Faecal biomarkers as tools to reconstruct land-use history in maar sediments in the Westeifel Volcanic Field, Germany

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The analysis of faecal biomarkers in lake sediments has been used to reconstruct human population densities and animal husbandry practices in an increasing number of studies in recent years. However, terrigenous biomarkers can decompose in soils, be stored and redeposited in colluvium and on flood-plains prior to their ultimate deposition in lakes. These and other effects can blur and distort biomarker signals. Therefore, we analysed sediments from two maars in Westeifel to evaluate whether signals of the faecal biomarkers (5β-stanols, bile acids) demonstrate statistically significant differences between contrasting periods in land-use intensity. In Holzmaar, palaeoenvironmental data showed evidence for agriculture including cereal cultivation and grassland during the pre-Roman Iron Age and Middle Ages compared with those from periods that were less influenced by land use and showed a higher abundance of broadleaf forest. However, the specific domesticated taxa of livestock in the locale from these periods remain speculative. We found statistically significantly different faecal biomarker signals, which we interpret to be related to an enhanced deposition of faeces of horses, pigs and ruminants in the core sections that represented periods of amplified land use. The analyses of grass- and broadleaf-tree characteristic n-alkanes supported the applicability of biomarkers for land-use reconstruction. Stanol data from a core section dating to the Mesolithic showed no clear results. Analyses of two core sections from Ullnener Maar, which covered periods before and after the decline of elm in the Neolithic, indicated input of pig faeces in the younger section. This study provides important evidence that faecal biomarkers can be used for land-use reconstruction in central European lakes with small catchment areas for time periods from the Neolithic onwards. The results underscore the importance of bile acid analyses in addition to stanol analyses for an identification of faeces inputs from different animals.

Evidence of past climate and landscape changes can be preserved in a number of bio-geo-archives (Bradley 2015). Deep, stratified lakes with good preservation conditions are especially good records of past environmental changes (Meyers 2003; Adrian et al. 2009; Sirocko 2016; Smol 2017) and laminated lake sediments allow precise dating (Zolitschka et al. 2015). Meaningful information on the palaeoenvironment has been obtained in lake sediments using, e.g. grain-size distributions, mineralogical composition, pollen and plant macro-remains.

There is a growing interest in the development of methods to precisely investigate early anthropogenic landscape changes (Schroeter et al. 2020). The resulting data sets can then be mapped into global syntheses (e.g. Ellis et al. 2021; Morrison et al. 2021) to identify past land management practices and provide connections between concepts of biodiversity and sustainability. Molecular biomarkers, such as those found in faeces, have been used as indicators of human presence, population densities and land use. For example, 5β-stanols were used as biomarkers for human faeces with the aim to reconstruct the onset of human settlement activities in New Zealand (Argiriadis et al. 2018), the first presence of humans in northern Alaska (Vachula et al. 2019) and human population fluctuations during the Mississippian period of the American Bottom region of North America (White et al. 2018, 2020). They were used as biomarkers for human and livestock faeces to investigate human population densities in combination with livestock in northern Norway (d’Anjou et al. 2012) and at a high-altitude site in western central Asia during the Holocene (Schroeter et al. 2020).

Bile acids are an important companion biomarker to 5β-stanols used to identify the specific taxa from which the faeces originated (Fig. 1). For example, they allow a differentiation between human faeces, the faeces of pigs (Sus domesticus) and distinct herbivores (Prost et al. 2017; Zocatelli et al. 2017). However, these biomarkers have heretofore been rarely investigated in sediments (Guillemot et al. 2017).

Frequently, faecal biomarkers are directly correlated with the depositional age of lake sediments to provide information about coeval cultural-environmental phenomena (d’Anjou et al. 2012; Guillemot et al. 2015; Argiriadis et al. 2018; White et al. 2018). However, working with these correlations has potential pitfalls: (i) In most cases, colluvial deposits are transported in flow cascades down from upland settings and sediments can...
Fig. 1. Steroid transformations yielding biomarkers in the gut of animals or in the environment (Bull et al. 1999, 2002) and a schematic synopsis of the faecal biomarker concentrations in the faeces of cow (Bos taurus), goat (Capra hircus), sheep (Ovis aries), horse (Equus caballus), pig (Sus domesticus), human (homo sapiens) and birds. The figure summarizes schematically data referred to dry and wet faeces from Gill et al. (2010), Leeming et al. (1996), Prost et al. (2017) and Tyagi et al. (2008). Concentrations of biomarkers in faeces analysed by these authors differed strongly; e.g. Leeming et al. (1996) found concentrations of 5β-stigmastanol of 184±41 μg g⁻¹ dry faeces in the faeces of cows and Prost et al. (2017) found concentrations of 5β-stigmastanol of 2440±665 μg g⁻¹ dry faeces in the faeces of cows.
be temporarily stored in flood-plains before they are deposited into lakes (Zolitschka et al. 2003; Fuchs et al. 2011). Therefore, there is a risk that biomarker signals in the sediments are blurred and distorted due to secondary deposition processes. (ii) It is possible that biomarkers can vertically move downwards into underlying older strata in sediments over time. (iii) The use of biomarker analyses is limited by insufficient knowledge about the conservation and degradation of biomarkers in soils and sediments.

In this study biomarkers were analysed in sediments of two maars in the Eifel (Germany). The maars in the Eifel are well-established archives for studies of the environment of the Lateglacial and the Holocene (Sirocko 2016). Their sediments have been dated independently using $^{137}\text{Cs}$, $^{210}\text{Pb}$, $^{14}\text{C}$, varve counting, luminescence and argon/argon dating techniques (Sirocko et al. 2013). Land-use reconstruction of the areas surrounding the maars is largely based on pollen data (Herbig & Sirocko 2013). Analyses of grain-size distributions, plant macro-remains, black carbon, lignin derivatives, polycyclic aromatic hydrocarbons and further analyses have also been carried out for detailed palaeoecological investigations in these sediments (Fuhrmann et al. 2004; Herbig & Sirocko 2013; Lehndorff et al. 2015; Sirocko 2016; Kappenberg et al. 2021). These previous investigations showed temporal changes of the vegetation cover with an inferred relationship to human land use in the Eifel. Briefly summarized, it was reconstructed that in the Mesolithic Period dense forests covered the Eifel (Litt et al. 2009; Herbig & Sirocko 2013), which were only inhabited by small groups of hunting and gathering communities (Löhr et al. 2012). The decline of elm (Ulmus sp.) in the Neolithic is well documented in the sediments of Eifel maars (Sirocko et al. 2016) and one reason for this decline is assumed to be animal husbandry (Parker et al. 2002; Sirocko et al. 2016). Extensive forest regression that was attributed to human activity occurred from the Iron Age onwards (Stockhausen & Zolitschka 1999; Prasad & Baier 2014) and the pre-Roman Iron Age was the pre-historic period with the most notable intensive land use in the Eifel (Litt et al. 2009). The vegetation cover in the Eifel did not considerably change during the Roman times (Herbig & Sirocko 2013). In the Migration Period (Völkerwanderung), archaeological evidence for settlements in the Eifel is again fragmentary (Herbig & Sirocko 2013) and it is assumed that the area was only sparsely populated (Litt et al. 2009; Bandowe et al. 2014). It was not until the Middle Ages that the region was subject to major economic growth when the population increase stabilized (David-Sirocko & Sirocko 2012). During this time, agriculture expanded and mountain landscapes such as the Eifel became attractive for settlement, as they offered potential for livestock farming and timber production (Herbig & Sirocko 2013). These changes between contrasting periods in land-use intensity in their catchment areas made the maars well-suited archives to investigate methods of land-use reconstruction.

Based on the existing palaeoenvironmental reconstructions, sections of sediment cores of Holzmaar and Ulmener Maar were selected for this study. They were used to test whether faecal biomarker signals in sediments with known land-use and vegetation changes can be used to gather information about animal breeding in lakes that lie in small catchment areas. The guiding hypothesis was that land-use changes were linked with statistically significant differences in the faecal biomarker patterns. The study aimed to: (i) Test whether sediment core sections of Holzmaar that represented periods of amplified land use with a clear abundance of grass pollen show faecal biomarker patterns that are characteristic for livestock. This was tested in comparison to biomarker patterns of core sections with a higher abundance of broad leaf tree pollen. (ii) Use the sediments of lake Holzmaar to test whether vegetation characteristic n-alkanes can be used to support faecal biomarkers. n-Alkanes were used as biomarkers that can show shifts between broadleaf forest and opened more grassy vegetation similar to pollen data (Cranwell 1973; Zech et al. 2009; Buggle et al. 2010) but have input paths into lake sediments that are comparable to those of faecal biomarkers. (iii) Use the sediments of Ulmener Maar to test if these sediments contain faecal biomarker patterns that are characteristic for livestock after the elm decline in comparison to the sediments that were deposited before this event.

Characteristics of the study sites

Holzmaar and Ulmener Maar from which sediments were used in this study (Table 1) have relatively consistent catchments (Fig. S1) and climate conditions. Both lakes are located within the Westeifel Quaternary Volcanic Field, Germany (Fig. S1), where the mean annual air temperature (1961–1990) was 7.8 °C, and the mean annual precipitation was 908 mm. The potential natural vegetation has been described as mesophytic deciduous broadleaf and mixed coniferous broadleaf forests of the collin-submontane zone (Litt et al. 2009).

Cores HM1 from Holzmaar and UM2 from Ulmener Maar were used in this study. They derive from the Eifel

| Table 1. Location and selected properties of Holzmaar and Ulmener Maar (Zolitschka 1998; Sirocko 2012). |
|---------------------------------|--------|--------|
| Catchment area (km$^2$)        | 2.06   | 4.01   |
| Latitude, longitude            | 50°7'N, 6°53'E  | 50°13'N, 6°59'E |
| Elevation (m a.s.l.)            | 425    | 420    |
| Lake surface area (m²)         | 58 000 | 55 000 |
| Maximum depth (m)              | 20     | 39     |
| Core identification            | HM1    | UM2    |
Laminated Sediment Archive (ELSA; Sirocko 2016). The selection of the sections of sediment cores was made based on data from previous studies. In this study, the stratigraphy of the cores is according to Sirocko et al. (2013) and pollen data were taken from Herbig & Sirocko (2013). The palaeoecological interpretations of the sediment sections from these and other previous studies are summarized as follows:

Five core sections from Holzmaar were analysed in this study (Fig. 2). Core HM1 represents the period from approximately 12 000 years BP until modern times. The two top core sections were sampled for statistical comparison. The pollen spectra of Herbig & Sirocko (2013) showed a difference between a more forest-dominated landscape in the lower section (HM-Fa; 2.5–2.9 m) and a more open-grassy landscape in the upper section (HM-LUa; 1.3–1.7 m). The core section of the more forest-dominated landscape (HM-Fa) dated to the Migration Period. The top core section (HM-LUa) extended into the High and Late Middle Ages and was characterized by a clear appearance of cereal and grass pollen that correlated with a decrease in pollen of alder (*Alnus* sp.), hazel (*Corylus* sp.), ash (*Fraxinus* sp.) and hornbeam (*Carpinus* sp.; Fig. 2). These pollen and archaeological data of the Eifel showed an expansion of areas for grain cultivation and pastures (Herbig & Sirocko 2013).

The two lower sections of the core also formed a statistically comparable data set (Fig. 2). Like the two upper sections of the core, the pollen spectra of Herbig & Sirocko (2013) for the two lower sections also showed a decline in forest pollen (e.g. beech (*Fagus* sp.), ash and hazel) in clear favour of grass and slight favour of domesticated cereal pollen from the older section, dating to the Bronze Age (HM-Fb; 4.3–4.7 m), to the younger section, dating to the pre-Roman Iron Age (HM-LUb; 3.5–3.9 m; Fig. 2). The decline of forest pollen coincided with high input of black carbon during the pre-Roman Iron Age that has been attributed to the use of fire for metal processing in Holzmaar (Lehndorff et al. 2015).

The oldest section in Holzmaar (HM-Fc; 7.5–8.0 m; Fig. 2) was not compared statistically with the other sections; it reached back to the Mesolithic Period. This was a period in which natural forest grew in the Westeifel region and no anthropogenic indicators were found in the pollen diagrams of maars in this region (Fig. 2; Litt et al. 2009; Herbig & Sirocko 2013).

Core UM2 from Ulmener Maar covers the period from the Late Mesolithic/Early Neolithic up to the Late Middle Ages. The two core sections of Ulmener Maar (Fig. 3), which were compared statistically, showed no clear differences in the abundance of cleared areas in the catchment in their pollen spectra (Herbig & Sirocko 2013).

Fig. 2. Characterization of the core sections of Holzmaar that were analysed in this study (stratigraphy according to Sirocko et al. 2013) and selected pollen data from Herbig & Sirocko (2013).
However, the older section pre-dated the elm decline in the Neolithic and the younger section post-dated this event. The pollen spectra of Herbig & Sirocko (2013) showed a decrease in elm in favour of alder, ash and beech pollen from the older (UM-F; 9.9–10.3 m) to the younger section of the core (UM-FP; 9.0–9.4 m; Fig. 3).

Material and methods

Sampling and analytical procedures

Seven core sections were sampled in 10-cm intervals, which corresponds to five samples of the section HM-Fc and four samples for all other sections. The samples were dried at 40 °C, sieved <2 mm and finely ground in an agate mill.

For the extraction, purification and measurement of n-alkanes, stanols, Δ5-sterols and bile acids the method from Birk et al. (2012) was used with small modifications. The n-alkane C36, pregnanol, isodeoxycholic acid and cholestane were used as internal standards. The extraction was done with a mixture of dichloromethane and methanol in a Soxhlet extractor. The extract was saponified and the n-alkanes, stanols, Δ5-sterols and bile acids were recovered from the saponification solution by a sequential lipid-lipid extraction. During this step the bile acids were purified from the other biomarkers. Stanols, Δ5-sterols and n-alkanes were purified by a solid phase extraction, which yielded n-alkanes and steroids in different fractions. The bile acids were purified by solid phase extraction after methylation of the carboxyl groups. All steroids were silylated and all biomarkers were quantified via gas chromatography-mass spectrometry (5975B, 6890N, DB-5ms UI, Agilent, Santa Clara, CA, USA). Measurements in scan mode were done to verify peak identity and measurements in selected ion monitoring mode (SIM) were performed for quantification. A detailed description of the analytical methods of the study and a table with the analysed substances, selected ions for the measurement of these substances, their retention times and further information are provided in the Supporting Information (Data S1, Table S1).

Calculations and statistics

For quantification, peak areas of the biomarkers in the samples and the substances in the external standards were divided by the peak area of cholestane, respectively. Using these ratios, calibration curves for each substance were calculated from the data of the external standards. The concentrations of each substance were divided by the amounts of total organic carbon (TOC) to correct for differences in TOC concentrations (ng g⁻¹ TOC; concentrations of TOC and the ratio of TOC to total nitrogen are shown in Table S2). Arithmetic means and standard errors were calculated for each parameter in each section. t-tests were calculated to test for significance of differences between sections that represented periods of amplified land use and the subjacent section below with a higher abundance of broadleaf forest in Holzmaar and between both core sections in Ulmener Maar (one tailed t-Tests for homoscedastic variables and Welch’s t-test for unequal variances according to the results of preceding F-tests). To test for relationships between variables, Spearman’s rank correlation coefficients were calculated. The level of significance was p < 0.05 for all statistical tests. Concentrations of each substance related to the sediment weight (ng g⁻¹) and to TOC concentrations (ng g⁻¹ TOC) in the individual samples, biomarker ratios in the individual samples, significance of differences between the core sections, and correlation matrices are shown in Tables S2–S11.

Results and discussion

n-Alkanes

The n-alkane pattern in Holzmaar was dominated by long-chain n-alkanes (C27–C33; ≤92 μg g⁻¹ TOC; Tables S3, S4), which are considered to be biomarkers for terrestrial plants (Eglinton & Hamilton 1967; Kolattukudy 1970;
Typical n-alkanes for algae and cyanobacteria with short chain lengths (C16–C18; Cranwell 1973; Glaser 2005) had concentrations ≤10 μg g⁻¹TOC (Table S2). Comparable concentrations and chain length distributions of n-alkanes were found by Fuhrmann et al. (2004), who analysed n-alkanes in samples from Holzmaar and Meierfelder Maar covering the Holocene and dating back until the Allerød (~13 000 years BP) and Late Pleniglacial (~14 500 years BP), respectively. The concentrations of n-alkanes did not statistically differ between the core sections in Holzmaar (HM-LUa vs. HM-Fa and HM-LUb vs. HM-Fb; p = 0.186–0.467).

The ratio of the concentrations of long-chain n-alkanes with odd carbon numbers to long-chain n-alkanes with even carbon numbers (odd-over-even predominance; OEP; (C27 + C29 + C31 + C33)/(C26 + C28 + C30 + C32)) can be used for both differentiation of aquatic and terrestrial biomass inputs into sediments and for determining the degree of degradation of n-alkane inputs of terrestrial plants (Eglinton & Hamilton 1967; Hoefs et al. 2002; Buggle et al. 2010; Bush & McInerney 2013). Ratios ≥4 are characteristic for n-alkane inputs from terrestrial plants (Hoefs et al. 2002). In Holzmaar the OEP values were between 2.5 and 10 and about half of the values were above 4 (Table S4). The OEP values in the core sections whose pollen spectra showed a more open landscape did not significantly differ from those in the respective lower sections with a clearer dominance of tree pollen (p ≥ 0.201; Fig. 4A). This suggests that there were no major differences in the degradation of the n-alkanes and/or the proportion of n-alkane inputs from aquatic plants between the pairwise compared sections.

In leaves of deciduous, broadleaf trees, the most abundant n-alkanes are C27 and C29 and in grasses and herbs the n-alkanes C31 and C33 dominate (Cranwell 1973; Zech et al. 2009; Buggle et al. 2010). Consequently, the ratio (C27 + C29)/(C31 + C33) allows differentiation between inputs from grasses/herbs (lower values) and broadleaf trees (higher values; Buggle et al. 2010). The ratio (C27 + C29)/(C31 + C33) differed statistically significantly between the core sections (p = 0.001–0.020). The ratio was 1.3–2.2 in the core sections whose pollen spectrum showed a more open landscape and was 1.5 times lower in these sections than in the respective lower sections, which demonstrated a clearer dominance of tree pollen (1.5–3.4; Fig. 4B, Table S4). A high ratio in the lowest core section (≤4.8) coincided with a high tree pollen abundance during the Mesolithic period in which a natural forest covered the area around the maars (Fig. 4B).

In lakes, pollen are partly transported through water inflow, as can be assumed for sediments containing n-alkanes and faecal biomarkers, but in contrast to biomarkers, some of them deposit into lakes through pollen rain (DeBusk 1997; Reitz & Shackley 2012; Frazer et al. 2020). More importantly, they can degrade in soils under optimal conditions within years (Dimbleby & Evans 1974) and their conservation is limited to soils with low biological activity due to low pH, redoximorphic conditions and cold or arid climate conditions (Pennington 1979; Reitz & Shackley 2012). With the analysis of n-alkanes a correlation between biomarkers and land-use changes, which were shown by pollen data, was shown. This indicated that the biomarkers entrained in sediments in Holzmaar did not suffer much from secondary deposition processes or pre- and postdepositional taphonomic effects.

The concentrations of n-alkanes in Ulmener Maar were in the same range as in Holzmaar. In Ulmener Maar long-chain n-alkanes (C27–C33) had concentrations ≤94 μg g⁻¹TOC and short-chain n-alkanes (C16–C18) had concentrations ≤5.8 μg g⁻¹TOC (Tables S2–S4). In Ulmener Maar, however, the two core sections significantly differed in their C18 and C29 alkane concentrations (p ≤ 0.020), which were about 1.3 times higher in UM-FP (C18: 2.2–3.0 μg g⁻¹TOC; C29: 82–94 μg g⁻¹TOC; Tables S2, S4) than in UM-F (C18: 1.4–2.2 μg g⁻¹TOC; C29: 56–85 μg g⁻¹TOC; Tables S2, S4). The OEP ratio was 10–14 (Table S4) and the two core sections also significantly differed in their OEP ratios (p = 0.001). The OEP ratio in UM-FP was 1.3 times higher than that in UM-F (Fig. 5A). The higher OEP in UM-FP indicated a higher input of n-alkanes from terrestrial plants and/or better preservation of the n-alkanes than in UM-F. The (C27 + C29)/(C31 + C33) ratio had values (1.8–2.2; Table S4) similar to those in Holzmaar. No significant differences were found in the values of this ratio between the two sections (p = 0.072), whose pollen data showed the Neolithic elm decline but no differences in the proportion of open land and tree pollen (Fig. 5B). Thus, even drastic changes in the tree composition and differences in OEP did not cause significant differences in the (C27 + C29)/(C31 + C33) ratio, which was used to differentiate between more open- and more closed-vegetation periods in the sediments of Holzmaar.

Stanols and Δ⁵-sterols

In Holzmaar the concentrations of the faecal biomarkers coprostanol, 5β-stigmastanol and their 3α-epimers (epi-coprostanol and epi-5β-stigmastanol) were ≤6 μg g⁻¹TOC (Table S6). These concentrations were slightly lower than the concentration reported by d’Anjou et al. (2012) from a lake in northern Norway (≤20 μg g⁻¹TOC) and were comparable to those of White et al. (2018) in a lake near the prehistoric mound complex of Cahokia in Illinois, USA. The concentrations of the 3α- and 3β-epimers positively correlated in the upper four sections (r = 0.5–0.74; p ≤ 0.049; Table S10), which corresponds to the assumption that 3α-epimers are formed from the 3β-epimers (Bull et al. 2002).
The amounts of the precursors of the stanols, the $\Delta^5$-sterols ($\leq 773 \mu g \cdot g^{-1} \text{TOC}$; Table S5; plant and animal membrane constituents; Fig. 1), and the concentrations of the $5\alpha$-stanols ($\leq 136 \mu g \cdot g^{-1} \text{TOC}$; Table S5) were higher than the concentrations of the faecal biomarkers and the concentrations of $5\alpha$-stanols positively correlated with those of the corresponding $\Delta^5$-sterols (Fig. 1) in the upper four core sections ($r = 0.54–0.8$; $p \leq 0.029$; Table S10). These correlations supported the hypothesis that $5\alpha$-stanols are formed in soils and sediments from $\Delta^5$-sterols (Fig. 1). The concentrations of cholestanol and cholesterol also positively correlated in these core sections with those of the corresponding $\Delta^5$-sterols (Fig. 1).

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the faecal biomarker coprosterol (indicative of omnivore faeces, e.g. humans and pigs; Fig. 1; $r_s = 0.50-0.59$; $p \leq 0.047$; Table S10). The correlations of coprosterol and cholestanol indicated that both were formed from the precursor cholesterol in soil or sediment and therefore a considerable input of coprosterol by faeces was unlikely. In contrast, no statistically significant correlations between the concentrations of 5β-stigmastanol (indicative of herbivore faeces; Fig. 1) and the amounts of 5α-stigmastanol and those of β-sitosterol were found ($r_s = 0.26-0.36$; $p \geq 0.165$; Table S10). The lack of these correlations indicated that it was unlikely that both stanols were of the same origin. Therefore, herbivore faeces were likely the source of 5β-stigmastanol, and of its 3α-epimer (Fig. 1). Epi-5β-stigmastanol was the only 5β-stanol that had significantly different concentrations in the core sections ($p \leq 0.018$; Table S6). The core section HM-LUb (2.2–4.9 μg g$^{-1}$TOC; Table S6) had on average 3.5 times higher content than HM-Fb (0.6–1.2 μg g$^{-1}$TOC; Table S6) and HM-LUa (2.9–5.5 μg g$^{-1}$TOC; Table S6) had 2.5 times higher content than HM-Fa (0.5–2.5 μg g$^{-1}$TOC; Table S6).

Background values of 5β-stanols were found in soils and sediments where no enhanced deposition of faeces was assumed (Bethel et al. 1994; Evershed et al. 1997; Bull et al. 2001). Therefore, the amounts of 5β-stanols were related to the amounts of 5α-stanols to correct for these background values (Bull et al. 2002). These ratios are based on the assumption that Δ3-sterols are mainly reduced to 5α-stanols and only a minor amount of 5β-stanols is produced in soils and sediments (Fig. 1). The ratio (coprosterol + epi-coprosterol)/cholesterol was used as a measure for the input of faeces of omnivores. This ratio did not demonstrate statistically significant differences between the upper four core sections with different land use (values: 0.1–0.5; $p = 0.074-0.299$; Table S7) and showed relatively high values ($\geq 0.6$; Table S7) in the lower core section. This corresponded to the correlations between coprosterol and cholesterol as well as cholesterol (see above), which indicated that coprosterol did not originate in considerable amounts from faecal inputs.

In relation to the faeces of farm animals, the faeces of humans contain coprosterol in higher concentrations (Fig. 1; e.g. Prost et al. 2017 measured mean concentration of coprosterol in human faeces of 6.9 mg g$^{-1}$ dry faeces and in faeces of farm animals ≤1.0 mg g$^{-1}$ dry faeces). Therefore, an input of enhanced amounts of human faeces during periods of intensified land use was not shown by the stanol data. However, studies that tested a correlation of faecal stanols with human population densities in the past were done in lakes with remains of settlements (d’Anjou et al. 2012; White et al. 2018). Such archaeological evidence of settlements is missing for Holzmaar and humans, who visited the catchment areas only for agricultural activities (and hunting) without building of settlements, may not be detectable by analyses of faecal stanols in lake sediments.

The (5β-stigmastanol + epi-5β-stigmastanol)/5α-stigmastanol ratio was calculated to investigate the potential inputs of herbivore faeces. The results yielded a ratio of ≤0.3 in Holzmaar (Table S7) and statistically significantly differed between the upper sections of the core (Fig. 4C; $p = 0.012$). The ratio was 2.2 times higher in the core section with high grass pollen abundance (HM-LUa) than in the sections below with higher tree pollen abundance (HM-Fb). In combination with the concomitant higher epi-5β-stigmastanol concentrations, there is a strong indication of faecal inputs by herbivores into the sediments of the upper core section, which represent a more open-grassy landscape. However, this ratio was relatively high in the lowest section of the core. Either there were high numbers of wild herbivores contributing faecal matter into this section or the ratio is not applicable for times so far back in this maar due to diagenesis or other taphonomic reasons.

As a measure to differentiate the faeces of herbivores and omnivores, the amounts of 5β-stigmastanols were divided by the amounts of 5β-cholestanols ((5β-stigmastanol + epi-5β-stigmastanol)/(coprosterol + epi-coprosterol)). This ratio significantly differed between the core sections in Holzmaar ($p \leq 0.009$). It was ≤3.7 (Table S7) and was approximately twice as high in the sections with higher grass pollen abundance as in the respective sections below with higher tree pollen abundance (Fig. 4D). This indicated an increased proportion of faecal matter input by herbivores in these core sections, which represent a more open-grassy landscape.

A relatively high abundance of the 3α-epimers of the 5β-stanols in relation to the 3β-epimers is typical for the faeces of horses (Equus caballus; Fig. 1; e.g. Prost et al. 2017 measured concentrations of epi-5β-stigmastanol of 0.8 mg g$^{-1}$ dry faeces and of 5β-stigmastanol of 1.0 mg g$^{-1}$ dry faeces in the faeces of horses and ratios of epi-5β-stigmastanol/5β-stigmastanol ≤0.25 in the faeces of other farm animals). Therefore, ratios of 3α- to 3β-epimers are suitable for the identification of the faeces of horses (Prost et al. 2017). The ratio epi-5β-stigmastanol/5β-stigmastanol significantly differed between the core sections ($p < 0.001$). The ratio was ≥1 in the core sections that represented a more open landscape and was more than 2.4 times higher in these sections than in the corresponding sections, which included sediments from more forested periods (Fig. 4E; Table S7). This ratio showed that the proportion of faeces of horses increased in the core sections with more open, grassier landscape conditions. This ratio did not show clear differences compared to the epi-5β-stigmastanol/5β-stigmastanol ratio between the core sections (up to 1.2 times higher in sections that represented a more open landscape than in the corresponding sections, which represented forested periods; $p = 0.017–0.399$). This can be explained by much lower concentrations of 5β-cholestanols in the faeces of horses.
(Fig. 1; e.g. Prost et al. 2017) measured concentrations of epi-coprostanol and of coprostanol of 0.1 mg $\text{g}^{-1}$ dry faeces and by a dominant input of 5β-cholestanoles from another source than faeces in Holzmaar (see above).

The amplification of land use was coeval to increases in biomarkers for livestock, but not those of humans. These results suggest more intensive cultivation practices were pursued as the technological strategies of Iron Age people expanded relative to earlier Mesolithic and Neolithic populations, but no settlements were located in the catchments or population numbers were evidently relatively stable in the Eifel. Changing settlement, subsistence and vegetation regimes of central Europe during the Holocene have heretofore been widely documented (Bandowe et al. 2014; Dreibrodt & Wiethold 2014; Lehndorff et al. 2015; Styring et al. 2017; Tserendorj et al. 2021); however, the use of stanols provides palaeodemographic context to the archaeological and ecological record.

In the lowest core section, the stanol ratios that were used to identify the proportion of herbivore and horse faeces had values that were between the upper sections representing a more open landscape and the upper sections representing a landscape predominated by forest taxa (Fig. 4D, E, Table S7). Therefore, these ratios did not provide information on the applicability of faecal biomarkers in this section.

In Ulmener Maar, the concentrations of 5β-stanols were 0.7–3.9 μg $\text{g}^{-1}$ TOC (Table S6). These concentrations were within the concentration ranges that were found in Holzmaar for these substances. The concentrations of 5α-stanols and Δ²-sterols were ≤78 μg $\text{g}^{-1}$ TOC (Table S5). In the core section UM-FP concentrations of coprostanol (≤1.6 μg $\text{g}^{-1}$ TOC; Table S6) were significantly higher than in UM-F (≤1.0 μg $\text{g}^{-1}$ TOC; 1.4 times higher in UM-FP than in UM-F; $p = 0.032$; Table S6). However, when the 5β-stanols were related to their 5β-epimers to correct for background values neither the ratio (coprostanol + epi-coprostanol)/cholesterol nor the ratio (5β-stigmastanol + epi-5β-stigmastanol)/5α-stigmastanol differed significantly between the two sections ($p ≥ 0.276$; Table S7).

The ratio (5β-stigmastanol + epi-5β-stigmastanol)/(coprostanol + epi-coprostanol) was ≤2.5 in UM-FP and the ratios of the 5β-epimers of the 5β-stanols to their 5β-epimers were ≤0.9 in UM-FP (Table S7). These ratios statistically significantly differed between the core sections ($p = 0.000–0.041$) and were about 0.7 times lower in UM-FP than in UM-F (Fig. 5C–E). These data indicated an enhanced input of faeces of omnivores in UM-FP, which is interpreted as having been forest pasture for pigs and cattle (Bos taurus), eventually combined with coppicing and gathering of elm bark, leaf and twigs for use as animal fodder (Parker et al. 2002; Herbig & Sirocko 2013; Sirocko et al. 2016).

**Bile acids**

In Holzmaar, hyodeoxycholic acid (HDCA) was the bile acid with the highest concentration (≤42 μg $\text{g}^{-1}$ TOC; Table S8). Deoxycholic acid (DCA) had the second highest concentration (≤37 μg $\text{g}^{-1}$ TOC; Table S8) and lithocholic acid (LCA) and Chenodeoxycholic acid (CDCA) had much lower concentrations (≤5 μg $\text{g}^{-1}$ TOC; Table S8). The concentrations of these bile acids positively correlated in the four upper sections ($r_s = 0.89–0.95$; $p < 0.001$; Table S11). Statistically significant differences in the concentration of bile acids were found between the core sections ($p ≤ 0.011$). The concentrations of HDCA, DCA and LCA were 3 to 24 times higher in the two core sections with higher abundance of grass pollen (HM-LUa and HM-Fa) than in the respective sections below with higher abundance of tree pollen (HM-LUb and HM-Fb; Table S8). CDCA concentrations only significantly differed between the two upper core sections ($p = 0.001$) and were three times higher in HM-LUa than in HM-Fa (Table S8).

HDCA predominates in the faeces of pigs and is not only in traces found in the faeces of other farm animals (Fig. 1). Due to the high concentrations of this bile acid in Holzmaar differences in the input of pig faeces between the core sections were analysed by calculating the ratios of HDCA to the other bile acids. The ratio of HDCA to DCA was ≤1.7 and the ratios of HDCA to LCA and CDCA were ≤14 (Table S9). All three ratios statistically significantly differed between the upper two core sections and reflected a land-use change ($p = 0.003–0.043$). Ratios in HM-LUa were up to eight times higher than those in HM-Fa (Fig. 6A–C). These data showed an increased input of pig faeces in HM-LUa compared to HM-Fa.

In the two core sections below, only the HDCA/CDCA ratio significantly differed ($p = 0.024$). This ratio was three times higher in HM-LUb than in HM-Fb (Fig. 6C). CDCA is the dominant bile acid in the faeces of birds (Fig. 1). The higher HDCA/CDCA ratio in the more deforested section of the core therefore indicated a higher input of pig faeces in relation to the faeces of birds. While the concentrations and ratios of the bile acids give a clear indication of an input of pig faeces in the core sections, which reflects enhanced land use, stanols only showed an increased input of faeces from herbivores, especially that of horses, in these sections. This seemingly contradicting result can be explained by different concentrations of bile acids and stanols in the faeces of the animals. HDCA concentrations are found in pig faeces in concentrations that are relatively high in relation to those of stanols (Fig. 1; e.g. Prost et al. 2017) found mean ratios of HDCA divided by the sum of 5β-stanols 0.35–0.79 in pig faeces). In contrast, stanol concentrations in the faeces of herbivores are much higher than those of the
bile acids (Fig. 1; e.g. Prost et al. 2017) found mean ratios of DCA + LCA + CDCA + HDCA divided by the sum of 5β-stanols 0.01–0.17 in faeces of herbivores). Therefore, the input of herbivore faeces was detected through the analysis of stanols. The analysis of bile acids in addition showed that also enhanced amounts of faeces of pigs were deposited in the core sections, which were more strongly influenced by land use than the respective sections below.

The DCA/LCA and DCA/CDCA ratios in Holzmaar were ≤17 (Table S9). Both ratios were significantly higher \((p = 0.000–0.029)\) in the core sections with amplified land use than in the respective sections with higher broadleaf tree dominance (2–10 times higher in HM-LUa and HM-LUb; Fig. 6D, E). Cattle and goats \((Capra hircus)\) show a pronounced dominance of DCA in their faeces in addition showed that also enhanced amounts of faeces of pigs were deposited in the core sections, which were more strongly influenced by land use than the respective sections below.

The DCA/LCA and DCA/CDCA ratios in Holzmaar were ≤17 (Table S9). Both ratios were significantly higher \((p = 0.000–0.029)\) in the core sections with amplified land use than in the respective sections with higher broadleaf tree dominance (2–10 times higher in HM-LUa and HM-LUb; Fig. 6D, E). Cattle and goats \((Capra hircus)\) show a pronounced dominance of DCA in their faeces in contrast to pigs and horses (Fig. 1; e.g. Prost et al. 2017) found mean ratios of DCA/LCA and DCA/CDCA 34–35 in faeces of goats, and a mean ratio of DCA/LCA of 9 in the faeces of cattle but only mean ratios of DCA/LCA and DCA/CDCA ≤3 in the faeces of horses and pigs. It should be noted that they did not find CDCA in the faeces of cattle and pigs). Consequently, these bile acid ratios indicated an increased input of faeces from ruminants in sections that were more dominated by land use. As a result, the DCA/LCA and DCA/CDCA ratios appeared to contradict the stanol data, which showed an increased input of horse faeces in these sections. An interpretive approach similar to that used to compare the concentrations of HDCA and the stanol data could also explain the apparent contradiction between these ratios and the stanol ratios. The proportion of bile acids in relation to stanols in the faeces of cattle and goats is higher than in the faeces of horses (Fig. 1; e.g. Prost et al. 2017) found mean ratios of DCA/LCA and HDCA divided by the sum of 5β-stanols ≥0.14 in faeces of cattle and goats and of 0.05 in the faeces of horses. The detection of horse faeces was therefore only possible with the help of the stanol analyses and the detection of additional ruminant faeces was only possible with the help of analyses of the bile acids. The bile acid ratios in the oldest sections had values that were comparable to the other more forested sections and do not contradict the interpretations above (Fig. 6).

The bile acid concentrations in Ulmener Maar were lower than in Holzmaar \((≤5 \mu g g^{-1} TOC; Table S8)\). A statistically significant difference between the two core sections was only found for the concentrations of HDCA \((p = 0.024)\). They were twice as high in the core section for which forest pasture was identified \((0.6–1.3 \mu g g^{-1} TOC; Table S8)\) as in the section below \((0.3–0.6 \mu g g^{-1} TOC; Table S8)\). The HDCA/DCA was 0.1 and HDCA/LCA was 1.3–2.9 in UM-FP (Table S9). These ratios significantly differed between the core sections \((p ≤ 0.015)\) and were at least twice as high in UM-FP as in UM-F (Fig. 7A), whereas the other bile acid ratios did not significantly differ between the sections \((p = 0.071–0.160; Table S9)\). These data indicate an input of pig faeces in the upper core section. Thus, the bile acid data agreed with the hypothesis that the elm decline was related to pig husbandry practices (Parker et al. 2002). However, the HDCA/DCA and HDCA/LCA ratios were lower than in
Holzmaar. The lower HDCA concentrations and ratios compared to the Holzmaar indicated that the input of pig faeces was low. A low input of pig faeces and the low concentrations of stanols in relation to HDCA in pig faeces (Fig. 1) may explain why an input of pig faeces was not clearly shown by the stanol ratio (coprostanol + epi-coprostanol)/cholestanol. The bile acid concentrations in combination with concentrations of coprostanol and the stanol ratio (5β-stigmastanol + epi-5β-stigmastanol)/(coprostanol + epi-coprostanol) showed input of pig faeces after the elm decline. The faecal biomarker data did not show that the human population also increased after the elm decline and therefore more intensive cultivation practices could be the reason for the higher input of livestock as discussed for Holzmaar. However, overall, in the sections of the cores studied in this investigation the data indicate strong correlations between the intensity of prehistoric livestock production and the decline of (specific) forest taxa, a trend found generally across Europe prior to industrialization (Kaplan et al. 2009; Kuneš et al. 2015; Marquer et al. 2017).

Conclusions

Statistically significant differences in the n-alkane and faecal biomarker data were in agreement with known land-use changes in the upper core sections of Holzmaar and the faecal biomarker patterns show statistical differences before and after the elm decline in Ulmener Maar. The data reported here are important evidence that 5β-stanols and bile acids are suitable for land-use reconstructions in central European lakes with small catchment areas from the Neolithic onwards. The data from these core sections additionally showed that a combined analysis of stanols and bile acids is necessary for the identification of specific taxa of livestock from faeces. The stanol data of the Mesolithic core section of Holzmaar raise questions about the applicability of 5β-stanols as faecal biomarkers in these lake sediments for older samples. However, reasonable data of bile acids in the Mesolithic core section underscored the importance of bile acids as faecal biomarkers.

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Supporting Information
Additional Supporting Information may be found in the online version of this article at http://www.boreas.dk.

Data S1. Detailed description of the analytical procedures.

Fig S1. Location of Holzmaar and Ulmener Maar and the surrounding topography (data from U.S. Department of the Interior U.S. Geological Survey. Shuttle Radar Topography Mission; WGS 84).

Table S1. Investigated compounds, suppliers, retention times (RT) and characteristic ion fragments (first ion fragment was used for quantification).

Table S2. Total organic carbon (TOC)\(^1\), ratio of TOC to total nitrogen (C/N)\(^1\) and short-chain n-alkane concentrations in Holzmaar (HM) and Ulmener Maar (UM). Concentrations are given based on the total weight of sediment (ng g\(^{-1}\)) and in relation to TOC (μg g\(^{-1}\) TOC). The abbreviations of the core sections are defined in Figs 2 and 3. * indicates significant differences (p < 0.05) between sections with amplified land use and the subjacent section below with less intense land use in Holzmaar and between the two core sections in Ulmener Maar.

Table S3. Concentrations of even-numbered long-chain n-alkanes in Holzmaar (HM) and Ulmener Maar (UM). Concentrations are given based on the total weight of sediment (ng g\(^{-1}\)) and in relation to TOC (μg g\(^{-1}\) TOC). The abbreviations of the core sections are defined in Figs 2 and 3. * indicates significant differences (p < 0.05) between sections with amplified land use and the subjacent section below with less intense land use in Holzmaar and between the two core sections in Ulmener Maar.

Table S4. Concentrations of odd-numbered long-chain n-alkanes, ratio (C27 + C29)/(C31 + C33) and odd-over-even predominance, OEP = (C27 + C29 + C31 + C33)/(C26 + C28 + C30 + C32) in Holzmaar (HM) and Ulmener Maar (UM). Concentrations are given based on the total weight of sediment (ng g\(^{-1}\)) and in relation to TOC (μg g\(^{-1}\) TOC). The abbreviations of the core sections are defined in Figs 2 and 3. * indicates significant differences (p < 0.05) between sections with amplified land use and the subjacent section below with less intense land use in Holzmaar and between the two core sections in Ulmener Maar.

Table S5. Δ\(^2\)-sterol and 5α-stanol concentrations in Holzmaar (HM) and Ulmener Maar (UM). Concentrations are given based on the total weight of sediment (ng g\(^{-1}\)) and in relation to TOC (μg g\(^{-1}\) TOC). The abbreviations of the core sections are defined in Figs 2 and 3. * indicates significant differences (p < 0.05) between sections with amplified land use and the subjacent section below with less intense land use in Holzmaar and between the two core sections in Ulmener Maar.

Table S6. 5β-stanol concentrations in Holzmaar (HM) and Ulmener Maar (UM). Concentrations are given
based on the total weight of sediment (ng g⁻¹) and in relation to TOC (μg g⁻¹_TOC). The abbreviations of the core sections are defined in Figs 2 and 3. * indicates significant differences (p < 0.05) between sections with amplified land use and the subjacent section below with less intense land use in Holzmaar and between the two core sections in Ulmener Maar.

*Table S7.* Stanol ratios in Holzmaar Maar (HM) and Ulmener Maar (UM). The abbreviations of the core sections are defined in Figs 2 and 3. * indicates significant differences (p < 0.05) between sections with amplified land use and the subjacent section below with less intense land use in Holzmaar and between the two core sections in Ulmener Maar.

*Table S8.* Bile acid concentrations in Holzmaar (HM) and Ulmener Maar (UM); LCA = lithocholic acid; DCA = deoxycholic acid; CDCA = chenodeoxycholic acid; HDCA = hyodeoxycholic acid. Concentrations are given based on the total weight of sediment (ng g⁻¹) and in relation to TOC (μg g⁻¹_TOC). The abbreviations of the core sections are defined in Figs 2 and 3. * indicates significant differences (p < 0.05) between sections with amplified land use and the subjacent section below with less intense land use in Holzmaar and between the two core sections in Ulmener Maar.

*Table S9.* Bile acid ratios in Holzmaar (HM) and Ulmener Maar (UM); LCA = lithocholic acid; DCA = deoxycholic acid; CDCA = chenodeoxycholic acid; HDCA = hyodeoxycholic acid. The abbreviations of the core sections are defined in Figs 2 and 3. * indicates significant differences (p < 0.05) between sections with amplified land use and the subjacent section below with less intense land use in Holzmaar and between the two core sections in Ulmener Maar.

*Table S10.* Correlations of Δ⁵-sterol and stanol concentrations in relation to TOC in the upper four core sections of Holzmaar (HM-LUa, HM-Fa, HM-LUb, HM-Fb). Numbers are Spearman’s rank correlation coefficients and * indicates significant correlations (p < 0.05).

*Table S11.* Correlations of bile acid concentrations in relation to TOC in the upper four core sections of Holzmaar (HM-LUa, HM-Fa, HM-LUb, HM-Fb); LCA = lithocholic acid; DCA = deoxycholic acid; CDCA = chenodeoxycholic acid; HDCA = hyodeoxycholic acid. Numbers are Spearman’s rank correlation coefficients and * indicates significant correlations (p < 0.05).