

Reply to Referee #1

by Marcel Lerch, Michael Zech & co-authors

Many thanks for considering me as reviewer of the manuscript entitled 'Human and livestock faecal biomarkers at the prehistorical encampment site of Ullafelsen in the Fotsch Valley, Stubai Alps, Austria – potential and limitations' by Lerch et al. The study is very interesting and addresses the analyses of faecal steroids in 37 soil samples and 2 faecal samples for the identification of the presence of cattle and sheep along humans at the Ullafelsen archaeological site. The Ullafelsen is an important (geo-)archaeological reference site and the analyses of faecal biomarkers gives further insights into human and/or animal occupation. The manuscript generally fits the scope of Biogeosciences, although I have major concerns about the methods, the results and the conclusions drawn from the analyses that need to be addressed by the authors before the manuscript could be considered for publication.

→ We are very grateful to anonymous Referee #1 for her/his encouraging words and the very constructive review helping us to further improve our manuscript. We replied to each individual comment below.

General comments:

My major concern is that there are apparently no analyses presented of nearby reference soil samples to compare the steroid analyses of the archaeological soil samples to the natural background. Without comparison, there is no possibility to actually identify the presence of cattle & sheep/humans at the Ullafelsen. Bile acids (especially deoxycholic acid) as well as 5 β -stigmastanol and coprostanol could be omnipresent in soil samples as wild animals contribute to low abundances of those faecal steroids in topsoils. Without reference samples you neither can characterise the changes in steroid patterns between archaeological samples and reference samples nor you can identify the enrichment of faecal steroids in the archaeological samples. Thus, how can you be sure, that the faecal steroids you find in your 37 soil samples are not just the natural background?

→ We agree with Referee #1 that additional faecal biomarker results for soil samples from reference soils will increase the impact of our manuscript. We therefore readily expanded our data set with samples of non-archaeological soil profiles from the Fotsch Valley and included those data during revision.

Furthermore, I don't understand the logic behind sampling two modern faecal samples of sheep and cattle. The data is compared to other studies, but the connection between the data of the faecal samples and the soil samples is not really discussed by the authors. The data from the analyses does neither give new insights into steroid patterns of herbivore faeces nor does it give any novel support of the steroid analyses of the soil samples. This issue must be either explained by the authors, why it was necessary to conduct analyses on modern faecal samples (How where the animals fed?), or it must be removed.

→ If you agree, we prefer not to remove the modern faeces part from our manuscript. First, it helps in our opinion introducing our readership into steroid analyses and results. Second, our results show the presence of low amounts of chenodeoxycholic acid in modern cattle and sheep faeces. Prost et al. (2017) did not report on this previously. We will readily emphasize this more explicitly in our manuscript during revision.

Furthermore, I don't think it is necessary to present the data of laboratory replicate analyses of the faecal samples. The results are just a quality check that the samples are homogenous and that the laboratory method is working correctly. It is much more advisable to present data of field replicates (several sheep/cattle individuals) to present the natural variability of steroid patterns between animals.

→ We agree with Referee #1 that field replicates are desirable. Nevertheless, laboratory replicate analyses show that the method of the faecal biomarker analyses works correctly and our results are robust. We therefore preferred not to remove these results from the supplementary material.

The other major concern is, that you proposed (when I understood correctly), that there were humans, cattle and sheep present at the Ullafelsen. Consequently, I would expect that there is a mixed signal of herbivore steroids (5 β -stigmastanol, deoxycholic acid) and human steroids (coprostanol, lithocholic acid), but your analyses show the presence of herbivore steroids. Is this a sign, that your steroid analyses presented in your study is the natural background of the region? I think, that this is very important for your study and it requires a discussion (along with the absence of reference samples) instead of one sentence in the conclusion ('Based on our faecal biomarker results, we cannot confirm the Mesolithic settlement of hunter-gatherers at the Ullafelsen').

→ Please allow us to raise the counter question "What is a natural background?" Undoubtedly, humans strongly influenced the Ullafelsen and its surroundings since the Mesolithic period. We therefore discuss/conclude from the absence of steroid contents and patterns indicating human faeces input that the Ullafelsen was at least not used as "toilet" (l. 382ff). With regard to the herbivore steroid contents and patterns, we readily included in the discussion part of our revised manuscript a comparison with the reference site results.

I also wondered, if you could provide the limit of detection/quantification of your steroids, because your concentrations are quite low.

→ Limit of detection was defined as signal-to-noise ratio of 3:1 and varied for the analytes between 0.6 ng g⁻¹ for all Δ^5 -sterols, stanols and stanones and 4 ng g⁻¹ soil for all bile acids. We included this information in the revised manuscript.

Could you also please provide an explanation, why you detect chenodeoxycholic acid in your 37 soil samples? According to Prost et al. 2017, there is no chenodeoxycholic acid in cattle and sheep faeces, but it is present in goat (also wild?) and human faeces. Furthermore, the presence of chenodeoxycholic acid contradicts your statement, that there is a strong indication that there were cattle and sheep faeces present? This should be discussed in your manuscript.

→ A very good point. During revision, we readily emphasized more explicitly that in contrast to Prost et al. (2017) our two modern faeces samples of cattle and sheep contain also low amounts of chenodeoxycholic acid. Furthermore, the results of our modern cattle and sheep faeces show also low contents of coprostanol and lithocholic acid, which are human-derived faecal biomarkers. However, given the low contents of these biomarkers combined with our evaluation of biomarker ratios (Fig. 7) we see no evidence for the input of human faeces in our soil samples.

Specific comments:

Abstract

Line 18-19: Instead of 'for contributing to a better understanding of pedogenesis and landscape evolution' highlight the main outcomes of your recent study.

→ Given the word limitations in the abstract, we prefer to maintain this sentence as it is. Further details are provided in the Introduction (ll. 58ff) and in the material and methods section (ll. 155ff).

Line 20: Why is it so important to look at faecal biomarkers at this specific site? Is it because it was unknown, if hunter-gathered also held animals during the Mesolithic period? Why is it important to identify animal biomarkers at this specific archaeological site? Please highlight this in your abstract.

→ We agree that “importance” is not the right terminology. We rewrote to “In order to study the human and/or livestock faeces input at this relevant geoarchaeological site ...”.

Line 22: 'dominance of 5β-stigmastanol and deoxycholic acid for ruminants' is quite a clear statement. I would suggest to write something comparable like 'dominance of 5β-stigmastanol and deoxycholic acid point to/suggest the input of ruminant faeces.

→ Changed

Introduction

Line 44: It is unclear to me, what 'human environment interaction' exactly means in this context?

→ Well, this is explained here in this introduction: Mesolithic hunter-gatherers used the Ullafelsen and its surrounding as summerly camp for hunting. The soils on the Ullafelsen are strongly impacted by this Mesolithic impact and latest since the Bronze Age alpine pastoralism changed the vegetation of the study area dramatically.

Line 61-65: this is an important information for the relevance of your study and should be highlighted in your abstract because it summarizes the reason, why you applied faecal biomarker analyses in your study.

→ Many thanks, we readily followed your suggestion and highlighted this relevant information in the abstract.

Line 68: This is not entirely correct, because bile acids are steroids. Please change this information to steroids (e.g. sterols, stanols, stanones and bile acids) or something similar.

→ Thank you very much, we readily followed your suggestion and changed the terminology of steroids.

Line 71: because you defined steroids previously, just use the term 'steroids' instead of 'steroid and bile acid.

→ Changed

Line 71: I would not write 'organisms' but would specify this information to 'animals.

→ Changed

Line 72-73: 'as well as their residues in soils and sediments' this part of the sentence does not make sense to me. Please rephrase.

→ Rephrased

Line 73: the correct surname is 'von der Lühe'. Please change all the citations accordingly and the correct order in the reference list.

→ Changed

Line 74: it is not only important to look at the plant $\Delta 5$ -sterols but it is much more important to look at reference samples that were not altered by human perturbations.

→ We carried out further analyses of reference soils from the Fotsch Valley and present our expanded findings in the results and discussion section.

Line 75: as already mentioned, please change this, because bile acids are also steroids.

→ Changed

Line 76: You forgot to explain the importance/formation of stanones.

→ Added

Line 81: I am not sure, if Prost et al. 2017 is the correct citation here. A much more important reference here is 'Lloyd et al. (2012) Tracing the flow-driven vertical transport of livestock-derived organic matter through soil using biomarkers'. Please add this reference and remove Prost et al. 2017. It might be also useful for the interpretation of your data.

→ Many thanks, we readily followed your suggestion. We added Lloyd et al. (2012) and removed Prost et al. (2017) at this position in the manuscript.

Line 76-87: this part is not well written and requires restructuring. In line 76 you introduce the 5β -stanol coprostanol and 5β -stigmastanol. In line 83 you explain how 5β -stanols are produced. Please restructure to the definition of your specific compounds and explain directly how they are formed and which $\Delta 5$ -sterol is the precursor molecule. In the end you can explain, that once 5β -stanols are introduced into the soil, they could be further transformed to epi- 5β -stanols.

→ Rephrased

Line 81: this sentence does not allow 'potential leaching of steroids', because steroids are known to be associated to organic matter. So I would recommend to add an uncertainty to this sentence such as 'it has been shown that they were not leached into deeper soil horizons (Lloyd et al. 2012).

→ Many thanks, we readily followed your suggestion and rephrased the sentence.

Line 85: please change to 'microbially transformed'. It is much more logic to start with 'bile acids are formed from cholesterol in the liver -> via the bile into the gut of mammals as primary bile acids -> could be further mediated to secondary bile acids by microorganisms. Please add some examples of secondary bile acids in brackets.

→ Changed

Line 101-107: The relevance is not clearly stated in the study. It is interesting to look at the faecal biomarkers at your archaeological site. What I still miss is the clear message that you assumed that hunter-gatherers at the Ullafelsen had animals with them.

→ Actually, it was never our assumption that hunter-gatherers at the Ullafelsen had animals with them. To make our motivation and hypotheses more clear, we added to our research question (iii) the sentence: “*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 overlaying OAh horizon.*”

Material and Methods

Line 113: Is it common to write plant names with capital letter? Otherwise, please change.

→ Changed

Line 132: ‘faeces samples from cattle and sheep’: this is not enough information. Are those samples from close-by herds? Natural samples? How were they collected and stored? How were the animals fed, because food does have an impact on the steroid pattern of faeces? This is important information for your interpretation. It is also known that steroid patterns could differ between individuals, so why did you sampled just one sample from one individual?

→ We readily added additional information about the two faeces samples during revision. We also agree that further field replicates would be desirable. However, given that the focus of our study is on the archaeological soils on the Ullafelsen, we currently do not plan field replicates for modern faeces samples.

Line 133: Do the soil samples from the trench serve as reference samples because they were not influenced by humans? Since the abstract, I was wondering how you are planning to compare your samples from the archaeological site and a reference sample would be very helpful for the interpretation of the data.

→ The soil samples from the close by trench profiles are also archaeological soil profiles on the Ullafelsen and cannot be used as reference samples. We added data of samples from reference soil profiles from the Fotsch Valley to our study.

Line 167: please change <2 mm to ≤ 2 mm because you also include particles with a size of 2 mm.

→ Changed

Line 167-168: I am confused, because in the section before, you mentioned that you already conducted TC and TN analyses which was published in Zech et al. (2021). How do these analyses differ from the analyses you mention here?

→ TC and TN values for the investigated soil samples were already published in Zech et al. (2021). We used these data also for our faecal biomarker analyses and included it in the material and methods section.

Line 172-173: Please remove this sentence ‘Analyses took place...’

→ Removed

Line 174-179: You weighed the samples before you applied the Soxhlet extraction. Please change the order of the sentences here.

→ Changed

Line 179: what do you mean with 'neutral and acid lipids'. It was not explained before and the information must be included here. You can also say ' α -pregnanol' served as IS for sterols and stanols, isodeoxycholic acid served as IS for bile acids. What is the IS for the stanones?

→ Neutral lipids mean the fraction of sterols, stanols and stanones, while acid lipids mean the fraction of bile acids. We used no IS for the stanones. For data analysis and interpretation, we consider no stanones in our calculated ratios. 5 α -cholestane was used as IS 2. We added this information in the manuscript.

Line 180: Please include the information how you removed the solvent. I guess it was rotary evaporation?

→ Yes, we removed the solvent using rotary evaporator and rephrased this information.

Line 181: Here is the definition of neutral lipids. This is too late, when you already mention them in line 179.

→ Changed

Line 181: acidic lipids do also include fatty acids.

→ Added

Line 186: I would say 'acidic fraction was methylated' instead of 'bile acids', because the fatty acids are included.

→ Rephrased

Line 189: I think, 'Merck' is enough information and you can remove 'Sigma-Aldrich and 10187' (Is this the ordering number?)

→ Removed

Line 190: you eluted the fatty acid methyl esters to waste, right?

→ Yes, this is correct.

Line 194: It is '5 α -cholestane'. Please change.

→ Changed

Line 194-195: You added the second internal standard to the reactivials? Or did you remove the derivatization agent with nitrogen and transferred the sample to GC-MS vials before you add ISII?

→ For the sterol, stanol and stanone fraction, we added IS2 after derivatization and drying under nitrogen. We transferred the sample solved in toluene together with IS2 into GC-vials.

→ For the bile acid fraction, we added IS2 into reactivials after derivatization and transferred it together with the derivatization agent into GC-vials.

Line 201: Merck' is enough information and you can remove 'Sigma-Aldrich and 10187'

→ Removed

Line 203: It is '5 α -cholestane'. Please change.

→ Changed

Line 206: what is the concentration of the stock solution of the external standard?

→ The stock solution concentration of the external standards is ~10 $\mu\text{g ml}^{-1}$.

Line 207-208: This step does not make sense to me. Why did you add the first internal standard to the external standards in the same concentration? To be able to calculate the recovery of the first internal standard in your samples you need the calibration line of the first internal standard. It is also not clear for what you used the second internal standard?

→ The external standard stock solution in our lab does not contain the recovery standard (IS1). To determine the recovery, we also need a calibration curve for the IS1 as you also pointed out. Therefore, we mixed the same volumes of IS1 solution (10 ng μL^{-1}) and the External Standard Mixture (10 ng μL^{-1} of each target substance) for each level of the calibration. IS2 was used to correct for injection variations (see explanation below).

→ We agree that this paragraph (line 205–209) was confusing and contained unnecessary information. Therefore, we changed it to:

“For external calibration, six concentrations from a stock solution (0.01 $\mu\text{g } \mu\text{L}^{-1}$ in hexane) containing all target sterols, stanols, and stanones as well as the recovery standard (IS1) were prepared ranging from 50 to 5000 ng per vial. After derivatization 500 ng 5 α -cholestane (dissolved in dry toluene, 50 μL , 0.01 $\mu\text{g } \mu\text{L}^{-1}$) were added as IS2. Peak areas of the analytes were divided by the peak area of IS2 and calibration curves for each substance were calculated using these ratios. Calibration curves for the bile acids were prepared accordingly. All calibration curves had coefficient of determination (R^2) > 0.98. Limit of detection was defined as signal-to-noise ratio of 3:1 and varied for the analytes between 0.6 ng g^{-1} and 4 ng g^{-1} soil. At last, recoveries of the first internal standard (IS1) were calculated for all samples. Recovery of IS1 of all soil samples ranged between 51-116 % for sterols, stanols and stanones as well as 50-122 % for bile acids. All results for the samples were corrected for the losses during extraction and purification with those individually calculated recoveries.”

Line 214: Please change to ‘injection in splitless mode’

→ Changed

Line 218-257: this section is part of the Introduction and a little bit misplaced here. There are also no references to the equations (Eqn. 1-5) in the text, instead you use ‘ratio 1-5’.

→ Many thanks, we readily followed your suggestion and added references to the equations. The unpublished equations 3 and 4 were calculated by own for a further interpretation of our results.

Line 246: ‘This transformation is induced by soil composting’ is not a general way how epi-5 β -stanols are formed. Composting requires specific aerobic conditions, while epi-5 β -stanols are preferably produced under anaerobic conditions. Please remove this statement.

→ Removed

Results and Discussion

Biomarker patterns of faeces from predominating animals

This whole paragraph was not really a discussion of the results and what kind of message you get from your calculated ratios. It was not clear to me, how the analyses of the faeces samples can be linked to the analyses of the soil samples and if there is any newly developed message you can draw from the ratios.

→ We compare our modern faeces results with data of Prost et al. (2017). Our results show similarities as well as differences in steroid patterns and ratios to Prost et al. (2017). We detected in our modern faeces samples a low content of chenodeoxycholic acid, while Prost et al. (2017) did not detect this bile acid compound in their samples of cattle and sheep faeces. Based on the steroid patterns of modern ruminant faeces from the Ullafelsen, we can evaluate the faecal biomarker results of our soil samples. Steroid ratios of modern faeces help us to identify the sources of dominant faeces input in soils.

Line 260-261: Again, bile acids are also steroids. You must either find a short term for sterols, stanols and stanones or you always name those three compounds.

→ Many thanks, we readily followed your suggestion. If we mean sterols, stanols and stanones, we named all three compounds in the manuscript.

Line 263: I am not sure what you mean with 'robustness of our results' by repeating the analyses. It is more a quality check, if your method is working and if your faecal samples are homogenous.

→ "Robustness of our results" describes a quality check of the faecal biomarker analyses. We demonstrate our readership that the applied method works correctly and our results are reproducible. We replaced "robustness" by "reproducibility".

Line 266: It depends also on how you treated your faeces samples after sampling.

→ Information was added in the material and methods section.

Line 274: How do you interpret the results of your samples of ratio 1?

→ Results of ratio 1 of our modern faeces show typical ratios for cattle and sheep faeces, which are also reported by Prost et al. (2017). We added this interpretation in the manuscript.

Line 273-278: This whole paragraph is difficult to read. Please rephrase. Also, it is not clear to me what is your interpretation of your calculated ratios? What kind of conclusion do you draw from your results of the calculated ratios?

→ We compare the ratios of our modern faeces results with data of Prost et al. (2017). In order to determine the origin of faeces input in soils, we calculated different ratios of modern ruminant faeces to get a reference ratio for our analysed soil samples. We readily followed to your suggestion and rephrased this paragraph.

Line 288: In my opinion, three measurements of the same sample is not a quality control of the natural fluctuations of steroids in faeces, it is rather a control if the method and analyses worked well. In this case, I think it is not necessary do discuss it. You can discuss natural variations when you took field replicates of several sheep/cattle individuals.

→ Thanks a lot, we considered your suggestion during revision and rephrased this paragraph.

Faecal biomarker contents and patterns in soils

Line 305: You 'assume' that the 'small contents of steroids in the subsoil horizons at the Ullafelsen are mainly caused by the influence of β -sitosterol due to the strong rooting in the soil matrix'. Please change.

→ Changed

Line 338: You can only indicate the strong input of faeces from cattle and sheep when you compare your results to reference samples. Furthermore, you also found chenodeoxycholic acid in your samples, which is also an indication of the input of other herbivore faeces than cattle and sheep (see Prost et al. 2017). How do you explain the presence of chenodeoxycholic acid?

→ Data of reference soil profiles were added during revision. In contrast to Prost et al. (2017), our faecal biomarker results of modern cattle and sheep faeces show also a low content of chenodeoxycholic acid. That's how we explain the presence of this bile acid compound in our soils. We assume that the input of chenodeoxycholic acid by other wild animals or herbivore species at the Ullafelsen is negligible. We emphasized this information better during revision.

Conclusion

Please remove citations in the conclusion section, because it is not common. Furthermore, the conclusion contains parts of the discussion which should be moved to the discussion section.

→ We removed citations in the conclusion section. In agreement with the co-authors, we decided to keep the conclusion chapter like it is if you agree. In this part of the manuscript we emphasize the main results and "take home" messages of our study, whereas we intend to discuss our results in the results and discussion section short and concise as possible.

Technical corrections

Please check if your references are in a correct order e.g. in line 53 it is 'Geitner et al., 2011; Schäfer, 2011a' and in line 56 it is the other way round.

→ Changed

Line 67: How do you order your citations here? After years? Please check your entire manuscript for the order of references and change accordingly (e.g. after years).

→ We changed the order of the citations after years.

Text is much easier to read when you include the common abbreviations of bile acids: deoxycholic acid = DCA, lithocholic acid = LCA, ...

→ Changed

All concentrations should be given in 'µg g⁻¹' instead of 'µg/g'.

→ Changed

Figures

Could you please provide graphs in higher quality? The axes are not readable and the lines of the bars are not consistent in thickness and color

→ Our figures and graphs were changed in a higher quality.

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 changed this sentence into "In order to study the human and/or livestock 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 emphasized 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 included 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 included 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 addressed and answered 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 followed your recommendation and omit "... agreement to literature data ...". Instead, we emphasized 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 for your recommendation. Given the high contents of plant-derived steroids in our soils, we preferred to keep this important finding in the abstract if you agree.

line 30 to 32: Shift Anthrosols and Amazonian Dark Earths to beginning of sentence. I like this comparison and it is more clear than 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.

→ We rewrote 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 partly rephrased 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 than 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 considered your suggestion during revision. If you agree, we rewrote these phrases as following: "We assume that sites close-by the archaeological excavation area were used as "toilet" from the Mesolithic hunter-gatherers. Future faecal biomarker analyses on further soil profiles at the Ullafelsen aim at more specific insight in this hypothesis."

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](https://doi.org/10.1016/S0160-4120(01)00124-6), 2002.
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