Reply to Prof. Dr. Eva Lehndorff (Referee #2)

by Marcel Lerch, Michael Zech & co-authors

Dear Marcel and Michael,

thanks for this interesting manuscript. It´s good to increase the number of high quality analyses in the field of faecal biomarkers. I have two major comments and a few minor ones.

→ Dear Eva, thank you very much for your encouraging words and your valuable suggestions how to further improve our manuscript. Please find our reply to your major and minor comments below.

Major comments:

The aim of your study was presented in the abstract of the manuscript as " study the importance of human and/or animals for occupation ... of this ... site". I suggest to simplify this aim to, e.g. " evaluate human and livestock biomarker signals at an archeological site". It doesn´t seem possible to evaluate an importance. Meaning of importance is not clear, if you maybe aimed at evaluating land use and settlements intensities, you should have other data sets to compare with. I hope that if such studies as yours continue we will come closer to such an evaluation.

→ We fully agree that "importance" is not the right terminology. We will change this sentence into "In order to study the human and/or animal faeces input at this relevant geoarchaeological site … ".

My second point is the selection of the sample set. You analyzed podsols of which the E horizon was found to be the former landscape surface by containing archeological artefacts. I wonder, why did you analyze the other soil horizons. Please provide a motivation for this. I cannot agree with your statement about water solubility and transport mechanisms of steroids in soils (line 81). Podsols have low pH (in your case <4). This enables transport of water insoluble organic substances via complexation with metals in soil. I suggest that you include this mechanism as a second aim in your study. You can then make perfect use of the depth resolved analyses and show whether complexation and leaching may affect the ratios of faecal markers or whether all the relevant marker substances are leached in the same way.

→ Readily, we emphasize our motivation a little bit more by adding after our research question (iii) Do the faecal biomarker patterns and ratios of the soil profiles on the Ullafelsen allow the reconstruction of the faeces input history during the holocene? *"We hypothesize that human faeces input is detectable in the E horizon representing the Mesolithic living floor (LL), whereas livestock faeces input dominates in the overlying OAh horizon."*

→ Many thanks for your very constructive feedback and suggestion. We will include in line 81 "Steroids have a low water solubility and are thus *usually* neither leached into deeper soil horizons (Bull et al., 2002; Prost et al., 2017) *nor detectable in soil leachates (Lloyd et al., 2012). It remains to be investigated whether this also holds true for very low soil pH values < 4 like in our study area, where organic substances can become mobile via complexation with metal ions."*

→ Following your suggestion, we will include this as research question at the end of the introduction section "*(iv) Is there any evidence for leaching of steroid biomarkers in soils with very low pH values such as in our study*

area?" During revision, we will address and answer this question more explicitly than hitherto in the discussion and the conclusion section.

Minor comments:

line 20 - 22: modern faeces analyses can be embedded more clearly. Maybe think of a hypothesis connecting to the transport and leaching problem, such as "inputs of modern livestock have been investigated for their potential to leach and mask ancient faecal marker signals". Better omit "... agreement to literature data ... " in the abstract.

→ Readily, we follow your recommendation and omit "... agreement to literature data ...". Instead, we will emphasize during the revision that low amounts of chenodeoxycholic acid are present in our modern cattle and sheep faeces samples and that this finding was not reported in literature previously.

line 29: a conclusion on plant-derived steroids and root inputs doesn´t seem to be relevant and may be excluded from the abstract to focus on the central hypotheses.

→ Thanks a lot, we will consider your recommendation during revision.

line 30 to 32: Shift Anthrosols and Amazonian Dark Earths to beginning of sentence. I like this comparison and it is more clear then how you evaluated the "strength of the faecal input" at your site. Still, it later has to be mentioned that you compare completely different soils from completely different climates.

→ Will be rewritten during revision.

line 37: delete "accordingly"

→ Deleted.

line 64-65: I cannot follow the reference to anthropozoology Zech et al 2021. There seems to be no evidence for grazing.

→ Admittedly, the evidence is not a direct one, but rather an evidence line discussed/presented in Zech, Lerch et al. 2021: (i) bulk and *n*-alkane radiocarbon dating as well as *n*-alkane contents suggest that a major vegetation change (from conifer dominance to grasses/herbs/shrubs) dating to the Mid Holocene is documented in the Ullafelsen soil profiles. (ii) In combination with the knowledge about the start of alpine pasturing during the Neolithic and intensification particular during the Bronze Age (including anthropozoological lowering of the upper treeline), this allows us to be confident about our statement concerning "anthropozoological impact on the Ullafelsen since the Bronze Age".

line 79: delete "which have also been detected in".

→ Deleted.

lines 101 ff.: re-consider your aims, include a reason for the analyses and report of all soil horizons. For archeological purposes the E horizon should be sufficient. Add a motivation or hypothesis for modern faeces sampling. Otherwise this part of sampling remains unexplained in lines 132 and finds its first explanation only in line 161.

→ We agree and will partly rewrite the aims during revision (see our replies above).

line 149 ff.: better use past tense "ranged" etc. for presentation of analytical results.

→ Done.

line 259: change to e.g. "biomarker patterns for modern livestock".

→ Changed.

line 295: change to e.g. "ancient faecal markers in soil".

→ Changed.

line 385: your conclusion for the use of some parts of the site as "toilet". This is rather speculative without discussion in a literature context. I suggest to add a brief chapter 3.4 "faecal marker intensities in context of previous archeological findings" (or include it in 3.3). You can try for a first and very careful comparison of faecal markers in archeological soil relicts and have a word on the comparability of signals. I think that this will be highly difficult for amounts and even faecal marker ratios may have much larger variability then we hope due to difference in climate, degradation of organic matter, input of different kinds of organic matter (plants, microbes, animals...).

→ Thanks a lot, we will consider your suggestion during revision.

References:

Bull, I. D., Lockheart, M. J., Elhmmali, M. M., Roberts, D. J. and Evershed, R. P.: The origin of faeces by means of biomarker detection, Environmental International, 27 (8), 647–654, https://doi.org/10.1016/S0160-4120(01)00124-6, 2002.

Lloyd, C. E. M., Michaelides, K., Chadwick, D. R., Dungait, J. A. J. and Evershed, R. P.: Tracing the flow-driven vertical transport of livestock-derived organic matter through soil using biomarkers, Organic Geochemistry, 43, 56–66, https://doi.org/10.1016/j.orggeochem.2011.11.001, 2012.

Prost, K., Birk, J. J., Lehndorff, E., Gerlach, R. and Amelung, W.: Steroid Biomarkers Revisited - Improved Source Identification of Faecal Remains in Archaeological Soil Material, PLoS ONE, 12 (1), 1–30, https://doi.org/10.1371/journal.pone.0164882, 2017.