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Renata Zocatelli, Marlène Lavrieux, Typhaine Guillemot, Léo Chassiot,  
Claude Le Milbeau, Jérémy Jacob

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1 **Fecal biomarker imprints as indicators of past human land uses: source**  
2 **distinction and preservation potential in archaeological and natural**  
3 **archives.**

4  
5 Renata ZOCATELLI<sup>1,2,3</sup>, Marlène LAVRIEUX<sup>4</sup>, Typhaine GUILLEMOT<sup>5</sup>, Léo  
6 CHASSIOT<sup>1,2,3</sup>, Claude Le MILBEAU<sup>1,2,3</sup>, Jérémy JACOB<sup>1,2,3</sup>.  
7

8 <sup>1</sup> Université d'Orléans, Institut des Sciences de la Terre d'Orléans (ISTO), UMR 7327, 45071 Orléans,  
9 France

10 <sup>2</sup> CNRS/INSU, ISTO, UMR 7327, 45071 Orléans, France

11 <sup>3</sup> BRGM, ISTO, UMR 7327, BP 36009, 45060 Orléans, France

12 <sup>4</sup>Environmental Geosciences, University of Basel, Bernoullistrasse 30, 4056 Basel, Switzerland

13 <sup>5</sup>Eawag – Swiss Federal Institute of Aquatic Science and Technology, Seestrasse 79, 6047  
14 Kastanienbaum, Switzerland  
15

16 **ABSTRACT**

17 This paper presents the potential of 5 $\beta$ -stanols and bile acids to act as fecal source biomarkers  
18 in order to identify and characterize past agropastoral activities in archaeological and natural  
19 archives. First of all, a molecular inventory of 5 $\beta$ -stanols and bile acids was made on fresh fecal  
20 human and domestic animal samples, using the same methodology to define the specificity of  
21 these molecular biomarkers. The selected species were cow, horse, pig and sheep as they are  
22 the major domestic species present in European archaeological sites. To our knowledge, our  
23 work constitutes the first report on fecal biomarkers in sheep feces. Bile acids can distinguish  
24 diet regime and species having the same diet with greater specificity than 5 $\beta$ -stanols. Fresh  
25 human fecal material and historical / archaeological fecal material were analyzed to assess their  
26 stability through time by calculating the coprostanol/epi-coprostanol (Cp/epi-Cp) and  
27 deoxycholic acid/cholic acid (DOC/C) ratios. Results show that bile acids are more resistant to  
28 diagenesis than 5 $\beta$ -stanols, at least on a 700-year time scale. Human and domestic animal fecal  
29 molecular imprints were then compared to the molecular content of 65 samples retrieved from  
30 archaeological sites, soils and lacustrine sediments to test their ability to trace past land-use  
31 dynamics. This study is the first to combine bile acids and 5 $\beta$ -stanols to identify a source of  
32 fecal material indifferent types of location (archaeological site, catchments and lacustrine  
33 sediments) and on different time scales. The combination of sterols and bile acids can be used  
34 in a variety of natural archives and archaeological contexts to define the origin of fecal material,  
35 to specify land-use, and to reconstruct past pastoral practices at various spatio-temporal scales.  
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Keywords: past agropastoral dynamics, fecal sterols, bile acids, archaeological and natural  
39 archives.  
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41 Corresponding author e-mail address: renata.zoccatelli1@univ-orleans.fr (R. Zocatelli).  
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47 **1. Introduction**

48 Land use change is known to be one of the main factors in global change, as it affects, inter  
49 alia, biodiversity (Smith and Zeder, 2013; Braje and Erlandson, 2013), biogeochemical cycles  
50 (Foley et al., 2005), geomorphological processes (Syvitski et al., 2005; Syvitski and Kettner,  
51 2011) and climate (IPCC, 2013; Ruddiman, 2013; Ellis et al., 2013). The development of  
52 agricultural practices, especially through animal domestication and land clearing, induced a  
53 fundamental shift in the interactions between humans and environments (Redman, 1999; Zeder  
54 et al., 2006), from local to regional and continental scales (Braje and Erlandson, 2013). To  
55 predict ecosystem responses to global changes, the characterization of past land-uses and their  
56 impacts on environment/climate systems are relevant (Ruddiman, 2003; Dearing, 2006; Hurtt  
57 et al., 2006; Jacob et al., 2009).

58 Fossil organic molecules or molecular biomarkers are compounds preserved in  
59 archaeological and natural (soil, sediment) archives, having an unequivocal biological source  
60 (e.g. van Bergen et al., 1997; Simoneit, 2002, Killops and Killops, 2005). Biomarker analysis  
61 can provide information on environmental conditions either in predominantly natural or in more  
62 anthropized contexts such as archaeological sites and urban areas, at local or catchment scale  
63 (e.g. Bull et al., 1998; Oldfield et al., 2003; Zolitschka and Ariztegui, 2007; Jacob et al., 2008;  
64 Battarbee and Bennion, 2011; Lavrieux et al., 2012, 2013a; Zocatelli et al., 2010, 2012, 2013).

65 Fecal biomarkers ( $C_{27}$ ,  $C_{28}$  and  $C_{29}5\beta$ -stanols and bile acids), produced in the digestive  
66 tracts of certain large mammals (Elhmmali et al., 2000; Bull et al., 2002; Tyagi et al., 2008) are  
67 commonly used to trace human or animal fecal inputs in current environments (Nes and Nes,  
68 1980; Elhmmali et al., 1997; Evershed et al., 1997; Bull et al., 1998, 1999b, 2001, 2002; Jardé  
69 et al., 2007a; Lavrieux et al., 2012; D'Anjouet al., 2012; Guillemot et al., 2015, 2016). However,  
70 to date, only a few studies have precisely distinguished human and animal sources in  
71 archaeological settlements (Bull et al., 2003) or in the surrounding area (Shillito et al., 2011a).  
72 Moreover, the fecal imprint of some large mammal species often encountered in archaeological

73 contexts, such as sheep, has never been investigated. Moreover, the published data were  
74 acquired using different protocols, which makes the comparison between the fecal imprints  
75 difficult.

76 To fill these gaps, this study proposes an integrated (“source to sink”) approach to fecal  
77 biomarkers (5 $\beta$ -stanols and bile acids), from the source organisms to the archives, at different  
78 spatial and temporal scales, based on a large set of new data. First, a complete inventory of  
79 fecal biomarkers was made in current human and domestic animal feces. Cow, horse, pig and,  
80 for the first time, sheep, were selected because they represent the most abundant animals  
81 described in European archaeological sites. To identify their fecal molecular distribution, the  
82 same protocol was used, allowing a comparison between them and a characterization of the  
83 potential specificity of these biomarkers. The early and selective degradation of fecal  
84 biomarkers was then studied in order to ascertain the stability of established relationships  
85 through time. This was achieved by analyzing the molecular content of fresh human feces,  
86 recent sediments in septic tanks and archaeological middens. Finally, the fecal molecular  
87 imprints previously proposed were tested in different archives. This includes septic tanks and  
88 soils in archaeological settlements (Orléans, France), soils (Lake Aydat catchment, Massif  
89 Central, France) and lake sediments (retrieved from Lake Montcineyre, Massif Central, France  
90 and Lake Igaliku, South Greenland). These archives enable past agropastoral dynamics to be  
91 assessed at different spatio-temporal scales.

92

## 93 **2. Materials and methods**

### 94 *2.1. Sampling*

#### 95 2.1.1. Reference samples

96 Fresh feces of humans, cows, horses, pigs and sheep constituted the reference samples.

97 Fresh animal feces were collected at the Institut National de la Recherche Agronomique (INRA,

98 Tours, France), whereas the human fecal material was obtained from an anonymous donor.  
99 Samples were placed in thermo coolers immediately after collection (-20 °C).

100

#### 101 2.1.2. Collection samples

102 An ancient septic tank, archaeological middens, soils and lacustrine sediments were  
103 sampled. First, they were used to assess the early diagenesis and preservation of the fecal  
104 biomarkers' distribution through time and then for the reconstruction of past agropastoral  
105 dynamics on various space and time scales.

106 A total of 60 samples were selected (Appendix 1):

107 - Four samples were taken to assess the possible early and preferential degradation of fecal  
108 biomarkers over the first centuries after deposition: a 19th century septic tank (St19) and three  
109 archaeological middens dated from the 14th, 15th and 16th centuries (St14, St15, St16,  
110 respectively; Joyeux, 2013) collected from undisturbed settlement layers in an excavated  
111 housing located in the medieval city of Orléans (Loire Valley, France).

112 - 10 soils (PS1-PS10), from fields pastured by bovines for at least 60 years, were sampled  
113 in the Lake Aydat catchment (Massif Central, France) in order to describe the fecal molecular  
114 distribution in grazing areas and their transfer to soils.

115 - A total of 46 samples were retrieved from two lacustrine sediment cores to reconstruct  
116 past and recent land uses at a catchment scale. Two catchments with distinct anthropogenic  
117 contexts were selected:

118 (i) Lake Montcineyre catchment (Massif Central, France) located twenty kilometers away  
119 from Aydat (Fig. 1). Archaeological and historical data attest to agropastoral activities  
120 since the Middle Ages (Charbonnier, 1999; Bayard, 2010 and references therein).  
121 However, despite the presence of archaeological settlements in the catchment since the  
122 Neolithic, agropastoral activities and their impact on the local ecosystems remain poorly  
123 understood at catchment scale (Chassiot, 2015; Chassiot et al., 2016). 13 samples were

124 retrieved from a sedimentary core from Lake Montcineyre (MO1-MO13), spanning the  
125 last 800 years (Chassiot et al., 2016).

126 (ii) Lake Igaliku catchment, located in South Greenland. Two distinct agropastoral  
127 periods are attested regionally: the Norse settlement, between AD 986 and ca. AD 1450  
128 and the modern period, since the 1920s (Gauthier et al., 2010; Massa et al., 2012). Located  
129 one and a half kilometers away from the lake, the village of Igaliku was the Norse  
130 episcopal capital of Greenland and one of the most important agropastoral centers,  
131 judging from archaeological structures (Arnebord et al., 2007). Nowadays, agropastoral  
132 activities are characterized by predominant sheep breeding (Austrheim et al., 2008). Two  
133 farms composed of stables, barns, and hay fields are located on the southern shore of Lake  
134 Igaliku. 33 sediment samples (Iga1-Iga33) spanning the last 2000 years were retrieved  
135 from Lake Igaliku (Guillemot et al., 2016).

136

## 137 2.2. Fecalbiomarkeranalyses

138 Samples were dried at 30 °C during 48h and then crushed. Ca. two grams of dried samples  
139 were extracted using an accelerated solvent extractor (Dionex® ASE 200, 100 °C and 1000 psi)  
140 with a mixture of CH<sub>2</sub>Cl<sub>2</sub>:MeOH (9:1 v/v). Total lipid extract was fractionated into neutral and  
141 acidic compounds using solid phase extraction on Supelclean LC-NH<sub>2</sub> SPE bulk packing  
142 (Zocatelli et al., 2012). Neutral compounds were eluted with CH<sub>2</sub>Cl<sub>2</sub>:isopropanol (2:1) and  
143 acidic compounds with ether:formic acid (9:1) after acidification. Total neutral and acid  
144 fractions were then dried under N<sub>2</sub>. As described in Lavrieux et al. (2011), the sterols were  
145 isolated from the total neutral fraction by flash chromatography on a Pasteur pipette filled with  
146 silica (activated 24h at 120°C, then deactivated with H<sub>2</sub>O, 5%), using a sequence of solvents of  
147 increasing polarity (heptane, heptane:toluene and heptane:ethyl acetate). The acidic fraction  
148 was methylated by adding a mixture of anhydrous MeOH and acetyl chloride (55°C for one  
149 hour). It was then separated into fatty acid methyl esters (FAMES) and hydroxy acid methyl

150 esters by flash chromatography with a Pasteur pipette filled with silica, using CH<sub>2</sub>Cl<sub>2</sub>,  
151 CH<sub>2</sub>Cl<sub>2</sub>:isopropanol and MeOH (Guillemot et al., 2015). Finally, sterol and bile acid fractions  
152 were silylated using BSTFA and pyridine (60°C for one hour). Prior to gas-chromatography  
153 and mass-spectrometry (GC-MS) analyses, 5 $\alpha$ -cholestane and nor-deoxycholic acid standards  
154 were added to all fractions for quantitation purposes.

155 Fractions were analyzed by gas chromatography-mass spectrometry (GC-MS) with a  
156 Polaris TRACE-GCQ. The chromatograph was fitted with an Rtx-5MS column (30 m, 0.25 mm  
157 i.d., 0.25  $\mu$ m film thickness). The GC operating conditions were: 40°C (hold 1 min) ramping  
158 from 40°C to 120°C at 30°C.min<sup>-1</sup>, then from 120°C to 300°C at 5°C.min<sup>-1</sup>, hold 30 min. The  
159 samples were injected automatically in splitless mode, with the injector temperature set at  
160 280°C. Helium was the carrier gas (1 ml.min<sup>-1</sup>). The MS was operated in the electron ionization  
161 (EI) mode at 70 eV and scanned from m/z 50-650. Identification of fecal biomarkers was based  
162 on published retention times and mass spectra (Elhmmali et al., 1997; Bull et al., 2001) and  
163 also on authentic standards of sterols and bile acids, obtained from Sigma Aldrich Chemical  
164 Co. and Alfa Aesar. Because of possible coelution with other components, compound  
165 concentrations were estimated by measuring peak areas on ion specific chromatograms. Fecal  
166 biomarker concentrations were estimated after calculation of a correction factor between the  
167 peak area on the ion specific chromatogram and the peak area on the total ion current (TIC)  
168 chromatogram for authentic standards. The minimum level of detection using GC-MS was  
169 about 10 ng.

170

### 171 **3. Results and Discussion**

#### 172 3.1. Fecal biomarker inventory

173 Nine sterols (*sensulato*): five fecal stanols [coprostanols or 5 $\alpha$ -stanols, coprostanol (Cp),  
174 epi-coprostanol (epi-Cp), methylcoprostanol (mCp), epi-ethylcoprostanol (epi-ethCp) and  
175 ethylcoprostanol (ethCp)]; two 5 $\alpha$ -stanols [(cholestanol (Chln) and ethylcholestanol (ethChln)]

176 and two sterols [*sensu stricto*; cholesterol (Chl) and sitosterol (Sit)] were detected in  
177 thereference samples and also in the samples. In addition, six bile acids were identified:  
178 lithocholic acid (LC), deoxycholic acid (DOC), chenodeoxycholic acid (CDOC), cholic acid  
179 (C), hyodeoxycholic acid (HDOC) and ursodeoxycholic acid (UDOC; Table 1 and 2).

180

### 181 3.2. Biomarker distribution in fresh fecal material

#### 182 3.2.1. Sterols

183 The distribution of sterols varied widely among the considered species (Table 1; Fig. 2a  
184 and e). In the herbivorous group, cow feces showed the highest values of total coprostanols  
185 (TC, 1817  $\mu\text{g/g}$  of dried fecal material; Table 1), followed by sheep (1302  $\mu\text{g/g}$ ) and horse  
186 (336  $\mu\text{g/g}$ ). In herbivorous fecal samples ethCp was the main compound followed by mCp (Table  
187 1). In the omnivorous group, TC concentrations were one order of magnitude greater than in  
188 the herbivorous group, with a predominance of Cp. Human and pig feces had rather similar  
189 fecal sterol profiles. Indeed, Cp and ethCp were the major compounds in pig (Cp = 3522  $\mu\text{g/g}$ ,  
190 48% of TC; ethCp = 3093  $\mu\text{g/g}$ ; 41% of TC) and human feces (Cp = 12251  $\mu\text{g/g}$ , 71% of TC;  
191 ethCp = 3929  $\mu\text{g/g}$ , 23% of TC). However, the proportion of TC was more than twice as high  
192 in human as in pig feces (Table 1). More especially, Cp contents in human feces were 3.5 times  
193 higher than in pig feces, which is consistent with previously published data (Leeming et al.,  
194 1996).

195 In the light of these results, the differences in fecal sterol distributions cannot distinguish  
196 between mammals having the same diet. Previous studies using ratios of fecal and non-fecal  
197 sterols barely succeeded in discriminating between mammals (e.g. Grimalt et al., 1990; Bull et  
198 al., 1999a, 2002, 2003; Jardé et al., 2007a,b; Shillito et al., 2011a; Baeten et al., 2012), assuming  
199 that the variations in coprostanol concentrations are not controlled by selective degradation.  
200 These ratios were either developed to attest to pollution ( $\text{Sr1} > 0.7$ ; Grimalt et al., 1990) or to  
201 identify animal sources ( $\text{Sr2}$ ; Bull et al., 1999a):



202  $Cp/(Cp + epi-Cp + Chln)$  (Sr1)

203  $(ethCp + epi-ethCp)/(Cp + epi-Cp)$  (Sr2)

204 We tested these two ratios in the reference samples. For all of them, Sr1 values were greater  
205 than 0.7 with  $Sr1_{human} > Sr1_{pig} > Sr1_{cow} > Sr1_{horse}$  and  $Sr1_{sheep}$  (Table 1) indicating that omnivorous  
206 animals produce more Cp than herbivorous animals. The Sr2 ratio, used to distinguish ruminant  
207 from pig/human material sources, was below 1.0 for human and pig feces (0.32 and 0.76,  
208 respectively), and above 1.0 for herbivorous feces (horse = 2.29, sheep = 2.67 and cow = 3.05).  
209 As expected, Sr1 and Sr2 ratios measured in the reference samples corroborated previous  
210 studies conducted by Grimalt et al. (1990) and Bull et al. (1999a,b,c, 2002) and can be  
211 considered reliable as long as there is no selective degradation and/or mixing of different animal  
212 imprints.

213

### 214 3.2.2. Bile acids

215 The distribution of bile acids also varied widely among the different animal species (Table  
216 1; Figure 2b and f). In the herbivorous group, all species produced DOC, whereas LC was only  
217 produced by cows and horses (Fig. 2b). It is therefore possible to distinguish them from sheep,  
218 whose feces only contained DOC (3.21  $\mu\text{g/g}$  of dried fecal material). Cows and horses can be  
219 distinguished by the total bile acid content, which was twice as high in cow (0.56  $\mu\text{g/g}$ ) as in  
220 horse feces (1.17  $\mu\text{g/g}$ ).

221 In the omnivorous group, human and pig fecal material can be discriminated by the bile  
222 acid composition. In human fecal samples, DOC was the major bile acid detected, followed by  
223 LC (Fig. 2f; 35.56 and 45.44  $\mu\text{g/g}$  respectively; Table 1). Other bile acids such as CDOC (2.15  
224  $\mu\text{g/g}$ ), C (1.79  $\mu\text{g/g}$ ) and UDOC (1.24  $\mu\text{g/g}$ ) were detected in lower contents. While DOC was  
225 the most abundant bile acid in human fecal material, it was not detected in pig fecal samples.  
226 In the latter, the major bile acids found were HDOC (32.01  $\mu\text{g/g}$ ) followed by LC (21.07  $\mu\text{g/g}$ )  
227 and UDOC in lower contents (2.31  $\mu\text{g/g}$ ). Thus, bile acid distributions allow a clear distinction

228 not only between herbivorous and omnivorous mammals, but also between animals having the  
229 same diet. The fecal molecular inventory of a large range of animal feces presented in this study  
230 not only confirms, but also completes previous studies (Bethell et al., 1994; Elhmmali et al.  
231 1997; Bull et al. 2002, 2003; Tyagi et al., 2008). Indeed, to our knowledge, our work constitutes  
232 the first report on fecal biomarkers in sheep feces.

233

### 234 3.3. *Fecal signal preservation*

235 Some studies attest to a rapid degradation of coprostanols by anaerobic bacteria present in  
236 sewage and natural waters (Gaskell and Eglinton, 1974; Grimalt et al., 1990), potentially  
237 masking their original sources (Mermoud et al., 1984; Rushdi et al., 2006). Nevertheless, in  
238 soils, other studies showed that these biomarkers are able to survive and highlight human  
239 activities during several millennia (Pepe and Dizabo, 1990; Bethell et al., 1994; Shillito et al.,  
240 2011a, b), assuming good preservation of these biomarkers. Few studies have attempted to  
241 assess such discrepancies. Elhmmali et al. (1997) showed that DOC was better preserved than  
242 Cp in sewage particulate organic matter before and after microbiological treatments, suggesting  
243 that bile acids are probably better preserved than sterols.

244 To better assess the respective preservation of fecal biomarkers, we compared the fecal  
245 imprint of the human reference sample to samples from the septic tank and from archaeological  
246 middens, covering the 19th to the 14th centuries (St19, St16, St15 and St14). All these samples  
247 were characterized by a major/exclusive human fecal input. In St19, St16, St15 and St14  
248 samples, Sr1 values ranged between 0.87 and 0.92 (Table 2) and Sr2 exhibited low values (0.33-  
249 0.84), all attesting to an omnivorous fecal imprint. In addition, Cp was the major sterol observed  
250 (between 0.58 and 10.54 mg/g), followed by ethCp (Fig. 2; Table 1). These indices, associated  
251 to the presence of CDOC and C, and the absence of HDOC, attest to a human fecal input.

252 Fecal sterols and bile acid contents decreased with the age of the samples (Table 2, Fig. 3).  
253 This trend could be attributed to a degradation of fecal biomarkers, but could also result from a

254 decreasing occupation of the site. Preservation of fecal biomarkers in human feces over time  
255 was assessed through the calculation of the Cp/epi-Cp and DOC/C ratios. The Cp/epi-Cp ratio is  
256 already used to overcome possible diagenetic biases related to the degradation of Cp into epi-  
257 Cp (Simpson et al., 1998; Bull et al., 1999a). In addition to this ratio, we used the DOC/C ratio  
258 because DOC is a secondary by-product of C formed in anaerobic environments via enzymatic  
259 reactions (Bull et al., 2003). A relatively low DOC/C ratio variability over time indicates good  
260 preservation. The DOC/C ratio can be used if it is assumed that the chemical difference between  
261 DOC and C is minor, implying that the polarity difference between the two compounds is  
262 negligible and thus would not affect the solubility and adsorption to soil/sediment particulates.  
263 From today to the 14th century, the DOC/C ratio remained constant in our samples (DOC/C  
264 ~20, Fig. 3e), whereas the Cp/epi-Cp ratio decreased during the first century (from ~70 to  
265 ~10). Assuming that this decrease in the Cp/epi-Cp ratio is not related to diet changes over  
266 centuries, it would suggest a greater degradation of fecal sterols than of bile acids. This better  
267 preservation of bile acids compared to 5 $\beta$ -stanols supports previous observations made by  
268 Elhmmali et al. (1997). Regarding these results, it appears that bile acids are better preserved,  
269 at least at the multi-centennial scale.

270

### 271 3.4. *Applications of fecal biomarkers to reconstruct agropastoral activities*

272 To trace and characterize past agropastoral activities in and outside archaeological sites,  
273 we applied the fecal biomarker distribution defined from our reference samples to several  
274 natural archives over various temporal scales.

275

276

277

278

279

#### 280 3.4.1. Aydat catchment soils

281 In soil samples retrieved from lake Aydat catchment (Massif Central, France), previous  
282 studies based on fecal biomarkers evidenced recent husbandry practices (Lavrieux et al., 2012;  
283 Zocatelli et al., 2012). Here, this was achieved by re-exploiting the dataset of fecal biomarker  
284 distribution and comparing it to the reference samples. The distribution of sterols and bile acids  
285 was quite similar over soil samples (Table 2, Fig. 4), with TC varying between 0.56 and  
286 26.9mg/g soil, and with 8-37% of coprostanols (ethCp and Cp; Table 2). Samples also contained  
287 significant amounts of LC and DOC (0.08-0.36 and 0.84-4.25  $\mu\text{g/g}$ ). No HDOC, which is  
288 specific of pig feces, was detected. Associated to the distribution of sterols, the identification  
289 of DOC and the absence of C and CDOC argue for a fecal input from cows to the soil, consistent  
290 with current husbandry in the catchment. In any case, this study shows that the recent  
291 fecal compounds distribution is transferred to soils and consistent with current agropastoral  
292 practices. For paleosoils located in archaeological sites fecal biomarkers are a promising tool  
293 in archaeological research regarding the use of space and human activities.

294

#### 295 3.4.2. Lake Montcineyre sediments

296 A high diversity of fecal biomarkers was inventoried in the 800-yr-long lacustrine core  
297 retrieved from Lake Montcineyre. Fecal biomarkers such as Cp, epi-Cp, mCp, ethCp, epi-  
298 ethCp, LC and DOC were detected all along the core. Fig.5b shows the evolution of total  
299 coprostanols, Sr2 ratio, LC and DOC. Although a small amount of Cp was detected, the absence  
300 of CDOC, C and UDOC indicates that there was no significant fecal human input (Table 2, Fig.  
301 5). Sr2 values above 1 together with DOC concentrations seven times higher than LC  
302 concentration over the whole archive are indicative of the presence of livestock composed  
303 mainly of cows over the last 800 years (Fig. 5b). Intervals with higher DOC and LC  
304 concentrations could indicate periods of increased agro-pastoral activities. Conversely, because  
305 TC did not exhibit the same trends, it probably illustrates differential degradation between bile

306 acids and fecal sterols. In view of the better preservation of bile acids compared to that of 5 $\beta$ -  
307 stanols confirmed and described in section 3.3., it is the evolution of LC and DOC  
308 biomarkers that describes agropastoral activities during the last 800 years in the Montcineyre  
309 catchment.

310

### 311 3.4.3. Lake Igaliku sediments

312 The regional historical context of South Greenland is very different from that of the  
313 Massif Central. In the lake Aydat and Montcineyre catchments, agropastoral activities occurred  
314 without interruption since the Neolithic. In South Greenland only two agropastoral periods (the  
315 Norse settlement between AD 986 to ~ AD 1450 and the last century) are attested. Figure 6  
316 shows the evolution of TC, Sr<sup>2</sup> ratio and DOC contents during the last two millennia. The Sr<sup>2</sup>  
317 ratio and DOC contents evidence the two phases synchronous with these two periods of  
318 agropastoralism. As here, Guillemot et al. (2015) identified two main peaks of DOC Igaliku  
319 lacustrine sediments. During the last century, Sr<sup>2</sup> values above 10 attest to the presence of  
320 herbivorous livestock grazing in the catchment. High contents of ethCp associated to the  
321 presence of DOC as the only bile acid suggest a predominant sheep fecal input. This is  
322 confirmed by current agropastoral activities which are mainly characterized by sheep breeding  
323 (Austrheim et al., 2008). In the Norse period, less information concerning agropastoral  
324 activities are available. In south Greenland, three archeological studies showed a predominance  
325 of ovine bones with, among others, cow and pig bones (McGovern, 1983; Edvardsson et al.,  
326 2007; Madsen, 2014). In the sediments of Lake Igaliku, a maximum of Sr<sup>2</sup> at 26 is recorded  
327 and evidences herbivorous livestock grazing around Lake Igaliku; low Cp contents and the  
328 absence of CDOC, C and UDOC indicate no significant human fecal contribution while a  
329 similar fecal biomarker imprint was identified during the last century. High contents of ethCp  
330 and the presence of DOC alone suggest that Norse livestock was mainly composed of sheep, as  
331 confirmed by archaeozoological data (McGovern, 1983; Edvardsson et al., 2007; Madsen,

332 2014). Considering the climatic and environmental conditions of south Greenland (positive  
333 temperatures only seven months a year and difficulties to cultivate fodders; Austrheim et al.,  
334 2008; Cappelen, 2014), as sheep are more resistant to cold conditions and needed less fodder  
335 than cows, it would be logical that the Norse Greenlandic livestock were mainly constituted  
336 of sheep, as nowadays. Between the two agropastoral phases of sheep breeding, TC and DOC  
337 contents drop, attesting to a decrease of pastoral activities around Igaliku.

338 This study has provided new insight in to the determination of fecal material sources from  
339 biomarkers in natural archives. A further step will be to interpret the varying concentrations of  
340 biomarkers in terms of livestock population changes. This should be achieved through a  
341 calibration aiming at establishing quantitative relationships and taking into account production,  
342 transport and sedimentation processes (Eglinton and Eglinton, 2008).

343

#### 344 **4. Conclusions**

345 Fecal molecular imprints of the most abundant big mammals encountered in archaeological  
346 sites in Europe were defined, using a unique extraction protocol on fresh cow, horse, sheep, pig  
347 and human feces. This study determined the fecal molecular distribution of sheep faeces. The  
348 sterol distribution in mammal feces clearly discriminates omnivorous (human and pig) from  
349 herbivorous (cow, horse and sheep) animals, but does not allow any differentiation at the  
350 species level. However, the bile acid patterns offer an unequivocal imprint for most of the  
351 species considered. Comparing fecal biomarkers in fresh human feces with an ancient septic  
352 tank and archaeological middens with known human fecal pollution showed that bile acids are  
353 better preserved over time than sterols, as suggested by previous studies. In the light of all these  
354 results, it is now possible to discriminate human and domestic animal species, using fecal  
355 molecular biomarkers and especially bile acids, showing a better specificity and preservation.

356 Thanks to the good preservation and specificity of fecal biomarkers, it was possible to test  
357 their ability to trace past land-use dynamics in various anthropication contexts, in natural soils

358 and lacustrine sediments, aged from the last decennia to millennia. A complete fecal molecular  
359 inventory in the Lake Aydat catchment soils, Lake Montcineyre and Lake Igaliku sediments is  
360 made and brings information about past land uses, allowing a reconstruction of local past  
361 pastoral practices and enlightening us on past interactions between human and environments.

362

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374

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563

#### 564 TABLE CAPTIONS

565 **Table 1:** Sterol and bile acids contents ( $\mu\text{g/g}$ ) and ratios of fecal sterols for reference samples.  
566 Values of  $\text{Sr1} > 0.7$  indicate fecal material detection (Grimalt et al., 1990). Values of  $\text{Sr2} > 1$   
567 indicate the predominance of herbivorous feces (Bull et al., 2002). Cp: coprostanol, epi-Cp:  
568 epi-coprostanol, Chl: cholesterol, Chln: cholestanol, mCp: methyl-coprostanol, ethCp: ethyl-  
569 coprostanol, Sit: sitosterol, ethChln: ethyl-cholestanol, LC: lithocholic acid, DOC: deoxycholic  
570 acid, CDOC: chenodeoxycholic acid, C: cholic acid, HDOC: hyodeoxycholic acid and UDOC:  
571 ursodeoxycholic acid.

572

573 **Table 2:** Sterol and bile acids contents ( $\text{mg/g}$  and  $\mu\text{g/g}$ , respectively) and ratios of fecal sterols  
574 for the samples. Values of  $\text{Sr1} > 0.7$  indicate feces samples. Values of  $\text{Sr2} > 1$  indicate the  
575 predominance of herbivorous feces (Bull et al., 2002). Cp: coprostanol, epi-Cp: epi-  
576 coprostanol, Chl: cholesterol, Chln: cholestanol, mCp: methyl-coprostanol, ethCp: ethyl-  
577 coprostanol, Sit: sitosterol, ethChln: ethyl-cholestanol, LC: lithocholic acid, DOC: deoxycholic  
578 acid, CDOC: chenodeoxycholic acid, C: cholic acid, HDOC: hyodeoxycholic acid and UDOC:  
579 ursodeoxycholic acid.

580

#### 581 FIGURE CAPTIONS

582 **Fig. 1:** Location of study sites: In Greenland (a), Igaliku catchment (b; dotted line, LI – Lake  
583 Igaliku), Orléans archaeological site and Massif Central(c) with, for the latter the Aydat  
584 catchment (dotted line, d; LA – Lake Aydat) and the Montcineyre catchment (dotted line, e; LM  
585 - Lake Montcineyre).

586

587 **Fig.2:** Distribution of sterols and bile acids in animal fecal materials in reference samples (a, b,  
588 e and f). Note the different scales used between sterols and bile acids, and between herbivorous  
589 and omnivorous fecal biomarkers. Example of total ion current (TIC) chromatogram of sheep  
590 (c and d) and human (g and h) feces. For the nomenclature of compounds refer to Section 3.1.

591

592 **Fig. 3:** Distribution of sterols (a) and bile acids (b) in a septic tank active during the 19<sup>th</sup> century  
593 (St19) and in the layers of a midden from a building dating from the Middle Ages with unknown

594 occupation (16<sup>th</sup>, 15<sup>th</sup> and 14<sup>th</sup> centuries). Specific ion mass (m/z) chromatograms of alcohol  
595 fraction (m/Z = 215+255+213+368+383+398+484) and acidic fraction (m/Z =  
596 208+215+253+255+368) from sample S16 (c, d). Cp/epi-Cp and DOC/C ratios from human  
597 fecal material, septic tank and middenlayers samples (e). For the nomenclature of compounds  
598 refer to Section 3.1.

599

600 **Fig. 4:** Relative average concentrations of sterols and bile acids in soil lipid extracts (a,b). GC–  
601 MS chromatograms (TIC) of sample PS8 for alcohol and acidic fractions (c,d). Nomenclature  
602 of compounds is presented in section 3.1. 24 $\alpha$ :C<sub>24</sub>  $\alpha$ -hydroxycarboxylic acid; 22 $\omega$ :C<sub>22</sub>  $\omega$ -  
603 hydroxycarboxylic acid; 24 $\omega$ :C<sub>24</sub>  $\omega$ -hydroxycarboxylic acid; 26 $\omega$ :C<sub>26</sub>  $\omega$ -hydroxycarboxylic  
604 acid; C<sub>30</sub>-OH:C<sub>30</sub> *n*-alkanol; C<sub>32</sub>-OH:C<sub>32</sub>*n*-alkanol.

605

606 **Fig.5:** Evolution of Total Coprostanols (Cp + epi-Cp + mCp + epi-ethCp +ethCp), Sr2 ratio,  
607 LC and DOC contents in lacustrine sediments (a). Specific ion mass (m/Z) chromatograms of  
608 alcohol fraction (m/Z = 215+255+213+368+383+398+484) and acidic fraction (m/Z =  
609 208+215+253+255+368) of sampleMO-1are shown (b,c). Nomenclature of compounds is  
610 presented in section 3.1.

611

612 **Fig.6:** Evolution of Total Coprostanols (Cp + epi-Cp + mCp + epi-ethCp +ethCp), Sr2 ratio  
613 and DOC contents over the last two millennia in lacustrine sediments (a). Specific ion mass  
614 (m/Z) chromatograms of alcohol fraction (m/z = 215+255+213+368+383+398+484) and acidic  
615 fraction (m/Z = 208+215+253+255+368) of sample IGA-82 are shown (b,c). Nomenclatures of  
616 compounds are given in section 3.1.

617

618

619

620

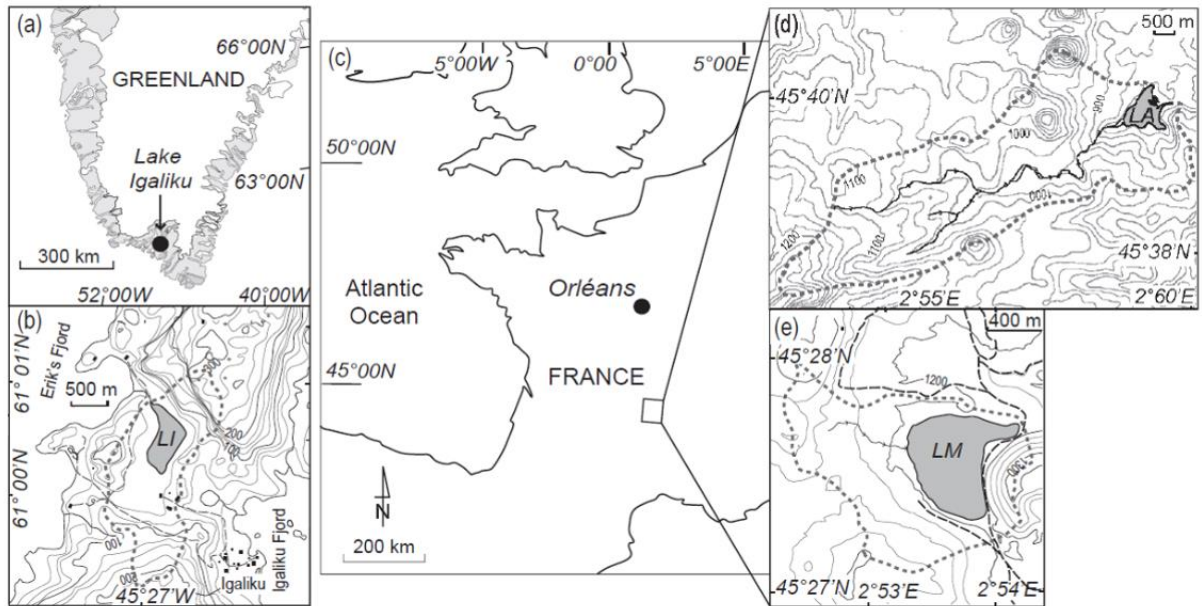
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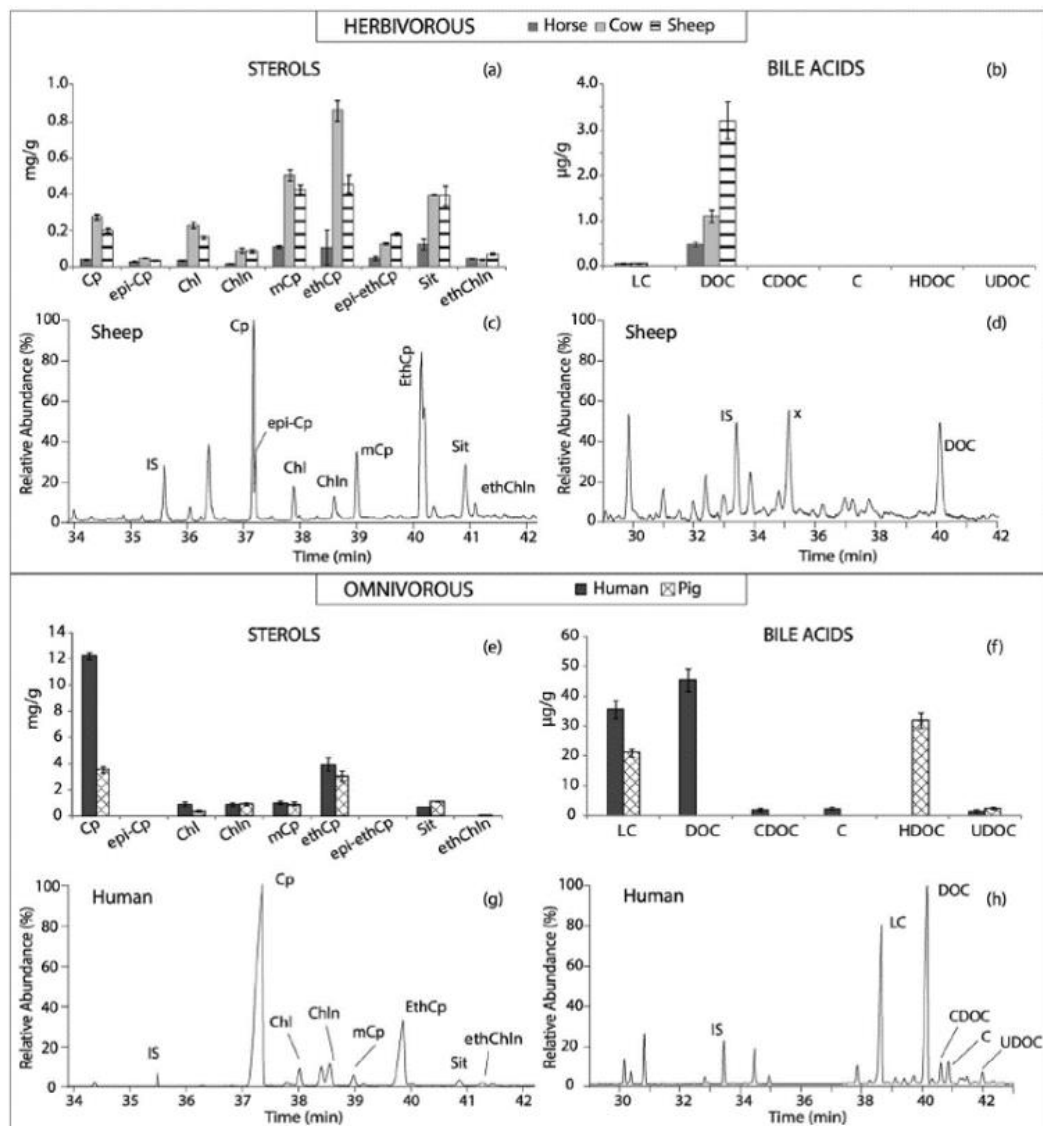
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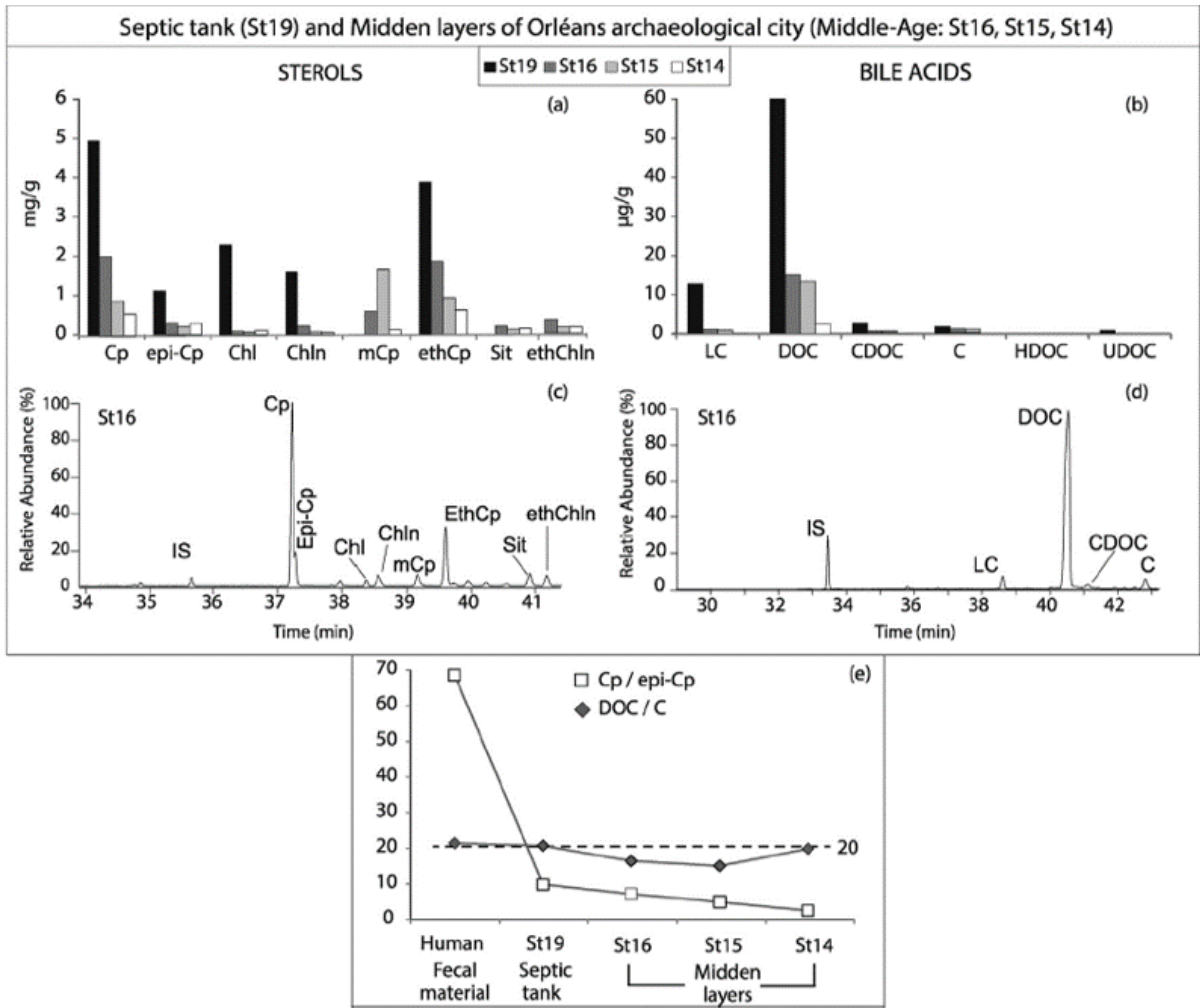
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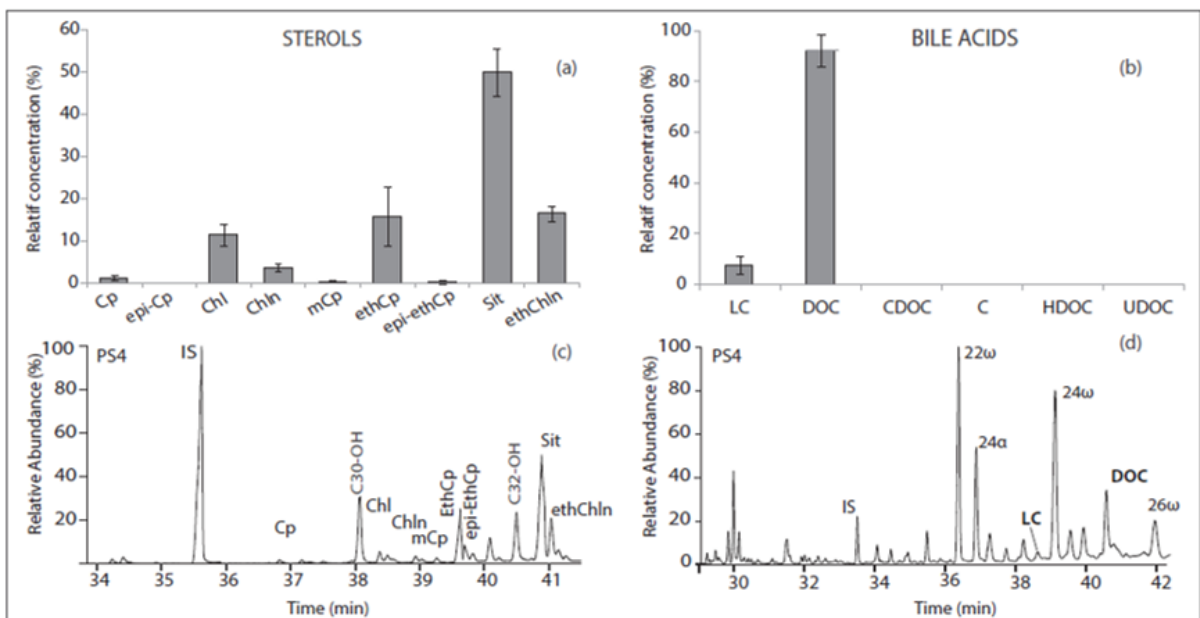
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629



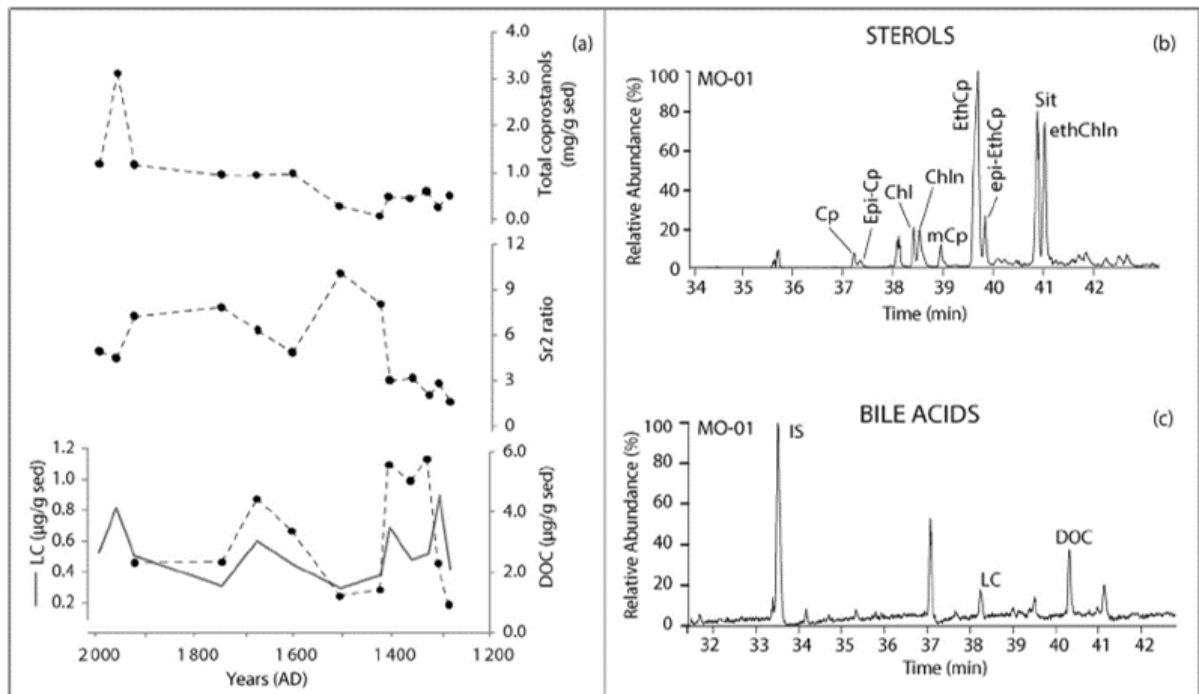
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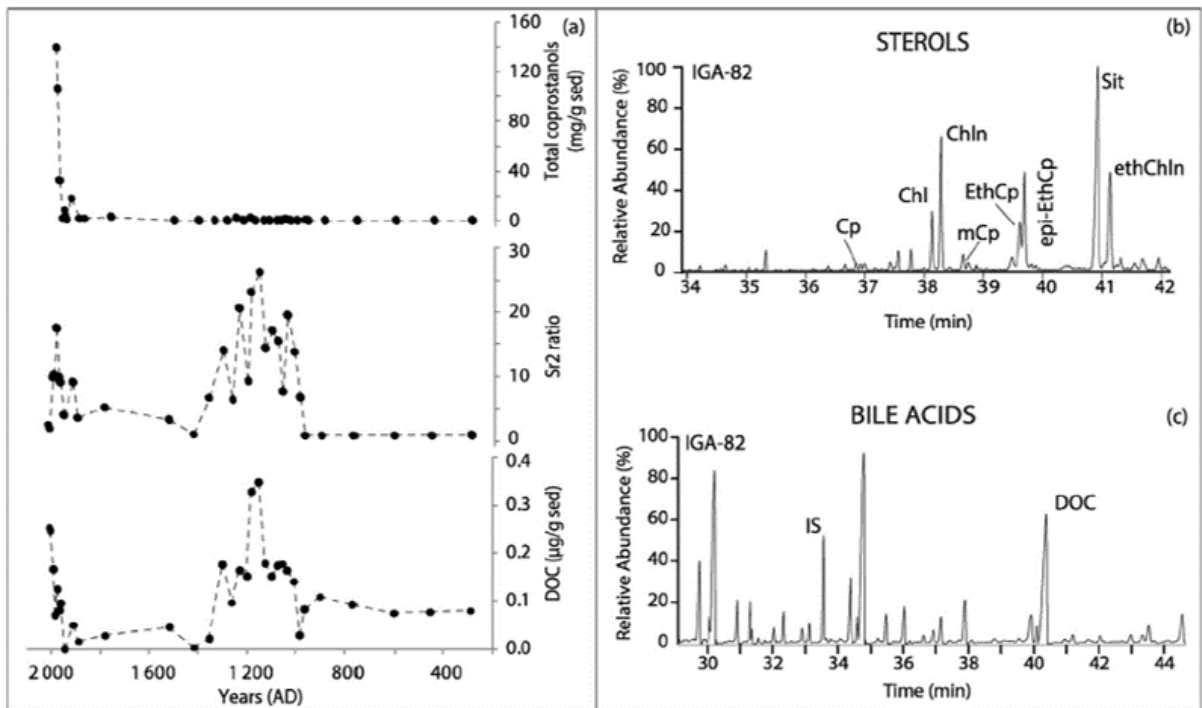
634

635 Fig. 5



636

637 Fig. 6



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639

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642

	Horse	Cow	Sheep	Human	Pig
<b>Sterols</b>					
Cp	40	275	203	12251	3552
epi-Cp	29	49	36	0	0
Chl	36	230	164	866	376
Chln	17	90	87	857	941
mCp	110	506	425	980	888
ethCp	109	859	456	3929	3093
epi-ethCp	48	128	182	0	0
Sit	124	395	392	707	1102
ethChln	44	40	72	0	80
Total Coprostanols	336	1817	1302	17160	7533
% coprostanols	60.33	70.64	64.55	87.70	76.29
Sr1	0.70	0.75	0.70	0.93	0.79
Sr2	2.29	3.05	2.67	0.32	0.76
<b>Bile Acids</b>					
LC	0.07	0.07	0.00	35.56	21.07
DOC	0.48	1.10	3.21	45.44	nd
CDOC	nd	nd	nd	2.15	nd
C	nd	nd	nd	1.79	nd
HDOC	nd	nd	nd	nd	32.01
UDOC	nd	nd	nd	1.24	2.31
Total Bile Acids	0.56	1.17	3.21	86.18	55.38

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658 Table 2:  
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	Cp	epi-Cp	Chl	Chln	mCp	ethCp	epi-ethCp	Sit	ethChln	Total Coprostanols	Coprostanols (%)	Sr1	Sr2	LC	DOC	CDOC	C	HDOC	UDOC
St19	10.54	1.12	2.29	1.61	0.00	3.89	0.00	0.00	0.00	15.55	79.98	0.88	0.33	12.85	60.12	0.00	2.95	0.00	0.10
St16	2.04	0.31	0.09	0.22	0.60	1.86	0.00	0.22	0.34	4.80	84.62	0.91	0.79	1.19	15.30	0.18	0.95	0.00	0.00
St15	0.91	0.20	0.06	0.08	0.55	0.94	0.00	0.14	0.15	2.60	85.80	0.94	0.84	1.03	13.45	0.17	0.92	0.00	0.00
St14	0.80	0.07	0.10	0.05	0.12	0.63	0.00	0.16	0.16	1.62	77.62	0.94	0.73	0.17	2.64	0.02	0.14	0.00	0.00
PS1	0.23	0.00	3.77	0.94	0.04	4.13	0.25	16.99	5.66	4.65	14.54	0.20	19.06	0.08	0.84	0.00	0.00	0.00	0.00
PS2	0.27	0.00	2.66	1.02	0.12	2.98	0.28	15.34	5.11	3.64	13.12	0.21	11.97	0.12	1.44	0.00	0.00	0.00	0.00
PS3	0.50	0.00	7.08	1.54	0.00	3.57	0.37	29.84	9.95	4.44	8.40	0.24	7.93	0.13	1.47	0.00	0.00	0.00	0.00
PS4	0.15	0.00	3.59	0.87	0.00	2.67	0.00	20.94	6.98	2.83	8.03	0.15	17.41	0.18	2.24	0.00	0.00	0.00	0.00
PS5	1.60	0.00	7.14	4.27	0.46	24.84	0.00	25.63	8.54	26.90	37.11	0.27	15.55	0.24	3.07	0.00	0.00	0.00	0.00
PS6	0.19	0.00	1.03	0.56	0.00	1.96	0.00	6.23	2.08	2.15	17.84	0.26	10.24	0.26	3.34	0.00	0.00	0.00	0.00
PS7	0.11	0.00	2.55	0.54	0.00	1.02	0.00	7.41	2.47	1.13	8.00	0.16	9.71	0.33	3.71	0.00	0.00	0.00	0.00
PS8	0.06	0.00	0.29	0.06	0.02	0.47	0.01	0.84	0.28	0.56	27.62	0.51	7.64	0.36	4.05	0.00	0.00	0.00	0.00
PS9	0.28	0.00	2.22	1.00	0.20	2.61	0.00	8.21	2.74	3.09	17.90	0.22	9.34	0.35	4.10	0.00	0.00	0.00	0.00
PS10	1.06	0.00	3.30	2.17	0.29	13.72	0.00	26.06	8.69	15.07	27.25	0.33	12.98	0.33	4.25	0.00	0.00	0.00	0.00
MO-0	0.11	0.08	8.88	1.40	0.06	0.81	0.13	14.05	5.19	1.19	3.88	0.12	4.92	0.53	5.05	0.00	0.00	0.00	0.00
MO-1	0.32	0.22	17.43	3.34	0.16	2.09	0.34	21.29	6.68	3.13	6.04	0.14	4.47	0.81	5.27	0.00	0.00	0.00	0.00
MO-2	0.09	0.05	7.75	1.20	0.06	0.83	0.13	13.91	4.84	1.16	4.01	0.10	7.22	0.51	2.33	0.00	0.00	0.00	0.00
MO-7	0.06	0.04	1.87	0.47	0.09	0.65	0.11	7.98	5.02	0.94	5.79	0.17	7.84	0.31	2.36	0.00	0.00	0.00	0.00
MO-9	0.06	0.05	2.43	0.45	0.15	0.59	0.10	15.69	11.58	0.95	3.06	0.19	6.31	0.60	4.45	0.00	0.00	0.00	0.00
MO-11	0.07	0.06	4.32	0.55	0.21	0.54	0.08	12.36	8.83	0.96	3.56	0.19	4.83	0.45	3.38	0.00	0.00	0.00	0.00
MO-14	0.01	0.01	0.81	0.08	0.02	0.21	0.03	2.95	2.24	0.29	4.53	0.24	10.11	0.30	1.21	0.00	0.00	0.00	0.00
MO-17	0.00	0.00	0.03	0.01	0.00	0.06	0.01	0.63	0.27	0.08	7.90	0.44	8.13	0.38	1.43	0.00	0.00	0.00	0.00
MO-25	0.02	0.04	1.58	0.35	0.20	0.16	0.04	6.51	3.32	0.46	3.80	0.16	3.02	0.69	5.59	0.00	0.00	0.00	0.00
MO-45	0.03	0.03	1.61	0.40	0.22	0.15	0.03	5.90	4.57	0.45	3.51	0.12	3.17	0.48	5.06	0.00	0.00	0.00	0.00

MO-60	0.03	0.05	1.81	0.45	0.35	0.14	0.02	6.73	2.25	0.58	4.94	0.15	2.09	0.52	5.77	0.00	0.00	0.00	0.00
MO-70	0.02	0.02	0.98	0.25	0.10	0.10	0.02	6.90	1.95	0.26	2.55	0.15	2.77	0.89	2.32	0.00	0.00	0.00	0.00
MO-80	0.04	0.06	1.62	0.37	0.21	0.15	0.02	6.61	1.64	0.48	4.50	0.22	1.61	0.42	0.94	0.00	0.00	0.00	0.00
IGA-01	38.61	0.00	154.59	130.56	0.00	100.60	0.00	89.55	14.92	139.20	26.32	0.23	2.61	0.00	0.25	0.00	0.00	0.00	0.00
IGA-03	34.87	0.00	103.57	106.38	0.00	71.71	0.00	75.08	12.51	106.58	26.37	0.25	2.06	0.00	0.25	0.00	0.00	0.00	0.00
IGA-07	3.04	0.00	53.52	57.13		29.63	0.00	66.45	11.07	32.67	14.79	0.05	9.75	0.00	0.17	0.00	0.00	0.00	0.00
IGA-09	0.23	0.00	2.91	3.81	0.00	2.41	0.00	4.06	0.68	2.64	18.73	0.06	10.42	0.00	0.07	0.00	0.00	0.00	0.00
IGA-11	0.50	0.00	12.23	11.55	0.00	8.64	0.00	12.86	2.14	9.14	19.07	0.04	17.44	0.00	0.13	0.00	0.00	0.00	0.00
IGA-12	0.28	0.00	3.52	6.36	0.00	2.71	0.00	3.47	0.58	2.98	17.63	0.04	9.79	0.00	0.08	0.00	0.00	0.00	0.00
IGA-13	0.14	0.00	1.56	2.84	0.00	1.31	0.00	2.45	0.41	1.45	16.66	0.05	9.09	0.00	0.09	0.00	0.00	0.00	0.00
IGA-15	3.73	0.00	27.75	53.70	0.00	14.88	0.00	33.49	5.49	18.61	13.39	0.07	3.98	0.00	0.00	0.00	0.00	0.00	0.00
IGA-19	0.28	0.00	5.74	7.74	0.00	1.02	1.50	4.76	0.77	2.80	12.86	0.03	9.08	0.00	0.05	0.00	0.00	0.00	0.00
IGA-21	0.60	0.00	12.20	12.69	0.00	0.91	1.30	15.19	2.47	2.81	6.19	0.05	3.69	0.00	0.02	0.00	0.00	0.00	0.00
IGA-29	0.51	0.00	6.44	6.48	0.00	1.22	1.50	5.34	0.87	3.23	14.46	0.07	5.30	0.00	0.03	0.00	0.00	0.00	0.00
IGA-45	0.01	0.00	0.05	0.05	0.00	0.01	0.02	0.05	0.01	0.04	20.08	0.16	3.22	0.00	0.05	0.00	0.00	0.00	0.00
IGA-52	0.01	0.00	0.03	0.02	0.00	0.00	0.01	0.02	0.00	0.02	26.27	0.40	1.13	0.00	0.00	0.00	0.00	0.00	0.00
IGA-57	0.01	0.00	0.15	0.14	0.00	0.02	0.03	0.13	0.02	0.07	13.00	0.06	6.79	0.00	0.02	0.00	0.00	0.00	0.00
IGA-62	0.02	0.00	0.31	0.34	0.00	0.12	0.15	0.43	0.07	0.29	20.09	0.05	13.96	0.00	0.18	0.00	0.00	0.00	0.00
IGA-66	0.30	0.00	6.57	8.77	0.00	0.76	1.13	5.56	0.93	2.19	9.13	0.03	6.27	0.00	0.10	0.00	0.00	0.00	0.00
IGA-70	0.01	0.00	0.37	0.34	0.00	0.06	0.09	0.38	0.06	0.16	12.24	0.02	20.60	0.00	0.16	0.00	0.00	0.00	0.00
IGA-74	0.20	0.00	4.77	6.83	0.00	1.49	0.37	4.17	0.69	2.06	11.13	0.03	9.23	0.00	0.15	0.00	0.00	0.00	0.00
IGA-77	0.01	0.00	0.25	0.36	0.00	0.11	0.16	0.45	0.07	0.28	20.07	0.03	23.19	0.00	0.33	0.00	0.00	0.00	0.00
IGA-82	0.02	0.00	0.07	0.08	0.00	0.22	0.31	0.12	0.02	0.56	65.53	0.21	26.28	0.00	0.35	0.00	0.00	0.00	0.00
IGA-87	0.01	0.00	0.33	0.38	0.00	0.09	0.13	0.34	0.06	0.23	17.06	0.04	14.35	0.00	0.18	0.00	0.00	0.00	0.00
IGA-92	0.01	0.00	0.50	0.43	0.00	0.08	0.12	0.60	0.10	0.22	11.90	0.03	17.12	0.00	0.15	0.00	0.00	0.00	0.00
IGA-96	0.01	0.00	0.26	0.28	0.00	0.04	0.07	0.26	0.04	0.12	12.27	0.02	15.40	0.00	0.17	0.00	0.00	0.00	0.00
IGA-99	0.11	0.00	2.63	2.69	0.00	0.35	0.48	1.76	0.29	0.93	11.22	0.04	7.55	0.00	0.18	0.00	0.00	0.00	0.00
IGA-102	0.01	0.00	0.47	0.40	0.00	0.07	0.10	0.56	0.09	0.18	10.67	0.02	19.47	0.00	0.16	0.00	0.00	0.00	0.00
IGA-106	0.02	0.00	0.41	0.55	0.00	0.09	0.12	0.50	0.08	0.22	12.72	0.03	13.89	0.00	0.14	0.00	0.00	0.00	0.00
IGA-109	0.22	0.00	3.45	6.27	0.00	0.63	0.87	3.77	0.63	1.73	10.89	0.03	6.70	0.00	0.03	0.00	0.00	0.00	0.00

IGA-111	0.01	0.00	0.21	0.45	0.00	0.03	0.05	0.14	0.01	0.09	9.48	0.01	13.06	0.00	0.08	0.00	0.00	0.00	0.00
IGA-118	0.01	0.00	0.28	0.45	0.00	0.06	0.08	0.23	0.01	0.15	13.29	0.02	19.80	0.00	0.11	0.00	0.00	0.00	0.00
IGA-132	0.01	0.00	0.33	0.45	0.00	0.07	0.10	0.38	0.02	0.18	13.21	0.03	14.22	0.00	0.09	0.00	0.00	0.00	0.00
IGA-147	0.02	0.00	0.54	0.52	0.00	0.07	0.10	0.43	0.02	0.19	11.12	0.03	10.66	0.00	0.07	0.00	0.00	0.00	0.00
IGA-161	0.01	0.00	0.54	0.51	0.00	0.10	0.14	0.46	0.03	0.25	14.16	0.03	17.68	0.00	0.08	0.00	0.00	0.00	0.00
IGA-175	0.03	0.00	0.62	0.57	0.00	0.07	0.10	0.54	0.03	0.20	10.05	0.05	6.33	0.00	0.08	0.00	0.00	0.00	0.00

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