Response to reviews of manuscript “Excess radiation exacerbates drought stress impacts on stomatal conductance along aridity gradients” bg-2022-50

**Dear editor,**

We would like to thank you for the thoughtful and valuable comments and suggestions on our manuscript entitled “Excess radiation exacerbates drought stress impacts on stomatal conductance along aridity gradients” (bg-2022-50). We have carefully revised our manuscript to take account of reviewers’ comments and suggestions. Meanwhile, we have rephrased our manuscript title as “Excess radiation exacerbates drought stress impacts on canopy conductance along aridity gradients”. Meanwhile, the manuscript has been edited and polished by Dr. Kathryn B. Piatek (kbpiatek@gmail.com), a US-based forest ecologist, to ensure that the quality of the language will be acceptable. Here are the point-to-point responses (responses in upright Roman in black front) to the comments (original queries in Italic in blue front).

**Response to the reviewer #1**

**General comments:**

*1) I think this paper would greatly benefit from the inclusion of a more open, thorough and detailed description of the raw bulk leaf d18O, LA and SLA data obtained in the different regions, including additional figures depicting this basic information. Readers interested in the oxygen isotope composition of plants in general will surely want to see the raw leaf d18O data, as well as more detailed data on the d18O composition of rainfall water in the different regions (amount-weighed annual averages, range of values, etc). These data were used to estimate D18Oenrichment in the different sampling sites, so it is important to report these basic raw data as well. I would also like to see the averages, ranges of values, standard deviations, etc of the leaf d18O, SLA and LA values of the different grass species sampled in each region, as well as a listing of grass species names in each plateau. The detailed species listing could be included as Supporting Information material, but it is still important to provide this basic information for each plateau/climate region separately. Ideally, all this important descriptive information could be synthesized in 1 or 2 additional figures (or tables) that should be provided at the beginning of the Results section. Also, please briefly comment in the Discussion how your leaf d18O and D18Oenrichment range of values compares to other datasets previously published in the literature, especially for arid and semiarid grasslands (in both China and elsewhere across world dryland ecosystems).*

Response: Thank you very much for your valuable comments. Major revisions have been made as follows:

(1) The patterns of raw leaf $\delta^{18}O$ and $\triangle^{18}O$ at species level along aridity gradient were added as an additional figure in "Supplementary 1" (Please see **Supplementary 1-Fig.S2**). Meanwhile, characteristics (e.g. values of average, maximum, minimum, standard deviation and coefficient of variation of co-occurring species) of leaf $\delta^{18}O$ and $\triangle^{18}O$ at species level for sampling sites in Loess (LP), Inner Mongolia (MP), and Tibetan (TP) Plateau were added as an additional Table in "Supplementary 1" (Please see **Supplementary 1-Table S2**). The values $\triangle^{18}O$ of each community and amount weighted $\delta^{18}O$ of precipitation have been listed in Table S1 (Please see **Supplementary 1-Table S1**). Patterns of community LA and SLA among transects have been presented in Fig.1.

(2) Information of coexisting species in each community have been listed in "Supplementary 2".

(3) In "Discussion" section, we compared the community $\triangle^{18}O$ with a study conducted in arid and semiarid grassland: "Community $\triangle^{18}O$ in this study was relatively high (from a low of 26.8‰ in Loess Plateau (LP) to a high of 42.4‰ in Tibetan Plateau (TP)) (Fig.2a, Table S1). A previous study conducted in a temperate grassland (mean annual precipitation was 753 mm) reported $\triangle^{18}O$ of 28.2~30.53‰ (Hirl et al. 2021). This indicated that the canopy conductance ($G_s$), presented by community $1/\triangle^{18}O$, was relatively low in this study, and community reduces $G_s$ in response to drought stress." (Please see **page 10 lines 226-229**).

*2) The paper would also benefit from a more open acknowledgement that rainwater d18O is only a (reasonable) proxy of topsoil water d18O, which is the real source of water used by most grass species. Evaporative isotopic enrichment of soil water in upper soil layers during prolonged rainless periods in dryland ecosystems usually results in heavy enrichment in the 18O in the remaining soil water used by plants. Longer rainless periods and heavier evaporative enrichment of soil water in the drier sites along the aridity gradient could be also contributing to the reported patterns, but this questions is not addressed in the paper. I would appreciate the inclusion of a few sentences in the Discussion to address this caveat of the study. Despite this criticism, I admit that the approach used by the authors to estimate D18Oenrichment is legitimate, in the absence of data on culm water isotopic composition in each species (which I am assuming is not available). However, the readers should be aware that interspecific differences in rooting and water acquisition depth and phenology among coexisting grass species can lead to substantial differences in the isotopic composition of their water sources, which cannot be detected with the approach used in the present study (even though they will certainly affect the real d18O and D18Ovalues of the different species). This should also be mentioned and discussed in the paper.*

Response: Thanks very much for your comments and suggestions. We respond these comments from two aspects.

(1) We firstly discussed the potential effect of the differences in water acquisition

depth on interspecific variation in leaf $\delta^{18}O$ in "Discussion" section: "Interspecific differences in rooting and water acquisition depth and phenology among coexisting species can lead to substantial differences in the $\delta^{18}O$ of their water sources (Moreno-Gutierrez *et al.* 2012). Previous studies found that the depth of water uptake of co-occurring species in grasslands commonly occurred in shallow soil layers throughout dry and wet periods (Bachmann et al. 2015; Hirl et al. 2019; Prieto et al. 2018). The differences in water acquisition depth could be ruled out as a major source of interspecific variation in leaf $\delta^{18}O$ in this study (Prieto *et al.* 2018)." (Please see **page 13 lines 299-303**).

And then, we clarified that rainwater $\delta^{18}O$ is only a (reasonable) proxy of topsoil water $\delta^{18}O$ in the study area: "Generally, data on long-term stem water isotopic composition in each species are not available. As precipitation is the only or the main source of water in dryland ecosystems, we assumed that the amount-weighted $\delta^{18}O$ of precipitation during the growing season can reflect $\delta^{18}O$ of source water (Guerrieri *et al.* 2019; Maxwell *et al.* 2018). $\delta^{18}O$ of monthly precipitation at each site was simulated using longitude, latitude, and elevation according to (Bowen *et al.* 2005). " (Please see **page 5 lines 124-128**).

(2) We discussed the effects of evaporative isotopic enrichment of soil water in upper soil layers on topsoil water $\delta^{18}O$, and its potential effects on our conclusion: "However, soil evaporation always exhibited increasing trends with increasing aridity, and usually resulted in heavy enrichment in $\delta^{18}O$ in the remaining soil water used by plants (Lyu *et al.* 2021). Longer rainless periods and heavier evaporative enrichment of soil water along the aridity gradient could also contribute to a decreasing trend in community $1/\triangle^{18}O$. Consequently, our results may overestimate the decreasing trend in $G_s$ along the aridity gradient." (Please see **page 13 lines 303-307**).

*3) Important data are missing from the M&M section, including the elevation/altitude, mean annual rainfall d18O, mean annual VPD, and LMA, LA (average, range of values) of the 3 different plateaus. This important information could be provided by adding additional panels to Figure 1. Please also add an additional panel for mean annual temperature (the one shown is for mean summer temperature). In panel f, please enhance the scale and resolution of the Y axis, as some of the drier sites in the Tibetan Plateau appear to have extremely low precipitation values that are hard to interpret in the graph.*

Response: Thank you very much for your comments and suggestions. We respond these comments from three aspects:

(1) Values of longitude, latitude, altitude, mean annual and growing season values of abiotic variables (e.g. temperature, precipitation, VPD and soil moisture), $\delta^{18}O$ of precipitation, and community $\triangle^{18}O$ for sampling sites along the aridity gradient were added in Table S1 (Please see **Supplementary 1-Table S1**).

(2) Values of average, maximum, minimum, standard deviation and coefficient of variation of geographic and climatic information for transects were presented in Table S2 (Please see **Supplementary 1-Table S2**).

(3) Changes in growing season climatic variables and community properties (leaf area and specific leaf area) among three transects were added as Figure 1. Meanwhile, changes in mean annual precipitation, VPD, solar radiation, and temperature among three transects were added as Figure S1 (Please see **Supplementary 1-Figure S1**).

*4) I would recommend the authors to discuss the influence of temperature on leaf d18O and D18Oenrichment data much more in depth, according to earlier findings of Brent Helliker and collaborators, which I think are very relevant here (Helliker & Richter 2008 Nature, Song et al.,2011 New Phytologist).*

Response: Thank you very much for your comment. The effect of temperature on $\triangle^{18}O$ has been discussed in section "4.4 Using community-weighted $1/\triangle^{18}O$ as an indicator of canopy conductance" :

"The decreasing trend in community $\triangle^{18}O$ along aridity may originate from temperature and VPD through their effects on evaporation and isotopic exchange between water and organic molecules (Barbour & Farquhar 2000; Helliker & Richter 2008; Song *et al.* 2011). For example, the equilibrium fractionation factor for water evaporation is dependent on temperature (Bottinga & Craig 1968). Temperature and VPD gradients between leaf and ambient air influence the evaporative gradient from leaf to air (Helliker & Richter 2008; Song *et al.* 2011). In addition, biochemical $^{18}O$-fractionation during cellulose synthesis is sensitive to temperature, and the proportion of oxygen in cellulose derived from source water was humidity-sensitive (Hirl *et al.* 2021).

The potential effects of temperature and VPD on $\triangle^{18}O$ via evaporation and isotopic exchange between water and organic molecules could be ruled out in this study. The growing season temperature variation was small along three transects (LP=3.3 °C, MP=4.9 °C, and TP=3.8 °C) (Table S1). However, community $\triangle^{18}O$ ranged from 3.89‰ in MP to 7.78‰ in LP (Table S1, Fig.2a). Previous studies demonstrated that the sensitivity of temperature to $\triangle^{18}O$ was approximately 0.23‰/°C (Helliker & Richter 2008; Song *et al.* 2011). It seems that the changes in temperature were not a main contributor to the large variability in community $\triangle^{18}O$. Meanwhile, a positive relationship between community $1/\triangle^{18}O$ and temperature was observed in LP ($P<0.05$), and negative between community $1/\triangle^{18}O$ and VPD in TP (Table 1). However, partial correlation analyses showed that community $1/\triangle^{18}O$ was not related to temperature ($P > 0.05$) and VPD after controlling for $G_s$ (data not shown). This indicated that the variability in community $1/\triangle^{18}O$ was mainly determined by $G_s$." (Please see **page 13 lines 308-323**).

**Specific comments:**

*1) L83-90: Some of the references cited in this section may not be very adequate if they refer to the d18O of tree rings, which is a more complicated process influenced by other factors (post-photosinthetic and photosynthate transport processes, ligning synthesis, etc). I would recommend to cite here only papers dealing specifically with the d18O and/or D18Oenrichment of bulk leaves, which is the topic of the present paper (e.g. see Ramirez et al 2009 Plant Cell Environ or the work by Margaret Barbour, Regina Hirl or Cabrera-Bosquet and Araus). Also, some of the references cited in this section appear to be missing from the References section (Levesque, Keitel?).*

Response: Thank you very much for your comments and suggestions. We rechecked the cited reference, corrected and rephrased this section as: "Given that leaf $\delta^{18}O$ at species level is affected by the leaf water evaporation process, variability in stomatal conductance ($g_s$) is expected to be evident in leaf $\delta^{18}O$ (Barbour 2007; Barbour & Farquhar 2000; Farquhar *et al.* 1998). A negative relationship between $\triangle^{18}O$ and gs has been observed at species (Barbour & Farquhar 2000; Cabrera-Bosquet *et al.* 2011; Grams *et al.* 2007; Moreno-Gutierrez *et al.* 2012) and canopy scales (Cabrera *et al.* 2021; Hirl *et al.* 2021), and among communities along soil (Ramirez *et al.* 2009) and climatic (Keitel *et al.* 2006) gradients. Consequently, we selected $1/\triangle^{18}O$ to be used as proxy for $g_s$ at species level in this study." (Please see **page 5 lines 130-135**).

*2) Lines 266-274: this section dealing with the relationships between SLA and leaf oxygen isotopes is very confusing and hard to interpret. Please try to better clarify the nature of this relationship in the different plateaus, preferably illustrating it with some additional graphs (1/D18Oenrichment vs SLA plots?). To the best of my knowledge, this relationship was first examined in depth by Prieto et al 2018 (Functional Ecology) in dry grassland species, so it would be interesting to compare and discuss the patterns encountered in both studies.*

Response: Thank you very much for your comment. We respond these comments from two aspects:

(1) This paragraph has been corrected and rephrased as: "Our earlier preliminary study demonstrated that $g_s$ at species level was significantly affected by LA in TP at species level (Wang & Wen 2022). However, the effect of community LA on $G_s$ was weak (P=0.061) (Fig.S5a), and variability in $G_s$ along an aridity gradient was controlled by specific leaf area (SLA) (Table 1, Fig.S5b). This highlighted the difference in biological drivers of $g_s$ at leaf and canopy scales. Contrary to the results from the dry grassland species in Mediterranean (Prieto *et al.* 2018) and karst communities in subtropical regions (Wang *et al.* 2021), community $1/\triangle^{18}O$ significantly decreased with SLA in this study (Table S1, Fig.S5). This indicated that the traditional leaf economic spectrum theory may not be supported at community level in TP due to multiple environmental stressors. SLA generally decreases with increasing solar radiation, and increases with temperature and water availability (Poorter *et al.* 2009). In this study, commumnity SLA was negatively related to soil moisture, and positively related to maximum temperature (Table S5) indicating that

changes in commumnity SLA were mainly controlled by maximum temperature. However, the direct effect of SLA on $G_s$ in the structural equation was not significant (Fig.5c). This effect may be obscured by drought stress. ” (Please see **page 12 lines 266-276**).

(2) Meanwhile, relationship between community $1/\triangle^{18}O$ and LA and SLA have been added as Figure S5 (Please see **Supplementary 1-Figure S5**).

*3) L226: “and viceversa” is confusing and hard to interpret, please elaborate and explain what you mean here.*

Response: Corrected and rephrased as: “In addition, a global meta-analysis demonstrated that ecosystem conductance was mainly limited by low SM in xeric sites, and by VPD in mesic sites (Novick et al. 2016).” (Please see **page 10 lines 233-234**).

*4) L269-270: I don’t understand the term “high heat capacity” used in this sentence, please clarify.*

Response: This sentence has been deleted.

*5) L16: this sentence is confusing and difficult to understand, please rephrase and clarify what you mean here.*

Response: Corrected and rephrased as: “$G_s$ in TP was lower than that in the other two plateaus for a given level of aridity due to low temperature and high radiation.” (Please see **page 1 lines 17-18**).

*(6) L51: I think this sentence is inaccurate, as it is indeed possible to measure the leaf gas exchange rates of whole canopies using the appropriate methods (e.g. see Liberati et al 2021 Global Change Biology).*

Response: This sentence has been deleted.

*7) L78-79: Please rephrase and clarify your second hypothesis, it is difficult to understand.*

Response: Thank you very much for your comment. We rephrased and clarified our second hypothesis from three aspects.

Firstly, we clarified that the effects of direction and intensity of solar radiation and temperature on gs at species level strongly depend on their distribution range and the relationship with aridity. Below the optimal values, $g_s$ at species level increased with increasing solar radiation and temperature, while excess radiation and high temperature-associated high VPD or low soil moisture would suppress $g_s$ (Please see **page 2 lines 53-57**).

Secondly, the basic climatic context for the three grassland transect has been added in the last paragraph of "**1 Instruction**" section. Meanwhile, we clarified that the three grassland transects experience different solar radiation and temperature conditions at a given aridity, due to the differences in geographical locations of the three plateaus. The order of mean annual temperature and solar radiation across the sites is LP>MP>TP and LP<MP<TP, respectively. (Please see **page 3 lines 77-79**).

Finally, we rephrased the second hypothesis as: "high solar radiation and low temperatures will jointly suppress $G_s$ at a given aridity among transects". (Please see **page 3 line 81**).

**Response to the reviewer #2**

**General comments:**

*1) The second hypothesis does not make sense to me. It is an abrupt hypothesis, as authors described in earlier paragraphs that solar radiation and temperature can have both positive and negative impacts on gs. It would be useful to added the basic climatic context for the three grassland transect.*

Response: Thank you very much for your comment. We rephrased and clarified our second hypothesis from three aspects.

Firstly, we clarified that the effects of direction and intensity of solar radiation and temperature on gs at species level strongly depend on their distribution range and the relationship with aridity. Below the optimal values, $g_s$ at species level increased with increasing solar radiation and temperature, while excess radiation and high temperature-associated high VPD or low soil moisture would suppress $g_s$ (Please see **page 2 lines 53-57**).

Secondly, the basic climatic context for the three grassland transect has been added in the last paragraph of "**1 Instruction**" section. Meanwhile, we clarified that the three grassland transects experience different solar radiation and temperature conditions at a given aridity, due to the differences in geographical locations of the three plateaus. The order of mean annual temperature and solar radiation across the sites is LP>MP>TP and LP<MP<TP, respectively. (Please see **page 3 lines 77-79**).

Finally, we rephrased the second hypothesis as: "high solar radiation and low temperatures will jointly suppress $G_s$ at a given aridity among transects". (Please see **page 3 line 81**).

*2) It is essential that the authors to discuss the influence of temperature and VPD on ∆18O spanning large altitudinal and/or latitudinal gradients, because temperature and VPD may lead to large inter-site offsets in leaf δ18O values.*

**Response**: Response: Thank you very much for your comment. The effect of temperature and VPD on $\triangle^{18}O$ has been discussed in section "4.4 Using community-weighted $1/\triangle^{18}O$ as an indicator of canopy conductance" :

"The decreasing trend in community $\triangle^{18}O$ along aridity may originate from temperature and VPD through their effects on evaporation and isotopic exchange between water and organic molecules (Barbour & Farquhar 2000; Helliker & Richter 2008; Song *et al.* 2011). For example, the equilibrium fractionation factor for water evaporation is dependent on temperature (Bottinga & Craig 1968). Temperature and VPD gradients between leaf and ambient air influence the evaporative gradient from leaf to air (Helliker & Richter 2008; Song *et al.* 2011). In addition, biochemical $^{18}O$-fractionation during cellulose synthesis is sensitive to temperature, and the proportion of oxygen in cellulose derived from source water was humidity-sensitive (Hirl *et al.* 2021).

The potential effects of temperature and VPD on $\triangle^{18}O$ via evaporation and isotopic exchange between water and organic molecules could be ruled out in this study. The growing season temperature variation was small along three transects (LP=3.3 °C, MP=4.9 °C, and TP=3.8 °C) (Table S1). However, community $\triangle^{18}O$ ranged from 3.89‰ in MP to 7.78‰ in LP (Table S1, Fig.2a). Previous studies demonstrated that the sensitivity of temperature to $\triangle^{18}O$ was approximately 0.23‰/°C (Helliker & Richter 2008; Song *et al.* 2011). It seems that the changes in temperature were not a main contributor to the large variability in community $\triangle^{18}O$. Meanwhile, a positive relationship between community $1/\triangle^{18}O$ and temperature was observed in LP ($P<0.05$), and negative between community $1/\triangle^{18}O$ and VPD in TP (Table 1). However, partial correlation analyses showed that community $1/\triangle^{18}O$ was not related to temperature ($P > 0.05$) and VPD after controlling for $G_s$ (data not shown). This indicated that the variability in community $1/\triangle^{18}O$ was mainly determined by $G_s$." (Please see **page 13 lines 308-323**).

**Specific comments:**

*1) Line 109: replace "plats" with " plots".*

**Response**: Corrected, thank you.

*2) Lines 175-177, partial correlation analyses can be used to examine the actual links between 1/△18O and soil moisture and vapor pressure deficit in Tibetan Plateau.*

**Response**: Corrected and rephrased as: "Partial correlation analyses showed that $1/\triangle^{18}O$ was not related to SM ($P > 0.05$) after controlling for VPD, indicating that variability in $1/\triangle^{18}O$ in TP was mainly determined by VPD." (Please see **page 8 lines 189-190**).

*3) Figure 4, please add the meaning of the asterisks and arrows.*

Response: To avoid confusion, we split Figure 4 into two graphs (Figure 4 and Figure 5). Meanwhile, the meaning of the asterisks and arrows have been added.

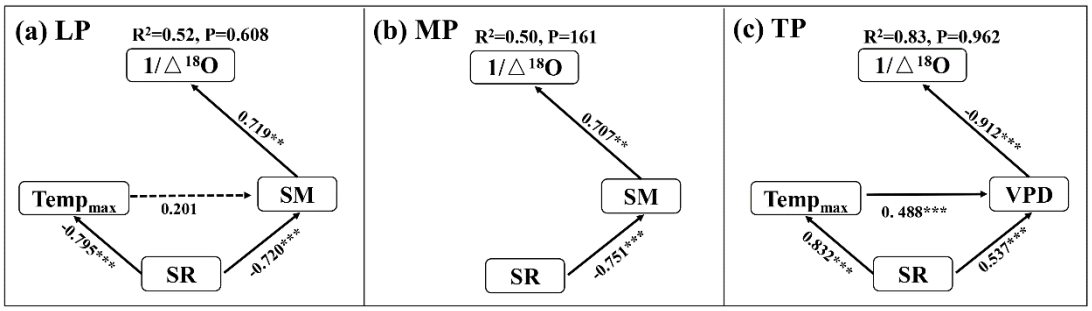


**Figure 4.** Structural equation models of abiotic factors explaining community $1/\triangle^{18}O$ in Loess Plateau (LP) (a), Inner Mongolia Plateau (MP) (b) and Tibetan Plateau (TP) (c). $\triangle^{18}O$, $^{18}O$ enrichment above source water of leaf organic matter; $Temp_{max}$: maximum temperature; SR, solar radiation; SM, soil moisture; VPD, vapor pressure deficit. Solid and dashed arrows represent significant and non-significant relationships in a fitted SEM, respectively. ***, P<0.001; **, P<0.01; *, P<0.05.

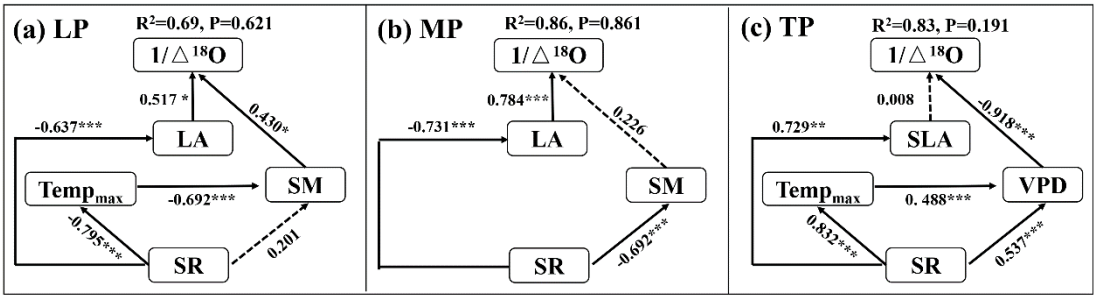


**Figure 5.** Structural equation models of abiotic and biotic factors explaining community $1/\triangle^{18}O$ in Loess Plateau (LP) (a), Inner Mongolia Plateau (MP) (b) and Tibetan Plateau (TP) (c). $\triangle^{18}O$, $^{18}O$ enrichment above source water of leaf organic matter; $Temp_{max}$: maximum temperature; SR, solar radiation; SM, soil moisture; VPD, vapor pressure deficit. LA, log-transformed leaf area; SLA, log-transformed specific leaf area. Solid and dashed arrows represent significant and non-significant relationships in a fitted SEM, respectively. ***, P<0.001; **, P<0.01; *, P<0.05.

*4) Line 270, SLA integrates leaf tissue density and thickness.*

**Response**: Thank you very much for your comment. This sentence has been removed, and this paragraph has been corrected and rephrased as: "Our earlier preliminary study demonstrated that $g_s$ at species level was significantly affected by LA in TP at species level (Wang & Wen 2022). However, the effect of community LA on $G_s$ was weak (P=0.061) (Fig.S5a), and variability in $G_s$ along an aridity gradient was controlled by specific leaf area (SLA) (Table 1, Fig.S5b). This highlighted the difference in biological drivers of $g_s$ at leaf and canopy scales. Contrary to the results from the dry grassland species in Mediterranean (Prieto *et al.* 2018) and karst

communities in subtropical regions (Wang *et al.* 2021), community $1/\triangle^{18}O$ significantly decreased with SLA in this study (Table S1, Fig.S5). This indicated that the traditional leaf economic spectrum theory may not be supported at community level in TP due to multiple environmental stressors. SLA generally decreases with increasing solar radiation, and increases with temperature and water availability (Poorter *et al.* 2009). In this study, commumnity SLA was negatively related to soil moisture, and positively related to maximum temperature (Table S5) indicating that changes in commumnity SLA were mainly controlled by maximum temperature. However, the direct effect of SLA on $G_s$ in the structural equation was not significant (Fig.5c). This effect may be obscured by drought stress. ” (Please see **page 12 lines 266-276**).

**Response to the reviewer #3**

**Specific comments:**

*1) Introduction: Please put the last paragraph (Line 81-91) before the penultimate paragraph (Line 72-80).*

Response: Thank you very much for your comment. This paragraph has been revised and removed to section “2.2.3 Stable isotope analysis”: “Given that leaf $\delta^{18}O$ at species level is affected by the leaf water evaporation process, variability in stomatal conductance ($g_s$) is expected to be evident in leaf $\delta^{18}O$ (Barbour 2007; Barbour & Farquhar 2000; Farquhar *et al.* 1998). A negative relationship between $\triangle^{18}O$ and gs has been observed at species (Barbour & Farquhar 2000; Cabrera-Bosquet *et al.* 2011; Grams *et al.* 2007; Moreno-Gutierrez *et al.* 2012) and canopy scales (Cabrera *et al.* 2021; Hirl *et al.* 2021), and among communities along soil (Ramirez *et al.* 2009) and climatic (Keitel *et al.* 2006) gradients. Consequently, we selected $1/\triangle^{18}O$ to be used as proxy for $g_s$ at species level in this study.” (Please see **page 5 lines 130-135**).

*2) Fig.1: Is the Y variable in (b) consistent with (f)? If yes, please unify them. Similarly, please modify the Y variable in (c) (d) (g) (h).*

Response: Thank you very much for your comment. Figure 1 has been changed as follows according to the comment of reviewer 1.

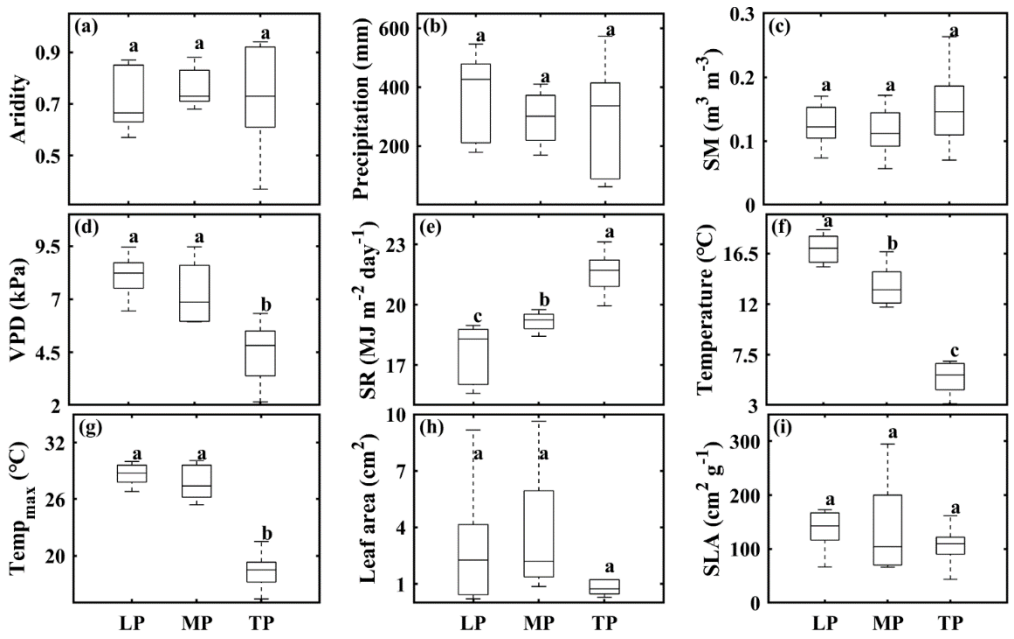


Figure 1. Comparison of aridity (a), growing season precipitation (b), soil moisture (SM) (c), vapor pressure deficit (VPD) (d), solar radiation (SR) (e), temperature (f), maximum temperature ($Temp_{max}$) (g), community leaf area (h), and specific leaf area (SLA) (i) among transects. LP: Loess Plateau; MP, Inner Mongolia Plateau; TP, Tibetan Plateau. Lowercase letters indicate significant differences among transects ($P<0.05$). Error bars indicate standard error of the mean; n=10.

*3) Fig.2: Please delete the "***" in Fig. 2(b).*

Response: Thank you very much for your comment. Change has been done.

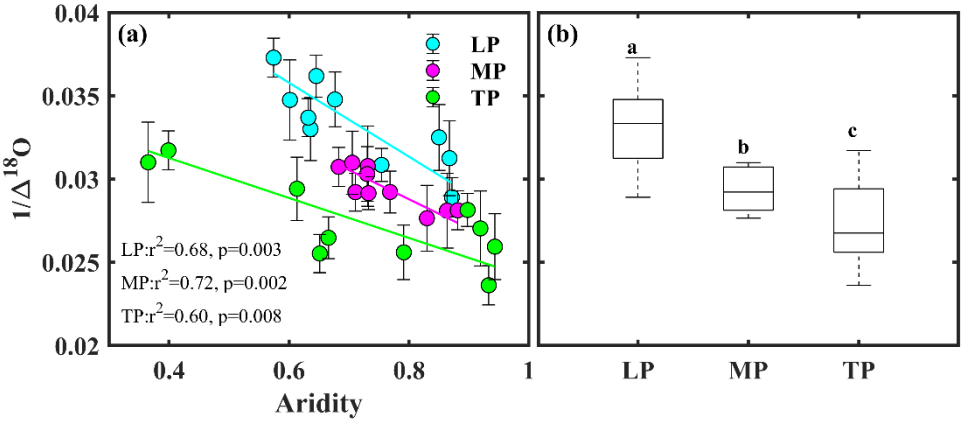


**Figure 2.** Patterns of community $1/\triangle^{18}O$ (a) along an aridity gradient within transects, and among (b) transects. Different letters indicate significant differences ($P < 0.001$) among transects. $\triangle^{18}O$, $^{18}O$ enrichment above source water of leaf organic matter; LP, Loess Plateau; MP, Inner Mongolia Plateau; TP, Tibetan Plateau.

*4) Fig.3: The X variable name in Fig.3(c) (TSR) is inconsistent with the name in the legend (SR). Please modify it.*

Response: Thank you very much for your comment. Change has been done.

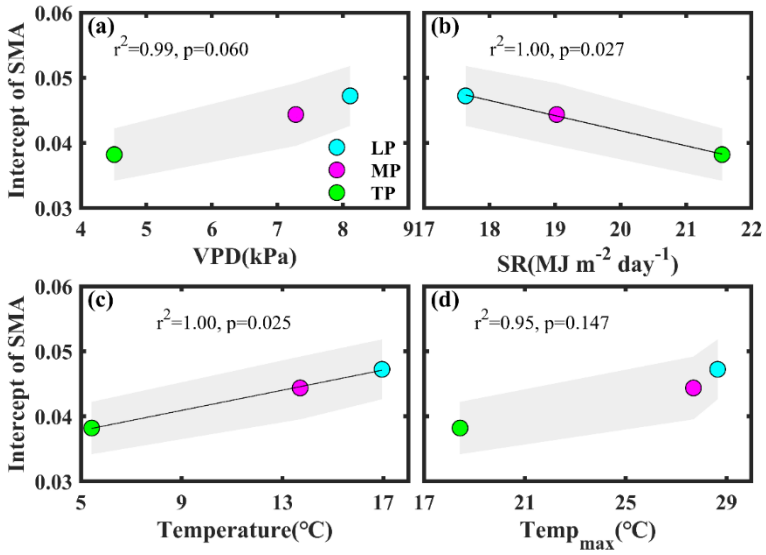


**Figure 3.** Patterns of the intercept obtained from standardized major axis analysis (SMA) among transects. VPD, vapor pressure deficit; SR, solar radiation; $Temp_{max}$, maximum temperature. LP, Loess Plateau; MP, Inner Mongolia Plateau; TP, Tibetan Plateau. Shaded area represents the 95% confidence interval of the SMA intercept.

*5) Table 1: The asterisk in the seventh row is inconsistent with other rows. Please revised them.*

Response: Thank you very much for your comment. Change has been done.

**Table 1** Pearson's coefficients among community $1/\triangle^{18}O$ and environmental factors and plant properties.

| | Loess Plateau | Inner Mongolia Plateau | Tibet Plateau |
|---|---|---|---|
| Aridity | -0.848** | -0.843** | -0.773** |
| SM | 0.719* | 0.707* | 0.659* |
| VPD | -0.554 | -0.384 | -0.912** |
| SR | -0.639* | -0.728* | -0.850** |
| $Temp_{mean}$ | 0.641* | 0.303 | -0.670* |
| $Temp_{max}$ | 0.678* | 0.038 | -0.852** |
| LA | 0.757* | 0.913** | 0.610 |
| SLA | -0.519 | -0.576 | -0.648* |

**, P<0.01; *, P<0.05. SM, soil moisture; VPD, vapor pressure deficit; SR, total solar radiation; $Temp_{mean}$, mean temperature; $Temp_{max}$, maximum temperature; LA, log-transformed leaf area; SLA, log-transformed specific leaf area.

*6) Fig.4: Please label the P value in each figure to ensure the reliability of the model. In addition, please add a priori model of effects of variables on the gs to Supplementary Information.*

Response: Thank you very much for your comment. To ensure the reliability of the model, P value of SEM model has been added in each sub-figure.

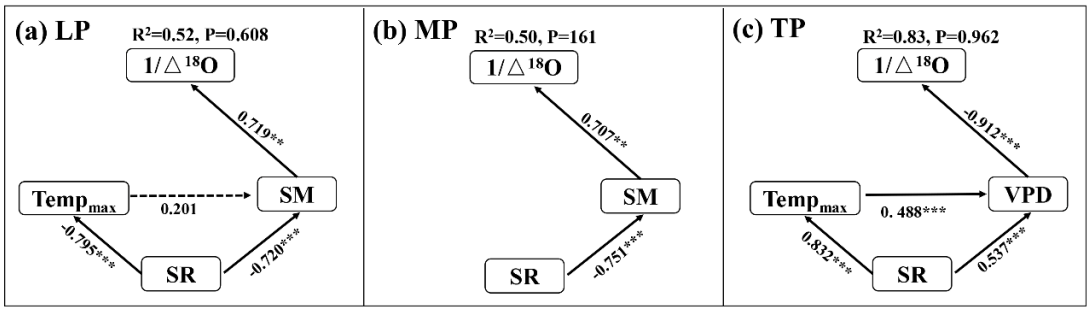


**Figure 4.** Structural equation models of abiotic factors explaining community $1/\triangle^{18}O$ in Loess Plateau (LP) (a), Inner Mongolia Plateau (MP) (b) and Tibetan Plateau (TP) (c). $\triangle^{18}O$, $^{18}O$ enrichment above source water of leaf organic matter; $Temp_{max}$: maximum temperature; SR, solar radiation; SM, soil moisture; VPD, vapor pressure deficit. Solid and dashed arrows represent significant and non-significant relationships in a fitted SEM, respectively. ***, P<0.001; **, P<0.01; *, P<0.05.

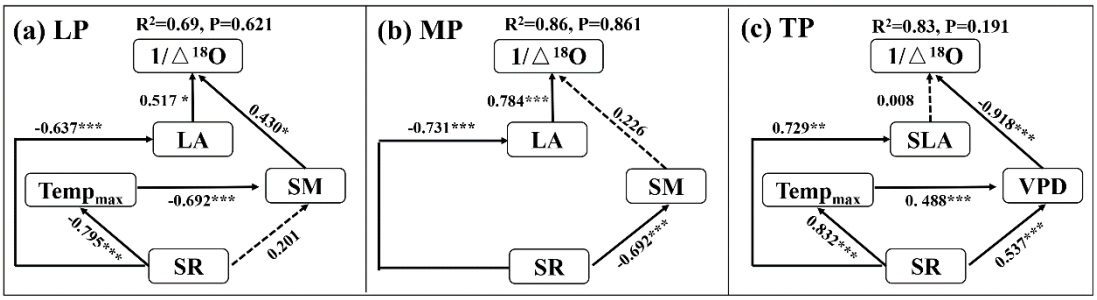


**Figure 5.** Structural equation models of abiotic and biotic factors explaining community $1/\triangle^{18}O$ in Loess Plateau (LP) (a), Inner Mongolia Plateau (MP) (b) and Tibetan Plateau (TP) (c). $\triangle^{18}O$, $^{18}O$ enrichment above source water of leaf organic matter; $Temp_{max}$: maximum temperature; SR, solar radiation; SM, soil moisture; VPD, vapor pressure deficit. LA, log-transformed leaf area; SLA, log-transformed specific leaf area. Solid and dashed arrows represent significant and non-significant relationships in a fitted SEM, respectively. ***, P<0.001; **, P<0.01; *, P<0.05.

*7) Fig.4: Why are there many types of SEMs for gs? Even in the same area, there are two SEMs for gs. How to determine which is the most accurate?*

Response: Thank you very much for your comment. To avoid confusion, we split Figure 4 into two graphs (Figure 4 and Figure 5). Figure 4 are the best-fitting models

illustrating the effects of abiotic variables on $1/\triangle^{18}O$, and Figure 5 are the best-fitting models illustrating the effects of abiotic and biotic variables on $1/\triangle^{18}O$.

*8) There are many problems in the manuscript. For example, (1) "s" of gs should be a subscript; (2) leaf area (Line 114); (3) as follows (Line 118); (4) "max" of Tempmax should be a subscript (Line 194); (5) All the abbreviations in the figures should be explained; etc. Please check the full text carefully*

Response: Change has been done according to your suggestions.

**Response to community comment #1**

**General comments:**

*1) The author should clarify why the radiation exhibited negative effect on gs, however, temperature exhibited positive or no effect on gs in different regions. Generally, radiation may influence plant gs through its influence air temperature, thus, the consistent effect of radiation and temperature on gs may be more reasonable.*

Response: Thank you very much for your comment. We respond this comment from three aspects.

(1) We corrected the structure equation method result about the effect of temperature on gs in Losses Plateau: "$Temp_{max}$ did not exert a significant effect on $1/\triangle^{18}O$ in LP ($P>0.05$)." (Please see **page 9 line 203**).

(2) We rephrased the discussion about the negative effect of solar radiation on canopy conductance (Gs, presented by community $1/\triangle^{18}O$) via drought stress: "Solar radiation and temperature regulated variability in $G_s$ within transect via drought stress (Fig.4). Solar radiation exhibited consistently negative effects on $G_s$ because it increased with increasing aridity within the three transects (Fig.1h, Table S1). These results were consistent with those of Fu et al. (2006), who demonstrated that the net $CO_2$ exchange in grasslands in MP and shrublands in TP was significantly reduced by high solar radiation. In this study, solar radiation exhibited a negative effect on $G_s$ via drought stressors (Fig.4 a-c). On one hand, increasing solar radiation would decrease SM by increasing energy partitioning into evaporation and transpiration (Zhang *et al.* 2019). In fact, solar radiation had negative effects on SM in the three transects in this study (Table S5). On the other hand, increasing solar radiation can increase VPD by increasing temperatures (Grossiord *et al.* 2020). However, a positive relationship between temperature and VPD was observed only in TP (Table S2). "(Please see **page 11 lines 239-247**).

(3) We rephrased the discussion about the negative effect of temperature on Gs via drought stress: "The drought stress on $G_s$ within transect was exacerbated by higher temperatures in TP (Fig.4c). In TP, temperature increased with increasing aridity

(Table S1), and was negatively related to SM and positively to VPD (Table S5, Fig.4c). As with solar radiation, increases in temperature tend to increase evaporation and transpiration, ultimately reducing SM, while VPD always increases with increasing temperatures (Grossiord *et al.* 2020; Oren *et al.* 1999). Consequently, increasing temperatures exacerbate soil and atmospheric drought, ultimately reducing $G_s$ along an aridity gradient in TP. However, temperature exhibited negative correlations with community $1/\triangle^{18}O$ in LP, while it did not exhibit significant effects on community $1/\triangle^{18}O$ in the SEM models (Fig.4a). The reason may be that temperature was significantly correlated with SM in LP (Table S5)." (Please see **page 11 lines 248-255**).

**Response to community comment #2**

**Specific comments:**

*1) There are expressions like "drought", "dryness", "low soil moisture" and "soil moisture stress" in this manuscript. I don't think these have the same meaning. Please check and use it properly. Similarly, this manuscript focused on gs, but sometimes there are expressions like "canopy gs".*

Response: Thank you very much for your comment. We have replaced "dryness" with "drought", and "soil moisture stress" with "low soil moisture" throughout the manuscript. Meanwhile, we used gs to present stomatal conductance at species level, and Gs to present canopy conductance.

*2) I think hypothesis should be based on the information provided in the introduction. In terms of the hypothesis 2 "excess solar radiation and low temperatures will result in differences in gs among transects", I don't understand how low temperatures will affect gs according to the information in introduction.*

Response: Thank you very much for your comment. We rephrased and clarified our second hypothesis from three aspects.

Firstly, we clarified that the effects of direction and intensity of solar radiation and temperature on gs at species level strongly depend on their distribution range and the relationship with aridity. Below the optimal values, $g_s$ at species level increased with increasing solar radiation and temperature, while excess radiation and high temperature-associated high VPD or low soil moisture would suppress $g_s$ (Please see **page 2 lines 53-57**).

Secondly, the basic climatic context for the three grassland transect has been added in the last paragraph of "**1 Instruction**" section. Meanwhile, we clarified that the three grassland transects experience different solar radiation and temperature conditions at a given aridity, due to the differences in geographical locations of the three plateaus. The order of mean annual temperature and solar radiation across the sites is LP>MP>TP and LP<MP<TP, respectively. (Please see **page 3 lines 77-79**).

Finally, we rephrased the second hypothesis as: “high solar radiation and low temperatures will jointly suppress $G_s$ at a given aridity among transects”. (Please see **page 3 line 81**).

*3) The last paragraph should be the last but one paragraph or in the methods.*

Response: Thank you very much for your comment. This paragraph has been revised and removed to section “2.2.3 Stable isotope analysis”: “Given that leaf $\delta^{18}O$ at species level is affected by the leaf water evaporation process, variability in stomatal conductance ($g_s$) is expected to be evident in leaf $\delta^{18}O$ (Barbour 2007; Barbour & Farquhar 2000; Farquhar *et al.* 1998). A negative relationship between $\triangle^{18}O$ and gs has been observed at species (Barbour & Farquhar 2000; Cabrera-Bosquet *et al.* 2011; Grams *et al.* 2007; Moreno-Gutierrez *et al.* 2012) and canopy scales (Cabrera *et al.* 2021; Hirl *et al.* 2021), and among communities along soil (Ramirez *et al.* 2009) and climatic (Keitel *et al.* 2006) gradients. Consequently, we selected $1/\triangle^{18}O$ to be used as proxy for $g_s$ at species level in this study.” (Please see **page 5 lines 130-135**).

*4) There may be interspecific difference in gs, so information on plant species and species composition of the three study sites should be provided.*

The species, genera and families of species occurred in each community have been listed in “**Supplementary 2**”.

*5) The headline of the first part in the discussion should be changed, because the patterns of gs among the tree transects are similar, but differ in magnitude. In addition, the authors attribute this difference to the temperature-induced changes in photosynthesis, which I don’t agree. Indeed, gs and photosynthesis are closely correlated, for example, to maximize carbon gain and minimize water loss according to the optimal stomatal behaviour. However, in my opinion, the correlation between gs and photosynthesis is regulated by stomatal behaviour.*

Response: Thank you very much for your comment. We respond this comment from two aspects.

(1) The headline has been change as: “ 4.3 Differences in canopy conductance among transects” (Please see **page 12 line 277**).

(2) The effects of VPD, solar radiation and temperature on the differences in canopy conductance among transects have been rephrased as:

“Significant differences in community $1/\triangle^{18}O$ for a given level of aridity were found among transects (Fig.2a). Among transects, only differences in VPD, solar radiation and temperature were significant ($P>0.05$) (Fig.1 and Fig.S1). In general, plants decrease their $g_s$ at species level to respond to increasing VPD (Grossiord *et al.* 2020). The intercept of linear regression between aridity and community $1/\triangle^{18}O$ decreased with decreasing VPD among transects ($P>0.05$) (Fig.3a). This indicated that the difference in VPD was not a contributor to the difference in $G_s$ among transects.

The differences in $G_s$ among transects may be attributed to the direct effects of solar radiation and temperature on $G_s$ and photosynthesis (Yu *et al.* 2002). Solar radiation exhibited a negative effect on the intercept of linear regression between aridity and community $1/\triangle^{18}O$ among transects ($P<0.05$) (Fig.3b). Excess ultraviolet-B radiation (Duan *et al.* 2008), insufficient thermal dissipation, and enhanced photorespiration under high solar radiation (Cui *et al.* 2003) can decrease photosynthesis, ultimately reducing $g_s$ at species level. For example, Yu et al. (2012) observed that photosynthesis in wheat in TP at leaf level was lower than that in North China Plain due to the high solar radiation in TP.

Transect with the low temperature exhibited a low intercept of linear regression between aridity and community $1/\triangle^{18}O$ (Fig.3c), indicating that $G_s$ differences among transects were also inhibited by low temperature. Generally, photosynthesis and $G_s$ increased with temperature below the optimum temperature (Xu *et al.* 2021). For example, the rate of photosynthesis in what was lower in a cold than in a warm environment (Yu *et al.* 2002). ” (Please see **page 12 lines 278-292**).

*6) line 25 delete “at leaf level”.*

Response: Change has been done.

*7) line 24 change “in one” and “in the other” into (1) and (2), respectively.*

Response: Change has been made.

*8) I suggest that “interaction effects” may be changed into “interactive effects”.*

Response: Change has been made.