

**Characterising life in settlements and structures: incorporating faecal lipid biomarkers within a multiproxy case study of a wetland village**

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**Author contributions:** HM and ACGH designed the study in collaboration with AC, GC and AGB. HM conducted the primary lipid data analysis and interpretation with guidance from IDB and ACGH. KLD conducted the primary insect data analysis and interpretation with NJW. JR and LR respectively conducted the primary macrofossil and micromorphology data. AC and GC facilitated sample collection and provided archaeological context for the study site. HM wrote the manuscript and all authors actively discussed the direction of the research and contributed to manuscript editing.

**Highlights:**

- First application of steroids in a multiproxy spatial study of wetland floor deposits.
- Multiproxy analyses refine characterisations of faecal sources and animal husbandry.
- Faecal proxies show changes in roundhouse use and conditions over time and space.
- Steroid biomarkers identify more subtle faecal sources than traditional proxies.
- Roundhouse has active inner area and flexible functionality.

## Abstract

Roundhouses are ubiquitous features of Iron Age landscapes across North West Europe, yet the way they were used internally is not well understood. We demonstrate how spatial analyses of steroid lipid biomarkers advances our understanding of household activities, living conditions and animal management associated with a well-preserved 5<sup>th</sup> century BCE roundhouse from Scotland's first Iron Age wetland village, Black Loch of Myrton, especially when combined with more traditional archaeological approaches. Faecal steroids (5 $\beta$ -stanols and bile acids) are well preserved within the wetland roundhouse floor deposits. Diffuse faecal inputs are identified within these deposits, limiting the resolution of faecal source discrimination compared with studies of concentrated faecal remains. However, analysis of both 5 $\beta$ -stanols and bile acids enables discrimination between ruminant (sheep, goat and cattle), pig and horse/human faecal remains. By integrating faunal data and entomological dung indicators we are able to characterise the on-site presence of animals associated with these archaeological structures. Steroids indicate short-lived and/or temporary pulses of dung deposition within the Iron Age roundhouse case study structure, which can be very difficult to determine using other archaeological proxies. Furthermore, our multiproxy results demonstrate the molecular preservation of steroids within deposits that have been subjected to regular floor cleaning, which is associated with the removal macrofossil proxies. Comparisons of multiproxy faecal signatures of the inner and outer sections of the structure show temporal and spatial heterogeneity in usage and living conditions. The faecal signature points to temporary sheltering of animals within the inner section of the structure. The multi-use and division of different activities within the roundhouse, determined by steroids, marks an important contribution to broader archaeological debates surrounding structures, their functions and re-use.

63 **Keywords:**

64 Faecal, Sterols, Bile acids, Palaeoecology, Settlement structures, Animal husbandry,

65 Wetland archaeology, Iron Age

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## 1. Introduction

A key advantage of analysing occupation sedimentary deposits, such as floor remains, is the retention of a wealth of information about the use of space in settlement sites (e.g. Manzanilla and Barba, 1990; Middleton and Price, 1996). The characteristics of these structural space uses, which may vary over time, can provide insights into social statuses and roles of houses, animal husbandry practises, food storage, and handcrafts etc. although, as is the case of Alpine Neolithic settlement houses, special functions are rare (Ebersbach, 2013). Almost all environmental proxies have been trialed to reconstruct the use of internal space including geochemistry, molecular proxies, pollen, insects, phytoliths as well as the standard analysis of micromorphology, plant macrofossils and faunal remains. The most effective characterisations rely on a combination of these proxies to provide multiple lines of evidence to support interpretations (Shillito, 2017). However, integration of multiproxy analyses can be complex and should be considered at the project design stage (Shillito, 2017) with clear considerations for the specificity of results obtained from each proxy (e.g. Middleton et al., 2010) as well as the role of the depositional environment as a record of activity (Shahack-Gross, 2011).

Of the biological proxies used to characterise occupation deposits, insects have been widely used due to their early synanthropism (Smith et al. 2020), and host-specific diversity related to almost all aspects of within structure activities as well as the external environment. Some of the best-known examples include the study of Norse North Atlantic farmsteads (Panagiotakopulu et al. 2007) and Viking age houses from 9<sup>th</sup> AD century Dublin (Reilly et al., 2016). Whilst pollen is less commonly used to characterise occupation deposits than insects, the case study of Pueblo houses in southwest USA demonstrates the ability of pollen spectra obtained from floors to

suggest different room uses such as food processing, ceremonial function or meeting rooms (Morris, 1986). A non-in situ example includes the high concentrations of cereal and grazing indicator pollen adjacent to *crannogs* (artificial island settlements) taken to indicate crop storage/processing (O'Brien et al., 2005) and animal tethering and slaughter (Brown et al. *subm.*). The use of phytoliths is more common in dryland settlement sites such as Çatalhöyük in Turkey (Ryan, 2011; Shillito and Ryan, 2013), but they have been used successfully in temperate European environments such as Williamson's Moss in Britain (Wade et al., 2019) and have great potential in tropical wetland sites such as the Kuk swamp in Papua New Guinea (Golson et al., 2017).

A commonly applied technique to assess function and use of space is micromorphological analysis of floors with soil phosphate and multi-elemental analysis (Middleton, 2004). Elevated soil phosphate is associated with animal use in a wide variety of environments (Holliday and Gartner, 2007). However, its equifinality has been demonstrated (Middleton et al., 2010) and accumulation patterns within soil requires careful interpretation (e.g. Nielsen and Kristiansen, 2014), particularly in wetland contexts that are impacted by changes in solubility, absorption, resorption, mobilization and leaching, at low pH and Eh, of sediments. In the classic Butser House, England, experiment, Macphail et al. (2004) showed how crust formation was important for phosphate retention and microscopic crust formation, the degree of floor compaction and its mineral content all related to the variability in phosphate depletion from floor surfaces exposed to pedestrian traffic and house cleaning. At the same experimental site, Evershed et al. (1997) highlighted that a manured area could not be clearly identified using concentrations of total phosphorus, but it was detectable using faecal lipid biomarkers (5 $\beta$ -stanols).

Recent developments in the refinement of faecal lipid biomarker signatures (Prost et al., 2017; Harrault et al., 2019) now facilitate the application of this approach within widely available bulk anthropic sediments, as well as concentrated faecal remains, to characterise animal husbandry and living conditions. These lipid compounds - termed steroids - have the ability to enhance characterisations of activity areas since they are direct markers of faecal matter produced by higher vertebrates and can identify human-animal interactions. The steroid composition of faecal matter produced by different animals varies according to their food sources, digestive processes and gut bacteria (Leeming et al., 1996). Therefore, diagnostic ratios of faecal and non-faecal sterols (e.g.  $5\beta$ -stanols vs  $5\alpha$ -stanols) and bile acids can discriminate between human, porcine and herbivore faecal matter (Bull et al., 2002; Prost et al., 2017; Harrault et al., 2019). Steroids have identified the presence and source of faeces in a range of archaeological settings including coprolites and manure (Evershed et al., 1997; Bull et al., 2001; Shillito et al., 2011; Prost et al. 2017; Ledger et al., 2019) and archaeological soils (Simpson et al., 1998; Bull et al., 1999; Harrault et al., 2019).

The utility of incorporating steroids within studies of activity areas has been demonstrated using sterol analyses of deposits obtained from experimental settlements and palaeosols. For example, the combined analyses of elements and sterols from an experimental Iron Age settlement identified separate activity areas and provided positive identification of activities in all except one area (Hjulström and Isaksson, 2009). The first spatial analysis of sterols obtained from paleosols identified patterns of animal husbandry from land adjacent to a 5<sup>th</sup>-11<sup>th</sup> c. AD Russian fortress-settlement (Harrault et al., 2019; Anderson et al., 2019). Whilst these studies showcase the ability of sterols to identify spatial patterns of activity

areas and animal husbandry, the use of both sterol and bile acid analyses within wetland archaeological settlement sediment deposits has yet to be tested.

We present the first multiproxy spatial study of Iron Age roundhouse wetland sedimentary deposits from the Black Loch of Myrton (BLM) in southwest Scotland, UK (Figure 1; Crone et al., 2018) using steroid lipid biomarkers (sterols and bile acids), ecofact analysis and micromorphology to investigate the use of space within a roundhouse structure (Structure 2). Excavations of waterlogged Iron Age roundhouses are rare, but other examples from the UK include Flag Fen (Pryor, 2001) and Glastonbury Lake Village (Hill et al., 2018). The BLM excavation offered an opportunity to investigate the usage of an Iron Age roundhouse since the nature of the wetland site means there was excellent structural integrity, providing insight into structural form and construction of the roundhouse (Crone et al., 2018), as well as good organic matter preservation within the archaeological soils. Structure 2 has well-stratified organic rich matrix, important for faecal steroids, which have low water solubility and are absorbed to particulate organic matter preventing vertical movement via leaching (Lloyd et al., 2012). As a result, steroids remain in situ at the point of deposition (Lloyd et al., 2012), are likely well preserved over the Iron Age timescale (Lin et al., 1978; Bull et al., 2001; Prost et al., 2017) and, in the case of coprolitic sources, are preserved in wetland settings (Ledger et al., 2019).

Two models for Iron Age roundhouse space use exist: (1) inner sections are areas of active communal domestic activity, with the outer section as a peripheral area for sleeping and storage (Hingley, 1990); and (2) outer sections of the roundhouse are reserved for stalling of animals (e.g. Kelly, 1988; Banks, 1995). The difference between these two models is dependent on region (Hill, 1995), with centrally focused roundhouse activity areas highlighted in the first model, generally found in northern



regions of Iron Age Britain. To determine the most appropriate model of roundhouse use and to establish whether livestock co-habited spaces with people we need to establish what these inner and outer spaces were used for by integrating multiproxy indicators of humans and animals.

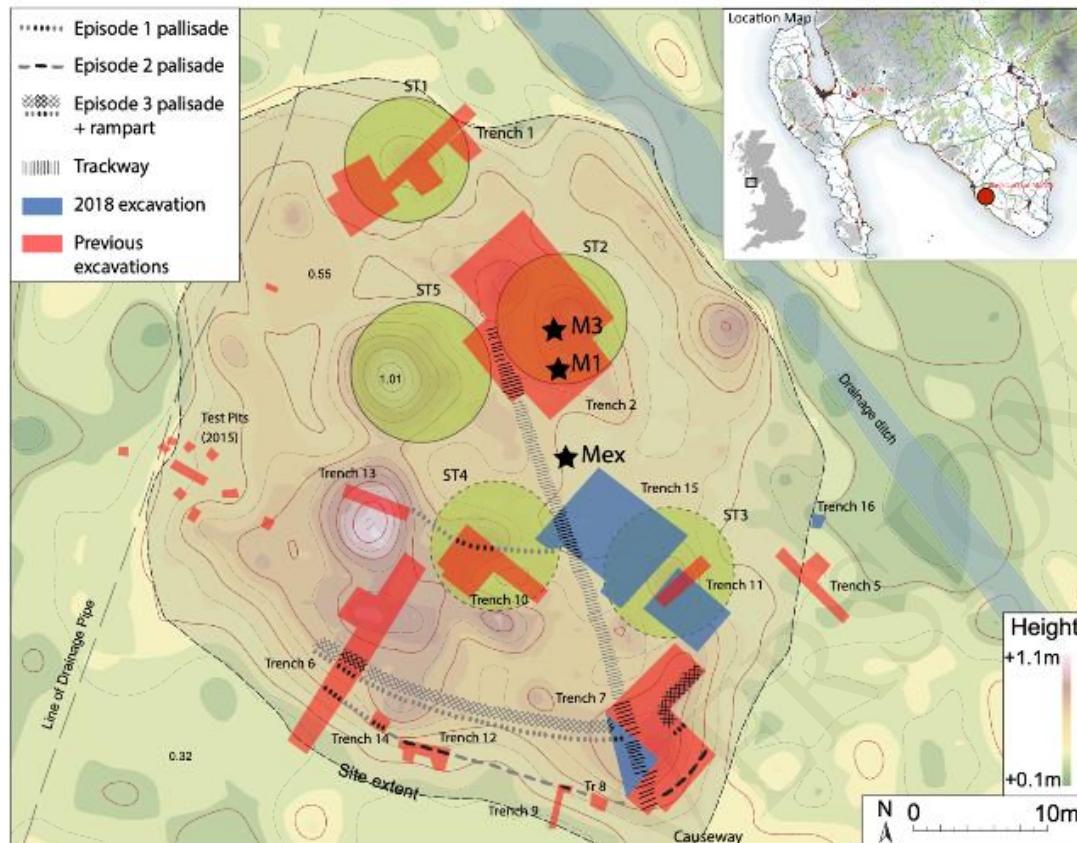
## **2. Methods**

### **2.1 Study site**

The Black Loch of Myrton (BLM) is a drained wetland in southwest Scotland, UK (54°45'13"N 4°32'53"W; Figure 1). Recent excavations show the settlement was constructed on top of a natural peaty island approximately 50 × 60 m within a shallow fen marshland (Crone and Cavers, 2015; 2016). Excavations and dating (radiocarbon-dating and dendrochronology) of five of the settlement mounds show that the date of settlement at BLM was the latter half of the 5<sup>th</sup> century BCE, ending in the 3<sup>rd</sup> century BCE with at least three phases of construction and renewal (Crone et al., 2018).

Structure 2 is a large roundhouse 12.8 m in diameter (Figure 1; Crone et al., 2018), the inner and outer sections of which were divided by a ring of posts proximal to the central stone hearth (Crone et al., 2018), likely reflecting a common, conscious organising principle of Iron Age roundhouse structures in Britain (Pope, 2007). The stratigraphy of the hearth, entrance and floor deposits indicate they have been refurbished at least twice, leading to the build-up of stratified acidic layers of plant litter (pH 5.3 ± 0.4), which were used to create the floor surfaces (Crone et al., 2018). Chronological evidence for the construction, occupation and abandonment of

189 Structure 2 brackets it to a 30 to 40-year period from *ca.* 435 BCE – 400 BCE (Crone  
190 et al., 2018). Preservation of structural and organic material is excellent due to  
191 waterlogging. Despite high-levels of organic matter preservation in BLM Structure 2,  
192 evidence for activities that took place within the roundhouse are limited: minimal  
193 material culture was recovered (Crone et al., 2018) and the micromorphology and  
194 macrofossil remains suggest regular cleaning within the structure, thereby removing  
195 anthropogenic activity signals (Robertson and Roy, 2019).

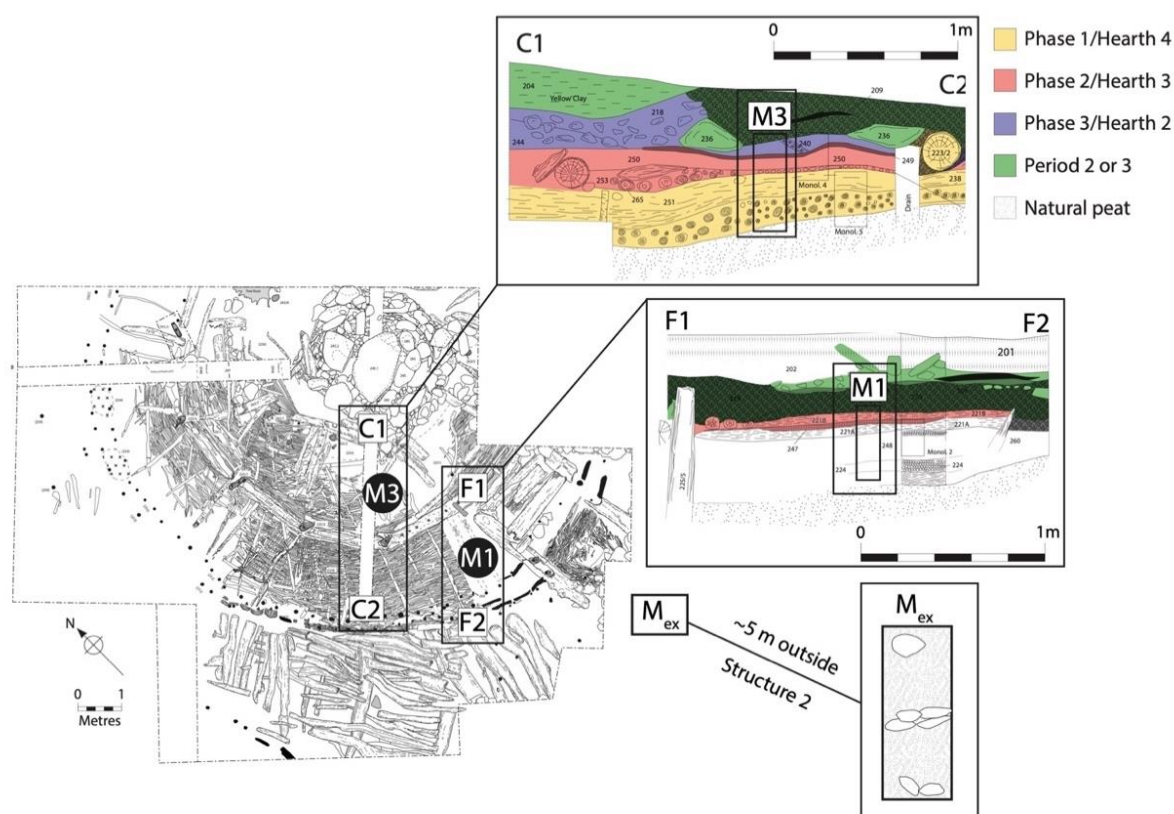


**Figure 1:** Location of Black Loch of Myrton in southwest Scotland. Digital terrain modelling characterizes the topography of the site, revealing seven-eight discrete mounds, five of which have been excavated. Black stars indicate Structure 2 (ST2) sampling locations: M3 = inner roundhouse, M1 = outer roundhouse, M<sub>ex</sub> = outside roundhouse entrance.

## 2.2 Sampling

Monolith tin samples were taken in summer 2015 from the inner and outer area of Structure 2 (Figure 2). An additional monolith for organic geochemical analysis was obtained *ca.* 5 m outside of the structure from contemporary archaeological deposits in front of the roundhouse entrance in January 2017, to characterise external dung deposits and/or trampled dung originating from animals entering and leaving the structure (M<sub>ex</sub>; Figure 2). Samples for steroid analysis and micromorphology were

extracted from the internal monoliths at depths corresponding to assigned contextual changes consisting of foundation deposits, primary floor layers and subfloor layers (Crone and Cavers, 2015; 2016).



**Figure 2:** Location of monolith samples obtained inside (M1, M3) and outside (M<sub>ex</sub>) Structure 2.

### 2.3 Faecal steroid analysis

Total lipids were extracted from approximately 1 g of dried, homogenised sediment, spiked with internal standards (androstanol and hyocholic acid), with solvents (DCM:MeOH, 2:1, v/v) using microwave assisted extraction (heated to 70 °C over 10 mins then held at 70 °C for 10 mins; Kornilova and Rosell-Melé, 2003) and

222 saponified using 5 M sodium hydroxide in MeOH. Following Bull et al. (2001),  
223 extracts were separated into neutral and acid fractions using aminopropyl SPE  
224 columns and these fractions were further split using silica gel column  
225 chromatography to isolate the sterol fraction and, following methylation using  
226 trimethylsilyldiazomethane (TMS-DAM) in toluene/methanol (4:1 v/v), the  
227 hydroxylated carboxylic acids (containing bile acids). The sterol and bile acid  
228 fractions were trimethylsilylated using *N,O*-bis(trimethylsilyl)trifluoroacetamide  
229 (BSTFA)+ trimethylchlorosilane (TMCS) (99:1 v/v).

230 Both derivatized sterol and bile acid fractions were dissolved in 50-100 µL of ethyl  
231 acetate prior to analysis by gas chromatography-flame ionisation detection (GC-FID)  
232 and gas chromatography-mass spectrometry (GC-MS). GC-MS analyses were  
233 performed using a ThermoScientific ISQ, with an ion source temperature of 300 °C  
234 and electron energy of 70 eV. The analyser was set to scan  $m/z$  50–650 with a duty  
235 cycle time of 0.2 s. Chromatographic separation was performed on an Agilent fused  
236 silica capillary column (HP-5, 60 m × 0.25 mm ID × 0.25 µm df). Sterol derivatives  
237 were analysed using the following temperature programme: 50 °C (held for 2 min) to  
238 200 °C at 10 °C min<sup>-1</sup> then to 300 °C at 4 °C min<sup>-1</sup> and held for 20 min. Bile acid  
239 derivatives were analysed using the following temperature programme: 40 °C (held  
240 for 1 min) to 230 °C at 20 °C min<sup>-1</sup> then to 300 °C at 2 °C min<sup>-1</sup> and held for 20 min.  
241 GC-MS peaks were identified through comparisons with known mass spectra  
242 (NIST08; Prost *et al.*, 2017 and a laboratory reference library), example  
243 chromatograms (Prost et al., 2017) and standards where possible. Analytes were  
244 quantified based on internal standards.

245 Potential faecal sources were identified from the sterol fraction using a ratio of the  
246 sum of faecally derived cholesterol reduction products (coprostanol +

epicoprostanol) to the sum of environmentally and faecally derived cholesterol reduction products (5 $\alpha$ -cholestanol + coprostanol + epicoprostanol) (Ratio 1; Bull et al., 1999) with ratio values *ca.*  $\geq 0.3$  indicative of potential faecal matter input (Prost et al., 2017).

$$\frac{(coprostanol+epicoprostanol)}{(5\alpha-cholestanol+coprostanol+epicoprostanol)} \quad (\text{Ratio 1})$$

Ratio 1 does not definitively identify faecal matter in isolation since small proportions of these compounds are also produced by the reduction of cholesterol in the natural environment (primarily to produce 5 $\alpha$ -cholestanol), thereby requiring comparative controls. The identification of herbivore faecal matter was indicated by the C<sub>27</sub> to C<sub>29</sub> 5 $\beta$ -stanol ratio (Ratio 2; Leeming et al., 1997), with values  $< 0.38$  indicative of herbivore faeces.

$$\frac{(coprostanol)}{(coprostanol+5\beta-stigmastanol)} \quad (\text{Ratio 2})$$

Evidence for the presence of faecal matter was also supported by the presence of bile acids and the dominant faecal matter source was identified using the ratio of deoxycholic acid (DCA) to lithocholic acid (LCA) ratio (Prost et al., 2017). Based on modern experimental data, the values of this ratio can be ascribed in the following way:  $< 0.4$  pigs and/or geese;  $0.6 - 4.5$  humans and/or horses;  $> 5$  ruminants (cattle, sheep and goats) (Prost et al., 2017). Whilst the dominant faecal source can be identified using these ratios, this does not preclude the presence of other faecal sources in smaller quantities.

## **2.4 Insect analysis**

Six bulk sediment samples (2 - 5 L) from floor contexts were processed using the standard paraffin floatation protocol (Coope, 1986). Briefly, sediments were wet-sieved through nested sieves (3 mm and 300 µm) to remove the inorganic clay and silt fraction, respectively. The collected float was washed with detergent then rinsed and stored in ethanol. Insect remains were picked using a large Bogorov sorting tray under a stereo microscope (10 – 60 × magnification) and the insects placed in ethanol for storage.

Beetle remains were identified using modern reference collections and standard published keys (e.g. Lindroth, 1974; Foster et al., 2014) and recorded as Minimum Numbers of Individuals (MNI). The species list and associated ecological information were generated using BUGSCEP (Buckland and Buckland, 2006), following the taxonomy of Duff (2008). Fly and ectoparasite remains were identified using reference materials and manuals (Skidmore, 1985; Smith, 1989; Whitaker, 2007); lice and fleas were identified to species level when heads were available. Muscidae fly puparia were identified to species level whilst remaining individuals could only be identified to genus level. Results presented here are a subset of the insect assemblage data, which are published elsewhere (Davies et al., in prep.), focusing on taxa that display an exclusivity for foul environments, are very common in dung and are closely associated with animals, following Hall and Kenward (1990) and Smith (2012).

## **2.5 Animal and plant macrofossil remains**

Bulk sediment samples were processed using the standard floatation method (Kenward et al., 1980), with waterlogged samples processed by hand to maximise

recovery of fragile plant remains. Macrofossils were examined under a microscope ( $\times 10$  -  $\times 100$  magnification) and identifications were made using modern reference material and seed atlases (Cappers et al., 2006; Jacomet, 2006). Charcoal samples containing two or more wood species were designated as fuel waste, whilst those containing larger concentrations of a single species were interpreted as burning events. Bone was identified to element and species with the aid of reference material and skeletal atlases (Schmid, 1972; Hillson, 1986). Where an element could not be identified to species level, it was categorised into large mammal (cattle/horse/deer), medium mammal (sheep/goat/pig) and small mammal (dog/cat/rodent).

## **2.6 Soil Micromorphology**

Eleven samples were extracted from the internal Structure 2 monoliths, corresponding to contexts targeted for steroid analysis, and prepared for micromorphological analysis after Murphy (1986). Thin section description was conducted using the identification and quantification criteria by Bullock et al. (1985) and Stoops (2003). Abundance of fabric constituents were estimated following categories outlined by Stoops (2003). Deposit types were identified based on particle size, shape and the composition of the coarse and fine fraction, particularly the frequency and type of organic matter, minerals and anthropogenic inclusions. Trampling was indicated by linear and parallel distributions, polyconcave voids and platy microstructures (Courty et al., 1989, Milek, 2012).



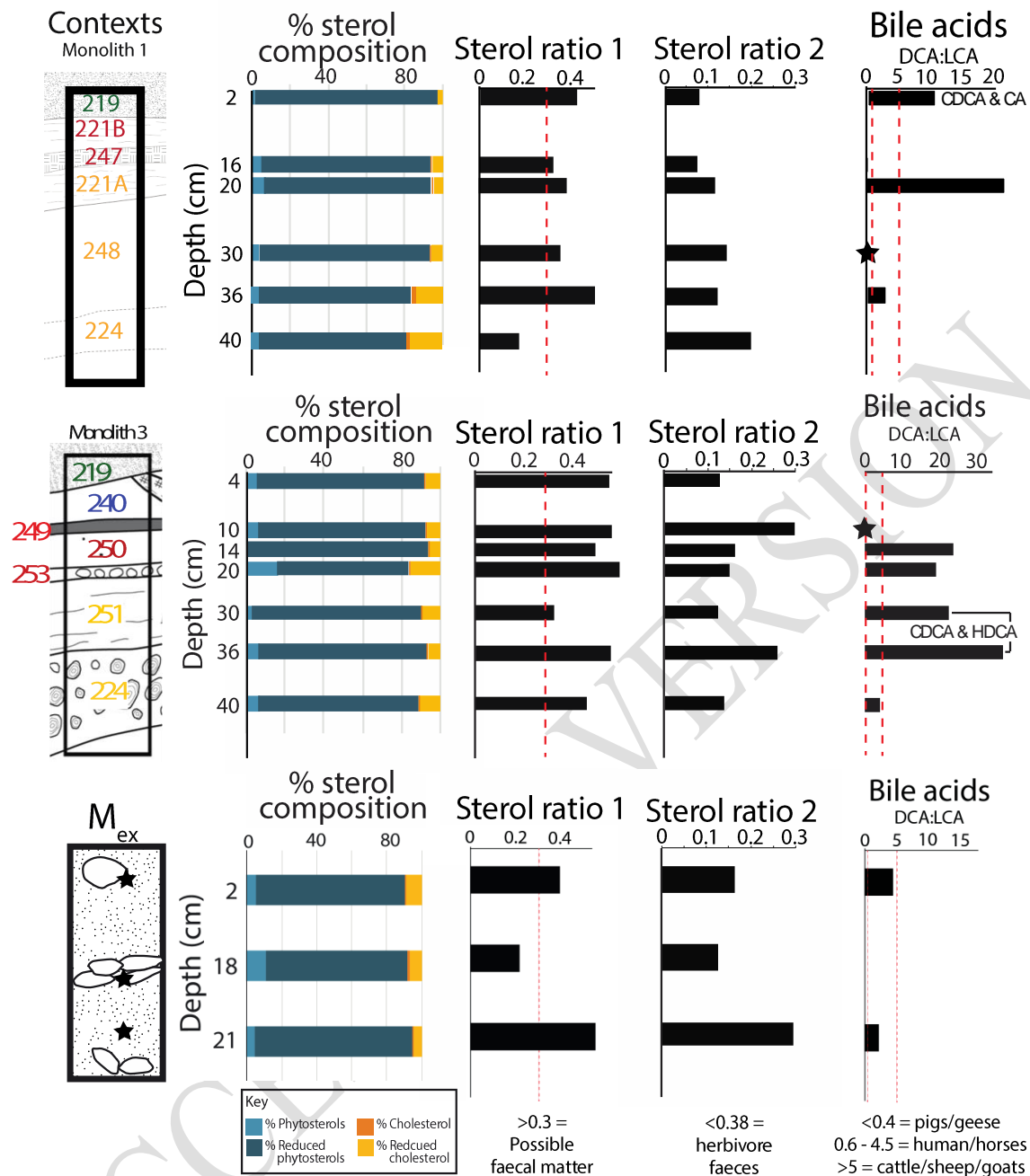
### 3. Results

#### 3.1 Faecal steroids

The total sterol composition of all samples is dominated by plant-derived compounds (phytosterols; campesterol and sitosterol and reduction products of phytosterols; C<sub>28</sub> and C<sub>29</sub> stanols), with cholesterol and its reduction products (C<sub>27</sub> stanols) accounting for <10% (Figure 3).

Ratio 1 returns values of >0.3, indicating a potential faecal input in all samples apart from two: [224] in M1 and 18 cm in M<sub>ex</sub> (Figure 3, Tables 2 and 3). The lack of faecal matter in these two samples is confirmed by the absence of detectable bile acids.

Ratio 1 of one sample ([219; M3]) suggests the possible presence of faecal matter but this is not supported by bile acids. Ratios of detected bile acids (DCA:LCA) constrain dominant animal faecal matter sources in Structure 2 deposits to pigs (<0.4), ruminants (cattle and/or sheep and/or goats (0.6-4.5) and human and/or horses (>5) (Figure 3, Tables 2 and 3). Ratio 2 <0.38, indicates herbivore faecal input in all samples.



**Figure 3:** M1 (outer), M3 (inner) and M<sub>ex</sub> (outside roundhouse entrance) steroid characteristics. Contexts are coloured by assigned phases (Phase 1: yellow; Phase 2: red; Phase 3: blue; Period 2 or 3: green). The phytosterols (campesterol and sitosterol: light blue) and their reduction products (C<sub>28</sub> and C<sub>29</sub> stanols; dark blue) and those of cholesterol (C<sub>27</sub> stanols: orange and yellow), reveal the degree of biohydrogenation of the  $\Delta^5$  unsaturated sterols. Sterol ratios refer to those numbered in the main text: (1) is indicative of the possible presence of faecal

336 matter when  $>0.3$  and (2) is indicative of herbivore faecal input when  $<0.38$ . Bile  
 337 acid DCA:LCA ratios indicate dominant faecal source  $<0.4$  pigs/geese, 0.6-4.5  
 338 human/horses,  $>5$  ruminants. Star symbols highlight where DCA was present in  
 339 isolation, therefore whilst the diagnostic source value cannot be calculated, faecal  
 340 matter is present within the sample. Samples containing chenodeoxycholic acid  
 341 (CDCA), cholic acid (CA) and hyodeoxycholic acid (HDCA) are also indicated using  
 342 bile acid abbreviations.

343

344 **Table 1:** Summary of faecal steroid results from within Structure 2 by location and  
 345 phase. Descriptions of floor layers from Crone and Cavers (2015). \*Dominant  
 346 faecal origin is based on bile acid profiles. All samples have sterol ratio 2 values  
 347 indicative of herbivore faecal matter, therefore a mixed faecal deposit is likely.

Context	Description	Location	Sample depth (cm)	Phase	Steroid characteristics	Faecal origin*
224	Plant litter subfloor	Inner (M3)	A1: 40 A2: 36	1	Faecal steroids present	A1: Humans A2: Mixed source: ruminants, pigs, humans and/or horses
251	Plant litter subfloor	Inner (M3)	30	1	Faecal steroids present	Mixed source: ruminants, pigs, humans and/or horses
253	Small branchwood	Inner (M3)	20	2	Faecal steroids present, reduced decay indicators	Ruminants
250	Plant litter subfloor	Inner (M3)	14 10	2	Faecal steroids present, reduced decay indicators	Ruminants
249	Carbonised plant litter	Inner (M3)	8	2	n/a (low organics)	None
240	Orange clay floor	Inner (M3)	4	3	No bile acids	n/a
224	Plant litter subfloor	Outer (M1)	40	1	No bile acids	n/a
248	Branchwood and brash	Outer (M1)	36 30	1	Faecal steroids present	Humans and/or horses
221A	Plant litter subfloor	Outer (M1)	20 16	1 & 2	Faecal steroids present	A1: Ruminants A2: Pigs
247	Grey clay, subfloor	Outer (M1)	12	2	n/a (low organics)	None
221B	Plant litter subfloor	Outer (M1)	5	2	n/a (low organics)	None
219	Peaty clay, decomposed floor	Outer (M1)	2	Period 2/3	Faecal steroids present	Mixed source: ruminants, pigs, humans and/or horses

348

349 **Table 2:** Summary of faecal steroid results from outside Structure 2 ( $M_{ex}$ ) by  
 350 depth. \*Dominant faecal origin is based on bile acid profiles. All samples have  
 351 sterol ratio 2 values indicative of herbivore faecal matter, therefore a mixed faecal  
 352 deposit is likely.

Depth	Description	Steroid characteristics	Faecal origin*
2 cm	Organic rich deposit with large stones	Faecal steroids present	Humans and/or horses
18 cm	Organic rich deposit with stones	No faecal steroids present	None
21 cm	Organic rich deposit	Faecal steroids present	Humans and/or horses

353

### 354 3.1.1 Spatial patterns in faecal steroids

355 Based on the DCA:LCA bile acid ratio (Prost et al., 2017) and sterol ratio 2 (Leeming  
 356 et al., 1997), the dominant source of faecal matter within the roundhouse originates  
 357 from ruminants (cattle, sheep and/or goats), with ruminant signals occurring more  
 358 frequently in the inner section of the roundhouse (M3) than the outer section (M1)  
 359 (Figure 3, Table 1). Faecal matter from pigs and humans and/or horse are identified  
 360 in some samples from both the inner and outer sections of Structure 2 based on the  
 361 presence of hyodeoxycholic acid (HDCA), diagnostic of pig faeces, and  
 362 chenodeoxycholic acid (CDCA), diagnostic of human and/or horse faeces (Prost et  
 363 al., 2017). While evidence for human and/or horse faeces is detected in  $M_{ex}$ , outside  
 364 of the roundhouse, there is no clear evidence of ruminant or pig faeces in this outside  
 365 area (Figure 3, Table 2).

### 366 3.1.2 Temporal patterns in steroid biomarkers

367 The first detection of faecal material occurs during Phase 1, registering earlier in the  
 368 inner section of the structure than the outer section (Figure 3, Table 1). The initial  
 369 bile acid ratios in both the inner and outer sections of the roundhouse originate from  
 370 humans and/or horses, then becomes mixed with input from humans and/or horses  
 371 (CDCA), pigs (HDCA) and ruminant input (DCA:LCA ratio). The DCA:LCA ratio in

372 Phase 2 indicates ruminant faecal matter and this is the only faecal source detected  
373 during this phase in M3 (inner). However, the source of faecal matter in the outer  
374 structure (in M1) switches to a human and/or horse dominated signal in latter  
375 contexts of Phase 2.

## 376 **3.2 Ecofacts**

### 377 **3.2.1 Insects**

378 Insect preservation is high with intact remains, although overall abundance is  
379 variable, with abundances ranging from 6 – 91 MNI l<sup>-1</sup> of sediment (Table 3). The  
380 highest concentrations of beetles and ectoparasites are present within [221A] (Figure  
381 4) from inner Phase 1, but concentrations between samples from the inner and outer  
382 sections of Structure 2 are comparable. There are low abundances of beetle taxa in  
383 the ‘foul rotting’ category e.g. *Aphodius spp.* and *Aphodius distinctus* (Müll) (Atty,  
384 1983), but they are present in samples from both the inner and outer sections in  
385 conjunction with species associated with the dung of large herbivores (e.g. *Cercyon*  
386 *quisquilius* (L.), *Aphodius prodromus/sphacelatus* (Panz)/(Brahm), *Cercyon*  
387 *melanocephalus* (L.), *Aphodius contaminatus* (Hbst.)(Koch 1989, Duff 1993)). Lice  
388 were found in both phases, *Bovicola bovis* (cattle louse) and *Pulex irritans* (human  
389 flea) [250] (Phase 2, inner section) and *Bovicola ovis* (sheep louse) (Phase 1, outer  
390 section). Fly puparia are common in both Phases 1 and 2 but are more abundant in  
391 samples from the outer areas of the roundhouse.

**Table 3:** Summary of common dung and animal-associated insects from Structure 2 by location and phase (n.d. = non detected). MNI = Minimum number of individuals per L<sup>-1</sup> (all taxa). Foul decomposers = beetle species primarily associated with foul, rotting organic matter (often dung) as defined by Hall and Kenward (1990) & Smith (2012).

Context	Description	Location	Phase	Conc. (MNI l <sup>-1</sup> )	Dung and Foul matter beetles (total counts)	Ectoparasites and flies (total counts)
251	Plant litter subfloor	Inner	1	24	<i>Aphodius Distinctus</i> (1)	<i>Musca domestica</i> (3) <i>Stomoxys calcitrans</i> (3)
250	Plant litter subfloor	Inner	2	32	<i>Cercyon quisquilius</i> (1) <i>Aphodius prodromus</i> / <i>sphacelatus</i> (1)	<i>Bovicola bovis</i> (3) <i>Pulex irritans</i> (1) Phthiraptera indet. (10) <i>Musca domestica</i> (13) <i>Stomoxys calcitrans</i> (4)
249	Carbonised plant litter	Inner	2	9	n.d.	n.d.
248	Branchwood and brash	Outer	1	48	<i>Cercyon melanocephalus</i> (1) <i>Aphodius contaminatus</i> (1) <i>Aphodius spp.</i> (1)	<i>Bovicola ovis</i> (1) Phthiraptera indet. (3) <i>Musca domestica</i> (4) <i>Stomoxys calcitrans</i> (4)
221A	Plant litter subfloor	Outer	1	108	<i>Cercyon pygmaeus</i> (1) <i>Cercyon unipunctatus</i> (1) <i>Aphodius distinctus</i> (1)	Lice spp. indet. (1) <i>Musca domestica</i> (37) <i>Stomoxys calcitrans</i> (10)
221B	Plant litter subfloor	Outer	2	32	<i>Aphodius ater</i> (1) <i>Aphodius spp.</i> (1)	<i>Musca domestica</i> (46) <i>Stomoxys calcitrans</i> (14)



**Figure 4:** Example of preservation level of beetle remains from context [221A]. A large number of elytra are from the hydrophilid genus *Cercyon* (especially *Cercyon analis*) alongside staphylinids and other hydrophilids.

### 3.2.2 Macroplant remains

Macroplant remains primarily consist of three categories: food and food processing waste; fuel debris and flooring materials (Table 4, Figure 5). The food waste consisted of cereals and wild food sources with the majority of this material carbonised but with small amounts that are waterlogged. The cereal and wild food remains were detected within contexts from both the inner and outer sections of the roundhouse: cereal remains were concentrated in inner Phase 1 [251] and outer Phase 3 [219], and wild food remains were most abundant in [251], from inner Phase 1, but were also present in [248] (outer Phase 1), [247] (outer Phase 2) and [250] (inner Phase 2). The main type of fuel used was wood, represented by a mixture of species, but there was also a

small quantity of charred peat. There was no evidence for other types of fuel such as dung in any of the contexts under discussion. The materials used for flooring consisted primarily of bracken, sedges, rushes, woodrush and woody brash.

**Table 4:** The waterlogged and carbonised macroplant assemblage for food and food processing waste and fuel debris categories. Key: \* $\leq 10$ , \*\*=10-29, \*\*\*=29-100, \*\*\*\* $\geq 100$ . All carbonised macroplants are recorded in brackets and all other plant remains are preserved through waterlogging.

Phase		1	1	1	2	2	2	2	3	3
Location (Out = outer, In = inner)		Out	Out	In	Out	In	In	In	Out	In
Context		221	248	251	247	249	250	253	219	240
Sample Vol (kg)		2.5	2.5	2.5	2	1.9	2.5	0.7	20	5
% Sorted		50	50	50	50	50	50	100	100	100
Vernacular name	Plant part									
<i>Hordeum vulgare</i> L.	Caryopses								(*)	
<i>Hordeum</i> var <i>nudum</i> L.	Caryopses								(*)	
<i>Hordeum</i> sp.	Caryopses			*					(**)	
<i>Triticum dicoccum</i> L.	Caryopses								(*)	
<i>Triticum dicoccum</i> L.	Glumes			**					*	
<i>Triticum dicoccum/spelta</i> L.	Caryopses								(**)	
cf. <i>Triticum aestivum/compactum</i> L.	Caryopses								(*)	
<i>Triticum</i> sp.	Caryopses								(**)	
<i>Triticum</i> sp.	Glume		*	***						
<i>Cerealia</i> indet.	Caryopses		**	* (*)					*	
<i>Cerealia</i> indet.	Glume								(*)	
Wild food										
<i>Corylus avellana</i> L.	Nutshell frgs		*(*)	*			*			
<i>Corylus avellana</i> L.	Buds and/or bud-scales				*					
<i>Rubus idaeus</i> L.	Seeds			***	*(*)					
<i>R. fruticosus</i> agg	Seeds				*					
Fuel										
Charcoal (weight in g)			5.5	2.5		2.5		11.5	56.2	91
Charred peat			*	**		****	**			





**Figure 5:** Examples of macroplant and faunal remains extracted from Structure 2 bulk samples. A: hazelnut [219], B: burnt bone [219], C: cereal grains [219], D: charcoal [219], E: chaff [240]

### 3.2.3 Faunal remains

The majority of faunal remains recovered from Structure 2 were small burnt bone fragments, 90% of which were not identifiable to species. There were small quantities of unburnt bone and teeth present. The number of identifiable specimens present in Structure 2 were cattle (61), sheep/goat (4), pig (2), large mammals (47) and medium mammals (164) (Table 5; Figure 5).

**Table 5:** Summary of burnt bone results from within Structure 2 by location and phase

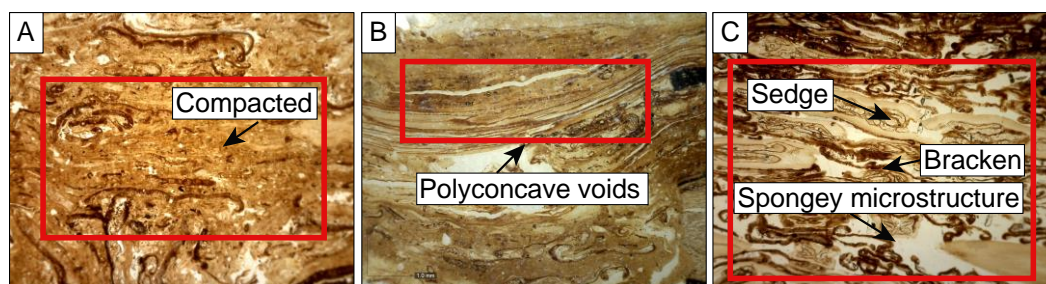
Context	Location	Phase	Bones present	Identifiable species/mammals
251	Inner	1	1.6g, 7 fragments	Large mammal long bone shaft Medium mammal rib (cut mark)
253	Inner	2	0.01g, 2 fragments	None identifiable
250	Inner	2	None	
249	Inner	2	2.8 g, 14 fragments (8 unburnt)	Cattle bone Cattle premolar, molar Medium mammal rib
240	Inner	3	51.7g, 56 fragments (10 unburnt)	Large and medium mammal long bone shafts Medium mammal mandibles, rib and vertebrae
248	Outer	1	5 g, 12 fragments (6 partly charred)	Medium mammal long bone shaft x 2 (burnt), phalanx and partly charred premolar
221	Outer	1 and 2	None	
247	Outer	2	None	
219	Outer	Period 2/3	44.5 g, 26 fragments (< 50 mm, burnt)	Cattle molar (unburnt) Sheep/goat humerus (burnt)

### 3.4 Micromorphology

The primary constituent of the floor material is plant organic matter, which is exceptionally preserved throughout the samples (Figure 6), but differences exist in the birefringence of organic matter with less degradation exhibited in samples from the outer area of the structure. Occasional thin excremental pedofeatures (<100 µm) caused by microfauna, indicative of limited bioturbation, are restricted to outer Phase 1 [248] and inner Phases 1 and 2 [250] and [251]. Anthropogenic indicators, based on micromorphology, are limited in most of the samples from the outer contexts as there are very few small wood and bark chips. Small amounts of coprolitic material, although not identified to species level, was observed in the inner section from context [251] (Phase 1) and possibility [240] (Phase 3) (Table 6).

The identification of trampling, a possible transfer mechanism of faecal material in organic sediments under waterlogged conditions is difficult to detect, as water and compression during burial causes swelling of sediment and masks trampling

indicators. However, the presence of loam and distinct microstructures suggests some trampling in both the inner and outer sections of the roundhouse during Phase 1 (contexts [224], [251] and [248]) but only in the inner section during Phase 2 [250] (Figure 6, Table 6).



**Figure 6:** Examples of morphology from Structure 2 samples. A: compacted/trampled organic layer [224], B: polyconcave voids [250] (inner), C: spongy microstructure and layers of bracken and sedge material [250] (outer)

**Table 6:** Summary of micromorphology results from within Structure 2 by location and phase (n.d = non detected)

Context	Location	Phase	Coprolitic material	Trampling indicators
224	Inner	1	n.d	Lenticular microstructure. Loam/soil clasts brought in from outside embedded within matrix.
251	Inner	1	Unknown source	Compacted, lenticular to massive microstructure. Loam/soil clasts embedded within matrix.
253	Inner	2	n.d	n.d
250	Inner	2	n.d.	Linear compaction and striation of coarse material. Polyconcave voids
249	Inner	2	n.d.	n.d.
240	Inner	3	Possible herbivore	n.d
224	Outer	1	n.d.	Parallel arrangement of inclusions, massive microstructures, polyconcave voids, low porosity, loam/soil clasts.
248	Outer	1	Soil microfauna only	Possible (dusty clay coatings to voids indicative of rotational movement of sediment caused by trampling)
221	Outer	1 and 2	Possible (yellow phosphatic filling)	n.d
247	Outer	2	n.d	n.d
219	Outer	Period 2/3	n.d	n.d

## 4. Discussion

### 4.1 Occupation floor deposits: detection of faecal matter and source organisms using steroid biomarkers

The majority of samples analysed from both the inner and outer sections of Structure 2 contained evidence of faecal matter as supported by the presence of bile acids, which are deposited in the excreta of vertebrates (Haslewood et al., 1967; Hofmann and Hagey, 2008). Comparisons of bile acids and  $5\beta$ -stanols within this study highlight the importance of considering context when applying sterol ratio threshold values (Grimalt et al., 1990; Bull et al., 1999) to definitively identify faecal sources within wetland settlement deposits: all ratio 1 values within this study were  $<0.7$ , which would only indicate the *possibility* of a faecal source based on Grimalt et al. (1990) thresholds, despite the majority of samples analysed within Structure 2 containing conclusive evidence of faecal matter deposition based on bile acids profiles. The ratio 1 threshold value was designed for modern sewage samples (Grimalt et al., 1990) and its validity in archaeological contexts has been critiqued (Bull et al., 1999, 2001, 2005; Simpson, 1998; Prost et al., 2017). The application of ratio thresholds to identify faecal inputs has been shown to be particularly challenging within organic rich soils, such as those obtained from Structure 2, owing to the abundance of  $5\alpha$ -stanols derived from plant remains (Birk et al., 2011); this drives faecal sterol ratio below the indicative thresholds even when faecal matter is present (e.g. Fritsmons et al., 1995; Birks et al., 2011).

Whilst the dominance of a  $5\alpha$ -stanol input in organic rich soils could call for a lowering of faecal sterol threshold values, one sample analysed from the inner section of the roundhouse contained a sterol ratio within the 'possible faecal matter'

range despite having no corresponding bile acids and therefore no supporting evidence for faecal matter. This example echoes findings from other studies reporting the presence of 5 $\beta$ -stanols despite no other evidence for faecal deposition (e.g. Bethel et al., 1994; Bull et al., 2001; Evershed et al., 1997). Multiple lines of faecal evidence, including both 5 $\beta$ -stanols and bile acids are therefore a more robust approach than changing threshold values when working with diffuse faecal sources in sedimentary settings.

Several studies have successfully circumvented sterol ratio threshold problems for faecal identification by comparing background sediment 5 $\beta$ -stanols concentrations with those from anthropic samples (e.g. Birk et al, 2011; Harrault et al., 2019). Alongside this contextual approach, our results demonstrate the importance of analysing both the sterol and bile acid lipid fractions from the same sediment sample when characterising diffuse faecal sources. This combined approach, also encouraged by Bull et al. (2002) and Prost et al. (2017) for more concentrated faecal inputs, not only provides greater confidence in faecal identification and constraining faecal sources, but also mitigates against possible difficulties in obtaining contemporary non-anthropoc sediment samples required to accurately establish background concentrations. An example of such difficulty within the Iron Age setting of this study, is achieving adequate chronological control for comparisons of different sampling locations when dates fall within the Hallstatt plateau (Becker and Kromer, 1993), a period of minimal discernible changes in radiocarbon calibration curve between 750 -400 cal BCE, when most radiocarbon determinations have calibrated age ranges in the order of several centuries (Crone et al., 2012).

Our steroid results demonstrate the presence of faecal matter from

cattle/sheep/goats, pigs and horse and/or humans in Structure 2. The presence of ruminants is supported by the faunal evidence which includes bones and teeth from cattle and sheep/goat. There is no evidence for pig or horse faunal remains in the contexts analysed, although the presence of pigs on site is supported by faunal remains from other contexts and insect remains are indicative of large herbivores contemporaneous with human/horse faecal signals (Figure 7). Steroid analyses of Structure 2 provide evidence for a greater diversity of animals associated with each context compared with faunal analysis where the acidity of the soils ( $\text{pH } 5.3 \pm 0.4$ ) hinders calcified bone survival.

The steroid results from Structure 2 have enhanced characterisation of animals associated with the roundhouse, however, the resolution of some identified faecal sources is lower than expected based on ratios obtained from modern reference material in concentrated archaeological faecal deposits (e.g. Prost et al., 2017; Harrault et al., 2019). This likely represents the difficulties of identifying faecal sources using diagnostic ratios and key indicator compounds when faecal inputs originate from a mix of source organisms (Prost et al., 2017) and are incorporated within organic-rich sedimentary archives (Birk et al., 2011). In such instances, the dominant faecal source organism(s) may be identified, but the refinement of faecal source identification relies on bile acid preservation (Prost et al., 2017). Faecal sterol distributions have also been used to refine differentiation of faecal sources, such as multivariate analyses of eleven  $5\beta$ -stanol compounds (Harrault et al., 2019).

However, the full suite of  $5\beta$ -stanol compounds required for faecal source differentiation are not present in all lipid extracts, including samples from this study and those analysed by Leeming et al. (1996), thereby limiting the resolution of faecal source identification. Such differences in  $5\beta$ -stanol characteristics between studies

may relate to differences in diet, which controls compound distributions (Prost et al., 2017; Harrault et al., 2019), or the dominance of plant-derived sterols and other polar lipid compounds within organic-rich sediments, which mask low  $5\beta$ -stanol concentrations despite extensive sample clean-up within the lipid analytical protocol.

Whilst multiple lines of steroid evidence must be considered when identifying faecal sources (Prost et al., 2017), an important consideration is the sensitivity of diagnostic ratios to different faecal sources. For example, since ruminants have a characteristic bile acid-derived DCA:LCA value, which is an order of magnitude higher than pigs and in some cases human and/or horses, their faecal signal has the potential to dominate a mixed source DCA:LCA ratio even if they were not the dominant faecal input. Therefore, whilst the dominance of ruminant faecal matter may be a robust feature within Structure 2 and is supported by sterol ratio 2, it may also be influenced by the sensitivity of the DCA:LCA ratio to ruminant faecal input. Experimental studies are essential to refine these diagnostic ratios and steroid distributions for diffuse faecal inputs within sedimentary deposits using approaches such as mixing models.

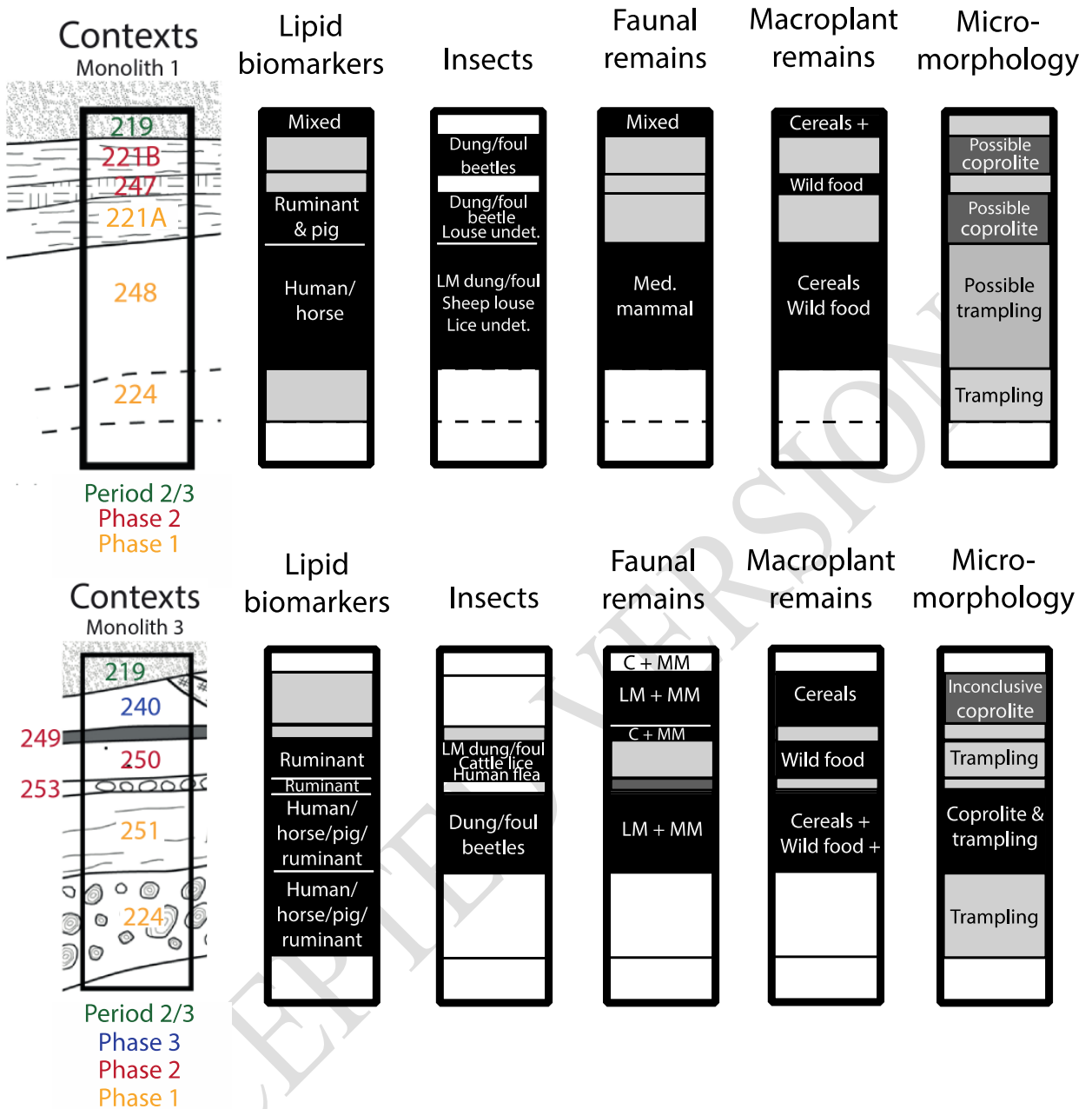
## **4.2 Multiproxy comparisons of faecal indicators**

Approximately 60% of analysed floor deposits contained dung indicators within the steroids compared with 50% from insect analyses and 10-20% from micromorphology (Figure 7). There are no conclusive dung indicators within the macroplant remains, although distinguishing between dung, fodder and floor deposits from macroplant remains is complex since plant assemblages are similar within these sources. Context [251] from Phase 1 of the inner section of the roundhouse does contain a high abundance of raspberry (*Rubus idaeus* L.) seeds,

555 which may originate from faecal deposition (e.g. Buckland, 1976; Miller and Smart,  
556 1984). Confirmation of faecal matter within this context is provided by the mixed  
557 steroid signal and the identification of coprolitic remains within the  
558 micromorphology (Figure 7), thus demonstrating the value of multiproxy  
559 comparisons, as also presented by Shillito et al. (2011).

560 Multiproxy dung comparisons across Structure 2 demonstrate the presence of  
561 steroids, low abundances of dung/foul indicator insect species and minimal  
562 micromorphological and macrofossil evidence, which suggests dung deposits that are  
563 transient or restricted (rather than persistent or large scale) within the roundhouse.  
564 The low quantities of domestic debris and sharp contacts between floor layers point  
565 towards active floor cleaning and/or removal of dung from Structure 2 and may  
566 explain the low insect signal throughout the structure. Despite the removal of floor  
567 material, the geochemical faecal signature has been preserved within the remaining  
568 floor surfaces. Similar practices of floor cleaning have been identified at other  
569 Scottish Iron Age structures e.g. Cnip in Lewis (Armit, 2006) and Cults Loch in  
570 Wigtownshire (Roy, 2018; Robertson, 2018), where removal and replacement of  
571 floor layers were identified from excavated stratigraphy. Incorporating steroid  
572 analysis of archaeological structures therefore has the potential to provide a more  
573 holistic insight in to occupation conditions of Iron Age roundhouses. This is  
574 especially true where floor clearing has occurred and many of the more traditional  
575 microscopic anthropic signals have been removed, or preservation conditions for  
576 macro-organic materials is poor.





**Figure 7:** M1 (outer) and M3 (inner) proxy comparisons. Black indicates clear evidence of large mammals, faecal sources or domestic food waste, dark grey indicates possible evidence of large mammals or faecal sources, light grey indicates no evidence of mammals, faecal matter or domestic food waste detected and white represents contexts with no data. Contexts with micromorphological evidence of trampling are also noted and source of dung/animal indicator listed (C=cattle, LM= large mammal, MM = medium mammals).

## 4.3 Multiproxy characterisation of Iron Age wetland roundhouse use

### 4.3.1 Spatial patterns of use associated with Structure 2

Our steroid results show clear spatial differences between inside and outside of Structure 2, with ruminant bile acid profiles detected in M3 and M1, but are absent from outside in M<sub>ex</sub> (Figure 3). The ruminant faecal signal also differs within the structure, with a stronger ruminant signal present in the inner section of the roundhouse (Figure 8). This faecal signal in the inner section is concomitant with *Bovicola bovis* (cattle louse) (Table 3, Figure 7) and is consistent with evidence from the micromorphology, since the inner section contained more contexts with confirmed trampling indicators and the only confirmed coprolitic remains were detected in context [251] from the inner section. The presence of faeces in context [251] is supported by the archaeobotanical evidence which contains the highest abundance of uncharred raspberry seeds, likely deposited within dung (e.g. Miller and Smart, 1984). The spatial distribution of faecal matter within Structure 2 could be related to (a) ruminant faecal matter being transferred into the inside of the roundhouse via trampling; (b) animal dung being used as hearth fuel and/or hide processing and (c) animals being kept within the structure.

The absence of evidence for ruminant faecal matter outside of the roundhouse based on M<sub>ex</sub> would suggest that trampling could not be a source of ruminant faecal matter into Structure 2. Proxy comparisons further support this: for example, context [244] in M1 contains micromorphological evidence of trampling, yet no faecal signal is detected in the steroids. Steroid dung signals are also more prominent in the inner sections of Structure 2 (i.e. in M3 next to the central hearth structure), but if

608 trampling was the key process then one would expect faecal matter to be widely  
609 distributed throughout the roundhouse.

610 Disentangling the causes of the stronger ruminant faecal signal near the hearth is  
611 difficult. It is possible this is related to dung storage, most likely for fuel, but we  
612 cannot rule out animal waste being produced in situ. If dung was kept close to the  
613 fire for ease of access, then this is likely to have accumulated on the floor surface  
614 surrounding the hearth. However, the main fuel identified from the macroplant  
615 analyses was charcoal and there is no clear evidence of burnt dung from the  
616 macroplant or micromorphological results or charred insect remains. Without  
617 geochemical analyses such as magnetism (e.g. Peters et al., 2004), XRF (e.g.  
618 Braadbaart et al., 2017) or phosphates (Macphail et al., 1997) on hearth deposits  
619 from Structure 2 it is difficult to eliminate dung as a fuel source. Based on the insect  
620 remains, the absence of charred dung and the wood charcoal in the macro-plant  
621 analysis, it is unlikely dung was a dominant source of fuel and therefore the faecal  
622 signal, within Structure 2.

623 The distribution of steroids most likely reflects the presence of designated livestock  
624 stalls within Structure 2. The more persistent faecal signal by the hearth could be  
625 explained by the deliberate placement of tethered animals proximal to the heat  
626 source to aid survival of the young or sick. Support for animal sheltering to improve  
627 the survival rates of new-born or unwell ruminants is evident from modern farming  
628 and veterinary studies as exposure is a key determinant of new-born mortality rates  
629 in wet and cold climatic conditions typical of Iron Age Scotland (e.g. Pollard, 2006;  
630 Hinch and Brien, 2014, Rawson et al., 1989). Placement by the hearth would also  
631 mimic the modern frost bite treatment for calves of rapid warming (Pelton et al.,  
632 2000).

#### 633 4.3.2 Temporal patterns of use within Structure 2

634 During Phase 1 of the occupation of the structure, the first source of faecal matter  
635 detected in both the inner and outer sections is horse and/or human and this  
636 indicates relatively foul living conditions, based on dung indicators across all proxies  
637 (Figure 7, Figure 8), suggesting greater persistence or abundance of faecal matter.  
638 With the detection of charcoal and food debris, such as bones and seeds, this  
639 suggests the inner section of Phase 1 contains household debris.

640 In Phase 2 of the occupation, the bile acid results suggest that the faecal source  
641 changed to ruminants in both sections of the structure. The insect assemblage of  
642 dung-associated taxa, flies and lice, and the resemblance of the micromorphology of  
643 context [250] to stabling environments, as well as the digested berry seeds identified  
644 within the macrofossils, also point to animal activity and the accumulation of dung.  
645 The number of dung-associated taxa and concentrations of fly puparia present a  
646 strong argument for the presence of dung in a foul deposit, but it remains difficult to  
647 conclude the specific activities from the insect evidence alone as is the case with  
648 other studies with greater insect numbers (Forbes and Milek 2014). The low  
649 concentrations and diversity of the dung insect community could be explained by  
650 regular removal and replacement of floor layers. The structure size would also limit  
651 the number of animals that could be housed (and thus the amount of dung produced)  
652 and the overall numbers of dung beetles would be reduced due to barriers to the  
653 outside created by walls (Smith *et al.*, 2014). The presence of *Bovicola bovis* in the  
654 inner section of the roundhouse highlights the complementary nature of faecal  
655 steroids and insect analyses, as the steroids confirm the presence of dung and insect  
656 indicator species refine the ruminant signal to confirm cattle and/or cattle hides  
657 were present.

### 4.3.3 Implications for use of Structure 2 and daily Iron Age life

Possible functions of Structure 2 include space for sleeping, storage, food preparation, craft working and/or animal stalling (Pope, 2007). Our multiproxy analyses show there is no overwhelming evidence for sleeping in this particular structure, as insect concentrations are low with only one human louse identified and there is minimal structural evidence for bedding. Similarly, there is no conclusive evidence for craft working because there is no debris associated with this activity in the micromorphology and macroplant remains. Evidence for food preparation in the inner section of the roundhouse comes from small quantities of domestic debris within the macroplant remains and micromorphology, as well as the presence of cereal caryopses and chaff, which suggest small scale grain processing was likely to have been occurring. Storage within the outer section of structure 2 is possible, but there is no evidence for storage remains and the presence of the large hearth structure indicates storage is likely be a secondary rather than primary function of Structure 2.

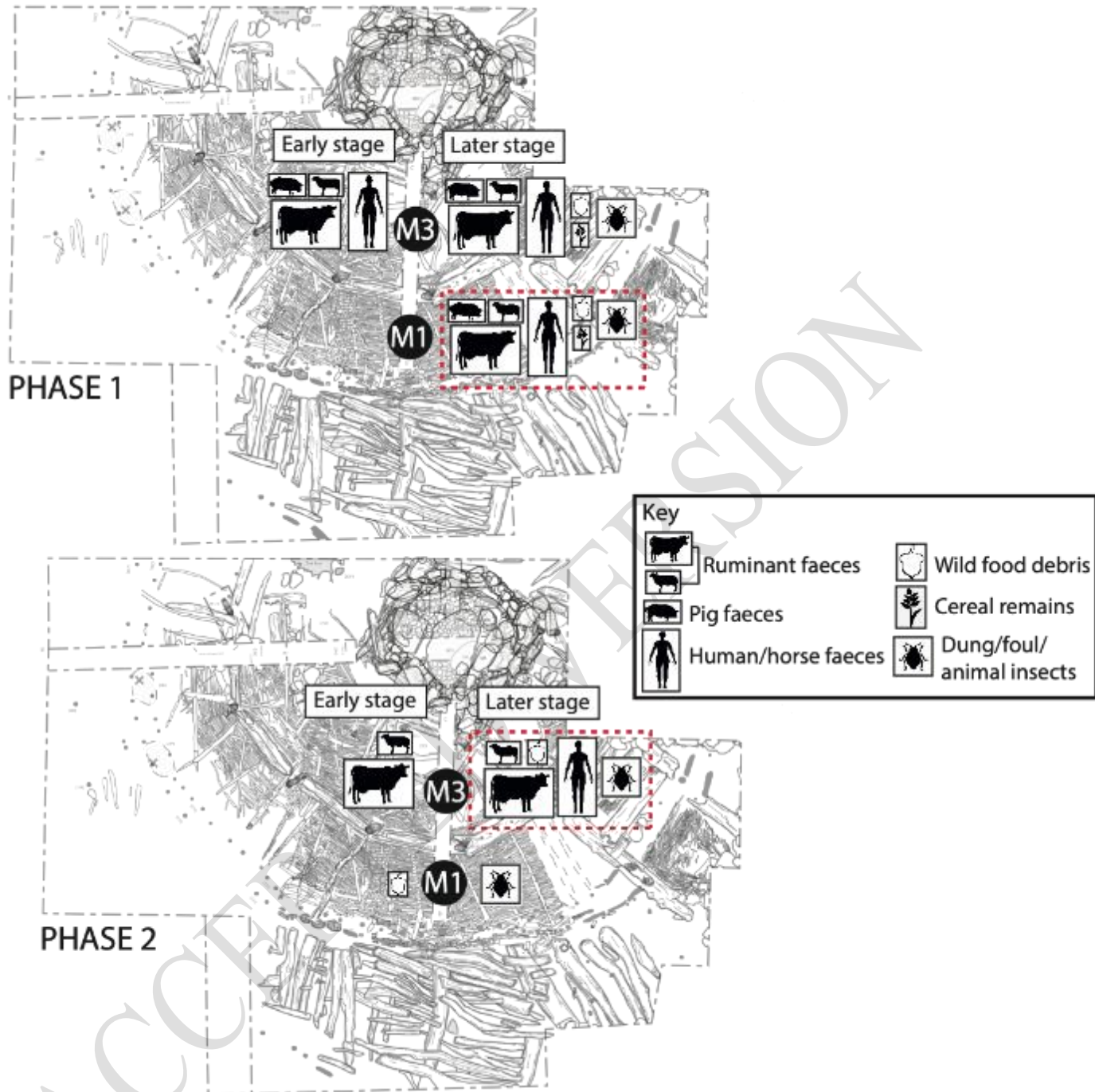
The steroid evidence from Structure 2 suggests animals were present within the roundhouse, however, the combined evidence across all proxies does not support a long-term and/or intensive stabling environment. The absence of animal-derived steroids from M<sub>ex</sub>, located outside of Structure 2, indicates that the roundhouse was used as a temporary or small scale area of human-animal cohabitation since we would expect to see a strong steroidal faecal signal outside the structure as a result of trampling and animal movement in and out of the roundhouse if this was a significant stabling environment. The ability to detect animal movement linked to stabling practises has been demonstrated using 5 $\beta$ -stanols analysed from the entrance of a stabling area in a modern experimental study of a reconstructed Iron

683 Age roundhouse, which, unlike results from Structure 2, reported the entrance had  
684 similar sterol signatures to deposits located within the stable (Hjulström and  
685 Isaksson, 2009).

686 There is a lack of evidence for dedicated stabling structures or permanent 'byre-  
687 houses' (*sensu* Harding, 2004; 2009) in British Iron Age sites (Sørensen, 2007). The  
688 clearest evidence for co-habitation comes from outside Britain, from Nørre Tranders,  
689 Denmark (Nielsen, 2007). Interior stalling has been inferred from structural  
690 evidence and high phosphate levels at Woodend Farm in Dumfries and Galloway  
691 (Banks, 2000; Duncan, 2000) and excavations at Dun Vulcan, South Uist suggest  
692 byre structures occurred within enclosures (Pearson and Sharples, 1999). The  
693 possible temporary presence of animals within occupied Iron Age structures has  
694 been identified from floor deposits after a rebuilding phase at Glastonbury Lake  
695 Village, England (Hill *et al.*, 2018). The results from both Glastonbury Lake Village  
696 and Black Loch of Myrton (this study) indicate associations between animals and  
697 Iron Age roundhouses likely changed over time, reflecting variability of roundhouse  
698 usage. Whilst both structures may have been used as temporary small-scale co-  
699 habitations of humans and animals, there is no evidence to suggest they were  
700 permanent byre-houses.

701 The internal activity within Structure 2 based on our steroids, micromorphology and  
702 archaeobotanical remains (Figure 8), follows Hingley's model of an active central  
703 area and peripheral outer area (Hingley, 1990) and supports the dominance of this  
704 model in Iron Age roundhouses in Northern Britain (*sensu* Hill, 1995, Pope, 2007). A  
705 peripheral, less frequently used outer area may also explain the abundance of flies  
706 detected in the outer section of the roundhouse since the reduced disturbance would  
707 facilitate fly larval pupation. Despite minimal evidence for activity in the outer

708 section of the roundhouse compared with the inner section, micromorphological  
709 insights into floor cleaning and rebuilding in the outer section highlight the  
710 importance of maintaining the cleanliness of this area even under difficult  
711 waterlogged conditions. Hawkes (1994) suggested outer roundhouse areas were not  
712 characterised by inactivity but rather served important 'cleaner' functions, such as  
713 storing foodstuffs and firewood or sleeping. Interpretations of the multiproxy results  
714 across Structure 2 highlight efforts to frequently clean and maintain this roundhouse  
715 and support use as a shelter, likely with different primary functions depending on  
716 requirements over time.



**Figure 8:** Summary of multiproxy results highlighting differences in spatial and temporal use within Structure 2. Red boxes represent periods of multiproxy evidence for dominant dung/animal presence and household debris.



## 5. Conclusions

Our study highlights the power of multiproxy approaches and the incorporation of steroids to advance insight into structure use, particularly when the sampling resolution facilitates characterisation of within-structure spatial and temporal patterns. Analyses of the Black Loch of Myrton's Structure 2 deposits provide evidence of floor cleaning and changes in use over time, demonstrating flexibility in roundhouse use over their short life cycle (*ca.* 30-40 years in this case). There is a more persistent faecal signal in the inner section compared with outer section of the roundhouse and this supports the 'active central area' roundhouse model (Hingley, 1990). In this case at Black Loch of Myrton, our data suggest small-scale temporary stabling within Structure 2, but likely only as a secondary function. Our results, however, highlight spatial complexity in roundhouse use as the outer area was less actively used and foul conditions persisted thus questioning the use of this space for 'clean conditions' (cf. Hingley, 1990).

Our application of steroids has successfully captured signals from short-lived/temporary pulses of faecal matter that are more difficult to extract from other traditional archaeological proxies. Our results also demonstrate steroids are particularly effective in archaeological settings with acidic soils, since they can identify the presence of animals where uncalcified bones do not preserve. Furthermore, faecal steroids provide valuable information about archaeological structures that have been subjected to the act of cleaning since they persist when visible indicators are removed or diminished.

The identification and characterisation of diffuse faecal input to the wetland settlement floor deposits within this study relies on analyses of both sterols and bile acids, supporting this combined analytical approach advocated by Bull et al., (2002)

747 and Prost et al. (2017) to effectively overcome known issues relating to sterol ratio  
748 threshold values and help refine faecal source characterisations. The diffuse faecal  
749 steroid input associated with this Iron Age roundhouse has limited the resolution of  
750 faecal source characterisation compared with that achieved in more concentrated  
751 faecal remains (e.g. Prost et al., 2017; Harrualt et al., 2019). However, the achieved  
752 source resolution is sufficient to advance understanding of human-animal  
753 interactions and cleanliness within the structure, and has benefited from further  
754 refinement through multiproxy comparisons.

755 The utility of incorporating steroid analysis is not restricted to wetland Iron Age  
756 structures, but is equally applicable to other periods and types of structures in  
757 different depositional settings. What is needed, however, are floors, inter-floor  
758 deposits, cleaning deposits or sealing layers contemporaneous with the  
759 abandonment of the structure. This study has highlighted the need for further  
760 experimental work focusing on diffuse faecal deposition in bulk occupation  
761 sediments to address questions raised about sensitivities of diagnostic ratios in such  
762 settings. A problem also highlighted here is where the controls should come from,  
763 especially within a settlement of several houses. An excavation of a small test pit  
764 outside the habitation area would seem most appropriate. As this study also shows,  
765 the combination of steroids with other proxies can help verify interpretations but  
766 may also raise new questions for investigation.

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