Dear Editor,

Thank you for your letter and for the reviewer's comments concerning our manuscript entitled "Controls on leaf water hydrogen and oxygen isotopes: A local investigation across seasons and altitude" (hess-2022-246). Those comments are all valuable and very helpful for improving our paper and for future studies. We have studied those comments carefully and have made corrections or explanations accordingly. Responses to the referee' comments are as follows:

Comments to the author:

We are pleased to inform you that the Editor report for the following HESS manuscript is now available:

hess-2022-246
Title: Controls on leaf water hydrogen and oxygen isotopes: A local investigation across seasons and altitude
Author(s): Jinzhao Liu et al.
MS type: Research article
Iteration: Major revision

Thank you. We read the reviewers' comments and carefully considered them. Many thanks to the reviewers for the helpful suggestions and comments, we have revised the paper accordingly and answered the questions carefully.

Reviewer #1:

Comment:

I appreciate the Editor to give me a chance to review the paper.
The manuscript "Controls on leaf water hydrogen and oxygen isotopes: A local investigation across seasons and altitude" presents a dataset on analysis of δ18Oleaf and δ2Hleaf together with isotopes from potential source waters and meteorological parameters along an elevation transect on the Chinese Loess Plateau. The research topic is important and within the scope of the journal. But it seems a bit simple and not systematic in the content. The manuscript at present lacks novel results or theory that would provide a significant advance in this field.

Response:

Thanks a lot for your comments. Our study have two significant novel points: 1) the previous studies have always emphasized on the combined $\delta^{18}O$ and $\delta^{2}H$ values of leaf water ($\delta^{18}O_{leaf}$ and $\delta^{2}H_{leaf}$), few studies considers the respective responses or variations of $\delta^{18}O_{leaf}$ and $\delta^{2}H_{leaf}$. A recent global meta-analysis indicate that the respective $\delta^{18}O_{leaf}$ and $\delta^{2}H_{leaf}$ reflected differently, seen details in Cernusak et al. (2022; NP). However, our local-study supported that $\delta^{2}H_{leaf}$ responds more closely to xylem water than $\delta^{18}O_{leaf}$, but both $\delta^{18}O_{leaf}$ and $\delta^{2}H_{leaf}$ responds comparatively to climatic factors (RH, T), challenging the global meta-analysis (Cernusak et al., 2022); 2) We proposed a framework that control the leaf water isotope line by using multivariate statistical methods (Hierarchical

clustering, Craig-Cordon model, Structural equation model, HYSPLIT, etc)

Reference

Cernusak, L. A., Barbeta, A., Bush, R., Eichstaedt R., Ferrio, J., Flanagan, L., Gessler, A., Martín-Gómez, P., Hirl, R., Kahmen, A., Keitel., C., Lai, C., Munksgaard, N., Nelson, D., Ogée J., Roden, J., Schnyder, H., Voelker, S., Wang L., Stuart-Williams, H., Wingate, L., Yu, W., Zhao, L., Cuntz, M., 2022. Do ^{2}H and ^{18}O in leaf water reflect environmental drivers differently? New Phytologist, DOI: 10.1111/nph.18113.

Comment:

1) *The main conclusion of this paper has been confirmed by previous studiesï¼ the first-order control on δ18O leaf and δ2Hleaf values was the source waterï¼and the second-order control was the enrichment associated with biochemical and environmental factorsï¼Cernusak et al., 2016; Barbour et al., 2017; Munksgaard et al., 2017). The experimental design and results of the paper are not innovative.*

Response:

Thanks. "The first-order control on $\delta^{18}O_{leaf}$ and $\delta^{2}H_{leaf}$ values was the source waterï¼and the second-order control was the enrichment associated with biochemical and environmental factorsï¼Cernusak et al., 2016; Barbour et al., 2017; Munksgaard et al., 2017)" is deed analyzed by previous studies, as discussed in Introduction section. Our studies analyzed the responses of respective ($\delta^{18}O_{leaf}$ and $\delta^{2}H_{leaf}$) and both to source waters (xylem water, soil water, and precipitation) and to meteorological parameters (temperature, RH). As above stated, we have two significant novel points.

Comment:

2) A large number of studies have shown that the enrichment associated with plant transpiration is an important factor affecting δ18Oleaf and δ2Hleaf values. However, the authors did not carry out research and discussion in this paper.

Response:

Thanks. We have added more discussion on transpiration.

Comment:

3)Plants and soils were sampled in May, July, and September 2020 (In the experimental design). Why only choose this three months? Is it persuasive?

Response:

Thanks. The growing season lasts from late April to Early October on the Chinese Loess Plateau, so we selected the pre- (May), peak (July), and post-(September) growing season. Also, the precipitation $\delta^{18}O$ and $\delta^{2}H$ varies across months (Fig. 5a, b), which was caused by different moisture transport routes from HYSPLIT (Fig, 5c).

Additionally, we sampled at the same plots (ten plots) along an elevation transect from ~600 m to ~3600 m, the three repeated sampling is OK. If more, the sampling will be a burdensome work and the plants is not available for more repeated sampling.

Comment:

4ï¼Besides, what is the specific sampling interval?

Response:

Thanks. The sampling plots were arranged for ten plots from ~600 m to ~3600 m along an elevation transect, which was detailed in Fig, 1 and supplementary Table S1.

The sampling plots were randomly selected in the first campaign from the bottom to top of mountain, then repeated by the next two sampling campaigns.

Comment:

5) Why only one or two deciduous and coniferous trees were chosen in each plot?

Response:

Thanks. There was a significant vegetation zone along an elevation transect of Mt. Taibai (Fig.1 and M&M), so we selected the dominant species at each zones.

Comment:

6) There are large differences in population and altitude between sampling points 5-8(Fig.1). But there is no weather station here.

Response:

Thanks. The weather stations along an elevation transect was very hard to settle up, the available weather stations were presented in Fig.1. We thank to Shaanxi Meteorological Bureau for supporting meteorological data. It is possible that more weather stations will be settled up along this elevation transects in the future.

Comment:

7) In 4.1ï¼these results argued with the recent global meta-analysis thatδ18Oleaf and δ2Hleaf values reflect climatic parameters (i.e., RH and temperature) differently. What are the reasons for the controversial conclusion?

Response:

Thanks. It is really a good question, I think it is probably due to the scale difference, e.g., global vs. local. The reason needs to be further explored in the future.

Comment:

8) It seems a bit simple in the conclusion. It needs a stronger ending for the conclusion. Besides, it is suggested to supplement the existing deficiencies and prospects.

Response:

Thanks. We have strengthened the conclusion.

Reviewer #2:

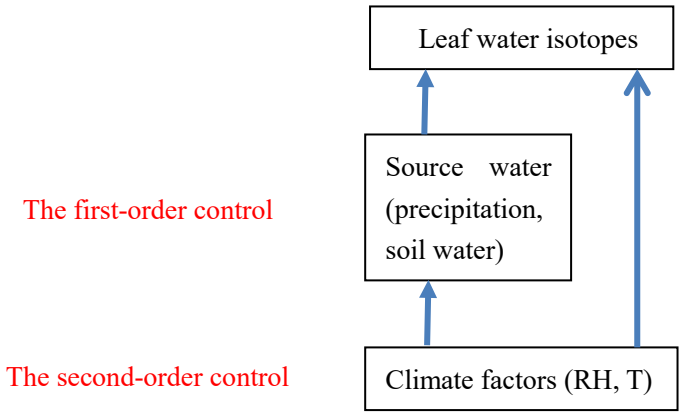
Comment:

Liu et al, based on field sampling of leaf water and measuring isotope composition (δ18O and δ2H) along an elevation transect on the Chinese Loess Plateau belt to illustrate controls on leaf water hydrogen and oxygen isotopes. The results point that the first-order control on δ18O and δ2H was the source water, and the second-order control was the enrichment associated with biochemical and environmental factors. Overall, this is an important and hard-working investigation for deepening the understanding of the control of leaf water isotopic composition in field conditions. However, the current analysis is mediocre, the scientific questions are poorly elaborated, and new discoveries are lacking. Secondly, the results show that source water is the main control of leaf water isotopes, which is contrary to the previous results which indicate that relative humidity is the main control of leaf water isotopes both under leaf and ecosystem scale. Therefore, there requires more evidence to support your conclusion. I recommend major revisions before considering publication in this journal.

Response:

Thanks a lot for your approvedness and suggestions. For the first question, we have two significant novel points: 1) the previous studies have always emphasized on the combined $\delta^{18}O$ and $\delta^{2}H$ values of leaf water ($\delta^{18}O_{leaf}$ and $\delta^{2}H_{leaf}$), few considering the respective responses or variations of $\delta^{18}O_{leaf}$ and $\delta^{2}H_{leaf}$. A recent global meta-analysis indicate that the respective $\delta^{18}O_{leaf}$ and $\delta^{2}H_{leaf}$ reflected differently, seen details in Cernusak et al. (2022; NP). However, our local-study supported that $\delta^{2}H_{leaf}$ responds more closely to xylem water than $\delta^{18}O_{leaf}$, but both $\delta^{18}O_{leaf}$ and $\delta^{2}H_{leaf}$ responds comparatively to climatic factors (RH, T), challenging the global meta-analysis (Cernusak et al., 2022); 2) We proposed a framework that control the leaf water isotope line by using multivariate statistical methods (Hierarchical clustering, Craig-Cordon model, Structural equation model, HYSPLIT, etc). The leaf water isotope line was generated by our analysis, which provides an important baseline for leaf-derived organic matter such as cellulose and leaf wax.

For the second question, our results were actually consistent with the previous conclusion. We proposed a hierarchical control on leaf water isotopes, as the following figure. The first-order control is source water, which is also affected by climate factors (e.g., T, RH). The climatic factors (e.g. RH) affect source water and directly affect leaf water isotopes. Without considering source water, the RH is the main control on leaf water isotopes.



Comment:

Minor revision:

Line 148-150, "For the plants, one or two deciduous and coniferous trees were chosen in each plot, and several large leaves and suberized twigs were collected for each species." Here should be describe in details.

Response:

Thanks. We have added more details.

Comment:

Line 214-215, How was the ð□□□ð□□ and ð□□□ð□□□ obtained? Here should be describe in details.

Response:

Thanks. We have added new citation, the kinetic fractionation equations for hydrogen and oxygen isotopes can be found in the reference.

Comment:

Line 264, predicted δ18O and δ2H values of leaf water were quiet simper, and need consider non stead state (NSS) under complex environment condition.

Response:

Thanks. It is a good question. We used the steady-state condition in this study because our sampling campaigns take place during the day when leaf water is generally near isotopic steady state because chloroplasts are mostly located near to the evaporative sites (Cernusak et al., 2016). The non-steady state effects on leaf water isotopes were expected at night because of low stomatal conductance (Cernusak et al., 2005; Cuntz et al., 2002; Cernusak et al., 2016).

Comment:

Line 456-459ï¼□there need more deep-seated analysis but not common knowledge in this area.

Response:

Thanks. We have depleted it. This needs to be further explored in the future.

Comment:

Line 466-469, The conclusion is too simple and needs to be further explored.

Response:

Thanks. We have strengthen the conclusion.