identified here can also be indicative of proteins^{6,11} and combined with the abundant related

1959 protein markers pyrrole, benzenepropanenitrile and indoles^{6,11} identified this is likely to be
1960 the main origin of these compounds in this sample, pointing to a substantial protein-derived
1961 component^{6,11}. This is also corroborated by the presence of aliphatic nitriles (carbon numbers
1962 C_{14:0}, C_{16:0} (max) and C_{18:0}) suggesting a lipid (fat/oil) in this sample which has combined
1963 with protein-derived nitrogen to produce these moieties⁵. In addition, the moderately
1964 abundant constituents indole and 3-methylindole (skatole) were also identified, which are
1965 consistent with a tryptophan amino acid source^{12,13,19,20}, although the presence of bacterial
1966 markers means the possibility that at least a partial origin could be microbial cannot be
1967 excluded.

1968 Far more diagnostic of a particular dietary source are the particular suite of alkyl pyrroles
1969 observed in the pyrograms of these samples, with an alkyl chain length of up to C₆. Both
1970 samples revealed significant amounts of these biomarkers. This included the 2- and 3-
1971 dimethylpyrroles, 2- and 3-ethylpyrroles, 2,4- and 2,3- dimethylpyrroles, three
1972 ethylmethylpyrroles, a trimethylpyrrole, two propylmethylpyrroles, five
1973 ethyldimethylpyrroles, three diethylpyrroles, 2,3,4,5-tetramethylpyrrole, an
1974 ethylpropylpyrrole, 3-ethyl-2,4,5-trimethylpyrrole, a diethylmethylpyrrole and an
1975 unidentified C₆ ethylpyrrole (trace). These specific C₁ to C₆ alkyl pyrroles are highly
1976 diagnostic of the presence of a significant tetrapyrrole component. Their porphyrin-derived
1977 origin, and the relative abundance of some of the key biomarkers, is indicative of an algal
1978 source observed in fossils and expected in similarly diagenetically altered archaeological
1979 material^{11,21,22}. Although C₁ and C₂ alkyl pyrroles can also derive from protein-derived
1980 amino acids such as glycine, proline, hydroxyproline, serine and glutamic acid^{12-14,20,40,41}, the
1981 C₃+ alkyl pyrroles identified in these six samples point to a tetrapyrrole source^{21,22,40,41}.
1982 Moreover, the same specific C₁ to C₅ alkylpyrroles identified in the Py-GC-MS of an algal
1983 kerogen^{21,22} were observed in these samples. More specifically, the highly unusual alkyl
1984 pyrroles, 3-methyl-4-isobutylpyrrole and 3-ethyl-4-isobutylpyrrole are known to derive from
1985 the C₈ side chain of bacteriochlorophylls c and d⁴⁷ and are biomarkers for the relatively rare
1986 bacteriochlorophylls c and d which are found in green bacteria such as *Chloroflexus* sp.⁴⁷ and
1987 are known to naturally occur in association with intertidal red seaweeds such as *Porphyra*
1988 *umbilicalis*⁴⁷⁻⁴⁹. Overall the two samples display a remarkably similar pyrolysis profile to
1989 algal-derived kerogen previously studied via very similar organic geochemical
1990 techniques^{21,22}.

1991 As discussed above, the bimodal distribution of *n*-1-alkenes/*n*-alkanes with maxima at
1992 C₁₀/C₁₁ and C₁₄, combined with abundant short chain (C₄-C₇) *n*-1-alkenes, suggests the
1993 original acyl lipids are likely to have been high in polyunsaturated fatty acids, which is
1994 consistent with a marine source. Moreover, the presence here of the specific C₁ to C₆ alkyl
1995 pyrroles, combined with the particular lipid profile identified, indicate the presence of a
1996 macroalgae, i.e. seaweed, in these samples^{21,22,24}. Seaweed is known to have been exploited
1997 for food in antiquity⁵⁶, although its consumption by humans in Orkney, for example (see
1998 below), seems only to have been tentatively considered to date^{2,57}.

1999

2000 **Raschoille Cave, Oban, Highland**

2001 Raschoille Cave was discovered in 1984 and was excavated by the local archaeological
2002 society⁸⁴. At least 57 individuals were recovered, several of whom have been radiocarbon
2003 dated, with dates ranging from 3942–3653 cal BC to 3487–3034 cal BC⁸⁵. Marine shells,
2004 fishbones, and animal bones were also recovered, some of these dating to within the
2005 Mesolithic period (6500–6000 BC). Clearly the cave was not used for the burial of human
2006 remains until the Early Neolithic period.

2007

2008 **24. Raschoille Cave, Oban, Highland (NMS unreg) ORC III 24.1: M1 right** 2009 **lingual (calculus: 2.94 mg) (4000-2900 BC)²⁸**

2010

2011 The thermal desorption total ion chromatogram (TIC) revealed carbon dioxide and 1-butene
2012 as the only detectable components, indicating the essential absence of free, thermally
2013 extractable organic components in this sample. It should be noted, however, that highly polar
2014 material could have been present, which would not have successfully eluted from the column,
2015 or indeed volatilised sufficiently in the probe. The 1-butene possibly derives from lipid or
2016 proteinaceous material in the sample¹¹, and perhaps produced at this relatively low
2017 temperature due to matrix effects. It is not possible to determine the significance of the
2018 carbon dioxide, beyond it being indicative of oxidised organic material.

2019 The pyrolysate TIC revealed carbon dioxide, C₄ to C₆ 1-alkenes, a C₇ diene, acetone, a
2020 methylcyclopentadiene, benzene, pyridine and toluene as the only detectable components.
2021 The dominance of carbon dioxide, combined with the relatively minor abundance of the few

small organic compounds present, suggests a highly oxidised sample. It is not possible to determine the significance of the carbon dioxide, beyond it being indicative of oxidised organic material and the other compounds are too low an abundance and too ubiquitous to provide any meaningful interpretation of their presence, beyond them being indicative of an organic origin. It should be noted, however, that highly polar material could have been present, which would not have successfully eluted from the column, or indeed volatilised sufficiently in the probe.

Discussion, Raschoille Cave, Oban, Highland (NMS unreg)

One sample of dental calculus from Raschoille Cave was analysed by sequential thermal desorption-gas chromatography-mass spectrometry (TD-GC-MS) and pyrolysis-gas chromatography-mass spectrometry (Py-GC-MS). The sample contained little free and bound/polymeric organic material, most likely reflecting unfavourable prevailing conditions for biomolecular preservation. Of those organic compounds identified, the dominance of carbon dioxide and the relatively minor abundance of only a few small organic compounds suggests that only highly oxidised organic material was present.

Boatbridge Quarry cist 2, Thankerton, S Lanarkshire

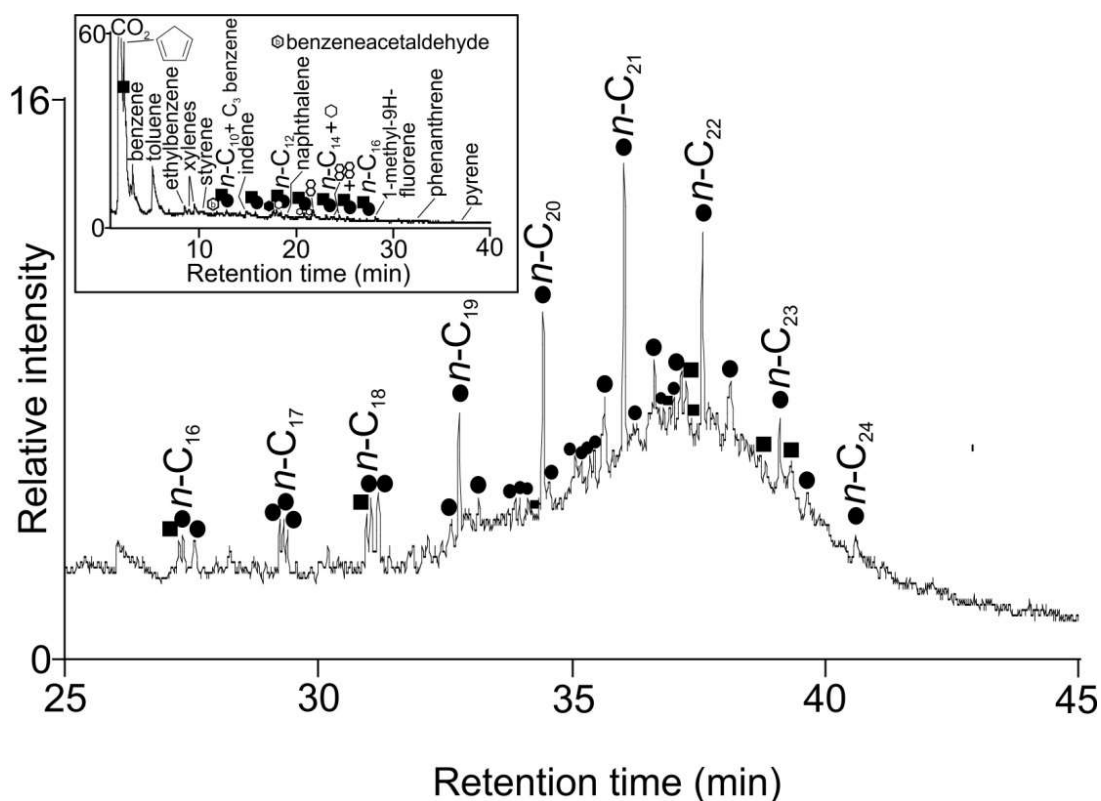
The human remains from Boatbridge cist 2 are those of an unusually tall adolescent male, radiocarbon dated to 2397–2138 cal BC and 2481–2040 cal BC^{1,86,87} The cist is one of two found in a sand and gravel quarry above the River Clyde⁸⁸.

25. Boatbridge Quarry cist 2, Thankerton, S Lanarkshire (associated with Beaker NMS X.EG 106): PM2 left labial S1 (calculus: 2.60 mg)

The four main compounds in the thermal desorption total ion chromatogram (TIC) (Fig. 19) are carbon dioxide, sulphur dioxide, benzene and acetic acid. In moderate abundance is an unresolved complex mixture (UCM; apparent as a ‘hump’ in the chromatogram) of alkenes and alkanes, carbon numbers C₁₆ to C₂₄, within which there is series of C₁₆ to C₂₄ *n*-alkanes (odd over even predominance) dominating this UCM profile and maximising at *n*-C₂₁.

Branched alkanes, primarily *iso*- and *anteiso*- with carbon numbers C₁₇ to C₂₄ are also present as minor constituents and are possibly indicative of microbially-derived biodegradation (but see summary below). These carbon numbers present in the TD chromatogram may suggest a submerged aquatic plant source (macrophyte)⁸⁹⁻⁹¹, possibly deriving from plant waxes in the original food consumed or reflecting prevailing environmental factors (but, again, see summary below). Naphthalene was also detected, albeit as a minor constituent. No characteristic protein or carbohydrate markers were observed meaning the significance of the non-diagnostic compounds carbon dioxide, benzene and acetic acid is uncertain. It should also be noted that highly polar material could have been present, which would not have successfully eluted from the column, or indeed volatilised sufficiently in the probe.

In contrast to the thermal desorption profile, the pyrolysis-GC-MS TIC (Fig. 19 inset) revealed a complex suite of organic compounds. These include a series of C₁₀ to C₁₆*n*-1-alkenes and *n*-alkanes, typical of unsaturated and saturated fat/oil-derived acyl lipids^{5,6}. The absence of these compounds in the TD profile provides molecular evidence for a polymeric/bound acyl lipid source. Importantly, there were no bacterially-derived hydrocarbons deriving from the original branched fatty acid acyl groups which are significant components of bacterial triglycerides, indicating this lipid component is not bacterial in origin. The pyrolysate TIC identified components indicative of 'black carbon', i.e. charcoal or soot⁷⁻¹⁰. Benzene and toluene were major compounds present in the pyrolysate (~15% and 30% of total quantified pyrogram peak area respectively), along with moderate quantities of ethyl benzene and *o*-, *m*- and *p*-xylenes (~5% total). The relative dominance of toluene, rather than the normally dominant aromatic hydrocarbon benzene, is somewhat atypical of chars⁷⁻¹⁰. However, the presence of significant amounts of naphthalene, methyl naphthalenes and methyl biphenyl, together with the polynuclear aromatic hydrocarbon combustion markers 1-methyl-9H-fluorene, phenanthrene, 3,6-dimethylphenanthrene, 1-methylanthracene, pyrene and 1-methylpyrene, confirm a 'char' component providing molecular evidence for exposure to fire/cooking. The lack of the attendant aromatic nitrogen-containing biomarkers pyrrole, benzonitrile, benzyl nitrile (phenylacetone nitrile), benzenepropanenitrile and indoles indicative of proteinaceous material^{6,11} suggests a collective origin as combustion compounds ('char') rather than a significant protein component. The presence of carbon dioxide is also consistent with a lipid component in this sample. It should also be noted that highly polar material could have been present, which would not have successfully eluted from the column, or indeed volatilised sufficiently in the probe.



2086

2087 **Supplementary Figure 19.** Reconstructed total ion chromatogram of the thermal desorption
 2088 profile (310 °C for 10 s) of sample X.EG 106, Boatbridge Quarry cist. Key to Fig. 19: Peak
 2089 identities (x indicates carbon chain length): filled squares indicates alkenes; filled circles, Cx
 2090 indicates alkanes. Inset displays a reconstructed total ion chromatogram of the pyrogram
 2091 (pyrolysis profile) (610 °C for 10) of this sample, after thermal desorption (310 °C for 10 s).
 2092 Peak identities: (x indicates carbon chain length): filled squares, Cx indicates alkenes; filled
 2093 circles, Cx indicates alkanes; CO₂ indicates carbon dioxide. Also shown are benzene, toluene,
 2094 ethyl benzene, xylenes (*o*-, *m*- and *p*-), styrene, benzeneacetaldehyde, an alkyl benzene,
 2095 indene, naphthalene, 1-methyl-9H-fluorene, phenanthrene and pyrene.

2096

2097 **26. Boatbridge Quarry cist 2, Thankerton, S Lanarkshire (associated with**
 2098 **Beaker NMS X.EG 106): M2 right labial S2 (calculus: 1.33 mg)^{1,86}**

2099 The four main compounds in the thermal desorption total ion chromatogram (TIC) are carbon
 2100 dioxide, sulphur dioxide, benzene and acetic acid. In minor abundance are a series of alkanes,
 2101 carbon numbers C₁₈ to C₂₂, maximising at *n*-C_{20/21}. Branched alkanes are also present and are

possibly indicative of microbially-derived biodegradation given the similarity to the hydrocarbon profile observed in the previous Boatbridge sample (but see summary below). The carbon numbers present in the TD chromatogram, when taken together with the hydrocarbons observed in Boatbridge S1, may suggest a submerged aquatic plant source (macrophyte)⁸⁹⁻⁹¹, possibly deriving from plant waxes in the original food consumed or reflecting prevailing environmental factors (but, again, see summary below). No characteristic protein or carbohydrate markers were observed meaning the significance of the non-diagnostic compounds carbon dioxide, benzene and acetic acid is uncertain. It should also be noted that highly polar material could have been present, which would not have successfully eluted from the column, or indeed volatilised sufficiently in the probe.

In contrast to the thermal desorption profile, the pyrolysis-GC-MS TIC revealed a complex suite of organic compounds. These include a series of C₁₀ to C₁₆ *n*-1-alkenes and *n*-alkanes, typical of unsaturated and saturated fat/oil-derived acyl lipids^{5,6}. The absence of these compounds in the TD profile provides molecular evidence for a polymeric/bound acyl lipid source. Importantly, there were no bacterially-derived hydrocarbons deriving from the original branched fatty acid acyl groups which are significant components of bacterial triglycerides, indicating this lipid component is not bacterial in origin. The pyrolysate TIC identified components indicative of 'black carbon', i.e. charcoal or soot⁷⁻¹⁰. Benzene and toluene were major compounds present in the pyrolysate (~15% and 30% of total quantified pyrogram peak area respectively), along with moderate quantities of ethyl benzene and *o*-, *m*- and *p*-xylenes (~5% total). The relative dominance of toluene, rather than the normally dominant aromatic hydrocarbon benzene, is somewhat atypical of chars⁷⁻¹⁰. However, the presence of significant amounts of naphthalene, methyl naphthalenes and a methylbiphenyl, together with the polynuclear aromatic hydrocarbon combustion markers 1-methyl-9H-fluorene and phenanthrene, confirm a 'char' component providing molecular evidence for exposure to fire/cooking. The lack of the attendant aromatic nitrogen-containing biomarkers pyrrole, benzonitrile, benzyl nitrile (phenylacetone nitrile), benzenepropanenitrile and indoles indicative of proteinaceous material^{6,11} suggests a collective origin as combustion compounds ('char') rather than a significant protein component. The presence of carbon dioxide is also consistent with a lipid component in this sample. It should also be noted that highly polar material could have been present, which would not have successfully eluted from the column, or indeed volatilised sufficiently in the probe.

2135

2136 **Discussion, Boatbridge Quarry, Thankerton**

2137 Two samples of dental calculus from Boatbridge Quarry, Thankerton, were analysed by
2138 sequential thermal desorption-gas chromatography-mass spectrometry (TD-GC-MS) and
2139 pyrolysis-gas chromatography-mass spectrometry (Py-GC-MS). The samples contained both
2140 free and bound/polymeric organic material.

2141 The thermal desorption total ion chromatogram (TIC) of both samples was dominated by
2142 carbon dioxide, sulphur dioxide, benzene and, more unusually, acetic acid. Although the
2143 similarity is notable and may suggest a common source, it is difficult to explain the
2144 significance of these common and non-diagnostic organic compounds, beyond indicating that
2145 these samples have undergone diagenetic changes of a highly oxidising nature. More
2146 interestingly, a series of *n*-alkanes, with carbon numbers notably similar were observed in the
2147 TD of both samples. More apparent in sample S1, which contained appreciably more
2148 thermally extractable organic material, these *n*-alkanes range from C₁₆ up to C₂₄, maximising
2149 at C₂₁. Despite evidence for some microbially-derived biodegradation, the odd over even
2150 carbon number predominance of the *n*-alkanes, which dominate the hydrocarbon fraction,
2151 suggest the biodegradation has not been extensive and therefore preserves the ‘chemical
2152 fingerprint’ of the original source. This is notable since the relatively unusual mid-chain
2153 carbon numbers present strongly suggests a submerged aquatic plant (macrophyte)⁸⁹⁻⁹¹ (albeit
2154 possibly with a small bacterial/algal component, i.e. C₁₆ to C₁₈ alkanes), since these typically
2155 have a plant wax component ranging from C₁₉ to C₃₁/C₃₃ and maximising at C₂₁/C₂₃, possibly
2156 deriving from the original food consumed in this context. The persistence of the *n*-alkanes is
2157 not unexpected given that they are known to be particularly resistant to diagenesis^{90,92}. In
2158 addition to the data above, an *n*-alkane proxy for the input/presence of submerged/floating
2159 freshwater aquatic macrophytes provides another corroborating measure of aquatic plant
2160 input⁸⁹ This proxy ratio, P_{aq}, gives values of 1.0 for this sample, which also indicates a
2161 submerged/floating aquatic plant⁸⁹.

2162 The C₂₁ maximum, combined with the relatively narrow *n*-alkane carbon number range in the
2163 calculus samples is indicative of biodegraded submerged aquatic plant (macrophyte) material.
2164 Although the identification of plant remains is challenging, the use of aquatic plants as food
2165 has been suggested. Specifically, the exploitation of the roots and tubers of aquatic plants in
2166 Mesolithic Europe has been hypothesised, with plant remains of eelgrass, horned pondweed

and beaked tassel weed identified at Argus Bank in Denmark⁹³ and a number of aquatic plants, including the submerged/floating species yellow water lily (*Nuphar lutea*), white water lily (*Nymphaea alba*) and pondweed (*Potamogeton pectinatus*), at Star Carr in England⁹⁴. Interestingly, the chemical profile of the dominant C₁₉ to C₂₄ *n*-alkanes, maximising at C₂₁ is consistent with the submerged aquatic plant genus *Nymphaea*⁸⁹. Further work corroborating this possibility using microscopy would be needed, but it would – particularly in light of the suggested earlier exploitation in the British Isles – certainly be worthy of further investigation.

The pyrolysis-GC-MS of both samples revealed a series of ~C₁₀ to C₁₆ *n*-1-alkenes and *n*-alkanes, typical of unsaturated and saturated fat/oil-derived acyl lipids^{5,6}. The absence of these compounds in the TD profile provides molecular evidence for a polymeric/bound acyl lipid source. Importantly, there were no bacterially-derived hydrocarbons deriving from the original branched fatty acid acyl groups which are significant components of bacterial triglycerides, indicating this lipid component is not bacterial in origin. However, it is not possible to be more precise about a possible source in these samples based on these lipid biomarkers alone. A suite of aromatic hydrocarbons observed in both samples from this site provides biomolecular evidence for exposure to fire/cooking.

Juniper Green, City of Edinburgh

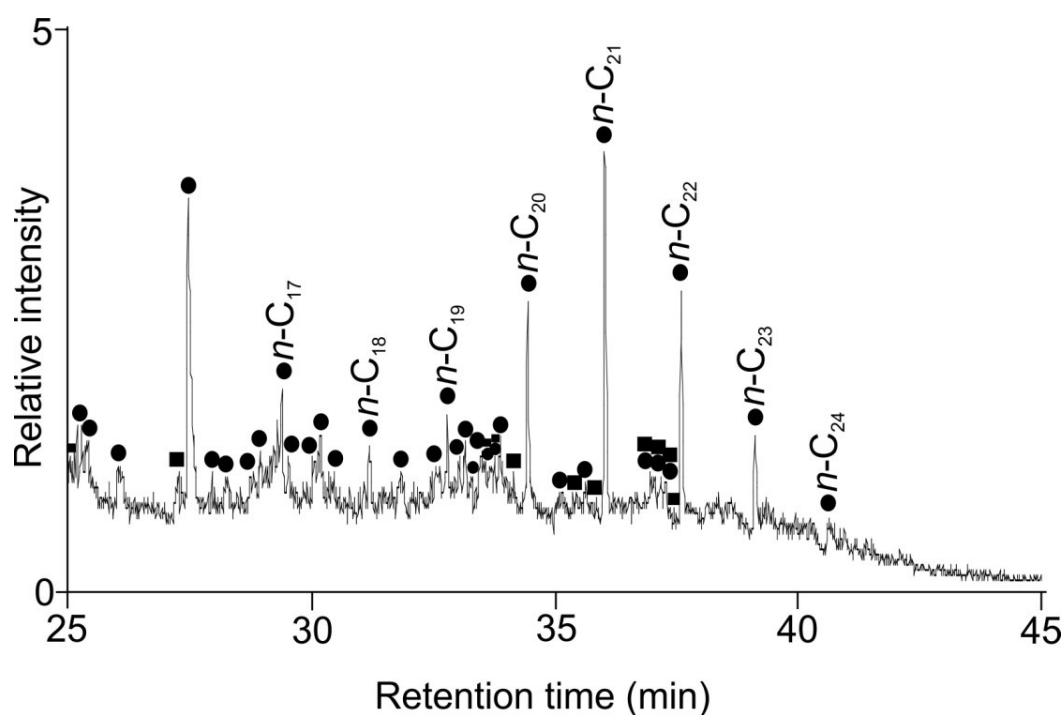
Juniper Green is located on the western outskirts of Edinburgh in east central Scotland. The remains of an adult male accompanied by a Beaker were found in a short stone cist in 1851. The individual has been dated to 2335–2135 cal BC⁸⁷ (see also <https://canmore.org.uk/site/50298>).

27. Juniper Green, City of Edinburgh (NMS X.ET 33): M3 right back of tooth (calculus: 2.48 mg)

The three main compounds in the thermal desorption total ion chromatogram (TIC) (Fig. 20) are carbon dioxide, sulphur dioxide and 1-butene. In minor abundance are a series of alkenes and alkanes, carbon numbers C₁₆ to C₂₄, within which there is series of C₁₇ to C₂₄ *n*-alkanes (odd over even predominance) dominating this and maximising at *n*-C₂₁. Branched alkanes with carbon numbers C₁₆ to C₂₂ are also present as very minor constituents and are possibly

indicative of limited microbially-derived biodegradation (but see summary below). These carbon numbers present in the TD chromatogram may suggest a submerged aquatic plant source (macrophyte)⁸⁹⁻⁹¹, possibly deriving from plant waxes in the original food consumed or reflecting prevailing environmental factors (but, again, see summary below). The 1-butene possibly derives from lipid or proteinaceous material in the sample¹¹, and perhaps produced at this relatively low temperature due to matrix effects. It is not possible to determine the significance of the carbon dioxide, beyond it being indicative of oxidised organic material. It should also be note that highly polar material could have been present, which would not have successfully eluted from the column, or indeed volatilised sufficiently in the probe.

The pyrolysate TIC revealed carbon dioxide, sulphur dioxide, the C₄ 1-alkene, a C₇ diene, acetone, benzene, toluene and pyrrole as the only detectable components. The dominance of carbon dioxide, combined with the relatively minor abundance of the few small organic compounds present, suggests a highly oxidised sample. It is not possible to determine the significance of the carbon dioxide, beyond it being indicative of oxidised organic material and the other compounds are too low an abundance and too ubiquitous to provide any meaningful interpretation of their presence, beyond them being indicative of an organic origin. It should be noted, however, that highly polar material could have been present, which would not have successfully eluted from the column, or indeed volatilised sufficiently in the probe.



Supplementary Figure 20. Reconstructed total ion chromatogram of the thermal desorption profile (310 °C for 10 s) of sample X.ET 33, Juniper Green. Key to Fig. 20: Peak identities (x indicates carbon chain length): filled squares indicates alkenes; filled circles, C_x indicates alkanes.

Discussion, Juniper Green, City of Edinburgh

One sample of dental calculus from Juniper Green was analysed by sequential thermal desorption-gas chromatography-mass spectrometry (TD-GC-MS) and pyrolysis-gas chromatography-mass spectrometry (Py-GC-MS). The sample contained both free and bound/polymeric organic material.

The thermal desorption total ion chromatogram (TIC) of the sample was dominated by carbon dioxide, sulphur dioxide and 1-butene. It is difficult to explain the significance of these common and non-diagnostic organic compounds, beyond indicating that these samples have undergone diagenetic changes of a highly oxidising nature. Although only minor constituents, a series of *n*-alkanes, ranging from C₁₇ up to C₂₄ and maximising at C₂₁, were also detected. There is no odd over even carbon number predominance suggesting the samples may have undergone biodegradation as a result of diagenetic changes (although see below). This is also indicated by the presence of branched alkanes, although they are very minor in abundance compared to the *n*-alkane putative biomarkers. In this context, the relatively unusual mid-chain carbon numbers present suggests a submerged aquatic plant (macrophyte)⁸⁹⁻⁹¹ (albeit possibly with a small bacterial/algal component, i.e. C₁₇ and C₁₈ alkanes), since these typically have a plant wax component ranging from C₁₉ to C₃₁/C₃₃ and maximising at C₂₁/C₂₃, possibly deriving from the original food consumed in this context. The persistence of the *n*-alkanes is not unexpected given that they are known to be particularly resistant to diagenesis^{90,92}. In addition to the data above, an *n*-alkane proxy for the input/presence of submerged/floating freshwater aquatic macrophytes provides another corroborating measure of aquatic plant input⁸⁹. This proxy ratio, P_{aq}, gives values of 1.0 for this sample, which also indicates a submerged/floating aquatic plant⁸⁹.

The C₂₁ maximum, combined with the relatively narrow *n*-alkane carbon number range in the calculus samples is indicative of biodegraded submerged aquatic plant (macrophyte) material. Although the identification of plant remains is challenging, the use of aquatic plants as food has been suggested. Specifically, the exploitation of the roots and tubers of aquatic plants in

Mesolithic Europe has been hypothesised, with plant remains of eelgrass, horned pondweed and beaked tassel weed identified at Argus Bank in Denmark⁹³ and a number of aquatic plants, including the submerged/floating species yellow water lily (*Nuphar lutea*), white water lily (*Nymphaea alba*) and pondweed (*Potamogeton pectinatus*), at Star Carr in England⁹⁴. Interestingly, the chemical profile of the dominant C₁₉ to C₂₄ *n*-alkanes, maximising at C₂₁ is consistent with the submerged aquatic plant genus *Nymphaea*⁸⁹. Further work corroborating this possibility using microscopy would be needed, but it would – particularly in light of the suggested earlier exploitation in the British Isles – certainly be worthy of further investigation.

Of those bound/polymeric organic compounds identified, the dominance of carbon dioxide and the relatively minor abundance of only a few small organic compounds confirms a highly oxidised bound/polymeric organic component was present although its significance beyond this is necessarily unclear.

Ruchlaw Mains, East Lothian (NMS unreg)

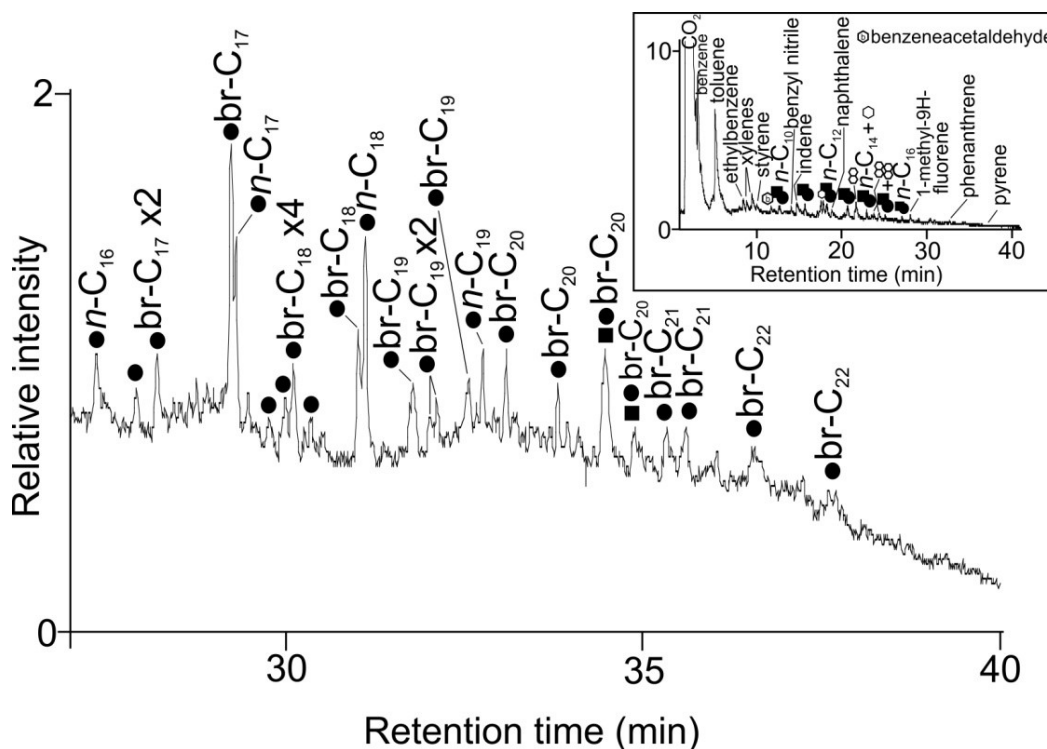
A cist containing an adult male skeleton and a Beaker was discovered in 1979⁹⁵. It has been radiocarbon dated to between 2340-2140 cal BC⁸⁷ (see also <https://canmore.org.uk/site/57808/>).

28. Ruchlaw Mains, East Lothian (NMS unreg): PM1,2 right lingual (calculus: 3.26 mg)

The six main compounds in the thermal desorption total ion chromatogram (TIC) (Fig. 21) are carbon dioxide, 1-butene, acetone, benzene, toluene and acetic acid. In minor abundance is an unresolved complex mixture (UCM; apparent as a ‘hump’ in the chromatogram) of alkenes and alkanes, carbon numbers C₁₆ to C₂₂, within which there is series of C₁₆ to C₁₉ *n*-alkanes (with no odd over even predominance) dominating this UCM profile and maximising at *n*-C₁₇. Branched alkanes with carbon numbers C₁₇ to C₂₂ are also present as minor constituents. These carbon numbers present in the TD chromatogram, combined with the relative abundance of branched hydrocarbons compared to the *n*-alkanes suggests a bacterial source and certainly significant microbially-derived biodegradation (see discussion below). Indene and naphthalene were also detected, albeit as a minor constituents. No characteristic

protein or carbohydrate markers were observed meaning the significance of the non-diagnostic compounds carbon dioxide, benzene and acetic acid is uncertain. It should also be noted that highly polar material could have been present, which would not have successfully eluted from the column, or indeed volatilised sufficiently in the probe.

In contrast to the thermal desorption profile, the pyrolysis-GC-MS TIC (Fig. 21 inset) revealed a complex suite of organic compounds. These include a series of C₁₀ to C₁₉ *n*-1-alkenes and *n*-alkanes, typical of unsaturated and saturated fat/oil-derived acyl lipids^{5,6}. The absence of these compounds in the TD profile provides molecular evidence for a polymeric/bound acyl lipid source. Importantly, there were no bacterially-derived hydrocarbons deriving from the original branched fatty acid acyl groups which are significant components of bacterial triglycerides, indicating this lipid component is not bacterial in origin. The pyrolysate TIC identified components indicative of 'black carbon', i.e. charcoal or soot⁷⁻¹⁰. Of these, benzene and toluene were major compounds present in the 'char' pyrolysate (~30% and 35% of total quantified pyrogram peak area respectively), along with moderate quantities of ethyl benzene and *o*-, *m*- and *p*-xylenes (~7% total). The relative dominance of toluene, rather than the normally dominant aromatic hydrocarbon benzene, is somewhat atypical of chars⁷⁻¹⁰. However, the presence of significant amounts of naphthalene, methyl naphthalenes and methyl biphenyl, together with the polynuclear aromatic hydrocarbon combustion markers 1-methyl-9H-fluorene, phenanthrene, 1-methylanthracene, pyrene and 1-methylpyrene, confirm a 'char' component providing molecular evidence for exposure to fire/cooking. The markers identified here can also be indicative of proteins^{6,11} and combined with the related protein markers pyrrole, benzyl nitrile (phenylacetonitrile) and methyl benzyl nitrile identified this is indicative of these compounds, at least in part, originating from a protein-derived component^{6,11}. The presence of carbon dioxide is also consistent with a lipid component in this sample. It should also be noted that highly polar material could have been present, which would not have successfully eluted from the column, or indeed volatilised sufficiently in the probe.



Supplementary Figure 21. Reconstructed total ion chromatogram of the thermal desorption profile (310 °C for 10 s) of sample NMS unreg, Ruchlaw Mains. Key to Fig. 21: Peak identities (x indicates carbon chain length): filled squares indicates alkenes; filled circles, Cx indicates alkanes. Inset displays a reconstructed total ion chromatogram of the pyrogram (pyrolysis profile) (610 °C for 10) of this sample, after thermal desorption (310 °C for 10 s). Peak identities: (x indicates carbon chain length): filled squares, Cx indicates alkenes; filled circles, Cx indicates alkanes; open hexagons indicate alkyl indenenes; conjoined open hexagons indicate alkyl naphthalenes; CO₂ indicates carbon dioxide. Also shown are benzene, toluene, ethyl benzene, xylenes (*o*-, *m*- and *p*-), styrene, benzeneacetaldehyde, benzyl nitrile, indene, naphthalene, 1-methyl-9H-fluorene, phenanthrene and pyrene.

Discussion, Ruchlaw Mains, East Lothian

One sample of dental calculus from Ruchlaw Mains was analysed by sequential thermal desorption-gas chromatography-mass spectrometry (TD-GC-MS) and pyrolysis-gas chromatography-mass spectrometry (Py-GC-MS). The sample contained both free and bound/polymeric organic material.

The thermal desorption total ion chromatogram (TIC) was dominated by carbon dioxide, 1-butene, acetone, benzene, toluene and, more unusually, acetic acid. It is difficult to explain

the significance of these common and non-diagnostic organic compounds, beyond indicating that these samples have undergone diagenetic changes of a highly oxidising nature. In minor abundance was an unresolved complex mixture of alkenes and alkanes, carbon numbers C₁₆ to C₂₂, within which there is a series of C₁₆ to C₁₉ *n*-alkanes (with no odd over even predominance) dominating this UCM profile and maximising at *n*-C₁₇. Branched alkanes with carbon numbers C₁₇ to C₂₂ are also present as minor constituents. These carbon numbers present in the TD chromatogram, combined with the relative abundance of branched hydrocarbons compared to the *n*-alkanes suggests a bacterial source and certainly significant microbially-derived biodegradation.

The pyrolysis-GC-MS revealed a series of ~C₁₀ to C₁₉ *n*-1-alkenes and *n*-alkanes, typical of unsaturated and saturated fat/oil-derived acyl lipids^{5,6}. The absence of these compounds in the TD profile provides molecular evidence for a polymeric/bound acyl lipid source. Importantly, there were no bacterially-derived hydrocarbons deriving from the original branched fatty acid acyl groups which are significant components of bacterial triglycerides, indicating this lipid component is not bacterial in origin. However, it is not possible to be more precise about a possible source in these samples based on these lipid biomarkers alone. A suite of aromatic hydrocarbons observed provides biomolecular evidence for exposure to fire/cooking.

Skateraw (Roy Richie 1958 cist), East Lothian (NMS unreg)

A short stone cist containing an adult male skeleton and a Beaker was discovered in 1958⁹⁶. The individual has been radiocarbon dated to 2340–2195 cal BC⁸⁷ (see also <https://canmore.org.uk/site/57808/>).

29. Skateraw (Roy Richie 1958 cist), East Lothian (NMS unreg): M1 right lingual (calculus: 3.28 mg)²⁸

One sample of dental calculus from Skateraw was analysed by sequential thermal desorption-gas chromatography-mass spectrometry (TD-GC-MS) and pyrolysis-gas chromatography-mass spectrometry (Py-GC-MS). The sample contained virtually no free and little bound/polymeric organic material, most likely reflecting unfavourable prevailing conditions for biomolecular preservation. Of those organic compounds identified, the dominance of

carbon dioxide and the relatively minor abundance of only a few small organic compounds suggests that only highly oxidised organic material was present.

The thermal desorption total ion chromatogram (TIC) revealed carbon dioxide as the only detectable component, indicating the essential absence of free, thermally extractable organic components in this sample. It should be noted, however, that highly polar material could have been present, which would not have successfully eluted from the column, or indeed volatilised sufficiently in the probe. It is not possible to determine the significance of the carbon dioxide, beyond it being indicative of oxidised organic material.

The pyrolysate TIC revealed carbon dioxide, C₄ to C₆ 1-alkenes, a methylcyclopentadiene, benzene and toluene as the only detectable components. The dominance of carbon dioxide, combined with the relatively minor abundance of the few small organic compounds present, suggests a highly oxidised sample. It is not possible to determine the significance of the carbon dioxide, beyond it being indicative of oxidised organic material and the other compounds are too low an abundance and too ubiquitous to provide any meaningful interpretation of their presence, beyond them being indicative of an organic origin. It should be noted, however, that highly polar material could have been present, which would not have successfully eluted from the column, or indeed volatilised sufficiently in the probe.

Lesmurdie cist B, Banff (NMS X.ET 14)

Several short stone cists containing individuals and Beakers were found at Lesmurdie on Scotland's north-east coast. One adult, probably male, aged 40–60 from cist B, associated with a Beaker and three flint chips, was radiocarbon dated to 2295–2040 cal BC⁹⁷ (see also <https://canmore.org.uk/site/17297>).

30. Lesmurdie cist B, Banff (NMS X.ET 14): R mandibular 2nd molar, labial (calculus: 1.47 mg)

One sample of dental calculus from Lesmurdie cist B was analysed by sequential thermal desorption-gas chromatography-mass spectrometry (TD-GC-MS) and pyrolysis-gas chromatography-mass spectrometry (Py-GC-MS). The sample contained little free and

bound/polymeric organic material, most likely reflecting unfavourable prevailing conditions for biomolecular preservation. Of those organic compounds identified, the dominance of carbon dioxide and the relatively minor abundance of only a few small organic compounds suggests that only highly oxidised organic material was present.

The thermal desorption total ion chromatogram (TIC) revealed carbon dioxide, sulphur dioxide and acetone as the only detectable components, indicating the essential absence of free, thermally extractable organic components in this sample. It should be noted, however, that highly polar material could have been present, which would not have successfully eluted from the column, or indeed volatilised sufficiently in the probe. It is not possible to determine the significance of the carbon dioxide, beyond it being indicative of oxidised organic material.

The pyrolysate TIC revealed carbon dioxide, sulphur dioxide, C₄ to C₆ 1-alkenes, cyclopentadiene, benzene and toluene as the only detectable components. The dominance of carbon dioxide, combined with the relatively minor abundance of the few small organic compounds present, suggests a highly oxidised sample. It is not possible to determine the significance of the carbon dioxide, beyond it being indicative of oxidised organic material and the other compounds are too low an abundance and too ubiquitous to provide any meaningful interpretation of their presence, beyond them being indicative of an organic origin. It should be noted, however, that highly polar material could have been present, which would not have successfully eluted from the column, or indeed volatilised sufficiently in the probe.

Lithuania

Twenty-one samples of dental calculus taken from twenty-one individuals from six sites in Lithuania ranging in date from the Mesolithic to the 5-6th centuries AD, were analysed by sequential thermal desorption-gas chromatography-mass spectrometry (TD-GC-MS) and pyrolysis-gas chromatography-mass spectrometry (Py-GC-MS). This technique facilitates the identification of both free/unbound and bound/polymeric organic components. The twenty-one calculus samples produced very little free organic material, with only seven samples containing free compounds if the essentially ubiquitous and thermally generated carbon

dioxide is excluded. In contrast, fourteen of the twenty-one samples revealed a very minor to significant amount of a bound/polymeric organic constituent.

Kretuonas, Early – Middle Neolithic

The Kretuonas settlement belongs to a group of Neolithic-Bronze Age settlements discovered along the shore of Lake Kretuonas (Švenčionys district) in eastern Lithuania. Investigation of settlements was carried in 1978-1989. Human skeletal remains are fragmentary; some graves were destroyed through ploughing. Burial items include all kinds of tools made from flint, stone, bone, antler, clay and adornments made of amber and animal bones. Remains of domestic animals (cattle, pig, horses and dogs), nuts, shells of molluscs were found⁹⁸.

31. Kretuonas SA1 (calculus: 0.65 mg) Early – Middle Neolithic, 25-30 years old.

The thermal desorption total ion chromatogram (TIC) revealed no detectable components, indicating the absence of free, thermally extractable organic components in this sample. It should be noted, however, that highly polar material could have been present, which would not have successfully eluted from the column, or indeed volatilised sufficiently in the probe.

The pyrolysate TIC revealed carbon dioxide as the only detectable component, indicating the essential absence of organic material in this sample. It should be noted, however, that highly polar material could have been present, which would not have successfully eluted from the column, or indeed volatilised sufficiently in the probe. It is not possible to determine the significance of the carbon dioxide, beyond it being indicative of oxidised organic material, although its presence as only a very minor component makes even this inference of very little meaningful value.

Donkalis and Spiginas

Mesolithic-Neolithic cemeteries are situated along the shores of Lake Biržulis (Telšiai district) in western Lithuania. Research excavations of the area were carried out between 1978 and 1993. Two archaeological complexes were discovered, namely the Mesolithic camp sites on the promontories of the lake peninsula (Spigino Ragas) and cemeteries and funeral feast and sacrifice pits on lake islands next to the camps (Donkalis and Spiginas). In total the remains of 24 individuals from 17 graves were uncovered. Most graves contained one

person but at Donkalnis there were at least two double graves. The dead were placed straight on their backs or in a bent posture⁹⁹.

32. Donkalnis SA4, around 50 years old (calculus: 0.62 mg), Neolithic.

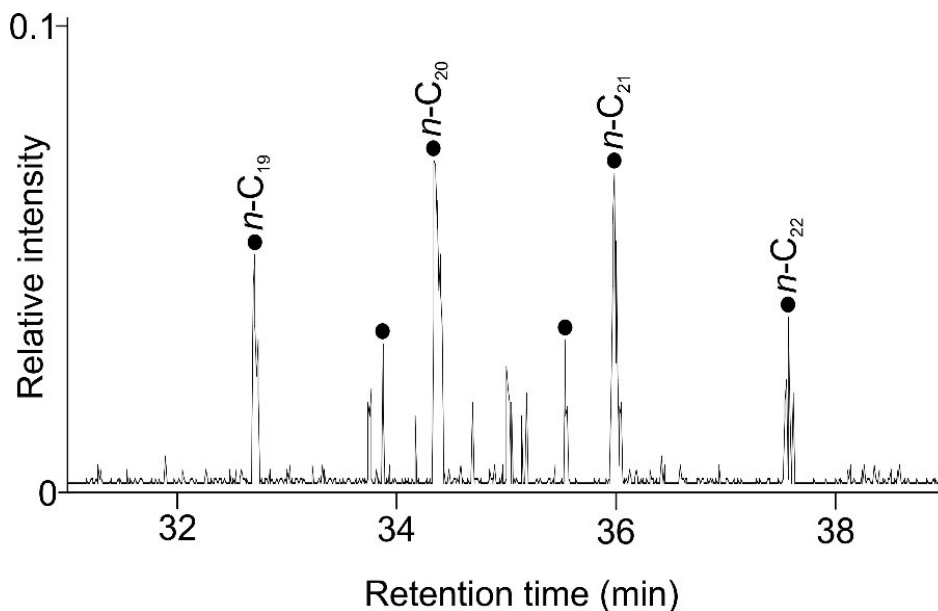
The thermal desorption total ion chromatogram (TIC) revealed carbon dioxide as the only detectable component, indicating the essential absence of free, thermally extractable organic components in this sample. It should be noted, however, that highly polar material could have been present, which would not have successfully eluted from the column, or indeed volatilised sufficiently in the probe. It is not possible to determine the significance of the carbon dioxide, beyond it being indicative of oxidised organic material.

The pyrolysate TIC revealed carbon dioxide as the only detectable component, indicating the essential absence of organic material in this sample. It should be noted, however, that highly polar material could have been present, which would not have successfully eluted from the column, or indeed volatilised sufficiently in the probe. It is not possible to determine the significance of the carbon dioxide, beyond it being indicative of oxidised organic material.

33. Spiginas SA5 (calculus: 1.63 mg). Middle Mesolithic (6412 – 6258 cal. BC), female, 30-35 years old.

The main compound in the thermal desorption total ion chromatogram (TIC) (Fig. 22) is carbon dioxide. In trace abundance are a series of *n*-alkanes, carbon numbers C₁₉ to C₂₂, maximising at C_{20/21}. There is no odd over even carbon number predominance suggesting the sample may have undergone biodegradation as a result of diagenetic changes. This is further corroborated by the presence of branched alkanes with carbon numbers C₂₀ and C₂₁ observed as relatively minor constituents and indicative of microbially-derived biodegradation (see discussion below). The relatively unusual mid-chain carbon numbers present in the TD chromatogram suggests a submerged aquatic plant (macrophyte)⁸⁹⁻⁹², possibly deriving from plant waxes in the original food consumed or reflecting prevailing environmental factors (again, see discussion below). It is not possible to determine the significance of the carbon dioxide, beyond it being indicative of oxidised organic material. It should also be note that highly polar material could have been present, which would not have successfully eluted from the column, or indeed volatilised sufficiently in the probe.

The pyrolysate TIC revealed carbon dioxide and 1-butene as the only detectable components. The dominance of carbon dioxide, combined with the relatively minor abundance of 1-butene, suggests a highly oxidised sample. It is not possible to determine the significance of the carbon dioxide, beyond it being indicative of oxidised organic material. 1-Butene can be indicative of lipid or proteinaceous material in the sample¹¹, although its relatively minor abundance and the absence of other related biomarkers prevents any meaningful interpretation of its significance. It should be noted, however, that highly polar material could have been present, which would not have successfully eluted from the column, or indeed volatilised sufficiently in the probe.



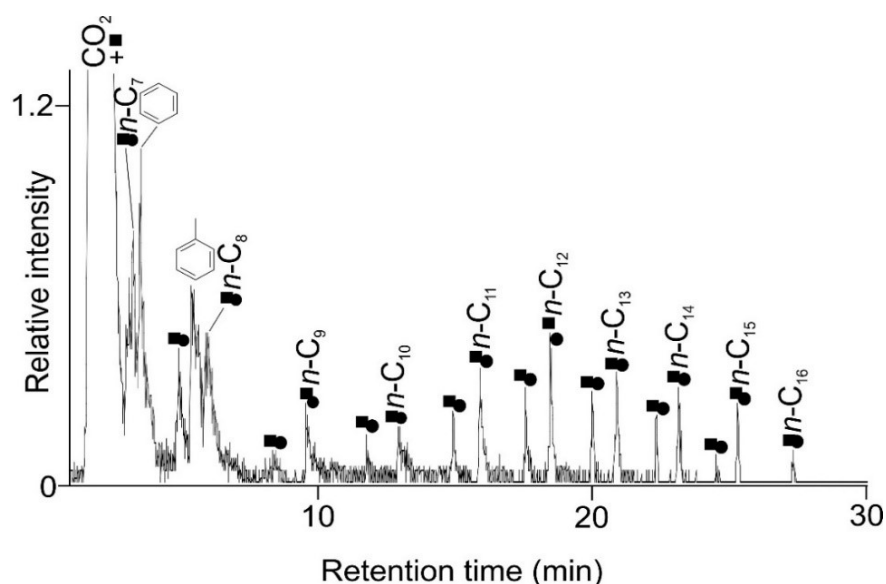
Supplementary Figure 22. Reconstructed total ion chromatogram of the thermal desorption profile (310 °C for 10 s) of sample 5A5 (Meso + Neo). Key to Fig. 22: Peak identities (x indicates carbon chain length): filled circles, Cx indicates alkanes.

34. Spiginas SA6 (calculus: 5.15 mg). Late Neolithic (2864 – 2488 cal. BC) Male, age 50-55 years old.

The thermal desorption total ion chromatogram (TIC) revealed carbon dioxide as the only detectable component, indicating the essential absence of free, thermally extractable organic components in this sample. It should be noted, however, that highly polar material could have been present, which would not have successfully eluted from the column, or indeed

volatilised sufficiently in the probe. It is not possible to determine the significance of the carbon dioxide, beyond it being indicative of oxidised organic material.

The pyrolysis-GC-MS TIC (Fig. 23) was dominated by carbon dioxide, 1-butene and 1-pentene, the alkenes being indicative of lipid or proteinaceous material in the sample¹¹. Cyclopentadiene and methylcyclopentadiene are also components of the pyrolysate although it is not possible to ascribe a specific origin here. Toluene was also detected, albeit as a very minor component. In contrast to the thermal desorption profile, the pyrogram also revealed a series of C₇ to C₁₆ *n*-1-alkenes and *n*-alkanes, typical of unsaturated and saturated fat/oil-derived acyl lipids^{5,6}. The absence of these compounds in the TD profile provides molecular evidence for a polymeric/bound acyl lipid source. However, in addition to *n*-1-alkenes and *n*-alkanes, typical of fat/oil derived lipids, the pyrogram also displayed a series of C₈ to C₁₅ isomethyl-1-alkenes (isoalkenes) and 2-methylalkanes (isoalkanes), presumably deriving from the branched fatty acids which are significant components of bacterial triglycerides. The dominance of carbon dioxide suggests a highly oxidised sample. It is not possible to determine the significance of the carbon dioxide, beyond it being indicative of oxidised organic material. It should be noted, however, that highly polar material could have been present, which would not have successfully eluted from the column, or indeed volatilised sufficiently in the probe.



Supplementary Figure 23. Reconstructed total ion chromatogram of the pyrogram (pyrolysis profile) (610 °C for 10s) of sample 5A6 (Meso + Neo), after thermal desorption

(310 °C for 10 s). Key to Fig. 23: Peak identities (x indicates carbon chain length): filled squares, C_x indicates alkenes; filled circles, C_x indicates alkanes. Also shown are the structures of two aromatic compounds identified: benzene and toluene. CO₂ indicates carbon dioxide.

35. Gyvakarai SA7. (calculus: 3.42 mg). Late Neolithic/Early Bronze Age, 2269 – 1977 cal. BC. Male, 35-40 years old.

The site of Gyvakarai (Kupiškis district) is situated in eastern Lithuania and is represented by one Late Neolithic - Bronze Age inhumation. The burial containing the skeletal remains of a middle-aged man was found accidentally in 2000. As the burial was disturbed the original position of the skeleton could not be reconstructed. Grave goods included a stone battle boat shaped axe and other flint items¹⁰⁰.

The thermal desorption total ion chromatogram (TIC) revealed carbon dioxide as the only detectable component, indicating the essential absence of free, thermally extractable organic components in this sample. It should be noted, however, that highly polar material could have been present, which would not have successfully eluted from the column, or indeed volatilised sufficiently in the probe. It is not possible to determine the significance of the carbon dioxide, beyond it being indicative of oxidised organic material.

The pyrolysate TIC revealed carbon dioxide as the only detectable component, indicating the essential absence of organic material in this sample. It should be noted, however, that highly polar material could have been present, which would not have successfully eluted from the column, or indeed volatilised sufficiently in the probe. It is not possible to determine the significance of the carbon dioxide, beyond it being indicative of oxidised organic material.

Discussion, Mesolithic and Neolithic samples, Lithuania.

Five samples of dental calculus from Mesolithic and Neolithic individuals were analysed by sequential thermal desorption-gas chromatography-mass spectrometry (TD-GC-MS) and pyrolysis-gas chromatography-mass spectrometry (Py-GC-MS). Three of the samples (SA1, SA4 and SA7) contained little free and bound/polymeric organic material, most likely reflecting their age and unfavourable prevailing conditions for biomolecular preservation.

2555 Carbon dioxide was the only compound identified in these samples suggesting that only
2556 highly oxidised organic material was present.

2557 Another sample, SA6, revealed only carbon dioxide in the TD-GC-MS analysis, indicating
2558 the essential absence of free, thermally extractable organic components in this sample. The
2559 carbon dioxide is also indicative of highly oxidised organic material. The pyrolysis-GC-MS
2560 revealed a series of C₇ to C₁₆ *n*-1-alkenes and *n*-alkanes, typical of unsaturated and saturated
2561 fat/oil-derived acyl lipids^{5,6}. The absence of these compounds in the TD profile provides
2562 molecular evidence for a polymeric/bound acyl lipid source. However, the additional
2563 presence of a series of C₈ to C₁₅ isoalkenes and isoalkanes, presumably deriving from the
2564 branched fatty acids, which are significant components of bacterial triglycerides, suggests a
2565 microbial origin for these lipid biomarkers, at least in part (also see below). The dominance
2566 of carbon dioxide again suggests a highly oxidised sample.

2567 The last Mesolithic/Neolithic sample, SA5, revealed both free and bound/polymeric organic
2568 material. The thermal desorption total ion chromatogram (TIC) of the sample was dominated
2569 by carbon dioxide; it is difficult to explain the significance of this non-diagnostic compound,
2570 beyond indicating that this sample has undergone diagenetic changes of a highly oxidising
2571 nature. Although only trace constituents, a series of *n*-alkanes, ranging from C₁₉ up to C₂₂ and
2572 maximising at C_{20/21}, were also detected. There is no odd over even carbon number
2573 predominance suggesting the samples may have undergone biodegradation as a result of
2574 diagenetic changes (although see below). This is also indicated by the presence of branched
2575 alkanes, although they are minor in abundance compared to the *n*-alkane putative biomarkers.
2576 In this context, the relatively unusual mid-chain carbon numbers present suggests a
2577 submerged aquatic plant (macrophyte)⁸⁹⁻⁹², since these typically have a plant wax component
2578 ranging from C₁₉ to C₃₁/C₃₃ and maximising at C₂₁/C₂₃, possibly deriving from the original
2579 food consumed in this context. The persistence of the *n*-alkanes is not unexpected given that
2580 they are known to be particularly resistant to diagenesis^{90,92}.

2581 In addition to the data above, an *n*-alkane proxy for the input/presence of submerged/floating
2582 freshwater aquatic macrophytes can provide another corroborating measure for an aquatic
2583 plant input⁸⁹. However, the *n*-alkane proxy ratio, P_{aq}, cannot be applied meaningfully here
2584 because of the lack of >C₂₃ *n*-alkanes. That being said, the C_{20/21} maximum, combined with
2585 the relatively narrow *n*-alkane carbon number range in the calculus sample is indicative of
2586 biodegraded submerged aquatic plant (macrophyte) material. Although the identification of

plant remains is challenging, the use of aquatic plants as food has been suggested. Specifically, the exploitation of the roots and tubers of aquatic plants in Mesolithic Europe has been hypothesised, with plant remains of eelgrass, horned pondweed and beaked tassel weed identified at Argus Bank in Denmark⁹³ and a number of aquatic plants, including the submerged/floating species yellow water lily (*Nuphar lutea*), white water lily (*Nymphaea alba*) and pondweed (*Potamogeton pectinatus*), at Star Carr in England⁹⁴. Interestingly, the chemical profile of the dominant C₁₉ to C_{22n}-alkanes, maximising at C₂₁ is consistent with the submerged aquatic plant genus *Nymphaea*⁸⁹, following biodegradation. Further work corroborating this possibility using microscopy would be needed, but it certainly be worthy of further investigation. With carbon preference indexes (CPIs) of 1.12 and a C_{20/21} maximum it is consistent with what would be expected for a (biodegraded) submerged aquatic plant. Notably similar values have been observed for submerged aquatic plant rhizomes from southern Europe: Greece, CPI = 1.13; France, CPI = 1.15¹⁰¹. Furthermore, the rhizomes in these studies had a far less pronounced odd-over-even carbon preference than leaves from the same plants, which is likely to reflect their microenvironment in the aquatic/marine sediments where they are far more exposed to biomolecular reworking and biodegradation from microbial inputs than leaves from the same plant. Of those bound/polymeric organic compounds identified, the dominance of carbon dioxide and the relatively minor abundance of 1-butene as the only other organic compound identified confirms a highly oxidised bound/polymeric organic component was present although its significance beyond this is necessarily unclear.

Obeliai, Central Lithuania flat burials. Lithuania People's Wandering (AD 5th-6th century).

Obeliai burial ground is situated on the shore of Lake Obeliai (Ukmergės district) in central Lithuania. Investigations carried in 1978-1983 revealed 65 inhumations, 6 cremated burials, and 5 horse graves, dated 5th-6th A.D. The western part of the Iron Age burial ground abuts 14th-15th A.D. Obeliai cemetery¹⁰².

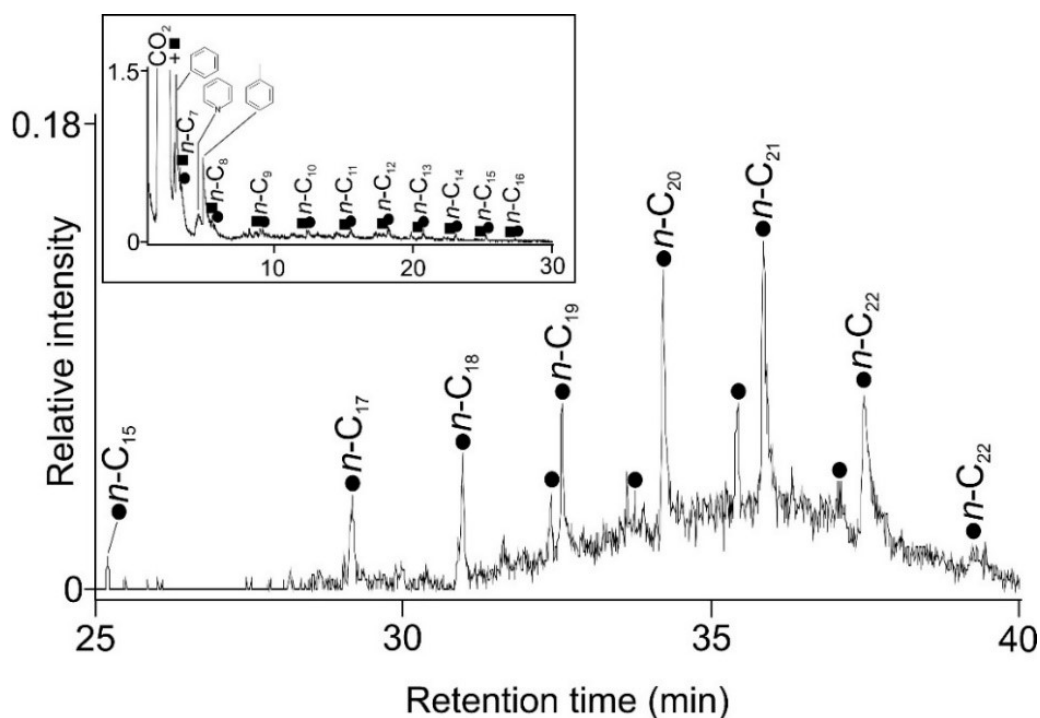
36. Obeliai, Central Lithuania flat burials. Sk1128 male 50-55 yrs T32 labial, 1A5 (calculus: 6.83 mg)

The main compound in the thermal desorption total ion chromatogram (TIC) (Fig. 24) is carbon dioxide. In minor abundance are a series of *n*-alkanes, carbon numbers C₁₅ to C₂₃, maximising at C₂₁. There is no odd over even carbon number predominance suggesting the sample may have undergone biodegradation as a result of diagenetic changes. This is further corroborated by the presence of branched alkanes with carbon numbers C₁₉ to C₂₂ observed as relatively minor constituents and indicative of microbially-derived biodegradation (see discussion below). The relatively unusual mid-chain carbon numbers present in the TD chromatogram suggests a submerged aquatic plant (macrophyte)⁸⁹⁻⁹² component, possibly deriving from plant waxes in the original food consumed or reflecting prevailing environmental factors (again, see discussion below), in addition to a bacterial input (C₁₅ to C₁₈ alkanes)^{92,103}. It is not possible to determine the significance of the carbon dioxide, beyond it being indicative of oxidised organic material. It should also be noted that highly polar material could have been present, which would not have successfully eluted from the column, or indeed volatilised sufficiently in the probe.

The pyrolysis-GC-MS TIC (Fig. 24, inset) was dominated by carbon dioxide, 1-butene and 1-pentene, the alkenes being indicative of lipid or proteinaceous material in the sample¹¹. Cyclopentadiene and methylcyclopentadiene are also significant components of the pyrolysate although it is not possible to ascribe a specific origin here. In contrast to the thermal desorption profile, the pyrogram also revealed a series of C₆ to C₁₆ *n*-1-alkenes and *n*-alkanes, typical of unsaturated and saturated fat/oil-derived acyl lipids^{5,6}. The absence of these compounds in the TD profile provides molecular evidence for a polymeric/bound acyl lipid source. However, in addition to *n*-1-alkenes and *n*-alkanes, typical of fat/oil derived lipids, the pyrogram also displayed a series of C₉ to C₁₆ iso-methyl-1-alkenes (isoalkenes) and 2-methylalkanes (isoalkanes), presumably deriving from the branched fatty acids which are significant components of bacterial triglycerides.

The pyrolysate TIC identified components indicative of 'black carbon', i.e. charcoal or soot⁷⁻¹⁰. Of these, benzene and toluene were major compounds present in the typical 'char' component pyrolysate (~50% and 30% of total quantified 'char' respectively), along with moderate quantities of ethyl benzene and o-, m- and p-xylenes (~7% total). The protein marker pyridine¹¹ was present in moderate abundance (~8%) and despite the absence of higher molecular weight 'char' organic compounds their collective presence provides at least

tentative molecular evidence for exposure to fire/cooking. The dominance of carbon dioxide suggests a highly oxidised sample. It is not possible to determine the significance of the carbon dioxide, beyond it being indicative of oxidised organic material. It should be noted, however, that highly polar material could have been present, which would not have successfully eluted from the column, or indeed volatilised sufficiently in the probe.

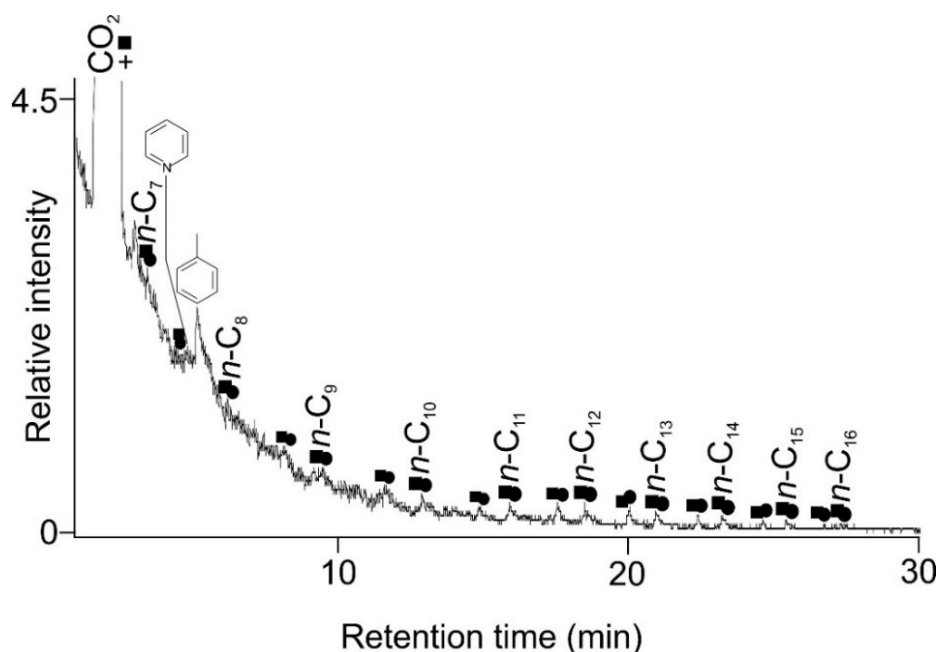


Supplementary Figure 24. Reconstructed total ion chromatogram of the thermal desorption profile (310 °C for 10 s) of sample 1A5, People's Wandering (AD 5th-6th century), Obeliai. Key to Fig. 24: Peak identities (x indicates carbon chain length): filled circles, Cx indicates alkanes. Inset displays a reconstructed total ion chromatogram of the pyrogram (pyrolysis profile) (610 °C for 10) of this sample, after thermal desorption (310 °C for 10 s). Peak identities: (x indicates carbon chain length): filled squares, Cx indicates alkenes; filled circles, Cx indicates alkanes. CO₂ indicates carbon dioxide. Also shown are the structures of three aromatic compounds identified: benzene, toluene and pyridine.

37. Obeliai, Central Lithuania flat burials. Lithuania People's Wandering (AD 5th-6th century), Sk1132 female 30-35 yrs T41 lingual, 1A6 (calculus: 2.91 mg)

The thermal desorption total ion chromatogram (TIC) revealed carbon dioxide as the only detectable component, indicating the essential absence of free, thermally extractable organic components in this sample. It should be noted, however, that highly polar material could have been present, which would not have successfully eluted from the column, or indeed volatilised sufficiently in the probe. It is not possible to determine the significance of the carbon dioxide, beyond it being indicative of oxidised organic material.

The pyrolysis-GC-MS TIC (Fig. 25) was dominated by carbon dioxide, 1-butene and 1-pentene, the alkenes being indicative of lipid or proteinaceous material in the sample¹¹. Cyclopentadiene and methylcyclopentadiene are also significant components of the pyrolysate although it is not possible to ascribe a specific origin here. Toluene was also detected, albeit as a minor component. In contrast to the thermal desorption profile, the pyrogram also revealed a series of C₇ to C₁₆ *n*-1-alkenes and *n*-alkanes, typical of unsaturated and saturated fat/oil-derived acyl lipids^{5,6}. The absence of these compounds in the TD profile provides molecular evidence for a polymeric/bound acyl lipid source. However, in addition to *n*-1-alkenes and *n*-alkanes, typical of fat/oil derived lipids, the pyrogram also displayed a series of C₈ to C₁₆ iso-methyl-1-alkenes (isoalkenes) and 2-methylalkanes (isoalkanes), presumably deriving from the branched fatty acids which are significant components of bacterial triglycerides. The dominance of carbon dioxide suggests a highly oxidised sample. It is not possible to determine the significance of the carbon dioxide, beyond it being indicative of oxidised organic material. It should be noted, however, that highly polar material could have been present, which would not have successfully eluted from the column, or indeed volatilised sufficiently in the probe.



Supplementary Figure 25. Reconstructed total ion chromatogram of the thermal desorption profile (310 °C for 10 s) of sample 1A6, People's Wandering (AD 5th-6th century), Obeliai. Key to Fig. 25: Peak identities (x indicates carbon chain length): filled circles, Cx indicates alkanes. Inset displays a reconstructed total ion chromatogram of the pyrogram (pyrolysis profile) (610 °C for 10) of this sample, after thermal desorption (310 °C for 10 s). Peak identities: (x indicates carbon chain length): filled squares, Cx indicates alkenes; filled circles, Cx indicates alkanes. CO₂ indicates carbon dioxide. Also shown are the structures of two aromatic compounds identified: pyridine and toluene.

38. Lithuania People's Wandering (AD 5th-6th century), Obeliai, Central Lithuania flat burials, Sk1289 male 30-35 yrs T32 labial, 1A8 (calculus: 5.09 mg)

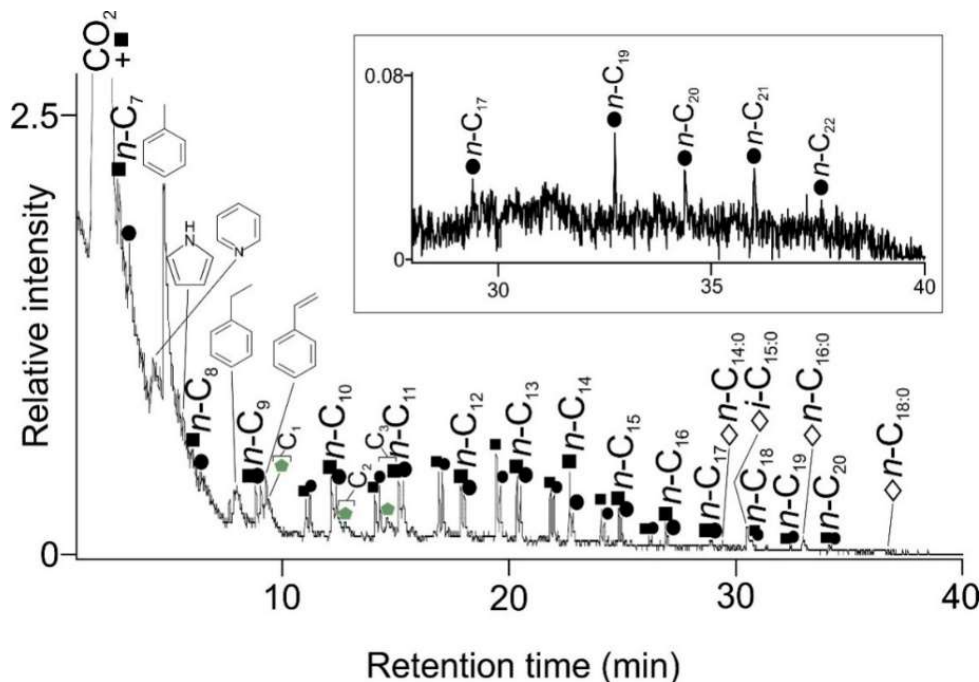
The main compound in the thermal desorption total ion chromatogram (TIC) (Fig. 26, inset) is carbon dioxide. In trace abundance are a series of *n*-alkanes, carbon numbers C₁₇ to C₂₂, maximising at C₁₉. There is an odd over even carbon number predominance, although the relative abundance of the C₂₀ and C₂₂ *n*-alkanes suggests the sample has undergone some biodegradation as a result of diagenetic changes. Although the trace quantities and evidence for biodegradation necessarily mean any inference must be tentative, the relatively unusual mid-chain carbon numbers present in the TD chromatogram, combined with the C₁₉ *n*-alkane being dominant and an odd over even carbon number predominance, suggests an algal source^{92,101,103} (see discussion below). It is not possible to determine the significance of the

2711 carbon dioxide, beyond it being indicative of oxidised organic material. It should also be note
2712 that highly polar material could have been present, which would not have successfully eluted
2713 from the column, or indeed volatilised sufficiently in the probe.

2714 The pyrolysis-GC-MS TIC (Fig. 26) was dominated by carbon dioxide, 1-butene and 1-
2715 pentene, the alkenes being indicative of lipid or proteinaceous material in the sample¹¹.
2716 Cyclopentadiene and methylcyclopentadiene are also significant components of the
2717 pyrolysate although it is not possible to ascribe a specific origin here. Acetone was also
2718 identified and can be indicative of the amino acid glycine or a carbohydrate component,
2719 although any interpretation should be considered tentative here. In contrast to the thermal
2720 desorption profile, the pyrogram also revealed a series of C₇ to C₂₀ *n*-1-alkenes and *n*-alkanes,
2721 typical of unsaturated and saturated fat/oil-derived acyl lipids^{5,6}. A distribution with maxima
2722 at C₁₃ suggests the original acyl lipids may have been high in unsaturated fatty acids. The
2723 absence of these compounds in the TD profile provides molecular evidence for a
2724 polymeric/bound acyl lipid source. However, in addition to *n*-1-alkenes and *n*-alkanes, typical
2725 of fat/oil derived lipids, the pyrogram also displayed a series of C₉ to C₁₆ iso-methyl-1-
2726 alkenes (isoalkenes) and 2-methylalkanes (isoalkanes), presumably deriving from the
2727 branched fatty acids which are significant components of bacterial triglycerides.

2728 A number of the biomarkers identified here can be indicative of proteins^{6,11}, i.e. toluene,
2729 pyridine, pyrrole. This is also corroborated by the presence of aliphatic nitriles (carbon
2730 numbers C_{14:0}, *i*-C_{15:0}, C_{16:0} (max) and C_{18:0}) suggesting a lipid (fat/oil) in this sample which
2731 has combined with protein-derived nitrogen to produce these moieties⁵. However, notable in
2732 this context is the additional presence of appreciable amounts of the more unusual 2-
2733 methylpyrroles, 2-ethylpyrrole and an ethylmethylpyrrole. Collectively, these C₁, C₂, and C₃
2734 C₄ are possibly not detected due to the low abundance of organic material present) alkyl
2735 pyrroles can be indicative of a tetrapyrrole/porphyrin-derived origin and here may tentatively
2736 suggest an algal source observed in fossils and expected in similarly diagenetically altered
2737 archaeological material^{11,21,22}. The presence here of the protein markers and specifically the
2738 C₁ to C₃ alkyl pyrroles, combined with the particular lipid profile, tentatively suggests the
2739 presence of a macroalgae, possibly a freshwater source, in this sample²¹⁻²³ (see discussion).
2740 The dominance of carbon dioxide suggests a highly oxidised sample. It is not possible to
2741 determine the significance of the carbon dioxide, beyond it being indicative of oxidised
2742 organic material. It should be noted, however, that highly polar material could have been

present, which would not have successfully eluted from the column, or indeed volatilised sufficiently in the probe.



Supplementary Figure 26. Reconstructed total ion chromatogram of the pyrogram (pyrolysis profile) (610 °C for 10s) of sample 1A8, People's Wandering (AD 5th-6th century), Obeliai, after thermal desorption (310 °C for 10 s). Key to Fig. 26: Peak identities (x indicates carbon chain length): filled squares, Cx indicates alkenes; filled circles, Cx indicates alkanes; open diamonds, Cx:y indicates acyclic nitriles; green filled pentagons indicates alkyl pyrroles (with alkyl chain length above, Cx). Also shown are the structures of five aromatic compounds identified: toluene, ethyl benzene, styrene, pyridine and pyrrole. CO₂ indicates carbon dioxide. Inset displays a reconstructed total ion chromatogram of the thermal desorption profile (310 °C for 10 s) of this sample. Peak identities: (x indicates carbon chain length): filled circles, Cx indicates alkanes.

39. Lithuania People's Wandering (AD 5th-6th century), Obeliai, Central Lithuania flat burials, Sk1294 male 50-55 yrs T43 lingual, 1A9 (calculus: 13.87 mg)

The main compound in the thermal desorption total ion chromatogram (TIC) (Fig. 27, inset) is carbon dioxide. In trace abundance are a series of *n*-alkanes, carbon numbers C₁₅ to C₂₂,

maximising at C₁₇. There is a slight odd over even carbon number predominance, although the relative abundance of the C₁₈ and C₂₀ *n*-alkanes and the presence of C₁₅ to C₂₁ branched chain alkanes suggests the sample has undergone some microbial-derived biodegradation as a result of diagenetic changes. Although the trace quantities and evidence for biodegradation necessarily mean any inference must be tentative, the relatively unusual mid-chain carbon numbers present in the TD chromatogram, combined with the C₁₇ *n*-alkane being dominant and a slight odd over even carbon number predominance, suggests an algal source^{92,101,103} (see discussion below). It is not possible to determine the significance of the carbon dioxide, beyond it being indicative of oxidised organic material. It should also be note that highly polar material could have been present, which would not have successfully eluted from the column, or indeed volatilised sufficiently in the probe.

The pyrolysis-GC-MS TIC (Fig. 27) was dominated by carbon dioxide, 1-butene and 1-pentene, the alkenes being indicative of lipid or proteinaceous material in the sample¹¹. Cyclopentadiene and methylcyclopentadiene are also significant components of the pyrolysate although it is not possible to ascribe a specific origin here. In contrast to the thermal desorption profile, the pyrogram also revealed a series of C₇ to C₂₀ *n*-1-alkenes and *n*-alkanes, typical of unsaturated and saturated fat/oil-derived acyl lipids^{5,6}. A distribution with maxima at C₁₃/C₁₄ suggests the original acyl lipids may have been high in unsaturated fatty acids. The absence of these compounds in the TD profile provides molecular evidence for a polymeric/bound acyl lipid source. However, in addition to *n*-1-alkenes and *n*-alkanes, typical of fat/oil derived lipids, the pyrogram also displayed a series of C₈ to C₁₆ iso-methyl-1-alkenes (isoalkenes) and 2-methylalkanes (isoalkanes), presumably deriving from the branched fatty acids which are significant components of bacterial triglycerides.

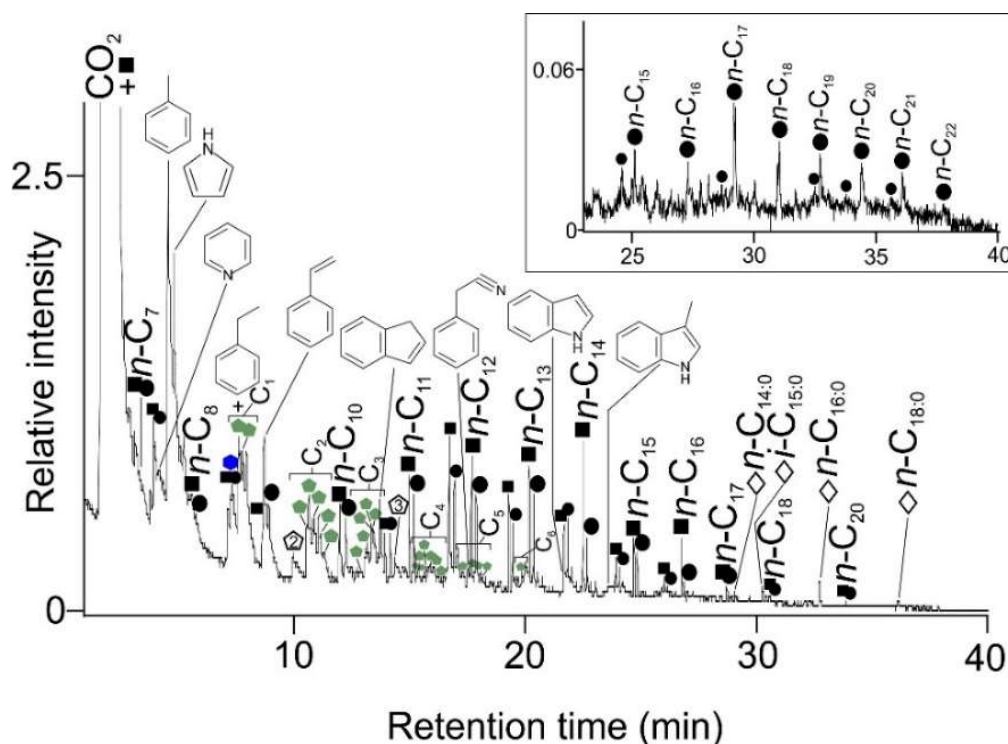
There were components identified in the pyrolysate TIC which can be indicative of 'black carbon', i.e. charcoal or soot⁷⁻¹⁰. However, the absence of naphthalenes and biphenyls and the dominance of tolu-ne, rather than the normally dominant aromatic hydrocarbon benzene, is notably atypical of chars⁷⁻¹⁰. The markers identified here can also be indicative of proteins^{6,11} and combined with the abundant related protein markers pyrrole, benzonitrile, benzyl nitrile (phenylacetone nitrile), benzenepropanenitrile and indoles^{6,11} identified this is likely to be the main origin of these compounds in this sample, pointing to a substantial protein-derived component^{6,11}. This is also corroborated by the presence of aliphatic nitriles (carbon numbers *n*-C_{14:0}, *i*-C_{15:0}, *n*-C_{15:0}, *i*-C_{16:0}, *n*-C_{16:0} (max) and *n*-C_{18:0}) suggesting a lipid (fat/oil) in this sample which has combined with protein-derived nitrogen to produce these moieties⁵. Taken

together, the presence of toluene, styrene and phenylacetonitrile (benzyl nitrile) can indicate the amino acid phenylalanine^{12,13}. In addition, the significant constituents indole and 3-methylindole (skatole), with a lesser amount of a C₂ indole (3-ethylindole?), were also identified, which are consistent with a tryptophan amino acid source^{12,13,19,20}. Proteins rich in the amino acids phenylalanine and tryptophan include meat, fish and dairy products, although the presence of bacterial markers means the possibility that at least a partial origin could be microbial cannot be excluded.

However, highly notable in this context is the additional presence of notably significant amounts of the more unusual alkyl pyrroles. C₁ alkyl pyrroles: 2- and 3-methylpyrroles; C₂ alkyl pyrroles: 2- and 3-ethylpyrroles, 2,4-, 2,3- and 3,4-dimethyl-pyrroles; C₃ alkyl pyrroles: 2-ethyl-4(?) -methylpyrrole, 2-ethyl-3(?) -methylpyrrole, 4-ethyl-2-methylpyrrole, 3-ethyl-4-methylpyrrole, and 2,3,5-trimethylpyrrole; C₄ alkyl pyrroles: two unidentified propylmethylpyrroles, four unidentified ethyldimethylpyrroles, 2,5(?) -diethylpyrrole, 2,4(?) -diethylpyrrole, 2,3(?) -diethylpyrrole, 3,4(?) -diethylpyrrole, 4-ethyl-2,3-dimethylpyrrole, 3-ethyl-2,4-dimethylpyrrole and 2,3,4,5-tetramethylpyrrole; C₅ alkyl pyrroles: 2,3(?) -diethyl-5?-methylpyrrole, 2,3-diethyl-4-methylpyrrole, 2,5?-diethyl-3-methylpyrrole and 3-ethyl-2,4,5-trimethylpyrrole; C₆ alkyl pyrroles: an unidentified C₆ ethylpyrrole. Collectively, these specific C₁, C₂, C₃, C₄, C₅ and C₆ alkyl pyrroles are characteristic of a significant tetrapyrrole/porphyrin-derived origin and are indicative of an algal source observed in fossils and expected in similarly diagenetically altered archaeological material^{11,21,22}. Notable also in this context were pyridine and its methyl-, and dimethyl derivatives (these pyridines can result from the pyrolysis of polypeptides containing the amino acid alanine¹⁹). The presence here of the abundant protein markers and specifically the C₁ to C₆ alkyl pyrroles, combined with the particular lipid profile and specific carbohydrate markers (see below) identified, indicate the presence of a macroalgae, i.e. seaweed or freshwater source, in this sample²¹⁻²³ (see discussion).

The thermally-derived carbohydrate markers 2-methylfuran, 2-methyl-2-cyclopenten-1-one and 2,3-dimethyl-2-cyclopenten-1-one were identified as significant components. That they were absent in the TD profile points to their originating from the original polymeric carbohydrate source (e.g. starch)²⁵. Although levoglucosan (1,6-anhydro-β-D-glucopyranose) is usually the major component in the thermally-derived products of carbohydrates analysed by TD/Py-GC-MS, its absence from the TD profile here is consistent with the removal of hydroxyl groups in the original carbohydrate polymer, so preventing its formation in the

probe. The additional absence of the normally significant carbohydrate markers 2-hydroxy-3-methyl-2-cyclopenten-1-one and 3-hydroxy-2-methyl-2-cyclopenten-1-one observed in pure standard material (glycogen, starch and cellulose) would also support this interpretation. This shows a strong matrix effect likely to be due to the inorganic component of the calculus and the microflora in the mouth. This dehydration/dehydroxylation has also been observed in dog calculus and 19th century human calculus⁶, where other markers, e.g. a prevalence of nitriles, with amides very minor, deriving from dehydrating conditions in the mouth of dogs and humans (though not sheep⁶) supporting this view⁶. The presence of carbon dioxide is also consistent with a lipid, amino acid/protein and carbohydrate component in this sample. Notably, these findings did not reveal key biomarkers typically observed in soils^{20,23}. It should also be noted that highly polar material could have been present, which would not have successfully eluted from the column, or indeed volatilised sufficiently in the probe.



Supplementary Figure 27. Reconstructed total ion chromatogram of the pyrogram (pyrolysis profile) (610 °C for 10s) of sample 1A9, People's Wandering (AD 5th-6th century), Obeliai, after thermal desorption (310 °C for 10 s). Key to Fig. 27: Peak identities (x indicates carbon chain length): filled squares, Cx indicates alkenes; filled circles, Cx indicates

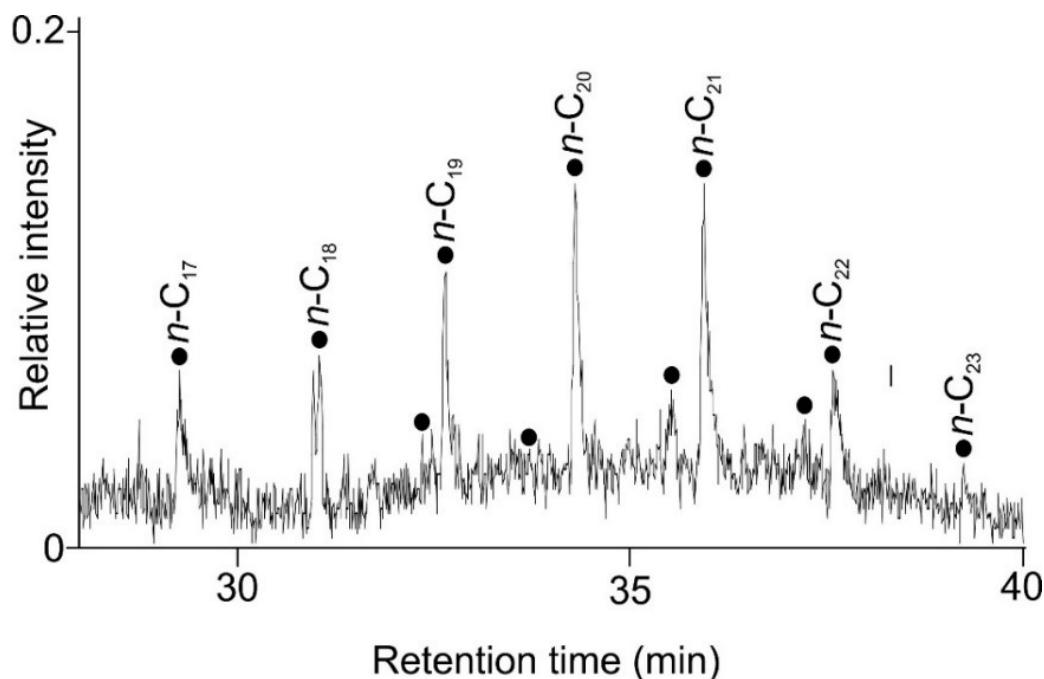
alkanes; open diamonds, C_x:y indicates acyclic nitriles; blue filled hexagon indicates alkyl pyridine; green filled pentagons indicates alkyl pyrroles (with alkyl chain length above, C_x); open pentagons with numerals inside indicates carbohydrate pyrolysis markers, 2 is 2-methyl-2-cyclopenten-1-one and 3 is 2,3-dimethyl-2-cyclopentene-1-one. Also shown are the structures of nine aromatic compounds identified: pyridine, pyrrole, toluene, ethylbenzene, styrene, indene, benzyl nitrile, indole and 3-methylindole (skatole). CO₂ indicates carbon dioxide. Inset displays a reconstructed total ion chromatogram of the thermal desorption profile (310 °C for 10 s) of this sample. Peak identities: (x indicates carbon chain length): filled circles, C_x indicates alkanes.

40. Lithuania People's Wandering (AD 5th-6th century), Obeliai, Central Lithuania flat burials, Sk0984 female >55 yrs T33 lingual, 1A10 (calculus: 3.74 mg)

The main compound in the thermal desorption total ion chromatogram (TIC) (Fig. 28) is carbon dioxide. In very minor abundance are a series of *n*-alkanes, carbon numbers C₁₇ to C₂₃, maximising at C₂₀/C₂₁. There is no odd over even carbon number predominance suggesting the sample may have undergone biodegradation as a result of diagenetic changes. This is further corroborated by the presence of branched alkanes with carbon numbers C₁₉ to C₂₂ observed as relatively minor constituents and indicative of microbially-derived biodegradation (see discussion below). The relatively unusual mid-chain carbon numbers present in the TD chromatogram suggests a submerged aquatic plant (macrophyte)⁸⁹⁻⁹² component, possibly deriving from plant waxes in the original food consumed or reflecting prevailing environmental factors (again, see discussion below), in addition to a bacterial input (C₁₇ and C₁₈ alkanes)^{92,103}. It is not possible to determine the significance of the carbon dioxide, beyond it being indicative of oxidised organic material. It should also be note that highly polar material could have been present, which would not have successfully eluted from the column, or indeed volatilised sufficiently in the probe.

The pyrolysis-GC-MS TIC was dominated by carbon dioxide, 1-butene, 1-pentene and hexene, the alkenes being indicative of lipid or proteinaceous material in the sample¹¹. Cyclopentadiene and methylcyclopentadiene are also significant components of the pyrolysate although it is not possible to ascribe a specific origin here. Toluene was also detected, albeit as a very minor component, preventing any meaningful interpretation of its presence, beyond it being indicative of an organic origin. The dominance of carbon dioxide

suggests a highly oxidised sample. It is not possible to determine the significance of the carbon dioxide, beyond it being indicative of oxidised organic material. It should be noted, however, that highly polar material could have been present, which would not have successfully eluted from the column, or indeed volatilised sufficiently in the probe.



Supplementary Figure 28. Reconstructed total ion chromatogram of the thermal desorption profile (310 °C for 10 s) of sample 1A9, People's Wandering (AD 5th-6th century), Obeliai. Key to Fig. 28: Peak identities (x indicates carbon chain length): filled circles, Cx indicates alkanes.

Discussion, People's Wandering (AD 5th-6th century), Obeliai, Central Lithuania flat burials

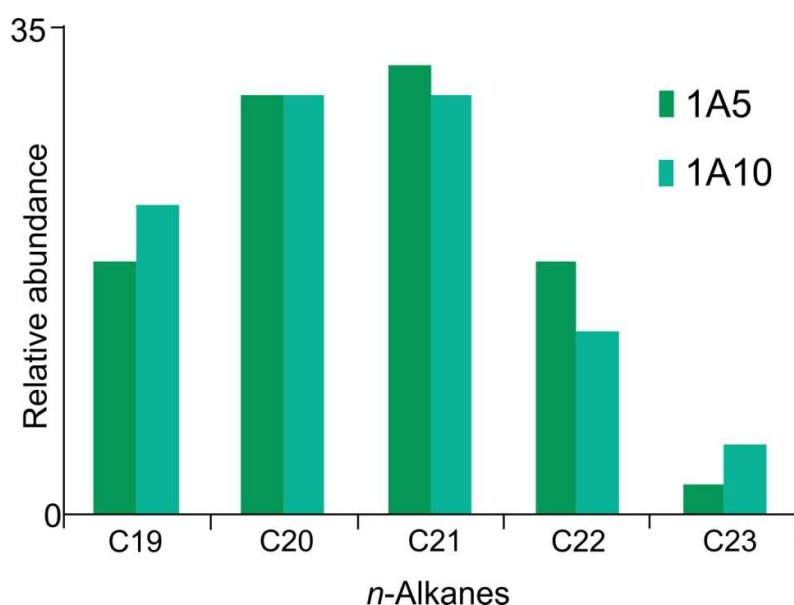
Five samples of dental calculus from individual burials at Obeliai dating to the People's Wandering (AD 5th-6th century) were analysed by sequential thermal desorption-gas chromatography-mass spectrometry (TD-GC-MS) and pyrolysis-gas chromatography-mass spectrometry (Py-GC-MS).

Two of the samples taken from two separate individuals (1A5 and 1A10) revealed a series of *n*-alkanes, ranging from C₁₇ up to C₂₃ and maximising at C₂₁, as notable, albeit minor,

constituents of the thermal desorption total ion chromatogram (TIC), which were both dominated by the ubiquitous carbon dioxide, indicative of highly oxidised organic material. There is no odd over even carbon number predominance suggesting the samples have undergone biodegradation as a result of diagenetic changes (although see below). This is also indicated by the presence of branched alkanes with carbon numbers C₁₉ to C₂₂ observed as relatively minor constituents compared to the *n*-alkane putative biomarkers and indicative of microbially-derived biodegradation. In this context, the relatively unusual mid-chain carbon numbers present suggests a submerged aquatic plant (macrophyte)⁸⁹⁻⁹², in addition to a bacterial input evidenced by the C₁₇ and C₁₈ *n*-alkanes^{92,103} since these typically have a plant wax component ranging from C₁₉ to C₃₁/C₃₃ and maximising at C₂₁/C₂₃, possibly deriving from plant waxes in the original food consumed in this context. The persistence of the *n*-alkanes is not unexpected given that they are known to be particularly resistant to diagenesis^{90,92}. In addition to the data above, an *n*-alkane proxy for the input/presence of submerged/floating freshwater aquatic macrophytes can provide another corroborating measure for an aquatic plant input⁸⁹. However, the *n*-alkane proxy ratio, P_{aq}, cannot be applied meaningfully here because of the lack of >C₂₃ *n*-alkanes. That being said, the C₂₁ maximum, combined with the relatively narrow *n*-alkane carbon number range in the two calculus samples is indicative of biodegraded submerged aquatic plant (macrophyte) material.

Interestingly, the chemical profile of the dominant C₁₉ to C₂₃ *n*-alkanes, maximising at C₂₁ is consistent with the submerged aquatic plant genus *Nymphaea*⁸⁹, following biodegradation. Further work corroborating this possibility using microscopy would be needed, but it certainly be worthy of further investigation. With a carbon preference indexes (CPIs) of 1.10 and 1.20 and C₂₁ maxima they are consistent with what would be expected for a (biodegraded) submerged aquatic plant. Notably similar values have been observed for submerged aquatic plant rhizomes from southern Europe: Greece, CPI = 1.13; France, CPI = 1.15¹⁰¹. Furthermore, the rhizomes in these studies had a far less pronounced odd-over-even carbon preference than leaves from the same plants, which is likely to reflect their microenvironment in the aquatic/marine sediments where they are far more exposed to biomolecular reworking and biodegradation from microbial inputs than leaves from the same plant. In this regard it is notable that the *n*-alkanes in the two dental calculus samples also display this unimodal ‘hump’; this could be indicative of the consumption of rhizomes from a submerged aquatic plant by these individuals, although it may also be explained by post-depositional diagenetic factors.

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2932

2933 **Supplementary Figure 29.** *n*-Alkane distribution of two calculus samples from Obeliai,
 2934 Lithuania: 1A5 male and 1A10 female. Source data are provided as a Source Data file.

2935 The pyrolysis-GC-MS for 1A5 revealed a series of C₆ to C₁₆ *n*-1-alkenes and *n*-alkanes,
 2936 typical of unsaturated and saturated fat/oil-derived acyl lipids^{5,6}. The absence of these
 2937 compounds in the TD profile provides molecular evidence for a polymeric/bound acyl lipid
 2938 source. However, the additional presence of a series of C₉ to C₁₆ isoalkenes and isoalkanes,
 2939 presumably deriving from the branched fatty acids, which are significant components of
 2940 bacterial triglycerides, suggests a microbial origin for these lipid biomarkers, at least in part,
 2941 and is consistent with the bacterial C₁₇ and C₁₈ *n*-alkanes observed in the TD. There were
 2942 components in the pyrolysate which can be indicative of 'black carbon', i.e. charcoal or soot<sup>7-
 2943 10</sup>. However, the dominance of toluene, rather than the normally dominant aromatic
 2944 hydrocarbon benzene, is notably atypical of chars⁷⁻¹⁰. That being said, despite the absence of
 2945 higher molecular weight 'char' organic compounds their collective presence provides at least
 2946 tentative molecular evidence for exposure to fire/cooking. In contrast to 1A5, the pyrolysis-
 2947 GC-MS for 1A10 revealed only C₄ to C₆ short chain alkenes, cyclopentadiene and its methyl-
 2948 derivative and toluene. In the absence of more diagnostic biomolecules their significance here
 2949 is uncertain. The dominance of carbon dioxide in both samples suggests highly oxidised
 2950 organic material.

Another sample, 1A6, revealed only carbon dioxide in the TD-GC-MS analysis, indicating the essential absence of free, thermally extractable organic components in this sample. The carbon dioxide is also indicative of highly oxidised organic material. The pyrolysis-GC-MS revealed a series of C₇ to C₁₆ *n*-1-alkenes and *n*-alkanes, typical of unsaturated and saturated fat/oil-derived acyl lipids^{5,6}. The absence of these compounds in the TD profile provides molecular evidence for a polymeric/bound acyl lipid source. However, the additional presence of a series of C₈ to C₁₆ isoalkenes and isoalkanes, presumably deriving from the branched fatty acids, which are significant components of bacterial triglycerides, suggests a microbial origin for these lipid biomarkers, at least in part (also see below). The dominance of carbon dioxide again suggests a highly oxidised sample.

The last two Obeliai samples, 1A8 and 1A9, revealed both free and bound/polymeric organic material. The thermal desorption total ion chromatogram (TIC) of the two samples were dominated by carbon dioxide; it is difficult to explain the significance of this non-diagnostic compound, beyond indicating that this sample has undergone diagenetic changes of a highly oxidising nature. Although only trace constituents, both samples revealed a series of *n*-alkanes, ranging from C₁₅/C₁₇ up to C₂₂. Sample 1A8 maximises at C₁₉, and 1A9 maximises at C₁₇. Both samples show an odd over even carbon number predominance, although the relative abundance of the C₁₆, C₁₈ and C₂₀ *n*-alkanes suggests the samples have undergone some biodegradation as a result of diagenetic changes. Although the trace quantities and evidence for biodegradation necessarily mean any inferences must be tentative, the narrow range (C₁₅/C₁₇ and C₂₂) and relatively unusual mid-chain carbon numbers, maximising at C₁₇ or C₁₉, combined with an odd over even predominance, is characteristic of an algal source^{92,101,103}. The presence of branched alkanes with carbon numbers C₁₅ to C₂₁ observed as relatively minor constituents is indicative of some microbially-derived biodegradation.

The pyrolysis-GC-MS analyses of these two samples revealed moderate amounts of organic material which were dominated by a complex suite of organic compounds indicative of lipid (fat/oil) and protein components. These include a series of C₇ to C₂₀ *n*-1-alkenes and *n*-alkanes, typical of unsaturated and saturated fat/oil-derived acyl lipids^{5,6}. A maxima C₁₃/C₁₄ combined with abundant short chain (C₄-C₇) *n*-1-alkenes suggests the original acyl lipids in these samples are likely to have been high in polyunsaturated fatty acids. The absence of these compounds in the TD profile provides molecular evidence for a polymeric/bound acyl lipid source. However, in addition to *n*-1-alkenes and *n*-alkanes, typical of fat/oil derived lipids, the pyrograms also displayed a series of C₈/C₉ to C₁₆ iso-1-alkenes and isoalkanes,

2984 presumably deriving from the branched fatty acids which are significant components of
2985 bacterial triglycerides (but see below).

2986 There were components in the pyrolysate of both samples which can be indicative of ‘black
2987 carbon’, i.e. charcoal or soot⁷⁻¹⁰. However, the dominance of toluene, rather than the normally
2988 dominant aromatic hydrocarbon benzene and the absence of naphthalenes and biphenyls, is
2989 notably atypical of chars⁷⁻¹⁰. The markers identified here can also be indicative of proteins^{6,11}
2990 and combined with the abundant related protein markers pyrrole, benzonitrile, benzyl nitrile
2991 (phenylacetonitrile), benzenepropanenitrile and indoles^{6,11} identified in sample 1A9 this is
2992 likely to be the main origin of these compounds, pointing to a substantial protein-derived
2993 component^{6,11} in this sample. This is also corroborated by the presence of aliphatic nitriles
2994 (carbon numbers C_{14:0}, C_{16:0} (max) and C_{18:0}) in both samples, suggesting a lipid (fat/oil) in
2995 this sample which has combined with protein-derived nitrogen to produce these moieties⁵.
2996 Taken together, the presence of toluene, styrene and phenylacetonitrile (benzyl nitrile) can
2997 indicate the amino acid phenylalanine^{12,13}. In addition, the significant constituents indole and
2998 3-methylindole (skatole), with a lesser amount of a C₂ indole (3-ethylindole?), in sample 1A9
2999 are consistent with a tryptophan amino acid source^{12,13,19,20}. Proteins rich in the amino acids
3000 phenylalanine and tryptophan include meat, fish and dairy products, although the presence of
3001 bacterial markers means the possibility that at least a partial origin could be microbial cannot
3002 be excluded.

3003 Far more diagnostic of a particular dietary source are the particular suite of alkyl pyrroles
3004 observed in the pyrograms of these samples, with an alkyl chain length of up to C₆. Both these
3005 samples revealed significant amounts of these biomarkers. This included the 2- and 3-
3006 dimethylpyrroles, 2- and 3-ethylpyrroles, 2,4-, 2,3- and 3,4-dimethylpyrroles, four
3007 ethylmethylpyrroles, a trimethylpyrrole, two propylmethylpyrroles, six
3008 ethyldimethylpyrroles, four diethylpyrroles, 2,3,4,5-tetramethylpyrrole, three
3009 diethylmethylpyrroles, an ethyltrimethylpyrrole and an unidentified C₆ ethylpyrrole. These
3010 specific C₁ to C₆ alkyl pyrroles are highly diagnostic of the presence of a significant
3011 tetrapyrrole component. Their porphyrin-derived origin, and the relative abundance of some
3012 of the key biomarkers, is indicative of an algal source observed in fossils and expected in
3013 similarly diagenetically altered archaeological material^{11,21,22}. Although C₁ and C₂ alkyl
3014 pyrroles can also derive from protein-derived amino acids such as glycine, proline,
3015 hydroxyproline, serine and glutamic acid^{12-14,20,40,41}, the C₃+ alkyl pyrroles identified in these
3016 two samples point to a tetrapyrrole source^{21,22,40,41}. Moreover, the same specific C₁ to C₅

alkylpyrroles identified in the Py-GC-MS of an algal kerogen^{21,22} were observed in these samples. Overall the two samples display a remarkably similar pyrolysis profile to algal-derived kerogen previously studied via very similar organic geochemical techniques^{21,22}.

Notable also in this context were pyridine and its methyl-, and dimethyl derivatives (these pyridines can result from the pyrolysis of polypeptides containing the amino acid alanine²⁵). The presence here of the abundant protein markers and specifically the C₁ to C₆ alkyl pyrroles, combined with the particular lipid profile and specific carbohydrate markers (see above) identified, indicate the presence of a macroalgae, i.e. seaweed or freshwater algal source, in this sample²¹⁻²³. Marine and freshwater algal sources are known to have been exploited for food in antiquity^{52,104}, although their consumption in this part of the world does not seem to have been seriously considered to date.

Lithuania People's Wandering (AD 5th-6th century), Pagrybis, North Lithuania barrows

Pagrybis burial ground represents a small hill located in the Pagrybis village near Akmena river (Šilalė region) in western Lithuania. During investigations carried in 1980-1982, 217 graves were uncovered containing 224 individuals. Adults and children were buried in grave pits in wooden coffins. Most graves were single, though some double graves were found. All had been buried on their backs with straight legs. Burial items include all kinds of tools, weapons, fragments of cloths and jewellery. 38 graves contained remains of sacrificed horses¹⁰⁵.

41. Lithuania People's Wandering (AD 5th-6th century), Pagrybis, North Lithuania barrows, Sk1410A female 40-45 yrs T43 labial, 1A17 (calculus: 4.11 mg)

The thermal desorption total ion chromatogram (TIC) revealed no detectable components, indicating the absence of free, thermally extractable organic components in this sample. It should be noted, however, that highly polar material could have been present, which would not have successfully eluted from the column, or indeed volatilised sufficiently in the probe.

The pyrolysate TIC revealed no detectable components, indicating the essential absence of organic material in this sample. It should be noted, however, that highly polar material could have been present, which would not have successfully eluted from the column, or indeed volatilised sufficiently in the probe.

3048

3049 **42. Lithuania People's Wandering (AD 5th-6th century), Pagrybis, North**
3050 **Lithuania barrows, Sk1409C male 30-35 yrs T31 labial, 1A18 (calculus: 4.14 mg)**

3051 The thermal desorption total ion chromatogram (TIC) revealed carbon dioxide as the only
3052 detectable component, indicating the essential absence of free, thermally extractable organic
3053 components in this sample. It should be noted, however, that highly polar material could have
3054 been present, which would not have successfully eluted from the column, or indeed
3055 volatilised sufficiently in the probe. It is not possible to determine the significance of the
3056 carbon dioxide, beyond it being indicative of oxidised organic material.

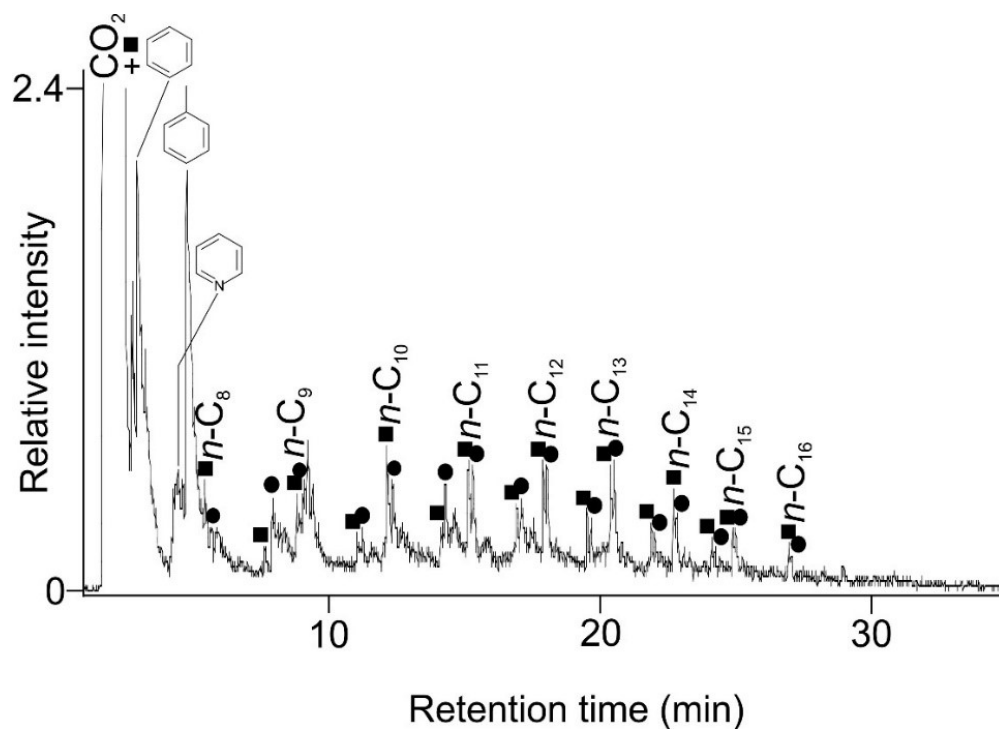
3057 The pyrolysis-GC-MS TIC was dominated by carbon dioxide, 1-butene, 1-pentene and
3058 hexene, the alkenes being indicative of lipid or proteinaceous material in the sample¹¹.
3059 Cyclopentadiene and methylcyclopentadiene are also significant components of the
3060 pyrolysate although it is not possible to ascribe a specific origin here. Toluene was also
3061 detected, albeit as a very minor component, preventing any meaningful interpretation of its
3062 presence, beyond it being indicative of an organic origin. The dominance of carbon dioxide
3063 suggests a highly oxidised sample. It is not possible to determine the significance of the
3064 carbon dioxide, beyond it being indicative of oxidised organic material. It should be noted,
3065 however, that highly polar material could have been present, which would not have
3066 successfully eluted from the column, or indeed volatilised sufficiently in the probe.

3067

3068 **43. Lithuania People's Wandering (AD 5th-6th century), Pagrybis, North**
3069 **Lithuania barrows, Sk1402 female 45-50 yrs T42 labial, 1A19 (calculus: 7.22 mg)**

3070 The thermal desorption total ion chromatogram (TIC) revealed carbon dioxide and 1-butene
3071 as the only detectable components, indicating the essential absence of free, thermally
3072 extractable organic components in this sample. It should be noted, however, that highly polar
3073 material could have been present, which would not have successfully eluted from the column,
3074 or indeed volatilised sufficiently in the probe. The 1-butene possibly derives from lipid or
3075 proteinaceous material in the sample¹¹, and perhaps produced at this relatively low
3076 temperature due to matrix effects. It is not possible to determine the significance of the
3077 carbon dioxide, beyond it being indicative of oxidised organic material.

The pyrolysis-GC-MS TIC (Fig. 30) was dominated by carbon dioxide, 1-butene and 1-pentene, the alkenes being indicative of lipid or proteinaceous material in the sample¹¹. Cyclopentadiene and methylenecyclopentadiene are also components of the pyrolysate although it is not possible to ascribe a specific origin here. Benzene, toluene and pyridine were also detected, albeit as minor components; their significance in the absence of other more diagnostic biomolecules is unclear. In contrast to the thermal desorption profile, the pyrogram also revealed a series of C₈ to C₁₆ *n*-1-alkenes and *n*-alkanes, typical of unsaturated and saturated fat/oil-derived acyl lipids^{5,6}. The absence of these compounds in the TD profile provides molecular evidence for a polymeric/bound acyl lipid source. However, in addition to *n*-1-alkenes and *n*-alkanes, typical of fat/oil derived lipids, the pyrogram also displayed a series of C₉ to C₁₅ iso-methyl-1-alkenes (isoalkenes) and 2-methylalkanes (isoalkanes), presumably deriving from the branched fatty acids which are significant components of bacterial triglycerides. The dominance of carbon dioxide suggests a highly oxidised sample. It is not possible to determine the significance of the carbon dioxide, beyond it being indicative of oxidised organic material. It should be noted, however, that highly polar material could have been present, which would not have successfully eluted from the column, or indeed volatilised sufficiently in the probe.

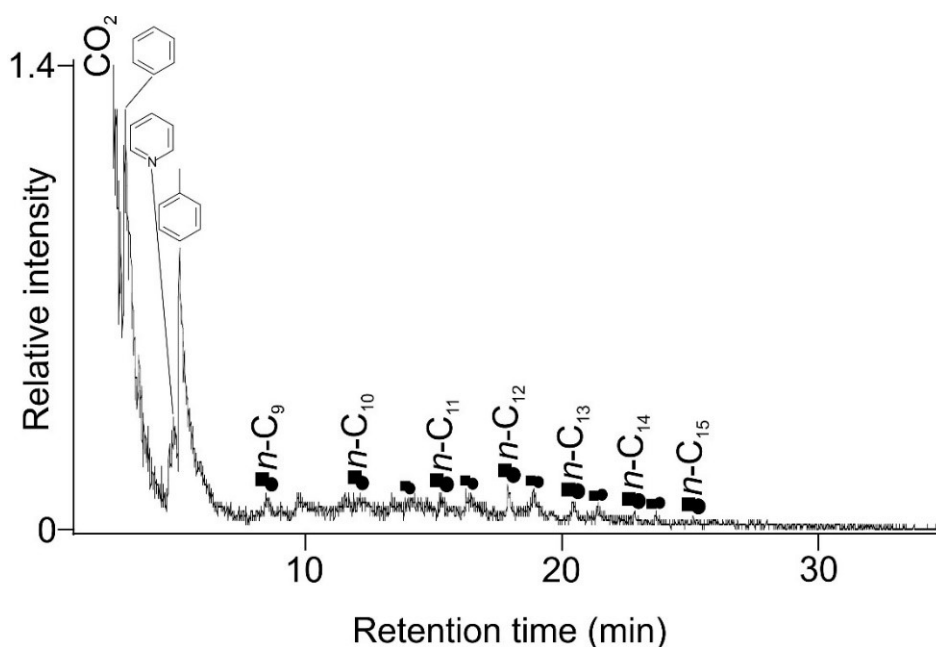


Supplementary Figure 30. Reconstructed total ion chromatogram of the pyrogram (pyrolysis profile) (610 °C for 10s) of sample 1A19, People's Wandering (AD 5th-6th century), Pagrybis, after thermal desorption (310 °C for 10 s). Key to Fig. 30: Peak identities (x indicates carbon chain length): filled squares, C_x indicates alkenes; filled circles, C_x indicates alkanes. Also shown are the structures of three aromatic compounds identified: benzene, toluene and pyridine. CO₂ indicates carbon dioxide.

44. Lithuania People's Wandering (AD 5th-6th century), Pagrybis, North Lithuania barrows, Sk1398 male >55 yrs T27 bucal, 1A20 (calculus: 3.83 mg)

The thermal desorption total ion chromatogram (TIC) revealed carbon dioxide as the only detectable component, indicating the essential absence of free, thermally extractable organic components in this sample. It should be noted, however, that highly polar material could have been present, which would not have successfully eluted from the column, or indeed volatilised sufficiently in the probe. It is not possible to determine the significance of the carbon dioxide, beyond it being indicative of oxidised organic material.

The pyrolysis-GC-MS TIC (Fig. 31) was dominated by carbon dioxide, 1-butene, 1-pentene and 1-hexene, the alkenes being indicative of lipid or proteinaceous material in the sample¹¹. Cyclopentadiene and methylcyclopentadiene are also components of the pyrolysate although it is not possible to ascribe a specific origin here. Benzene, toluene and pyridine were also detected, albeit as very minor components; their significance in the absence of other more diagnostic biomolecules is unclear. In contrast to the thermal desorption profile, the pyrogram also revealed trace amounts of a series of C₉ to C₁₅ *n*-1-alkenes and *n*-alkanes, typical of unsaturated and saturated fat/oil-derived acyl lipids^{5,6}. The absence of these compounds in the TD profile provides molecular evidence for a polymeric/bound acyl lipid source. However, in addition to *n*-1-alkenes and *n*-alkanes, typical of fat/oil derived lipids, the pyrogram also displayed a series of C₁₁ to C₁₅ branched alkenes and alkanes, presumably deriving from branched fatty acids which are significant components of bacterial triglycerides. The dominance of carbon dioxide suggests a highly oxidised sample. It is not possible to determine the significance of the carbon dioxide, beyond it being indicative of oxidised organic material. It should be noted, however, that highly polar material could have been present, which would not have successfully eluted from the column, or indeed volatilised sufficiently in the probe.



3130

3131 **Supplementary Figure 31.** Reconstructed total ion chromatogram of the pyrogram
 3132 (pyrolysis profile) (610 °C for 10s) of sample 1A20, People's Wandering (AD 5th-6th
 3133 century), Pagrybis, after thermal desorption (310 °C for 10 s). Key to Fig. 31: Peak identities
 3134 (x indicates carbon chain length): filled squares, Cx indicates alkenes; filled circles, Cx
 3135 indicates alkanes. Also shown are the structures of three aromatic compounds identified:
 3136 benzene, toluene and pyridine. CO₂ indicates carbon dioxide.

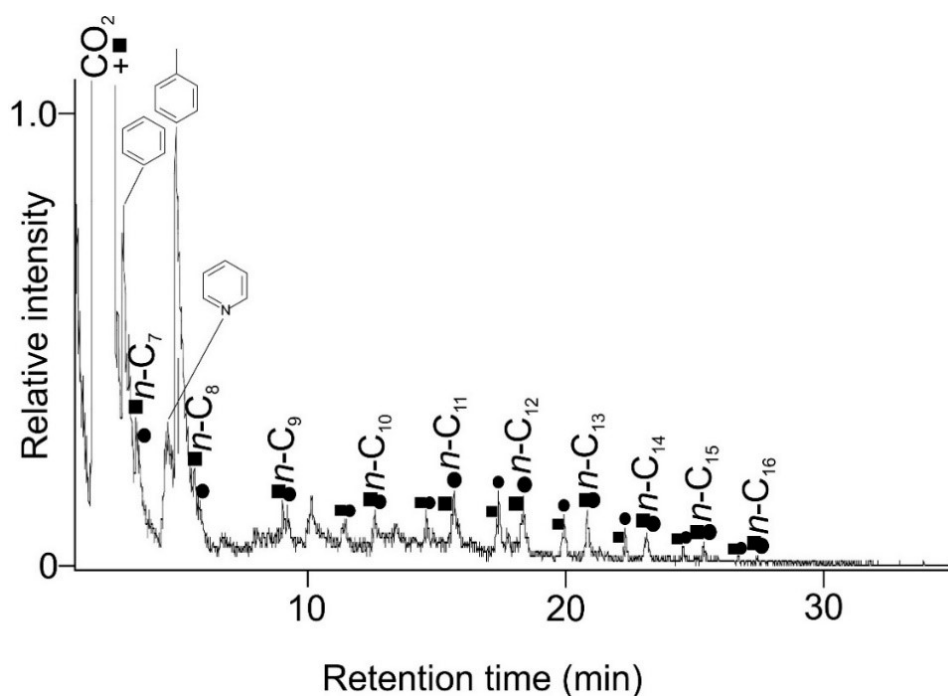
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3138 **45. Lithuania People's Wandering (AD 5th-6th century), Pagrybis, North**
 3139 **Lithuania barrows, Sk1058 male 30-35 yrs T41 labial, 1A21 (calculus: 4.46 mg)**

3140 The thermal desorption total ion chromatogram (TIC) revealed carbon dioxide as the only
 3141 detectable component, indicating the essential absence of free, thermally extractable organic
 3142 components in this sample. It should be noted, however, that highly polar material could have
 3143 been present, which would not have successfully eluted from the column, or indeed
 3144 volatilised sufficiently in the probe. It is not possible to determine the significance of the
 3145 carbon dioxide, beyond it being indicative of oxidised organic material.

3146 The pyrolysis-GC-MS TIC (Fig. 32) was dominated by carbon dioxide, 1-butene, 1-pentene
 3147 and 1-hexene, the alkenes being indicative of lipid or proteinaceous material in the sample¹¹.
 3148 Cyclopentadiene and methylcyclopentadiene are also components of the pyrolysate although

it is not possible to ascribe a specific origin here. Benzene, toluene and pyridine were also detected, albeit as very minor components; their significance in the absence of other more diagnostic biomolecules is unclear. In contrast to the thermal desorption profile, the pyrogram also revealed trace amounts of a series of C₇ to C₁₆ *n*-1-alkenes and *n*-alkanes, typical of unsaturated and saturated fat/oil-derived acyl lipids^{5,6}. The absence of these compounds in the TD profile provides molecular evidence for a polymeric/bound acyl lipid source. However, in addition to *n*-1-alkenes and *n*-alkanes, typical of fat/oil derived lipids, the pyrogram also displayed a series of C₁₀ to C₁₆ iso-methyl-1-alkenes (isoalkenes) and 2-methylalkanes (isoalkanes), presumably deriving from the branched fatty acids which are significant components of bacterial triglycerides. The dominance of carbon dioxide suggests a highly oxidised sample. It is not possible to determine the significance of the carbon dioxide, beyond it being indicative of oxidised organic material. It should be noted, however, that highly polar material could have been present, which would not have successfully eluted from the column, or indeed volatilised sufficiently in the probe.



Supplementary Figure 32. Reconstructed total ion chromatogram of the pyrogram (pyrolysis profile) (610 °C for 10s) of sample 1A21, People's Wandering (AD 5th-6th century), Pagrybis, after thermal desorption (310 °C for 10 s). Key to Fig. 32: Peak identities (x indicates carbon chain length): filled squares, C_x indicates alkenes; filled circles, C_x

indicates alkanes. Also shown are the structures of three aromatic compounds identified: benzene, toluene and pyridine. CO₂ indicates carbon dioxide.

Discussion, Lithuania People's Wandering (AD 5th-6th century), Pagrybis, North Lithuania barrows

Five samples of dental calculus from individual burials at Pagrybis dating to the People's Wandering (AD 5th-6th century) were analysed by sequential thermal desorption-gas chromatography-mass spectrometry (TD-GC-MS) and pyrolysis-gas chromatography-mass spectrometry (Py-GC-MS).

One sample (1A17) contained no detectable free or bound/polymeric organic material, most likely reflecting unfavourable prevailing conditions for biomolecular preservation. A second sample (1A18) contained little free and bound/polymeric organic material, again most likely reflecting unfavourable prevailing conditions for biomolecular preservation. Of those organic compounds identified, the dominance of carbon dioxide and the relatively minor abundance of only a few small organic compounds suggests that only highly oxidised organic material was present.

The three remaining samples (1A19, 1A20 and 1A21) showed a notably similar biomolecular fingerprint for the sequential analyses. The TD-GC-MS analyses revealed only carbon dioxide indicating the essential absence of free, thermally extractable organic components in these samples. The carbon dioxide is also indicative of highly oxidised organic material. The pyrolysis-GC-MS revealed a series of C₇/C₈ to C₁₆ *n*-1-alkenes and *n*-alkanes, typical of unsaturated and saturated fat/oil-derived acyl lipids^{5,6}. The absence of these compounds in the TD profiles provides molecular evidence for a polymeric/bound acyl lipid source. However, the additional presence of a series of C₈ to C₁₅/C₁₆ isoalkenes and isoalkanes, presumably deriving from the branched fatty acids, which are significant components of bacterial triglycerides, strongly suggests a microbial origin for these lipid biomarkers, at least in part. The dominance of carbon dioxide again suggests a highly oxidised sample.

Lithuania People's Wandering (AD 5th-6th century), Vėluikiai, North Lithuania barrows

Vėluikiai cemetery is situated on the shore of Ančia river (Tauragė district) in western Lithuania. Investigations carried in 2006 and 2020 revealed that a part of a cemetery was

destroyed by floods. In total, 5 inhumations were found. They had all been buried on their backs, with their arms in various positions and their legs straight. The burial pits had rounded ends. Artefacts recovered at the Vėluikiai cemetery suggest it belongs to the final stage of the Old (Roman) Iron Age and the beginning of the early Middle Ages¹⁰⁶.

46. Lithuania People's Wandering (AD 5th-6th century), Vėluikiai, North Lithuania barrows, Sk1421 female 25-30 yrs T46 lingual, 1A28 (calculus: 2.33 mg)

The thermal desorption total ion chromatogram (TIC) revealed carbon dioxide as the only detectable component, indicating the essential absence of free, thermally extractable organic components in this sample. It should be noted, however, that highly polar material could have been present, which would not have successfully eluted from the column, or indeed volatilised sufficiently in the probe. It is not possible to determine the significance of the carbon dioxide, beyond it being indicative of oxidised organic material.

The pyrolysate TIC revealed carbon dioxide, 1-butene, 1-pentene, and cyclopentadiene as the only detectable components. The dominance of carbon dioxide, combined with the relatively minor abundance of the few small organic compounds present, suggests a highly oxidised sample. It is not possible to determine the significance of the carbon dioxide, beyond it being indicative of oxidised organic material and the other compounds are too low in abundance and too ubiquitous to provide any meaningful interpretation of their presence, beyond them being indicative of an organic origin. It should be noted, however, that highly polar material could have been present, which would not have successfully eluted from the column, or indeed volatilised sufficiently in the probe.

Discussion, Lithuania People's Wandering (AD 5th-6th century), Vėluikiai, North Lithuania barrows

One sample of dental calculus from an individual burial at Vėluikiai dating to the People's Wandering (AD 5th-6th century) was analysed by sequential thermal desorption-gas chromatography-mass spectrometry (TD-GC-MS) and pyrolysis-gas chromatography-mass spectrometry (Py-GC-MS). The sample contained little free and bound/polymeric organic material, most likely reflecting unfavourable prevailing conditions for biomolecular preservation. Of those organic compounds identified, the dominance of carbon dioxide and

the relatively minor abundance of only a few small organic compounds suggests that only highly oxidised organic material was present.

Lithuania Roman period (AD 1st-3th century), Pašatrijis, North Lithuania barrows

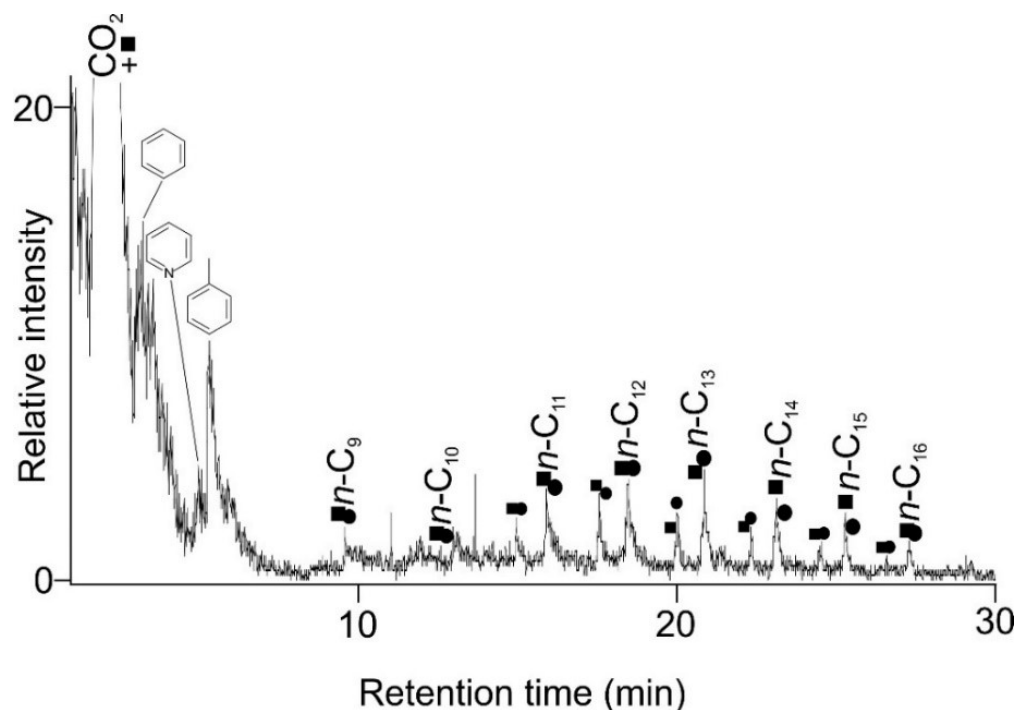
The Pašatrija hillfort and the settlement at its foot is situated in Telšiai district in western Lithuania. Excavations carried in 2012 uncovered an area totalling 1320 m². Materials including pottery, iron smelting remnants, slag, animal bones, as well as metal ornaments and tools were also found. The complex was dated to the 1st/4th – late 14th / early 15th centuries AD. The earliest inhumations discovered belonged to nine individuals buried in five graves: three single graves, one double grave with two adult individuals, and one grave which contained four individuals (two adult males and two children). Most of the skeletons were in bent position¹⁰⁷.

47. Lithuania Roman period (AD 1st-3th century), Pašatrijis, North Lithuania barrows, Sk7224 male 40-45 yrs T27 bucal, 1A29 (calculus: 3.47 mg)

The thermal desorption total ion chromatogram (TIC) revealed carbon dioxide as the only detectable component, indicating the essential absence of free, thermally extractable organic components in this sample. It should be noted, however, that highly polar material could have been present, which would not have successfully eluted from the column, or indeed volatilised sufficiently in the probe. It is not possible to determine the significance of the carbon dioxide, beyond it being indicative of oxidised organic material.

The pyrolysis-GC-MS TIC (Fig. 33) was dominated by carbon dioxide, 1-butene, 1-pentene and 1-hexene, the alkenes being indicative of lipid or proteinaceous material in the sample¹¹. Cyclopentadiene and methylcyclopentadiene are also components of the pyrolysate although it is not possible to ascribe a specific origin here. Benzene, toluene and pyridine were also detected, albeit as very minor components; their significance in the absence of other more diagnostic biomolecules is unclear. In contrast to the thermal desorption profile, the pyrogram also revealed trace amounts of a series of C₉ to C₁₆ *n*-1-alkenes and *n*-alkanes, typical of unsaturated and saturated fat/oil-derived acyl lipids^{5,6}. The absence of these compounds in the TD profile provides molecular evidence for a polymeric/bound acyl lipid source. However, in addition to *n*-1-alkenes and *n*-alkanes, typical of fat/oil derived lipids, the pyrogram also displayed a series of C₁₁ to C₁₆ iso-methyl-1-alkenes (isoalkenes) and 2-methylalkanes

(isoalkanes), presumably deriving from the branched fatty acids which are significant components of bacterial triglycerides. The dominance of carbon dioxide suggests a highly oxidised sample. It is not possible to determine the significance of the carbon dioxide, beyond it being indicative of oxidised organic material. It should be noted, however, that highly polar material could have been present, which would not have successfully eluted from the column, or indeed volatilised sufficiently in the probe.



Supplementary Figure 33. Reconstructed total ion chromatogram of the pyrogram (pyrolysis profile) (610 °C for 10s) of sample 1A29, Roman period (AD 1st-3th century), Pašatrijis, after thermal desorption (310 °C for 10 s). Key to Fig. 33: Peak identities (x indicates carbon chain length): filled squares, Cx indicates alkenes; filled circles, Cx indicates alkanes. Also shown are the structures of three aromatic compounds identified: benzene, toluene and pyridine. CO₂ indicates carbon dioxide.

48. Lithuania Roman period (AD 1st-3th century), Pašatrijis, North Lithuania barrows, Sk7227 male 20-25 yrs T32 labial, 1A30 (calculus: 2.67 mg)

The thermal desorption total ion chromatogram (TIC) revealed no detectable components, indicating the absence of free, thermally extractable organic components in this sample. It

should be noted, however, that highly polar material could have been present, which would not have successfully eluted from the column, or indeed volatilised sufficiently in the probe.

The pyrolysate TIC revealed carbon dioxide, 1-butene, 1-pentene, and 1-hexene as the only detectable components. The dominance of carbon dioxide, combined with the relatively minor abundance of the few small organic compounds present, suggests a highly oxidised sample. It is not possible to determine the significance of the carbon dioxide, beyond it being indicative of oxidised organic material and the other compounds are too low in abundance to provide any meaningful interpretation of their presence, beyond them being indicative of an organic origin. It should be noted, however, that highly polar material could have been present, which would not have successfully eluted from the column, or indeed volatilised sufficiently in the probe.

Discussion, Lithuania Roman period (AD 1st-3th century), Pašatrijis, North Lithuania barrows

Two samples of dental calculus from individual burials at Pašatrijis dating to the Roman period (AD 1st-4th century) were analysed by sequential thermal desorption-gas chromatography-mass spectrometry (TD-GC-MS) and pyrolysis-gas chromatography-mass spectrometry (Py-GC-MS).

One sample (1A30) contained virtually no free and bound/polymeric organic material, most likely reflecting unfavourable prevailing conditions for biomolecular preservation. Of those organic compounds identified, the dominance of carbon dioxide and the very minor abundance of only a very few small organic compounds suggests that only highly oxidised organic material was present. The other sample (1A29) revealed only carbon dioxide in the TD-GC-MS indicating the essential absence of free, thermally extractable organic components in these samples. The carbon dioxide is also indicative of highly oxidised organic material. The pyrolysis-GC-MS revealed a series of C₉ to C₁₆ *n*-1-alkenes and *n*-alkanes, typical of unsaturated and saturated fat/oil-derived acyl lipids^{5,6}. The absence of these compounds in the TD profiles provides molecular evidence for a polymeric/bound acyl lipid source. However, the additional presence of a series of C₁₁ to C₁₆ isoalkenes and isoalkanes, presumably deriving from the branched fatty acids, which are significant components of bacterial triglycerides, strongly suggests a microbial origin for these lipid biomarkers, at least in part. The dominance of carbon dioxide in the pyrogram again suggests a highly oxidised sample.

3314

3315 **Lithuania Viking period (AD 7th-12th century), Jakštaičiai-Meškiai, North Lithuania**
3316 **barrows**

3317 The Jakštaičiai-Meškiai cemetery is situated on the shore of Dubysa river, between villages
3318 Jakštaičiai and Meškiai (Šiauliai district) in northern Lithuania. Investigations carried in
3319 1973-1983 revealed 102 inhumations, 9 cremated burials and 1 horse grave. Preservation of
3320 skeletons was poor. Some graves had been destroyed through ploughing. Burial items include
3321 various kinds of tools, weapons, fragments of cloths and adornments¹⁰².

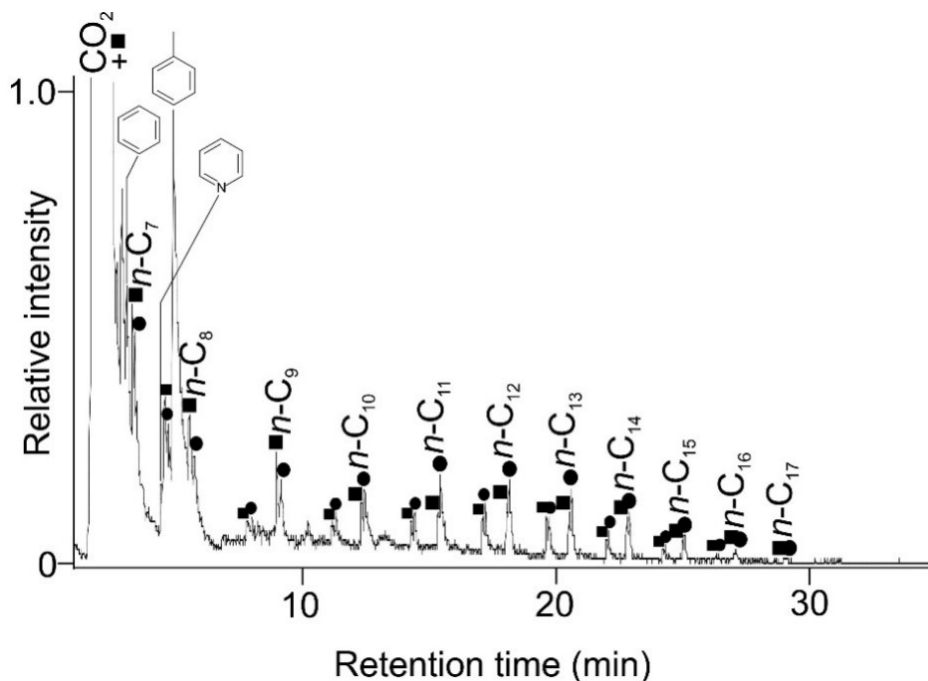
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3323 **49. Lithuania Viking period (AD 7th-12th century), Jakštaičiai-Meškiai, North**
3324 **Lithuania barrows, Sk1388B male 20-25 yrs T36 lingual, 1A31 (calculus: 4.33 mg)**

3325 The thermal desorption total ion chromatogram (TIC) revealed carbon dioxide as the only
3326 detectable component, indicating the essential absence of free, thermally extractable organic
3327 components in this sample. It should be noted, however, that highly polar material could have
3328 been present, which would not have successfully eluted from the column, or indeed
3329 volatilised sufficiently in the probe. It is not possible to determine the significance of the
3330 carbon dioxide, beyond it being indicative of oxidised organic material.

3331 The pyrolysis-GC-MS TIC (Fig. 34) was dominated by carbon dioxide, 1-butene, 1-pentene
3332 and 1-hexene, the alkenes being indicative of lipid or proteinaceous material in the sample¹¹.
3333 Cyclopentadiene and methylcyclopentadiene are also components of the pyrolysate although
3334 it is not possible to ascribe a specific origin here. Acetone was also identified and can be
3335 indicative of the amino acid glycine or a carbohydrate component, although any
3336 interpretation should be considered tentative. Benzene, toluene and pyridine were also
3337 detected, albeit as minor components; their significance in the absence of other more
3338 diagnostic biomolecules is unclear. In contrast to the thermal desorption profile, the pyrogram
3339 also revealed trace amounts of a series of C₇ to C₁₇ *n*-1-alkenes and *n*-alkanes, typical of
3340 unsaturated and saturated fat/oil-derived acyl lipids^{5,6}. The absence of these compounds in the
3341 TD profile provides molecular evidence for a polymeric/bound acyl lipid source. However, in
3342 addition to *n*-1-alkenes and *n*-alkanes, typical of fat/oil derived lipids, the pyrogram also
3343 displayed a series of C₈ to C₁₆ iso-methyl-1-alkenes (isoalkenes) and 2-methylalkanes
3344 (isoalkanes), presumably deriving from the branched fatty acids which are significant
3345 components of bacterial triglycerides. The dominance of carbon dioxide suggests a highly

oxidised sample. It is not possible to determine the significance of the carbon dioxide, beyond it being indicative of oxidised organic material. It should be noted, however, that highly polar material could have been present, which would not have successfully eluted from the column, or indeed volatilised sufficiently in the probe.



Supplementary Figure 34. Reconstructed total ion chromatogram of the pyrogram (pyrolysis profile) (610 °C for 10s) of sample 1A31, Viking period AD 7th-12th century), Jakštaičiai-Meškia, after thermal desorption (310 °C for 10 s). Key to Fig. 34: Peak identities (x indicates carbon chain length): filled squares, Cx indicates alkenes; filled circles, Cx indicates alkanes. Also shown are the structures of three aromatic compounds identified: benzene, toluene and pyridine. CO₂ indicates carbon dioxide.

50. Lithuania Viking period (AD 7th-12th century), Jakštaičiai-Meškia, North Lithuania barrows, Sk1386 female 20-25 yrs T46 lingual, 1A32 (calculus: 8.47 mg)

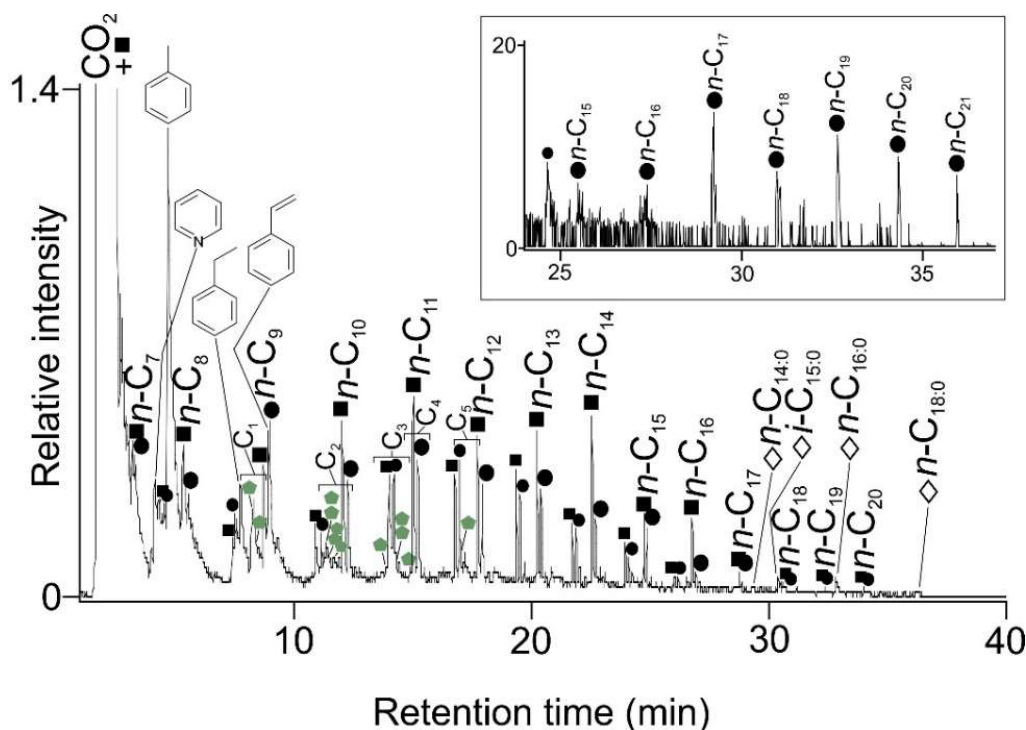
The thermal desorption total ion chromatogram (TIC) (Fig. 35, inset) revealed carbon dioxide as the main detectable (trace) component, indicating very little free, thermally extractable organic components in this sample. However, also in trace abundance are a series of *n*-alkanes, carbon numbers C₁₅ to C₂₁, maximising at C₁₇. There is a slight odd over even

3365 carbon number predominance, although the relative abundance of the C₁₈ and C₂₀ *n*-alkanes
3366 suggests the sample has undergone some biodegradation as a result of diagenetic changes.
3367 Although the trace quantities and evidence for biodegradation necessarily mean any inference
3368 must be tentative, the relatively unusual mid-chain carbon numbers present in the TD
3369 chromatogram, combined with the C₁₇ *n*-alkane being dominant and a slight odd over even
3370 carbon number predominance, suggests an algal source^{92,101,103} (see discussion below). It is
3371 not possible to determine the significance of the carbon dioxide, beyond it being indicative of
3372 oxidised organic material. It should also be note that highly polar material could have been
3373 present, which would not have successfully eluted from the column, or indeed volatilised
3374 sufficiently in the probe.

3375 The pyrolysis-GC-MS TIC (Fig. 35) was dominated by carbon dioxide, 1-butene, 1-pentene
3376 and 1-hexene, the alkenes being indicative of lipid or proteinaceous material in the sample¹¹.
3377 Cyclopentadiene and methylcyclopentadiene are also components of the pyrolysate although
3378 it is not possible to ascribe a specific origin here. Acetone was also identified and can be
3379 indicative of the amino acid glycine or a carbohydrate component, although any
3380 interpretation should be considered tentative. In contrast to the thermal desorption profile, the
3381 pyrogram also revealed a series of C₇ to C₂₀ *n*-1-alkenes and *n*-alkanes, typical of unsaturated
3382 and saturated fat/oil-derived acyl lipids^{5,6}. A distribution with maxima at C₁₁ and C₁₃/C₁₄
3383 suggests the original acyl lipids may have been high in polyunsaturated fatty acids. The
3384 absence of these compounds in the TD profile provides molecular evidence for a
3385 polymeric/bound acyl lipid source. However, in addition to *n*-1-alkenes and *n*-alkanes, typical
3386 of fat/oil derived lipids, the pyrogram also displayed a series of C₈ to C₁₆ iso-methyl-1-
3387 alkenes (isoalkenes) and 2-methylalkanes (isoalkanes), presumably deriving from the
3388 branched fatty acids which are significant components of bacterial triglycerides.

3389 A number of the biomarkers identified here can be indicative of proteins^{6,11}, i.e. benzene,
3390 toluene, pyridine, pyrrole. This is also corroborated by the presence of aliphatic nitriles
3391 (carbon numbers C_{14:0}, *i*-C_{15:0}, C_{16:0} (max) and C_{18:0}) suggesting a lipid (fat/oil) in this sample
3392 which has combined with protein-derived nitrogen to produce these moieties⁵. However,
3393 notable in this context is the additional presence of appreciable amounts of the more unusual
3394 alkyl pyrroles. C₁ alkyl pyrroles: 2- and 3-methylpyrroles; C₂ alkyl pyrroles: 2- and 3-
3395 ethylpyrroles, 2,4-, 2,3- and 3,4-dimethyl-pyrroles; C₃ alkyl pyrroles: 2-ethyl-4(?)
3396 methylpyrrole, 2-ethyl-3(?) methylpyrrole and 4-ethyl-2-methylpyrrole; C₄ alkyl pyrrole: an
3397 unidentified propylmethylpyrrole; C₅ alkyl pyrrole: 2-ethyl-5?-propylpyrrole. Collectively,

these specific C₁, C₂, C₃, C₄ and C₅ alkyl pyrroles are characteristic of a tetrapyrrole/porphyrin-derived origin and are indicative of an algal source observed in fossils and expected in similarly diagenetically altered archaeological material^{11,21,22}. The presence here of protein markers and specifically the C₁ to C₅ alkyl pyrroles, combined with the particular lipid profile identified, indicate the presence of a macroalgae, i.e. seaweed or freshwater source, in this sample²¹⁻²³ (see discussion). The dominance of carbon dioxide suggests a highly oxidised sample. It is not possible to determine the significance of the carbon dioxide, beyond it being indicative of oxidised organic material. It should be noted, however, that highly polar material could have been present, which would not have successfully eluted from the column, or indeed volatilised sufficiently in the probe.



Supplementary Figure 35. Reconstructed total ion chromatogram of the pyrogram (pyrolysis profile) (610 °C for 10s) of sample 1A32, period (AD 7th-12th century), Jakštaičiai-Meškia, after thermal desorption (310 °C for 10 s). Key to Fig. 35: Peak identities (x indicates carbon chain length): filled squares, Cx indicates alkenes; filled circles, Cx indicates alkanes; open diamonds, Cx:y indicates acyclic nitriles; green filled pentagons indicates alkyl pyrroles (with alkyl chain length above, Cx). Also shown are the structures of four aromatic compounds identified: pyridine, toluene, ethylbenzene and styrene. CO₂ indicates carbon

dioxide. Inset displays a reconstructed total ion chromatogram of the thermal desorption profile (310 °C for 10 s) of this sample. Peak identities: (x indicates carbon chain length): filled circles, C_x indicates alkanes.

51. Lithuania Viking period (AD 7th-12th century), Jakštaičiai-Meškia, North Lithuania barrows, Sk1382 male 35-40 yrs T36 lingual, 1A33 (calculus: 11.05 mg)

The main compound in the thermal desorption total ion chromatogram (TIC) (Fig. 36, inset) is carbon dioxide. In very minor abundance are a series of *n*-alkanes, carbon numbers C₁₆ to C₂₂, maximising at C₁₉. There is a slight odd over even carbon number predominance, although the relative abundance of the C₁₈ and C₂₀ *n*-alkanes and the presence of C₁₅, C₁₇ and C₁₉ to C₂₂ branched chain alkanes suggests the sample has undergone some microbial-derived biodegradation as a result of diagenetic changes. Although the trace quantities and evidence for biodegradation necessarily mean any inference must be tentative, the relatively unusual mid-chain carbon numbers present in the TD chromatogram, combined with the C₁₉ *n*-alkane being dominant and a slight odd over even carbon number predominance, suggests an algal source^{92,101,103} (see discussion below). It is not possible to determine the significance of the carbon dioxide, beyond it being indicative of oxidised organic material. It should also be noted that highly polar material could have been present, which would not have successfully eluted from the column, or indeed volatilised sufficiently in the probe.

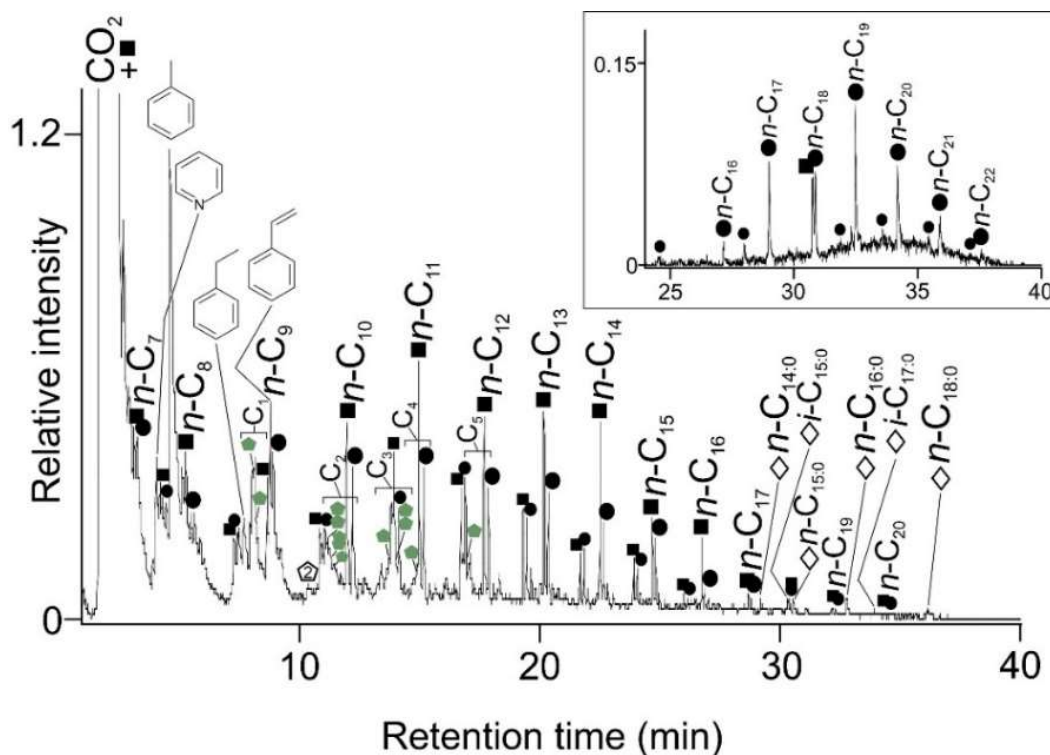
The pyrolysis-GC-MS TIC (Fig. 36) was dominated by carbon dioxide, 1-butene, 1-pentene and 1-hexene, the alkenes being indicative of lipid or proteinaceous material in the sample¹¹. Cyclopentadiene and methylcyclopentadiene are also components of the pyrolysate although it is not possible to ascribe a specific origin here. Acetone was also identified and can be indicative of the amino acid glycine or a carbohydrate component, although any interpretation should be considered tentative. In contrast to the thermal desorption profile, the pyrogram also revealed a series of C₇ to C₁₈ *n*-1-alkenes and *n*-alkanes, typical of unsaturated and saturated fat/oil-derived acyl lipids^{5,6}. A distribution with maxima at C₁₁ and C₁₃/C₁₄ suggests the original acyl lipids may have been high in polyunsaturated fatty acids. The absence of these compounds in the TD profile provides molecular evidence for a polymeric/bound acyl lipid source. However, in addition to *n*-1-alkenes and *n*-alkanes, typical of fat/oil derived lipids, the pyrogram also displayed a series of C₈ to C₁₆ iso-methyl-1-

3448 alkenes (isoalkenes) and 2-methylalkanes (isoalkanes), presumably deriving from the
3449 branched fatty acids which are significant components of bacterial triglycerides.

3450 A number of the biomarkers identified here can be indicative of proteins^{6,11}, i.e. benzene,
3451 toluene, pyridine and pyrrole. This is also corroborated by the presence of aliphatic nitriles
3452 (carbon numbers C_{14:0}, *i*-C_{15:0}, *ai*-C_{15:0}, C_{15:0}, *i*-C_{16:0}, C_{16:0} (max), *i*-C_{17:0} and C_{18:0}) suggesting
3453 a lipid (fat/oil) in this sample which has combined with protein-derived nitrogen to produce
3454 these moieties⁵. However, notable in this context is the additional presence of appreciable
3455 amounts of the more unusual alkyl pyrroles. C₁ alkyl pyrroles: 2- and 3-methylpyrroles; C₂
3456 alkyl pyrroles: 2- and 3-ethylpyrroles, 2,4-, 2,3- and 3,4-dimethyl-pyrroles; C₃ alkyl pyrroles:
3457 2-ethyl-4(?) -methylpyrrole, 2-ethyl-3(?) -methylpyrrole and 4-ethyl-2-methylpyrrole; C₄ alkyl
3458 pyrrole: an unidentified propylmethylpyrrole; C₅ alkyl pyrrole: 2-ethyl-5?-propylpyrrole.
3459 Collectively, these specific C₁, C₂, C₃, C₄ and C₅ alkyl pyrroles are characteristic of a
3460 tetrapyrrole/porphyrin-derived origin and are indicative of an algal source observed in fossils
3461 and expected in similarly diagenetically altered archaeological material^{11,21,22}. The presence
3462 here of protein markers and specifically the C₁ to C₅ alkyl pyrroles, combined with the
3463 particular lipid profile and carbohydrate markers (see below) identified, indicate the presence
3464 of a macroalgae, i.e. seaweed or freshwater source, in this sample²¹⁻²³ (see discussion).

3465 The thermally-derived carbohydrate markers 2-methylfuran and 2-methyl-2-cyclopenten-1-
3466 one were identified as moderately significant components. That they were absent in the TD
3467 profile points to their originating from the original polymeric carbohydrate source (e.g.
3468 starch)²⁷. Although levoglucosan (1,6-anhydro-β-D-glucopyranose) is usually the major
3469 component in the thermally-derived products of carbohydrates analysed by TD/Py-GC-MS,
3470 its absence from the TD profile here is consistent with the removal of hydroxyl groups in the
3471 original carbohydrate polymer, so preventing its formation in the probe. The additional
3472 absence of the normally significant carbohydrate markers 2-hydroxy-3-methyl-2-
3473 cyclopenten-1-one and 3-hydroxy-2-methyl-2-cyclopenten-1-one observed in pure standard
3474 material (glycogen, starch and cellulose) would also support this interpretation. This shows a
3475 strong matrix effect likely to be due to the inorganic component of the calculus and the
3476 microflora in the mouth. This dehydration/dehydroxylation has also been observed in dog
3477 calculus and 19th century human calculus⁶, where other markers, e.g. a prevalence of nitriles,
3478 with amides very minor, deriving from dehydrating conditions in the mouth of dogs and
3479 humans (though not sheep⁶) supporting this view⁶. The presence of carbon dioxide is also
3480 consistent with a lipid, amino acid/protein and carbohydrate component in this sample.

Notably, these findings did not reveal key biomarkers typically observed in soils^{20,23}. It should also be noted that highly polar material could have been present, which would not have successfully eluted from the column, or indeed volatilised sufficiently in the probe.



Supplementary Figure 36. Reconstructed total ion chromatogram of the pyrogram (pyrolysis profile) (610 °C for 10s) of sample 1A33, period (AD 7th-12th century), Jakštaičiai-Meškia, after thermal desorption (310 °C for 10 s). Key to Fig. 36: Peak identities (x indicates carbon chain length): filled squares, Cx indicates alkenes; filled circles, Cx indicates alkanes; open diamonds, Cx:y indicates acyclic nitriles; green filled pentagons indicates alkyl pyrroles (with alkyl chain length above, Cx); open pentagons with numerals inside indicates carbohydrate pyrolysis marker, 2 is 2-methyl-2-cyclopenten-1-one. Also shown are the structures of four aromatic compounds identified: pyridine, toluene, ethylbenzene and styrene. CO₂ indicates carbon dioxide. Inset displays a reconstructed total ion chromatogram of the thermal desorption profile (310 °C for 10 s) of this sample. Peak identities: (x indicates carbon chain length): filled square, Cx indicates alkene; filled circles, Cx indicates alkanes.

Discussion, Viking period (AD 7th-12th century), Jakštaičiai-Meškia, North Lithuania barrows

Three samples of dental calculus from individual burials at Jakštaičiai-Meškia dating to the Viking period (AD 7th-12th century) were analysed by sequential thermal desorption-gas chromatography-mass spectrometry (TD-GC-MS) and pyrolysis-gas chromatography-mass spectrometry (Py-GC-MS).

One sample (1A31) revealed only carbon dioxide in the TD-GC-MS indicating the essential absence of free, thermally extractable organic components in these samples. The carbon dioxide is also indicative of highly oxidised organic material. The pyrolysis-GC-MS revealed a series of C₇ to C₁₇ *n*-1-alkenes and *n*-alkanes, typical of unsaturated and saturated fat/oil-derived acyl lipids^{5,6}. The absence of these compounds in the TD profiles provides molecular evidence for a polymeric/bound acyl lipid source. However, the additional presence of a series of C₈ to C₁₆ isoalkenes and isoalkanes, presumably deriving from the branched fatty acids, which are significant components of bacterial triglycerides, strongly suggests a microbial origin for these lipid biomarkers, at least in part. The dominance of carbon dioxide in the pyrogram again suggests a highly oxidised sample.

The other two Jakštaičiai-Meškia samples, 1A32 and 1A33, revealed both free and bound/polymeric organic material. The thermal desorption total ion chromatogram (TIC) of the two samples were dominated by carbon dioxide; it is difficult to explain the significance of this non-diagnostic compound, beyond indicating that this sample has undergone diagenetic changes of a highly oxidising nature. Although only trace constituents, both samples revealed a series of *n*-alkanes, ranging from C₁₅/C₁₆ to C₂₁/C₂₂. Sample 1A32 maximises at C₁₇, and 1A33 maximises at C₁₉. Both samples show an odd over even carbon number predominance, although the relative abundance of the C₁₈ and C₂₀ *n*-alkanes suggests the samples have undergone some biodegradation as a result of diagenetic changes. Although the trace quantities and evidence for biodegradation necessarily mean any inferences must be tentative, the narrow range (between C₁₅ and C₂₂) and relatively unusual mid-chain carbon numbers, maximising at C₁₇ or C₁₉, combined with an odd over even predominance, is characteristic of an algal source^{92,101,103}. The presence of branched alkanes with carbon numbers C₁₇ and C₁₉ to C₂₂ observed in 1A33 as relatively minor constituents is indicative of some microbially-derived biodegradation.

The pyrolysis-GC-MS analyses of these two samples revealed appreciable amounts of organic material which were dominated by a complex suite of organic compounds indicative of lipid (fat/oil) and protein components. These include a series of C₇ to C₂₀ *n*-1-alkenes and *n*-alkanes, typical of unsaturated and saturated fat/oil-derived acyl lipids^{5,6}. A bimodal distribution with maxima at C₁₁ and C₁₃/C₁₄ combined with abundant short chain (C₄-C₇) *n*-1-alkenes suggests the original acyl lipids in these samples are likely to have been high in polyunsaturated fatty acids. The absence of these compounds in the TD profile provides molecular evidence for a polymeric/bound acyl lipid source. However, in addition to *n*-1-alkenes and *n*-alkanes, typical of fat/oil derived lipids, the pyrograms also displayed a series of C₈ to C₁₆ iso-1-alkenes and isoalkanes, presumably deriving from the branched fatty acids which are significant components of bacterial triglycerides (but see below).

A number of the biomarkers identified here can be indicative of proteins^{6,11}, i.e. benzene, toluene, pyridine, pyrrole. This is also corroborated by the presence of aliphatic nitriles (carbon numbers C_{14:0}, *i*-C_{15:0}, C_{16:0} (max) and C_{18:0}) in both samples, suggesting a lipid (fat/oil) in this sample which has combined with protein-derived nitrogen to produce these moieties⁸. Far more diagnostic of a particular dietary source are the particular suite of alkyl pyrroles observed in the pyrograms of these samples, with an alkyl chain length of up to C₅. Both these samples revealed appreciable amounts of these biomarkers. This included the 2- and 3-dimethylpyrroles, 2- and 3-ethylpyrroles, 2,4-, 2,3- and 3,4-dimethylpyrroles, three ethylmethylpyrroles, a propylmethylpyrrole and an ethylpropylpyrrole. These specific C₁ to C₅ alkyl pyrroles are highly diagnostic of the presence of an appreciable tetrapyrrole component. Their porphyrin-derived origin, and the relative abundance of some of the key biomarkers, is indicative of an algal source observed in fossils and expected in similarly diagenetically altered archaeological material^{11,21,22}. Although C₁ and C₂ alkyl pyrroles can also derive from protein-derived amino acids such as glycine, proline, hydroxyproline, serine and glutamic acid^{12-14,20,40,41}, the C₃+ alkyl pyrroles identified in these two samples point to a tetrapyrrole source^{21,22,40,41}. Moreover, the same specific C₁ to C₅ alkylpyrroles identified in the Py-GC-MS of an algal kerogen^{21,22} were observed in these samples. Overall the two samples display a remarkably similar pyrolysis profile to algal-derived kerogen previously studied via very similar organic geochemical techniques^{21,22}.

The presence here of protein markers and specifically the C₁ to C₅ alkyl pyrroles, combined with the particular lipid profile and specific carbohydrate markers (see above) identified, indicate the presence of a macroalgae, i.e. seaweed or freshwater source, in this sample²¹⁻²³.

Marine and freshwater algal sources are known to have been exploited for food in antiquity^{52,104}, although their consumption in this part of the world does not seem to have been seriously considered to date.

Kanaljorden, Sweden. Mesolithic

Kanaljorden is a Mesolithic ritual wetland-site located at Motala, Östergötland, South Sweden. Ritual depositions, including human crania, had been placed on a stone-packing built on the bottom of a small lake. The human assemblage comprises the remains of 10 adults and one infant. The calculus samples discussed here were taken from individual 2 and 4. Sample 122 represent individual 4, a female 30 – 45 years old, dated to 7834-7573 cal BP (Ua-42118). Sample 126 represent a male 50+ years old, dated to 7664-7518 cal BP (Ua-51722). The depositions also include remains of forest game including wild boar, brown boar, elk and red deer, fish like pike, perch, pikeperch and salmonidae as well as pond-turtle. There are also depositions of hazelnuts, raspberries and tree fungus. The small lake at Kanaljorden was situated close to the large lake Vättern, and the river Motala Ström. The distance to the sea was about 30 km^{1,2}.

Two samples of dental calculus taken from two individuals from Kanaljorden, Motala, Sweden were analysed by sequential thermal desorption-gas chromatography-mass spectrometry (TD-GC-MS) and pyrolysis-gas chromatography-mass spectrometry (Py-GC-MS). This technique facilitates the identification of both free/unbound and bound/polymeric organic components. Both calculus samples produced very little free organic material, yet a significant amount of a bound/polymeric organic constituent.

52. Kanaljorden 122 (calculus: 0.25 mg)

The thermal desorption total ion chromatogram (TIC) revealed carbon dioxide as the only detectable component, indicating the virtual absence of free, thermally extractable organic components in this sample. It should be noted, however, that highly polar material could have been present, which would not have successfully eluted from the column, or indeed volatilised sufficiently in the probe. It is not possible to determine the significance of the carbon dioxide, beyond it being indicative of oxidised organic material.

In contrast to the thermal desorption profile, the pyrolysis-GC-MS TIC was dominated by C₈ to C₁₆ *n*-1-alkenes and *n*-alkanes, typical of unsaturated and saturated fat/oil-derived acyl lipids^{5,6}. The absence of these compounds in the TD profile provides molecular evidence for a polymeric/bound acyl lipid source. However, in addition to *n*-1-alkenes and *n*-alkanes, typical of fat/oil derived lipids, the pyrogram also displayed a series of C₁₁ to C₁₅ 2-methyl *n*-1-alkenes and 2-methyl *n*-alkanes, presumably deriving from the branched fatty acids which are significant components of bacterial triglycerides. There were components identified in the pyrolysate TIC which can be indicative of 'black carbon', i.e. charcoal or soot^{7,10}. However, naphthalenes and biphenyl present in 'chars'^{7,10} were absent and the dominance of toluene, rather than the normally dominant aromatic hydrocarbon benzene, is notably atypical of chars^{7,10}. The markers identified here can also be indicative of proteins^{6,11} and combined with the protein markers benzonitrile and indole¹¹ identified this is a more likely origin for these compounds in this sample. This is also corroborated by the relatively significant abundance of phenol, 2-methylphenol (*o*-cresol), 4-methylphenol (*p*-cresol) (with the 4-methyl isomer being markedly predominant), and minor amounts of 2,4-dimethylphenol and 4-ethylphenol, all of which, collectively, are indicative of the amino acid tyrosine¹²⁻¹⁴. With guaiacol detected only as a trace constituent, and an absence of other guaiacol moieties characteristic of lignin^{15,16}, the possibility that the phenols may originate from wood or woody plant material can be excluded. Alkyl phenols have also been observed in wood smoke and smoked foods^{17,18}, however the absence of methoxyphenols, diagnostic of lignin-derived smoke, means this possibility can also be discounted. Proteins rich in tyrosine include meat, fish and dairy products, although the presence of bacterial markers means the possibility that at least a partial origin could be microbial cannot be excluded. However, taken together, the presence of toluene, styrene and phenylacetonitrile (benzyl nitrile) can indicate the amino acid phenylalanine^{12,13}, which is also high in proteins from meat, fish or dairy. In addition, although minor constituents, indole and 2-methylindole were also identified which are consistent with a tryptophan amino acid source^{12,13,20}, also notably high in meat, fish or dairy. The presence of carbon dioxide is also consistent with both a lipid and amino acid/protein component in this sample. Notably, these findings revealed no polysaccharide markers or higher alkyl aromatics typically observed in soils²⁰, nor polynuclear aromatic hydrocarbons (PAHs) indicative of combustion processes.

3623

3624 **53. Kanaljorden 126 (calculus: 0.83 mg)**

3625 The thermal desorption total ion chromatogram (TIC) revealed carbon dioxide as the only
3626 detectable component, indicating the virtual absence of free, thermally extractable organic
3627 components in this sample. It should be noted, however, that highly polar material could have
3628 been present, which would not have successfully eluted from the column, or indeed
3629 volatilised sufficiently in the probe. It is not possible to determine the significance of the
3630 carbon dioxide, beyond it being indicative of oxidised organic material.

3631 In contrast to the thermal desorption profile, the pyrolysis-GC-MS TIC was dominated by C₉
3632 to C₁₇ *n*-1-alkenes and *n*-alkanes, typical of unsaturated and saturated fat/oil-derived acyl
3633 lipids^{5,6}. The absence of these compounds in the TD profile provides molecular evidence for
3634 a polymeric/bound acyl lipid source. Notably, the series of C₁₁ to C₁₅ 2-methyl *n*-1-alkenes
3635 and 2-methyl *n*-alkanes observed in sample 122 were absent; the lack of branched alkenes
3636 and alkanes in this sample suggest this lipid component is not bacterial in origin. There were
3637 components identified in the pyrolysate TIC which were indicative of ‘black carbon’, i.e.
3638 charcoal or soot⁷⁻¹⁰, and although toluene was the major component, rather than the normally
3639 dominant benzene, the associated ‘char’ markers naphthalene, methylnaphthalene and
3640 biphenyl were present in sufficient abundance to suggest a ‘char’ component in the sample⁷⁻
3641 ¹⁰. Benzene constituted ~33% of the ‘char’ markers, with ~46% toluene and moderate
3642 quantities of ethyl benzene, xylenes and styrene (~15% total). However, the markers
3643 identified here can also be indicative of proteins^{6,11}, and their presence, combined with the
3644 phenolic and nitrogen-containing protein markers¹¹⁻¹⁴ (see below), is consistent with a protein
3645 component in this sample. The relatively significant abundance of phenol, 2-methylphenol
3646 (*o*-cresol), 4-methylphenol (*p*-cresol) (with the 4-methyl isomer being notably predominant),
3647 and minor amounts of 2,4-dimethylphenol and 4-ethylphenol are, collectively, indicative of
3648 the amino acid tyrosine¹²⁻¹⁴. With guaiacol detected only as a very minor constituent, and an
3649 absence of other guaiacol moieties characteristic of lignin^{15,16}, the possibility that the phenols
3650 may originate from wood or woody plant material can be excluded. Alkyl phenols have also
3651 been observed in wood smoke and smoked foods^{17,18}, however the absence of
3652 methoxyphenols, diagnostic of lignin-derived smoke, means this possibility can also be
3653 discounted. Proteins rich in tyrosine include meat, fish and dairy products and the absence of
3654 bacterial markers in this sample suggests it may have a dietary origin. Moreover, taken
3655 together, the presence of toluene, styrene and phenylacetonitrile (benzyl nitrile) can indicate

the amino acid phenylalanine^{12,13}, which is also high in proteins from meat, fish or dairy. In addition, although very minor constituents, indole and 2-methylindole were also identified which are consistent with a tryptophan amino acid source^{12,13,20} also notably high in meat and fish, or dairy. The presence of carbon dioxide is also consistent with both a lipid and amino acid/protein component in this sample. Notably, these findings revealed no polysaccharide markers or higher alkyl aromatics typically observed in soils²⁰. Despite chemical evidence for a ‘char’ component, polynuclear aromatic hydrocarbons (PAHs) indicative of combustion processes could not be detected, which may reflect the moderate amount of organic material in the sample.

Discussion, Kanaljorden

The results revealed a moderate amount of organic material in the two samples possibly reflecting prevailing environmental conditions for biomolecular preservation. The results revealed not only evidence of bacterially-derived components as one might expect from material taken from the microbially active human mouth, but also evidence for food-based fats and proteins, and in one sample evidence for a char suggesting possible smoke inhalation or cooking. More specifically, both samples revealed chemical evidence for a protein component high in tyrosine and phenylalanine, which may be indicative of a meat or fish source [no dairy likely here]. The profile of the fat markers is indicative of a source with a moderate degree of unsaturation, which is consistent with a meat or fish origin, as indicated by the protein markers.

Iberia

La Corona (Casa Corona), Alicante, Spain

Casa Corona is located in Villena, Alicante, Spain, by the Vinalopó River on the margin of an endorheic basin, around 80 km from the coast. Archaeological material from Late Mesolithic and Chalcolithic periods has been recovered, with the north sector of the site comprising the Late Mesolithic phase. The Late Mesolithic phase consists of several archaeological deposits formed in a grey-brown dark sandy matrix with ashes and organic material and 2 pit graves

with individual inhumations. The site was reoccupied during the Chalcolithic period; this phase comprises pit structures¹¹⁰.

Three samples of dental calculus taken from one individual from La Corona, Alicante, Spain were analysed by sequential thermal desorption-gas chromatography-mass spectrometry (TD-GC-MS) and pyrolysis-gas chromatography-mass spectrometry (Py-GC-MS). This technique facilitates the identification of both free/unbound and bound/polymeric organic components. Two calculus samples produced no free organic material, and only very minor amounts of bound/polymeric organic constituents, whereas one sample produced a relatively complex suite of both free and bound/polymeric organic constituents.

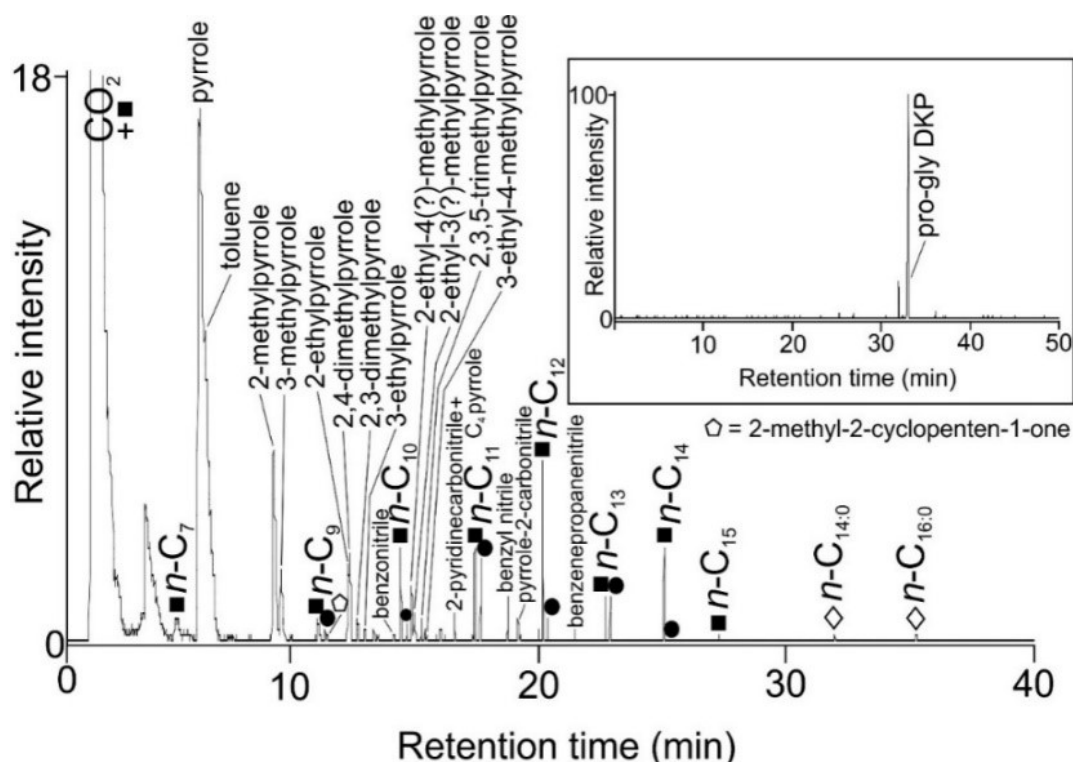
54. La Corona, Alicante, Spain: 25 Mesolithic (calculus: 5.68 mg)

The thermal desorption total ion chromatogram (TIC) (Fig. 37, inset) is dominated by a 2,5-diketopiperazine derivative of proline-glycine (pro-gly). This is thermally generated in the pyroprobe from the amino acids proline and glycine and is indicative of proteinaceous material containing these two protein constituents^{5,12,13}. No other compounds were observed indicating little free, thermally extractable, organic components in this sample. It should be noted, however, that highly polar material could have been present, which would not have successfully eluted from the column, or indeed volatilised sufficiently in the probe.

The Py-GC-MS TIC (Fig. 37) is dominated by C₉ to C₁₅ *n*-1-alkenes and *n*-alkanes, typical of unsaturated and saturated fat/oil derived lipids^{5,6}. A bimodal distribution with maxima at C₁₂ and C₁₄ suggests the original acyl lipids may have been high in polyunsaturated fatty acids. The absence of these compounds in the TD profile provides molecular evidence for a polymeric/bound acyl lipid source in this sample. Notably, there were no bacterially-derived hydrocarbons deriving from the original branched fatty acid acyl groups which are significant components of bacterial triglycerides, indicating this lipid component is not bacterial in origin. Other significant components included the aromatic hydrocarbons toluene, ethyl benzene and styrene and although these markers can be indicative of chars when other combustion markers such as benzene, xylenes, naphthalenes and biphenyls are present⁷⁻¹⁰, the absence of these additional 'char' markers, combined with the abundant nitrogen-containing biomolecules pyrrole, benzonitrile, phenylacetonitrile (benzyl nitrile) and benzenepropanenitrile, is indicative of the presence of a significant protein component in this sample^{6,11}. This is also corroborated by the presence, albeit as minor components, of aliphatic

nitriles (carbon numbers C_{14:0} and C_{16:0}) also suggesting a lipid (fat/oil) in this sample, which has combined with protein-derived nitrogen to produce these moieties⁵. Taken together, the presence of toluene, styrene and phenylacetonitrile (benzyl nitrile) can indicate the amino acid phenylalanine^{12,13}, which is high in proteins from meat or fish.

However, highly notable in this context is the additional presence of appreciable amounts of the more unusual 2- and 3-methylpyrroles, 2- and 3-ethylpyrroles, 2,4- and 2,3-dimethylpyrroles, 2-ethyl-4(?) -methylpyrrole, 2-ethyl-3(?) -methylpyrrole, 2,3,5-trimethylpyrrole, 3-ethyl-4-methylpyrrole and an unidentified C₄ alkyl pyrrole. These specific C₁, C₂, C₃ and C₄ alkyl pyrroles suggest a significant porphyrin-derived origin and are indicative of an algal source observed in fossils and expected in similarly diagenetically altered archaeological material^{11,21,22}. Notable, given their observance in remarkably similar pyrolysis profiles on dental calculus from Isbister and Quanterness in Orkney and Distillery Cave, Oban (see relevant samples above), were the presence, albeit as moderate to minor components, of the nitrogen containing aromatic compounds 1-H-pyrazole-4-carbonitrile, ethylcyanobenzene, 2-pyridinecarbonitrile and 1H-pyrrole-2-carbonitrile, and two carbohydrate markers; 2-methyl-2-cyclopenten-1-one and an unidentified derivative. The presence here of the abundant protein markers and specifically the C₁ to C₄ alkyl pyrroles, combined with the particular lipid profile and specific carbohydrate markers identified and observed in other 'seaweed samples' (see Isbister, Quanterness and Distillery Cave above), suggests the presence of a macroalgae, i.e. seaweed, in this sample. The presence of carbon dioxide is also consistent with both a lipid and amino acid/protein component in this sample. Notably, these findings did not reveal key markers typically observed in soils^{20,23}, nor polynuclear aromatic hydrocarbons (PAHs) indicative of combustion processes. It should also be note that highly polar material could have been present, which would not have successfully eluted from the column, or indeed volatilised sufficiently in the probe.



Supplementary Figure 37. Reconstructed total ion chromatogram of the pyrogram (pyrolysis profile) (610 °C for 10) of sample 25, La Corona, Alicante, Spain, after thermal desorption (310 °C for 10 s). Key to Fig. 37: Peak identities (x indicates carbon chain length): filled squares, Cx indicates alkenes; filled circles, Cx indicates alkanes; open diamonds, Cx:0 indicates nitriles; open pentagon indicates the carbohydrate pyrolysis marker, 2-methyl-2-cyclopenten-1-one; CO₂ indicates carbon dioxide. Also shown are toluene, pyrrole and its C₁ to C₄ derivatives, and five aromatic nitriles. Inset displays a reconstructed total ion chromatogram of thermal desorption profile (310 °C for 10 s) this sample. Peak identities: pro-gly DKP indicates the 2,5-diketopiperazine derivative of the amino acids proline and glycine.

55. La Corona 2 M1 mfieq (lingual) 21-05-13 (calculus: 2.58 mg)

The thermal desorption total ion chromatogram (TIC) revealed carbon dioxide as the only detectable component, indicating the essential absence of free, thermally extractable organic components in this sample. It should be noted, however, that highly polar material could have been present, which would not have successfully eluted from the column, or indeed

3761 volatilised sufficiently in the probe. It is not possible to determine the significance of the
3762 carbon dioxide, beyond it being indicative of oxidised organic material.

3763 The pyrolysate TIC also contained relatively little detectable organic material, and when
3764 taken together with the TD indicates a very low abundance of organic material in this
3765 calculus sample, although it should be said that this may be due to diagenetic changes.
3766 However, the components which were identified in the pyrolysate TIC were indicative of
3767 'black carbon', i.e. charcoal or soot⁷⁻¹⁰. Excluding the carbon dioxide, benzene was the major
3768 compound present in the pyrolysate (~53% of total quantified pyrogram peak area), along
3769 with a significant amounts of toluene (~22%) and minor to moderate quantities of ethyl
3770 benzene, xylenes and styrene (~5% total). A moderate amount of naphthalene and very minor
3771 amount of biphenyl were also detected, which are also typical of chars⁷⁻¹⁰. The protein marker
3772 benzonitrile¹¹ was present in minor abundance (~3%) fairly typical of previously
3773 characterised protein-containing chars⁷⁻¹⁰. Indene and two methyl indene derivatives were
3774 also identified.

3775

3776 **56. La Corona 4 M3 mf dch (lingual) 21-05-13 (calculus: 1.60 mg)**

3777 The thermal desorption total ion chromatogram (TIC) revealed carbon dioxide as the only
3778 detectable component, indicating the virtual absence of free, thermally extractable organic
3779 components in this sample. It should be noted, however, that highly polar material could have
3780 been present, which would not have successfully eluted from the column, or indeed
3781 volatilised sufficiently in the probe. It is not possible to determine the significance of the
3782 carbon dioxide, beyond it being indicative of oxidised organic material.

3783 The pyrolysate TIC also contained relatively little detectable organic material, and when
3784 taken together with the TD indicates a very low abundance of organic material in this
3785 calculus sample, although it should be said that this may be due to diagenetic changes.
3786 However, the components which were identified in the pyrolysate TIC were indicative of
3787 'black carbon', i.e. charcoal or soot⁷⁻¹⁰. Excluding the carbon dioxide, benzene was the major
3788 compound present in the pyrolysate (~44% of total quantified pyrogram peak area), along
3789 with a significant amounts of toluene (~22%) and moderate quantities of ethyl benzene,
3790 xylenes and styrene (~8% total). A moderate amount of naphthalene was also detected, which
3791 is also typical of chars⁷⁻¹⁰. The protein marker benzonitrile¹¹ was present in minor abundance
3792 (~2%) fairly typical of previously characterised protein-containing chars⁷⁻¹⁰ and very similar

3793 to the other calculus sample analysed. Indene and two methyl indene derivatives were also
3794 identified.

3795

3796 **La Corona sediment ‘control’ (sediment/soil: 10.92 mg)**

3797 A sample of sediment from the area surrounding the remains at the La Corona site was also
3798 analysed. The thermal desorption total ion chromatogram (TIC), like the two calculus
3799 samples, revealed carbon dioxide as the only detectable component, indicating the virtual
3800 absence of free, thermally extractable organic components in this sample. It is not possible to
3801 determine the significance of the carbon dioxide, beyond it being indicative of oxidised
3802 organic material.

3803 The pyrolysate TIC contained very little detectable organic material, and when taken together
3804 with the TD indicates an extremely low abundance of organic material in the sediment, likely
3805 to be due to diagenetic changes. The only components identified in the pyrolysate TIC were
3806 carbon dioxide and trace amounts of the aromatic hydrocarbons, benzene and toluene, well
3807 below the levels identified in the two dental calculus samples. The other regarded char
3808 markers observed in the two calculus samples were not detected in the sediment.

3809

3810 **Discussion, La Corona**

3811 In sample 54, the TD-GC-MS analysis revealed only the 2,5-diketopiperazine derivative
3812 (DKP) of proline-glycine (pro-gly) as the only ‘free’ organic compound detected. No other
3813 compounds were observed indicating little free, thermally extractable, organic components in
3814 this sample. It is perhaps notable, however, that this DKP, along with a far lesser amount of
3815 the DKP of proline-alanine (pro-ala), dominated the thermal desorption profile of samples
3816 with an essentially identical complex chemical profile in the Py-GC-MS, where tetrapyrroles
3817 deriving from [macro]algae were observed (see below). Pyrroles can derive from proteins
3818 containing glycine, proline, hydroxyproline and glutamic acids^{20,111} (although see below),
3819 which may partially explain their presence here and suggest a protein high in these four
3820 amino acids. Interestingly, these four protein constituents, along with alanine, are notably
3821 high in seaweeds⁴⁸ (see below).

3822 The C₉ to C₁₅ *n*-1-alkenes and *n*-alkanes observed in the Py-GC-MS is typical of unsaturated
3823 and saturated fat/oil derived acyl lipids^{5,6}. The bimodal distribution with maxima at C₁₂ and

C₁₄ suggests the original acyl lipids may have been high in polyunsaturated fatty acids, which is consistent with a marine source. Although the long chain moieties characteristic of a marine input (eicosapentaenoic acid, C_{20:5} [EPA] and docosahexaenoic acid, C_{22:6} fatty acid [DHA]) were not detected, these compounds are particularly labile and susceptible to degradation. Perhaps notably, the expected biopolymeric products generated during pyrolysis if these biomolecules were incorporated into a bound organic fraction would be expected to maximise at C₁₁/C₁₂ and C₁₄ *n*-1-alkene/*n*-alkanes, i.e. promote a bimodal distribution of alkene/alkanes, as is observed in this sample, although other possible food sources high in polyunsaturated fatty acids could also explain this molecular profile. Importantly, no microbial biomarkers (e.g. branched hydrocarbons) were observed, indicating this lipid component is not microbial in origin.

Far more diagnostic of a particular dietary source are the particular suite of alkyl pyrroles observed in the pyrogram. The presence of appreciable amounts of 2-methylpyrrole, 3-methylpyrrole, 2-ethylpyrrole, 3-ethylpyrrole, 2,4-dimethylpyrrole, 2,3-dimethylpyrroles, 2-ethyl-4(?) -methylpyrrole, 2-ethyl-3(?) -methylpyrrole, 2,3,5-trimethylpyrrole, 3-ethyl-4-methylpyrrole and an unidentified C₄ alkyl pyrrole is highly significant. These specific C₁ to C₄ alkyl pyrroles are diagnostic of the presence of a significant tetrapyrrole component. Their porphyrin-derived origin are indicative of an algal source observed in fossils and expected in similarly diagenetically altered archaeological material^{11,21,22}. Overall the sample displays a remarkably similar pyrolysis profile to dental calculus samples from Isbister and Quanterness in Orkney and Distillery Cave, Oban (see relevant samples above). The aromatic hydrocarbons toluene, ethyl benzene and styrene, combined with pyrrole, benzonitrile, phenylacetone nitrile and benzenepropanenitrile, are also collectively indicative of the presence of a significant protein component^{6,9}, with the aliphatic nitriles identified suggesting a source high in both protein and fat/oil. The additional presence of specific nitrogen-containing organic compounds, such as the carbonitriles and two carbohydrate markers observed, is highly notable. These exact same compounds were also identified, in approximately the same relative proportions, in dental calculus from Isbister and Quanterness in Orkney and Distillery Cave, Oban (see relevant samples above). Collectively, the presence here of the abundant protein markers and specifically the C₁ to C₄ alkyl pyrroles, combined with the particular lipid profile and exact same protein and carbohydrate markers identified and observed in other 'seaweed samples' (see Isbister, Quanterness and Distillery Cave sections above), strongly suggests the presence of a macroalgae, i.e. seaweed, in this sample. Seaweed

is known to have been exploited for food in antiquity⁵², although its consumption in this part of the world does not seem to have been seriously considered to date.

That being said, due to the relative distance between the La Corona site and the Alicante coast (~50km) it is sensible to consider other possible sources for this algal chemical signature in the dental calculus. There are endorheic lagoons and ponds in the area¹¹², which may have had the potential to provide sufficient nutrients for freshwater algae such as spirulina. Freshwater algae is known to have been used as a source of food in antiquity^{52,113}, but current evidence suggests the environmental conditions, and likely absence of the appropriate taxa in any significant abundance necessary for it to have been considered as a useful and viable food source, makes this a remote possibility at La Corona. With this considered, the consumption of seaweed as part of the diet is the most credible explanation, and if this was a seasonal component the relatively short distance to the coast means the exploitation of marine resources, particularly at this time, i.e. late Mesolithic, would not be particularly surprising.

That being said, stable isotopic analyses have not detected a marine component in the diet at this time^{112,114}. The same research also concluded there was no lagoonal food source in the diet at this Late Mesolithic site^{112,114}. The research has emphasised a wholly terrestrial diet, despite marine shellfish being identified the site, and whose presence has been explained as body adornment, with no exploitation of these marine invertebrates as a food resource¹¹². Moreover, it is suggested that not only is there no isotopic evidence, but there is no archaeological evidence for the consumption of marine resources¹¹⁴. This interpretation is interesting given the biomolecular evidence presented here as part of this current study. Importantly, it should be noted that if there was any marine component, such as fish or shellfish, it would be expected that the isotopic data would reveal this if it was a significant dietary component, i.e. greater than ~20%. However, if marine algae, i.e. seaweed (or other marine resources), was being consumed, particularly as a seasonal food resource, the presence of this component, even if significant in the diet, i.e. up to 20%, would not be detected. This serves to emphasise the potential of this biomarker approach to provide key additional, and often complimentary, palaeodietary evidence.

To conclude, in direct contradiction to the previous conclusion that: "...this population did not have any direct and regular dietary contact with the sea."¹¹², the organic chemistry of the dental calculus in the individual analysed here points to a macroalgal constituent in the diet at

3889 La Corona. When taken with the stable isotopic evidence, which would suggest that a
3890 freshwater [micro]algal source is indeed less likely, it points to the consumption of an
3891 appreciable amount of seaweed in this individual, albeit possibly a seasonal food resource at
3892 this Late Mesolithic inland site. This strongly, if not unequivocally, suggests that at least part
3893 of the population at La Corona did indeed have dietary contact with the sea, even if limited
3894 by seasonal factors, although further research on a larger sample size of individuals would be
3895 needed to better understand the significance for the wider population at this site.

3896 The results from the other two samples, revealed very little organic material in the samples
3897 possibly reflecting unfavourable prevailing environmental conditions for biomolecular
3898 preservation. Of those organic molecules identified they were indicative of a char of soot,
3899 possibly suggesting smoke inhalation or cooking, although the identification of trace levels of
3900 the two main char markers in the sediment, which was also analysed, necessarily make this
3901 inference somewhat tentative.

3902

3903 **Bòbila Madurell, Catalonia, Spain**

3904 Bòbila Madurell is a Neolithic cemetery in north east Iberia. It contains 179 documented pit
3905 burials¹¹⁵. Stable isotope analysis suggests the diet was based on terrestrial domesticated
3906 plants and animals¹¹⁶.

3907 One sample of dental calculus from Bòbila Madurell was analysed by sequential thermal
3908 desorption-gas chromatography-mass spectrometry (TD-GC-MS) and pyrolysis-gas
3909 chromatography-mass spectrometry (Py-GC-MS).

3910

3911 **57. Bòbila Madurell, Catalonia, Spain: MF18 male, Neolithic (calculus: 11.82 mg)**

3912 The thermal desorption total ion chromatogram (TIC) revealed carbon dioxide as the only
3913 detectable (trace) component, indicating the essential absence of free, thermally extractable
3914 organic components in this sample. It should be noted, however, that highly polar material
3915 could have been present, which would not have successfully eluted from the column, or
3916 indeed volatilised sufficiently in the probe. It is not possible to determine the significance of
3917 the carbon dioxide, beyond it being indicative of oxidised organic material.

The pyrolysate TIC contained very little detectable organic material. The only components identified were carbon dioxide and a trace amount of the aromatic hydrocarbon toluene. Given the wide natural distribution of toluene and the absence of other organic compounds its origin here remains uncertain. Nor is it possible to determine the significance of the carbon dioxide, beyond it being indicative of oxidised organic material.

Discussion, Bòbila Madurell, Catalonia

The results revealed a virtual absence of organic material, most likely reflecting unfavourable prevailing environmental conditions for biomolecular preservation. The extremely low level of carbon dioxide and toluene as the only compounds detected make any meaningful interpretation impossible, beyond indicating the unfavourable environmental conditions already mentioned.

Muge and Sado

Mesolithic shells middens found along the Tagus River in Portugal have been known about for over 150 years¹¹⁷ while Mesolithic shell middens in the nearby and Sado Valley were first recorded in the 1930s. Since this time, several periods of excavation have been undertaken, and well over 100 human skeletons have been recovered. Perforated shell jewellery and the use of ochre have been found in association with some of the burials. Radiocarbon dates suggest that the Muge middens were in use between 6100-5600 cal BC¹¹⁸. The development of the middens has been linked to the 8.2ka cold event, as a response to a decline in marine resources and an increase in sea level leading the hunter gatherer fishers to move inland. Stable isotope analysis on samples from the Sado River middens suggest that the diet comprised terrestrial animals, a small amount of terrestrial plants, and around a 20% component comprising marine resources⁷⁹.

Eleven samples of dental calculus taken from eleven individuals from the sites Moita do Sebastião and Cabeço da Arruda in the Muge Valley, Portugal and Cabeço do Pez in the Sado Valley, Portugal, were analysed by sequential thermal desorption-gas chromatography-mass spectrometry (TD-GC-MS) and pyrolysis-gas chromatography-mass spectrometry (Py-GC-MS). This technique facilitates the identification of both free/unbound and bound/polymeric

3948 organic components. The calculus samples revealed variable amounts of both free organic
3949 material and bound/polymeric organic constituents.

3950 **58. Moita do Sebastião, Muge, Portugal: MSD male (calculus: 2.59 mg)**

3951 The thermal desorption total ion chromatogram (TIC) revealed no detectable components,
3952 indicating the absence of free, thermally extractable organic components in this sample. It
3953 should be noted, however, that highly polar material could have been present, which would
3954 not have successfully eluted from the column, or indeed volatilised sufficiently in the probe.

3955 The pyrolysate TIC revealed carbon dioxide as the only detectable (trace) component,
3956 indicating the essential absence of organic material in this sample. It should be noted,
3957 however, that highly polar material could have been present, which would not have
3958 successfully eluted from the column, or indeed volatilised sufficiently in the probe. It is not
3959 possible to determine the significance of the carbon dioxide, beyond it being indicative of
3960 oxidised organic material, although its presence as only a very trace component makes even
3961 this inference of no meaningful value.

3962

3963 **59. Moita do Sebastião, Muge, Portugal: MSY female (calculus: 2.39 mg)**

3964 The thermal desorption total ion chromatogram (TIC) revealed no detectable components,
3965 indicating the absence of free, thermally extractable organic components in this sample. It
3966 should be noted, however, that highly polar material could have been present, which would
3967 not have successfully eluted from the column, or indeed volatilised sufficiently in the probe.

3968 The pyrolysate TIC revealed carbon dioxide as the only detectable (minor trace) component,
3969 indicating the essential absence of organic material in this sample. It should be noted,
3970 however, that highly polar material could have been present, which would not have
3971 successfully eluted from the column, or indeed volatilised sufficiently in the probe. It is not
3972 possible to determine the significance of the carbon dioxide, beyond it being indicative of
3973 oxidised organic material, although its presence as only a very trace component makes even
3974 this inference of no meaningful value.

3975

3976 **60. Moita do Sebastião, Muge, Portugal: MS7.3 male (calculus: 2.37 mg)**

3977 The thermal desorption total ion chromatogram (TIC) revealed carbon dioxide as the only
3978 detectable component, indicating the essential absence of free, thermally extractable organic
3979 components in this sample. It should be noted, however, that highly polar material could have
3980 been present, which would not have successfully eluted from the column, or indeed
3981 volatilised sufficiently in the probe. It is not possible to determine the significance of the
3982 carbon dioxide, beyond it being indicative of oxidised organic material.

3983 The pyrolysate TIC revealed carbon dioxide as the only detectable component, indicating the
3984 essential absence of organic material in this sample. It should be noted, however, that highly
3985 polar material could have been present, which would not have successfully eluted from the
3986 column, or indeed volatilised sufficiently in the probe. It is not possible to determine the
3987 significance of the carbon dioxide, beyond it being indicative of oxidised organic material.

3988

3989 **61. Moita do Sebastião, Muge, Portugal: MS29 child F.1-6 (female) (calculus:**
3990 **0.67 mg)**

3991 The thermal desorption total ion chromatogram (TIC) revealed no detectable components,
3992 indicating the absence of free, thermally extractable organic components in this sample. It
3993 should be noted, however, that highly polar material could have been present, which would
3994 not have successfully eluted from the column, or indeed volatilised sufficiently in the probe.

3995 The pyrolysate TIC revealed no detectable components, indicating the essential absence of
3996 organic material in this sample. It should be noted, however, that highly polar material could
3997 have been present, which would not have successfully eluted from the column, or indeed
3998 volatilised sufficiently in the probe.

3999

4000 **62. Moita do Sebastião, Muge, Portugal: MS32 male (calculus: 1.85 mg)**

4001 The thermal desorption total ion chromatogram (TIC) revealed carbon dioxide as the only
4002 detectable (trace) component, indicating the essential absence of free, thermally extractable
4003 organic components in this sample. It should be noted, however, that highly polar material
4004 could have been present, which would not have successfully eluted from the column, or
4005 indeed volatilised sufficiently in the probe. It is not possible to determine the significance of
4006 the carbon dioxide, beyond it being indicative of oxidised organic material.

The pyrolysate TIC revealed carbon dioxide as the only detectable (trace) component, indicating the essential absence of organic material in this sample. It should be noted, however, that highly polar material could have been present, which would not have successfully eluted from the column, or indeed volatilised sufficiently in the probe. It is not possible to determine the significance of the carbon dioxide, beyond it being indicative of oxidised organic material.

63. Moita do Sebastião, Muge, Portugal: MS56 male (calculus: 3.97 mg)

The thermal desorption total ion chromatogram (TIC) revealed no detectable components, indicating the absence of free, thermally extractable organic components in this sample. It should be noted, however, that highly polar material could have been present, which would not have successfully eluted from the column, or indeed volatilised sufficiently in the probe.

The pyrolysate TIC revealed carbon dioxide as the only detectable (trace) component, indicating the essential absence of organic material in this sample. It should be noted, however, that highly polar material could have been present, which would not have successfully eluted from the column, or indeed volatilised sufficiently in the probe. It is not possible to determine the significance of the carbon dioxide, beyond it being indicative of oxidised organic material.

64. Moita do Sebastião, Muge, Portugal: MS60 female (calculus: 1.30 mg)

The thermal desorption total ion chromatogram (TIC) revealed no detectable components, indicating the absence of free, thermally extractable organic components in this sample. It should be noted, however, that highly polar material could have been present, which would not have successfully eluted from the column, or indeed volatilised sufficiently in the probe.

The pyrolysate TIC revealed no detectable components, indicating the essential absence of organic material in this sample. It should be noted, however, that highly polar material could have been present, which would not have successfully eluted from the column, or indeed volatilised sufficiently in the probe.

Discussion, Moita do Sebastião, Muge

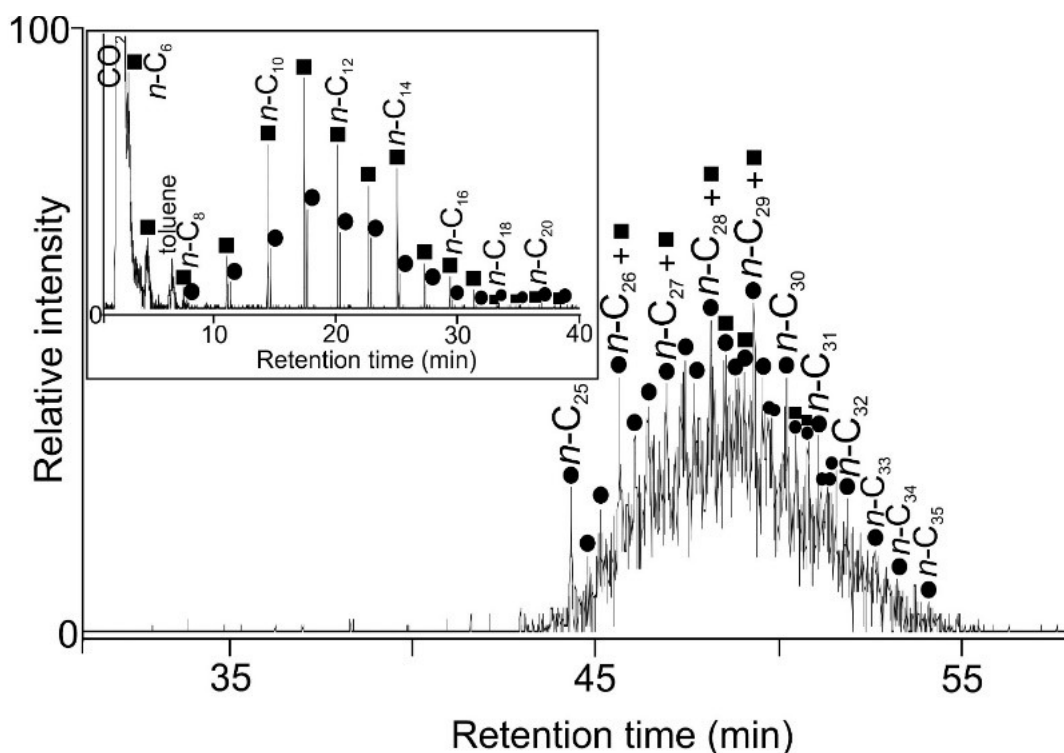
Seven samples of dental calculus taken from seven individuals from Moita do Sebastião were analysed by sequential thermal desorption-gas chromatography-mass spectrometry (TD-GC-MS) and pyrolysis-gas chromatography-mass spectrometry (Py-GC-MS). The results revealed a virtual absence of organic material in all samples, most likely reflecting unfavourable prevailing environmental conditions for biomolecular preservation. The extremely low levels of carbon dioxide as the only compound identified make any meaningful interpretation impossible, beyond indicating the unfavourable environmental conditions already mentioned.

65. Cabeço da Arruda, Muge, Portugal: ARR XIII (113) female (calculus: 5.70 mg)

The thermal desorption total ion chromatogram (TIC) (Fig. 38) is dominated by an unresolved complex mixture (UCM; apparent as a ‘hump’ in the chromatogram) of alkenes and alkanes, carbon numbers C₂₅ to C₃₅, within which there is series of C₂₅ to C₃₅ *n*-alkanes (odd over even predominance) dominating this UCM profile and maximising at *n*-C₂₉. Branched alkanes, primarily *iso*- and *anteiso*- with carbon numbers C₂₇, C₂₉ and C₃₁, are also notable and are possibly indicative of microbially-derived biodegradation (but see discussion below). These unusually high carbon numbers present in the TD chromatogram may suggest a higher plant source, possibly deriving from plant waxes in the original food consumed⁶ (but, again, see summary below). No lipid, protein or carbohydrate markers were observed, with the only other compound detected being the non-diagnostic carbon dioxide. It should also be note that highly polar material could have been present, which would not have successfully eluted from the column, or indeed volatilised sufficiently in the probe.

The pyrolysate TIC (Fig. 38, inset) identified components indicative of fat/oil-derived lipids or higher plant waxes. Toluene was also present, albeit a relatively minor compound in the pyrolysate. No protein or carbohydrate markers were observed. The Py-GC-MS profile was dominated by C₇ to C₂₁ *n*-1-alkenes and *n*-alkanes, typical of unsaturated and saturated fat/oil derived lipids and higher plant waxes^{5,6}. An essentially unimodal distribution with a clear maximum at C₁₁, and a secondary lesser maximum at C₁₄ may indicate the presence of polyunsaturated fatty acids in the original acyl lipids, although the ‘chemical fingerprint’ is notably analogous to that previously observed in archaeological beeswax¹¹⁹⁻¹²¹ (see

discussion below). Importantly, there were no bacterially-derived hydrocarbons deriving from the original branched fatty acid acyl groups which are significant components of bacterial triglycerides, indicating this lipid component is not bacterial in origin. There was no convincing evidence of a char suggesting smoke inhalation or cooking. It should also be note that highly polar material could have been present, which would not have successfully eluted from the column, or indeed volatilised sufficiently in the probe.



Supplementary Figure 38. Reconstructed total ion chromatogram of the thermal desorption profile (310 °C for 10 s) of sample ARR XIII (113) female, Cabeço da Arruda, Muge, Portugal. Key to Fig. 38: Peak identities (x indicates carbon chain length): filled squares indicates alkenes; filled circles, Cx indicates alkanes. Inset displays a reconstructed total ion chromatogram of the pyrogram (pyrolysis profile) (610 °C for 10) of this sample, after thermal desorption (310 °C for 10 s). Peak identities: (x indicates carbon chain length): filled squares, Cx indicates alkenes; filled circles, Cx indicates alkanes; CO₂ indicates carbon dioxide. Also shown is toluene.

Discussion, Cabeço da Arruda, Muge

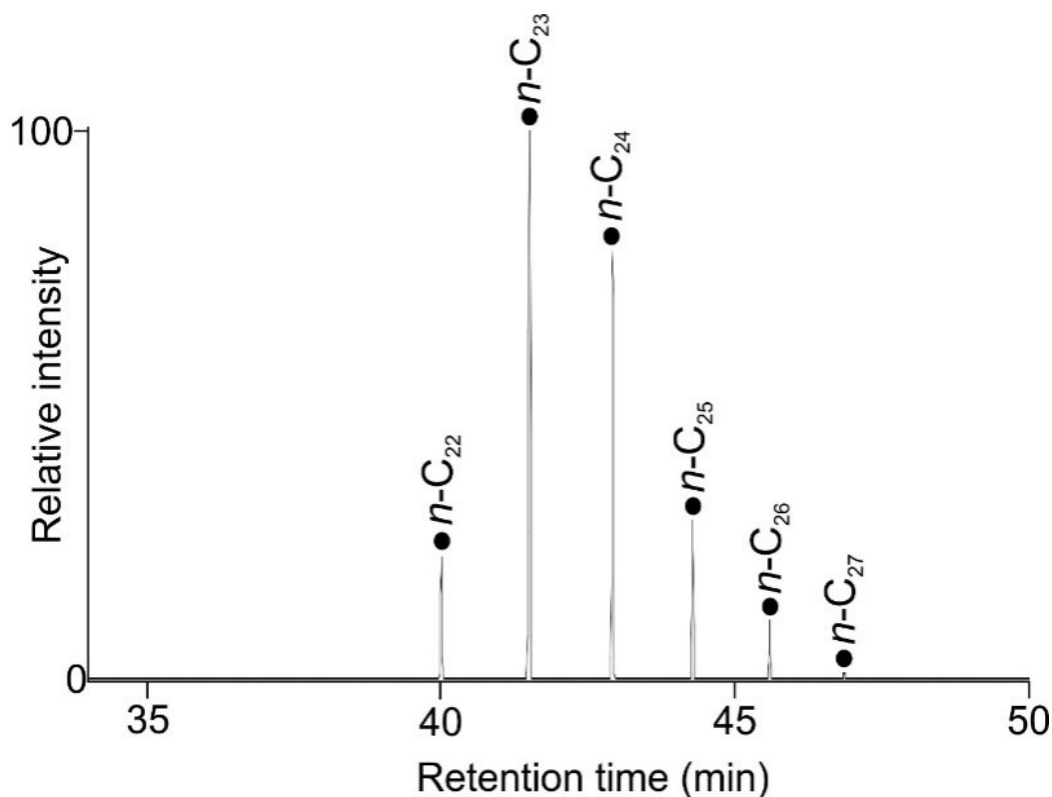
One sample of dental calculus from Cabeço da Arruda was analysed by sequential thermal desorption-gas chromatography-mass spectrometry (TD-GC-MS) and pyrolysis-gas chromatography-mass spectrometry (Py-GC-MS). Although not abundant, the sample contained both free and bound/polymeric organic material indicative of a fat/oil or wax. When taken together, the TD and Py chromatograms provide two conceivable interpretations of the chemical evidence presented in this sample. 1. It is possible that the biomolecules indicate a biodegraded higher plant wax deriving from the original higher plant consumed (e.g. leafy green vegetables). 2. Both the TD-GC-MS and Py-GC-MS chemical profiles are notably similar to biodegraded/archaeological beeswax¹¹⁹⁻¹²¹ and have interesting similarities with the closely chemically related bee product propolis¹²². The *n*-alkane distribution in beeswax is typically C₂₃ to C₃₅/C₂₅ to C₃₃, maximising at C₂₇¹¹⁹⁻¹²¹. In the dental calculus sample the C₂₅ to C₃₅, maximising at C₂₉, is notably similar, and in archaeological samples where beeswax has been positively identified C₂₉ has been observed as the major *n*-alkane as a result biodegradation¹¹⁹⁻¹²¹. The C₂₇, C₂₉ and C₃₁ *iso*-branched alkanes, maximising at C₂₉, also identified may be telling, since the C₂₉ and C₃₁ *iso*-branched alkanes, also maximising at C₂₉, have been observed as significant components in propolis wax¹²². Moreover, the ‘chemical fingerprint’ obtained from Py-GC-MS analysis is distinctive for beeswax given its particular chemistry, and although palmitic acid (possibly due to diagenetic changes) and the long-chain hydrocarbon moieties from C₂₄ to C₃₀ (even over odd predominance) were not observed (conceivably due to the small amount of organic material present; these are in relatively low abundance in archaeological samples compared to the shorter chain hydrocarbons^{119,120}), the chemical profile has notable similarities with pyrolysed fresh and archaeological beeswax^{119,120}. Taken together, the chemical evidence from both the TD-GC-MS and Py-GC-MS is consistent with a biodegraded beeswax or propolis wax, which would support previous evidence for its widespread use across Europe in prehistory¹²³⁻¹²⁶. However, corroborating archaeological/scientific evidence for beeswax or propolis would be needed in order to support this currently tentative, albeit potentially very interesting, inference.

66. Cabeço do Pez, Sado Valley, Portugal: b251-b female (calculus: 1.77 mg)

The thermal desorption total ion chromatogram (TIC) (Fig. 39) is dominated by a series of *n*-alkanes, carbon numbers C₂₂ to C₂₇, maximising at C₂₃. There is no odd over even carbon

number predominance suggesting the sample may have undergone biodegradation as a result of diagenetic changes. The relatively unusual mid-chain carbon numbers present in the TD chromatogram suggests a submerged aquatic plant (macrophyte)⁸⁹⁻⁹², presumably deriving from the original food consumed. No acyl lipid (including bacterially-derived), protein or carbohydrate markers were observed, and nor were any other compounds identified. It should also be note that highly polar material could have been present, which would not have successfully eluted from the column, or indeed volatilised sufficiently in the probe.

The pyrolysate TIC revealed carbon dioxide as the only detectable (trace) component, indicating little organic material in this sample, beyond that identified in the TD. It should be noted, however, that highly polar material could have been present, which would not have successfully eluted from the column, or indeed volatilised sufficiently in the probe. It is not possible to determine the significance of the carbon dioxide; theoretically it is indicative of oxidised organic material, although its presence as only a barely detectable trace component makes even this inference of no meaningful value.



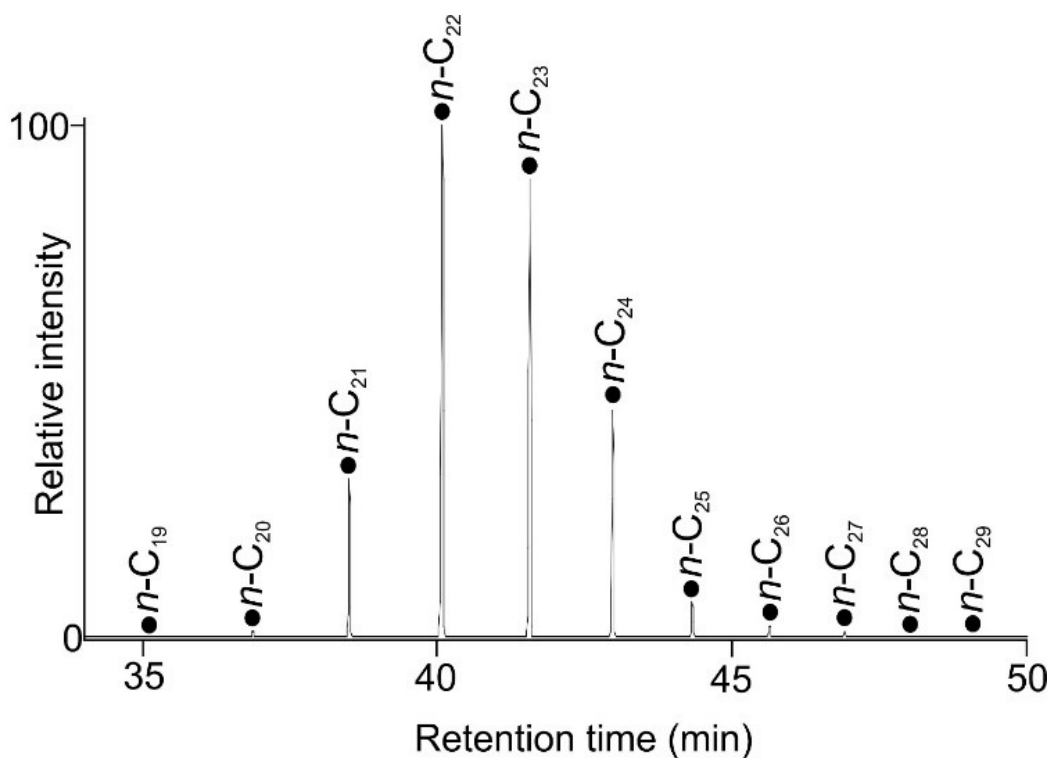
Supplementary Figure 39. Reconstructed total ion chromatogram of the thermal desorption profile (310 °C for 10 s) of sample b251-b female, Cabeço do Pez, Sado Valley, Portugal.

Key to Fig. 39: Peak identities (x indicates carbon chain length): filled circles, C_x indicates alkanes.

67. Cabeço do Pez, Sado Valley, Portugal: Esq27 Esquina male (calculus: 4.06 mg)

The thermal desorption total ion chromatogram (TIC) (Fig. 40) is dominated by a series of *n*-alkanes, carbon numbers C₁₉ to C₂₉, maximising at C₂₂. There is no odd over even carbon number predominance suggesting the sample may have undergone biodegradation as a result of diagenetic changes. The relatively unusual mid-chain carbon numbers present in the TD chromatogram suggests a submerged aquatic plant (macrophyte)⁸⁹⁻⁹², presumably deriving from the original food consumed. No acyl lipid (including bacterially-derived), protein or carbohydrate markers were observed, with the only other compound detected being carbon dioxide as a minor constituent. It is not possible to determine the significance of the carbon dioxide, beyond it being indicative of a small amount of oxidised organic material. It should also be note that highly polar material could have been present, which would not have successfully eluted from the column, or indeed volatilised sufficiently in the probe.

The pyrolysate TIC revealed carbon dioxide as the only detectable (trace) component, indicating little organic material in this sample, beyond that identified in the TD. It should be noted, however, that highly polar material could have been present, which would not have successfully eluted from the column, or indeed volatilised sufficiently in the probe. It is not possible to determine the significance of the carbon dioxide, beyond it being indicative of oxidised organic material, although its presence as only a trace component makes even this inference of very little value.



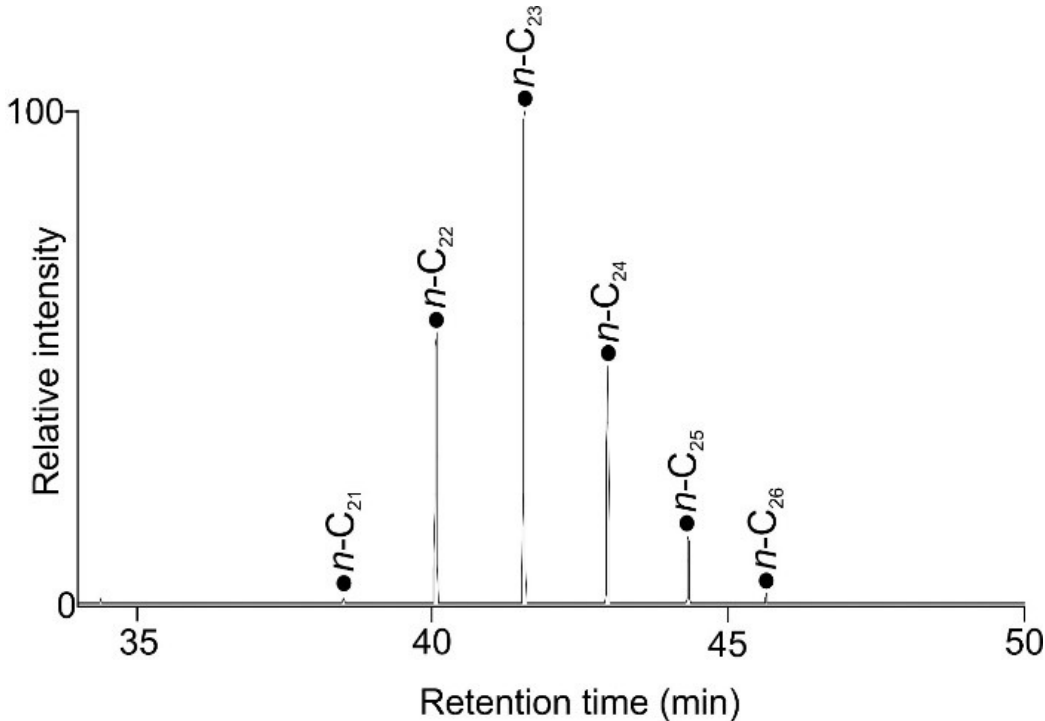
Supplementary Figure 40. Reconstructed total ion chromatogram of the thermal desorption profile (310 °C for 10 s) of sample Esq27 Esquina male, Cabeço do Pez, Sado Valley, Portugal. Key to Fig. 40: Peak identities (x indicates carbon chain length): filled circles, Cx indicates alkanes.

68. Cabeço do Pez, Sado Valley, Portugal: 6250-D Esquina A-B Jordi male (calculus: 2.65 mg)

The thermal desorption total ion chromatogram (TIC) (Fig. 41) is dominated by a series of *n*-alkanes, carbon numbers C₂₁ to C₂₆, maximising at C₂₃. There is no odd over even carbon number predominance suggesting the sample may have undergone biodegradation as a result of diagenetic changes. The relatively unusual mid-chain carbon numbers present in the TD chromatogram suggests a submerged aquatic plant (macrophyte)⁸⁹⁻⁹², presumably deriving from the original food consumed. No acyl lipid (including bacterially-derived), protein or carbohydrate markers were observed, with the only other compound detected being carbon dioxide. It is not possible to determine the significance of the carbon dioxide, beyond it being indicative of a small amount of oxidised organic material. It should also be note that highly

polar material could have been present, which would not have successfully eluted from the column, or indeed volatilised sufficiently in the probe.

The pyrolysate TIC revealed carbon dioxide as the only detectable (trace) component, indicating little organic material in this sample, beyond that identified in the TD. It should be noted, however, that highly polar material could have been present, which would not have successfully eluted from the column, or indeed volatilised sufficiently in the probe. It is not possible to determine the significance of the carbon dioxide, beyond it being indicative of oxidised organic material, although its presence as only a trace component makes even this inference of very little value.



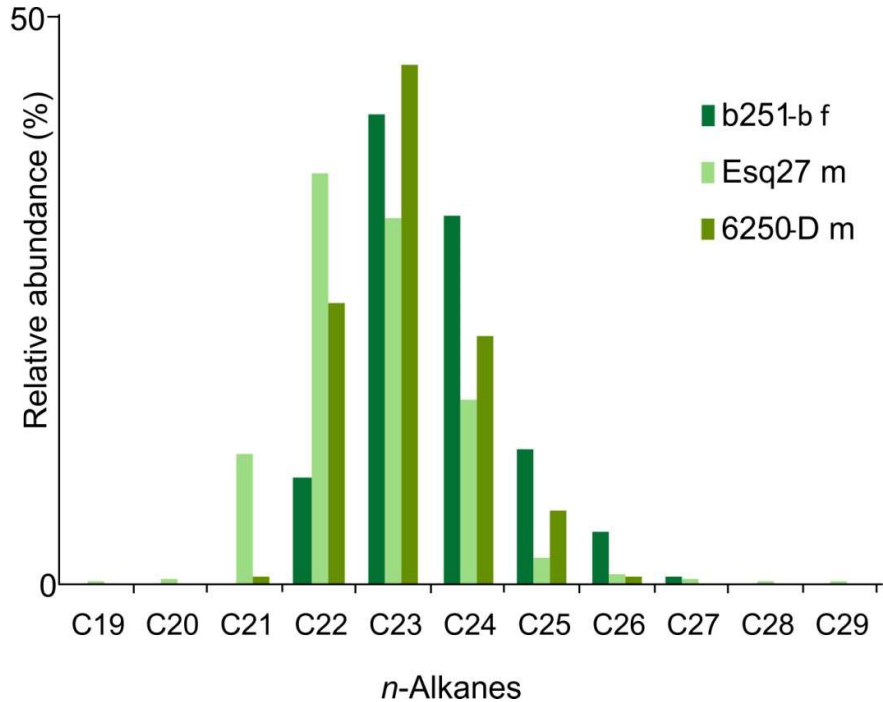
Supplementary Figure 41. Reconstructed total ion chromatogram of the thermal desorption profile (310 °C for 10 s) of sample 6250-D Esquina A-B Jordi male, Cabeço do Pez, Sado Valley, Portugal. Key to Fig. 41: Peak identities (x indicates carbon chain length): filled circles, Cx indicates alkanes.

Discussion. Cabeço do Pez, Sado

Three samples of dental calculus taken from three individuals from Cabeço do Pez, Sado were analysed by sequential thermal desorption-gas chromatography-mass spectrometry (TD-GC-MS) and pyrolysis-gas chromatography-mass spectrometry (Py-GC-MS). The Py-GC-MS results revealed trace quantities of carbon dioxide as the only compound detected in all three samples, indicating an essential absence of a preserved macromolecular organic component in these samples, and most likely reflecting relatively unfavourable prevailing environmental conditions for biomolecular preservation. Despite the virtual absence of organic material in the pyrolysate, however, the thermal desorption total ion chromatogram (TIC) of all three samples was dominated by a series of *n*-alkanes, with carbon numbers notably similar, ranging from C₁₉ up to C₂₉, maximising at C₂₂/C₂₃ (see Fig. 42). There is no odd over even carbon number predominance suggesting the samples may have undergone biodegradation as a result of diagenetic changes (although see below). The relatively unusual mid-chain carbon numbers present strongly suggests a submerged aquatic plant (macrophyte)⁸⁹⁻⁹², since these typically have a plant wax component ranging from C₁₉ to C₃₁/C₃₃ and maximising at C₂₃, presumably deriving from the original food consumed in this context. No acyl lipid (including bacterially-derived), protein or carbohydrate markers were observed, which is consistent with a significantly biodegraded organic component, as is the persistence of the *n*-alkanes which are known to be particularly resistant to diagenesis^{90,92}. In addition to the data above, an *n*-alkane proxy for the input/presence of submerged/floating freshwater aquatic macrophytes provides another corroborating measure of aquatic plant input³⁴. This proxy ratio, P_{aq}, gives values of 1.0 for all three samples, which also indicates a submerged/floating aquatic plant⁸⁹.

The average C₂₃ maximum, combined with the variable and relatively narrow *n*-alkane carbon number range (C₂₁ to C₂₆ >2%) in the calculus samples, with no branched alkanes detected, identifies biodegraded submerged aquatic plant (macrophyte) material. With carbon preference indexes (CPIs) of 1.17 and 1.12 for both samples with a C₂₃ maximum (suggesting less of a diagenetic impact in these samples), they are entirely consistent with what would be expected for a (biodegraded) submerged aquatic plant. Notably similar values have been observed for submerged aquatic plant rhizomes from southern Europe: Greece, CPI = 1.13; France, CPI = 1.15¹⁰¹. Furthermore, the rhizomes in these studies had a far less pronounced odd-over-even carbon preference than leaves from the same plants, which is likely to reflect their microenvironment in the marine sediments where they are far more exposed to biomolecular reworking and biodegradation from microbial inputs than leaves from the same

plant. In this regard it is notable that the *n*-alkanes in the dental calculus samples also display this unimodal ‘hump’; this could be indicative of the consumption of rhizomes from a submerged aquatic plant by these individuals, although it may also be explained by post-depositional diagenetic factors.



Supplementary Figure 42. *n*-Alkane distribution of three calculus samples from Cabeço do Pez, Sado: b251-b female; Esq27 Esquina male; 6250-D Esquina A-B Jordi male. Source data are provided as a Source Data file.

Although the identification of plant remains is challenging, exploitation of aquatic plants as food in the Iberian Peninsula has been suggested in the Mesolithic period, where aquatic plant storage tissue has been identified¹²⁷; Alismataceae (water plantains), Butomaceae (flowering rushes), Typhaceae (reedmaces) and Sparganiaceae (bur-reeds) were considered possible sources. The exploitation of the roots and tubers of aquatic plants elsewhere in Mesolithic Europe has also been hypothesised, with plant remains of eelgrass, horned pondweed and beaked tassel weed identified at Argus Bank in Denmark⁹³ and a number of aquatic plants, including the submerged/floating species yellow water lily (*Nuphar lutea*), white water lily (*Nymphaea alba*) and pondweed (*Potamogeton pectinatus*), at Star Carr in

England⁹⁴. Interestingly, the chemical profile of C₁₉ to C₂₉ *n*-alkanes, maximising at C₂₃ is consistent with a submerged aquatic plant (e.g. *Potamogeton* spp. (pondweed)^{89,91} at Cabeço do Pez, rather than an emergent macrophyte such as those in an Iberian context cited above. Further work would be needed to establish the macrophytes, such as *Potamogeton* spp., likely to have been available at Cabeço do Pez in order to determine possible sources.

The stable isotope data from Cabeço do Pez have been interpreted as indicating a largely terrestrial diet (animals and vegetables), with some marine protein⁷⁹. Notably here, the isotope data did not reveal evidence for freshwater protein⁷⁹ and from this it was inferred that there was no contribution of freshwater resources⁷⁹. However, aquatic plants have previously been shown to exhibit significant variations in their stable isotopic composition ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$), particularly submerged species such as *Potamogeton* spp.¹²⁸. With typical $\delta^{13}\text{C}$ values of ~ -23 to -31‰ and $\delta^{15}\text{N}$ values of ~ -1 to $+7\text{‰}$, these can be equivalent to a C₃ terrestrial fauna input¹²⁹ and when combined with a small (~ 20 - 30%) marine protein component in the diet, as reasonably inferred⁷⁹, can produce values consistent with those observed at Cabeço do Pez, including the individual No.27 ($\delta^{13}\text{C} = -19.5$ and $\delta^{15}\text{N} = 10.0$) who was also part of the study presented here. This perhaps illustrates the value of a multiple approach to dietary reconstruction, and the dangers of an overreliance on stable isotopes alone (whether bulk or compound specific data). Significantly, perhaps, the stable isotope study at Cabeço do Pez showed no significant differences between males and females⁷⁹, which is consistent (regardless of the exact composition of the diet) with the similarity of the biomolecular profiles obtained from the calculus samples from the female and two males, i.e. the dominance of the *n*-alkanes from the presumed epicuticular wax component of the same submerged aquatic plant.

[Due to the common use of paraffin wax in archaeological excavations in the 19th and 20th centuries during the periods excavations at Cabeço do Pez took place, combined with the known use during the excavations at archaeological sites in the Sado Valley^{79,128}, it is sensible to take this into account when interpreting the biomolecular data. In this context, it should be said that paraffin wax can have a similar chemical profile, but with a CPI of ≤ 1.0 and typically C₂₀ to C₃₃ *n*-alkanes, maximising at C₂₄-C₂₆¹³⁰, with slightly more volatile fractions typically C₁₉/C₂₀ to C₃₂/C₃₃, maximising at C₂₃^{130,131}, they are significantly different in molecular composition to those observed in the calculus samples. Most notably, paraffin

waxes have a wider carbon number range than those seen in the calculus samples, with a more even distribution, and small but identifiable quantities of branched alkanes. The *n*-alkanes are highly resilient and would not be expected to degrade to the point of their absence in a matter of decades, as might be the case post-excavation and subsequent museum storage if employed in excavations. Indeed, in practice, even when exposed to heat and high light levels, paraffin wax employed in early 20th century archaeological excavations, notably less environmentally favourable than can be expected in the Sado Valley, is chemically unchanged after almost a century¹¹⁹. The average C₂₃ maximum, combined with the variable and relatively narrow *n*-alkane carbon number range in the calculus samples (C₂₁ to C₂₆ >2% *cf.* C₂₀ to C₂₉ >2% for paraffin wax), with no branched alkanes detected, identifies biodegraded submerged aquatic plant (macrophyte) material. In addition, it should be noted that the 'P_{aq} values' for paraffin waxes of ≤0.91 are significantly different from those for the three calculus samples, which also excludes it as a possible source here. It is also important to note that carbon stable isotopic analysis was carried out successfully on material from Cabeço do Pez⁷⁹, specifically on one of the samples presented here (No.27), presumably because those burials with paraffin wax applied during the excavations were avoided, which would, necessarily, also apply to the calculus samples, thereby avoiding any possible contamination.]

Netherlands

Schipluiden and Hardinxveld, Netherlands

Schipluiden is located some 7 km beyond the present coastline – in an extensive beach plain in between the oldest beach barrier (Voorburg-Rijswijk in Zuid Holland) and the back marshes. The settlement of Schipluiden lies on the top of a low dune. The absolute chronology of the settlement was established by means of stratified radiocarbon dates (obtained for samples of various materials) – giving a time span of 3650 until 3380 cal. years BC and as such associated with the Middle Neolithic. Many thousands of features including: wells, hearths, postholes, graves and fences reflect the long-term, intensive use of the entire dune. Wells or watering places were the most numerous. Diversity in the landscape was demonstrated throughout the occupation phases. The high salt marsh and possibly low dunes in the surroundings offered possibilities for small-scale agriculture. Emmer and naked barley

were the two cultivated crops. Besides these cereals, the diet comprised a broad spectrum of gathered wild plants, including fruits and berries, and roots and tubers. Two species were collected to a greater extent, namely sloe plums and crab apples¹³².

Hardinxveld-Giessendam De Bruin is one of many river dune sites located in the Lower Rhine-Meuse river delta in the western Netherlands. This site and the neighbouring Hardinxveld-Giessendam Polderweg were inhabited during the Late Mesolithic (5th millennium BC), but the use of De Bruin, in contrast to Polderweg, continued into the Early Neolithic (Early Swifterbant). Three phases of occupation were distinguished at the De Bruin site. Phase 1, the oldest settlement, dated to c. 5500 to 5300 cal BC, Phase 2, witnessing the introduction of pottery, dated to c. 5100 to 4800 cal BC, and Phase 3, when the first domestic animals appeared at the site, dated to c. 4700 to 4450 cal BC^{133,134}.

The river dunes of De Bruin and Polderweg were situated in an area with slowly flowing rivers with low banks and extensive marsh vegetation dominated by reed and sedges. A variety of nuts, fruits, and berries were collected and processed for food, as indicated by their charred remains from Hardinxveld-Giessendam Polderweg and Hardinxveld-Giessendam De Bruin, including hazelnut, water chestnut, acorn, wild apple, hawthorn, dogwood, and possibly guelder rose. Numerous (uncharred) seeds of yellow water-lily (*Nuphar lutea*) and white water-lily (*Nymphaea alba*) indicate the presence of both species in the waters near the sites, and suggest that seeds of both species might have been processed for food. Starchy root tubers of lesser celandine (*Ranunculus ficaria*, syn. *Ficaria verna*) were also likely used as food, as suggested by the charred remains from Hardinxveld-Giessendam Polderweg. The human skeletal remains are dated between the end of Phase 1 and beginning of Phase 2^{133,134}.

Ten samples of dental calculus taken from seven individuals from two sites in the Netherlands were analysed by sequential thermal desorption-gas chromatography-mass spectrometry (TD-GC-MS) and pyrolysis-gas chromatography-mass spectrometry (Py-GC-MS). Four samples of soil/burial sediment from several of the graves were also analysed. This technique facilitates the identification of both free/unbound and bound/polymeric organic components. All ten calculus samples and four soil/sediment samples produced greater amounts of a bound/polymeric organic material compared to the free organic material present, although both fractions were highly oxidised in nature.

69. Schipluiden Grave 1 Individual 1 TMS0081: maxilla labial molar (calculus: 15.19 mg)

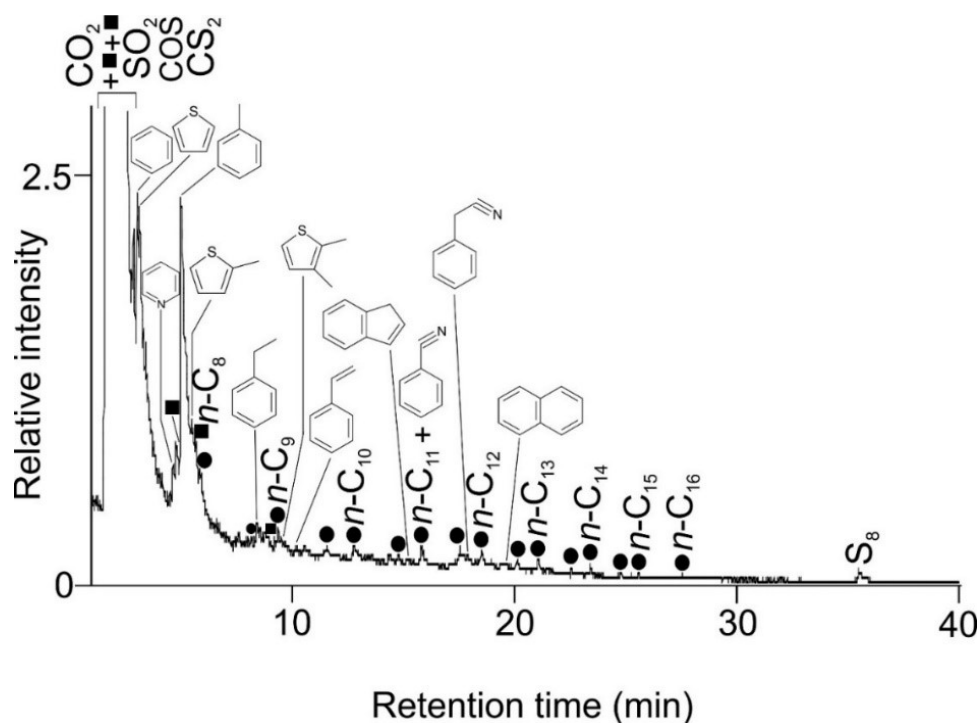
The thermal desorption total ion chromatogram (TIC) revealed carbon dioxide, sulphur dioxide and carbon disulphide as the only detectable, albeit relatively abundant, components. No other free, thermally extractable, organic components could be detected in this sample. It should be noted, however, that highly polar material could have been present, which would not have successfully eluted from the column, or indeed volatilised sufficiently in the probe. It is not possible to determine the significance of the carbon dioxide, beyond it being indicative of oxidised organic material. The sulphur dioxide and carbon disulphide are also difficult to interpret, although these species can be indicative of oxidised and pyrolysed sulphur-containing amino acids or sulphones^{135,136} (see discussion). Sulphur dioxide is also known to be a major pyrolysis product of lignosulphonates¹³⁷, and its organic nature would facilitate sulphur dioxide generation at this relatively low temperature, although this possibility in this context is probably less likely (again, see discussion).

The pyrolysis-GC-MS TIC (Fig. 43) was dominated by carbon dioxide, 1-butene and 1-pentene, the alkenes being indicative of lipid or proteinaceous material in the sample¹¹. As observed in the TD profile, sulphur dioxide and carbon disulphide were also major components. However, these were also accompanied by carbonyl sulphide and the aromatic sulphur compound thiophene and its methyl and dimethyl derivatives. The presence of thiophene with the sulphur dioxide, carbonyl sulphide and carbon disulphide can be indicative of pyrolysed sulphur-containing amino acids^{135,136,138} (see discussion). The presence of acetic acid as a major component is also consistent with sulphur-containing amino acids¹³⁵. Notably, sulphur as its cyclic octaatomic S₈ form was also detected. More specifically, thiophene and its C₁ and C₂ alkyl derivatives are pyrolysis products of cysteine and cystine^{135,136,138}, and elemental sulphur as S₈ has also been posited as a pyrolysis product of the sulphur-rich cystine¹³⁹.

In contrast to the thermal desorption profile, the pyrogram also revealed a complex suite of organic compounds, albeit in relatively minor abundance. These include a series of C₁₀ to C₁₆ *n*-alkanes, typical of saturated fat/oil-derived acyl lipids^{5,6}, although the absence of the *n*-1-alkene counterparts may well reflect diagenetic changes, rather be indicative of a dominance of saturated acyl lipids in the original fat/oil. Indeed, the presence of C₅ to C₉ *n*-1-alkenes in relatively significant abundance compared to the longer carbon chain *n*-alkane moieties is indicative of an appreciable unsaturated acyl lipid component. The absence of these

compounds in the TD profile provides molecular evidence for a polymeric/bound acyl lipid source. However, in addition to *n*-1-alkenes and *n*-alkanes, typical of fat/oil derived lipids, the pyrogram also displayed a series of C₉ to C₁₅ 2-methylalkanes (isoalkanes), presumably deriving from the branched fatty acids which are significant components of bacterial triglycerides (again, the absence of their iso-methyl-1-alkenes (isoalkenes) counterparts may well reflect diagenetic changes, rather be indicative of a dominance of saturated branched acyl lipids in the original lipid source). Although non-specific, the presence of carbon dioxide is also consistent with a (highly oxidised) lipid component in this sample.

The pyrolysate TIC identified components indicative of 'black carbon', i.e. charcoal or soot⁷⁻¹⁰. Of these, benzene and toluene were major compounds present in the 'char' pyrolysate (~30% and 60% of total quantified pyrogram peak area respectively), along with moderate quantities of ethyl benzene and o-, m- and p-xylenes (~8% total). The relative dominance of toluene, rather than the normally dominant aromatic hydrocarbon benzene, is somewhat atypical of chars⁷⁻¹⁰. However, the presence of naphthalene, combined with their relative abundances, is indicative of a 'char' component providing molecular evidence for likely exposure to fire/cooking. The markers identified here can also be indicative of proteins⁶⁻¹¹ and combined with the related protein markers pyridine, benzonitrile and benzyl nitrile (phenylacetone nitrile) identified this is indicative of these compounds, at least in part, originating from a protein-derived component⁶⁻¹¹. It should also be noted that highly polar material could have been present, which would not have successfully eluted from the column, or indeed volatilised sufficiently in the probe.



Supplementary Figure 43. Reconstructed total ion chromatogram of the pyrogram (pyrolysis profile) (610 °C for 10s), after thermal desorption (310 °C for 10 s) of sample TMS0081 Individual 1 Schipluiden Grave 1. Key to Fig. 43: Peak identities (x indicates carbon chain length): filled squares, Cx indicates alkenes; filled circles, Cx indicates alkanes. Also shown are the structures of thirteen aromatic compounds identified: benzene, thiophene, pyridine, toluene, methylthiophene, ethylbenzene, dimethylthiophene, styrene, indene, benzonitrile, benzyl nitrile and naphthalene. CO₂ indicates carbon dioxide, SO₂ indicates sulphur dioxide, COS indicates carbonyl sulphide, CS₂ indicates carbon disulphide and S₈ indicates cyclic octaatomic sulphur.

70. Schipluiden Grave 1 Individual 2 TMS0077: mandible labial right PM1 (calculus: 4.22 mg)

The thermal desorption total ion chromatogram (TIC) revealed carbon dioxide, 1-butene, sulphur dioxide and carbon disulphide as the only detectable components. No other free, thermally extractable, organic components could be detected in this sample. It should be noted, however, that highly polar material could have been present, which would not have successfully eluted from the column, or indeed volatilised sufficiently in the probe. It is not possible to determine the significance of the carbon dioxide, beyond it being indicative of

oxidised organic material. The dominance of 1-butene can be indicative of lipid or proteinaceous material in the sample¹¹, and perhaps produced at this relatively low temperature due to matrix effects. The sulphur dioxide is difficult to interpret, although it can be indicative of oxidised and pyrolysed sulphur-containing amino acids or sulphones^{135,136} (see discussion). Sulphur dioxide is also known to be a major pyrolysis product of lignosulphonates¹³⁷, and its organic nature would facilitate sulphur dioxide generation at this relatively low temperature, although this possibility in this context is probably less likely (again, see discussion).

The pyrolysis-GC-MS TIC was dominated by carbon dioxide, 1-butene and 1-pentene, the alkenes being indicative of lipid or proteinaceous material in the sample¹¹. As observed in the TD profile, sulphur dioxide was a significant component. However, like the pyrogram of the previous sample, it was accompanied by carbonyl sulphide (trace) and carbon disulphide, which collectively can be indicative of sulphur-containing amino acids or sulphones^{135,136} (see discussion). Thiophene was also detected, albeit only as a trace component; this can again can be indicative of pyrolysed sulphur-containing amino acids^{135,136,138} although the relative abundance here makes any interpretation very tentative. The presence of acetic acid as a major component in this sample is also consistent with sulphur-containing amino acids¹³⁵. The pyrolysate TIC identified benzene and toluene as the only other components detected although there very minor abundance and an absence of other potentially more diagnostic biomarkers mean their significance is unclear. It should also be noted that highly polar material could have been present, which would not have successfully eluted from the column, or indeed volatilised sufficiently in the probe.

71. Schipluiden Grave 1 Individual 2 TMS0077: maxilla/mandible? lingual left incisor 1-2 (calculus: 5.89 mg)

The thermal desorption total ion chromatogram (TIC) revealed carbon dioxide, 1-butene (trace) and sulphur dioxide as the only detectable components. No other free, thermally extractable, organic components could be detected in this sample. It should be noted, however, that highly polar material could have been present, which would not have successfully eluted from the column, or indeed volatilised sufficiently in the probe. It is not possible to determine the significance of the carbon dioxide, beyond it being indicative of oxidised organic material. The 1-butene can be indicative of lipid or proteinaceous material in

the sample¹¹, and perhaps produced at this relatively low temperature due to matrix effects. The sulphur dioxide is difficult to interpret, although it can be indicative of oxidised and pyrolysed sulphur-containing amino acids or sulphones^{135,136} (see discussion). Sulphur dioxide is also known to be a major pyrolysis product of lignosulphonates¹³⁷, and its organic nature would facilitate sulphur dioxide generation at this relatively low temperature, although this possibility in this context is probably less likely (again, see discussion).

The pyrolysis-GC-MS TIC was dominated by carbon dioxide, 1-butene and 1-pentene, the alkenes being indicative of lipid or proteinaceous material in the sample¹¹. As observed in the TD profile, sulphur dioxide was a significant component. However, like the pyrogram of the two previous samples, it was accompanied by carbonyl sulphide and carbon disulphide, which collectively can be indicative of sulphur-containing amino acids or sulphones^{135,136} (see discussion). Thiophene was also detected, albeit only as a very minor component; this can again can be indicative of pyrolysed sulphur-containing amino acids^{135,136,138} although the relative abundance here makes any interpretation somewhat tentative. The pyrolysate TIC identified benzene and toluene as the only other components detected although their very minor abundance and an absence of other potentially more diagnostic biomarkers mean their significance is unclear. It should also be noted that highly polar material could have been present, which would not have successfully eluted from the column, or indeed volatilised sufficiently in the probe.

72. Schipluiden Grave 1 Individual 2 TMS0077: maxilla/mandible? lingual right M2-3 (calculus: 19.28 mg)

The thermal desorption total ion chromatogram (TIC) revealed carbon dioxide, 1-butene, acetone, sulphur dioxide, carbonyl sulphide and carbon disulphide as the only detectable components. No other free, thermally extractable, organic components could be detected in this sample. It should be noted, however, that highly polar material could have been present, which would not have successfully eluted from the column, or indeed volatilised sufficiently in the probe. It is not possible to determine the significance of the carbon dioxide, beyond it being indicative of oxidised organic material. The 1-butene can be indicative of lipid or proteinaceous material in the sample¹¹, and perhaps produced at this relatively low temperature due to matrix effects. Collectively, the sulphur dioxide, carbonyl sulphide and carbon disulphide can be indicative of oxidised and pyrolysed sulphur-containing amino

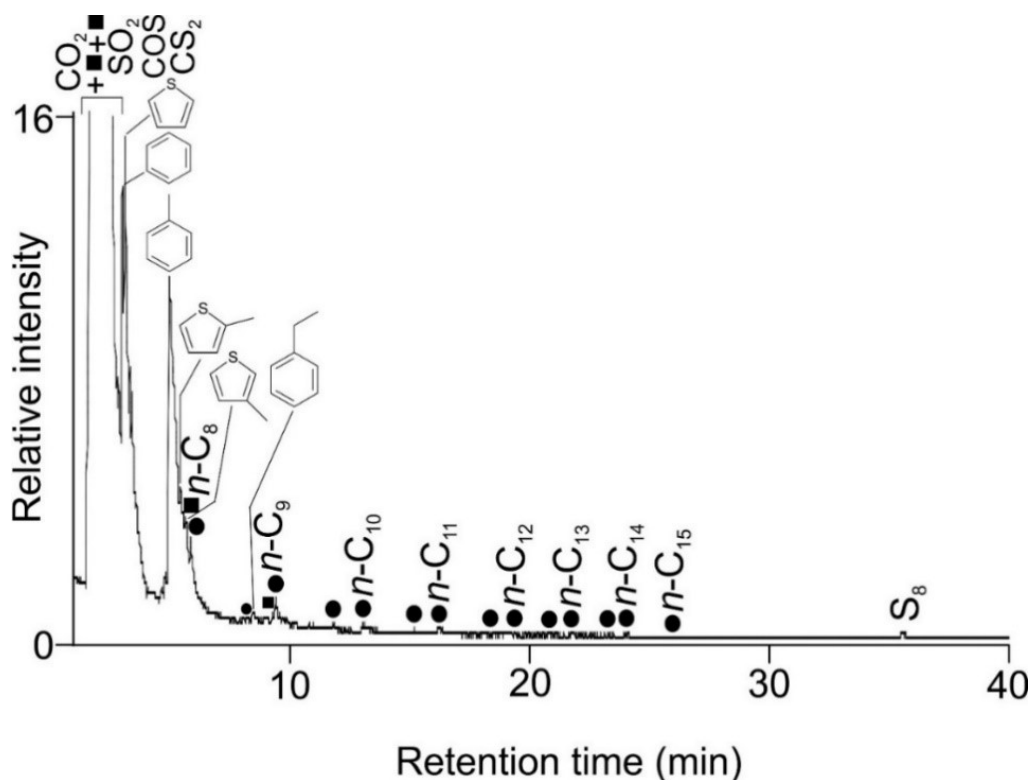
4479 acids or sulphones^{135,136} (see discussion). Sulphur dioxide is also known to be a major
4480 pyrolysis product of lignosulphonates¹³⁷, and its organic nature would facilitate sulphur
4481 dioxide generation at this relatively low temperature, although this possibility in this context
4482 is probably less likely (again, see discussion).

4483 The pyrolysis-GC-MS TIC (Fig. 44) was dominated by carbon dioxide, 1-butene and 1-
4484 pentene, the alkenes being indicative of lipid or proteinaceous material in the sample¹¹. As
4485 observed in the TD profile, sulphur dioxide, carbonyl sulphide and carbon disulphide were
4486 also significant components. However, these were also accompanied by the aromatic sulphur
4487 compound thiophene and two methyl derivatives. The presence of thiophene with the sulphur
4488 dioxide, carbonyl sulphide and carbon disulphide can be indicative of pyrolysed sulphur-
4489 containing amino acids^{135,136,138} (see discussion). The presence of acetic acid as a major
4490 component is also consistent with sulphur-containing amino acids¹³⁵. Notably, sulphur as its
4491 cyclic octaatomic S₈ form was also detected. More specifically, thiophene and its C₁ and C₂
4492 alkyl derivatives are pyrolysis products of cysteine and cystine^{135,136,138}, and elemental
4493 sulphur as S₈ has also been posited as a pyrolysis product of the sulphur-rich cystine¹³⁹.

4494 In contrast to the thermal desorption profile, the pyrogram also revealed a complex suite of
4495 organic compounds, albeit in minor abundance. These include a series of C₁₀ to C₁₅ *n*-
4496 alkanes, typical of saturated fat/oil-derived acyl lipids^{5,6}, although the absence of the *n*-1-
4497 alkene counterparts may well reflect diagenetic changes, rather be indicative of a dominance
4498 of saturated acyl lipids in the original fat/oil. Indeed, the presence of C₅ to C₉ *n*-1-alkenes in
4499 relatively significant abundance compared to the longer carbon chain *n*-alkane moieties is
4500 indicative of an appreciable unsaturated acyl lipid component. The absence of these
4501 compounds in the TD profile provides molecular evidence for a polymeric/bound acyl lipid
4502 source. However, in addition to *n*-1-alkenes and *n*-alkanes, typical of fat/oil derived lipids,
4503 the pyrogram also displayed a series of C₉ to C₁₄ 2-methylalkanes (isoalkanes), presumably
4504 deriving from the branched fatty acids which are significant components of bacterial
4505 triglycerides (again, the absence of their iso-methyl-1-alkenes (isoalkenes) counterparts may
4506 well reflect diagenetic changes, rather be indicative of a dominance of saturated branched
4507 acyl lipids in the original lipid source). Although non-specific, the presence of carbon dioxide
4508 is also consistent with a (highly oxidised) lipid component in this sample.

4509 The pyrolysate TIC identified components indicative of 'black carbon', i.e. charcoal or soot⁷⁻
4510 ¹⁰. Of these, benzene and toluene were major compounds present in the 'char' pyrolysate

(~30% and 65% of total quantified pyrogram peak area respectively), along with moderate quantities of ethyl benzene and o-, m- and p-xylenes (~4% total). The relative dominance of toluene, rather than the normally dominant aromatic hydrocarbon benzene, is somewhat atypical of chars⁷⁻¹⁰. However, their relative abundances are very similar to those observed above and so are at least tentatively indicative of a ‘char’ component providing molecular evidence for exposure to fire/cooking. It should also be noted that highly polar material could have been present, which would not have successfully eluted from the column, or indeed volatilised sufficiently in the probe.



Supplementary Figure 44. Reconstructed total ion chromatogram of the pyrogram (pyrolysis profile) (610 °C for 10s), after thermal desorption (310 °C for 10 s) of sample TMS0077 (M2-3) Individual 2 Schipluiden Grave. Key to Fig. 44: Peak identities (x indicates carbon chain length): filled squares, Cx indicates alkenes; filled circles, Cx indicates alkanes. Also shown are the structures of six aromatic compounds identified: benzene, thiophene, toluene, methylthiophene (x2) and ethylbenzene. CO₂ indicates carbon dioxide, SO₂ indicates sulphur dioxide, COS indicates carbonyl sulphide, CS₂ indicates carbon disulphide and S₈ indicates cyclic octaatomic sulphur.

Schipluiden soil ‘control’ from Grave 1, Individuals 1 and 2 (soil/sediment: 5.28 mg)

The thermal desorption total ion chromatogram (TIC) revealed carbon dioxide, sulphur dioxide, carbonyl sulphide and carbon disulphide as the only detectable components. No other free, thermally extractable, organic components could be detected in this sample. It should be noted, however, that highly polar material could have been present, which would not have successfully eluted from the column, or indeed volatilised sufficiently in the probe. It is not possible to determine the significance of the carbon dioxide, beyond it being indicative of oxidised organic material. The sulphur dioxide, carbonyl sulphide and carbon disulphide can be indicative of oxidised and pyrolysed sulphur-containing amino acids or sulphones^{135,136} (see discussion). Sulphur dioxide is also known to be a major pyrolysis product of lignosulphonates¹³⁷, and its organic nature would facilitate sulphur dioxide generation at this relatively low temperature, although this possibility in this context is probably less likely (again, see discussion). In this context, these sulphur-containing moieties derive from the burial soil/sediment (see discussion).

The pyrolysis-GC-MS TIC was dominated by carbon dioxide, with sulphur dioxide, carbonyl sulphide and carbon disulphide the only other significant components. Collectively these latter three compounds can be indicative of sulphur-containing amino acids or sulphones^{135,136} (see discussion), which suggests they derive from the burial soil/sediment (see discussion). The pyrolysate TIC identified benzene as the only other component detected although its trace abundance means its significance is unclear. It should also be noted that highly polar material could have been present, which would not have successfully eluted from the column, or indeed volatilised sufficiently in the probe.

73. Schipluiden Grave 3 TMS0058: lower jaw-mandible labial molar (calculus: 13.58 mg)

The thermal desorption total ion chromatogram (TIC) revealed carbon dioxide, sulphur dioxide, carbonyl sulphide and carbon disulphide as the only detectable, albeit relatively abundant, components. No other free, thermally extractable, organic components could be detected in this sample. It should be noted, however, that highly polar material could have been present, which would not have successfully eluted from the column, or indeed volatilised sufficiently in the probe. It is not possible to determine the significance of the carbon dioxide, beyond it being indicative of oxidised organic material. Collectively, the

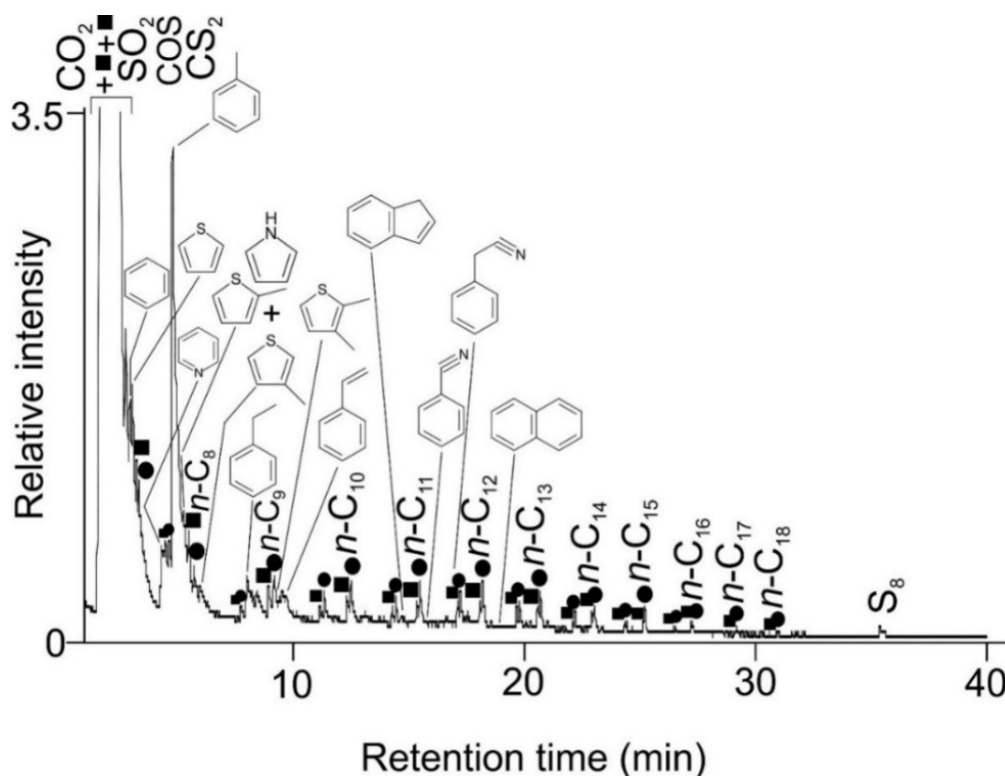
4561 sulphur dioxide, carbonyl sulphide and carbon disulphide can be indicative of oxidised and
4562 pyrolysed sulphur-containing amino acids or sulphones^{135,136} (see discussion). Sulphur
4563 dioxide is also known to be a major pyrolysis product of lignosulphonates¹³⁷, and its organic
4564 nature would facilitate sulphur dioxide generation at this relatively low temperature, although
4565 this possibility in this context is probably less likely (again, see discussion).

4566 The pyrolysis-GC-MS TIC (Fig. 45) was dominated by carbon dioxide, 1-butene and 1-
4567 pentene, the alkenes being indicative of lipid or proteinaceous material in the sample¹¹. As
4568 observed in the TD profile, sulphur dioxide, carbonyl and carbon disulphide were also
4569 significant components. However, these were also accompanied by the aromatic sulphur
4570 compound thiophene and its methyl and dimethyl derivatives. The presence of thiophene with
4571 the sulphur dioxide, carbonyl sulphide and carbon disulphide can be indicative of pyrolysed
4572 sulphur-containing amino acids^{135,136,138} (see discussion). The presence of acetic acid as a
4573 significant component is also consistent with sulphur-containing amino acids¹³⁵. Notably,
4574 sulphur as its cyclic octaatomic S₈ form was also detected. More specifically, thiophene and
4575 its C₁ and C₂ alkyl derivatives are pyrolysis products of cysteine and cystine^{135,136,138}, and
4576 elemental sulphur as S₈ has also been posited as a pyrolysis product of the sulphur-rich
4577 cystine¹³⁹.

4578 In contrast to the thermal desorption profile, the pyrogram also revealed a complex suite of
4579 organic compounds, albeit in relatively minor abundance. These include a series of C₇ to C₁₈
4580 *n*-1-alkenes and *n*-alkanes, typical of unsaturated and saturated fat/oil-derived acyl lipids^{5,6}.
4581 The absence of these compounds in the TD profile provides molecular evidence for a
4582 polymeric/bound acyl lipid source. However, in addition to *n*-1-alkenes and *n*-alkanes, typical
4583 of fat/oil derived lipids, the pyrogram also displayed a series of C₈ to C₁₆ iso-methyl-1-
4584 alkenes (isoalkenes) and 2-methylalkanes (isoalkanes), presumably deriving from the
4585 branched fatty acids which are significant components of bacterial triglycerides. Although
4586 non-specific, the presence of carbon dioxide is also consistent with a (highly oxidised) lipid
4587 component in this sample.

4588 The pyrolysate TIC identified components indicative of 'black carbon', i.e. charcoal or soot⁷⁻
4589 ¹⁰. Of these, benzene and toluene were major compounds present in the 'char' pyrolysate
4590 (~25% and 65% of total quantified pyrogram peak area respectively), along with moderate
4591 quantities of ethyl benzene and o-, m- and p-xylenes (~10% total). The relative dominance of
4592 toluene, rather than the normally dominant aromatic hydrocarbon benzene, is somewhat

atypical of chars⁷⁻¹⁰. However, the presence of naphthalene, combining with their relative abundances, is indicative of a ‘char’ component providing molecular evidence for exposure to fire/cooking. The markers identified here can also be indicative of proteins⁶⁻¹¹ and combined with the related protein markers pyridine, benzonitrile and benzyl nitrile (phenylacetone nitrile) identified this is indicative of these compounds, at least in part, originating from a protein-derived component⁶⁻¹¹. It should also be noted that highly polar material could have been present, which would not have successfully eluted from the column, or indeed volatilised sufficiently in the probe.



Supplementary Figure 45. Reconstructed total ion chromatogram of the pyrogram (pyrolysis profile) (610 °C for 10s), after thermal desorption (310 °C for 10 s) for sample TMS0058 (lower labial molar) Schipluiden Grave 3. Key to Fig. 45: Peak identities (x indicates carbon chain length): filled squares, Cx indicates alkenes; filled circles, Cx indicates alkanes. Also shown are the structures of fourteen aromatic compounds identified: benzene, thiophene, pyridine, toluene, methylthiophene (x2), pyrrole, ethylbenzene, dimethylthiophene, styrene, indene, benzonitrile, benzyl nitrile and naphthalene. CO₂ indicates carbon dioxide, SO₂ indicates sulphur dioxide, COS indicates carbonyl sulphide, CS₂ indicates carbon disulphide and S₈ indicates cyclic octaatomic sulphur.

4611

4612 **74. Schipluiden Grave 3 TMS0058: upper jaw-maxilla labial molar (v. worn)**
4613 **(calculus: 14.64 mg)**

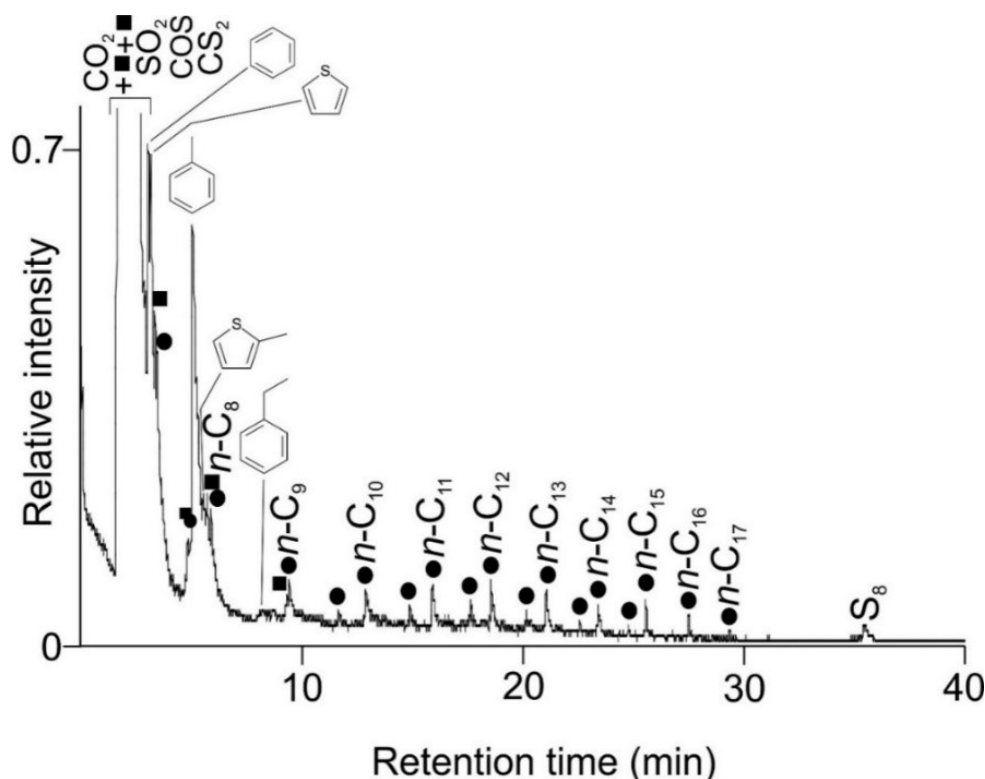
4614 The thermal desorption total ion chromatogram (TIC) revealed carbon dioxide, sulphur
4615 dioxide, carbonyl sulphide and carbon disulphide as the only detectable components. No
4616 other free, thermally extractable, organic components could be detected in this sample. It
4617 should be noted, however, that highly polar material could have been present, which would
4618 not have successfully eluted from the column, or indeed volatilised sufficiently in the probe.
4619 It is not possible to determine the significance of the carbon dioxide, beyond it being
4620 indicative of oxidised organic material. Collectively, the sulphur dioxide, carbonyl sulphide
4621 and carbon disulphide can be indicative of oxidised and pyrolysed sulphur-containing amino
4622 acids or sulphones^{135,136} (see discussion). Sulphur dioxide is also known to be a major
4623 pyrolysis product of lignosulphonates¹³⁷, and its organic nature would facilitate sulphur
4624 dioxide generation at this relatively low temperature, although this possibility in this context
4625 is probably less likely (again, see discussion).

4626 The pyrolysis-GC-MS TIC (Fig. 46) was dominated by carbon dioxide, 1-butene and 1-
4627 pentene, the alkenes being indicative of lipid or proteinaceous material in the sample¹¹. As
4628 observed in the TD profile, sulphur dioxide, carbonyl sulphide and carbon disulphide were
4629 also significant components. However, these were also accompanied by the aromatic sulphur
4630 compound thiophene and a methyl derivative. The presence of thiophene with the sulphur
4631 dioxide, carbonyl sulphide and carbon disulphide can be indicative of pyrolysed sulphur-
4632 containing amino acids^{135,136,138} (see discussion). The presence of acetic acid as a major
4633 component is also consistent with sulphur-containing amino acids¹³⁵. Notably, sulphur as its
4634 cyclic octaatomic S₈ form was also detected. More specifically, thiophene and its C₁ and C₂
4635 alkyl derivatives are pyrolysis products of cysteine and cystine^{135,136,138}, and elemental
4636 sulphur as S₈ has also been posited as a pyrolysis product of the sulphur-rich cystine¹³⁹.

4637 In contrast to the thermal desorption profile, the pyrogram also revealed a complex suite of
4638 organic compounds, albeit in relatively minor abundance. These include a series of C₁₀ to C₁₇
4639 *n*-alkanes, typical of saturated fat/oil-derived acyl lipids^{5,6}, although the absence of the *n*-1-
4640 alkene counterparts may well reflect diagenetic changes, rather be indicative of a dominance
4641 of saturated acyl lipids in the original fat/oil. Indeed, the presence of C₅ to C₉ *n*-1-alkenes in
4642 relatively significant abundance compared to the longer carbon chain *n*-alkane moieties is

indicative of an appreciable unsaturated acyl lipid component. The absence of these compounds in the TD profile provides molecular evidence for a polymeric/bound acyl lipid source. However, in addition to *n*-1-alkenes and *n*-alkanes, typical of fat/oil derived lipids, the pyrogram also displayed a series of C₁₀ to C₁₅ 2-methylalkanes (isoalkanes), presumably deriving from the branched fatty acids which are significant components of bacterial triglycerides (again, the absence of their iso-methyl-1-alkenes (isoalkenes) counterparts may well reflect diagenetic changes, rather be indicative of a dominance of saturated branched acyl lipids in the original lipid source). Although non-specific, the presence of carbon dioxide is also consistent with a (highly oxidised) lipid component in this sample.

The pyrolysate TIC identified components indicative of 'black carbon', i.e. charcoal or soot⁷⁻¹⁰. Of these, benzene and toluene were major compounds present in the 'char' pyrolysate (~40% and 55% of total quantified pyrogram peak area respectively), along with moderate quantities of ethyl benzene and *o*-, *m*- and *p*-xylenes (~3% total). The relative dominance of toluene, rather than the normally dominant aromatic hydrocarbon benzene, is somewhat atypical of chars⁷⁻¹⁰. However, their relative abundances are similar to those observed above and so are at least tentatively indicative of a 'char' component providing molecular evidence for exposure to fire/cooking. It should also be noted that highly polar material could have been present, which would not have successfully eluted from the column, or indeed volatilised sufficiently in the probe.



Supplementary Figure 46. Reconstructed total ion chromatogram of the pyrogram (pyrolysis profile) (610 °C for 10s), after thermal desorption (310 °C for 10 s) of sample TMS0058 (upper labial molar) Schipluiden Grave 3). Key to Fig. 46: Peak identities (x indicates carbon chain length): filled squares, Cx indicates alkenes; filled circles, Cx indicates alkanes. Also shown are the structures of five aromatic compounds identified: benzene, thiophene, toluene, methylthiophene and ethylbenzene. CO₂ indicates carbon dioxide, SO₂ indicates sulphur dioxide, COS indicates carbonyl sulphide, CS₂ indicates carbon disulphide and S₈ indicates cyclic octaatomic sulphur.

75. Schipluiden Grave 5 child TMS0051: maxilla labial PM1 (calculus: 3.54 mg)

The thermal desorption total ion chromatogram (TIC) revealed carbon dioxide, sulphur dioxide, carbonyl sulphide and carbon disulphide as the only detectable components. No other free, thermally extractable, organic components could be detected in this sample. It should be noted, however, that highly polar material could have been present, which would not have successfully eluted from the column, or indeed volatilised sufficiently in the probe. It is not possible to determine the significance of the carbon dioxide, beyond it being indicative of oxidised organic material. Collectively, the sulphur dioxide, carbonyl sulphide

and carbon disulphide can be indicative of oxidised and pyrolysed sulphur-containing amino acids or sulphones^{135,136} (see discussion). Sulphur dioxide is also known to be a major pyrolysis product of lignosulphonates¹³⁷, and its organic nature would facilitate sulphur dioxide generation at this relatively low temperature, although this possibility in this context is probably less likely (again, see discussion).

The pyrolysis-GC-MS TIC revealed carbon dioxide, cyclopentadiene, sulphur dioxide and carbonyl sulphide as the only detectable components. The presence of sulphur dioxide and carbonyl sulphide can be indicative of pyrolysed sulphur-containing amino acids or sulphones^{135,136}, although the absence of more diagnostic sulphur-containing organic species makes any connection somewhat tentative. It is not possible to determine the significance of the carbon dioxide, beyond it being indicative of oxidised organic material. It should also be noted that highly polar material could have been present, which would not have successfully eluted from the column, or indeed volatilised sufficiently in the probe.

Schipluiden soil ‘control’ from Grave 5, child (soil/sediment: 8.75 mg)

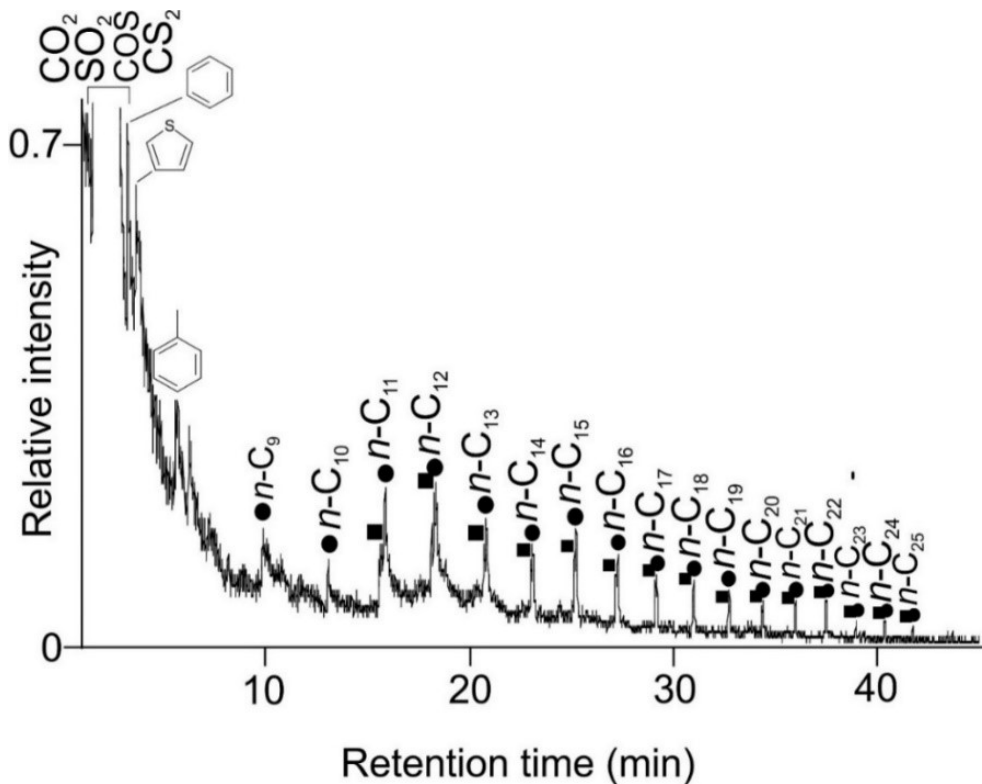
The thermal desorption total ion chromatogram (TIC) was dominated by carbon dioxide, sulphur dioxide and carbon disulphide. The presence of sulphur dioxide and carbonyl sulphide can be indicative of pyrolysed sulphur-containing amino acids or sulphones^{135,136}. The presence of acetic acid as a major component is also consistent with sulphur-containing amino acids¹³⁵. Notably, sulphur as its cyclic octaatomic S₈ form was also detected as a very minor constituent, which has been posited as a pyrolysis product of the sulphur-rich cystine¹³⁹. 3(2H)-pyridazinone was also detected as a minor constituent. It should be noted, however, that highly polar material could have been present, which would not have successfully eluted from the column, or indeed volatilised sufficiently in the probe. It is not possible to determine the significance of the carbon dioxide, beyond it being indicative of oxidised organic material.

The pyrolysis-GC-MS TIC (Fig. 47) was dominated by carbon dioxide, with sulphur dioxide, carbonyl sulphide and carbon disulphide the only other major components. Collectively these latter three compounds can be indicative of sulphur-containing amino acids or sulphones^{135,136} (see discussion), which suggests they derive from the burial soil/sediment (see discussion). These sulphur compounds were also accompanied by the aromatic sulphur compound thiophene. The presence of thiophene with the sulphur dioxide, carbonyl sulphide

and carbon disulphide can be indicative of pyrolysed sulphur-containing amino acids^{135,136,138} (see discussion).

In contrast to the thermal desorption profile, the pyrogram also revealed a complex suite of organic compounds, albeit in relatively minor abundance. These include a series of C₁₁ to C₂₅ *n*-1-alkenes and *n*-alkanes, typical of unsaturated and saturated fat/oil-derived acyl lipids^{5,6}. The absence of these compounds in the TD profile provides molecular evidence for a polymeric/bound acyl lipid source in the sediment of Grave 5. Interestingly, there were no bacterially-derived hydrocarbons deriving from the original branched fatty acid acyl groups which are significant components of bacterial triglycerides, indicating this lipid component is not bacterial in origin. Although non-specific, the presence of carbon dioxide is also consistent with a (highly oxidised) lipid component in this sample.

The pyrolysate TIC identified benzene and toluene as the only other components detected although in the absence of more diagnostic compounds their significance here is unclear. It should also be noted that highly polar material could have been present, which would not have successfully eluted from the column, or indeed volatilised sufficiently in the probe.



Supplementary Figure 47. Reconstructed total ion chromatogram of the pyrogram (pyrolysis profile) (610 °C for 10s), after thermal desorption (310 °C for 10 s) of the soil (control) from Schipluiden Grave 5. Key to Fig. 47: Peak identities (x indicates carbon chain length): filled squares, C_x indicates alkenes; filled circles, C_x indicates alkanes. Also shown are the structures of three aromatic compounds identified: benzene, thiophene and toluene. CO₂ indicates carbon dioxide, SO₂ indicates sulphur dioxide, COS indicates carbonyl sulphide and CS₂ indicates carbon disulphide.

Discussion, Schipluiden burials

Seven samples of dental calculus and two burial ‘control’ samples of soil from Schipluiden were analysed by sequential thermal desorption-gas chromatography-mass spectrometry (TD-GC-MS) and pyrolysis-gas chromatography-mass spectrometry (Py-GC-MS). The samples contained both free and bound/polymeric organic material, albeit highly oxidised.

Beyond significant abundances of carbon dioxide, carbonyl sulphide, carbon disulphide and sulphur dioxide, the calculus and soil samples contained little free and bound/polymeric organic compounds, most likely reflecting unfavourable prevailing conditions for good biomolecular preservation. The dominance of only a few small organic compounds, primarily carbon dioxide, suggests that only highly oxidised sulphur-rich organic material was present.

Notably, the Netherlands has one of the highest sulphur contents in subsoils^{140,141}. The site of Schipluiden is in close proximity to salt marsh and peat¹⁴², which are also known to be high in organic and inorganic sulphur^{143,144}. The alkyl thiophenes, in addition to elemental sulphur (as S₈), may be indicative of the amino acids cysteine and cystine, although the presence of other pyrolysis markers consistent with these amino acids in the soil samples (carbonyl sulphide, carbon disulphide and sulphur dioxide) means their presence here may be due to the burial matrix rather than deriving from the dental calculus itself. It is also possible that the sulphur dioxide may, at least in part, derive from the thermal decomposition of sulphuric acid in the probe, which can be expected to take place at the thermal desorption and pyrolysis temperatures employed.

The bound/polymeric acyl lipid component observed in four of the calculus samples from three individuals does not appear in the soil samples from Schipluiden and therefore this indicates a bacterial lipid constituent present in the calculus, which may well derive from the

individuals' own oral microbiomes. Although biomolecular preservation was poor, there was at least tentative organic chemical evidence for exposure to fire/cooking, and within this 'char' the indication of a protein component. Beyond this, there was nothing which could be indicative of dietary input or environmental exposure in any of the Schipluiden samples.

**76. Hardinxveld de Bruin, Brui 0011014 p.22 VI.35: Elvis F1004 (Box 0307)
mandible lingual left PM2 (calculus: 10.77 mg)**

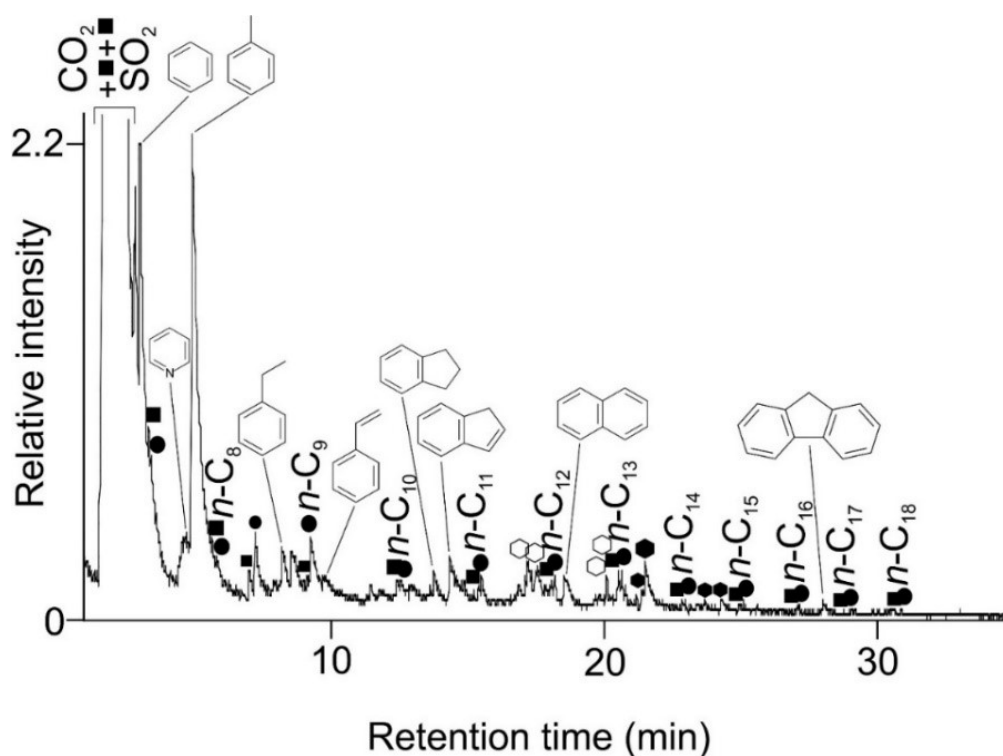
The thermal desorption total ion chromatogram (TIC) revealed carbon dioxide and sulphur dioxide (trace) and benzene as the only detectable components. No other free, thermally extractable, organic components could be detected in this sample. It should be noted, however, that highly polar material could have been present, which would not have successfully eluted from the column, or indeed volatilised sufficiently in the probe. It is not possible to determine the significance of the carbon dioxide, beyond it being indicative of oxidised organic material. The sulphur dioxide is in too low relative abundance to make and meaningful interpretation of its presence here and benzene is too ubiquitous to assign any origin in the absence of other associated and more diagnostic biomarkers.

The pyrolysis-GC-MS TIC (Fig. 48) was dominated by carbon dioxide, 1-butene and 1-pentene, the alkenes being indicative of lipid or proteinaceous material in the sample¹¹. As observed in the TD profile, sulphur dioxide was a significant component, although in the absence of other sulphur compounds any interpretation of its significance has to be very tentative, beyond an organic sulphur source. Cyclopentadiene and methylcyclopentadiene are also significant components of the pyrolysate although it is not possible to ascribe a specific origin here. Acetone was also identified and can be indicative of the amino acid glycine or a carbohydrate component, although any interpretation should be considered tentative here.

In contrast to the thermal desorption profile, the pyrogram also revealed a complex suite of organic compounds, albeit in relatively minor abundance. These include a series of C₇ to C₁₈ *n*-1-alkenes and *n*-alkanes, typical of unsaturated and saturated fat/oil-derived acyl lipids^{5,6}. The absence of these compounds in the TD profile provides molecular evidence for a polymeric/bound acyl lipid source. However, in addition to *n*-1-alkenes and *n*-alkanes, typical of fat/oil derived lipids, the pyrogram also displayed a several branched alkenes and alkanes (C₉, C₁₃, C₁₅ and C₁₈), presumably deriving from the branched fatty acids which are

significant components of bacterial triglycerides. Although non-specific, the presence of carbon dioxide is also consistent with a (highly oxidised) lipid component in this sample.

The pyrolysate TIC identified components indicative of 'black carbon', i.e. charcoal or soot⁷⁻¹⁰. Benzene and toluene were moderately abundant compounds present in the pyrolysate (~30% and 45% of total quantified pyrogram peak area respectively), along with significant quantities of ethyl benzene and o-, m- and p-xylenes (~10% total). The relative dominance of toluene, rather than the normally dominant aromatic hydrocarbon benzene, is somewhat atypical of chars⁷⁻¹⁰. However, the presence of significant amounts of naphthalene, methyl naphthalenes and dimethyl naphthalenes, together with the polynuclear aromatic hydrocarbon combustion marker fluorene, confirm a 'char' component providing molecular evidence for exposure to fire/cooking. The lack of the attendant aromatic nitrogen-containing biomarkers pyrrole, benzonitrile, benzyl nitrile (phenylacetone nitrile), benzenepropanenitrile and indoles indicative of proteinaceous material⁶⁻¹¹ suggests a collective origin as combustion compounds ('char') rather than a significant protein component, although pyridine, styrene, indene and C₁ and C₂ alkyl indenenes are indicative of a protein constituent and so diagenetic processes may explain the lack of nitrogenous organic molecules. The presence of carbon dioxide is also consistent with a lipid component in this sample. It should also be noted that highly polar material could have been present, which would not have successfully eluted from the column, or indeed volatilised sufficiently in the probe.



Supplementary Figure 48. Reconstructed total ion chromatogram of the pyrogram (pyrolysis profile) (610 °C for 10s), after thermal desorption (310 °C for 10 s) of sample Elvis F1004 from Brui 0011014 p.22 VI.35 Hardinxveld de Bruin. Key to Fig. 48: Peak identities (x indicates carbon chain length): filled squares, Cx indicates alkenes; filled circles, Cx indicates alkanes. Also shown are the structures of nine aromatic compounds identified: benzene, pyridine, toluene, ethylbenzene, styrene, indane, indene, naphthalene and fluorene. Open hexagons indicates alkyl indenenes; filled hexagons indicates alkyl naphthalenes. CO₂ indicates carbon dioxide and SO₂ indicates sulphur dioxide.

Hardinxveld de Bruin soil ‘control’ from Brui 0011014 p.22 VI.35 (soil/sediment: 10.48 mg)

The thermal desorption total ion chromatogram (TIC) revealed carbon dioxide as the only detectable (trace) component, indicating the essential absence of free, thermally extractable organic components in this sample. It should be noted, however, that highly polar material could have been present, which would not have successfully eluted from the column, or indeed volatilised sufficiently in the probe. It is not possible to determine the significance of the carbon dioxide, beyond it being indicative of oxidised organic material.

The pyrolysate TIC revealed carbon dioxide and sulphur dioxide (trace) as the only detectable components, indicating very little organic material in this sample. It should be noted, however, that highly polar material could have been present, which would not have successfully eluted from the column, or indeed volatilised sufficiently in the probe. It is not possible to determine the significance of the carbon dioxide, beyond it being indicative of oxidised organic material.

**77. Hardinxveld de Bruin (Meso), Box 0308 mandible lingual right PM1 (Henk)
(calculus: 0.23 mg)**

The thermal desorption total ion chromatogram (TIC) revealed no detectable components, indicating the absence of free, thermally extractable organic components in this sample. It should be noted, however, that highly polar material could have been present, which would not have successfully eluted from the column, or indeed volatilised sufficiently in the probe.

The pyrolysate TIC revealed carbon dioxide as the only detectable (minor trace) component, indicating the essential absence of organic material in this sample. It should be noted, however, that highly polar material could have been present, which would not have successfully eluted from the column, or indeed volatilised sufficiently in the probe. It is not possible to determine the significance of the carbon dioxide, beyond it being indicative of oxidised organic material, although its presence as only a very trace component makes even this inference of no meaningful value.

Hardinxveld de Bruin (Meso) Box 0308 soil 'control' from burial (soil/sediment: 11.47 mg)

The thermal desorption total ion chromatogram (TIC) revealed carbon dioxide and sulphur dioxide as the only detectable components. No other free, thermally extractable, organic components could be detected in this sample. It should be noted, however, that highly polar material could have been present, which would not have successfully eluted from the column, or indeed volatilised sufficiently in the probe. It is not possible to determine the significance of the carbon dioxide, beyond it being indicative of oxidised organic material. Sulphur dioxide is also known to be a major pyrolysis product of lignosulphonates¹³⁷, and its organic

nature would facilitate sulphur dioxide generation at this relatively low temperature, although this possibility in this context is probably less likely (again, see discussion/summary).

The pyrolysis-GC-MS TIC was dominated by carbon dioxide, with sulphur dioxide and carbon disulphide the only other major components. Collectively these latter two compounds can be indicative of sulphur-containing amino acids or sulphones^{135,136} (see discussion), which suggests they derive from the burial soil/sediment (see discussion). It is not possible to determine the significance of the carbon dioxide, beyond it being indicative of oxidised organic material.

78. Hardinxveld Trintjuite, Box 0279 F1004 Put 25 mandible incisor lingual (female) (calculus: 0.15 mg)

The thermal desorption total ion chromatogram (TIC) revealed carbon dioxide as the only detectable (trace) component, indicating the essential absence of free, thermally extractable organic components in this sample. It should be noted, however, that highly polar material could have been present, which would not have successfully eluted from the column, or indeed volatilised sufficiently in the probe. It is not possible to determine the significance of the carbon dioxide, beyond it being indicative of oxidised organic material.

The pyrolysate TIC revealed carbon dioxide as the only detectable (minor trace) component, indicating the essential absence of organic material in this sample. It should be noted, however, that highly polar material could have been present, which would not have successfully eluted from the column, or indeed volatilised sufficiently in the probe. It is not possible to determine the significance of the carbon dioxide, beyond it being indicative of oxidised organic material, although its presence as only a very trace component makes even this inference of no meaningful value.

Discussion, Hardinxveld de Bruin burials

Three samples of dental calculus and two burial ‘control’ samples of soil from Hardinxveld were analysed by sequential thermal desorption-gas chromatography-mass spectrometry (TD-GC-MS) and pyrolysis-gas chromatography-mass spectrometry (Py-GC-MS). The samples contained both free and bound/polymeric organic material, albeit highly oxidised.

Beyond the relatively major abundance of carbon dioxide, with minor amounts of carbon disulphide and sulphur dioxide, the calculus and soil samples contained very little free and bound/polymeric organic compounds, most likely reflecting particularly unfavourable prevailing conditions for biomolecular preservation. The dominance of only a very few small organic compounds, primarily carbon dioxide, suggests that only highly oxidised sulphur-containing organic material was present.

Notably, the Netherlands has one of the highest sulphur contents in subsoils^{140,141}, which includes the site of Hardinxveld. The site is also in close proximity to marshland and peat¹⁴⁵, which are also known to be high in organic and inorganic sulphur^{143,144}. At this site the alkyl thiophenes and elemental sulphur (as S₈) were not observed and so no meaningful interpretation of the significance of the carbon disulphide and sulphur dioxide can be given beyond being indicative of an organic, and possibly inorganic, sulphur source, although their presence here may be due to the burial matrix rather than having any direct connection with the dental calculus itself. It's also possible that the sulphur dioxide may, at least in part, derive from the thermal decomposition of sulphuric acid in the probe, which can be expected to take place at the thermal desorption and pyrolysis temperatures employed.

The bound/polymeric acyl lipid component observed in the Hardinxveld de Bruin Brui 0011014 calculus sample does not appear in the soil samples from the same burial site and therefore this indicates a bacterial lipid constituent present in the calculus, which may well derive from the individual's own oral microbiome. Although biomolecular preservation was poor, there was organic chemical evidence for exposure to fire/cooking, and within this 'char' the indication of an appreciable protein component, which correlates with the abundance of the protein marker 1-butene in this calculus sample. Beyond this, there was nothing which could be indicative of dietary input or environmental exposure. The Mesolithic samples (calculus and soil) and Hardinxveld Trintjuite sample revealed a virtual absence of evidence for an organic constituent, suggesting the age and particular archaeological context at these sites were extremely unfavourable for biomolecular preservation.

Overall, it is worth noting that the limited biomolecular information obtained from the Schuipluden and Hardinxveld samples here may well reflect poor environmental conditions for the chemical preservation of an organic component in dental calculus, and indeed other organic residues.

4922 **Kruszyn, Poland**

4923 **Introduction**

4924 One sample of dental calculus from an individual from the Linear Band Pottery culture was
4925 taken from the site of Kruszyn. Kruszyn is situated on the left bank of Vistula river
4926 (Włocławek district, central Poland). Rescue excavations conducted in 2004, 2007-2009
4927 revealed multicultural settlement and graves from Neolithic, Early Bronze Age, Roman
4928 Period, Medieval Age and Modern Times. Grave goods of LBK included fragments of
4929 pottery¹⁴⁶. The sample was analysed by sequential thermal desorption-gas chromatography-
4930 mass spectrometry (TD-GC-MS) and pyrolysis-gas chromatography-mass spectrometry (Py-
4931 GC-MS). This technique facilitates the identification of both free/unbound and
4932 bound/polymeric organic components.

4933

4934 **79. Kruszyn, Poland: Grave G93, Site 13, male 35-40/45 years old Linear Band**
4935 **Pottery culture (calculus: 9.73 mg)**

4936 The thermal desorption total ion chromatogram (TIC) revealed carbon dioxide as the only
4937 detectable (trace) component, indicating the essential absence of free, thermally extractable
4938 organic components in this sample. It should be noted, however, that highly polar material
4939 could have been present, which would not have successfully eluted from the column, or
4940 indeed volatilised sufficiently in the probe. It is not possible to determine the significance of
4941 the carbon dioxide, beyond it being indicative of oxidised organic material.

4942 The pyrolysate TIC revealed carbon dioxide and the C₄ and C₅ 1-alkenes as the only
4943 detectable components. It is not possible to determine the significance of the carbon dioxide,
4944 beyond it being indicative of oxidised organic material and the two short chain hydrocarbons
4945 are too low an abundance and too ubiquitous to provide any meaningful interpretation of their
4946 presence, beyond being indicative of an organic origin. It should be noted, however, that
4947 highly polar material could have been present, which would not have successfully eluted
4948 from the column, or indeed volatilised sufficiently in the probe.

4949

4950 **Discussion, Kruszyn**

4951 The sample contained very little free and bound/polymeric organic material, most likely
4952 reflecting unfavourable prevailing conditions for biomolecular preservation. Of the organic

compounds identified, the dominance of carbon dioxide and the relatively minor abundance of only two small organic compounds suggests that only highly oxidised organic material was present.

Rønstenen, Denmark

Rønstenen is a Middle Mesolithic settlement currently submerged to around two meters in marine water. It is well known among local divers, as a site rich in lithic items, while, occasionally, organic materials have also been recovered. It has been regularly inspected by the Danish Authority for Cultural Heritage who salvaged material of scientific importance. Among these finds was a right mandibula with three extant teeth that dates to the same period as the lithics: 7542 ± 42 ^{14}C years BP (AAR-11355)¹⁴⁷.

One sample of dental calculus taken from one individual from the site of Rønstenen was analysed by sequential thermal desorption-gas chromatography-mass spectrometry (TD-GC-MS) and pyrolysis-gas chromatography-mass spectrometry (Py-GC-MS). This technique facilitates the identification of both free/unbound and bound/polymeric organic components.

80. Rønstenen, Denmark: AS 15/06 (calculus: 0.60 mg)

The thermal desorption total ion chromatogram (TIC) revealed carbon dioxide as the only detectable (trace) component, indicating the essential absence of free, thermally extractable organic components in this sample. It should be noted, however, that highly polar material could have been present, which would not have successfully eluted from the column, or indeed volatilised sufficiently in the probe. It is not possible to determine the significance of the carbon dioxide, beyond it being indicative of oxidised organic material.

The pyrolysate TIC contained very little detectable organic material. The only components identified were carbon dioxide, sulphur dioxide, C_4 to C_6 1-alkenes, cyclopentadiene and a trace amount of the aromatic hydrocarbon benzene. The dominance of carbon dioxide, combined with the relatively minor abundance of the few small organic compounds present, suggests a highly oxidised sample. It is not possible to determine the significance of the carbon dioxide, beyond it being indicative of oxidised organic material and the other compounds are too low an abundance and too ubiquitous to provide any meaningful interpretation of their presence, beyond being indicative of an organic origin. It should be

noted, however, that highly polar material could have been present, which would not have successfully eluted from the column, or indeed volatilised sufficiently in the probe.

Discussion, Rønstenen

The sample contained very little free and bound/polymeric organic material, most likely reflecting unfavourable prevailing conditions for biomolecular preservation. Of those organic compounds identified, the dominance of carbon dioxide and the relatively minor abundance of only a few small organic compounds suggests that only highly oxidised organic material was present.

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