REPLY TO REVIEWER #1:

We are grateful for the detailed review and these useful suggestions provided by Reviewer #1. The provided comments will contribute substantially to improving the paper. We will implement most of the suggestions in the revised version of the manuscript. Please, find below in black the comments of the reviewer, in blue our responses to the comments and how these comments will be addressed in the revised manuscript.

The study by Voigt et al. focuses on the main drivers of temporal variations in the triple oxygen isotopic composition of leaf water and phytoliths. The authors performed a grassland plot experiment and measured key physiological, isotopically, and environmental drivers over the seasonal course and over the course of the diel cycle during two days of the experiment. The authors then compared the measured leaf water isotope values with predicted ones derived from Craig-Gordon based steady state and non-steady isotope models, as well as performed a sensitivity analysis to infer the main cause of isotopic variations in leaf water of grasses. As a novel part, they included high-resolution measurements of water vapour isotopes and measured leaf temperature data to improve the models. Besides, the authors found a relationship between daytime RH and 17O-excess of grass phytoliths.

The paper is overall nicely written, and the methods and results are well presented. Yet, after reading through the complete manuscript, I felt that the conclusions of the paper remain vague and that the discussion on the phytolith part, which is highlighted in the title of the paper, falls brief and short, while the leaf water isotope part, which is not highlighted in the title, is well discussed. Given the imbalance, I feel that some parts of the paper should be revised to match the title or that the title itself should be revised.

We thank both reviewers for pointing out that the discussion on the phytolith part falls short in the current version of the manuscript. We agree on this and will extend the discussion on our results in relation to previously published data, better highlight the advantage of ^{17}O-excess in comparison to the traditional isotope variables (δ^{18}O, δ^{2}H, d-excess) and providing tracks for the application of ^{17}O-excess of phytoliths to reconstruct paleo-RH in the revised version. We will additionally revise the title.

Major

Discussion 4.3: I think the start of the paragraph reads nice, but then it's not clear how the RH signal in phytoliths 17O-excess in the current study is linked to previous observations and applications. Did the authors expect to find phytoliths' 17O-excess values to be close to daytime than to daily RH values?

In previous experiments carried out in growth chambers environmental parameters were set constant, without considering day-night cycles. Here, under natural conditions, we investigate whether daytime or daily RH should be considered in the equation linking ^{17}O-excess$_{phyto}$ and RH. This will be further detailed in the revised manuscript.

How well does 17O-excess perform compared to d18O, which was also measured and is well known to carry RH and VPD signals if derived from plant water and material?

This is an important point, and we thank the reviewer for pointing out that this is not clearly explained in the manuscript. ^{17}O-excess is little variable in atmospheric water vapor and in rainfall at the seasonal or yearly scale, in contrast to δ^{18}O. ^{17}O-excess of leaf water and phytoliths is

therefore a more direct tracer for changes in RH. We will discuss the advantages of ^{17}O-excess compared to $\delta^{18}O$ in relation to our observations in the revised manuscript.

Would it also make sense to measure d2h and d-excess in phytoliths (maybe not possible)?

Phytoliths are made of amorphous silica (SiO_2 $(H_2O)_n$) in which the hydroxyls (OH) and water molecules (H_2O) are exchangeable with the surrounding environment. Thus, these molecules are removed by heating (1100°C) under a N_2 flow prior to the isotope analysis. There is no more H or H_2 to analyze after this dehydration step. This will be detailed in the revised manuscript.

Despite, combination of phytolith with other established proxies, e.g. δ^2H in leaf waxes or $\delta^{18}O$ in cellulose, can be part of future work. Combining multiple proxies will allow to achieve more robust estimates of past RH, and open perspectives to obtain a bigger picture of relations between climate and vegetation in the past.

Are there field studies with spatial or temporal resolution on phytoliths 17O-excess values, providing similar results?

The only data from natural sites are from phytoliths extracted from soils sampled along an aridity transect in Central and West Africa (Alexandre et al., 2018). These data better reflect the daily average RH over the growing season rather than RH in the afternoon. However, the scatter is large, the soil phytoliths assemblages represent a mixture of different vegetation sources and we have no information on leaf-to-air temperature gradients that may significantly bias the RH estimate. In the revised manuscript, we will extend the discussion on the implications of our results for the application of this proxy on soil phytoliths.

The discussion should also consider that the authors have only 3 values for phytoliths 17O-excess, which makes it difficult to set up a relationship with RH.

The objective was to verify that the obtained data are consistent with the equation obtained from the growth chamber calibrations, which is the case. More data is needed, in particular from regions with contrasting daytime vs daily RH, and different natural contexts (tropical forest, savannah grassland, steppe, temperate regions, etc.) to generalize our conclusions. We will provide tracks for future studies in the revised version of the manuscript.

The discussion on leaf transpiration and development should be combined with the one in the result part Line 406-412. I would also recommend incorporating the information in Figure 5.

We agree. This section will be implemented in the discussion in revised manuscript.

Minor:

Introduction: I am wondering whether it would be worth highlighting that the 17O-excess approach in leaf water is still rather novel compared to d18O and d2H application In my opinion, that is one of the novel parts of the study, but it does not clearly drop out of the introduction.

We agree. In the revised manuscript, we will better highlight the advantages of ^{17}O-excess in leaf water and phytoliths in comparison to traditional isotopes ($\delta^{18}O$, δ^2H, d-excess).

Line 21 and 23: If “the” is used, its not clear to which specific model (e.g. the CraigGordon Model) it refers to.

We will specify this in the revised version.

Line 24-25. I think these results are not clearly illustrated in the discussion part and figures.

In the revised manuscript, we will extend the discussion on when we expect ^{17}O-excess of phytoliths to reflect daily vs daytime and provide tracks for the application of this proxy on paleo-records.

Line 27: Yes, but it provides also new knowledge regarding the climate-sensitivity of leaf water stable isotope variations and their models, which I think is not well highlighted so far.

Our study shows that ^{17}O-excess of atm water vapor and inflow varies little from diurnal to monthly timescale and thus has little influence in ^{17}O-excess in leaf water. The ^{17}O-excess in leaf water is mainly driven by RH. In the revised manuscript, we will highlight these advantages of ^{17}O-excess in comparison to δ^{18}O.

Line 55: Not all abbreviations of the model are explained here, e.g. Rs, aeq, adiff

All used abbreviations will be explained in the revised manuscript.

Line 43: It might be worth adding the multiplication factor that changes “per mil” into “per meg” for 17O-excess.

In the revised manuscript, we will remove the multiplication factor of 1000 from the equation and specify that ^{17}O-excess is reported in “per meg”, which is 0.001 per mil.

Line 62: Written like that it implies that some of the previously cited publications “neglected” the two-pool idea. I think this statement really depends on the plant species, which is quite diverse through all these studies. Maybe rather highlight, that the two-pool idea is important for grass species (but see Liu et al 2017, doi: 10.1111/nph.14549), where parts of the bulk leaf water pool in grasses do not experience evaporation and thus isotopic enrichment. It should also be highlighted that grasses have large isotopic leaf water gradients from the bottom to the top and that “bulk leaf water” is integrating this gradient. Further, the leaf water isotope gradients are integrated into the d18O of plant compounds (but see various papers from Sternberg, Helliker, and Lehmann on plant carbohydrates). In this regard, do we know whether phytolith formation/synthesis is equal along the grass blades? The link between water and phytolith formation during leaf development and growing season could also be an interesting discussion point, particularly if Figure 5 would be considered for the discussion.

Thank you for pointing out this. Silicification and isotope composition of phytoliths along the grass leaf blade has been investigated in Alexandre et al. (2019). Although the ^{17}O-excess$_{phyto}$ increase with the distance to the leaf base, the ^{17}O-excess of the bulk phytoliths can be correctly modeled. In the revised manuscript, we will further discuss the differences between phytoliths with an isotope composition that reflects a bulk water content integrating variations in space and time and the leaf water isotope composition which reflects discrete conditions in space and time.

Line 68-70: I strongly assume this statement refers to the “Peclet” effect. I would thus suggest introducing this “term” here so that discussion and introduction are better “linked” to each other.

Yes, we refer here to the “Péclet” effect. We will specify this in the revised manuscript.

Line 70: Rleaf is not defined yet, right?

All used abbreviations will be explained in the revised manuscript.

Line 80: While I agree with the temporal separation, the example is not 100% clear to me. Assuming that sugars are produced under low RH, then they should carry this climatic information in their oxygen isotopic composition. It these sugars are later on used in the night/during rain, their isotopic composition formed under low RH should be transferred (at least partially) to the cellulose which is synthesized under high RH conditions. Maybe the moss example is a bit out of place. There are many studies on grass species and the isotopic composition of plant carbohydrates, which could be highlighted to make this point clearer (but see papers from groups of Schnyder, Helliker, and Lehmann).

We agree that using an example from a grass species may be more appropriate in view of our manuscript objective. We will address this in the revised manuscript.

Line 110: Maybe define “season” here, because it could be a growing season, but I think the authors mean “spring, summer, autumn”.

In the revised version, we will clarify that we refer here to different seasons of the year, and not to the growing season.

Line 120: What is the full name of the species? Is it a C3 or C4 plant? Mono or Dicot? This is important information because d18O in leaf water and cellulose and d2H in n-alkanes have been observed to depend on the physiological and biochemical background (but see different papers from Helliker & Ehleringer 2000 and 2002, and Gamarra et al, 2016, PCE).

We used the C3 grass species *Festuca arundinacea*, the same species that has been used for calibration of the $^{17}O\text{-excess}_{phyto}$ vs RH relationship in growth chamber experiments. As all grasses, it is a monocot species. We will add these details in the revised manuscript.

Line 120: Why was the study performed within a woodland, which I assume is a forest? How do I need to imagine this plot? A grass plot surrounded by trees? I also assume that the grasses were grown on the topsoil in the woodland and that the topsoil was fertilized, is this correct? Does the grass plot include any replicates?

As outlined in line 105, the grass plot was setup in the understory of a natural Mediterranean downy oak forest. The site was chosen, as it provides the necessary facilities for this extensive monitoring study, including meteorological and plant physiological measurements, and the infrastructure to install the Picarro CRDS instrument for on-site atmospheric water vapor measurements. As outlined in line 121-124, the grasses were grown on the shallow topsoil to which we added potting soil. The plot was fertilized to ensure a sufficient amount of nutriments and bio-available silica. No replicates of the grass plot were conducted. We think that this information is sufficiently covered in the current manuscript, so that no changes will be done during revision.

Line 125: The mean isotope value of the tap water could be provided here.

We prefer to show the mean isotope value of tap water in the results section, as it is done in the current version of the manuscript (line 265). The variability of the tap water isotope composition over the experimental period is illustrated in Fig. A2 of the current version.

Line 145: Maybe add the exact period after "over the day".

The period varied a bit from a sampling day to another, mainly from sunrise to sunset, except for the 24h monitoring. The data is illustrated in Fig. A3 and A4.

Line 158: Where the sampled leaf material fully developed and intact?

Yes, we sampled only fully developed, not senescent leaves. We will specify this in the revised version.

Line 163: What does "grass leaves" reflect? Only grass blades? How much material was harvested by end of the season?

Yes, we refer to grass blades. We will specify this in the revised manuscript. The whole grass plot was harvested by the end of each regrowth, resulting in 120 -150 g dry mass.

Table 1 and 2: I assume that providing the raw d17O results of water and phytoliths does not give any additional information and interpretation is only feasible for 17O-excess?

Yes, the $\delta^{17}O$ doesn't provide directly additional information to $\delta^{18}O$ as deviations from GMWL are small. The $\delta^{17}O$ may be back-calculated with high precision from ^{17}O-excess and $\delta^{18}O$ using the equation given in the introduction section.

Table 2: How many replicates were measured for the phytolith isotopic composition and what does the standard deviation reflect? I assume the plots were not "repeated" and that the SD is "technical replicates". Finally, how exactly was the SiO2 concentration determined? BTW, I think "rate" is the wrong word here because a change over time was not measured, right? Does the SiO2 concentration reflect the amount of phytoliths per gram biomass (i.e, SiO2 = phytoliths)? Do the long and short-cell phytoliths refer to the different "types" as stated in the method (Line 224)?

The SD is determined based on 4 replicates. We will specify this in table caption in the revised version. The silica contents of harvested grass blades were determined by inductively coupled plasma-atomic emission spectroscopy (ICP-AES). The silicification rate is calculated from the measured SiO_2 concentration at the end of the regrowth and the length of the regrowth period, assuming a linear production rate (av. rate). Yes, we assume that SiO_2 = phytolith, and LC = refers to the proportion of long cell phytoliths on the sum of short and long cell phytoliths in the sample. We will clarify this in the revised manuscript.

Line 207: Plant water refers to leaf water, or did I miss something, e.g. another plant tissue?

Yes, we refer here to leaf water. We will use "leaf water" throughout the revised manuscript.

Line 208: Maybe consider/acknowledge that isotope fractionation can occur during CVD extraction, but it affects d2h more than the d18O (Chen et al, PNAS). To my knowledge, not much is known for d17O. Yet, also amount effects can change the isotopic composition of CVD-extracted water (Diao et al. 2022, HESS). Here I would simply double-check whether the extracted water content was high enough (e.g. difference of weight of exetainers before and after CVD extraction?).

Water yield after leaf water extraction was 103 ± 5% in average and always higher than 94%. We will add this information in the revised manuscript.

Line 223: The extraction of the phytoliths should be explained in more detail. Its not clear how the phytoliths have been taken or extracted from the leaf material. How much biomass is needed in order to get a sufficient amount of phytoliths? I assume this is time-consuming and difficult work, which then also explains/justifies the low number of replicates in this study.

Phytoliths were extracted following the "wet digestion" protocol in Table 2 of Corbineau et al. (2013). We will specify this in the revised manuscript but will not provide details on the protocol in the manuscript. Instead, we outline the individual steps here: 1) Grass leaves were cut in cm-sized pieces and dried overnight. 2) Dried samples were immersed in 1 N HCl and heated to 80ºC for 2h, subsequently rinsed with deionized water and dried. 3) Concentrated H_2SO_4 was added, and the solution was heated for 5h at 80ºC. 4) 30% H_2O_2 is slowly added and again heated for 8h. 5) 65% HNO_3 and a pinch of $KClO_3$ is added and the solution is heated at 80ºC for 7h. 6) Before being decanted and rinsed with deionized water, the solution remains unheated overnight. 7) The phytoliths are immersed in 0.001 M KOH solution and heated to 130ºC for 10 min. 8) Finally, samples are rinsed with deionized water and dried.

Line 244: Which values were used for gs and gb and were these derived from own measurements or from the literature?

As described in line 146-147, stomatal and boundary layer conductance were measured continuously on one selected leaf using a Li-64000 XT gas exchange system. Values averaged over 30 min before grass leaf sampling are provided in Table S3. In addition, stomatal conductance was measured hourly on ten randomly selected leaves to assess the spatial variability using an AP4 porometer (Line 148-149). The Li-COR measurements are generally within the range of stomatal conductances observed with the porometer (cf. Fig. A4). We will clarify this when describing the model approach.

Line 259: So no leaf water content data is available for this study?

We determined water yield during leaf water extraction, but unfortunately do not have enough data to calculate absolute leaf water content (W m^{-2}).

Line 260: Maybe slightly rephrase, and state that a best-fit model was used to set W.

We will rephrase this sentence in the revised manuscript.

Figure 2, Line 322: add "red" to "Dashed circle". Throughout I would avoid using "yellow" in the figures, because its hardly visible.

We will revise the color schemes for all figures to allow readers with color vision deficiencies to correctly interpret our findings.

Line 335-345: How exactly were d-excess and 17O-excess modeled with the Craig-Gordon model? I assume one needs the input of d2h, d17O, and d18O data, then run the model once for each isotope ratio and combine the data to gain excess estimates (e.g d18O and d2h for d-excess, and d17O and d18O for 17O-excess). Please clarify all this in the method part.

The model calculations performed according to Eq. 1-2 for the C-G steady state model and to Eq. 5a,b for the non-steady state model are presented in the supplement tables S3 and S4, respectively. Model calculations are performed for $\delta^{2}H$, $\delta^{17}O$ and $\delta^{18}O$, and secondary parameters d-excess and ^{17}O-excess are derived from predicted primary isotope values. All model input variables have been measured. We will clarify this in the modeling section.

Figure 2, 3: What about d2h? The data is shown in the diurnal cycle, but not in the seasonal cycle.

We will add $\delta^{2}H$ in the figures in the revised manuscript.

358: I would suggest linking the results more clearly to each panel of Figure 4 a – x.

We agree. In the revised version, we will change the order of the panels according to the main text and add the references to each of the panels in the main text.

Figure 4: It appears that a change in temperature is stronger than a change in RH, but this sounds not right to me. From my own experience, the effect of temperature on d18O values in water and organics is rather secondary and induced via the equilibrium fractionation factor, which typically only varies around 2 per mil for d18O between 10 and 30°C or so. Moreover, why have these values been chosen for the sensitivity analysis, e.g. 5% RH and 2°C? Can we really compare them? Does it reflect an x-percent change per mean of each variable?

It is correct that temperature has generally only little effect on $\delta^{18}O$. However, here, we do not change only temperature, but modify the leaf-to-air temperature gradients. This affects the effective relative humidity (h), which is the ratio of atmospheric water vapor pressure over saturation vapor pressure at leaf temperature. 2ºC change in $\Delta T_{leaf\text{-}air}$ are common in nature and can have a strong effect on leaf water isotope composition, as illustrated in Fig. 4. We will emphasize this in the revised manuscript and justify the used values for RH.

Line 406-412: This is an interpretation of the results and should be moved to discussion 4.3.

We agree. This section will be implemented in the discussion in revised manuscript.

Figure 5, Line 413-421: This part comes a bit out of the blue and it is not yet nicely incorporated in the discussion on the phytoliths. I would also suggest adding more information on the forming water (FW) model and isotope fractionation factor (alpha and lambda values) in the result part. In which space does the alpha value vary and can they be given for each of the examples? If the purpose of figure 5 is to link 17O-excess and d18O of leaf water with those in the "phytoliths forming water", this should be discussed in more detail.

We thank both reviewers for pointing out that figure 5 is not clear and not well implemented in the discussion of the current manuscript version. In the revised version, we will provide more details on the variability of temperature-dependent isotope equilibrium fractionation factors. Further, we will discuss the implications of these results for RH reconstruction from ^{17}O-excess of fossil phytolith assemblages.

Line 450: So it seems that the Peclet effect could play a role for the leaf water model in the case of high transpiration. Why not provide some values of the Peclet corrected CG-model for the discussion? I assume all the parameters are available to run this model, right?

We will do some model calculations considering Péclet effect and implement the results in the discussion section in the revised manuscript.

Line 489ff: I agree with the paragraph. Maybe it should be more clearly considered that changes in the water vapour isotopic composition are more rapidly affecting the leaf water isotopic composition, within hours (but Lehmann et al. 2020, PCE for grasses), while the isotopic composition of precipitation has to go through the soil before its taken up by the plant and transported to the leaf.

We thank both reviewers for pointing out that the impact of observed isotope variability in the atmospheric water vapor on leaf water is not well discussed in the current version of the manuscript. We will address this point in the revised version.

Figure 6: The irrigation water point could be dropped for decreasing the x-axis scale and reduce the large space on the left side in the figure.

We think that showing the irrigation water is important to get an impression of the evaporative effect of transpiration on the leaf water isotope composition. We will keep this figure as it is in the revised manuscript.

Line 512-516: Please clarify whether the cited papers and equations are derived from grasses or from other species too. Please also clarify how many measurements of phytoliths 17O-excess were taken to generate the linear models, as well as the RH conditions of this study, to have some context. I assume that the RH conditions were similar to those in the current study.

These data are derived from the same grass species *Festuca arundinacea*. The linear model is based on a total of 16 measurements of ^{17}O-excess of phytoliths at RH of 40,60 and 80%. We will provide these details in the revised manuscript.

Line 528-531: I think the discussion on the nighttime transpiration/stomatal conductance on tropical tree species goes a bit too far, as the current study focuses on grass species.

We agree. We will revise this section in the revised manuscript.

Line 561: I would be surprised if this is really the "first continuous record" given that the laser spectrometer measuring d2h, d18O, d17O are available for some years. How novel is the data? Please clarify this.

The ^{17}O-excess of water vapor has been monitored in laboratory experiments (Brady and Hodell, 2021; Outrequin et al., 2021). However, here we present the first continuous record of ^{17}O-excess of atmospheric water vapor in the natural environment. Continuous high-precision measurements of ^{17}O-excess atm water vapor measurements by CRDS are complex, highly laborious, and cost-intense (cf. Voigt et al., 2021), and rarely been used so far.

572-574: Maybe I missed it, but how was the temperature non-sensitivity of phytoliths 17O-excess determined? Can the authors refer to a table or figure? Maybe move this to discussion point 4.3.

The impact of leaf-to-air temperature gradients on the ^{17}O-excess of phytoliths is assessed by comparing atmospheric relative humidity (RH) and the effective relative humidity (h), which is the water vapor pressure ratio between the leaf and the atmosphere (Fig. 7). The difference between reconstructed RH and h is lower than the uncertainty on the reconstructed values (>4%). Thus, we conclude that the small leaf-to-air temperature gradients observed in our study (<1.1ºC) do not significantly impact the RH estimates in our case. In the revised manuscript, we will discuss in more detail how leaf-to-air temperature gradients can affect phytolith isotope composition and when we expect this effect to become significant.

576: "RH proxy that is 17O-excess" reads a bit strange

We will rephrase this in the revised manuscript.

576-579: That's a good point, but this has not been discussed yet. See my comments on Figure 5.

See reply to comment on Figure 5.

Discussion 4.1: Is the CG steady-state model also working for d2H of leaf water in the current study? Maybe its worth adding a sentence on that.

The C-G steady state model prediction also agrees within uncertainty with measured δ^2H. We will implement the δ^2H in figure 2 and 3 in the revised manuscript.

Discussion 4.2: The results suggest that there is a difference in the water vapor influence on the temporal changes in d18O vs. 17O-excess, right (Figure 4)? If correct, maybe this is worth briefly discussing.

This comment builds on previous comments regarding the advantage of 17O-excess vs d18O and the link between isotope variability in atm water vapor and leaf water. Our data show that high variability in $\delta^{18}O_{vapor}$ strongly influence $\delta^{18}O_{leaf}$ (hourly timescale, Fig. 3). However, $^{17}O\text{-excess}_{vapor}$ varies little compared to the large variations in $^{17}O\text{-excess}_{leaf}$ with changes in RH (from hourly to monthly timescale). Thus, RH changes estimated from $^{17}O\text{-excess}_{leaf/phyto}$ are more robust than from $\delta^{18}O_{leaf/phyto}$ as $\delta^{18}O_{leaf}$ also depends strongly on source water / atm water vapor isotope composition. In the revised version, we will discuss the advantages of using $^{17}O\text{-excess}_{phyto}$ instead of $\delta^{18}O_{phyto}$ to estimate paleo-RH.