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

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ABSTRACT

This paper presents the potential of 5β-stanols and bile acids to act as fecal source biomarkers in order to identify and characterize past agropastoral activities in archaeological and natural archives. First of all, a molecular inventory of 5β-stanols and bile acids was made on fresh fecal human and domestic animal samples, using the same methodology to define the specificity of these molecular biomarkers. The selected species were cow, horse, pig and sheep as they are the major domestic species present in European archaeological sites. To our knowledge, our work constitutes the first report on fecal biomarkers in sheep feces. Bile acids can distinguish diet regime and species having the same diet with greater specificity than 5β-stanols. Fresh human fecal material and historical / archaeological fecal material were analyzed to assess their stability through time by calculating the coprostanol/epi-coprostanol (Cp/epi-Cp) and deoxycholic acid/cholic acid (DOC/C) ratios. Results show that bile acids are more resistant to diagenesis than 5β-stanols, at least on a 700-year time scale. Human and domestic animal fecal molecular imprints were then compared to the molecular content of 65 samples retrieved from archaeological sites, soils and lacustrine sediments to test their ability to trace past land-use dynamics. This study is the first to combine bile acids and 5β-stanols to identify a source of fecal material indifferent types of location (archaeological site, catchments and lacustrine sediments) and on different time scales. The combination of sterols and bile acids can be used in a variety of natural archives and archaeological contexts to define the origin of fecal material, to specify land-use, and to reconstruct past pastoral practices at various spatio-temporal scales.
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Keywords: past agropastoral dynamics, fecal sterols, bile acids, archaeological and natural archives.

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1. Introduction

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 (Foley et al., 2005), geomorphological processes (Syvitski et al., 2005; Syvitski and Kettner, 2011) and climate (IPCC, 2013; Ruddiman, 2013; Ellis et al., 2013). The development of agricultural practices, especially through animal domestication and land clearing, induced a fundamental shift in the interactions between humans and environments (Redman, 1999; Zeder et al., 2006), from local to regional and continental scales (Braje and Erlandson, 2013). To predict ecosystem responses to global changes, the characterization of past land-uses and their impacts on environment/climate systems are relevant (Ruddiman, 2003; Dearing, 2006; Hurtt et al., 2006; Jacob et al., 2009).

Fossil organic molecules or molecular biomarkers are compounds preserved in 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 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 (e.g. Bull et al., 1998; Oldfield et al., 2003; Zolitschka and Ariztegui, 2007; Jacob et al., 2008; Battarbee and Bennion, 2011; Lavrieux et al., 2012, 2013a; Zocatelli et al., 2010, 2012, 2013).

Fecal biomarkers (C_{27}, C_{28} and $C_{29}5\beta$-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 commonly used to trace human or animal fecal inputs in current environments (Nes and Nes, 1980; Elhmmali et al., 1997; Evershed et al., 1997; Bull et al., 1998, 1999b, 2001, 2002; Jardé et al., 2007a; Lavrieux et al., 2012; D'Anjouet al., 2012; Guillemot et al., 2015, 2016). However, to date, only a few studies have precisely distinguished human and animal sources in archaeological settlements (Bull et al., 2003) or in the surrounding area (Shillito et al., 2011a). 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.

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

2. Materials and methods

2.1. *Sampling*

2.1.1. Reference samples

Fresh feces of humans, cows, horses, pigs and sheep constituted the reference samples. Fresh animal feces were collected at the Institut National de la Recherche Agronomique (INRA,

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

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.

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

2.2. *Fecalbiomarkeranalyses*

Samples were dried at 30 °C during 48h and then crushed. Ca. two grams of dried samples were extracted using an accelerated solvent extractor (Dionex® ASE 200, 100 °C and 1000 psi) with a mixture of CH_2Cl_2:MeOH (9:1 v/v). Total lipid extract was fractionated into neutral and acidic compounds using solid phase extraction on Supelclean LC-NH_2 SPE bulk packing (Zocatelli et al., 2012). Neutral compounds were eluted with CH_2Cl_2:isopropanol (2:1) and acidic compounds with ether:formic acid (9:1) after acidification. Total neutral and acid fractions were then dried under N_2. As described in Lavrieux et al. (2011), the sterols were isolated from the total neutral fraction by flash chromatography on a Pasteur pipette filled with silica (activated 24h at 120°C, then deactivated with H_2O, 5%), using a sequence of solvents of increasing polarity (heptane, heptane:toluene and heptane:ethyl acetate). The acidic fraction was methylated by adding a mixture of anhydrous MeOH and acetyl chloride (55°C for one hour). It was then separated into fatty acid methyl esters (FAMEs) and hydroxy acid methyl

esters by flash chromatography with a Pasteur pipette filled with silica, using CH_2Cl_2, CH_2Cl_2:isopropanol and MeOH (Guillemot et al., 2015).Finally, sterol and bile acid fractions were silylated using BSTFA and pyridine (60°C for one hour). Prior to gas-chromatography and mass-spectrometry (GC-MS) analyses, 5α-cholestaneand nor-deoxycholic acid standards were added to all fractions for quantitation purposes.

Fractions were analyzed by gas chromatography-mass spectrometry (GC-MS) with a Polaris TRACE-GCQ. The chromatograph was fitted with an Rtx-5MS column (30 m, 0.25 mm 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 samples were injected automatically in splitless mode, with the injector temperature set at 280°C. Helium was the carrier gas (1 ml.min^{-1}).The MS was operated in the electron ionization (EI) mode at 70 eV and scanned from m/z50-650. Identification of fecal biomarkers was based on published retention times and mass spectra (Elhmmali et al., 1997; Bull et al., 2001) and also on authentic standards of sterols and bile acids, obtained from Sigma Aldrich Chemical Co. and Alfa Aesar. Because of possible coelution with other components, compound concentrations were estimated by measuring peak areas on ion specific chromatograms. Fecal biomarker concentrations were estimated after calculation of a correction factor between the peak area on the ion specific chromatogram and the peak area on the total ion current (TIC) chromatogram for authentic standards. The minimum level of detection using GC-MS was about 10 ng.

3. Results and Discussion

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 alsoin the samples. In addition, six bile acidswere identified: lithocholic acid (LC), deoxycholic acid (DOC), chenodeoxycholic acid (CDOC), cholic acid (C), hyodeoxycholic acid (HDOC) and ursodeoxycholic acid (UDOC; Table 1 and 2).

3.2. *Biomarker distribution in fresh fecal material*

3.2.1. Sterols

The distribution of sterols varied widely among the considered species (Table 1; Fig. 2a and e). In the herbivorous group, cow feces showed the highest values of total coprostanols (TC, 1817 µg /g of dried fecal material; Table 1), followed by sheep (1302 µg/g) and horse (336µ/g). In herbivorous fecal samples ethCp was the main compound followed by mCp (Table 1). In the omnivorous group, TC concentrations were one order of magnitude greater than in the herbivorous group, with a predominance of Cp. Human and pig feces had rather similar fecal sterol profiles. Indeed, Cp and ethCp were the major compounds in pig (Cp = 3522 µg/g, 48% of TC; ethCp = 3093µg/g; 41% of TC) and human feces (Cp = 12251µg/g, 71% of TC; ethCp = 3929µg/g, 23% of TC). However, the proportion of TC was more than twice as high in human asin pig feces (Table 1). More especially, Cp contents in human feces were 3.5 times higher than in pig feces, which is consistent with previously published data (Leeming et al., 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 (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 µg/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 µg/g) as in
220 horse feces (1.17 µg/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 45.44µg/g respectively; Table 1). Other bile acids such as CDOC (2.15
224 µg/g), C (1.79 µg/g) and UDOC (1.24 µg/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 µg/g) followed by LC (21.07 µg/g)
227 and UDOC in lower contents (2.31 µg/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.

3.3. *Fecal signal preservation*

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

To better assess the respective preservation of fecal biomarkers, we compared the fecal imprint of the human reference sample to samples from the septic tank and from archaeological middens, covering the 19th to the 14th centuries (St19, St16, St15 and St14). All these samples were characterized by a major/exclusive human fecal input. InSt19, St16, St15 and St14 samples, Sr1 values ranged between 0.87 and 0.92 (Table 2) and Sr2 exhibited low values (0.33-0.84), all attesting toan omnivorous fecal imprint. In addition,Cp was the major sterol observed (between 0.58 and 10.54 mg/g), followed by ethCp (Fig. 2; Table 1). These indices,associated to the presence of CDOC and C, and the absence of HDOC, attest to a human fecal input.

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

decreasing occupation of the site. Preservation of fecal biomarkers in human feces over time was assessed through the calculation of theCp/epi-Cpand DOC/C ratios.The Cp/epi-Cp ratio is 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 because DOC is a secondary by-product of C formed in anaerobic environments via enzymatic reactions (Bull et al., 2003). A relatively low DOC/C ratio variability over time indicates good preservation. The DOC/C ratio can be used if it is assumed that the chemical difference between 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. From today to the 14th century, the DOC/C ratio remainedconstant in our samples (DOC/C ~20, Fig. 3e), whereas the Cp/epi-Cp ratiodecreasedduring the first century (from ~70 to ~10).Assuming that this decrease in the Cp/epi-Cp ratio is not related to diet changes over centuries, it would suggest a greater degradation of fecal sterols than of bile acids. This better preservation of bile acids compared to 5β-stanols supports previous observations made by Elhmmali et al. (1997). Regarding these results, it appears that bile acids are better preserved, at least at the multi-centennial scale.

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.

3.4.1. Aydat catchment soils

In soil samples retrieved from lake Aydat catchment (Massif Central, France), previous studies based on fecal biomarkers evidenced recent husbandry practices (Lavrieux et al., 2012; Zocatelli et al., 2012). Here, this was achieved by re-exploiting the dataset of fecal biomarker 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 26.9mg/g soil, and with 8-37% of coprostanols (ethCp and Cp; Table 2). Samples also contained significant amounts of LC and DOC (0.08-0.36 and 0.84-4.25 µg/g). No HDOC, which is specific of pig feces, was detected. Associated to the distribution of sterols, the identification of DOC and the absence of C and CDOC argue for a fecal input from cows to the soil, consistent with current husbandry in the catchment. In any case, this study shows that the recent fecalcompoundsdistribution istransferred to soils andconsistent with current agropastoral practices. For paleosoils located in archaeological sites fecal biomarkers are a promising tool inarchaeological research regarding the use of space and human activities.

3.4.2. Lake Montcineyre sediments

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

306 acidsand fecal sterols. In view ofthe better preservation of bile acids compared to that of 5β-
307 stanols confirmed and described in section 3.3., it isthe evolution of LC and DOC
308 biomarkersthat 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 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,

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).

4. Conclusions

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

Thanks to the good preservation and specificity of fecal biomarkers, it was possible to test 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 inventory in the Lake Aydat catchment soils, Lake Montcineyre and Lake Igaliku sediments is made and brings information about past land uses, allowing a reconstruction of local past pastoral practices and enlightening us on past interactions between human and environments.

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TABLE CAPTIONS

Table 1: Sterol and bile acids contents (µg/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: ethyl-coprostanol, Sit: sitosterol, ethChln: ethyl-cholestanol, LC: lithocholic acid, DOC: deoxycholic acid, CDOC: chenodeoxycholic acid, C: cholic acid, HDOC: hyodeoxycholic acid and UDOC: ursodeoxycholic acid.

Table 2: Sterol and bile acids contents (mg/g and µg/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: epi-coprostanol, Chl: cholesterol, Chln: cholestanol, mCp: methyl-coprostanol, ethCp: ethyl-coprostanol, Sit: sitosterol, ethChln: ethyl-cholestanol, LC: lithocholic acid, DOC: deoxycholic acid, CDOC: chenodeoxycholic acid, C: cholic acid, HDOC: hyodeoxycholic acid and UDOC: ursodeoxycholic acid.

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).

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

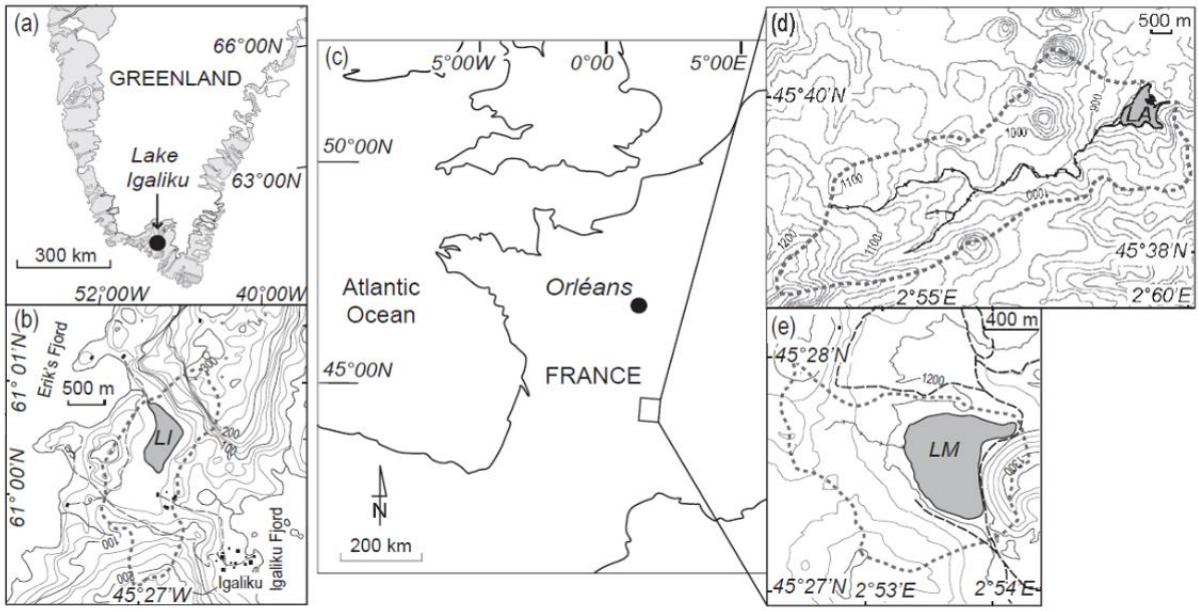
occupation (16^{th}, 15^{th} and 14^{th} centuries). Specific ion mass (m/z) chromatograms of alcohol fraction (m/Z = 215+255+213+368+383+398+484) and acidic fraction (m/Z = 208+215+253+255+368) from sample S16 (c, d). Cp/epi-Cp and DOC/C ratios from human fecal material, septic tank and middenlayers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

Fig. 4: Relative average concentrations of sterols and bile acids in soil lipid extracts (a,b). GC–MS chromatograms (TIC) of sample PS8 for alcohol and acidic fractions (c,d). Nomenclature of compounds is presented in section 3.1. 24α:C_{24} α-hydroxycarboxylic acid; 22ω:C_{22} ω-hydroxycarboxylic acid; 24ω:C_{24} ω-hydroxycarboxylic acid; 26ω:C_{26} ω-hydroxycarboxylic acid; C_{30}-OH:C30 *n*-alkanol; C_{32}-OH:C_{32}*n*-alkanol.

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.

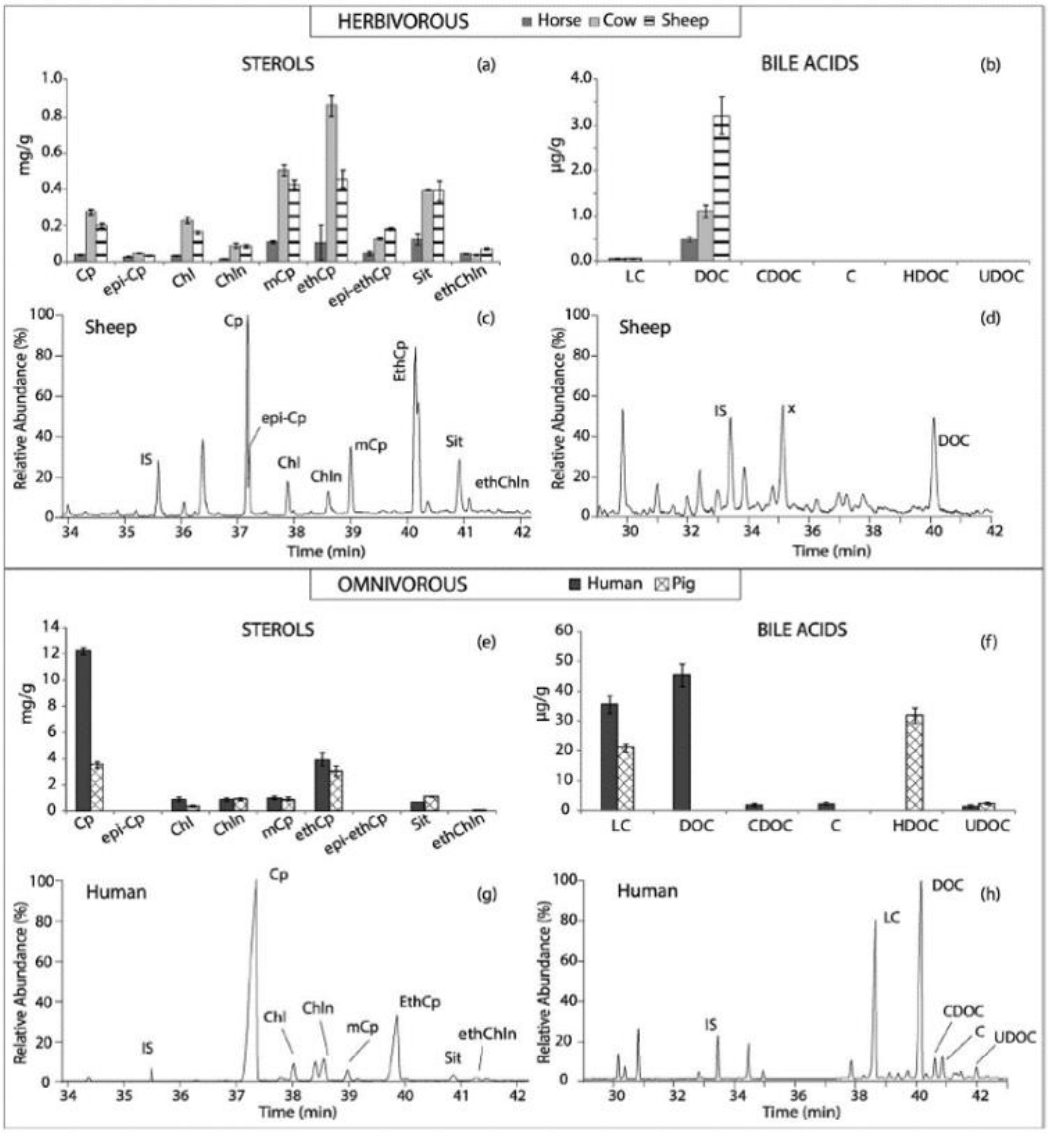
Fig.6: Evolution of Total Coprostanols (Cp + epi-Cp + mCp + epi-ethCp +ethCp), Sr2 ratio and DOC contents over the last two millennia in lacustrine sediments (a). Specific ion mass (m/Z) chromatograms of alcohol fraction (m/z = 215+255+213+368+383+398+484) and acidic fraction (m/Z = 208+215+253+255+368)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

626 Fig.1



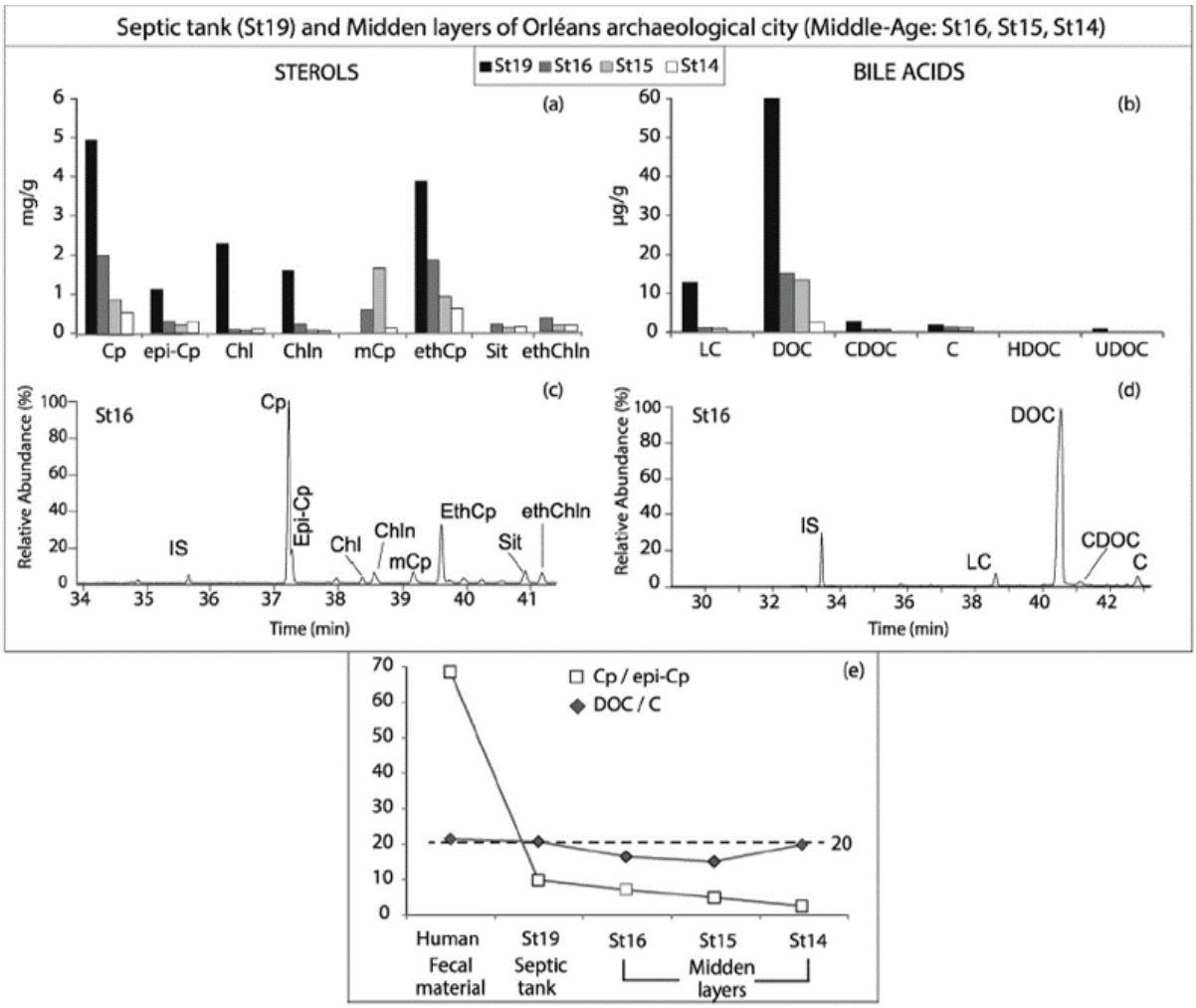
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628 Fig.2



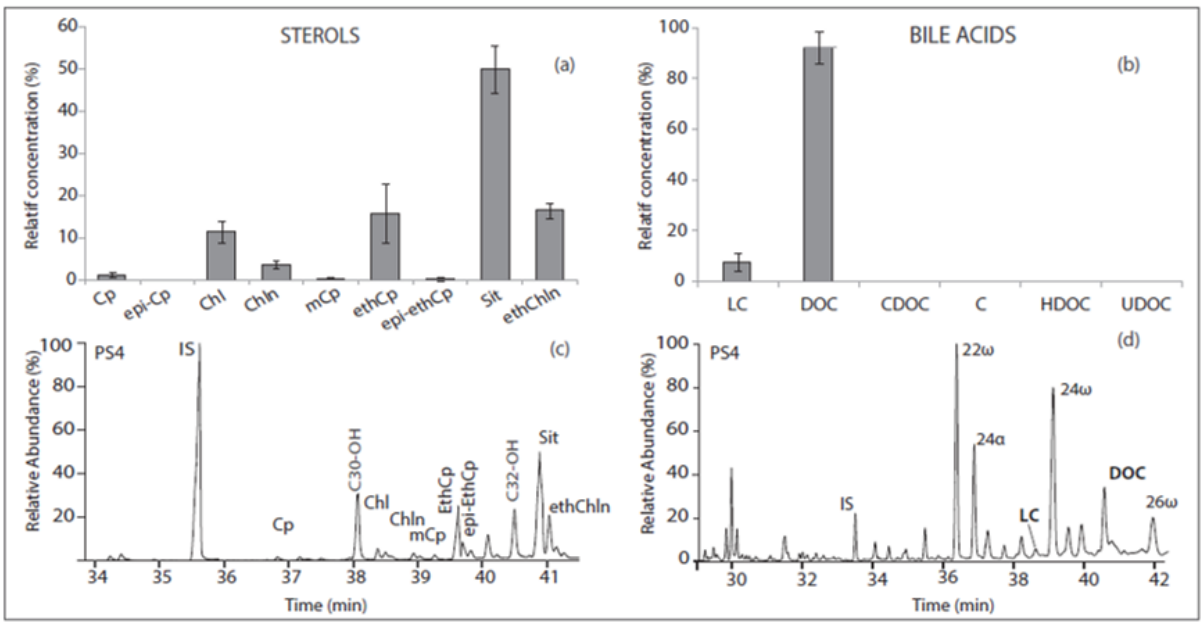
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630 Fig. 3



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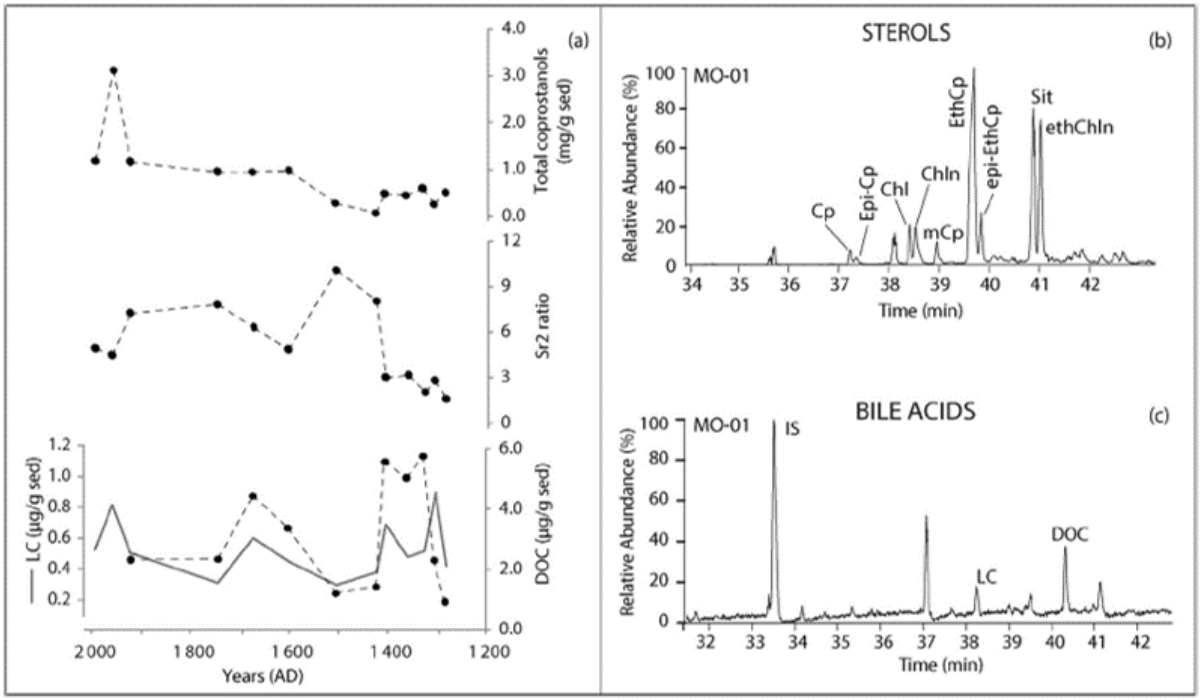
632 Fig. 4



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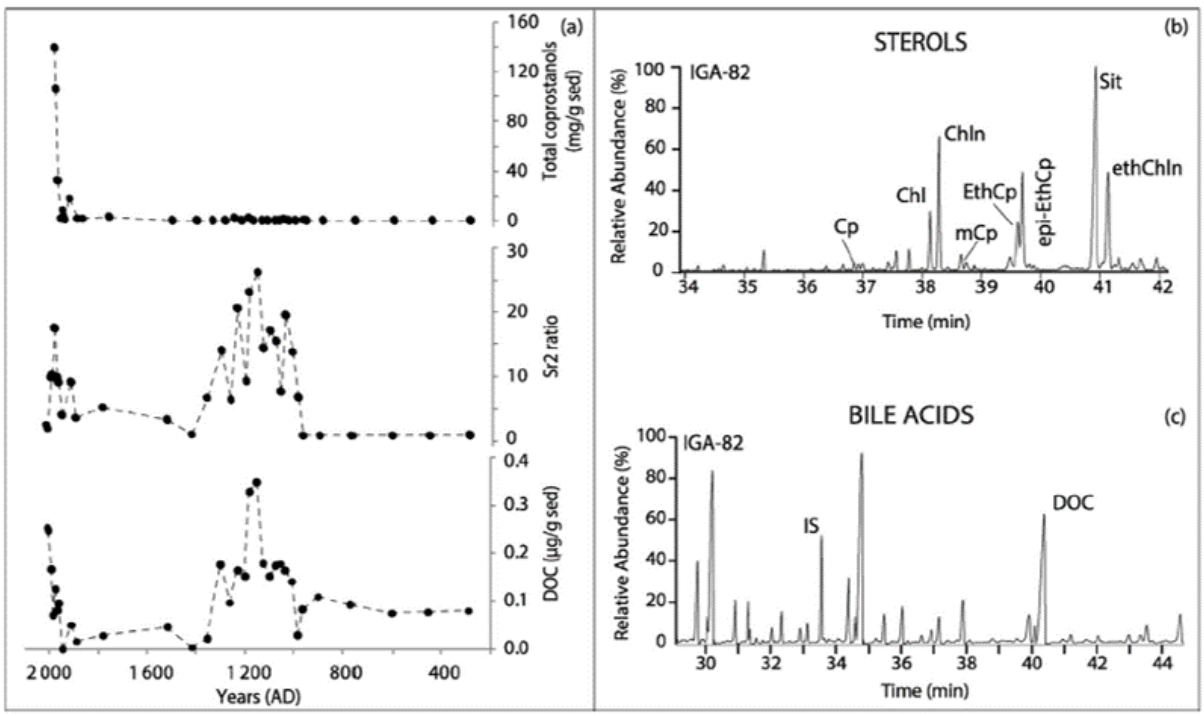
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635 Fig. 5



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637 Fig. 6



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643 Table 1:

	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

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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