

RESPONSE TO THE ANONYMOUS REVIEWERS

We thank the reviewers for their positive and helpful comments on our manuscript. We proposed to revise the paper as suggested.

Response to anonymous reviewer 2

General comments:

...However, the paper is too descriptive, the interpretation of lower-concentration CO₂ impacts on vegetation is overly general, and some key information is missing.

Response: We appreciate the reviewer's insightful comments and will conduct a thorough proofread to enhance clarity and reduce excessive descriptiveness, particularly in relation to the data presentation.

Regarding the effects of low atmospheric CO₂ on vegetation, we recognize the need to strengthen our discussion. We will build on the points already raised in the manuscript,

- a) we highlight the impact of CO₂ limitations on photosynthesis and plant growth (e.g., L134, 161), and we also discuss the reduced water-use efficiency (WUE) associated with lower CO₂ levels, explaining how decreased CO₂ concentrations affect stomatal regulation, leading to increased water loss and heightened vulnerability to drought stress.
- b) we acknowledge the role of CO₂ in shaping plant community composition. Although, we agree that this aspect could be further developed (e.g., L453), particularly in explaining why heathlands may have dominated LGM landscapes. We plan to expand on this by emphasizing that **lower CO₂ levels, combined with cooler conditions, would have favored the establishment of vegetation types adapted to these constraints. The heathland species are resilient, making them well-suited to glacial environments.**

Finally, if the comment "some key information is missing" pertains to the need for updated references (Rev#2 highlights that "recent papers about pollen-based climate reconstructions (e.g., Izumi and Bartlein, 2016; Chevalier et al., 2020; Wei et al., 2021; Prentice et al., 2022; and Izumi et al., 2023)" we fully acknowledge this and will incorporate this relevant, recent literature in the revised version to strengthen our discussion.

Specific comments:

The authors described that "The study of increased plant growth and global vegetation greening under higher concentrations of pCO₂ (CO₂ fertilisation) is very topical within discussions of current global climate change (e.g., Piao et

al., 2019), whilst the inverse scenario (low pCO₂) has received less attention.” at line 116-119. However, recent papers about pollen-based climate reconstructions (e.g., Izumi and Bartlein, 2016; Chevalier et al., 2020; Wei et al., 2021; Prentice et al., 2022; and Izumi et al., 2023) have discussed the impacts of lower atmospheric CO₂ on vegetation. The reference papers listed here are pollen-based climate reconstructions using an inverse-modeling approach related to the papers the authors already cited, Guiot et al. (2000), Wu et al. (2007), and Prentice et al. (2017). Pollen-based climate reconstructions using inverse modeling methods have not differed significantly from temperature reconstructions using conventional statistical methods such as regression analysis and modern analogue techniques. On the other hand, large differences are produced in reconstructing hydrological climate from the traditional methods that tend to overestimate dryness during glacial periods. This may influence the authors’ interpretation of climate from vegetation. As a result, the authors potentially need to largely rewrite the “Abstract”, “Introduction”, “Discussion”, and “Conclusion”. Moreover, the paper, Piao et al. (2019) is not in the reference.

Response: We greatly appreciate the reviewer’s comment, as it highlights an important and evolving area of research. The development of improved new methodologies and the continuous refinement of climate reconstructions from pollen records, particularly under low CO₂ conditions, are indeed crucial for improving our understanding of past environments.

To address the reviewer’s concerns, we will integrate the suggested references into our manuscript, ensuring a more comprehensive discussion of the various approaches used in pollen-based climate reconstructions. We recognize that the primary issue related to low CO₂ is its influence on moisture availability rather than temperature. Indeed, this is in agreement with what we have already stated, in different parts of the manuscript:

*“The Mediterranean region, with its characteristic **annual hydrological deficit and seasonal water stress**, is a key place for exploring the potential role of pCO₂ limitation on vegetation growth. Therefore, past vegetation dynamics in this region may be considered as a significant (inverse) analogue to understand the current impact of increasing temperature and pCO₂ within semi-arid and arid ecosystems.”*

We recognise a need to further our discussion to reflect how different reconstruction techniques, particularly inverse modeling methods, have contributed to understanding past climate conditions. To give some context about the exploration done regarding WA-PLS (noting this exercise was done prior to the published article of Wei et al., 2021, pers. comm.), we acknowledge the limitations of this approach, and we have previously tested it on approximately ten Iberian marine and coastal records spanning the last 23 ka. Our findings indicated two key challenges: a) The technique systematically pushes reconstructions away from extreme values, a known issue that we were able to confirm through our tests.

b) Systematic biases were observed in coastal regions, affecting the reliability of the reconstructions.

For those cores, whilst trend comparisons were possible, the presence of modern biases complicated direct interpretation, and the lack of a consistent systematic bias made it difficult to develop an approach to correct the data. In the study of those cores, the use of WA-PLS as a method was not the primary focus of our research, therefore that particular work did not progress further. However, we acknowledge that addressing these methodological challenges here in this current paper is essential for improving the robustness of marine pollen-based reconstructions. The articles of Wei et al., 2021, Prentice et al, 2022 and Cruz-Silva 2023 are able to provide improvements and a better understanding about the pCO₂ role.

In summary, we acknowledge the challenges associated with pollen-based climate reconstructions, particularly in the context of low CO₂, and we will update our manuscript to integrate recent references and methodological discussions. While our findings align with broader trends reported in the literature, we recognize the need for continued refinement of reconstruction techniques and will ensure that our study appropriately contextualizes these methodological considerations. At the same time we recognize the importance and high value of qualitative datasets, and its information.

Comment L49-54. The purpose of the authors' study should be to investigate vegetation changes and the effects of pCO₂ changes on vegetation on the Iberian Peninsula margin, not to track and compare them with global pCO₂ changes.

Response: We appreciate the valuable comment from Reviewer 2. Our intention was to investigate vegetation changes on the Iberian Peninsula margin and assess their relationship with global pCO₂ variations. In doing so, we utilized multiple proxies and compared them with global pCO₂ records to evaluate how vegetation dynamics responded to these changes—hence our use of the term "tracking." However, we acknowledge that our wording may have led to a different interpretation. To enhance clarity and readability, we will revise the sentence accordingly.

“The high-resolution analysis of terrestrial (pollen, C₂₉:C₃₁ organic biomarker) and marine (alkenone-derived Sea Surface Temperature, C₃₇:4%, and long-chain n-alkane ratios) indicators, using a direct land-sea comparison at the Iberian margin site Integrated Ocean Drilling Program (IODP) U1385 ("Shackleton site") throughout the Last Glacial Maximum (LGM) and last deglaciation, allowed us to investigate .the Iberian Peninsula vegetation response to major global pCO₂ changes/fluctuations.”

Comment L130-132. About stomatal conductance and stomatal density under low pCO₂ concentration: Is this correct? Does stomatal density change with application to climate over short periods? Is this the author's idea? If not, please put the paper cited.

Response: We appreciate this valuable comment from Reviewer 2, in fact it misses a reference to back it up. And yes, it is correct there are in fact several studies showing that changes pCO₂ can play a strong role by affecting the initiation of stomata, which in turn could impact stomatal density. In this study they present an interesting experiment with elevated and sub-ambient CO₂, with durations between 14 days to five years. in which it is possible to detect differences in the number of stomata. I will add for completeness the reference Royer, D. L. (2001). [Stomatal density and stomatal index as indicators of paleoatmospheric CO₂ concentration. *Review of Palaeobotany and Palynology*, 114(1-2), 1-28.] to the sentence highlighted.

L152-154. I disagree with this sentence, at least about climate reconstructions. In section 3.3, the authors need to describe ecological groups other than the temperate and Mediterranean forests in Figure 3. Which pollen taxa belong to the “semi-desertic taxa” and “heathland”, respectively?

Response: We appreciate the valuable comment from Reviewer 2, considering the references given we will update this sentence regarding the contributions highlight in the suggested papers. We will include this information in the Figure 3 caption.

Semi desertic-plants includes Amaranthaceae (Chenopodiaceae), Artemisia and Ephedra.

Heathland includes all the Ericaceae (which includes all the types of Erica sps.) and Calluna sp.

To be more cautious and avoiding overgeneralization we can rephrase it taking out the word “majority”. As a note regarding the disagreement, we can just mention that Prentice et al. (2022) as well as Cruz-Silva et al., (2023) are reiterating in their papers there still exist some models which do not contemplate the effect of CO₂ in their reconstructions.

Why do the authors apply a Generalised Additive Model (GAM) to TMF alone? Why not use it for the other ecological groups?

Response: We appreciate this insightful comment.

The decision to apply a Generalized Additive Model (GAM) exclusively to the temperate Mediterranean forest (TMF) group GAM to help reduce noise (counting, chronological) when integrating pollen data from multiple sites into a single curve. We only performed that exercise for the TMF. For the other groups it doesn't seem necessary.

L309-316. Is the high production of C₂₉ n-alkanes by trees and shrubs interpreted as a high distribution of these vegetations? The explanations in this text (L309-312) do not help for interpretation for the result.

Response: We thank the reviewer 2 for this question and comment. The understanding of C₂₉ alkanes is that they reflect the production of leaf waxes, generally to protect plants from harsh conditions, hence not necessarily representing the distribution of the species/groups of plants.

We will rephrase this to: "Index values >1 are typically considered to reflect higher quantities of C₂₉ *n*-alkanes **produced** by trees and shrubs, while values of the index <1 are generally considered to indicate the production of higher quantities of C₃₁ *n*-alkanes by grasses and herbaceous plants (Cranwell, 1973; Ortiz et al., 2010; Rodrigues et al., 2009)."

Overall, we will check and revise for clarity the description of the n-alkane results.

The content in L313-316 should be put before the description of index < 1 or index > 1. L415.

Response: We agree with the reviewer comment; we will implement this change.

What are the +/- and up/down arrow values for each period (the YD, BA, HS1, and LGM) in the figure (Fig. 4) compared to? Wouldn't it be better to have a quantitative discussion?

Response: The arrows or values are compared to the preceding period, in relation to the referred parameter (we will add this information to the caption of the fig.). A quantitative discussion is not possible because the temperature (except for the SST) and moisture are based on % pollen-based ecological groups. We can instead talk about relative change. Fig. 3 could give a perception of the % values for each parameter.

L423-426. The authors need to elaborate more, especially on how to read the S.M. Fig.2. Moreover, the figure is difficult to read because of unclear, and its caption is inadequate.

This approach seems to work if the relationship between vegetation and climate is constant regardless of CO₂ concentration. How would we use this figure if the relationship between vegetation and climate changes with changes in CO₂ concentration?

Response: We sincerely appreciate the reviewer's insightful comments regarding the clarity of **Supplementary Figure 2 (SM Fig.2)**. We acknowledge that the figure's readability could be improved, and we will enhance the visual representation by clearly indicating the parameters analyzed and reinforcing the color contrast to ensure better

comprehension. Also, we will revise the figure caption to provide a clearer explanation of these insights and ensure that readers can more easily interpret the relationships presented.

In Section 5.1, the authors need to discuss the influences of lower concentration CO₂ on vegetation changes, including the more recent papers, especially pollen-based climate reconstruction during the LGM and last glacial periods.

Response: We appreciate the reviewer's suggestion and acknowledge the importance of incorporating recent studies on the influence of low CO₂ concentrations on vegetation changes, particularly in pollen-based climate reconstructions of the LGM and last glacial periods.

As noted in our previous responses, we will integrate the suggested references into our discussion to provide a more up-to-date perspective on the role of CO₂ in shaping past vegetation dynamics. While our current discussion (L493) already considers the impact of moisture and temperature changes, the additional references will strengthen our argument, particularly in highlighting how LGM conditions were likely wetter than previously inferred based on pollen records alone. Given that these recent studies reinforce the idea that pollen-based climate reconstructions often overestimate aridity due to the omission of physiological constraints imposed by low CO₂ on plant water-use efficiency (Wei et al., 2021; Prentice et al., 2022; Cruz-Silva et al., 2023), we recognize the value of including them for a more comprehensive discussion.

In Section 6, what are the key messages of this study? The authors could have described them more briefly and effectively. This is also true in the Abstract.

Response: We appreciate your comment; we will address it by summarizing and including the key messages in bullet points. The key messages for this study will be updated in the manuscript.

- **Vegetation Dynamics Across Key Climate Transitions:** HS1, B-A and YD (to be synthesized)
- **Influence of Low pCO₂ on vegetation changes during the LGM:** relatively cold conditions, low seasonality, and wetter but with exacerbated drought stress under low pCO₂ further restricted tree growth while promoting heathland expansion, likely due to the moisture-adapted traits of Mediterranean Ericaceae.
- **Critical pCO₂ Threshold for Forest Expansion (~225 ppmv):** A) Our study proposes that pCO₂ values of ~225 ppmv acted as a critical threshold for forest expansion in the Iberian Peninsula during the last deglaciation. This value is

quite similar with peak in the Mediterranean forest pollen percentages, during MIS13 a decrease in CO₂ and a value of 216 ppmv in Oliveira et al. (2020), despite the different insolation conditions. Future modeling efforts should explore the amplitude and thresholds of pCO₂ impacts on regional vegetation, including during past cold periods.

Oliveira, D., Desprat, S., Yin, Q., Rodrigues, T., Naughton, F., Trigo, R. M., ... & Goñi, M. F. S. (2020). Combination of insolation and ice-sheet forcing drive enhanced humidity in northern subtropical regions during MIS 13. *Quaternary Science Reviews*, 247, 106573.

- **Relevance for Present and Future Climate Change:** the findings provide a critical baseline for understanding how arid and semi-arid ecosystems might evolve under rapidly changing pCO₂ levels in the present and future.

The other comments

L57. “(HS1)the” to “(HS1), the”

Response: We have adjusted the space as suggested.

L60. “condition” to “conditions”

Response: We have added an “s” as suggested.

L64. “mosaic,” to “mosaic;”

Response: We have added an “;” as suggested.

L77. The authors should define the period of “the last deglaciation” here (not L90). Moreover, the authors’ definition, from 21 to 6 ka, is not true. The last deglaciation does not include the mid Holocene period.

Response: We thank you the reviewer for this comment. There was a mistake while considering all the period presented in the paper with the deglaciation itself. The beginning of the deglaciation occurs at 20-19 ka (e.g. Denton et al 1981; Toucanne et al., 2008; Denton, 2010) spanning to the final episode of Laurentide ice sheet at 7/6.8

ka (e.g. Dyke, 1987; Carlson et al., 2008). Other authors have considered climate during the last deglaciation (20 to 6 cal ka BP)”
<https://doi.org/10.5194/cp-6-245-2010>, 2010

L104. “in Northern Hemisphere” to “in the Northern Hemisphere”

Response: We have added “the” as suggested.

L184. “at centennial-scale resolution” to “at the centennial-scale resolution”

Response: Will change it to “at a centennial-scale resolution” in the middle way that sounds better.

L202. “the the” to “the”

Response: We have deleted the extra “the” as suggested.

L205-207. “Köppen classification Csa, with warm summers (around 22°C as the average temperature of the warmest month) mean annual temperatures between 12.5°C and 17.5°C and mean annual precipitation from 400 to 1000 mm/yr.”

Response: Not clear about the comment. I will address it if there is more detail.

L235 and L236. What are “HCl” and “HF”

Response: We understand that some people should not be so familiar with chemistry, including the name of certain acids, as such we have wrote the name of the acids for a better understanding. HCl stands for hydrochloric acid and HF stands for hydrofluoric acid (which is the solution of hydrogen fluoride in water, being a liquid at room temperature).

L251-252. It is an unclear sentence to me.

Response: This is a normal procedure/calculation, but the explanation may not be clear. When calculating the main sum, we use most of the specimens excluding some taxa (which for different reasons- high production, transport, local and /or aquatic taxa) appeared overrepresented.

Pollen percentages were calculated on the number of pollen grains from terrestrial plants excluding some taxa because of their natural over-representation (Pollen X1 /Main sum). **The percentages of over-represented taxa were calculated on the basis of the main sum plus the counts for that particular individual taxa Pinus / (Main sum + Pinus) and Cedrus / (main sum + Cedrus).** If it helps the understanding I could add the equations for a better understanding.

L258. Remove “(CONISS)”

Response: We will remove it.

L258. “(U 1385-1 to 5)” to “(U 1385-1 to 5 in Fig. 3 and Table S1)”

Response: We will edit it.

L265. “eight pollen records” to “eight marine cores” (?)

Response: We will re-write to “eight marine records”

L311. “value” to “values”

Response: We will add the “s”.

L392. “inin” to “in”

Response: We will delete the “in”

L636-643. Basic information on Poaceae and Cyperaceae (Fig. 3g) should be described in Section 3.3 first. It is unclear in the result section why the authors treated Poaceae and Cyperaceae with Fig.3.

Response: I will include this information on section 3. Basically, the Poaceae + Cyperaceae group was because they could be potentially representative of C₄ plants (generally better adapted to low CO₂ environments), and the curve was to assess if there were any trends compatible with changes with CO₂ and C₂₉/C₃₁ ratios, being C₃₁ related with grasses. In a recent article Casas-Gallego et al. (2025) refers that the actual percentage of C₄ is relatively low and contains just ~2.4-5.6% of the vascular plants in SW of Iberia.

Casas-Gallego, M., Postigo-Mijarra, J. M., Sánchez-de Dios, R., Barrón, E., Bruch, A. A., Hahn, K., & Sainz-Ollero, H. (2025). Changes in distribution of the Iberian vegetation since the Last Glacial Maximum: A model-based approach. *Quaternary Science Reviews*, 351, 109162.

In Table S1, “1105” to “11050” about the U1385-4 period

Response: We will correct it.