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Fecal biomarker imprints as indicators of past human land uses: source 1 distinction and preservation potential in archaeological and natural 2 3 archives. 4 5 Renata ZOCATELLI^{1,2,3}, Marlène LAVRIEUX⁴, Typhaine GUILLEMOT⁵, Léo CHASSIOT^{1,2,3}.Claude Le MILBEAU^{1,2,3}. Jérémy JACOB^{1,2,3}. 6 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 ⁴Environmental 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 5β -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 5β-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 5β-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 5β-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 alia, biodiversity (Smith and Zeder, 2013; Braje and Erlandson, 2013), biogeochemical cycles 49 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 (e.g. van Bergen et al., 1997; Simoneit, 2002, Killops and Killops, 2005). Biomarker analysis 60 61 can provide information on environmental conditions either in predominantly natural or in more anthropized contexts such as archaeological sites and urban areas, at local or catchment scale 62 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 (C27, C28 and C295β-stanols and bile acids), produced in the digestive tracts of certain large mammals (Elhmmali et al., 2000; Bull et al., 2002; Tyagi et al., 2008) are 66 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

contexts, such as sheep, has never been investigated. Moreover, the published data were
acquired using different protocols, which makes the comparison between the fecal imprints
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 soils in archaeological settlements (Orléans, France), soils (Lake Aydat catchment, Massif 88 89 Central, France) and lake sediments (retrieved from Lake Montcinevre, 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

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

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

Four samples were taken to assess the possible early and preferential degradation of fecal
biomarkers over the first centuries after deposition: a19thcentury septic tank (St19) and three
archaeological middens dated from the 14th, 15th and 16th centuries (St14, St15, St16,
respectively; Joyeux, 2013) collected from undisturbed settlement layers in an excavated
housing located in the medieval city of Orléans (Loire Valley, France).

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

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

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

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

(ii) Lake Igaliku catchment, located in South Greenland. Two distinct agropastoral 126 127 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 -cholestaneand 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 µm film thickness). The GC operating conditions were: 40°C (hold 1 min) ramping 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 158 159 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/z50-650. 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

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

and two sterols [*sensustricto*; cholesterol (Chl) and sitosterol (Sit)] were detected in
thereference samples and also in the samples. In addition, six bile acidswere identified:
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 μ g/g of dried fecal material; Table 1), followed by sheep (1302 μ g/g) and horse 186 $(336\mu/g)$. 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 = $3522 \mu g/g$, 190 48% of TC; ethCp = $3093\mu g/g$; 41% of TC) and human feces (Cp = $12251\mu g/g$, 71% of TC; 191 ethCp = $3929\mu g/g$, 23% of TC). However, the proportion of TC was more than twice as high 192 in human asin 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).

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

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

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

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} (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

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

In the omnivorous group, human and pig fecal material can be discriminated by the bile acid composition. In human fecal samples, DOC was the major bile acid detected, followed by LC (Fig. 2f; 35.56 and 45.44 μ g/g respectively; Table 1). Other bile acids such as CDOC (2.15 μ g/g), C (1.79 μ g/g) and UDOC (1.24 μ g/g) were detected in lower contents. While DOC was the most abundant bile acid in human fecal material, it was not detected in pig fecal samples. In the latter, the major bile acids found were HDOC (32.01 μ g/g) followed by LC (21.07 μ g/g) and UDOC in lower contents (2.31 μ g/g). Thus, bile acid distributions allow a clear distinction not only between herbivorous and omnivorous mammals, but also between animals having the
same diet. The fecal molecular inventory of a large range of animal feces presented in this study
not only confirms, but also completes previous studies (Bethell et al., 1994; Elhmmali et al.
1997; Bull et al. 2002, 2003; Tyagi et al., 2008). Indeed, to our knowledge, our work constitutes
the first report onfecal 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. InSt19, 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 theCp/epi-Cpand 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-Cp (Simpson et al., 1998; Bull et al., 1999a). In addition to this ratio, we used the DOC/C ratio 257 258 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 negligible and thus would not affect the solubility and adsorption to soil/sediment particulates. 262 263 From today to the 14th century, the DOC/C ratio remainedconstant in our samples (DOC/C 264 ~20, Fig. 3e), whereas the Cp/epi-Cp ratiodecreasedduring 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 5β-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.

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271 3.4. Applications of fecal biomarkers to reconstruct agropastoral activities

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

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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 was quite similar over soil samples (Table 2, Fig. 4), with TC varying between 0.56 and 285 286 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 µg/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 fecalcompounds 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 inarchaeological 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

acidsand 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 SouthGreenland is very different from that of the 313 Massif Central. In the lake Avdat and Montcinevre 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,

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

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

343

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

- 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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564 TABLE CAPTIONS

- **Table 1**: Sterol and bile acids contents ($\mu g/g$) and ratios of fecal sterols for reference samples. Values of Sr1 > 0.7 indicate fecal material detection (Grimalt et al., 1990). Values of Sr2 > 1 indicate the predominance of herbivorous feces (Bull et al., 2002). Cp: coprostanol, epi-Cp: epi-coprostanol, Chl: cholesterol, Chln: cholestanol, mCp: methyl-coprostanol, ethCp: ethylcoprostanol, Sit: sitosterol, ethChln: ethyl-cholestanol, LC: lithocholic acid, DOC: deoxycholic acid, CDOC: chenodeoxycholic acid, C: cholic acid, HDOC: hyodeoxycholic acid and UDOC: ursodeoxycholic acid.
- 572

Table 2: Sterol and bile acids contents (mg/g and μ g/g, respectively) and ratios of fecal sterols for the samples. Values of Sr1 > 0.7 indicate feces samples. Values of Sr2 > 1 indicate the predominance of herbivorous feces (Bull et al., 2002). Cp: coprostanol, epi-Cp: epicoprostanol, Chl: cholesterol, Chln: cholestanol, mCp: methyl-coprostanol, ethCp: ethylcoprostanol, Sit: sitosterol, ethChln: ethyl-cholestanol, LC: lithocholic acid, DOC: deoxycholic acid, CDOC: chenodeoxycholic acid, C: cholic acid, HDOC: hyodeoxycholic acid and UDOC: ursodeoxycholic acid.

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581 FIGURE CAPTIONS

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

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

Fig. 3: Distribution of sterols (a) and bile acids (b) in a septic tank active during the 19th century
(St19) and in the layers of a midden from a building dating from the Middle Ageswith unknown

594 occupation (16^{th} , 15^{th} and 14^{th} 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.

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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. 24α :C₂₄ α-hydroxycarboxylic acid; 22ω :C₂₂ ω-603 hydroxycarboxylic acid; 24ω :C₂₄ ω-hydroxycarboxylic acid; 26ω :C₂₆ ω-hydroxycarboxylic 604 acid; C₃₀-OH:C₃₀ *n*-alkanol; C₃₂-OH:C₃₂*n*-alkanol.

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Fig.5: Evolution of Total Coprostanols (Cp + epi-Cp + mCp + epi-ethCp + ethCp), Sr2 ratio,
 LC and DOC contents in lacustrine sediments (a). Specific ion mass (m/Z) chromatograms of

alcohol fraction (m/Z = 215+255+213+368+383+398+484) and acidic fraction (m/Z = 208+215+253+255+368) of sampleMO-1are shown (b,c). Nomenclature of compounds is presented in section 3.1.

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

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626 Fig.1









630 Fig. 3

















643	Table 1:	
015	14010 11	

	Horse	Cow	Sheep	Human	Pig
Sterols					
Ср	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
С	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

658 Table 2:

	C		Chi	Chla		-th Ca	epi-	S :4	-thChlm	Total	Coprostanols	C1	S-2		DOC	CDOC	C	UDOC	UDOC
St19	<u>Cp</u> 10 54	epi-Cp	2 29	1.61	<u>mCp</u>	3 89	$\frac{\text{ethCp}}{0.00}$	<u>Sit</u>	0.00	Loprostanois	<u>(%)</u> 79.98	0.88	<u>Sr2</u> 0.33	12.85	60.12	0.00	2.95	0.00	0.10
St17	2.04	0.21	0.00	0.22	0.60	1.96	0.00	0.00	0.00	19.55	91.60	0.00	0.55	1 10	15.20	0.00	0.05	0.00	0.10
5110	2.04	0.51	0.09	0.22	0.00	1.60	0.00	0.22	0.34	4.80	84.02	0.91	0.79	1.19	13.30	0.16	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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