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Faecal biomarkers as evidence of human presence in the caves of Kraków-Częstochowa Upland

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ABSTRACT

This study investigates past human presence at cave sites by analysing faecal biomarkers—specifically sterols, bile acids and phosphorus—and compares the results with available archaeological data. The research concentrates on seven sites in the Kraków-Częstochowa Upland (S Poland): Biśnik Cave, Bramka Rockshelter, Ciasna Cave, Łabajowa Cave, Łokietka Cave, Sąspowska Zachodnia Cave, and Shelter in Smoleń III. It involved analysing sediment samples from the stratigraphic sequences of each site. The application of faecal biomarkers allowed us to detect human faecal matter at several distinct stratigraphic levels, aligned with and independent of archaeological evidence. Our findings contributed to existing archaeological data on human occupation in the region and revealed distinct patterns in the presence and distribution of faecal markers. The results highlight the potential of targeting deeper cave areas, particularly Palaeolithic layers, while also identifying key limitations—most notably the low concentrations of bile acids, which hinder interpretation, highlighting the need for further research into the factors influencing biomarker stability in cave contexts.

1. Introduction

Sediments at archaeological sites were once seen primarily as the context for artefacts and human or animal remains. Later, they became a key source for environmental reconstruction, including molecular analyses, for example, of lipids (Argiriadis et al., 2018; Buonasera et al., 2015; Connolly et al., 2021; Holtvoeth et al., 2010; Kovaleva et al., 2019; Ludgate, 2012; Patalano et al., 2020; Schwark et al., 2002; Xie et al., 2002). Recent advancements in molecular methods, such as sedaDNA (Brown et al., 2021; Curtin et al., 2021; Slon et al., 2017; Zetter et al., 2025) or soil proteomics (Oonk et al., 2012), have revealed new possibilities for sediment analysis, enabling the detection of previously hidden evidence of human presence and activity at archaeological sites. This study aims to follow this approach by exploring the potential of faecal marker analysis—specifically, faecal sterols, bile acids, and phosphorus—to provide direct evidence of human presence in caves. These compounds have rarely been explored in caves to detect human faecal matter. However, the stable conditions and good organic matter preservation offer significant potential for uncovering traces of human activity.

Sterols are lipids that play an essential role in the structure of cell and organelle membranes in higher organisms (Malainey, 2011). In animal and human tissues, the predominant sterol is cholesterol, while in plants, it includes sitosterol, stigmasterol, and brassicasterol, all classified as Δ5-sterols. Sterols in humans and animals can be synthesised internally or absorbed from food and are later excreted in faeces (Cuevas-Tena et al., 2017). The composition of sterols in faecal matter reflects variations in diet and metabolic pathways, allowing for the differentiation of herbivores, carnivores, and omnivores (Bull et al., 1999; Gryczewska et al., 2025; Harrault et al., 2019; Leeming et al., 1996; Prost et al., 2017; Shillito et al., 2020).

Among sterols, 5β-stanols, such as coprostanol (derived from cholesterol) and 24-ethyl-coprostanol (derived from sitosterol), are particularly significant for archaeological research. These compounds

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are produced in the digestive tracts of humans and animals and excreted alongside other faecal sterols (Björkhem and Gustafsson, 1971; Gérard, 2013; Harrault et al., 2019). After deposition, they can undergo epimerisation to form compounds such as epi-5β-coprostanol but remain stable over time, making them reliable biomarkers for detecting faecal remains (Bull et al., 2002; Prost et al., 2017). In contrast, Δ5-sterols, such as cholesterol, can originate in sediments from various sources, including decomposing plant or animal material, root exudates or fungal presence (Bethell et al., 1994; von der Lühe et al., 2013; Weete et al., 2010). These sterols can also transform into 5α-stanols, such as cholestanol, and to a lesser extent, into 5β-stanols (Bull et al., 2002; Gaskell and Eglinton, 1975). Analysing the complete spectrum of sterols in sediment allows for identifying faecal matter and its specific source, including human-derived inputs (Battistel et al., 2015; Prost et al., 2017).

Bile acids (BAs) are 24-carbon amphipathic molecules synthesised from cholesterol in vertebrates (Dutta et al., 2019). They are classified into primary, secondary, and tertiary BAs (Dutta et al., 2019; Gérard, 2013; Hofmann and Hagey, 2008). Primary BAs, such as cholic acid (CA) and chenodeoxycholic acid (CDCA), are produced in the liver and metabolised in the intestine into secondary BAs, including deoxycholic acid (DCA), lithocholic acid (LCA), and hyodeoxycholic acid (HDCA). Tertiary BAs, such as ursodeoxycholic acid (UDCA), are produced through further modifications of secondary BAs in the liver and gut, with UDCA being derived from LCA (Di Ciaula et al., 2017). BAs facilitate the digestion and absorption of dietary fats while influencing the gut microbiota's composition and function (Hofmann and Hagey, 2008; Panek-Jeziorna and Mulak, 2017). Like 5β-stanols, BAs are excreted in faeces, with their composition and concentration varying between taxa due to differences in metabolism and, to some extent, diet and health of the individual (Panek-Jeziorna and Mulak, 2017; Porru et al., 2022; Prost et al., 2017). However, unlike 5β-stanols, BAs in archaeological contexts originate exclusively from faeces or decomposing bodies.

Faecal biomarkers have been used at archaeological sites to trace human presence and activity, settlement patterns, land use history, and husbandry practices. First studied at the Roman fort of Bearsden (Knights et al., 1983), they have since been extensively researched in latrines, middens, and cesspits (Baeten et al., 2012; Bethell et al., 1994; Bull et al., 2005), as well as in contexts related to manuring (Battistel et al., 2015; Bull et al., 1999). More recently, their application has expanded to lacustrine sediments to reconstruct settlement history around lakes, beginning with Lake Liland in Norway (D'Anjou et al., 2012), and followed by other studies focused on human arrival or husbandry practices (Battistel et al., 2015; Brown et al., 2021, 2022; Guillemot et al., 2015; Schroeter et al., 2020; Vachula et al., 2019). At open-air sites, they provide insights into spatial use and the function of specific structures (Bemmann et al., 2014; Mackay et al., 2020; March, 2018; Scherer et al., 2021; Tomé et al., 2024). In caves, they are used to identify burnt dung deposits ("fumiers") associated with livestock enclosures (Fernández-Palacios et al., 2024; Gea et al., 2017; Vallejo et al., 2022, 2024). Research on human faeces in caves remains limited but includes two Middle Palaeolithic sites (Krajcarz et al., 2013; Sistiaga et al., 2014a,b) and studies of coprolites preserved in cave environments (Lin et al., 1978; Shillito et al., 2020; Sistiaga et al., 2014a,b).

Additionally, phosphorus is included here as another potential marker of faecal matter. While linked to a variety of human activities like food preparation, waste disposal, and manuring (Devos, 2018; Fernández et al., 2002; Holliday and Gartner, 2007), its combination with other methods can help identify specific activities (Fernández et al., 2002; Homsey and Capo, 2006; Misarti et al., 2011; Wilson et al., 2009). Phosphorus has also been used in caves as a proxy for human presence (Goldberg and Nathan, 1975; Schiegl et al., 1996), though bat guano and decomposing animals are important natural sources at many sites (Audra et al., 2019; Shahack-Gross et al., 2004; Sokol et al., 2022).

Despite their potential, faecal biomarkers are still underutilised in cave sediments. Although cave environments are generally stable, the preservation of organic compounds varies due to regional climate and cave microbiota (Biagioli et al., 2024; Ravn et al., 2020). Cave stratigraphy is often discontinuous (Krajcarz et al., 2020), complicating reconstructions compared to well-stratified lake sediments (Brown et al., 2021; D'Anjou et al., 2012; Vachula et al., 2020). Post-depositional disturbances, such as burrowing animals, further hinder interpretation. Additionally, the changing use and function of caves over time (Angelucci et al., 2009; Bailey and Galanidou, 2009; Bergsvik and Skeates, 2012; Kot et al., 2021) can limit the presence of human faecal matter. Nevertheless, we aim to demonstrate the significant potential of faecal biomarker analysis through a regionally focused approach.

This study applies faecal biomarkers—specifically sterols and bile acids—to investigate past human presence in cave environments, focusing on seven cave sites in the Kraków-Częstochowa Upland, Poland. Additionally, total phosphorus (TP), total organic carbon (TOC), and total nitrogen (TN) were measured, and the TOC/TN ratio was calculated.

Specifically, our research focuses on two questions.

(1) Can we obtain new data on the occupational history of the studied caves, particularly in layers where human presence remains unconfirmed, through faecal biomarkers?
(2) Can we observe any patterns in the presence and consistency of faecal biomarkers over time or across the studied caves, and what insights does this offer into the limitations and potential of using faecal markers in cave settings?

We seek to supplement traditional archaeological evidence, such as artefacts or skeletal remains, with chemical signatures indicating the presence of humans. This approach is particularly valuable in cases where direct evidence is sparse or absent, offering a new method to reconstruct past human activities in cave environments.

2. Materials

Cave sediment samples were collected from seven sites situated in the Kraków-Częstochowa Upland: Biśnik Cave, Bramka Rockshelter, Ciasna Cave, Łabajowa Cave, Łokietka Cave, Sąspowska Zachodnia Cave, and Shelter in Smoleń (Fig. 1). Sampling was typically conducted on uncovered and cleaned profile sections. The study focuses on analysing the most complete stratigraphic sequences available (Fig. 1B). Additionally, samples from the uppermost layers were excluded in some cases as current contamination was anticipated. Details concerning the archaeological sites included in the project and data on the sediment samples included in the research can be found in **Supplementary Materials (S1-S2).**

3. Methods

3.1. Faecal sterols and bile acids analysis

Faecal biomarkers were analysed using the method proposed by Birk et al. (2012) with several modifications. A detailed description is provided in the **Supplementary Materials (S3)**. The compounds studied included faecal sterols, specifically coprostanol (cop), epicoprostanol (epicop), cholesterol, cholestanol, brassicasterol, stigmasterol, 24-ethyl-coprostanol (24ethylcop), 24-ethyl-epicoprostanol (24ethylepicop), β-sitosterol, and sitostanol, as well as bile acids (BAs), specifically deoxycholic acid (DCA), lithocholic acid (LCA), cholic acid (CA), chenodeoxycholic acid (CDCA), hyodeoxycholic acid (HDCA), and ursodeoxycholic acid (UDCA). The reagents used in the analysis were of at least analytical grade, with a detailed list provided in **Supplementary Materials (S4).**

Sediment samples were dried, ground, and sieved (1 mm mesh). Approximately 10 g of each sample was spiked with D6-cholesterol and nordeoxycholic acid (NDCA), then extracted using Dionex ASE 350 with

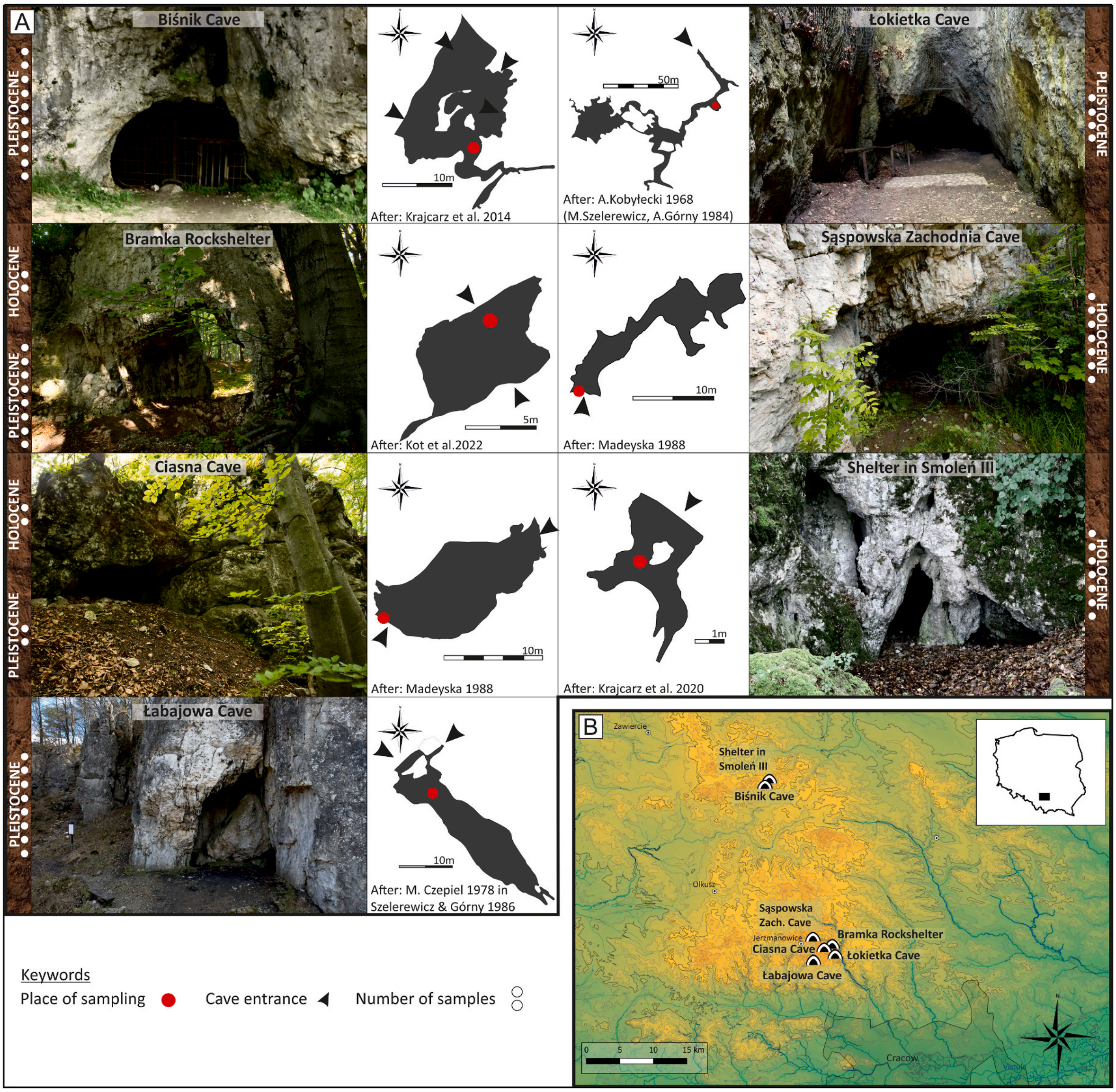

Fig. 1. A. Photos and outlines of cave sites included in the study with information concerning general chronology and number of sediment samples analysed. Phot. M.Myszkowski, M.Bogacki, K.Lorek. B. Map of Kraków-Częstochowa Upland with marked location of the sites included in the study. Prepared by C.Berto.

dichloromethane (DCM)/methanol (2:1, v) at 100 °C. The solution was dried under nitrogen after each step. Extracts were saponified overnight in 0.7M KOH in methanol, followed by liquid-liquid extraction with chloroform to separate sterols (neutral) and bile acids (acidic). BAs were methylated using 1.25 M HCl in methanol at 80 °C for 2 h and later neutralised using columns with $KHCO_3$. Solid-phase clean-up used 5 % deactivated silica gel for sterols, with hexane, DCM and DCM/acetone (2:1, v) for elution. The sample was spiked with 5-α-Cholestane, dried, and derivatised using a commercially obtained silylating mixture I according to Sweeley (HMDS + TMCS + Pyridine 2:1:10). Activated silica gel columns were used for BAs, with sequential elution using hexane, DCM/hexane (2:1, v) and DCM/methanol (2:1, v). The sample was spiked with 5-α-cholestane, dried, and derivatised with a silylating mixture of dry toluene, BSTFA, and TSIM (25:49:1).

Samples were analysed via Gas Chromatography-Mass Spectrometry (GC-MS) using an Agilent 7890B GC coupled with a 5977A MSD Mass Spectrometer. Steroids were separated on an Agilent HP-5ms Ultra Inert column (30 m × 250 μm × 0.25 μm) with helium (99.999 % purity) as the carrier gas. External standards were used to identify retention times and mass spectra **(S5- S6).**

3.2. Analysis of organic carbon, nitrogen and phosphorus

Total Organic Carbon (TOC), Total Nitrogen (TN), and Total Phosphorus (TP) concentrations were measured following standard methodologies ISO 10694:1995, ISO 15681–2:2018, ISO 3051A, EPA 3051A (International Organization for Standardisation, 2018, 1995; United States Environmental Protection Agency, 2007).

TOC and TN concentrations were measured using a Thermo Flash 2000 Elemental Analyzer. The inorganic carbon fraction was removed by adding 3 M HCl, neutralising the sample with distilled water, and drying at 40 °C for 24h.

TP samples were first mineralised with nitric acid (HNO_3) in a microwave mineraliser Berghof Speedwave. After mineralisation, Total Phosphorus was measured using a Continuous Flow Analyzer (SAN++).

All the laboratory analyses were performed in the Laboratory of Biogeochemistry and Environmental Protection, Faculty of Biology, University of Warsaw, Poland.

3.3. Data analysis and interpretation

The method for detecting and identifying traces of human faecal matter was developed at the project's outset, based on the current state of research and a reference dataset created for this purpose, as described in Gryczewska et al. (2025). Ten sterol ratios were then applied to assess the feasibility of distinguishing human inputs. These ratios are also calculated for the current dataset, with particular emphasis on those designed for archaeological contexts and incorporating epimers—-products of environmental stanol reduction (Bull et al., 2002). First, Ratios 1–7 were used to confirm the presence of faecal traces (Table 1). In particular, Ratio 2 identifies human (or omnivore) faeces, while Ratio 5 indicates herbivore input (Bull et al., 1999; Prost et al., 2017). Ratios 8–9 further differentiate between herbivores and omnivores, with Ratio 9 designed for archaeological contexts. Ratio 10 targets human faecal matter (Table 1).

Many of these ratios (Ratios 1–3, 6–10) are often explicitly used to confirm human faecal matter (Furtula et al., 2012; Vázquez et al., 2021), mainly because humans are frequently the only omnivorous input expected. Pig, the only other omnivore in such studies, can be distinguished by Ratio 10 (Prost et al., 2017). In cave settings, however, other wild omnivores are expected. Our prior study (Gryczewska et al., 2025) confirmed that applying these 10 selected ratios to faeces from wild animals does not lead to the false identification of human faecal matter. Since some carnivores and some wild omnivores produce little to no 5β-stanols—key biomarkers of faecal matter (Bull et al., 2002; Harrault et al., 2019; Leeming et al., 1996)- their identification remains challenging. Therefore, this study focused specifically on detecting human faecal input.

Table 1
Faecal ratios used in the study, table after Gryczewska et al., 2025.

No.	Ratio	The most common interpretation of the results	References
1	cop/(cop + cholestanol)	>0.7 faecal material 0.7-0.3 inconclusive 0.1-0.3 no faecal material	Grimalt et al. (1990)
2	(cop + epicop)/(cop + epicop + cholestanol)	>0.7 faecal material 0.7-0.3 inconclusive 0.1-0.3 no faecal material	Bull et al. (1999)
3	cop/cholestanol	>0.5 faecal material 0.5-0.3 inconclusive <0.3 no faecal material	Leeming et al. (1998)
4	24ethylcop/sitostanol	>0.5 faecal material	Leeming et al. (1998)
5	(24ethylcop+ 24ethylepicop)/(24ethylcop+ 24ethylepicop + sitostanol)	>0.7 faecal material 0.7-0.3 inconclusive 0.1-0.3 no faecal material	Prost et al. (2017)
6	cop/(cholesterol + cholestanol)	>0.2 faecal material 0.2–0.15 inconclusive <0.15 no faecal material	Chan et al. (1998)
7	(cop + epicop)/(cholesterol + cholestanol)	>0.2 faecal material 0.2–0.15 inconclusive <0.15 no faecal material	based on ratio 6
8	coprostanol/24ethylcop	>1 human/ omnivore <1 herbivore	Evershed and Bethell (1996)
9	(cop + epicop)/(24ethylcop+24ethylepi)	>1 human, pig or canine/carnivore <1 herbivore	Bull et al. (1999)
10	cop/(cop+24ethylcop)	>0.75 human 0.75–0.30 mixed <0.30 herbivore	Leeming et al. (1997)

Conclusions were drawn primarily based on **Ratios 2, 5, 7, 9, 10** (using threshold ≥0.7 for Ratios 2 and 5). Some ratios included an "inconclusive" threshold, reflecting that standard cut-offs for identifying faecal traces may be overly strict (Prost et al., 2017).

The composition of BAs is well studied: DCA dominates in herbivores and some carnivores, humans show a combination of DCA and LCA, while pigs have high HDCA with no DCA (Bull et al., 2002; Prost et al., 2017; Vázquez et al., 2021). Gryczewska et al. (2025) reported that wild omnivores, carnivores, and insectivores (e.g., bats) exhibit more diverse bile acid profiles, sometimes with CA or CDCA dominating. None resemble human profiles, which show DCA ≈ LCA, with minor UDCA and CDCA (Ridlon et al., 2006; Zhang et al., 2022).

Lastly, TP and the TOC/TN ratio were analysed alongside faecal biomarkers. While TP can derive from faeces, burials, or animal remains (Audra et al., 2019; Shahack-Gross et al., 2004), we assessed its correlation with sterol and bile acid levels. The TOC/TN ratio, influenced by organic matter's source and degradation state (Riddell et al., 2023), may help determine the origin of the markers and assess organic matter preservation. Higher ratios may indicate advanced decomposition or higher plant material contribution, while lower values could reflect better-preserved nitrogenous compounds, possibly linked to faecal inputs (Riddell et al., 2023; Wurster et al., 2015). Following Riddell et al. (2023), we use average TOC/TN values of amino acids (3.15) as a reference for microbially-based sources, such as faecal matter, and the TOC/TN range of humic and fulvic acids (6.23–147) to represent more decomposed organic material, such as plant matter.

4. Results

A total of 53 sediment samples from seven cave sites were analysed. Detailed results, including faecal sterols, bile acids, TP, TOC, and TN compositionas well as faecal markers calculated relative to TOC are presented in **Supplementary Materials (S7-S10)**. Ten sterol ratios (Table 1) were applied, with particular emphasis on Ratios 2, 5, 7, 9, and 10 **(S11).** Exemplary chromatographs are presented in **Supplementary Materials (S12).**

Sterols were detectable in almost all samples, with total content ranging from 21.4 to 16913.3 ng/g. The total content of 5β-stanols (including epimers) varied from 0.0 to 7615.7 ng/g. Bile acids were detectable in most samples, with total content ranging from 0.0 to 33904.7 ng/g. Results are discussed relative to the seven studied caves.

4.1. Biśnik Cave

Ten samples from nine Middle Palaeolithic layers in Chamber III of Biśnik Cave were analysed. These layers include 11, 12, 12a, 13, 13a (two samples), 14, 15, and 18 (with two samples - 18 and 18a) (Fig. 2). All layers contained archaeological materials spread throughout the whole cave, ranging from 54 lithics in layer 11 to 161 lithics in layer 15, with excavated area covering more than 280 sqm (Cyrek et al., 2014, 2016). No human bones were found. In the majority of the studied layers, remnants of hearths or large concentrations of charcoals were noted, further confirming repeated human presence at the site.

Total sterol content and the sum of 5β-stanols and epi-5β-stanols were relatively high throughout the sequence (148.3–1790.1 ng/g), except for the lowermost layers (18 and 18a), which had lower concentrations (Fig. 2). The main compounds were coprostanol and cholesterol. Sterol ratios indicate human faecal matter in most layers (11, 12, 12a, 13, 13a, 14, and 15; Fig. 2), with faecal matter in layers 18 and 18a inconclusive. This correlates with archaeological data, as all layers with human faecal traces contained artefacts. Bile acid results were less consistent, with detectable compounds in only three samples (layers 11, 13, and 13a), ranging from 2 to 11.1 ng/g. Only layer 13 showed a human-specific bile acid profile (DCA and LCA), confirming human faecal matter.

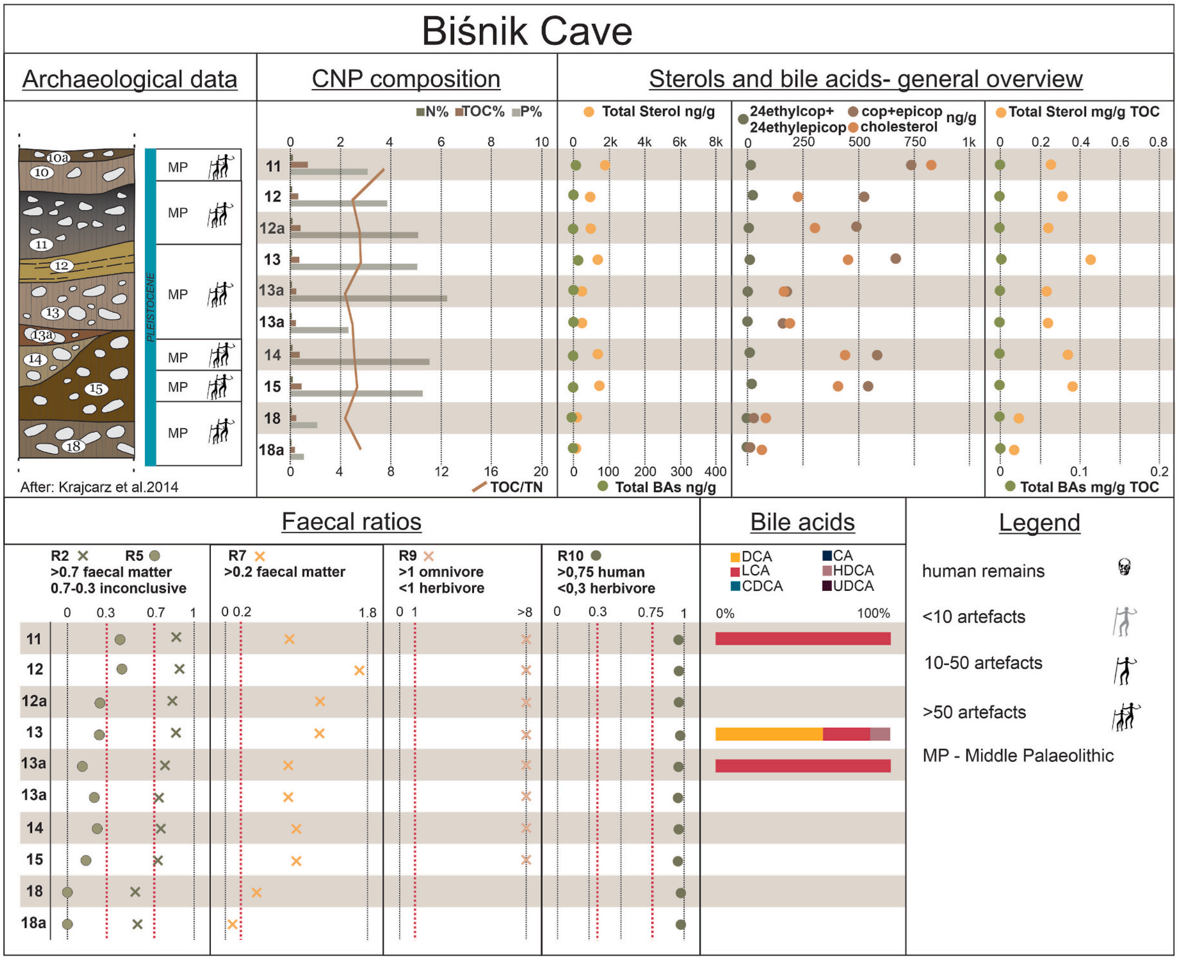

Fig. 2. Integration of molecular proxy results with archaeological data from Biśnik Cave. Ratios sourced from Table 1.

TP levels (0.5–6.2 %) were relatively high compared to samples from other sites. It was particularly elevated in layers 11–15, with a decrease observed in the lower layers (18 and 18a). The TOC/TN ratio (4.4–7.6) remains relatively stable and low throughout this sequence section but shows fluctuations between layers, following a similar pattern to Total Sterol content. This may suggest varying contributions of human faecal matter within the sequence. However, TP and the TOC/TN ratio fluctuations could also be influenced by animal remains and plant material presence.

4.2. Bramka Rockshelter

Ten samples from nine different layers (1, 1a, 2, 3, 4, 6 (two samples), 7, 8, and 9) were analysed from Bramka Rockshelter (Fig. 3). Layer 1 is a mixed level with traces from the Neolithic, Late Bronze Age, and 19th-20th centuries. The most intensive occupation was in layer 1a, which yielded Early Mesolithic materials (Chmielewski, 1988; Kot et al., 2022) and human bones, dated recently to the Bronze Age (Kot et al., 2021). In lower layers – 2, 3, 4, 6 – few artefacts connected to the Late Upper Palaeolithic were found. In the lowermost levels – 7 and 8 – only single flint lithics were found, and no artefacts were found in layer 9. Layer 7, identified in recent excavations, was previously mistaken for regolith (Chmielewski, 1988; Kot et al., 2022). Middle Palaeolithic artefacts, including a discoid core in layer 1a and heavily patinated flakes in layer 5, were also noted. Evidence of fire activity at the site is linked to layers 1a (heat-damaged lithics) and possibly to layers 7 or 5, as noted in archival field documentation (Kot et al., 2022).

Total sterol content (151.1–6966.9 ng/g) varied across the profile, with the highest concentrations in layer 1 (Fig. 3), relatively high levels in layer 1a, two samples from layer 6, and a sample from layer 7. 5β-stanols and epi-5β-stanols increased only in layer 7 (260.7 ng/g). Faecal ratios indicate the presence of human faecal matter in layer 7. However, these results could not be reliably compared to archaeological data because layer 7 was only identified during profile cleaning at the end of the fieldwork and was not excavated. In several layers (1, 3, 4, 6, 9) sterol ratios are inconclusive.

Bile acids (4.3–258.4 ng/g) were present in most samples, except for one from layer 6 and one from layer 7 (Fig. 3). The highest concentrations were in layers 1 and 1a, with minor peaks in layers 3, 6, 8, and 9. The bile acid profile was dominated by DCA, with smaller amounts of LCA, CA, and HDCA. In layers 1, 4, 6, and 9, the composition resembled human faecal matter, but with relatively low LCA and without sterol confirmation, these results remain inconclusive.

TP concentrations were generally low throughout the sequence, with a noticeable peak in layers 6 and 7 (1.4 % and 2.4 %, respectively). This peak in layer 7 corresponds to the human faecal matter identified through sterol analysis. The top layers' TOC/TN ratios are relatively high (10.8 and 13.1). From layer 7 onwards, the ratio drops, indicating reduced microbial activity and/or the presence of animal remains or faecal matter.

4.3. Ciasna Cave

Four samples from three layers (2, 3, and two from 4) were analysed from Ciasna Cave (Fig. 4). Layer 2 contained sparse but mixed traces of human occupation, including Palaeolithic, Neolithic, and Early Iron Age

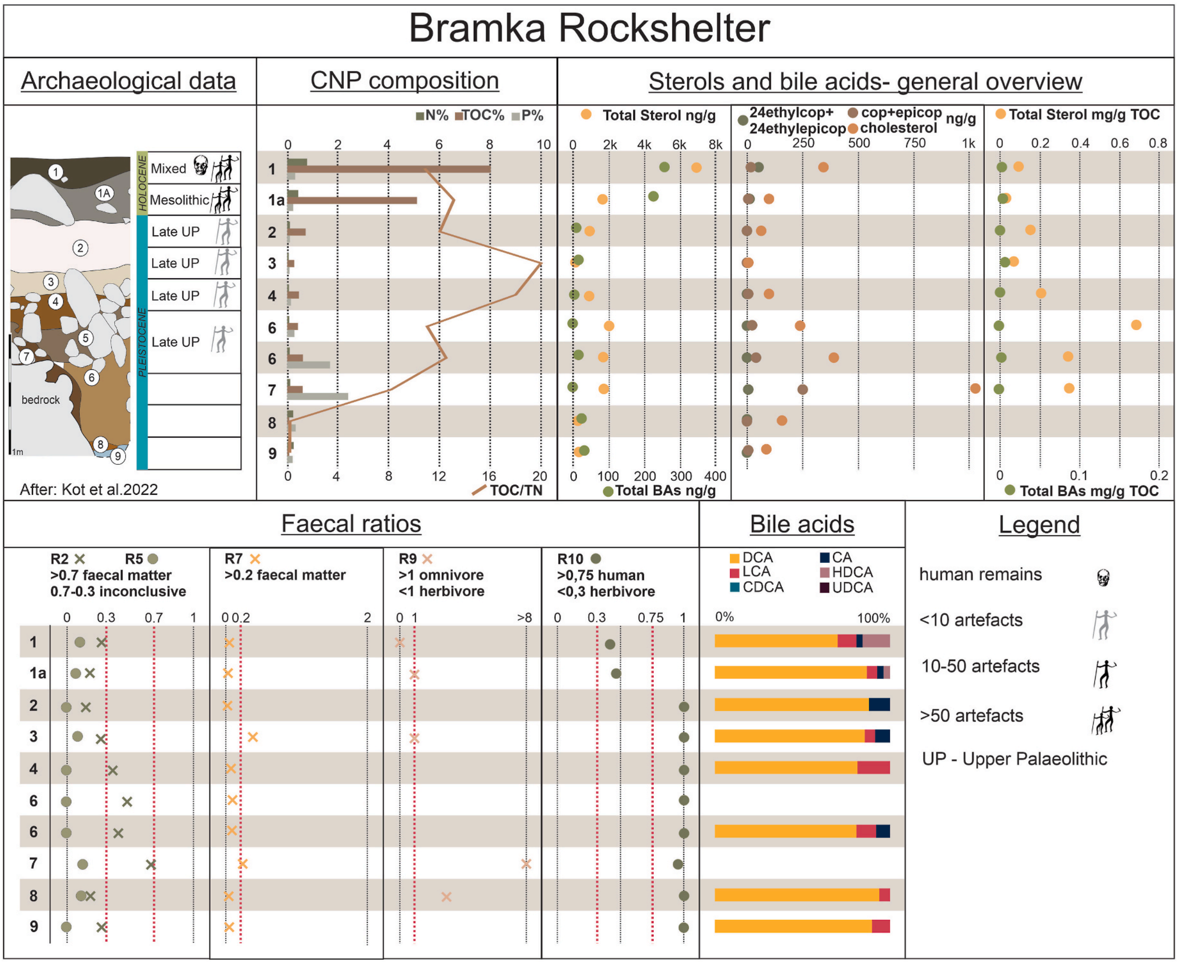

Fig. 3. Integration of molecular proxy results with archaeological data from Bramka Rockshelter. Ratios sourced from Table 1.

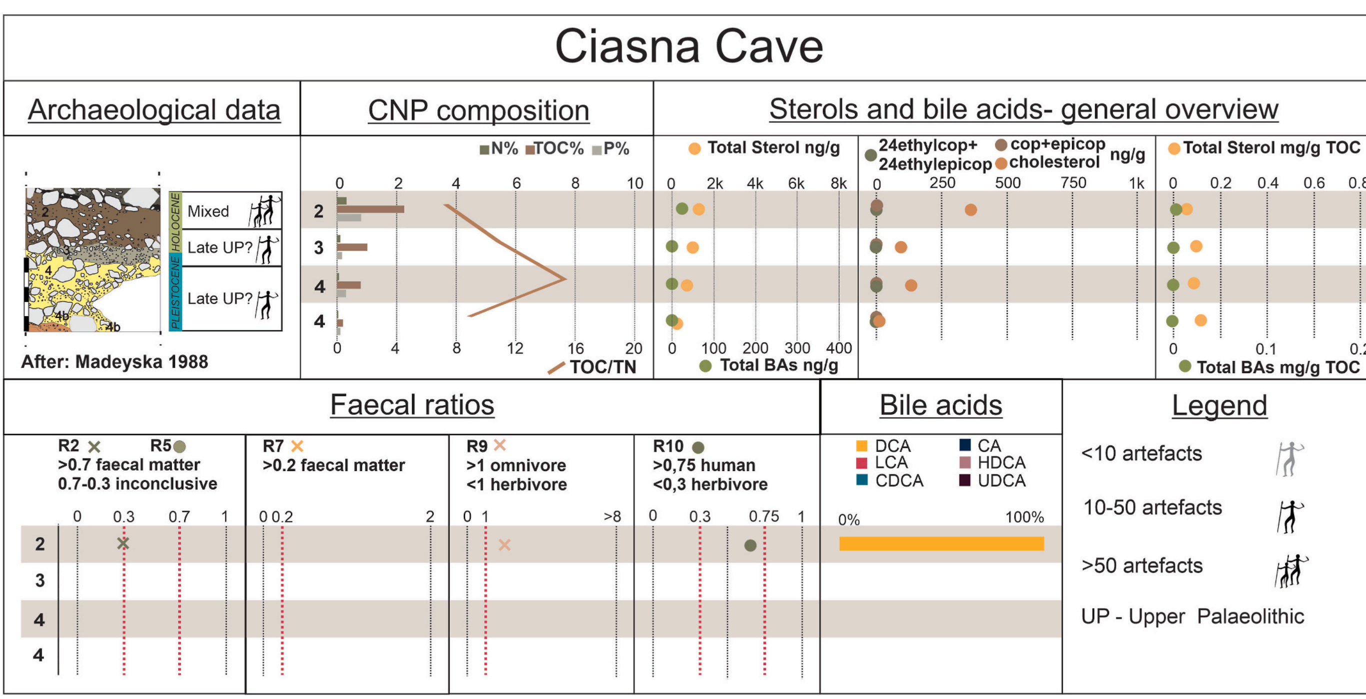

Fig. 4. Integration of molecular proxy results with archaeological data from Ciasna Cave. Ratios sourced from Table 1.

artefacts (Chmielewski, 1988; Czajka, 2019). Layer 3 was identified as a cultural level with hearth traces and lithics, possibly linked to the Late Palaeolithic (Chmielewski, 1988). The upper part of layer 4 yielded a small assemblage of artefacts, while no finds were recorded in its lower part. A possible burial was noted in layer 2, suggested by archival records and characteristic Złocka culture pottery, though unconfirmed as the bones are lost. Human presence is evident in all layers except the lower part of layer 4.

The total sterol content (236–1277.3 ng/g) peaked in the uppermost analysed layer (layer 2) and gradually decreased down the sequence (Fig. 4). 5β-stanols and epi-5β-stanols were detected only in layer 2, with coprostanol present at very low levels (9.2 ng/g). Faecal ratios pointed to layer 2 as containing possible traces of faecal matter. Bile acids, specifically DCA, were found only in layer 2 (23.7 ng/g) (Fig. 4), aligning with sterol results, as both showed the highest concentrations in this layer while lacking clear evidence of human faecal input.

TP concentrations peaked in layer 2 (0.8 %) but were relatively low compared to other sites, gradually decreasing down the sequence. TOC/TN (7.4–15.4) peaked in the upper part of layer 4 and remained relatively high throughout, indicating organic matter degradation or input from plant material rather than faecal matter.

4.4. Łabajowa Cave

Eight samples from eight layers (H3, H4, H6, H8, H9, H8', H10, H12) were analysed from Łabajowa Cave (Fig. 5). Layers H3 and H4 contained scarce artefacts likely from the Neolithic and 19th century, most likely redeposited from earlier excavations. Few artefacts were found in the lower layers, but the site remains under study. Limited records of 19th-century exploration and the discovery of a Jerzmanowician leaf point suggest Palaeolithic occupation (Gryczewska et al., 2023). No human remains were found, but significant charcoal deposits in layer H9 may indicate past human activity.

Sterol content (89.6–653,81 ng/g) showed a slight peak in layer H9 but remained low elsewhere (Fig. 5). Low concentrations of 24-ethylepicoprostanol and 24-ethylcoprostanol were detected in layers H7, H8, H9, and H12 (1.1–4.0 ng/g). Faecal ratios did not indicate human faecal matter. Bile acids (15.5–86.4 ng/g) were found in layers H3, H8, and H9 (Fig. 5), mainly composed of DCA. In layer H9, LCA and HDCA were also detected, but not in proportions indicative of human faecal matter.

TP levels (0.1–0.2 %) were relatively low across all samples, with a slight peak in H9, aligning with sterol and bile acid results. TOC/TN (6–17) varied throughout the sequence without a clear correlation to the studied markers. Layer H8 had a relatively low TOC/TN ratio, with higher values in H6, H9, and H8'.

4.5. Łokietka Cave

Four samples from four distinct layers (20, 22, 23, 24) were analysed from Łokietka Cave (Fig. 6). In layers 20 and 24 no archaeological materials were found, while in layers 22 and 23 artefacts connected to Upper and Middle Palaeolithic were found, among others Mousterian

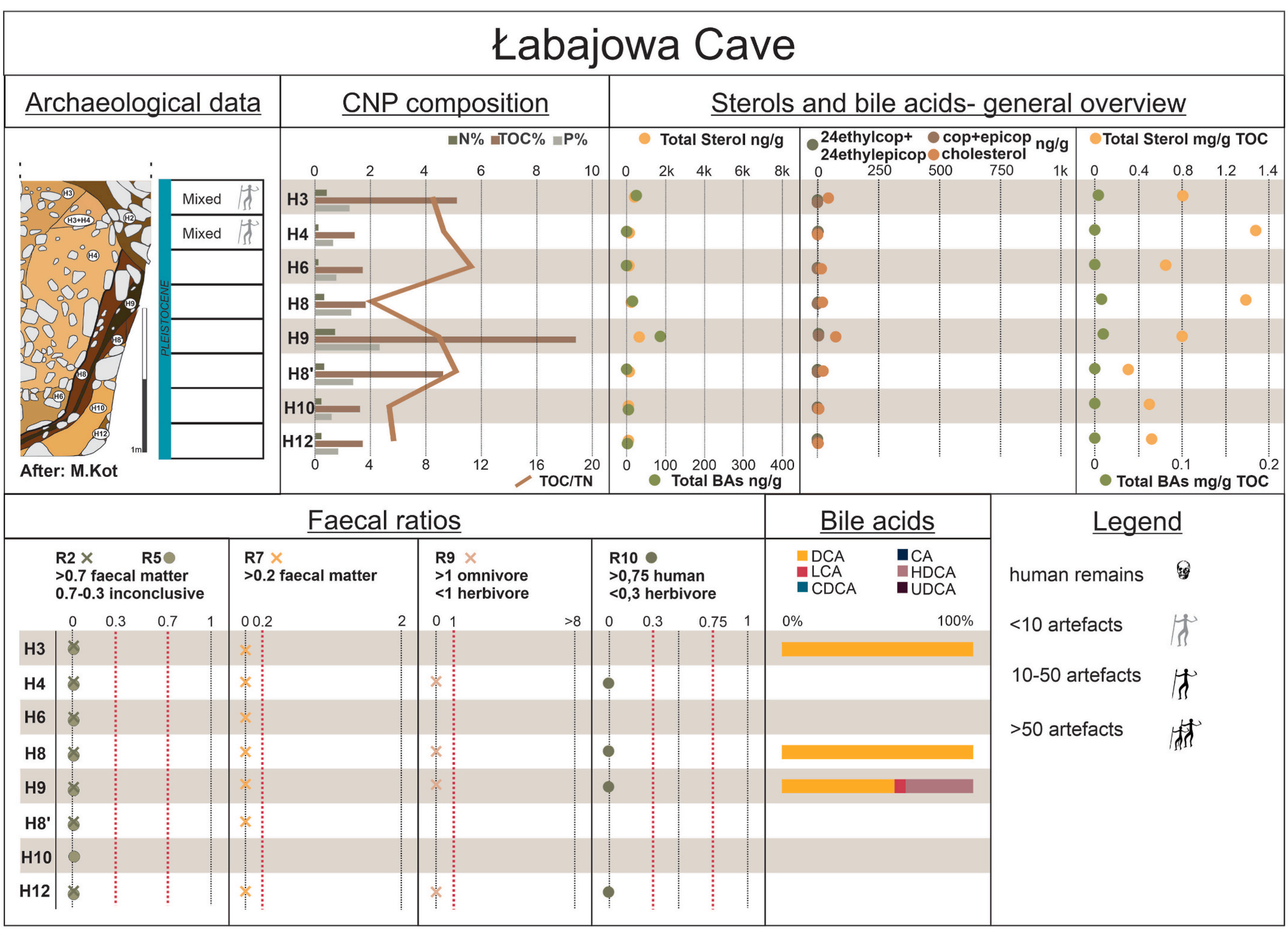

Fig. 5. Integration of molecular proxy results with archaeological data from Łabajowa Cave. Ratios sourced from Table 1. Note the differing scale used for Total Sterol (mg/g TOC) in this figure.

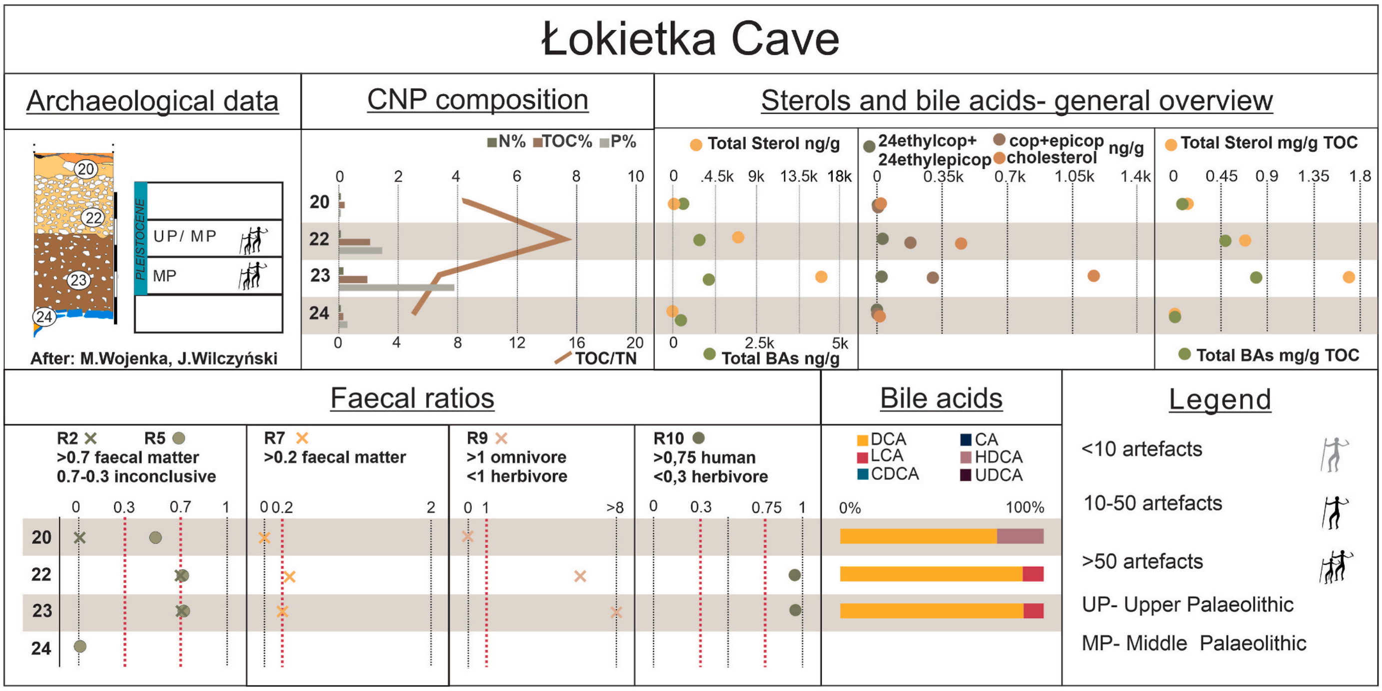

Fig. 6. Integration of molecular proxy results with archaeological data from Łokietka Cave. Ratios sourced from Table 1. Note the differing scale used for a general overview of sterols and bile acids in this figure.

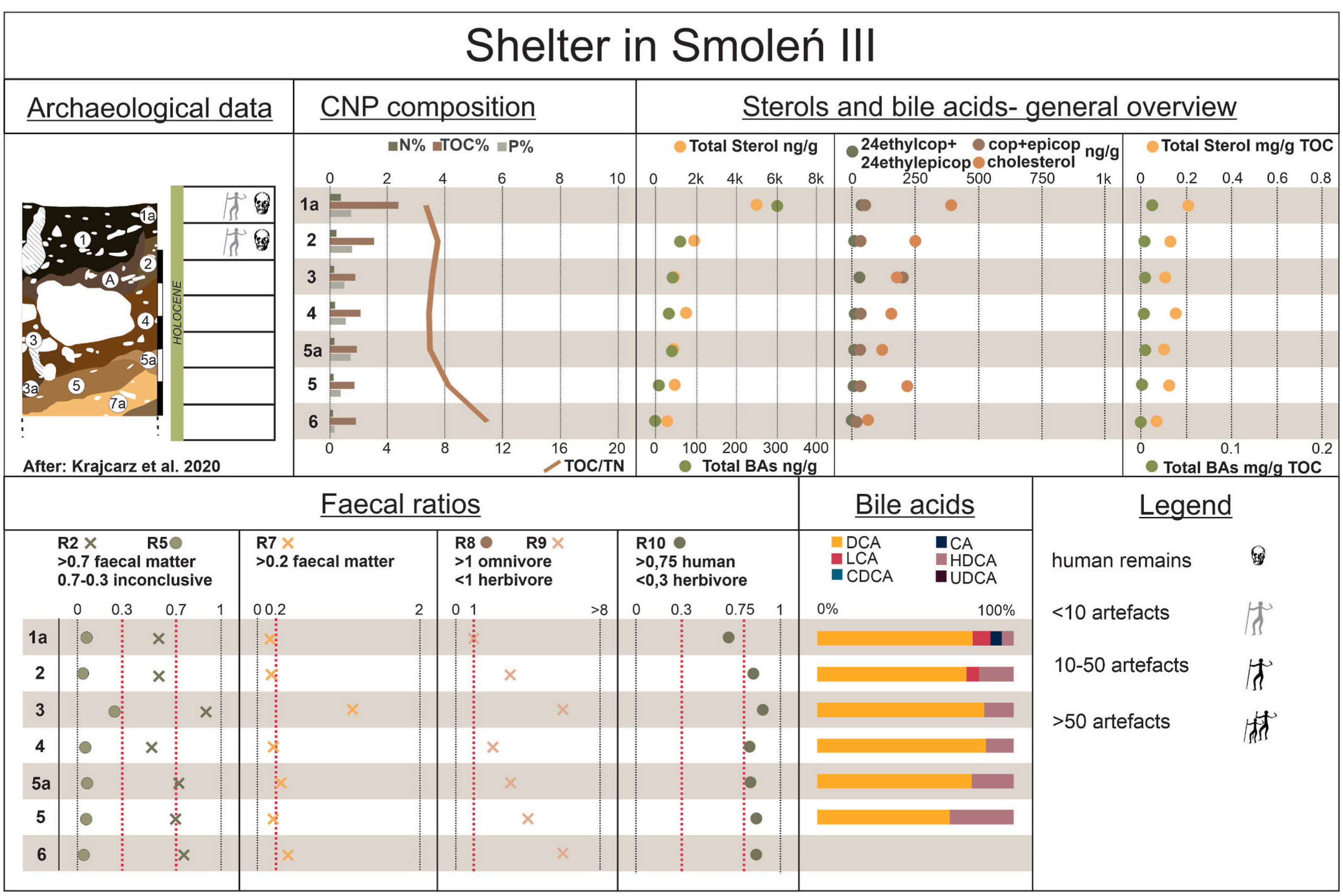

Fig. 7. Integration of molecular proxy results with archaeological data from Sąspowska Zachodnia Cave. Ratios sourced from Table 1.

and Jerzmanowician, thus indicating several episodes of human presence at the site (Sobczyk and Sitlivy, 2001). No human remains or traces of hearth were noted in this part of the section.

The total sterol content (21.4–16913.3 ng/g) was notably higher than in other sites, peaking in layers 22 and 23, where cholesterol and coprostanol dominated (Fig. 6). In contrast, sterol concentrations were very low in layers 20 and 24. Sterol ratios indicated human faecal matter in layers 22 and 23, where archaeological data also point to human presence.

Bile acids (305.7–884.9 ng/g) were detected in layers 20, 22, and 23 (Fig. 6). Layers 22 and 23 contained DCA and LCA, consistent with human faecal matter, while layer 20 contained HDCA instead of LCA. These results align with sterols.

TP concentrations (0.1–3.9 %) were notably higher in layers 22 and 23, supporting faecal markers and archaeological evidence. TOC/TN (5–15.3) was relatively high in layer 22 but dropped significantly in layer 23. This contrasts with the peak in total sterols and bile acids observed in layer 23.

4.6. *Sąspowska Zachodnia Cave*

Ten samples from distinct layers (1A–1E, 2, 3, 4, 5, 6) were analysed from Sąspowska Zachodnia Cave (Fig. 7). Holocene layers (1A–3) contained evidence of human presence, including rich Neolithic (1D) and Bronze Age (1A) flint lithic assemblages (Berto et al., 2022; Chmielewski, 1988). Human bones in layer 1D suggest a possible burial. No artefacts were found in layer 4, while layers 5 and 6 contained a few Late Palaeolithic finds. Evidence of fire activity was recorded in layers 1A and 1E. The site remains under study.

Sterol content (84.5–709.9 ng/g) remained stable, except for notably lower concentrations in layer 6 and relatively low levels in 1D (Fig. 7). 5β-stanols and epi-5β-stanols (2.7–136.0 ng/g) were generally low, with slightly higher levels in layers 1B and 5, mainly as 24-ethylepicoprostanol. Faecal ratios did not provide strong evidence of human faecal input in any layer, though they suggested a possible presence of faecal matter in all layers except the lowermost 6.

Bile acids (34.1–305.7 ng/g) were present in most samples except layers 1D and 6 (Fig. 7). DCA predominated, with HDCA and LCA also present. Still, the composition was inconclusive regarding human faecal sources, as HDCA exceeded LCA in most layers except 3. While BAs did not conclusively indicate human faecal matter in any layer, similar inconclusive results were observed in layers 1C–3.

TP concentrations (0.1–1.2 %) varied, peaking in the upper layers (1A–E) and layer 6. The TOC/TN ratio (9.8–100.5) was generally high, particularly in 1A and 1E, where traces of hearths were recorded.

4.7. *Shelter in Smoleń III*

Seven samples from distinct layers (1a, 2, 3, 4, 5a, 5, 6) were analysed from Shelter in Smoleń III (Fig. 8). Human activity in the studied section is primarily linked to Feature A, a large pit in the upper layers (Krajcarz et al., 2020; Sudoł et al., 2015). Sparse artefacts found outside Feature A likely resulted from post-depositional disturbance. No human remains were identified in the analysed layers, though burials were recorded in Feature A. No hearths were found.

Sterol content (619.5–5003.5 ng/g) was relatively high compared to

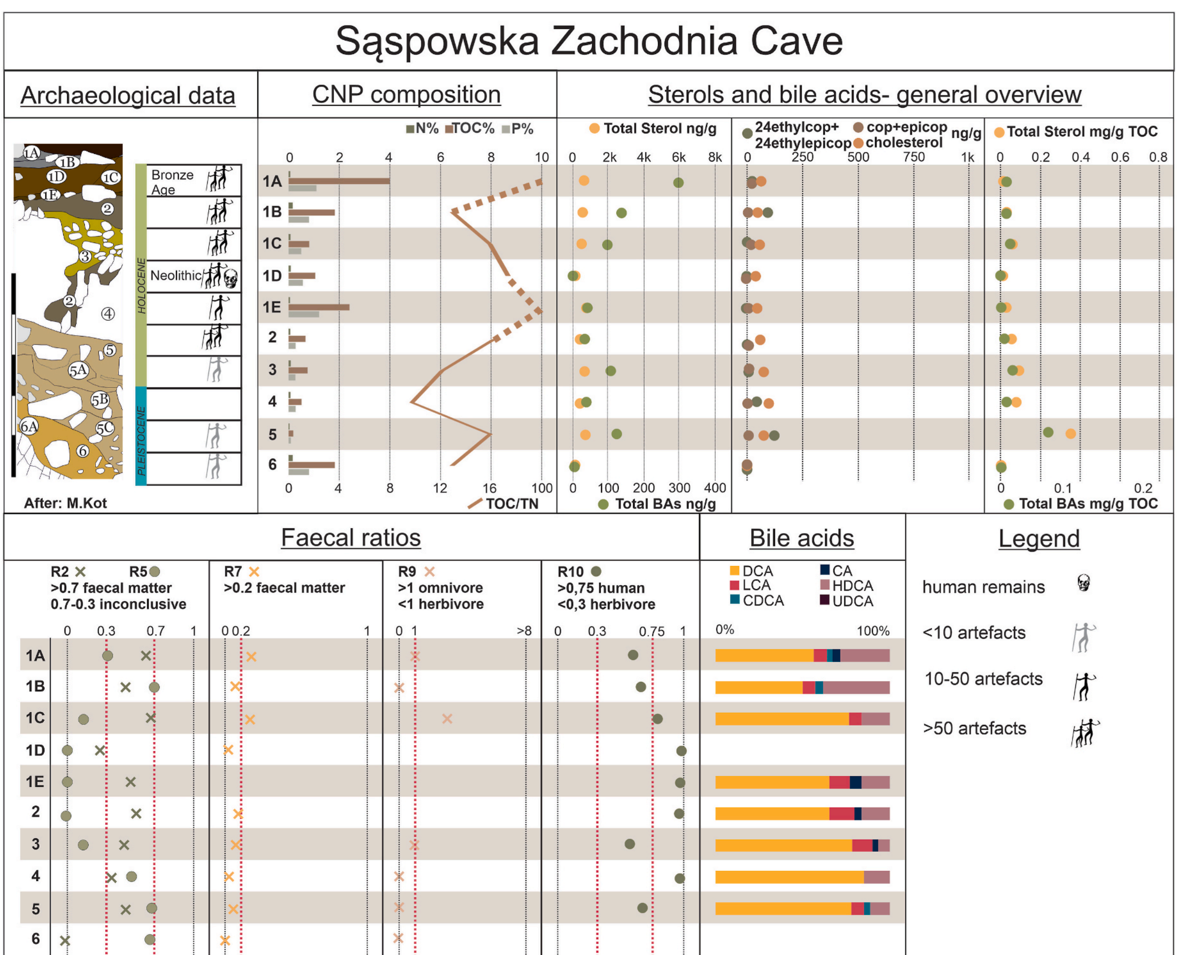

Fig. 8. Integration of molecular proxy results with archaeological data from Shelter in Smoleń III. Ratios sourced from Table 1.

Sąspowska Zachodnia and Łabajowa caves, peaking in layer 1a, with additional increases in layers 2 and 4 (Fig. 8). 5β-stanols and epi-5β-stanols were present in all samples, with the highest coprostanol concentration in layer 3 (201.2 ng/g). Faecal ratios indicated human presence in layers 3, 5a, 5, and 6, with inconclusive results for other layers. Bile acids (9.7–300.9 ng/g) were detected in all layers except 6 (Fig. 8), with the highest concentration in 1A, decreasing down the sequence. DCA predominated, with HDCA as the second most abundant, except in layer 1A, where LCA was higher. This could indicate human faecal matter, but the relatively low LCA and sterol results make the interpretation inconclusive.

TP levels (0.1–0.7 %) were low and stable, decreasing slightly in layer 6, showing no correlation with faecal markers. TOC/TN ratios (6.7–11) increased towards the bottom of the sequence, suggesting highly degraded organic matter; it did not align with sterol or bile acid results.

5. Discussion

5.1. Investigating human presence in the caves of the Kraków-Częstochowa Upland through their faecal matter

At several sites in this study, faecal markers directly indicate human presence (Fig. 9), even in layers where archaeological evidence is inconclusive. While faecal biomarkers and archaeological data (e.g., artefacts or hearths) represent different aspects of human activity, some correlation is expected, making them valuable reference points for interpretation. From this, four scenarios emerge.

(1) Faecal markers and archaeological evidence point to human presence at the site

The first scenario pertains to most samples from Biśnik Cave and two samples from Łokietka Cave (Fig. 9). Still, only one sample from the first site was confirmed by both sterols and bile acids. TP was elevated in all samples. Notably, these samples are associated with the Middle or Middle/Upper Palaeolithic, and the samples were collected from within the caves rather than the entrance areas (Cyrek et al., 2014, 2016; Sobczyk and Sitlivy, 2001). Furthermore, Biśnik Cave is the only site where faecal sterols were previously studied (Krajcarz et al., 2013), enabling us to compare findings and affirm their consistency.

(2) Faecal markers suggest human presence in layers without archaeological artefacts

This scenario includes layer 7 at Bramka Rockshelter and four layers (3, 5a, 5, and 6) from Shelter in Smoleń III. Here, once again, sterols—but not BAs—indicate human presence, with TP levels elevated at

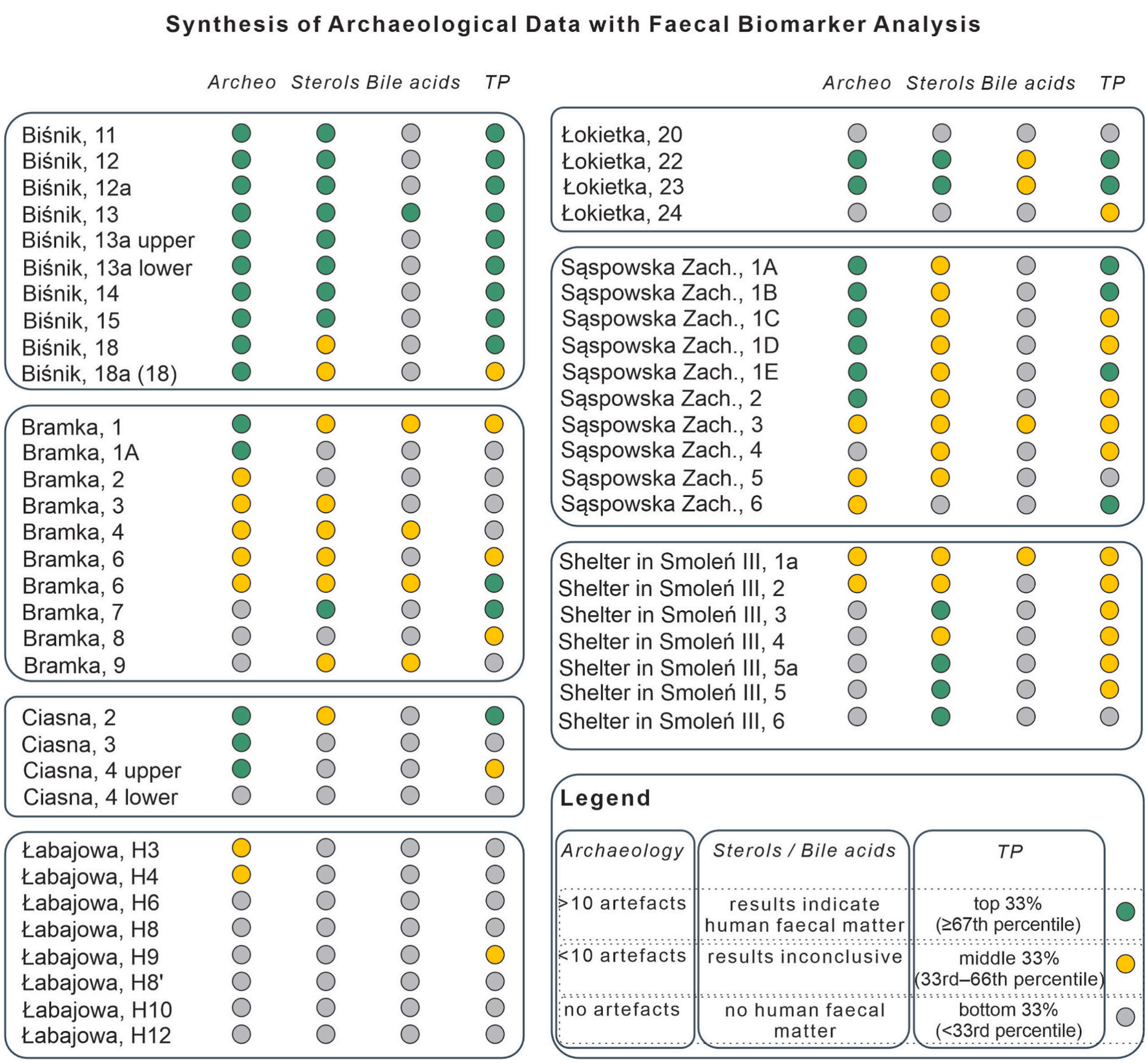

Fig. 9. Synthesis of archaeological data and faecal biomarker data.

Bramka and to a lesser extent at Shelter in Smoleń III. Bramka Rockshelter presents a particularly intriguing case, as early excavations mistakenly identified the debris as regolith/bedrock, leaving layer 7 unexcavated. Although Middle Palaeolithic artefacts were recovered, such as a core in layer 1a and patinated flakes in layer 5, no layer was conclusively linked to the Middle Palaeolithic (Chmielewski, 1988; Kot et al., 2022). The lithology and dating of adjacent layers within the stratigraphic sequence suggest that layer 7 is a probable candidate for Middle Palaeolithic occupation, although direct layer dating was unsuccessful (Kot et al., 2022). The composition of faecal sterols and a significant increase in phosphorus levels strongly indicate human presence at the site.

In contrast, the four samples from Shelter in Smoleń III are more challenging to confirm archaeologically as no human occupation was indicated, even though a large part of the site was excavated (Sudoł et al., 2015). The rock shelter is small, with narrow galleries and a low roof, providing no space for a convenient stay. Our results suggest that the main human activities during the deposition of layers 3, 5a, 5, and 6, could have happened outside the rock shelter, at the terrace or a slope in front of it, while the rock shelter interior served as a toilet place. These results should be treated with caution.

(3) Archaeological data confirm human presence, but faecal markers are inconclusive or disagreeable

Several layers yield inconclusive faecal marker results. For faecal sterols, this is due to values falling within inconclusive thresholds. For BAs composition, this occurs because, although DCA and LCA generally predominate, the LCA concentrations are not high enough to indicate human presence. These inconclusive results could then be related to the expected mixture of faecal traces from humans, wild animals, and domesticated animals in cave settings. However, this category includes samples from various chronologies and localities within the caves, reflecting different intensities of human presence. This could then also suggest that for these levels, human activity at the site did not involve defecation or that such activities took place farther from the sampling area.

This category includes samples from Biśnik Cave (layers 18, 18a), Bramka Rockshelter (layers 1, 3, 4, 6), Ciasna Cave (layer 2), Sąspowska Zach. (layers 1A-3, 5) and Shelter in Smoleń III (layer 1a, 2). As for TP concentrations, they are elevated in only a few samples from Biśnik (18), Bramka (6), Ciasna (2), and Sąspowska Zachodnia (1A, 1B, 1E).

(4) Neither archaeological evidence nor faecal markers clearly indicate human presence:

This final scenario concerns a majority of the sequence from Łabajowa Cave, and several samples from various caves (Fig. 9). Layer H9 in Łabajowa (Gryczewska et al., 2023), initially thought to be a cultural level associated with the Jerzmanowician leafpoint based on archival data, large amounts of charcoal, and early dating, was not confirmed as related to human activity through faecal markers.

Overall, this study demonstrates how faecal markers can contribute to our understanding of past human presence in the region. The analysis provides evidence that caves were used for physiological needs across multiple periods and sites. At the same time, it offers new insights into human occupation itself, most notably indicating human presence in layer 7 of Bramka Rockshelter, supporting the interpretation of Middle Palaeolithic activity despite limited archaeological evidence (Chmielewski, 1988; Kot et al., 2022). Similarly, results from Shelter in Smoleń III suggest ephemeral human activity that have gone undetected during excavation (Krajcarz et al., 2020; Sudoł et al., 2015). Inconclusive results from the lower layers of Sąspowska Zachodnia—where artefacts are scarce but present—point to the value of further research beyond the well-studied Holocene levels (Berto et al., 2022; Chmielewski, 1988). Lastly, the absence of faecal markers in Łabajowa Cave helps clarify the debate over a proposed Jerzmanowician occupation, which is not supported by recent fieldwork (Gryczewska et al., 2023).

5.2. Human behaviour and the changing morphology of the cave

Despite certain limitations, such as the ongoing investigation of the analysed sites and the reliance on single samples per layer, the study allows for identifying some patterns related to the presence of human faecal markers. The results suggest that the most important factors determining the presence of chemical traces of human activity may be the sampling location within the cave and the chronology and/or lithology of the analysed layer.

Each cave is a unique natural structure with a morphology that changes over time. These changes can be attributed to natural karstic processes, weathering, and rock falls, thereby altering the cave's size, entrance location, and overall morphology (Farrand, 2001). Each cave included in the research looked significantly different during the Pleistocene compared to the beginning of the Holocene or today. A remarkable example is Biśnik Cave, featuring a sequence over 7 m long, with an extensive analysis of artefact distribution at each level, and the discovery of 'fossil entrances', indicating significant changes in morphology (Cyrek et al., 2016). This changing morphology might have influenced the spatial use of the site, activity areas within the site, and consequently, the distribution of faecal biomarkers on the "living floor" of each layer.

A most significant example of the impact of changed morphology may be the findings from Bramka Rockshelter. Faecal markers indicate human presence in layer 7. This prompts an inquiry into the absence of chemical traces of human activity in layer 1a, which resulted in over 5000 lithics and traces of hearth (Kot et al., 2022). Two key factors may explain this discrepancy: the alteration in the morphology of Bramka Rockshelter and the differing matrix in which faecal matter was searched. During the deposition of layer 1a, the site likely resembled its current form—a rock window with a limited area beneath the cave roof and steep slopes of the Sąspowska Valley in front of both entrances. During the deposition of layer 7, the site was probably part of a more extensive karstic system (Madeyska, 1988), and the area of the 2018 trench (in which sampling took place) may have been then located far inside the cave. This is based on the lithology of the layer, possibly the second important factor, as layer 7 is a clayey loam (Kot et al., 2022), a characteristic sediment type for a cave setting. Similarly, all studied layers from Biśnik Cave belong to a sequence of cave loams and loess-like sediments (layers 12 and 15) (Krajcarz et al., 2014).

Another site worth examining in terms of its location is Ciasna Cave. All layers here contained archaeological materials, suggesting repeated episodes of human occupation (Chmielewski, 1988). Fieldwork, during which sampling took place, was conducted in a small trench in the corner at the very entrance of the cave. This area is so narrow that entering it today requires crawling. As a result, the sampling likely did not capture the part of the cave where humans actively lived and used the space.

Three sites with possible traces of human faecal matter (Biśnik Cave, Łokietka Cave, and Shelter in Smoleń) were sampled within the caves, with the first two also being linked to Palaeolithic occupation. The question remains whether deeper localities are more suitable for faecal marker analysis and whether this is related to human behaviour or natural conditions and processes affecting deposition and preservation. The study was based on the assumption that chemical compounds were likely to spread on the living floor, i.e. through human and animal trampling. However, areas used for hygienic practices, if separate, would result in the highest concentrations of human faecal markers, as in the case of latrines or cesspits at open-air sites (Baeten et al., 2012; Bull et al., 2005) and thus be distinct and more straightforward to detect. Furthermore, the area around the entrance might be more susceptible to erosion, weathering, and general disturbance due to vegetation, wind, humidity and human and animal activity, while in the deeper part of the cave, the conditions are more stable (Moldovan et al., 2018; White, 2007).

This issue warrants further research, including analysis of different cave areas (e.g., entrance vs. interior), spatial variation in faecal marker concentrations, and detailed examination of the lithology and chemistry of each layer. The need for more studies on lipid biomarker taphonomy in various sedimentary matrices has recently been emphasized, as the use of faecal markers in archaeology increases and differences in the degradation pathways of specific compound groups have been observed (Mallol et al., 2025).

5.3. Comparing faecal sterols and bile acids

Previous research confirmed the stability of faecal biomarkers, with sterols being particularly extensively studied (Bull et al., 1998, 2000, 1999; Evershed et al., 1997; Simpson et al., 1998, 1999). Bile acids (BAs) are also stable, with degradation primarily occurring under aerobic conditions, although factors such as salinity, temperature, and pH can influence this process (Feller et al., 2021; Obuseng et al., 2013). Studies in manure and soils indicate a loss of steroid content over time, particularly in bile acids, but without significant changes in relative compound ratios (Bull et al., 1998, 2000; Prost et al., 2018). Bile acids are much less abundant than sterols, with only 5 % excreted (0.2–0.6 g/day) (Zhang et al., 2022). Furthermore, some studies found BAs absent where sterols were present (Battistel et al., 2015; March, 2018).

In our study, BAs were most concentrated in top, young layers (Bramka, Ciasna, and Sąspowska Zachodnia) and reduced in older (Biśnik and Łabajowa), suggesting significant degradation over time. However, comparing BA concentrations to 5β-stanols and their epimers at the same levels **(see S13)**, shows no significant differences in overall preservation. Łabajowa's bile acid absence likely reflects lack of faeces, not preservation bias. Biśnik and Bramka layer 7 show unusually poor preservation, unlike Łokietka, suggesting local factors. Air-dried, room-temp storage may have also affected preservation (Ulmer et al., 2021).

Further research is needed, especially as BAs are used to differentiate pigs from humans based on high HDCA concentrations (Bull et al., 2002). Larger samples may improve results. Still, sterol analysis is frequently used independently (Bemmann et al., 2014; Bethell et al., 1994; Keenan et al., 2021; Vachula et al., 2020), thus can be considered reliable even with no BAs. Analytical approaches to detect and identify human faecal matter were primarily developed for human and domesticated animal faeces and are usually applied to much younger deposits than here (Battistel et al., 2015; Bull et al., 2002; Prost et al., 2017). A regional reference dataset focused on wild animals (Gryczewska et al., 2025) confirms human sterol ratios apply in caves, though non-human inputs remain difficult to confirm. Bile acids could improve differentiation, but more data is needed. Based on current data (Gryczewska et al., 2025), we assumed herbivore inputs differ enough, and other species either lack 5β-stanols or have distinct sterol profiles. Therefore, we focused on human inputs—especially since sterol ratio thresholds vary. For instance, Battistel et al. (2015) noted that ratio coprostanol/cholesterol can be interpreted $\geq$0.2 for faecal matter and $\geq$1 for human. Shillito et al. (2020) used the latter to distinguish carnivores and humans, supported by bile acid profiles. Here, we used the 0.2 threshold, as few wild carnivores exceeded it (Gryczewska et al., 2025). In cave contexts, cholesterol may originate from non-faecal sources, therefore we prioritized 5β-stanol-based ratios.

Non-faecal sterol sources must be carefully considered in cave contexts. For sterols, established ratios help account for non-faecal contributions. In contrast, BAs are vertebrate-exclusive and typically derived from faecal matter in archaeological contexts, can also originate from the biliary system—for example, in sediments linked to decomposing bodies (von der Lühe et al., 2020). BA profiles vary by source; human bile contains CDCA, CA, DCA, and trace LCA (Ridlon et al., 2006; Zhang et al., 2022; Zheng et al., 2024). Background data on bile acids in caves is also limited, but studies show that DCA is widespread in topsoils, control samples, and some carnivore faeces (Birk et al., 2012; Gryczewska et al., 2025; Prost et al., 2017; von der Lühe et al., 2020), which is reflected in our results, as DCA was the most common bile acid detected.

For a more integrated approach, total phosphorus (TP) was applied here as an additional faecal marker. Although it can originate from multiple sources in cave environments—including faecal matter, burials, and decomposing animal carcasses (Goldberg and Nathan, 1975; Shahack-Gross et al., 2004), the results aligned well with the faecal marker evidence, particularly sterols. The TOC/TN ratio also offered insight into organic matter degradation and supported the interpretation of faecal matter. Overall, we can conclude that, integrating multiple faecal markers strengthens the identification of human presence and activity in archaeological sites.

Lastly, possible differences between Modern Human and Neanderthal should be considered as an additional interpretative challenge. Existing data (Rampelli et al., 2019; Sistiaga et al., 2014a,b) suggest similar gut microbiota and diets, supporting current detection methods for Middle Palaeolithic contexts. However, diet influences faecal profiles—sterol concentration and composition vary with intake and absorption (Mitry et al., 2019). Bile acid data are less clear, with some studies linking higher secondary bile acid levels to meat-rich diets, while others find no correlation (Mitry et al., 2019; Reddy et al., 1980).

6. Conclusion

This study shows that molecular markers, particularly faecal sterols and bile acids, can effectively trace human presence in cave sites. Among 53 sediment samples from seven caves, human faecal markers were found in 10 of the 35 samples with archaeological evidence, and in five additional samples without it. Eleven more showed possible traces. Considering that not every episode of human presence in a cave—especially brief—would necessarily leave distinct faecal traces, the number of positive samples is promising. These results suggest that faecal biomarkers can reveal human activity even without traditional archaeological indicators.

The distribution of markers varied across cave contexts, with deeper areas and Palaeolithic layers yielding the most consistent results. Sterols proved especially stable. Continued research on preservation conditions across cave environments is needed to better separate human signals from non-anthropogenic ones. Overall, the findings highlight both the potential and limitations of faecal marker analysis in archaeological contexts and point to several directions for future studies.

CRediT authorship contribution statement

N. Gryczewska: Writing – original draft, Visualization, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **M. Sulwiński:** Writing – review & editing, Methodology, Formal analysis. **M. Kot:** Writing – review & editing, Resources. **M.T. Krajcarz:** Writing – review & editing, Resources. **K. Cyrek:** Writing – review & editing, Resources. **M. Sudoł-Procyk:** Writing – review & editing, Resources. **J. Wilczyński:** Writing – review & editing, Resources. **M. Wojenka:** Writing – review & editing, Resources. **K. Szymczak:** Writing – review & editing, Supervision. **M. Suska-Malawska:** Writing – review & editing, Supervision, Conceptualization.

Data availability

The data that support the findings of this study are openly available in RepOD at https://doi.org/10.18150/R0SVUX and in the Supplementary Materials.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Natalia Gryczewska reports financial support was provided by National Science Centre, Poland. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jas.2025.106311.

References

Angelucci, D.E., Boschian, G., Fontanals, M., Pedrotti, A., Vergès, J.M., 2009. Shepherds and karst: the use of caves and rock-shelters in the Mediterranean region during the Neolithic. World Archaeol. 41, 191–214. https://doi.org/10.1080/00438240902843659.

Argiriadis, E., Battistel, D., McWethy, D.B., Vecchiato, M., Kirchgeorg, T., Kehrwald, N. M., Whitlock, C., Wilmshurst, J.M., Barbante, C., 2018. Lake sediment fecal and biomass burning biomarkers provide direct evidence for prehistoric human-lit fires in New Zealand. Sci. Rep. 8, 2–10. https://doi.org/10.1038/s41598-018-30606-3.

Audra, P., De Waele, J., Bentaleb, I., Chroňáková, A., Krištůfek, V., D'Angeli, I., Carbone, C., Madonia, G., Vattano, M., Scopelliti, G., Cailhol, D., Vanara, N., Temovski, M., Bigot, J.-Y., Nobécourt, J.-C., Crespe, Vence, Galli, E., Rull, F., Sanz-Arranz, A., 2019. Guano-related phosphate-rich minerals in European caves. IJS 48, 75–105. https://doi.org/10.5038/1827-806X.48.1.2252.

Baeten, J., Marinova, E., de Laet, V., Degryse, P., de Vos, D., Waelkens, M., 2012. Faecal biomarker and archaeobotanical analyses of sediments from a public latrine shed new light on ruralisation in Sagalassos, Turkey. J. Archaeol. Sci. 39, 1143–1159. https://doi.org/10.1016/j.jas.2011.12.019.

Bailey, G., Galanidou, N., 2009. Caves, palimpsests and dwelling spaces: examples from the Upper Palaeolithic of south-east Europe. World Archaeol. 41, 215–241. https://doi.org/10.1080/00438240902843733.

Battistel, D., Piazza, R., Argiriadis, E., Marchiori, E., Radaelli, M., Barbante, C., 2015. GC-MS method for determining faecal sterols as biomarkers of human and pastoral animal presence in freshwater sediments. Anal. Bioanal. Chem. 407, 8505–8514. https://doi.org/10.1007/s00216-015-8998-2.

Bemmann, J., Lehndorff, E., Klinger, R., Linzen, S., Munkhbayar, L., Oczipka, M., Piezonka, H., Reichert, S., 2014. Biomarkers in archaeology-land use around the Uyghur capital Karabalgasun, Orkhon Valley, Mongolia. Prahistorische Zeitschrift 89, 337–370. https://doi.org/10.1515/pz-2014-0022.

Bergsvik, K.A., Skeates, R., 2012. Caves in Context: the Cultural Significance of Caves and Rockshelters in Europe. Oxbow Books, Oxford.

Berto, C., Szymanek, M., Blain, H.A., Pereswiet-Soltan, A., Krajcarz, M., Kot, M., 2022. Small vertebrate and mollusc community response to the latest Pleistocene-Holocene environment and climate changes in the Kraków-Częstochowa Upland (Poland, Central Europe). Quat. Int. 633. https://doi.org/10.1016/j.quaint.2021.09.010.

Bethell, P.H., Goad, L.J., Evershed, R.P., Ottaway, J., 1994. The Study of molecular markers of human activity: the use of coprostanol in the soil as an indicator of human faecal material. J. Archaeol. Sci. 21, 619–632. https://doi.org/10.1006/jasc.1994.1061.

Biagioli, F., Coleine, C., Delgado-Baquerizo, M., Feng, Y., Saiz-Jimenez, C., Selbmann, L., 2024. Outdoor climate drives diversity patterns of dominant microbial taxa in caves worldwide. Sci. Total Environ. 906, 167674. https://doi.org/10.1016/j.scitotenv.2023.167674.

Birk, J.J., Dippold, M., Wiesenberg, G.L.B., Glaser, B., 2012. Combined quantification of faecal sterols, stanols, stanones and bile acids in soils and terrestrial sediments by gas chromatography-mass spectrometry. J. Chromatogr. A 1242, 1–10. https://doi.org/10.1016/j.chroma.2012.04.027.

Björkhem, I., Gustafsson, J., 1971. Mechanism of microbial transformation of cholesterol into coprostanol. Eur. J. Biochem. 21, 428–432. https://doi.org/10.1111/j.1432-1033.1971.tb01488.x.

Brown, A.G., Van Hardenbroek, M., Fonville, T., Davies, K., Mackay, H., Murray, E., Head, K., Barratt, P., McCormick, F., Ficetola, G.F., Gielly, L., Henderson, A.C.G., Crone, A., Cavers, G., Langdon, P.G., Whitehouse, N.J., Pirrie, D., Alsos, I.G., 2021. Ancient DNA, lipid biomarkers and palaeoecological evidence reveals construction and life on early medieval lake settlements. Sci. Rep. 11, 1–13. https://doi.org/10.1038/s41598-021-91057-x.

Brown, A.G., Fonville, T., Van Hardenbroek, M., Cavers, G., Crone, A., McCormick, F., Murray, E., Mackay, H., Whitehouse, N.J., Henderson, A.C.G., Barratt, P., Davies, K., Head, K., Langdon, P., Alsos, I.G., Pirrie, D., 2022. New integrated molecular approaches for investigating lake settlements in north-western Europe. Antiquity 96, 1179–1199. https://doi.org/10.15184/aqy.2022.70.

Bull, I.D., Van Bergen, P.F., R Poulton, P., Evershed, R.P., 1998. Organic geochemical studies of soils from the Rothamsted classical experiments - II, soils from the Hoosfield Spring Barley experiment treated with different quantities of manure. Org. Geochem. 28, 11–26. https://doi.org/10.1016/S0146-6380(97)00114-9.

Bull, I.D., Simpson, I.A., Van Bergen, P.F., Evershed, R.P., 1999. Muck "n" molecules: organic geochemical methods for detecting ancient manuring. Antiquity 73, 86–96. https://doi.org/10.1017/S0003598X0008786X.

Bull, I.D., Bergen, P.F.V., Nott, C.J., Poulton, P.R., Evershed, R.P., 2000. Organic geochemical studies of soils from the Rothamsted classical experiments - V. The fate of lipids in different long-term experiments. Org. Geochem. 31, 389–408. https://doi.org/10.1016/S0146-6380(00)00008-5.

Bull, I.D., Lockheart, M.J., Elhmmali, M.M., Roberts, D.J., Evershed, R.P., 2002. The origin of faeces by means of biomarker detection. Environ. Int. 27, 647–654. https://doi.org/10.1016/S0160-4120(01)00124-6.

Bull, I.D., Elhmmali, M.M., Perret, V., Matthews, W., Roberts, D.J., Evershed, R.P., 2005. Biomarker evidence of faecal deposition in archaeological sediments at çatalhöyük. In: Hodder, I. (Ed.), Inhabiting Çatalhöyük: Reports from the 1995-1999 Seasons. British Institute at Ankara. McDonald Institute for Archaeological Research, London, UK, pp. 415–420.

Buonasera, T.Y., Tremayne, A.H., Darwent, C.M., Eerkens, J.W., Mason, O.K., 2015. Lipid biomarkers and compound specific δ13C analysis indicate early development of a dual-economic system for the Arctic Small Tool tradition in northern Alaska. J. Archaeol. Sci. 61, 129–138. https://doi.org/10.1016/j.jas.2015.05.011.

Chan, K.H., Lam, M.H.W., Poon, K.F., Yeung, H.Y., Chiu, T.K.T., 1998. Application of sedimentary fecal stanols and sterols in tracing sewage pollution in coastal waters. Water Res. 32, 225–235. https://doi.org/10.1016/S0043-1354(97)00175-9.

Chmielewski, W., 1988. Jaskinie Doliny Sąspowskiej: Tło Przyrodnicze Osadnictwa Pradziejowego: Praca Zbiorowa, Prace Instytutu Archeologii UW. Wydawnictwa Uniwersytetu Warszawskiego, Warszawa.

Connolly, R., Jambrina-Enríquez, M., Herrera-Herrera, A.V., Mallol, C., 2021. Investigating hydrogen isotope variation during heating of n-Alkanes under limited oxygen conditions: implications for palaeoclimate reconstruction in archaeological settings. Molecules 26, 1830. https://doi.org/10.3390/molecules26071830.

Cuevas-Tena, M., Alegría, A., Lagarda, M.J., 2017. Determination of fecal sterols following a diet with and without plant sterols. Lipids 52, 871–884. https://doi.org/10.1007/s11745-017-4286-6.

Curtin, L., D'Andrea, W.J., Balascio, N.L., Shirazi, S., Shapiro, B., De Wet, G.A., Bradley, R.S., Bakke, J., 2021. Sedimentary DNA and molecular evidence for early human occupation of the Faroe Islands. Commun. Earth Environ. 2, 253. https://doi.org/10.1038/s43247-021-00318-0.

Cyrek, K., Sudoł, M., Czyzewski, Ł., Osipowicz, G., Grelowska, M., 2014. Middle Palaeolithic cultural levels from Middle and Late Pleistocene sediments of Biśnik Cave, Poland. Quat. Int. 326–327, 20–63. https://doi.org/10.1016/j.quaint.2013.12.014.

Cyrek, K., Sudoł, M., Czyżewski, Ł., 2016. The record of changes in the Middle Palaeolithic settlement zone of the Biśnik Cave. Anthropologie (Brno) 54, 5–20.

Czajka, G., 2019. Źródła Archeologiczne Z Holoceńskich Nawarstwień Sedymentu Jaskini Ciasnej Z Badań W Latach 1969–70 I 2018 (Bachelor Degree). Uniwersytet Warszawski, Warszawa.

Devos, Y., 2018. Near total and inorganic phosphorus concentrations as a proxy for identifying ancient activities in urban contexts: the example of dark earth in Brussels, Belgium. Geoarchaeology 33, 470–485. https://doi.org/10.1002/gea.21665.

Di Ciaula, A., Garruti, G., Lunardi Baccetto, R., Molina-Molina, E., Bonfrate, L., Wang, D. Q.-H., Portincasa, P., 2017. Bile acid physiology. Ann. Hepatol. 16, S4–S14. https://doi.org/10.5604/01.3001.0010.5493.

Dutta, M., Cai, J., Gui, W., Patterson, A.D., 2019. A review of analytical platforms for accurate bile acid measurement. Anal. Bioanal. Chem. 411, 4541–4549. https://doi.org/10.1007/s00216-019-01890-3.

D'Anjou, R.M., Bradley, R.S., Balascio, N.L., Finkelstein, D.B., 2012. Climate impacts on human settlement and agricultural activities in northern Norway revealed through sediment biogeochemistry. Proc. Natl. Acad. Sci. U. S. A 109, 20332–20337. https://doi.org/10.1073/pnas.1212730109.

Evershed, R.P., Bethell, P.H., 1996. Application of multimolecular biomarker techniques to the identification of fecal material in archaeological soils and sediments. In: Archaeological Chemistry: Organic, Inorganic, and Biochemical Analysis, ACS Symposium Series. American Chemical Society, Washington, DC. https://doi.org/10.1021/bk-1996-0625.

Evershed, R.P., Bethell, P.H., Reynolds, P.J., Walsh, N.J., 1997. 5β-Stigmastanol and related 5β-Stanols as biomarkers of manuring: analysis of modern experimental material and assessment of the archaeological potential. J. Archaeol. Sci. 24, 485–495.

Farrand, W.R., 2001. Sediments and stratigraphy in rockshelters and Caves: a personal perspective on principles and pragmatics. Geoarchaeol.–Int. J. 16, 537–557. https://doi.org/10.1002/gea.1004.

Feller, F.M., Holert, J., Yücel, O., Philipp, B., 2021. Degradation of bile acids by soil and water bacteria. Microorganisms 9, 1759.

Fernández, F.G., Terry, R.E., Inomata, T., Eberl, M., 2002. An ethnoarchaeological study of chemical residues in the floors and soils of Q'eqchi' Maya houses at Las Pozas, Guatemala: chemical Residues in Soils of Q'eqchi' Maya Houses, Guatemala. Geoarchaeology 17, 487–519. https://doi.org/10.1002/gea.10026.

Fernández-Palacios, E., Herrera-Herrera, A.V., Gilson, S.-P., Égüez, N., Jambrina-Enríquez, M., Santana, J., Mallol, C., 2024. Distinguishing between sheep and goat in archaeological fumiers through faecal lipid biomarkers: the case of Belmaco Cave (Canary Islands, Spain). Quat. Int. 683–684, 135–144. https://doi.org/10.1016/j.quaint.2023.08.012.

Furtula, V., Liu, J., Chambers, P., Osachoff, H., Kennedy, C., Harkness, J., 2012. Sewage treatment plants efficiencies in removal of sterols and sterol ratios as indicators of fecal contamination sources. Water Air Soil Pollut. 223, 1017–1031. https://doi.org/10.1007/s11270-011-0920-8.

Gaskell, S.J., Eglinton, G., 1975. Rapid hydrogenation of sterols in a contemporary lacustrine sediment. Nature 254, 209–211. https://doi.org/10.1038/254209b0.

Gea, J., Sampedro, M.C., Vallejo, A., Polo-Díaz, A., Goicolea, M.A., Fernández-Eraso, J., Barrio, R.J., 2017. Characterization of ancient lipids in prehistoric organic residues: chemical evidence of livestock-pens in rock-shelters since early neolithic to bronze age. J. Separ. Sci. 40, 4549–4562. https://doi.org/10.1002/jssc.201700692.

Gérard, P., 2013. Metabolism of cholesterol and bile acids by the gut microbiota. Pathogens 3, 14–24. https://doi.org/10.3390/pathogens3010014.

Goldberg, P.S., Nathan, Y., 1975. The phosphate mineralogy of et-Tabun cave, Mount Carmel, Israel. Mineral. Mag. 40, 253–258. https://doi.org/10.1180/minmag.1975.040.311.06.

Grimalt, J.O., Fernández, P., Bayona, J.M., Albaigés, J., 1990. Assessment of fecal sterols and ketones as indicators of urban sewage inputs to coastal waters. Environ. Sci. Technol. 24, 357–363. https://doi.org/10.1021/es00073a011.

Gryczewska, N., Kot, M., Berto, C., Brancaleoni, G., Krajcarz, M.T., Cyrek, K., Sudoł-Procyk, M., Wojenka, M., Wilczyński, J., Chmielewska, M., Sulwiński, M., Suska-Malawska, M., 2023. Tracing ephemeral human occupation through archaeological, palaeoenvironmental and molecular proxies at Łabajowa Cave. Antiquity 97, e31. https://doi.org/10.15184/aqy.2023.147.

Gryczewska, N., Sulwi, M., Krajcarz, M.T., Zegarek, M., Pereswiet-Soltan, A., Szymczak, K., 2025. Applying sterols and bile acids as biomarkers for identifying human versus wild animals' faecal traces in cave sediments at archaeological sites. Archaeometry.

Guillemot, T., Zocatelli, R., Bichet, V., Jacob, J., Massa, C., Le Milbeau, C., Richard, H., Gauthier, E., 2015. Evolution of pastoralism in Southern Greenland during the last two millennia reconstructed from bile acids and coprophilous fungal spores in lacustrine sediments. Org. Geochem. 81, 40–44. https://doi.org/10.1016/j.orggeochem.2015.01.012.

Harrault, L., Milek, K., Jardé, E., Jeanneau, L., Derrien, M., Anderson, D.G., 2019. Faecal biomarkers can distinguish specific mammalian species in modern and past environments. PLoS One 14, 1–26. https://doi.org/10.1371/journal.pone.0211119.

Hofmann, A.F., Hagey, L.R., 2008. Bile acids: chemistry, pathochemistry, biology, pathobiology, and therapeutics. Cell. Mol. Life Sci. 65, 2461–2483. https://doi.org/10.1007/s00018-008-7568-6.

Holliday, V.T., Gartner, W.G., 2007. Methods of soil P analysis in archaeology. J. Archaeol. Sci. 34, 301–333. https://doi.org/10.1016/j.jas.2006.05.004.

Holtvoeth, J., Vogel, H., Wagner, B., Wol, G.A., Wolff, G.A., 2010. Lipid biomarkers in Holocene and glacial sediments from ancient Lake Ohrid (Macedonia, Albania). Biogeosciences 7, 4607–4640. https://doi.org/10.5194/bgd-7-4607-2010.

Homsey, L.K., Capo, R.C., 2006. Integrating geochemistry and micromorphology to interpret feature use at Dust Cave, a Paleo-Indian through middle-archaic site in Northwest Alabama. Geoarchaeology 21, 237–269. https://doi.org/10.1002/gea.20103.

International Organization for Standardization, 1995. Soil Quality — Determination of Organic and Total Carbon After Dry Combustion (Elementary Analysis). ISO No. 10694). Geneva.

International Organization for Standardization, 2018. Water Quality — Determination of Orthophosphate and Total Phosphorus Contents by Flow Analysis (FIA and CFA). Part 2: Method by Continuous Flow Analysis (CFA). No. ISO 15681-2). Geneva.

Keenan, B., Imfeld, A., Johnston, K., Breckenridge, A., Gélinas, Y., Douglas, P.M.J., 2021. Molecular evidence for human population change associated with climate events in the Maya lowlands. Quat. Sci. Rev. 258, 106904. https://doi.org/10.1016/j.quascirev.2021.106904.

Knights, B.A., Dickson, C.A., Dickson, J.H., Breeze, D.J., 1983. Evidence concerning the roman military diet at Bearsden, Scotland, in the 2nd Century AD. J. Archaeol. Sci. 10, 139–152. https://doi.org/10.1016/0305-4403(83)90048-1.

Kot, M., Czajka, G., Jaskulska, E., Szeliga, M., Kontny, B., Marciszak, A., Mazur, M., Wojenka, M., 2021. Sepulchral use of caves in Lusatian culture: evidence from the Sąspówka Valley in the Polish Jura. Archeol. Rozhl. 73, 200–227. https://doi.org/10.35686/AR.2021.7.

Kot, M., Gryczewska, N., Szymanek, M., Moskal del-Hoyo, M., Szeliga, M., Berto, C., Wojenka, M., Krajcarz, M., Krajcarz, M.T.M., Wertz, K., Fedorowicz, S., Jaskulska, E., Pilcicka-Ciura, H., 2022. Bramka Rockshelter: an Early Mesolithic cave site in Polish Jura. Quat. Int. 610, 44–64. https://doi.org/10.1016/j.quaint.2021.08.015.

Kovaleva, N.O., Stolpnikova, E.M., Kovalev, I.V., 2019. n-Alkane distribution in buried soils: implication for paleoecology 35–35. https://doi.org/10.36291/hit.2019.kovaleva.035.

Krajcarz, M.T., Cyrek, K., Gola, M., 2013. Osadnictwo paleolityczne w Jaskini Biśnik w zapisie antropogenicznych biomarkerów. Pr. Stud. Geogr. 51, 57–68.

Krajcarz, M.T., Bosák, P., Šlechta, S., Pruner, P., Komar, M., Dresler, J., Madeyska, T., 2014. Sediments of Biśnik Cave (Poland): lithology and stratigraphy of the Middle Palaeolithic site. Quat. Int. 326–327, 6–19. https://doi.org/10.1016/j.quaint.2013.10.017.

Krajcarz, M.M.T., Szymanek, M., Krajcarz, M.M.T., Pereswiet-Soltan, A., Alexandrowicz, W.P., Sudoł-Procyk, M., 2020. Shelter in Smoleń III – a unique example of stratified Holocene clastic cave sediments in Central Europe, a lithostratigraphic stratotype and a record of regional paleoecology. PLoS One 15, e0228546. https://doi.org/10.1371/journal.pone.0228546.

Leeming, R., Ball, A., Ashbolt, N., Nichols, P., 1996. Using faecal sterols from humans and animals to distinguish faecal pollution in receiving waters. Water Res. 30, 2893–2900. https://doi.org/10.1016/S0043-1354(96)00011-5.

Leeming, R., Latham, V., Rayner, M., Nichols, P., 1997. Detecting and distinguishing sources of sewage pollution in Australian inland and coastal waters and sediments. In: Eganhouse, R. (Ed.), Molecular Markers in Environmental Geochemistry ACS Symposium Series. American Chemical Society, Washington, DC, pp. 306–319. https://doi.org/10.1021/bk-1997-0671.ch020.

Leeming, R., Ashbolt, N.J., Nichols, P.D., 1998. Distinguishing sources of faecal pollution in Australian inland and coastal waters using sterol biomarkers and microbial faecal indicators. Res. Rep. (204) Water Services Association of Australia, Melbourne.

Lin, D.S., Connor, W.E., Napton, L.K., Heizer, R.F., 1978. The steroids of 2000-year-old human coprolites. JLR (J. Lipid Res.) 19, 215–221.

Ludgate, N., 2012. The use of lipid biomarkers from cave sediments as palaeoenvironmental indicators in South-East Asia. Quat. Int. 279–280, 293–294. https://doi.org/10.1016/j.quaint.2012.08.789.

Mackay, H., Davies, K.L., Robertson, J., Roy, L., Bull, I.D., Whitehouse, N.J., Crone, A., Cavers, G., McCormick, F., Brown, A.G., Henderson, A.C.G., 2020. Characterising life in settlements and structures: incorporating faecal lipid biomarkers within a multiproxy case study of a wetland village. J. Archaeol. Sci. 121, 105202. https://doi.org/10.1016/j.jas.2020.105202.

Madeyska, T., 1988. Osady jaskiń i schronisk Doliny Sąspowskiej. In: Chmielewski, W. (Ed.), Jaskinie Doliny Sąspowskiej. Tło Przyrodnicze Osadnictwa Pradziejowego. Wydawnictwa Uniwersytetu Warszawskiego, Warszawa, pp. 77–173.

Malainey, M.E., 2011. A Consumer's Guide to Archaeological Science, Manuals in Archaeological Method, Theory and Technique. Springer, New York, New York, NY. https://doi.org/10.1007/978-1-4419-5704-7.

Mallol, C., Égüez, N., Jambrina-Enríquez, M., Herrera-Herrera, A.V., 2025. Advancing archaeological sedimentary lipid biomarker analysis: a review of recent developments and methodological guidelines. iScience, 112064.. https://doi.org/10.1016/j.isci.2025.112064.

Misarti, N., Finney, B.P., Maschner, H., 2011. Reconstructing site organization in the eastern Aleutian Islands, Alaska using multi-element chemical analysis of soils. J. Archaeol. Sci. 38, 1441–1455. https://doi.org/10.1016/j.jas.2011.02.007.

Mitry, P., Wawro, N., Sharma, S., Kriebel, J., Artati, A., Adamski, J., Heier, M., Meisinger, C., Thorand, B., Grallert, H., Peters, A., Linseisen, J., 2019. Associations between usual food intake and faecal sterols and bile acids: results from the Cooperative Health Research in the Augsburg Region (KORA FF4) study. Br. J. Nutr. 122, 309–321. https://doi.org/10.1017/S000711451900103X.

Moldovan, O.T., Kováč, Ľ., Halse, S. (Eds.), 2018. Cave Ecology, Ecological Studies. Springer International Publishing, Cham. https://doi.org/10.1007/978-3-319-98852-8.

Obuseng, V.C., Moshoeshoe, M., Nareetsile, F., 2013. Bile acids as specific faecal polllution indicators in watre and sediments. Eur. Sci. J. 9, 273–286.

Oonk, S., Cappellini, E., Collins, M.J., 2012. Soil proteomics: an assessment of its potential for archaeological site interpretation. Org. Geochem. 50, 57–67. https://doi.org/10.1016/j.orggeochem.2012.06.012.

Panek-Jeziorna, M., Mulak, A., 2017. The role of bile acids in the pathogenesis of bowel diseases. Postepy Hig. Med. Dosw. 71. https://doi.org/10.5604/01.3001.0010.3852, 0–0.

Patalano, R., Zech, J., Roberts, P., 2020. Leaf wax lipid extraction for archaeological applications. Curr. Protocol. Plant Biol. 5, e20114. https://doi.org/10.1002/cppb.20114.

Porru, E., Scicchitano, D., Interino, N., Tavella, T., Candela, M., Roda, A., Fiori, J., 2022. Analysis of fecal bile acids and metabolites by high resolution mass spectrometry in farm animals and correlation with microbiota. Sci. Rep. 12, 2866. https://doi.org/10.1038/s41598-022-06692-9.

Prost, K., Birk, J.J., Lehndorff, E., Gerlach, R., Amelung, W., 2017. Steroid biomarkers revisited – improved source identification of faecal remains in archaeological soil material. PLoS One 12, e0164882. https://doi.org/10.1371/journal.pone.0164882.

Prost, K., Bradel, P.L., Lehndorff, E., Amelung, W., 2018. Steroid dissipation and formation in the course of farmyard manure composting. Org. Geochem. 118, 47–57. https://doi.org/10.1016/j.orggeochem.2017.12.006.

Rampelli, S., Turroni, S., Mallol, C., Hernandez, C., Galvan, B., Sistiaga, A., Biagi, E., Astolfi, A., Brigidi, P., Benazzi, S., Lewis, C.M., Warinner, C., Hofman, C.A., Schnorr, S.L., Candela, M., 2019. Components of a neanderthal gut microbiome recovered from fecal sediments from El salt. SSRN Electron. J. https://doi.org/10.2139/ssrn.3497736.

Ravn, N.R., Michelsen, A., Reboleira, A.S.P.S., 2020. Decomposition of organic matter in caves. Front. Ecol. Evol. 8, 554651. https://doi.org/10.3389/fevo.2020.554651.

Reddy, B.S., Hanson, D., Mangat, S., Mathews, L., Sbaschnig, M., Sharma, C., Simi, B., 1980. Effect of high-fat, high-beef diet and of mode of cooking of beef in the diet on fecal bacterial enzymes and fecal bile acids and neutral sterols. J. Nutr. 110, 1880–1887. https://doi.org/10.1093/jn/110.9.1880.

Riddell, J.L., Downey, A.R., Vesper, D.J., Padilla, I.Y., 2023. Total organic carbon concentrations in clastic cave sediments from Butler Cave, Virginia, USA: implications for contaminant fate and transport. Environ. Earth Sci. 82, 231. https://doi.org/10.1007/s12665-023-10893-4.

Ridlon, J.M., Kang, D.-J., Hylemon, P.B., 2006. Bile salt biotransformations by human intestinal bacteria. JLR (J. Lipid Res.) 47, 241–259. https://doi.org/10.1194/jlr.R500013-JLR200.

March, R.J., 2018. Searching for the functions of fire structures in Eynan (Mallaha) and their formation processes: a geochemical approach. In: Natufian Foragers in the Levant, pp. 227–283. https://doi.org/10.2307/j.ctv8bt33h.

Scherer, S., Höpfer, B., Deckers, K., Fischer, E., Fuchs, M., Kandeler, E., Lechterbeck, J., Lehndorff, E., Lomax, J., Marhan, S., Marinova, E., Meister, J., Poll, C., Rahimova, H., Rösch, M., Wroth, K., Zastrow, J., Knopf, T., Scholten, T., Kühn, P., 2021. Middle Bronze Age land use practices in the northwestern Alpine foreland – a multi-proxy study of colluvial deposits, archaeological features and peat bogs. SOIL 7, 269–304. https://doi.org/10.5194/soil-7-269-2021.

Schiegl, S., Goldberg, P., Bar-Yosef, O., Weiner, S., 1996. Ash deposits in Hayonim and Kebara Caves, Israel: macroscopic, microscopic and mineralogical observations, and their archaeological implications. J. Archaeol. Sci. 23, 763–781. https://doi.org/10.1006/jasc.1996.0071.

Schroeter, N., Lauterbach, S., Stebich, M., Kalanke, J., Mingram, J., Yildiz, C., Schouten, S., Gleixner, G., 2020. Biomolecular evidence of early human occupation of a high-altitude site in Western Central Asia during the Holocene. Front. Earth Sci. 8, 1–13. https://doi.org/10.3389/feart.2020.00020.

Schwark, L., Zink, K., Lechterbeck, J., 2002. Reconstruction of postglacial to early Holocene vegetation history in terrestrial Central Europe via cuticular lipid biomarkers and pollen records from lake sediments. Geology 30, 463–466. https://doi.org/10.1130/0091-7613(2002)030<0463:ROPTEH>2.0.CO;2.

Shahack-Gross, R., Berna, F., Karkanas, P., Weiner, S., 2004. Bat guano and preservation of archaeological remains in cave sites. J. Archaeol. Sci. 31, 1259–1272. https://doi.org/10.1016/j.jas.2004.02.004.

Shillito, L.M., Whelton, H.L., Blong, J.C., Jenkins, D.L., Connolly, T.J., Bull, I.D., 2020. Pre-Clovis occupation of the Americas identified by human fecal biomarkers in coprolites from Paisley Caves, Oregon. Sci. Adv. 6, eaba6404. https://doi.org/10.1126/sciadv.aba6404.

Simpson, I.A., Dockrill, S.J., Bull, I.D., Evershed, R.P., 1998. Early anthropogenic soil Formation at Tofts ness, Sanday, orkney. J. Archaeol. Sci. 25, 729–746. https://doi.org/10.1006/jasc.1997.0216.

Simpson, I.A., Van Bergen, P.F., Perret, V., Elhmmali, M.M., Roberts, D.J., Evershed, R.P., 1999. Lipid biomarkers of manuring practice in relict anthropogenic soils. Holocene 9, 223–229. https://doi.org/10.1191/095968399666898333.

Sistiaga, A., Berna, F., Laursen, R., Goldberg, P., 2014a. Steroidal biomarker analysis of a 14,000 years old putative human coprolite from Paisley Cave, Oregon. J. Archaeol. Sci. 41, 813–817. https://doi.org/10.1016/j.jas.2013.10.016.

Sistiaga, Ainara, Mallol, C., Galván, B., Summons, R.E., 2014b. The Neanderthal meal: a new perspective using faecal biomarkers. PLoS One 9, e101045. https://doi.org/10.1371/journal.pone.0101045.

Slon, V., Hopfe, C., Weiß, C.L., Mafessoni, F., Rasilla, M.D., Lalueza-Fox, C., Rosas, A., Soressi, M., Knul, M.V., Miller, R., Stewart, J.R., Derevianko, A.P., Jacobs, Z., Li, B., Roberts, R.G., Shunkov, M.V., de Lumley, H., Perrenoud, C., Gušić, I., Kućan, Ž., Rudan, P., Aximu-Petri, A., Essel, E., Nagel, S., Nickel, B., Schmidt, A., Prüfer, K., Kelso, J., Burbano, H.A., Pääbo, S., Meyer, M., 2017. Neandertal and Denisovan DNA from Pleistocene sediments. Science 608, 605–608.

Sobczyk, K., Sitlivy, V., 2001. Badania wykopaliskowe w Jaskini Łokietka w Ojcowskim Parku Narodowym w latach 1998-2000. In: Lech, J., Partyka, J. (Eds.), Z Archeologii Ukrainy I Jury Ojcowskiej. Ojcowski Park Narodowy, Ojców, p. 592.

Sokol, E.V., Kozlikin, M.B., Kokh, S.N., Nekipelova, A.V., Kulik, N.A., Danilovsky, V.A., Khvorov, P.V., Shunkov, M.V., 2022. Phosphate record in Pleistocene-Holocene sediments from Denisova Cave: formation mechanisms and archaeological implications. Minerals 12. https://doi.org/10.3390/min12050553.

Sudoł, M., Bokiniec, E., Krajcarz, M.M., Krajcarz, M.M., Trojan, A., Grafka, O., 2015. Human activity traces from shelter in Smoleń III (central part of Kraków-Częstochowa Upland) from the last centuries of antiquity. Przegląd Archeologiczny 63, 177–193.

Tomé, L., Iriarte, E., Blanco-González, A., Jambrina-Enríquez, M., Égüez, N., Herrera-Herrera, A.V., Mallol, C., 2024. Searching for traces of human activity in earthen floor sequences: high-resolution geoarchaeological analyses at an early Iron Age village in Central Iberia. J. Archaeol. Sci. 161, 105897. https://doi.org/10.1016/j.jas.2023.105897.

Ulmer, C.Z., Koelmel, J.P., Jones, C.M., Garrett, T.J., Aristizabal-Henao, J.J., Vesper, H.W., Bowden, J.A., 2021. A review of efforts to improve lipid stability during sample preparation and standardization efforts to ensure accuracy in the reporting of lipid measurements. Lipids 56, 3–16. https://doi.org/10.1002/lipd.12263.

United States Environmental Protection Agency, 2007. Method 3051A: microwave assisted acid digestion of sediments. Sludges, Soils, and Oils (No. EPA SW-846 3051A). U.S. Environmental Protection Agency.

Vachula, R.S., Huang, Y., Longo, W.M., Dee, S.G., Daniels, W.C., Russell, J.M., 2019. Evidence of Ice Age humans in eastern Beringia suggests early migration to North America. Quat. Sci. Rev. 205, 35–44. https://doi.org/10.1016/j.quascirev.2018.12.003.

Vachula, R.S., Huang, Y., Russell, J.M., Abbott, M.B., Finkenbinder, M.S., O'Donnell, J.A., 2020. Sedimentary biomarkers reaffirm human impacts on northern Beringian ecosystems during the last glacial period. Boreas 49, 514–525. https://doi.org/10.1111/bor.12449.

Vallejo, A., Gea, J., Gorostizu-Orkaiztegi, A., Vergès, J.M., Martín, P., Sampedro, M.C., Sánchez-Ortega, A., Goicolea, M.A., Barrio, R.J., 2022. Hormones and bile acids as biomarkers for the characterization of animal management in prehistoric sheepfold caves: el Mirador case (Sierra de Atapuerca, Burgos, Spain). J. Archaeol. Sci. 138. https://doi.org/10.1016/j.jas.2022.105547.

Vallejo, A., Forgia, V., Vergès, J.M., Gorostizu-Orkaiztegi, A., Alday-Izaguirre, A., Elejaga-Jimeno, A., Sampedro, M.C., Sánchez-Ortega, A., Barrio, R.J., 2024. Identification of animal species housed and herding practices in ancient sediments from the Vallone Inferno rock-shelter (Scillato, Sicily, Italy) using faecal biomarkers, hormones, and their metabolites. Quat. Int. 683–684, 123–134. https://doi.org/10.1016/j.quaint.2023.08.003.

Vázquez, C., Vallejo, A., Vergès, J.M., Barrio, R.J., 2021. Livestock activity biomarkers: estimating domestication and diet of livestock in ancient samples. J. Archaeol. Sci.: Report 40. https://doi.org/10.1016/j.jasrep.2021.103220.

von der Lühe, B., Dawson, L.A., Mayes, R.W., Forbes, S.L., Fiedler, S., 2013. Investigation of sterols as potential biomarkers for the detection of pig (S. s. domesticus) decomposition fluid in soils. Forensic Sci. Int. 230, 68–73. https://doi.org/10.1016/j.forsciint.2013.03.030.

von der Lühe, B., Prost, K., Birk, J.J., Fiedler, S., 2020. Steroids aid in human decomposition fluid identification in soils of temporary mass graves from World War II. J. Archaeol. Sci.: Report 32, 102431. https://doi.org/10.1016/j.jasrep.2020.102431.

Weete, J.D., Abril, M., Blackwell, M., 2010. Phylogenetic distribution of fungal sterols. PLoS One 5, e10899. https://doi.org/10.1371/journal.pone.0010899.

White, W.B., 2007. Cave sediments and paleoclimate. J. Cave Karst Stud. 78.

Wilson, C.A., Davidson, D.A., Cresser, M.S., 2009. An evaluation of the site specificity of soil elemental signatures for identifying and interpreting former functional areas. J. Archaeol. Sci. 36, 2327–2334. https://doi.org/10.1016/j.jas.2009.06.022.

Wurster, C.M., Munksgaard, N., Zwart, C., Bird, M., 2015. The biogeochemistry of insectivorous cave guano: a case study from insular Southeast Asia. Biogeochemistry 124, 163–175. https://doi.org/10.1007/s10533-015-0089-0.

Xie, S., Wang, Z., Wang, H., Chen, F., An, C., 2002. The occurrence of a grassy vegetation over the Chinese Loess Plateau since the last interglacier: the molecular fossil record. Sci. China Earth Sci. 45, 53–62. https://doi.org/10.1007/BF02879696.

Zetter, S., Garcés-Pastor, S., Lammers, Y., Brown, A.G., Walsh, K., Goslar, T., Lavergne, S., Coissac, E., Consortium, P., Tribsch, A., Heintzman, P.D., Greve Alsos, I., 2025. SedaDNA shows that transhumance of domestic herbivores has enhanced plant diversity over the Holocene in the Eastern European Alps. Holocene 35, 383–396. https://doi.org/10.1177/09596836241307304.

Zhang, X., Liu, X., Yang, J., Ren, F., Li, Y., 2022. Quantitative profiling of bile acids in feces of humans and rodents by ultra-high-performance liquid chromatography-quadrupole time-of-flight mass spectrometry. Metabolites 12, 633. https://doi.org/10.3390/metabo12070633.

Zheng, D., Ge, K., Qu, C., Sun, T., Wang, J., Jia, W., Zhao, A., 2024. Comparative profiling of serum, urine, and feces bile acids in humans, rats, and mice. Commun. Biol. 7, 641. https://doi.org/10.1038/s42003-024-06321-3.