

Neolithic pastoralism and plant community interactions at high altitudes of the Pyrenees, Southern Europe

Corresponding Author: Ms Irene Julián-Posada

This file contains all editorial decision letters in order by version, followed by all author rebuttals in order by version.

Attachments originally included by the reviewers as part of their assessment can be found at the end of this file.

Version 0:

Decision Letter:

**** Please ensure you delete the link to your author home page in this e-mail if you wish to forward it to your coauthors ****

Dear Ms Julián-Posada,

Your manuscript titled "Sedimentary ancient DNA reveals Neolithic pastoralism and plant community interactions at Southern European high altitudes" has now been seen by 3 reviewers, and we include their comments at the end of this message. They find your work of interest, but some important points are raised. We are interested in the possibility of publishing your study in Communications Earth & Environment, but would like to consider your responses to these concerns and assess a revised manuscript before we make a final decision on publication.

We therefore invite you to revise and resubmit your manuscript, along with a point-by-point response that takes into account the points raised. Please highlight all changes in the manuscript text file.

In particular, please ensure that the revised manuscript meets the following editorial thresholds:

**** Clarify the relationship between the sedimentological and chronological data.**

**** Provide additional archaeological context about the studied area.**

Please submit your point-by-point responses as a separate file, distinct from your cover letter where you can add responses to the Editors' comments that you do not want to be made available to the reviewers. Word files are preferred. We recommend that any figures, tables or graphs that are included in the response to reviewers are also included in the main article or Supplementary Information.

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Please use the following link to submit your revised manuscript, point-by-point response to the referees' comments (which should be in a separate document to any cover letter), a tracked-changes version of the manuscript (as a PDF file) and the completed checklist:

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We hope to receive your revised paper within six weeks; please let us know if you aren't able to submit it within this time so

that we can discuss how best to proceed. If we don't hear from you, and the revision process takes significantly longer, we may close your file. In this event, we will still be happy to reconsider your paper at a later date, as long as nothing similar has been accepted for publication at Communications Earth & Environment or published elsewhere in the meantime.

Please do not hesitate to contact us if you have any questions or would like to discuss these revisions further. We look forward to seeing the revised manuscript and thank you for the opportunity to review your work.

Best regards,

Carolina Ortiz Guerrero, Ph.D.
Associate Editor
Communications Earth & Environment

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REVIEWER COMMENTS:

Reviewer #1 (Remarks to the Author):

The article by Julián-Posada et al. highlights the complex interactions of pastoralism, climate and associated landscape interactions during the Neolithic in the Mediterranean mountains. The comprehensive study of sedimentary ancient DNA from a lake record from the central Pyrenees spanning the Holocene revealed the earliest continuous presence of domesticated sheep and cattle in the mountains of southern Europe, confirming Neolithic pastoralist use of mountain ecosystems.

The study provides novel insights into Neolithic pastoralism in European mountain ecosystems, revealing the earliest continuous presence of domesticates.

Therefore, the paper will be of great interest to the field, providing novel insights of a currently understudied area, addressing the interaction between human landscape management and past climate changes in the Pyrenees.

The paper provides important information about the environmental transformations during the Holocene and the influence human presence had on high altitude areas. A subject which is currently underrepresented in the field. Therefore, the paper will influence the thinking in the field and hopefully inspire future studies on this subject.

The scientific claims are based on a robust and well-established methodology and the results are presented in a convincing and understandable way. Furthermore, the authors make appropriate use of previous literature in the discussion of their findings.

The manuscript is well written with a clear structure and informative figures. I highly appreciate the authors using colour schemes suitable for colour blindness.

The authors of this study have done themselves justice without overselling their claims. Their research is based on a robust methodology and they have presented a thorough discussion of their study, by using previous literature appropriately. The statistical analysis is sound and all scripts are provided by the authors.

I would really like to compliment the authors on their incredibly detailed and well-structured methodological description and provided supplementary information. Unfortunately, it is really rare to review a submitted manuscript with already such a wealth of information. The filtering criteria applied to the datasets and the provided scripts should be detailed enough to be reproducible. This manuscript and the provided scripts are a stellar example for good scientific conduct, open access and reproducibility.

Here are some very minor comments that I hope you might find useful to improve your manuscript further:

l. 86: The authors mention core TRAM21. However, in the results, method and supplementary sections they detail that four sediment cores were acquired in 2020 and 2021 of which only two have been used for the age-depth model reported in this paper and only one of the two has been used for the sedaDNA analysis. To avoid misunderstandings, please clarify this very briefly in the objectives or use the appropriate core ID. Otherwise, I suggest removing the ID in line 86.

Please explain why the dating was done on two cores instead on just the genetically analysed core. Wouldn't it have been more efficient to just use one core for the complete analysis? Also why were the other two cores excluded?

l. 92: Similarly to the point made above... how is it important to mention the overall timespan of the archive if you then never refer to it again. I can only assume that the authors are planning to publish follow up studies focussing on the remaining time period, why not mention that in the conclusions as an outlook? Otherwise, I would recommend only presenting the data relevant to the here described study.

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l. 310: please correct ca to ca.

I would like to ask the authors to provide a brief discussion regarding their excluded sample TRAM_1b_3U_136.137. Why do you think the sample is such an outlier? Is this due to a methodological error, low DNA input or possible due to environmental factors influencing the DNA preservation? Depending on the reasoning and explanations of the authors this additional part of the discussion could easily also be included in the supplementary part to avoid lengthening of the main manuscript.

I highly recommend this manuscript for publication after minor revision.

Reviewer #2 (See attached document):

Reviewer #3 (Remarks to the Author):

The paper by Irene Julián-Posada et al. is well-written, clear, and, in my opinion, suitable for publication in Communications Earth & Environment. It provides the oldest continuous record of domestic animal presence at a mountain site in Europe. The high quality of the data, combined with a robust age model, reinforces confidence in the temporal continuity of the record.

This study is particularly significant because it presents the oldest sedimentary sequence from which DNA has been extracted, revealing both domestic animals and the plants that once grew around the site. The remarkable preservation of sedimentary DNA in this lake allows for an extensive and diverse reconstruction of the surrounding flora and fauna.

This paper will undoubtedly attract interest within the field, as it strengthens existing archaeological studies in the region, even though it does not challenge any major existing theories. The results are convincing, and the authors carefully avoid overextending their interpretations beyond the lake's immediate catchment area. Given the nature of pastoralism, which often concentrates around water sources, the findings are reasonably representative of the broader region. In fact, they are no less representative than the evidence typically gleaned from bones found in caves.

While the study has many strengths, a few limitations should be noted. The absence of the uppermost sedimentary layers makes it harder to fully understand the spatial representation of the lake's DNA, as more recent sediments often provide insight into the taphonomy of deposits. This would help to further assess the spatial representativeness of the DNA record. Additionally, it is regrettable that no further sedimentological analyses were conducted, particularly to verify whether changes in mineralogical composition could explain the marked diversity shift observed around 6000 years BP. The lithology hints at such a change, but XRF analyses could have clarified this.

Finally, Figure 1 could be enhanced by including clear demarcations of the lake's catchment area, which would improve the overall clarity of the data presentation.

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Version 1:

Decision Letter:

<*** REMEMBER TO ATTACH REVISIONS CHECKLIST (WORD)***>

** Please ensure you delete the link to your author home page in this e-mail if you wish to forward it to your coauthors **

Dear Ms Julián-Posada,

Your manuscript titled "Sedimentary ancient DNA reveals Neolithic pastoralism and plant community interactions at Southern European high altitudes" has now been seen by our reviewers, whose comments appear below. In light of their advice we are delighted to say that we are happy, in principle, to publish a suitably revised version in Communications Earth & Environment.

We therefore invite you to revise your paper one last time to address the remaining concerns of our reviewer #2. At the same time we ask that you edit your manuscript to comply with our format requirements and to maximise the accessibility and therefore the impact of your work.

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Please review our specific editorial comments and requests regarding your manuscript in the attached "Editorial Requests Table".

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We hope to hear from you within two weeks; please let us know if you need more time.

Best regards,

Carolina Ortiz Guerrero, Ph.D.
Associate Editor
Communications Earth & Environment

REVIEWERS' COMMENTS:

Reviewer #1 (Remarks to the Author):

I would like to thank the authors of the article "Sedimentary ancient DNA reveals Neolithic pastoralism and 1 plant community interactions at Southern European high altitudes" for addressing all remarks. I have no further suggestions and I highly recommend this manuscript for publication.

Reviewer #2 (Remarks to the Author):

I appreciate the clarifications and revisions you made that have enhanced my understanding of your work. I attached a separate document with few questions and some reflections following your responses to my comments. I hope they could help with this and your future research.

Reviewer #3 (Remarks to the Author):

I didn't have many comments; the corrections made by the authors are acceptable.

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Note: point-by-point responses to referees' comments are written in purple in this document, and all references to line numbers of modifications in the manuscript refer to the marked up file (**Julian-Posada et al_manuscript marked up_review.pdf**).

Reviewer #1 (Remarks to the Author)

The article by Julián-Posada et al. highlights the complex interactions of pastoralism, climate and associated landscape interactions during the Neolithic in the Mediterranean mountains. The comprehensive study of sedimentary ancient DNA from a lake record from the central Pyrenees spanning the Holocene revealed the earliest continuous presence of domesticated sheep and cattle in the mountains of southern Europe, confirming Neolithic pastoralist use of mountain ecosystems.

The study provides novel insights into Neolithic pastoralism in European mountain ecosystems, revealing the earliest continuous presence of domesticates. Therefore, the paper will be of great interest to the field, providing novel insights of a currently understudied area, addressing the interaction between human landscape management and past climate changes in the Pyrenees.

[...]

I would really like to compliment the authors on their incredibly detailed and well-structured methodological description and provided supplementary information. Unfortunately, it is really rare to review a submitted manuscript with already such a wealth of information. The filtering criteria applied to the datasets and the provided scripts should be detailed enough to be reproducible. This manuscript and the provided scripts are a stellar example for good scientific conduct, open access and reproducibility.

We are extremely grateful for comments provided by Referee 1, which have undoubtedly contributed to enhancing the clarity, scientific rigor, and scope of the current manuscript. We are particularly pleased that the referee has acknowledged our efforts to ensure the reproducibility and inclusivity of our research, particularly with respect to colour blindness.

Here are some very minor comments that I hope you might find useful to improve your manuscript further:

l. 86: The authors mention core TRAM21. However, in the results, method and supplementary sections they detail that four sediment cores were acquired in 2020 and 2021 of which only two have been used for the age-depth model reported in this paper and only one of the two has been used for the sedaDNA analysis. To avoid misunderstandings, please clarify this very briefly in the objectives or use the appropriate core ID. Otherwise, I suggest removing the ID in line 86.

Please explain why the dating was done on two cores instead on just the genetically analysed core. Wouldn't it have been more efficient to just use one core for the complete analysis? Also why were the other two cores excluded?

We collected four replicated cores in different years due to: 1) constraints arising from the pandemic; and 2) limitations related to fieldwork timing and weather. From these four cores, two of them (one collected in 2020 and other in 2021) were discarded as they lacked certain sediment intervals, making them discontinuous and unsuitable for both analyses and building a chronological model. Regarding the two selected cores, the one obtained in 2020 (TRAM20-1B) was exposed to suboptimal conditions for *sedaDNA* analyses, although it was sufficient for other proxies that are still under investigation. Consequently, we relied on the core collected in 2021 (TRAM21-1B) for these and additional analyses. A chronology was established for TRAM20-1B, as it was necessary for the ongoing analyses of other proxies, and we proceeded to date new samples from TRAM21-1B. We combine part of both dating datasets based on sedimentological criteria to enable correlation between both cores (eg. facies correlation) and thus combining ^{14}C dates from both cores in one sedimentary sequence (TRAM21-1B). By integrating dates from both cores, we were able to develop a coherent chronology for TRAM21-1B. This methodology has been described in **lines 360-362 and 372-374** of the manuscript, and thoroughly explained in **Supplementary Material 1 (text, Fig. 1 and Table 1)**. In addition, we would like to retain the TRAM21 notation for enhanced traceability of our research in the future. Last, we have improved **Figure 1 in Supp. Mat.** by highlighting the transferred dates in both cores and in **Table 1**, as well as expanding the given explanation.

I. 92: Similarly to the point made above... how is it important to mention the overall timespan of the archive if you then never refer to it again. I can only assume that the authors are planning to publish follow up studies focussing on the remaining time period, why not mention that in the conclusions as an outlook? Otherwise, I would recommend only presenting the data relevant to the here described study.

Similarly, presenting the integrity of the retrieved cores facilitates future reference to: 1) the appropriate sections within each core; and 2) emerging questions related to composite cores, while being fully transparent about the available sediment and the constraints encountered in conducting various analyses. Conversely, failing to disclose the information we currently possess would imply concealing pertinent data that could be valuable for future research by ourselves and our colleagues. We are uncertain about the feasibility of incorporating additional biological proxies in Section 5U, as the siliciclastic nature of the sediment hampers the preservation of organic matter. Consequently, we remain unsure about the future implications we may be able to derive from it. Nevertheless, we found this comment very useful and we have modified the text in **lines 93-95** in order to improve the explanation.

I. 277: However,

I. 310: please correct ca to ca.

Both corrections were included.

I would like to ask the authors to provide a brief discussion regarding their excluded sample

TRAM_1b_3U_136.137. Why do you think the sample is such an outlier? Is this due to a methodological error, low DNA input or possible due to environmental factors influencing the DNA preservation? Depending on the reasoning and explanations of the authors this additional part of the discussion could easily also be included in the supplementary part to avoid lengthening of the main manuscript.

In examining the content of this sample, we observed that it contains only a limited number of taxa, particularly aquatic ones. This suggests that its status as an outlier may primarily be attributable to restricted DNA input, particularly of terrestrial origin, which hinders a comprehensive interpretation of the basin-level observations. The lack of diverse DNA sequences may point to either methodological limitations or environmental factors that could be influencing the preservation of DNA.

I highly recommend this manuscript for publication after minor revision.

Many thanks again to reviewer 1 for all the comments, which have undoubtedly helped to improve the manuscript.

Reviewer #2

General overview

The manuscript titled “Sedimentary ancient DNA reveals Neolithic pastoralism and plant community interactions at Southern European high altitudes” addresses two important objectives: 1) determining the archaeological timing of the arrival of the first domesticated animals in the central Pyrenees, and 2) assessing the potential impact of these animals on plant communities in the subalpine zone of the Pyrenees.” [...]

I found this study highly interesting, as it tackles an issue of great relevance to the scientific community, particularly those studying Neolithization processes in high-altitude environments and their ecological consequences. The use of sedaDNA is noteworthy, as it represents a cutting-edge technique that, while still not widely applied in archaeological research, offers great potential.

Formal Aspects

The manuscript is generally well-organized, with the aims and research perspective clearly stated in the introduction. The results are clearly presented, and the text is well-structured, making it easy and engaging to read. The supplementary material is extensive and well-organized, which enhances the overall quality of the work.

We would like to express our sincere gratitude to the referee for their insightful and encouraging comments on our manuscript. We greatly appreciate the recognition of our study's relevance to the scientific community, particularly in relation to the Neolithization processes in high-altitude environments. The positive feedback on our use of sedaDNA and the overall organisation of the manuscript is invaluable, and we are grateful for the referee's acknowledgment of the extensive

supplementary material, which we believe enhances the quality of our work.

Please check these small formal inaccuracies:

- Line 99: "Polymerase Chain Reaction (PCR)". Please specify the abbreviation PCR. Not everyone is clear what PCR is.

- Line 132: bibliographic reference number 39 must be placed in superscript

Both corrections were included.

Content and Purpose

Despite its strengths, certain areas require clarification to enhance the overall clarity and impact of the paper:

- **Title:** The current title is somewhat misleading as it suggests a broader geographical focus, encompassing "Southern European high altitudes". However, the discussion is primarily centered on the Iberian Peninsula, specifically the central Pyrenees. The manuscript does not provide sufficient discussion at the Mediterranean or European level regarding the effects of early pastoralism on ecosystems. I recommend revising the title to more accurately reflect the geographical scope of the study. This adjustment would not diminish the value of the research but would more precisely align it with its regional focus, while still contributing to the broader discussion on early pastoralism's impact on ecosystems.

We appreciate this suggestion, although we believe that the broader geographical scope of the initial title may be valuable for future research. In our view, the findings can likely be extended to other high altitude areas in southern Europe, and the title's inclusivity may help reach additional researchers in related fields. However, we remain open to Referee 2's suggestion of adjusting the title if this change is crucial for publication. This would specify the Pyrenees as the focus of study: "Sedimentary ancient DNA reveals Neolithic pastoralism and plant community interactions at high altitudes of the Pyrenees, Southern Europe".

- **Bibliography:** Some of the references, particularly references 2 and 3 (line 42), do not seem to be the most relevant or comprehensive for discussing the long-term ecosystem effects of domesticated plants and animals. Additionally, since the article attempts to make comparisons with other Pyrenean and north Mediterranean mountain regions, the bibliography should be expanded to include more relevant sources. I will suggest some specific references at the end of this review to help strengthen the discussion, particularly regarding early pastoralism in the Pyrenees and north Mediterranean mountains.

We are grateful for this comment that has helped us to expand the context in several sections of the introduction and the discussion. Various references suggested by reviewer 2 (numbers 2, 3 and 4) have now been included in **lines 42, 44, 56, 216, 332**. Besides, we included references 39 and 40 in **lines 99 and 100**.

- **Supplementary Material 1 – Sedimentological and Chronological Correlation:** The chronology of the levels sampled in the TRAM21-1B core for DNA analysis is based on sedimentological correlation with the TRAM20-1B core. However, no direct radiocarbon dating was performed

on the TRAM21-1B core levels. Given that the novelty of the findings hinges on the chronological framework, it would have been preferable to directly date at least a couple of levels from TRAM21-1B. This would increase the robustness of the results.

We thank you for this comment, as it points us to realize that, maybe, in the previous version of the manuscript it was not clear enough the efforts we made to explain how the chronological model had been built, despite we explained it at the Supplementary Material section. You can now read an expanded version of it in the new **Supp. Mat. 1**. Just to clarify it in this answer, and as we detailed to referee 1, we collected two replicated cores in different years due to: 1) constraints arising from the pandemic; and 2) limitations related to time and weather. The core obtained in 2020 (TRAM20-1B) was exposed to suboptimal conditions for *sedaDNA* analyses, although it was sufficient for other proxies that are still under investigation. Consequently, we relied on the core collected in 2021 (TRAM21-1B) for *sedaDNA* and additional analyses. A chronology was established for TRAM20-1B, as it was necessary for the ongoing analyses of other proxies, and we proceeded to date 7 new samples from TRAM21-1B, combining these based on sedimentological criteria (facies correlation) to enable correlation between both cores. In other words, we created a coherent chronology based on 7 dates from TRAM21-1B and 6 dates from TRAM20-1B. This methodology is thoroughly explained in **Supplementary Material 1 (first paragraph, Figure 1 and Table 1)**.

- **TRAM21-1B - Superficial Levels:** *The decision not to analyze the superficial levels (2U and 1U) is not fully explained. The authors mention in line 354 that “Sections 1U and 2U exhibit sedimentary features that preclude their use in building a reasonable chronology,” but the specific nature of these sedimentary features is unclear. Analyzing superficial levels is crucial in ecological studies, as they provide a reference point close to the present day, allowing comparisons with more recent (and controllable) archaeological and environmental data. If these levels were not analyzed, the authors should provide a clearer explanation for this decision.*

Thank you for pointing this out, it has helped us better explain why we opted not to use the uppermost sections of the sediment for the chronological model or any analyses. We have now included a clarification on this aspect in **lines 365-371**. Sections 1U and 2U contain laminated sediments that represent high-energy depositional events that can disturb the regular deposition of lacustrine sediments, disrupting the chronological sequence and therefore leading to inaccuracies in age-depth models if included. While we acknowledge that uppermost samples may indeed contribute to addressing taphonomic questions, we believe that the origin and sources of *sedaDNA* are ideally investigated through dedicated monitoring programmes, which fall beyond the scope of our current research.

- **Sedimentation Dynamics:** *The sedimentation dynamics of the lake basin should be further clarified. The information presented in Figure 1 of the supplementary material is insufficient to fully contextualize the *sedaDNA* results. A better understanding of the sedimentation processes is essential for interpreting the provenance and scale of representation of the *sedaDNA*. For example, what is the size of the lake's catchment area?*

Thank you for raising this point. We have indeed selected the analyzed sedimentary interval for *sedaDNA* based on the stability of sedimentation dynamics throughout the studied time period.

Lacustrine sedimentation at Tramacastilla began around 25 ka BP, following the retreat of the glacier from the lake's position. The basal 1.5 m of recovered sediments consist of massive, greyish siliciclastic silts with cm-sized clasts, characteristic of high-energy detrital fluxes in a recently deglaciated, vegetation-free landscape prone to erosion (Unit 5). In contrast, Units 3 and 4 are dominated by massive to banded silty sediments with organic carbon concentrations exceeding 10%, indicating a stable depositional environment shaped by a mix of allochthonous material from runoff and autochthonous material, primarily organic matter produced within the lake. The upper layers of the sequence are laminated, corresponding to high-energy depositional events, where no sedaDNA analyses were performed. The catchment area of Tramacastilla Lake is relatively small, covering approximately 50 ha, and is shown in **Fig. 1d** of the manuscript to clarify the data. Importantly, both the catchment area and sedimentary processes remained stable during the deposition of the sediments analysed (Units 3 and 4), spanning from the onset of the Holocene to around 1500 BP. We have now included a note on the analysed units' choice criteria in **lines 364-365**.

In addition, tracing the origin of sedaDNA and how it is affected by mineralogic changes is indeed a fascinating question that remains unresolved, and ongoing research by sedaDNA specialists is aimed at addressing this challenge (see Sand et al., 2024, also quoted in the text, **line 99**). However, within the scope of our project, which does not involve monitoring programs, it is not feasible to precisely trace the origin of the sedaDNA we have analysed. Instead, we adopt the generally accepted view in the current literature that sedaDNA is most likely derived from the lake basin, which is typically quite localised (Edwards et al., 2020, **line 100** of the manuscript). Please refer to our explanation in the revised manuscript on **lines 97-100**.

- **Fire practices and grazing:** At line 332, the authors mention that “The synergic effect of climate and grazing likely contributed to the gradual clearing of woodlands and the emergence of open landscape and novel pasture communities.” It is well-documented that fire has historically played a significant role in opening woodlands and promoting the growth of herbaceous species in various parts of the Pyrenees. Did the authors consider the use of fire in their analysis? Were charcoal fragments identified in the cores? If not, this should be addressed in future research, but it could also be mentioned as a consideration in the present study.

We thank you for the meaningful comment. We have added in **lines 329-332** a future-perspectives section, where we include the study of fire regime due to its historically role in clearing dense forests.

- **Supplementary Material 9 – Archaeological Context:** The manuscript lacks specific archaeological data for the basin studied. Have any archaeological excavations or investigations been conducted in the area? If so, were any settlements or other human activities identified that could be correlated with the different chronological periods identified in the cores? Including such information would enrich the interpretation of the sedaDNA results and strengthen the overall conclusions.

Thank you for the relevant comment. There is no much archaeological information in the surroundings of Tramacastilla lake. The only known prehistoric remains are megalithic and lack any direct dating, although the phenomenon started 6000 years ago, during the Neolithic period. This information has now been added in the last paragraph of **Supplementary Material 9**.

[...]

We cannot finish this response to the comments of reviewer 2 without thanking again for all the suggestions and clarifications raised because they have been essential to improve our manuscript.

Reviewer #3 (Remarks to the Author)

The paper by Irene Julián-Posada et al. is well-written, clear, and, in my opinion, suitable for publication in Communications Earth & Environment. It provides the oldest continuous record of domestic animal presence at a mountain site in Europe. The high quality of the data, combined with a robust age model, reinforces confidence in the temporal continuity of the record.

This study is particularly significant because it presents the oldest sedimentary sequence from which DNA has been extracted, revealing both domestic animals and the plants that once grew around the site. The remarkable preservation of sedimentary DNA in this lake allows for an extensive and diverse reconstruction of the surrounding flora and fauna.

This paper will undoubtedly attract interest within the field, as it strengthens existing archaeological studies in the region, even though it does not challenge any major existing theories. The results are convincing, and the authors carefully avoid overextending their interpretations beyond the lake's immediate catchment area. Given the nature of pastoralism, which often concentrates around water sources, the findings are reasonably representative of the broader region. In fact, they are no less representative than the evidence typically gleaned from bones found in caves.

We are grateful for the relevant comments that referee three has made to our work. It has expanded our scope and helped us to produce a clearer version. We answer each suggestion in depth in the following lines.

While the study has many strengths, a few limitations should be noted. The absence of the uppermost sedimentary layers makes it harder to fully understand the spatial representation of the lake's DNA, as more recent sediments often provide insight into the taphonomy of deposits. This would help to further assess the spatial representativeness of the DNA record. Additionally, it is regrettable that no further sedimentological analyses were conducted, particularly to verify whether changes in mineralogical composition could explain the marked diversity shift observed around 6000 years BP. The lithology hints at such a change, but XRF analyses could have clarified this. Finally, Figure 1 could be enhanced by including clear demarcations of the lake's catchment area, which would improve the overall clarity of the data presentation.

Thank you for this valuable suggestion. As we have detailed to Referee 2, we have now clarified in **lines 365-371** why we opted not to use the uppermost sections. Sections 1U and 2U contain laminated sediments that result from high-energy transport fluxes associated to runoff, sedimentary features that render them unsuitable for establishing a reliable chronology (i.e. these sediments may mobilize organic remains from previous events thus resulting in an unreliable chronology). These sediments represent high-energy depositional events that can disrupt the usual sequence of

lacustrine sediment deposition, thereby potentially introducing inaccuracies into age-depth models if included. While we acknowledge that uppermost samples may indeed contribute to addressing taphonomic questions, we believe that the origin and sources of *sedaDNA* are ideally investigated through dedicated monitoring programmes, which fall beyond the scope of our current research.

Additionally, we would like to emphasize the lack of important mineralogical or geochemical changes occurring from Unit 4 to Unit 3 (ca. 6000 years BP). Tramacastilla sediments were described based on smear slides and organized in sedimentary facies and units. We include in this document (**Fig. 1**) geochemical analyses that are still in progress; in those it becomes patent that units 4 and 3 were deposited under the same depositional environments with higher organic content as indicated by both the high TOC (Total Organic Carbon) and redox activity (Fe/Mn) values (**Fig 1** in this document). There is a progressive change from unit 4 to 3 in reducing organic carbon that, however, does occur after 6 ka BP. In addition, we observe that there is a general dominance of the same minerals within both units, namely quartz, feldspars and clays (K and Si profiles **Fig 1** in this document).

Finally, we are grateful for the relevant comment regarding the lake's catchment area. We have included it in **Figure 1d** of the manuscript, and hope it improves the overall clarity of the data.

Once again, we would like to reiterate our gratitude to reviewer 3 for all the comments and suggestions, which have undoubtedly served to improve the revised version of our manuscript.

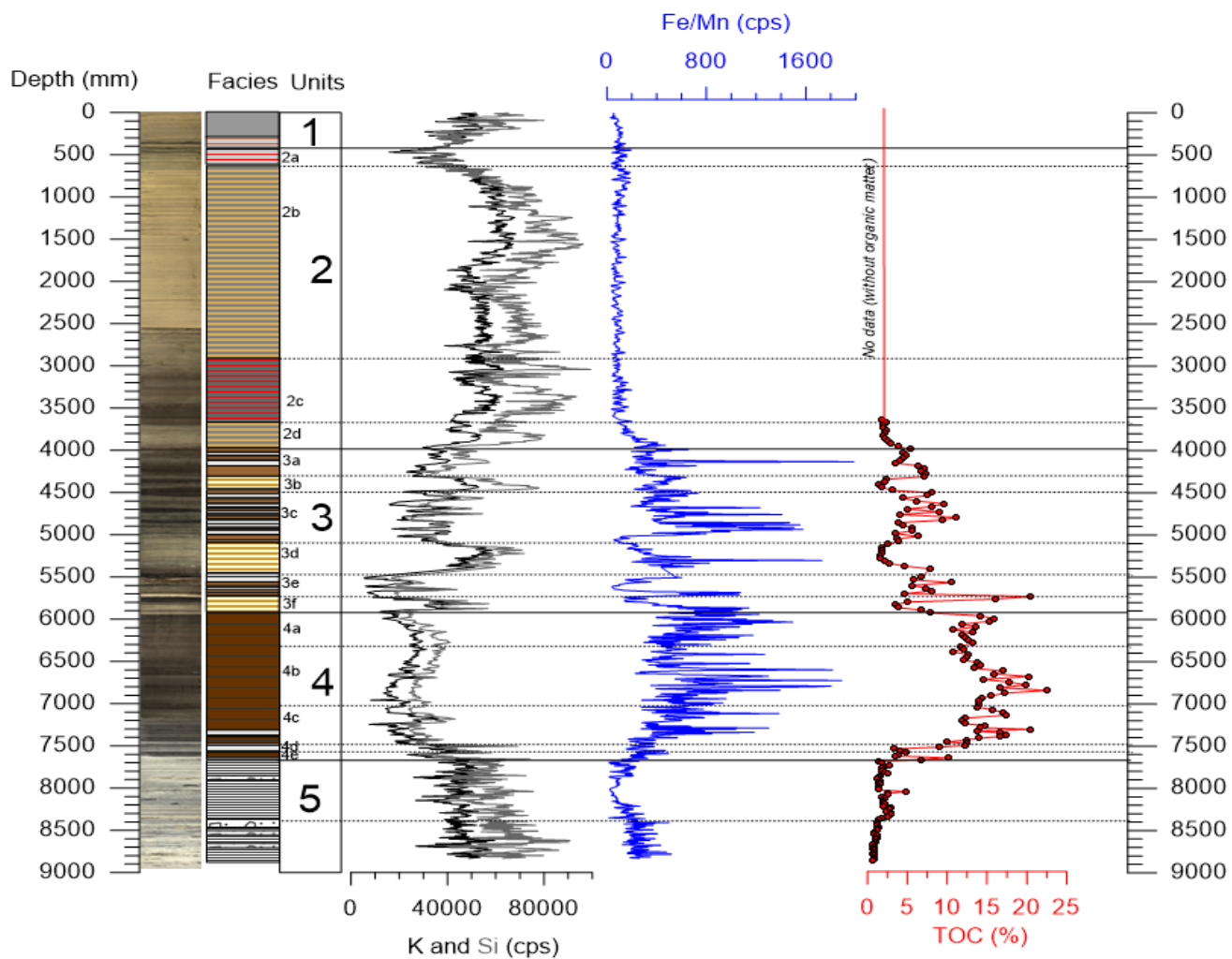


Figure 1. TRAM20-1B record with sedimentary facies and units, including geochemical elements measured using a XRF core scanner (K, Si, Fe/Mn) in counts per second (cps) and the percentage of Total Organic Carbon (TOC).

Note: new responses to reviewer 2 are written in purple in this document.

Thank you for your detailed responses to my comments on your manuscript. I appreciate the clarifications and revisions that have enhanced my understanding of your work. It has been a pleasure to contribute to the review process for such an interesting and well-conceived study. I look forward to seeing the final version and its impact on the field. Wishing you all the best in the next steps of your research

Thank you very much for your kind words and thoughtful revisions. We sincerely appreciate the time and effort you have dedicated to reviewing our work and for providing insightful feedback that has improved the clarity and quality of our study. We, too, look forward to the final publication and hope that the study will make a meaningful contribution to the field.

Reviewer #2

Content and Purpose

Despite its strengths, certain areas require clarification to enhance the overall clarity and impact of the paper:

- **Title:** *The current title is somewhat misleading as it suggests a broader geographical focus, encompassing "Southern European high altitudes". However, the discussion is primarily centered on the Iberian Peninsula, specifically the central Pyrenees. The manuscript does not provide sufficient discussion at the Mediterranean or European level regarding the effects of early pastoralism on ecosystems. I recommend revising the title to more accurately reflect the geographical scope of the study. This adjustment would not diminish the value of the research but would more precisely align it with its regional focus, while still contributing to the broader discussion on early pastoralism's impact on ecosystems.*

We appreciate this suggestion, although we believe that the broader geographical scope of the initial title may be valuable for future research. In our view, the findings can likely be extended to other high altitude areas in southern Europe, and the title's inclusivity may help reach additional researchers in related fields. However, we remain open to Referee 2's suggestion of adjusting the title if this change is crucial for publication. This would specify the Pyrenees as the focus of study: "Sedimentary ancient DNA reveals Neolithic pastoralism and plant community interactions at high altitudes of the Pyrenees, Southern Europe".

Compared to the first proposal, I believe that the title suggested by the authors in this comment better reflects the specific contents of their work while remaining of value for future research and of help in reaching additional researchers in related fields.

We appreciate this comment, that has helped us adjust the title to the geographical focus of the study. According to your suggestion, and adjusting to the journal requirements, the final title for the paper is "Neolithic pastoralism and plant community interactions at high altitudes of the Pyrenees, Southern Europe".

- **TRAM21-1B - Superficial Levels:** *The decision not to analyze the superficial levels (2U and 1U) is not fully explained. The authors mention in line 354 that “Sections 1U and 2U exhibit sedimentary features that preclude their use in building a reasonable chronology,” but the specific nature of these sedimentary features is unclear. Analyzing superficial levels is crucial in ecological studies, as they provide a reference point close to the present day, allowing comparisons with more recent (and controllable) archaeological and environmental data. If these levels were not analyzed, the authors should provide a clearer explanation for this decision.*

Thank you for pointing this out, it has helped us better explain why we opted not to use the uppermost sections of the sediment for the chronological model or any analyses. We have now included a clarification on this aspect in **lines 365-371**. Sections 1U and 2U contain laminated sediments that represent high-energy depositional events that can disturb the regular deposition of lacustrine sediments, disrupting the chronological sequence and therefore leading to inaccuracies in age-depth models if included. While we acknowledge that uppermost samples may indeed contribute to addressing taphonomic questions, we believe that the origin and sources of *sedaDNA* are ideally investigated through dedicated monitoring programmes, which fall beyond the scope of our current research.

Do the authors offer any explanation or insights regarding the change in sedimentation dynamics observed in section 2U? Could this shift be related to transformations in the environmental resource management system?

Considering what we know about the sediment, we cannot explain that change in sedimentation dynamics observed in section 2U. Your hypothesis could be true, and this change could be related to the environmental resource management system. Nevertheless, further research is needed in order to address this question.

- **Sedimentation Dynamics:** *The sedimentation dynamics of the lake basin should be further clarified. The information presented in Figure 1 of the supplementary material is insufficient to fully contextualize the *sedaDNA* results. A better understanding of the sedimentation processes is essential for interpreting the provenance and scale of representation of the *sedaDNA*. For example, what is the size of the lake's catchment area?*

Thank you for raising this point. We have indeed selected the analyzed sedimentary interval for *sedaDNA* based on the stability of sedimentation dynamics throughout the studied time period. Lacustrine sedimentation at Tramacastilla began around 25 ka BP, following the retreat of the glacier from the lake's position. The basal 1.5 m of recovered sediments consist of massive, greyish siliciclastic silts with cm-sized clasts, characteristic of high-energy detrital fluxes in a recently deglaciated, vegetation-free landscape prone to erosion (Unit 5). In contrast, Units 3 and 4 are dominated by massive to banded silty sediments with organic carbon concentrations exceeding 10%, indicating a stable depositional environment shaped by a mix of allochthonous material from runoff and autochthonous material, primarily organic matter produced within the lake. The upper layers of

the sequence are laminated, corresponding to high-energy depositional events, where no *sedaDNA* analyses were performed. The catchment area of Tramacastilla Lake is relatively small, covering approximately 50 ha, and is shown in **Fig. 1d** of the manuscript to clarify the data. Importantly, both the catchment area and sedimentary processes remained stable during the deposition of the sediments analysed (Units 3 and 4), spanning from the onset of the Holocene to around 1500 BP. We have now included a note on the analysed units' choice criteria in **lines 364-365**.

In addition, tracing the origin of *sedaDNA* and how it is affected by mineralogic changes is indeed a fascinating question that remains unresolved, and ongoing research by *sedaDNA* specialists is aimed at addressing this challenge (see Sand et al., 2024, also quoted in the text, **line 99**). However, within the scope of our project, which does not involve monitoring programs, it is not feasible to precisely trace the origin of the *sedaDNA* we have analysed. Instead, we adopt the generally accepted view in the current literature that *sedaDNA* is most likely derived from the lake basin, which is typically quite localised (Edwards et al., 2020, **line 100** of the manuscript). Please refer to our explanation in the revised manuscript on **lines 97-100**.

If Units 2 and 3 represent a stable depositional environment, partially shaped by organic matter produced within the lake (autochthonous material), what might explain the apparent absence of organisms typically inhabiting aquatic ecosystems?

The most superficial levels could certainly be studied through dedicated monitoring programs, but an interdisciplinary approach that integrates *sedaDNA* analysis with traditional environmental archaeology and bioarchaeology tools (e.g., pollen, phytoliths, charcoals, etc.) would likely yield more comprehensive results. Cross-referencing these diverse sources of data could contribute significantly to characterizing agro-sylvo-pastoral systems and understanding their historical dynamics, even in complex sedimentary contexts such as levels 1 and 2. This type of integrative research seems essential to the objectives of this study, as it addresses specific topics (e.g., Neolithic pastoralism and its effects on plants community) that can only be fully explored using such methodologies. Without this comprehensive approach, the results, despite their potential, risk to be trapped in too broad generalizations about ancient rangeland management practices to which this *sedaDNA* work should precisely contribute. I hope that these reflections can be incorporated by the authors into their future research works.

Regarding your comment, we assume you are referring to Units 3 and 4 when mentioning in the first sentence, "*If Units 2 and 3 represent a stable depositional environment [...]*", as Units 3 and 4 are the ones associated with a stable depositional environment in terms of detrital input and runoff. Based on this, there is no absence of organisms typically found in aquatic ecosystems, as *sedaDNA* analysis identified several such organisms, including algae, hygrophytes, and hydrophytes (listed in **Supp. Data 3** under the 'group' column), as well as animals like frogs, toads, and newts (listed in **Supp. Data 8** under the 'Wild animal' category).

We appreciate your insights regarding monitoring the uppermost units. We are currently analysing various proxies, and we hope that future results will address this information gap, enhancing our understanding of the ecosystem dynamics inferred from *sedaDNA* in the context of management practices. Thank you for your valuable recommendations.