Response to comments from Anonymous Referee #2

Thank you for taking the time to review our manuscript and the constructive comments. Below you find a point-by-point response to each of the comments and revisions suggested. The line numbers refer to the original preprint version that you reviewed.

- Referee comments are in black color.
- Replies to referee comments are in blue.
- <u>The new paragraphs, sentences or words added to the manuscript are underlined</u> <u>and in orange.</u>

The manuscript contains a dataset of $\delta^2 H_{wax}$ values from different terrestrial, riverine and marine sediment samples across environments with different aridity indexes in Chile. These $\delta^2 H_{wax}$ values are accompanied by $\delta^2 H$ values of precipitation, and a large set of different environmental characteristics, with the aim of identifying how well sediment derived $\delta^2 H_{wax}$ values track $\delta^2 H$ values of precipitation, and with that provide new insights into the validity of using $\delta^2 H_{wax}$ values for paleoclimatic reconstructions. The results show that on a global scale, the obtained $\delta^2 H_{wax}$ values follow $\delta^2 H$ values of precipitation quite well. However, within the dataset itself, across the aridity gradient, other environmental drivers also appear to become important in shaping $\delta^2 H_{wax}$ values. The latter even seems to differ for $\delta^2 H$ values of the two studied leaf wax *n*-alkane C-chain lengths, possibly related to changes in vegetation types. Lastly, the $\delta^2 H_{wax}$ values found in marine sediment samples reflect the $\delta^2 H_{wax}$ values from the terrestrial and riverine sediments.

I enjoyed reading the manuscript, which is written well with clear explanations of objectives and implications. The data presented here provides interesting new insight into how well $\delta^2 H_{wax}$ values track $\delta^2 H$ values of precipitation on a global scale, but also considers in more detail deviation of $\delta^2 H_{wax}$ from the expected $\delta^2 H_{pre}$ pattern by different drivers, like changes in evapotranspiration and vegetation type, along the aridity gradient. I think this is a valuable new approach to gain more insights into the drivers of $\delta^2 H_{wax}$ values. Although I am not an expert in the modelling approach and therefore cannot judge its accuracy very well, the explanation of the model was clear enough that I could follow what was being done. I only have a few minor comments that may help further strengthen the manuscript, and I think this paper is suitable for publication after these minor points have been addressed.

Thank you for your thoughtful and positive comments on our manuscript.

Minor comments:

Although changes in δ²H_{wax} values as an effect of differences in vegetation type are discussed in section 4.2, it is not addressed in the manuscript introduction. In L47-L59 I believe it might be valuable to already introduce the possible effects of species variation on δ²H_{wax} along the aridity gradient where changes in plant community composition may occur. Additionally, for the discussion section 4.2, it could be considered that even within taxonomically/physiologically constrained groups like herbaceous plants or eudicots, species differences in ε_{wax/pre} can still be very large (Chikaraishi et al., 2004, *Phytochem*.; Gao et al., 2014, *PLoS ONE*; He et al., 2020, *Geochim. Cosmochim. Acta*; Baan et al., 2023, *Geochim. Cosmochim. Acta*). This complicates the interpretation of the effect of the broad term 'vegetation type' changes on δ²H_{wax} values and requires more detailed knowledge on the integration of

n-alkanes and their δ^2 H values from different plant species into the sediment (as youstate in L540).

We appreciate the feedback provided on this point. We did not originally include text in the introduction about the effects of plant species variation along Chile, because there is limited detailed information on the plant species found along Chile. Nevertheless, this comment highlights the necessity to add context to the readers that introduces this topic. In a revised version, we have now extended the paragraph to include new sentences starting in line 52 that introduce how general changes in plant communities can cause changes in $\varepsilon_{wax/pre}$ and consequently $\delta^2 H_{wax}$.

Sentences added in line 52: Yet, $\varepsilon_{wax/pre}$ values can be affected by the type of plant communities sourcing the *n*-alkanes. Generally, $\varepsilon_{wax/pre}$ values are higher in C₃ plants than in C₄ plants (Chikaraishi et al., 2004; Smith & Freeman, 2006, Kahmen et. al 2013, Sachse et al. 2010, Sachse et al. 2012). It has been suggested that these differences originate due to specific discrimination against ²H between distinct photosynthetic pathways (Chikaraishi et al., 2004), as well as due to different pools of biosynthetic source waters fed by different mixtures of enriched leaf water and unenriched soil water (Kahmen et al. 2013), or due to both processes. Moreover, studies analyzing plants by growth form (Griepentrog et al., 2019; Liu et al., 2016) or even at the species level (Gao et al., 2014) show a strong control of vegetation type or species on $\varepsilon_{wax/pre}$, explained by physiological and biochemical factors that vary among different plant taxa (Gao et al., 2014; Liu et al., 2016). In addition to the vegetation effects,

Regarding the discussion section 4.2, we agree with the view expressed by the reviewer, that even within the same plant growth form (i.e., herbaceous, or woody), differences in $\varepsilon_{wax/pre}$ between different plant species can still be very large. We acknowledge that the use of plant growth forms might not be ideal over short spatial scales of meters, as it might oversimplify all the complexities introduced by individual plant species. But at the scale of our study, we believe it is currently the best approach in terms of feasibility. At the moment, to our knowledge there are not open and accurate datasets of plant species distribution at the scale of the catchments we studied in Chile. Therefore, we have made the decision to use plant growth forms instead of plant species distribution datasets because of the availability of the current remote sensing datasets. We hope that soon accurate plant species distribution become openly available. This would allow future studies to investigate the effects of individual plant species on $\delta^2 H_{wax}$ values at a catchment scale, and to improve the accuracy of paleoenvironmental reconstructions.

To address the reviewer's comment, we have rearranged the paragraph between lines 439 and 453, also changed the wording of some sentences in the paragraph between lines 454 and lines 459, and finally added new sentences starting on line 452 that mention the possible effects of distinct plant species of the same growth form and the need for further research to investigate the universality of the findings discussed in section 4.2.

Sentences added in line 452: or particular plant species. In this study we focused on the level of plant growth forms due to the lack of plant species datasets at the scale of our study areas. However, it is important to note that within the same plant growth form, different plant species might exhibit distinct apparent and/or biosynthetic fractionation values and consequently affect $\delta^2 H_{wax}$ differently. The influence of both plant growth

forms and plant species on sedimentary $\delta^2 H_{wax}$ over large spatial scales is unclear, and more studies are needed to fully understand these relationships.

2. Even though in Fig. 2A & B all of the datapoints from the Chilean dataset would be considered to fall within error among the datapoints in the global dataset (i.e. roughly falls on the expected line in a global $\delta^2 H_{pre}$ gradient), it seems that once the Chilean dataset is isolated, there is no longer a strong relationship between $\delta^2 H_{wax}$ and $\delta^2 H_{pre}$ Can the authors comment on the relation between $\delta^2 H_{wax}$ and $\delta^2 H_{pre}$ within the Chilean dataset?

We thank the reviewer for the thoughtful comment. We fully agree with this observation about the Chilean dataset looking disperse around the global relationship. We refer here to Table 2. There, we present the statistical parameters of the linear regressions obtained between $\delta^2 H_{pre}$ and $\delta^2 H_{wax}$ both for the global dataset and the isolated Chilean dataset, separating it further into only soil samples, only river samples, and soils plus rivers samples together. The results from Table 2, which are described in the manuscript, between lines 293 and 296 indicate that the relationship between $\delta^2 H_{pre}$ and $\delta^2 H_{wax}$ is strong and significant when considering the river samples, as well as rivers and soil samples together, but for the dataset of only soils it is not significant. Furthermore, the results indicate that for the *n*-C₂₉ homologue, the combined dataset of Chilean soils and rivers shows slope and intersect values (Slope = 0.76; intercept = -118) that are equivalent to the slope and intersect values from the global dataset (Slope = 0.79; intercept = -124), while all the other datasets have significant differences for the slope and intersect values relative to the global dataset.

The implications of Table 2 results are discussed in section 4.4. However, we acknowledge that Table 2 could be improved, and the results should be directly referenced in the discussion of the manuscript so that the findings are not overlooked. Therefore, we did three things, first we modified the formatting of the p-value numbers to provide more clarity. Second, we added an extra column called "significance" that represents the significance of the correlations obtained between $\delta^2 H_{pre}$ and $\delta^2 H_{wax}$ using asterisks symbols. If a p-value is greater than 0.05 there are no asterisks; if a p-value is between 0.05 and 0.01, it is represented with one asterisk (*), a p-value is between 0.01 and 0.001, it is represented with 2 asterisks (**) and a p-value less than 0.001 is represented with three asterisks (**). Third, we changed the topic sentence starting section 4.4 and added a new one in line 491.

Sentence added in line 491: Analysing the Chilean dataset at the differential spatial scales, we found that $\delta^2 H_{pre}$ values showed better correlation with $\delta^2 H_{wax}$ values from river sediments than from soils (Table 2).

From Fig. 2A & B it looks like this relationship is not very strong, and if this is the case, could this be an effect of uncertainty in $\delta^2 H_{pre}$ values, or an effect of additional environmental/biological control on $\delta^2 H_{wax}$ values?

We thank you for the opportunity to clarify some of the findings of our study by asking what could control $\delta^2 H_{wax}$ values beyond $\delta^2 H_{pre}$? And questioning if this is an effect of uncertainties in $\delta^2 H_{pre}$ values or is due to further environmental/biological controls. In the manuscript we tackle these questions in section 4.1 and the subsections that are contained therein. The uncertainties in $\delta^2 H_{pre}$ values are discussed in subsection 4.1.1,

where we validate the accuracy of $\delta^2 H_{pre}$ values derived from the Online Isotopes in Precipitation Calculator (OIPC) by comparing them to $\delta^2 H_{pre}$ measurements from the IAEA in 9 stations along Chile. The results are displayed in figures S1.A and S1.B, together with the statistical parameters of the linear regressions. The data presented provides strong evidence that the OIPC-derived $\delta^2 H_{pre}$ values are accurate along the Chilean gradient. The high and significant correlation values between the predicted and the long-term measured values suggest that the OIPC model is accurately capturing the variations in $\delta^2 H_{pre}$. Although there are some uncertainties associated with the OIPC model, these uncertainties do not appear to be systematic and do not bias the results towards any particular aridity zone.

With respect to the environmental controls on $\delta^2 H_{wax}$ values, in the manuscript we divide this discussion in two. In subsection 4.1.2 we first discuss the controls on $\delta^2 H_{wax}$ values for samples from the humid, semiarid and arid zone. In subsection 4.1.3, we discuss the controls on $\delta^2 H_{wax}$ values for samples from the hyperarid zone. We followed this approach based on the residuals analysis showed in Fig. 2B and 2D and the results in Table 3. These results show that in the arid, semiarid and humid zone, the $\delta^2 H_{wax}$ values do not significantly deviate from what is predicted by the global regression between $\delta^2 H_{wax}$ and $\delta^2 H_{pre}$. This suggest that, in these regions, variability in $\delta^2 H_{wax}$ values is primarily driven by variability in $\delta^2 H_{pre}$ values. However, in the hyperarid zone, residuals do significantly deviate from the global regression between $\delta^2 H_{wax}$ and $\delta^2 H_{pre}$, indicating that additional factors besides $\delta^2 H_{pre}$ values affect $\delta^2 H_{wax}$ values. We suggest that in the hyperarid zone $\delta^2 H_{wax}$ values are heavily controlled by evapotranspirative processes, in addition to $\delta^2 H_{pre}$ values. We reach this conclusion based on the analysis of apparent fractionation values against climatic parameters that we discuss between lines 381 and 406, as well as the mechanistic modelling of ²H enrichment in soils and leaves that is discussed between lines 413 and 436.

The discussion about biological controls on $\delta^2 H_{wax}$ values is presented in section 4.2. Initially we only discussed how plant growth forms could control the differences in $\delta^2 H_{wax}$ values identified between the *n*-C₂₉ and *n*-C₃₁ homologues. However, after the first comment of your review, we added further sentences in line 452 to discuss the effect of plant species on $\delta^2 H_{wax}$ values. The sentences added are mentioned above as the reply to the first comment.

As a result of this, what magnitude of error could be introduced when reconstructing $\delta^2 H_{pre}$ from $\delta^2 H_{wax}$ for a given site that may be subject to additional environmentally/ biologically induced variation in $\delta^2 H_{wax}$ values over time? I suppose the latter is difficult to answer quantitatively, but perhaps the authors can comment on this.

Thank you for this comment. As you mention, the uncertainties on reconstructed $\delta^2 H_{pre}$ values are hard to quantify. Nonetheless, we acknowledge that it is necessary to provide more clarity and context about these potential uncertainties to the readers. Thus, based on this and on your third comment, we have added a new paragraph after line 541 and additionally revised and modified the paragraphs between lines 524 and 541. The new paragraph as well as the new additions to the paragraphs between lines 524 and 541 are shown below as part of the response to the third comment.

Overall, I find the different comparisons made in Fig. 2 very interesting, but it might be valuable to clear up the interpretation and implications of the results on different spatial scales.

We appreciate this comment, and we also consider important to interpret and discuss the implications of the results at the level of the different spatial scales. The discussion of the interpretations and implications of this on the different spatial scales is done in section 4.4, where we added a new sentence at line 491 based on the first question of this comment, which can be seen above, we additionally modified Table 2, as explained above, to bring more clarity to the results obtained at the different spatial scales. We believe that these additions to the manuscript address both the first question (about isolating the Chilean dataset) and the last question (about the interpretations and implications on different spatial scales) of this comment.

3. L527-L533: The results presented suggest that changes in the hydrological and vegetation characteristics of a given study site over time (i.e. irrespective of its current aridity state) can introduce some error in the reconstructed $\delta^2 H_{pre}$ from sedimentary $\delta^2 H_{wax}$ values, which is somewhat in contrast to the statement made in L527. As such, the continuation of this paragraph seems to be slightly opposing the initial statement, as hydrological changes may not be reflected in $\delta^2 H_{pre}$ Perhaps this paragraph could be slightly revised to provide a better overview of the nuances required for paleoclimate reconstructions from $\delta^2 H_{wax}$ values.

Thank you for your comment. We agree that the results of our study suggest that changes in the hydrological and vegetation characteristics of an area over time can introduce additional errors in the reconstructed $\delta^2 H_{pre}$ from sedimentary $\delta^2 H_{wax}$ values. However, we also believe that the aridity state of an area is an important factor to consider when interpreting $\delta^2 H_{wax}$ values. In our study, we found that the significant evapotranspirative effects on $\delta^2 H_{wax}$ only have been identified in hyperarid zones. In contrast, the humid, semiarid, and arid zones are less affected by evapotranspirative fractionation, and $\delta^2 H_{wax}$ generally reflects $\delta^2 H_{pre}$ in these zones. Thus, we believe that it is important to consider the aridity state of an area when using $\delta^2 H_{wax}$ values as a paleoenvironmental proxy.

To clarify this further, and as part of the response to one of the questions in your second comment above, we have revised and rephrased the paragraph starting in line 527. Additionally, we combined this paragraph with the paragraph starting in line 524, to make it more cohesive and consistent. The new combined, revised, and rephrased version of the paragraph is as follows (the changes to the text are in orange): Our results demonstrate the potential of $\delta^2 H_{wax}$ as a proxy for $\delta^2 H_{pre}$ in the humid to arid zones of Chile. We found that $\delta^2 H_{wax}$ values in marine sediments are consistent with those in river sediments and soils from the adjacent continent, supporting the use of marine sedimentary $\delta^2 H_{wax}$ as a tracer of continental $\delta^2 H_{pre}$. However, our analysis also revealed that hyperaridity can cause strong ²H enrichment (i.e., smaller <u>ewax/pre_values</u>) and nonlinear relationships between hydrological variables and Ewax/pre. These findings suggest that $\delta^2 H_{wax}$ is highly sensitive to the onset of extreme aridity. While $\delta^2 H_{wax}$ values largely reflect $\delta^2 H_{pre}$ values in humid, semiarid, and arid settings, in hyperarid regions, the strong evapotranspirative effects on $\delta^2 H_{wax}$ could lead to an overestimation of $\delta^2 H_{pre}$ values, and consequently of hydrological changes, as also discussed in Hou et al. (2018). Because of this, it is crucial to consider the aridity states that an area may have <u>experienced when using $\delta^2 H_{wax}$ as a paleoenvironmental proxy.</u>

We also revised and changed some wordings to the paragraph starting in line 534. The new revised version of the paragraph is as follows (the changes to the text are in orange): Furthermore, our analysis revealed differential responses of the *n*-C₂₉ and *n*-C₃₁ homologues under strong aridity conditions, likely due to different vegetation sources. We found that *n*-C₂₉ is more sensitive to aridity and exhibits less negative $\delta^2 H_{wax}$ values relative to *n*-C₃₁ in sites with high aridity (Fig. 5). Similar findings have been reported in previous studies (Chen et al., 2022; Garcin et al., 2012; Wang et al., 2013). We found that the difference between $\delta^2 H_{wax}$ values in *n*-C₂₉ and *n*-C₃₁ is particularly pronounced in arid settings, especially where herbaceous vegetation dominates. This differential sensitivity could be useful in detecting the onset of high aridity, and thus could help avoiding overestimation about the *n*-alkanes of the vegetation from the source areas.

Finally, we added a new paragraph after line 541: In summary, along the Chilean humid to arid zones, our results support the use of $\delta^2 H_{wax}$ as a proxy for $\delta^2 H_{pre}$ and to study changes in paleohydrological conditions. However, with the onset of hyperaridity, $\delta^2 H_{wax}$ values can become decoupled from $\delta^2 H_{pre}$ and be controlled by evapotranspirative processes.

Phrasing/textual comments:

L30: Italicize 'n' in 'n-alkanes'. This is not consistently done throughout the manuscript.

This was revised and fixed throughout the whole manuscript.

L52: although 'less negative' is not incorrect, I find that this can be a somewhat confusing term. More straightforward referencing between different δ and ϵ values could simply be 'higher' or 'lower' than (in this specific case 'higher'). Also goes for further on in the manuscript (e.g. L286 and L287).

This was revised and fixed throughout the whole manuscript.

L65: superscript of '13' and subscript of 'wax' should be fixed.

This was fixed following the same recommendation from reviewer 1.

L93: Was the internal standard also used as a recovery standard to account for losses during sample processing? This is not mentioned later in the paragraph regarding *n*-alkane quantification (L107).

Strictly, we only used the internal standard to normalize the peak areas of the n-alkanes in the chromatogram using the peak area from the standard and the known concentration of standard added. This allowed us to compare the relative abundance of the n-alkanes in different samples. We spiked the sample after extracting the total lipid extract (TLE) from the sediments, but before performing the separation of the TLE through solid phase extraction (SPE). This could be seen as a recovery standard to some researchers, but others would suggest naming it otherwise.

To strive for clarity, we added the following paragraph in line 107: <u>We used the internal</u> standard to normalize the peak areas of the n-alkanes in the chromatogram. The internal

standard was spiked into the sample after extracting the TLE from the sediments, but before performing the separation of the TLE through SPE. This allowed us to compare the relative abundance of the n-alkanes in different samples, while accounting for any losses that may have occurred during SPE.

L198: Reference format: should not be in separate brackets?

Corrected following the same suggestion made by reviewer 1.

L445: 'They' refers to herbaceous plants? 'Deeper rooting depths' relative to what (other vegetation types or with aridity, I presume the latter, but it is not entirely clear from this sentence)?

Thank you for bringing this to our attention, we rephrased the sentence in line 445 to increase clarity: Also, in desert ecosystems, herbaceous plants generally have deeper rooting depths in comparison to woody plants like shrubs,

L493-494: '..., as they integrate over larger regions.' seems a bit confusing at the end of the sentence since '... as they average both vegetation and climatic variability to a greater extent ...' is already mentioned before (i.e. last part of the sentence is redundant I think). Perhaps change to something like: '... as they integrate both vegetation and climatic variability over larger regions than soil samples.'

Thanks for this suggestion, it was implemented directly as suggested.

Table 4: This table seems somewhat redundant, as the p values are already shown in Fig. 7. The table itself could perhaps be moved to a supplemental info document or somehow processed into the text, if manuscript length would be in issue.

After revising it, we agree with this comment and see no relevant new information presented in Table 4. Since the p-values are shown in Fig. 7, it can be considered redundant, therefore we move it into the supplement of the manuscript where it can be found as Table S9.

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