



HAL
open science

Fecal biomarker imprints as indicators of past human land uses: Source distinction and preservation potential in archaeological and natural archives

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

► To cite this version:

Renata Zocatelli, Marlène Lavrieux, Typhaine Guillemot, Léo Chassiot, Claude Le Milbeau, et al.. Fecal biomarker imprints as indicators of past human land uses: Source distinction and preservation potential in archaeological and natural archives. *Journal of Archaeological Science*, 2017, 81, pp.79 - 89. 10.1016/j.jas.2017.03.010 . insu-01503523

HAL Id: insu-01503523

<https://insu.hal.science/insu-01503523v1>

Submitted on 7 Apr 2017

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial - NoDerivatives 4.0 International License

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^{1,2,3}, Marlène LAVRIEUX⁴, Typhaine GUILLEMOT⁵, Léo
6 CHASSIOT^{1,2,3}, Claude Le MILBEAU^{1,2,3}, Jérémy JACOB^{1,2,3}.

7
8 ¹ Université d'Orléans, Institut des Sciences de la Terre d'Orléans (ISTO), UMR 7327, 45071 Orléans,
9 France

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

11 ³ BRGM, ISTO, UMR 7327, BP 36009, 45060 Orléans, France

12 ⁴ Environmental Geosciences, University of Basel, Bernoullistrasse 30, 4056 Basel, Switzerland

13 ⁵ 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 β -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 β -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 β -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 β -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 β -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.

36 .
37
38

Keywords: past agropastoral dynamics, fecal sterols, bile acids, archaeological and natural
39 archives.

40
41 Corresponding author e-mail address: renata.zoccatelli1@univ-orleans.fr (R. Zocatelli).

42

43

44

45

46

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 β -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 CH₂Cl₂:MeOH (9:1 v/v). Total lipid extract was fractionated into neutral and
141 acidic compounds using solid phase extraction on Supelclean LC-NH₂ SPE bulk packing
142 (Zocatelli et al., 2012). Neutral compounds were eluted with CH₂Cl₂: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 N₂. 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 H₂O, 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 CH₂Cl₂,
151 CH₂Cl₂: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 α -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 μ 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⁻¹, then from 120°C to 300°C at 5°C.min⁻¹, 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⁻¹). 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 α -stanols, coprostanol (Cp),
174 epi-coprostanol (epi-Cp), methylcoprostanol (mCp), epi-ethylcoprostanol (epi-ethCp) and
175 ethylcoprostanol (ethCp)]; two 5 α -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 (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 β -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 β -
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, Sr² ratio and DOC contents during the last two millennia. The Sr²
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, Sr² 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 Sr² 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

363 **Acknowledgments**

364 The work received financial support from the OTARIE Project (Région Centre, European
365 Council) and the ERODE Project (EC2CO, CNRS/INSU). We thank the Institut National de la
366 Recherche Agronomique (INRA) for the reference samples, Pascal Joyeux (INRAP), and
367 Emmanuel Chapron (GEODE UMR 5602 CNRS, University of Toulouse). We also thank the
368 French Polar Institute (IPEV) and the ANR CEP&S “Green Greenland” project (ANR-10-
369 CELP-0008) and particularly Vincent Bichet, Emilie Gauthier, Hervé Richard (Chrono-
370 environnement UMR 6249, University Franche-Comté) and anonymous donors for sampling
371 authorizations and/or providing some of the collection and reference samples. We would also
372 like to thank the two anonymous reviewers for suggestions and comments which have helped
373 to improve the quality of this manuscript.

374

375 **5. References**

- 376 Arneborg, J., 2007. Saga trails. In: Brattahlid G (ed.) Hvalsey Fjord’s Church and Heljolfnesnes:
377 Four Chieftain’s Framsteads in the North Settlements of Greenland. A Visitor’s
378 Guidebook. Copenhagen: The National Museum of Denmark, 94 pp.
- 379 Austrheim, G., Asheim, L.-J., Bjarnason, G., Feilberg, J., Fosaa, A. M., Holand, Ø., Jónsdóttir,
380 I. S., Magnússon, B., Mortensen, L. E., Mysterud, A., Olsen, E., Skonhoft, A.,
381 Steinheim, G., Thórhallsdóttir, A. G. 2008: Sheep grazing in the North-Atlantic region – a
382 longterm perspective on management, resource economy and ecology. Norges Teknisk-
383 Naturvitenskapelige Universitet, Vitenskapsmuseet, Trondheim, Rapport Zoologisk Serie
384 2008-3, 1–86.
- 385 Baeten, J., Marinova, E., De Laet, V., Degryse, P., De Vos, D., Waelkens, M., 2012. Fecal
386 biomarker and archaeobotanical analyses of sediments from a public latrine shed new light
387 on ruralisation in Sagalassos, Turkey. *Journal of Archaeological Science* 39, 1143–1159.
- 388 Battarbee, R.W., Bennion, H., 2011. Palaeolimnology and its developing role in assessing the
389 history and extent of human impact on lake ecosystems. *Journal of Paleolimnology* 45,
390 399–404.
- 391 Bayard, A., 2010. Les lieux élitaires du premier Moyen-âge en Auvergne: vecteurs de création
392 de réseaux et de production de territoires? Apports croisés des données textuelles et
393 matérielles. PhD Thesis. Université Paris I-Panthéon-Sorbonne, 81–104.
- 394 Bethell, P.H., Goad, L.J., Evershed, R.P., Ottaway, J., 1994. The study of molecular markers
395 of human activity: the use of coprostanol in the soil as an indicator of human fecal
396 material. *Journal of Archaeological Science* 21, 619–632.
- 397 Braje, T.J., Erlandson, J.M., 2013. Human acceleration of animal and plant extinctions: A Late
398 Pleistocene, Holocene and Anthropocene continuum. *Anthropocene* 4, 14–23.
- 399 Bull, I.D., Elhmmali, M.M., Roberts, D.J., Evershed, R.P., 2003. The application of steroidal
400 biomarkers to track the abandonment of a Roman waste water course at the agora (Athens,
401 Greece). *Archaeometry* 45, 149–161.
- 402 Bull, I.D., Lockheart, M.J., Elhmmali, M.M., Roberts, D.J., Evershed, R.P., 2002. The origin
403 of faeces by means of biomarker detection. *Environment International* 27, 647–654.

- 404 Bull, I.D., Betancourt, P.P., Evershed, R.P., 2001. An organic geochemical investigation of the
405 practice of manuring at a Minoan site on Pseira Island, Crete. *Geoarchaeology* 16, 223–242.
- 406 Bull, I.D., Betancourt, P.P., Evershed, R.P., 1999a. Chemical evidence for a structured
407 agricultural manuring regime on the island of Pseira, Crete during the Minoan Period,
408 *Aegaeum* 20, 69–74.
- 409 Bull, I.D., Simpson, I.A., Dockrill, S.J., Evershed, R.P., 1999b. Organic geochemical evidence
410 for the origin of ancient anthropogenic soil deposits at Tofts Ness, Sanday, Orkney. *Organic*
411 *Geochemistry* 30, 535–556.
- 412 Bull, I.D., Simpson, I.A., Van Bergen, P.F., Evershed, R.P., 1999c. Muck ‘n’ molecules:
413 Organic geochemical methods for detecting ancient manuring. *Antiquity* 73: 86–96.
- 414 Bull, I.D., van Bergen, P.F., Poulton, P.R., Evershed, R.P., 1998. Organic geochemical studies
415 of soil from the Rothamsted Classical Experiments II, soils from Hoosfield Spring Barley
416 experiment treated with different quantities of manure. *Organic Geochemistry* 28, 11–26.
- 417 Cappelen, J., 2014. Weather observations from Greenland 1958–2013. Observations data with
418 description Technical report, No. 14-08, 24 pp. Danish Meteorological Institute,
419 Copenhagen.
- 420 Charbonnier, P., 1999. Histoire de l’Auvergne des origines à nos jours: Haute et Basse-
421 Auvergne, Bourbonnais et Velay. Editions de Borée, 540pp.
- 422 Chassiot, L., Chapron, E., Di Giovanni, C., Lajeunesse, P., Tachikawa, K., Garcia, M., Bard,
423 E., 2016. Historical seismicity of the Mont Dore volcanic province (Auvergne, France)
424 unraveled by a regional lacustrine investigation: New insights about lake sensitivity to
425 earthquakes. *Sedimentary Geology* 339, 134–150.
- 426 Chassiot, L., 2015. Forçages naturels et anthropiques sur la sédimentation holocène en domaine
427 lacustre. Application aux lacs naturels d’Auvergne et aux réservoirs des bassins versants de
428 la Loire et d’Adour-Garonne. PhD Thesis, Université d’Orléans, 500pp.
- 429 D’Anjou, R.M., Bradley, R.S., Balascio, N.L., Finkelstein, D.B., 2012. Climate impacts on
430 human settlement and agricultural activities in northern Norway revealed through sediment
431 biogeochemistry. *Proceedings of the National Academy of Sciences* 109, 20332–20337.
- 432 Dearing, J.A., Battarbee, R.W., Dikau, R., Larocque, I., Oldfield, F., 2006. Human-
433 environment interactions: learning from the past. *Regional Environmental Change* 6:1, 1-
434 16.
- 435 Edvardsson, R., Paulsen, C., Church, M., Simpson, I., Adderly, P., Palsdottir, A., McGovern,
436 T.H., 2007. Archaeological excavations at Qassiarsuk 2005 - 2006. Field and data structure
437 report 03–07, 108 pp.
- 438 Elhmmali, M.M., Roberts, D.J., Evershed, R.P., 2000. Combined analysis of bile acids and
439 sterols/stanols from riverine particulates to assess sewage discharges and other fecal
440 sources. *Environmental Science and Technology* 34, 39–46.
- 441 Elhmmali, M.M., Roberts, D.J., Evershed, R.P., 1997. Bile acid as a new class of sewage
442 pollution indicator. *Environmental Science Technology* 31, 3663–3668.
- 443 Ellis, E.C., Fuller, D.Q., Kaplan, J.O., Lutters, W.G., 2013. Dating the Anthropocene: Towards
444 an empirical global history of human transformation of the terrestrial biosphere. *Elementa: Science of the Anthropocene* 1, 1–6. doi: 10.12952/journal.elementa.000018.
- 445
446 Evershed, R.P., Bethell, P.H., Reynolds, P.J., Walsh, N.J., 1997. 5 β -stigmastanol and related
447 5 β -stanols as biomarkers of manuring: analysis of modern experimental material and
448 assessment of the archaeological potential. *Journal of Archaeological Science* 24, 485–495.
- 449 Foley, J.A., Defries, R., Asner G.P., Barford, C., Bonan, G., 2005. Global consequences of land
450 use. *Science* 309, 570–574.
- 451 Gaskell, S.J., Eglinton, G., 1974. Short-term diagenesis of sterols. In: *Advances in organic*
452 *geochemistry*, 1973. Tissot, B., Bienner, F. (Eds), Rueil-Malmaison, France, 963–976.
- 453 Gauthier, E., Bichet, V., Massa, C., Petit, ., Vannièrè, B., Richard, H., 2010. Pollen and non-
454 pollen palynomorph evidence of medieval farming activities in southwestern Greenland.
455 *Vegetation History and Archaeobotany* 19, 427–438.

- 456 Grimalt, J.O., Fernandez, P., Bayona, J.M., Albaiges, J., 1990. Assessment of fecal sterols and
457 ketones as indicators of urban sewage inputs to coastal waters. *Environmental Science*
458 *Technology* 24, 357–363.
- 459 Guillemot, T., Bichet, V., Gauthier, E., Zocatelli, R., Massa, C., Richard, H., 2016.
460 Environmental responses of past and recent agropastoral activities on south Greenlandic
461 ecosystems through molecular biomarkers. *The Holocene* 27:6, 1–13.
- 462 Guillemot, T., Zocatelli, R., Bichet, V., Jacob, J., Gauthier, E., Massa, C., Le Milbeau, C.,
463 Richard, H., 2015. Evolution of pastoralism in Southern Greenland during the last two
464 millennia reconstructed from bile acids and coprophilous fungal spores in lacustrine
465 sediments. *Organic Geochemistry* 81, 40–44.
- 466 Hurtt, G.C., Frohling, S., Fearon, M.G., Moore, B., Shevliakova, E., Malyshev, S., Pacala S.W.,
467 Houghton, R.A., 2006. The underpinnings of land-use history: three centuries of global
468 gridded land-use transitions, wood harvest activity, and resulting secondary lands. *Global*
469 *Change Biology* 12, 1208–1229.
- 470 IPCC 2013. *Climate Change 2013: The Physical Science Basis: A Report of Working Group I*
471 *of the Intergovernmental Panel on Climate Change*. IPCC.
- 472 Jacob, J., Disnar, J.-R., Arnaud, F., Gauthier, E., Billaud, Y., Chapron, E., Bardoux, G., 2009.
473 Impacts of new agricultural practices on soil erosion during the Bronze Age in the French
474 Prealps. *The Holocene* 19, 241–249.
- 475 Jacob, J., Disnar, J.-R., Arnaud, F., Chapron, E., Debret, M., Lallier-Vergès, E., Desmet, M.,
476 Revel-Rolland, M., 2008. Millet cultivation history in the French Alps as evidenced by a
477 sedimentary molecule. *Journal of Archaeological Sciences* 35, 814–820.
- 478 Jardé, E., Gruau, G., Mansuy-Huault, L., 2007a. Detection of manure-derived organic
479 compounds in rivers draining agricultural areas of intensive manure spreading. *Applied*
480 *Geochemistry* 22, 1814–1824.
- 481 Jardé, E., Gruau, G., Mansuy-Huault, L., Peu, P., Martinez, J., 2007b. Using sterols to detect
482 pig slurry contribution to soil organic matter. *Water, Air and Soil Pollution* 178, 169–178.
- 483 Joyeux, P., 2013. *Mémoire de fouilles : Une nouvelle Orléans, Des secrets bien enfouis sous la*
484 *2de ligne de tramway*. INRAP, Institut national de recherches archéologiques préventives.
485 40pp.
- 486 Killops, S.D., Killops, V.J., 2004. *An Introduction to Organic Geochemistry*, second ed.
487 Blackwell Publishing, Oxford, 408 pp.
- 488 Lavrieux, M., Jacob, J., Disnar, J.-R., Bréheret, J.-G., Le Milbeau, C., Miras, Y., Andrieu-
489 Ponel, V., 2013a. Sedimentary cannabiniol tracks the history of hemp retting. *Geology* 41,
490 751–754.
- 491 Lavrieux, M.L., Bréheret, J.G., Disnar, J.-R., Jacob, J., Le Milbeau, C., Zocatelli, R., 2012.
492 Preservation of an ancient grassland biomarker signature in a forest soil from the French
493 Massif Central. *Organic Geochemistry* 51, 1–10.
- 494 Lavrieux, M., Jacob, J., Le Milbeau, C., Zocatelli, R., Masuda, K., Bréheret, J.G., Disnar, J.-
495 R., 2011. Occurrence of triterpenyl acetates in soil and their potential as chemotaxonomical
496 biomarkers of Asteraceae. *Organic Geochemistry* 42, 1315–1323.
- 497 Leeming, R., Ball, A., Ashbolt, N., Nichols, P., 1996. Using fecal sterols from humans and
498 animals to distinguish fecal pollution in receiving waters. *Water Research* 30, 2893–2900.
- 499 Madsen, C.K., 2014. *Pastoral settlement, farming and hierarchy in Norse Vatnahverfi, South*
500 *Greenland*. PhD Thesis, University of Copenhagen, 440 pp.
- 501 Massa, C., Bichet, V., Gauthier, E., Perren, B.B., Mathieu, O., Petit, C., Monna, F., Giraudeau,
502 J., Losno, R., Richard, H., 2012. A 2500 year record of natural and anthropogenic soil
503 erosion in South Greenland. *Quaternary Science Reviews* 32, 119–130.
- 504 McGovern, T.H., 1983. The zooarcheology of Ø17a. *Meddelelsero Grønland Man and Society*
505 18, 58–74.

506 Mermoud, F., Wunsche, L., Clerc, O., Gulacar, F.O. & Buchs, A., 1984. Steroidal ketones in
507 the early diagenetic transformations of Δ^5 sterols in different types of sediments. *Organic*
508 *Geochemistry* 6, 25–29.

509 Nes, W.R., Nes, W.D., 1980. *Lipids in evolution*. New York: Plenum Press, 244pp.

510 Oldfield, F., Wake, R., Boyle, J., Jones, R., Nolan, S., Gibbs, Z., Appleby, P., Fisher, E., Wolff,
511 G., 2003. The late-Holocene history of Gormire Lake (NE England) and its catchment: a
512 multiproxy reconstruction of past human impact. *The Holocene* 13, 677–690.

513 Pepe, C., Dizabo, P., 1990. Étude d'une fosse du 13^{ème} siècle par les marqueurs
514 biogéochimiques: chantier archéologique du Louvre (Paris). *Revue d'Archéométrie* 14, 23–
515 28.

516 Redman, C.L., 1999. *Human Impact on Ancient Environments*. University of Arizona Press,
517 Tucson, 288pp.

518 Ruddiman, W.F., 2013. The Anthropocene. *Annual Review of Earth and Planetary Sciences*
519 41, 45–68.

520 Ruddiman, W.F., 2003. The Anthropogenic greenhouse era began thousands of years ago.
521 *Climate Change* 61, 261–293. (doi:10.1023/B:CLIM.0000004577.17928.fa)

522 Rushdi, A.I., Douabul, A.A., Mohammed, S.S., Simoneit, B.R.T., 2006. Compositions and
523 sources of extractable organic matter in Mesopotamian marshland surface sediments of
524 Iraq: II. Polar compounds. *Environmental Geology* 50, 1171–1181.

525 Shillito, L.-M., Bull, I.D., Matthew, W., Almond, M.A., Williams, J.M., Evershed, R.P., 2011a.
526 Biomolecular and micromorphological analysis of suspected fecal deposits at Neolithic
527 Çatalhöyük, Turkey. *Journal of Archaeological Science* 38, 1869–1877.

528 Shillito, L.-M., Matthews, W., Almond, M.J., Bull, I.D., 2011b. The microstratigraphy of
529 middens: capturing daily routine in rubbish at Neolithic Çatalhöyük, Turkey. *Antiquity* 85,
530 1024–1038.

531 Simoneit, B.R.T., 2002. Molecular indicators (biomarkers) of past life. *The Anatomical Record*
532 268, 186–195.

533 Simpson, I.A., Bull, I.D., Dockrill, S.J., Evershed, R.P., 1998. Early anthropogenic soil
534 formation at Tofts Ness, Sanday, Orkney. *Journal of Archaeological Science* 25, 729–746.

535 Smith, B.D., Zeder, M.A., 2013. The onset of the Anthropocene. *Anthropocene*,
536 <http://dx.doi.org/10.1016/j.ancene.2013.05.001>.

537 Syvitski, J.P.M., Kettner, A. 2011. Sediment flux and the Anthropocene. *Philosophical*
538 *Transactions of the Royal Society A: Mathematical, Physical and Engineering Sciences*
539 369, 957–975.

540 Syvitski, J.P.M., Kettner, A., Peckham, S.D., Kao, S.J. 2005. Predicting the flux of sediment to
541 the coastal zone: application to the Lanyang watershed, northern Taiwan. *J. Coast. Res.* 21,
542 580–587. (doi:10.2112/04-702A.1).

543 Tyagi, P., Edwards, D.R., Coyne, M.S., 2008. Use of Sterol and Bile Acid Biomarkers to
544 Identify Domesticated Animal Sources of Fecal Pollution. *Water Air Soil Pollution* 187,
545 263–274.

546 van Bergen, P., Bull, I., Poulton, P.R., Evershed, R.P., 1997. Organic geochemical studies of
547 soils from the Rothamsted Classical Experiments – I. Total lipid extracts, solvent insoluble
548 residues and humic acids from Broadbalk Wilderness. *Organic Geochemistry* 26, 117–135.

549 Zeder, M.A., Bradley, D.G., Emshwiller, E., Smith, B.D. (Eds.), 2006. *Documenting*
550 *Domestication: New Genetic and Archaeological Paradigms*. University of California
551 Press, Berkeley.

552 Zocatelli, R., Moreira-Turcq, P., Bernardes, M., Turcq, B., Cordeiro, R.C., Gogo, S., Disnar,
553 J.-R., Boussafir, M., 2013. Sedimentary evidence of soil organic matter input to the Curuai
554 Amazonian floodplain. *Organic Geochemistry* 63, 40–47.

555 Zocatelli, R., Lavrieux, M., Disnar, J.-R., Le Milbeau, C., Jacob, J., Bréheret, J.-G., 2012. Free
556 fatty acids in Lake Aydat catchment soils (French Massif Central): sources, distributions
557 and potential use as sediment biomarkers. *Journal of Soils and Sediments* 12, 734–748.

558 Zocatelli, R., Jacob, J., Turcq, B., Boussafir, M., Sifeddine, A., Bernardes, M.C., 2010.
559 Molecular evidence for recent turf cultivation in Northeast Brazil (Lagoa do Boqueirão, RN
560 State). *Organic Geochemistry* 41, 427–430.
561 Zolitschka, B. and Ariztegui, D., 2007. Lacustrine and marine archives of environmental
562 variability across South America. *Quaternary International* 161, 1–3.
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 (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 19th century
593 (St19) and in the layers of a midden from a building dating from the Middle Ages with unknown

594 occupation (16th, 15th and 14th 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 α :C₂₄ α -hydroxycarboxylic acid; 22 ω :C₂₂ ω -
603 hydroxycarboxylic acid; 24 ω :C₂₄ ω -hydroxycarboxylic acid; 26 ω :C₂₆ ω -hydroxycarboxylic
604 acid; C₃₀-OH:C₃₀ *n*-alkanol; C₃₂-OH:C₃₂*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

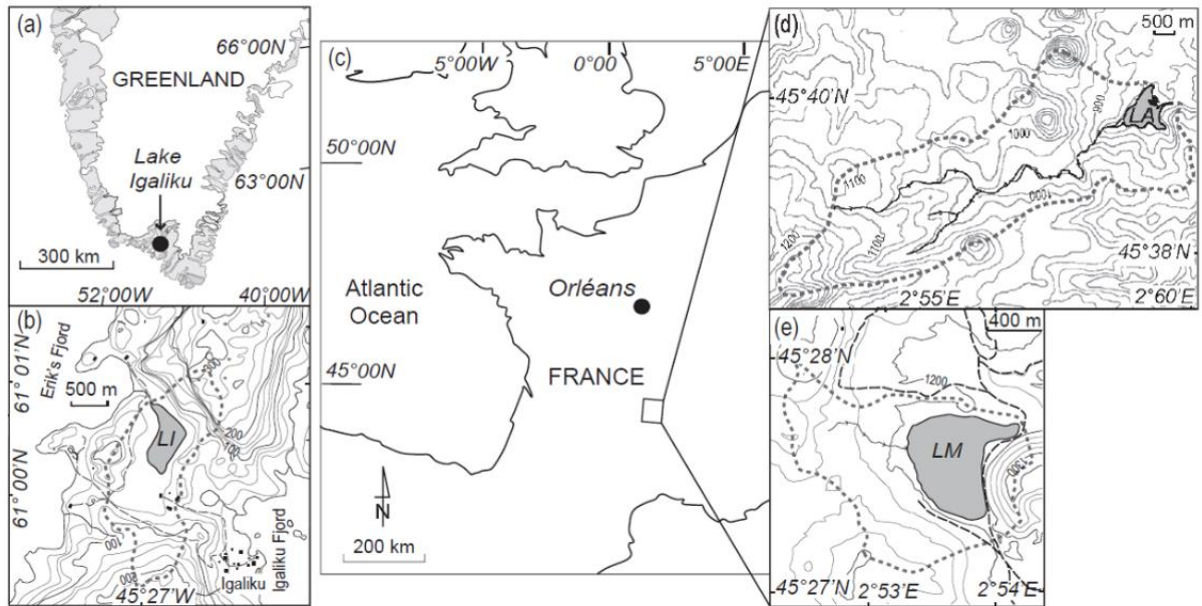
621

622

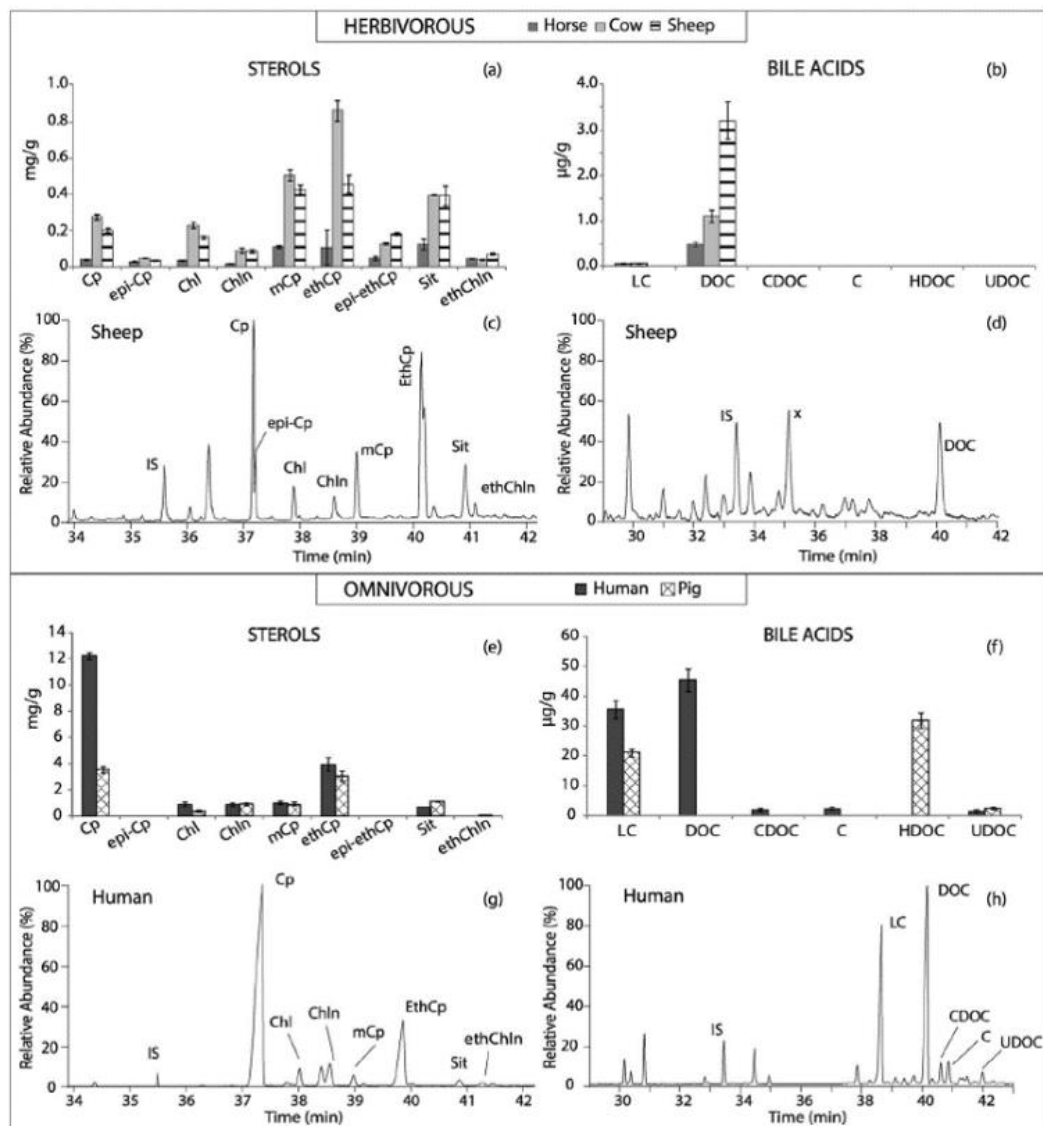
623

624

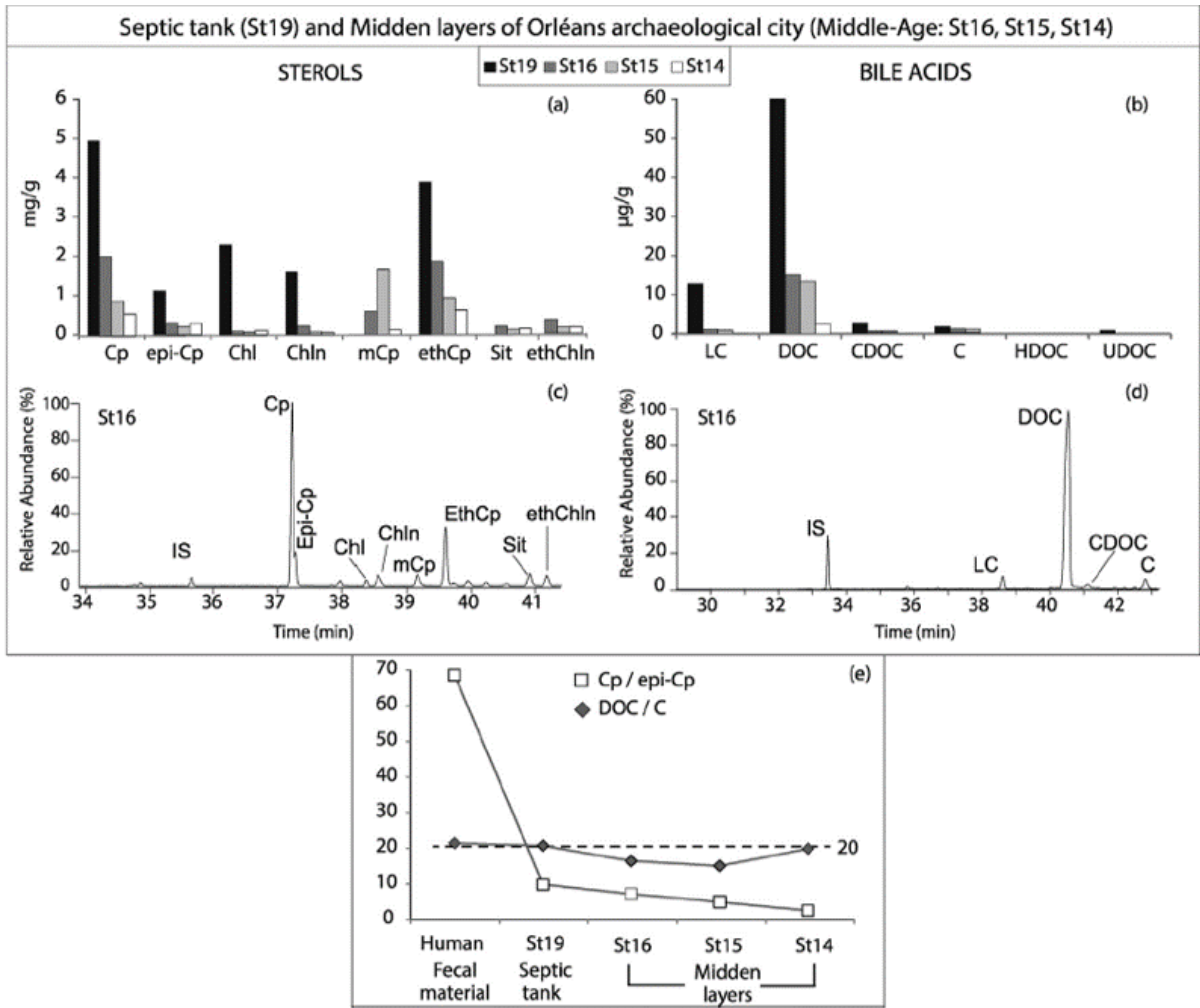
625



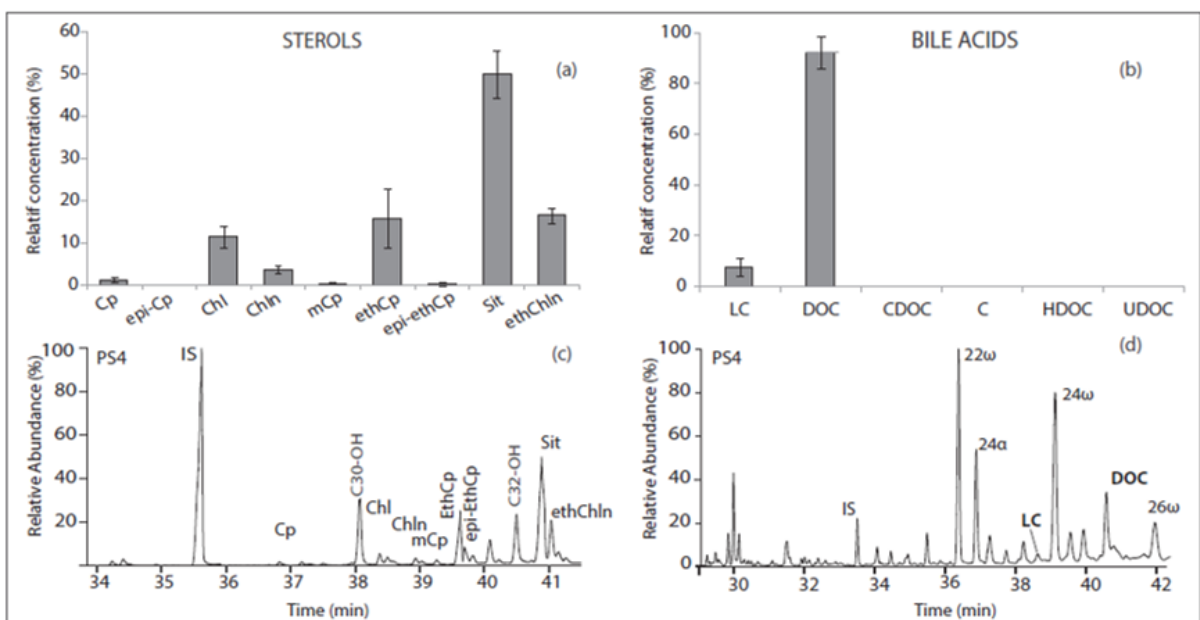
627



629



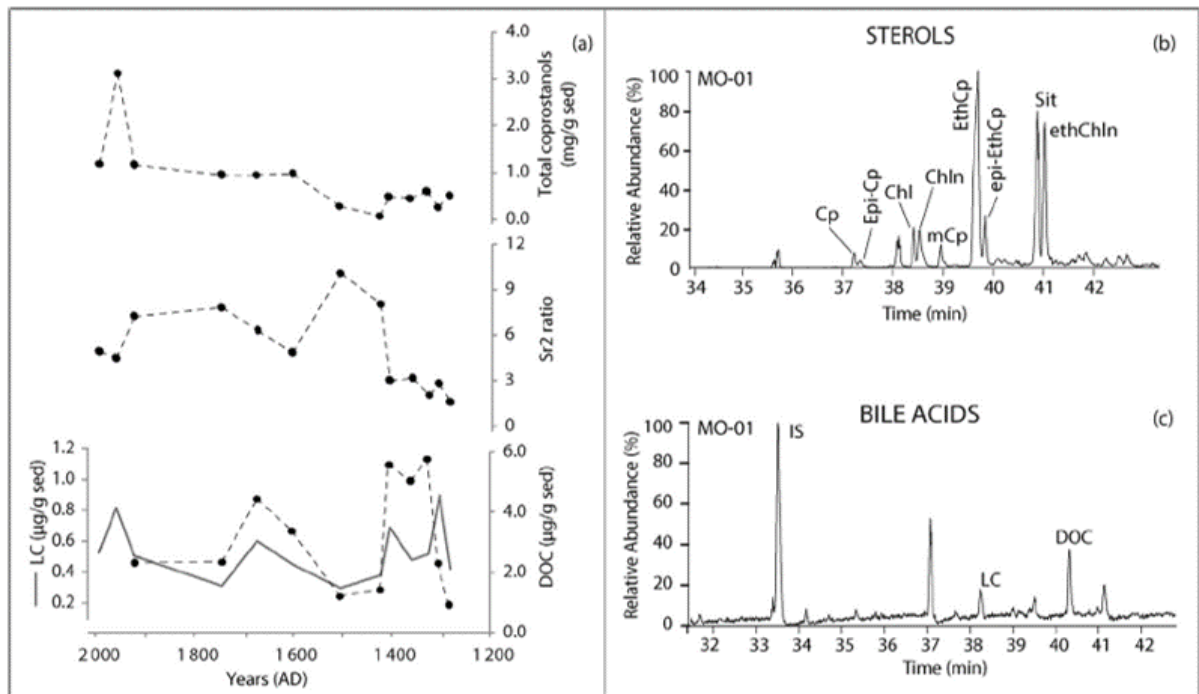
631



633

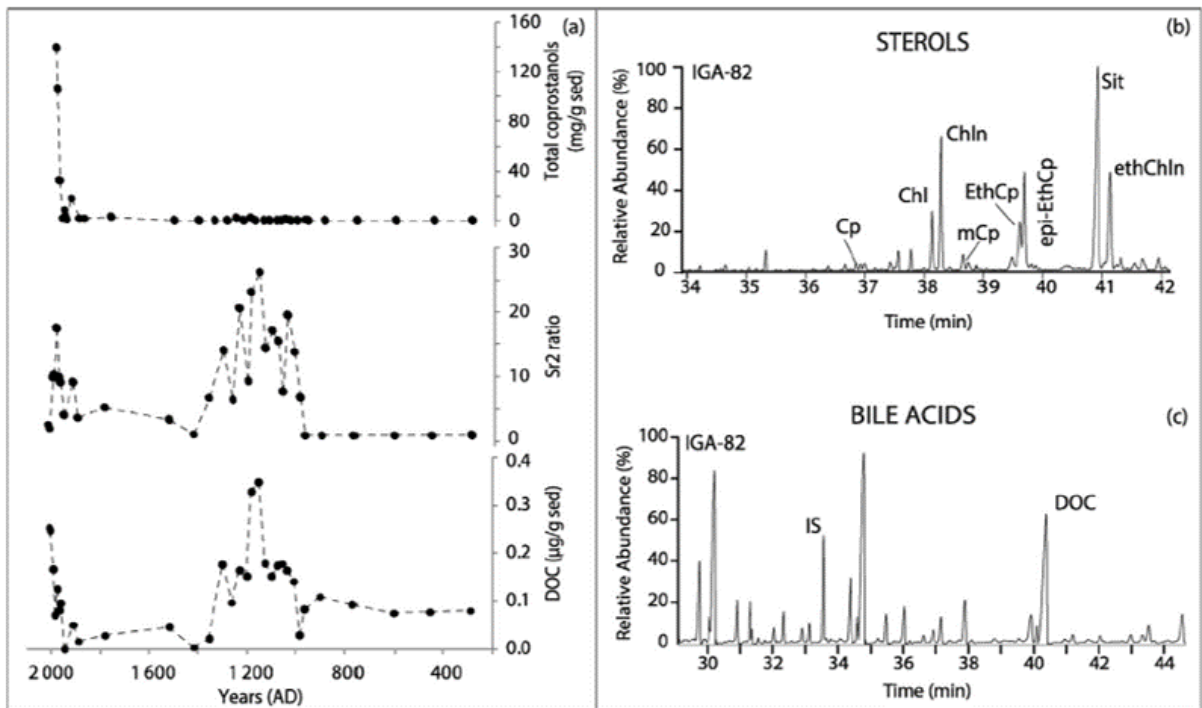
634

635 Fig. 5



636

637 Fig. 6



638

639

640

641

642

	Horse	Cow	Sheep	Human	Pig
Sterols					
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
Bile Acids					
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

644

645

646

647

648

649

650

651

652

653

654

655

656

657

Table 2:

	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

660

661

662

663

664