Response list to the reviewers' comments

Ref: doi:10.5194/bg-2016-372
Title: **Differences in instantaneous water use efficiency derived from post-carboxylation fractionation respond to the interaction of CO_2 concentrations and water stress in semi-arid areas**
Authors: Na Zhao, Ping Meng, Yabing He, Xinxiao Yu*

Dear Editor,

Thanks for your thoughtful and constructive comments that provide scientific guidance for our writing and future research. We have been carefully considering your suggestions and revising the manuscript in the revised manuscript (marked in red color) accordingly. The following below in blue are our point-to-point responses for the referees' questions and your comments.

We are looking forward to your further comments and a possible publication in the BG special issue (Ecosystem processes and functioning across current and future dryness gradients in arid and semi-arid lands).

Kind regards,

Xinxiao Yu

Referee #1

General comments

*In this work, Zhao et al. present an experimental study on the interactive effects of CO2 and water availability on instantaneous water-use efficiency (iWUE) and the carbon isotope composition (d13C) of leaf water-soluble organic matter (LWSOM). Although the study of the interaction between CO2 and drought and its effects on d13C and iWUE is not new (Picon, Ferhi, & Guehl 1997), there is no clear consensus on the interpretation of d13C changes in response to increasing CO2 (Schubert & Jahren 2012). In this context, the comprehensive dataset here presented may contribute to understand the limitations of d13C as a surrogate for iWUE, and to better predict the response of tree species to increasing CO2, particularly in drought-prone environments. This is particularly relevant for the proper interpretation of long-term trends in d13C in relation to changes in water use efficiency, particularly in drought-prone environments, e.g. based on tree-ring records (Duquesnay et al. 1998; Saurer, Siegwolf, & Schweingruber 2004; Voltas et al. 2013), or from herbarium and sub-fossil material (Peñuelas & Azcón-Bieto 1992;Beerling 1996;Köhler et al. 2010). The experiment is well-designed and the data is generally well presented, although some details on the methodology are missing (see technical corrections). However, the manuscript requires some improvements, particularly on the interpretation of results.

Response: Thank you for the careful review and constructive comments. According your helpful suggestions, revisions throughout the whole article have been made and the results have been improved and supplemented with the related contents.

Specific comments

*My main concern about the manuscript is that it relies on the assumption that the only source of divergence between gas-exchange iWUE and d13C of recent assimilates could be post-photosynthetic fractionation. Although this is likely to play a role, the authors should consider that what actually defines carbon isotope discrimination (D13C) is the CO2 concentration in the chloroplast (Cc), not in the intercellular space, as used in the simplified equation of the Farquar's model (Evans et al. 1986; Farquhar, Ehleringer, & Hubick 1989). Indeed, the difference between gas-exchange derived values and online measurements of D13C has been widely used to estimate Ci-Cc and mesophyll conductance for CO2 (Le Roux et al. 2001;Warren & Adams 2006;Flexas et al. 2006;Evans et al. 2009;Flexas et al. 2012;Evans & von Caemmerer 2013). In this regard, changes in mesophyll conductance could be partly responsible for the observed variations, as it generally increases in the short term in response to elevated CO2 (Flexas et al. 2007;Flexas et al. 2014), whereas it tends to decrease under drought (Flexas et al. 2004;Ferrio et al. 2012;Hommel et al. 2014;Théroux-Rancourt, Éthier, & Pepin 2014). Hence, the manuscript would be greatly improved by considering both post-photosynthetic fractionation and mesophyll conductance as potential sources of variation. With the data available, the authors may be able to estimate changes in mesophyll conductance, based on the Evans method, which can be adapted to recent assimilates (Pons et al. 2009). Even without alternative estimates for mesophyll conductance, this would provide a useful ground for a deeper discussion.

Response: Thanks for your helpful comments about our research. The consensus has been reached that the routine of CO_2 diffusion into photosynthetic site in plant includes two main procedures, which are CO_2 moving from ambient environment surrounding the leaf (C_a) to the sub-stomatic cavities (C_i) through stomata, and from there to the site of carboxylation within the chloroplast stroma (C_c) of leaf mesophyll. The latter diffusion is defined as mesophyll conductance (g_m) (Flexas et al., 2008; Evans et al. 2009). Moreover, g_m has been identified to coordinate with environmental variables at the faster rate than that of stomatal conductance (Galmés et al., 2007; Tazoe et al., 2011; Flexas et al., 2007). g_m as the important factor that could improve water use efficiency under drought pretreatment (Han et al., 2016). There has been a dispute how g_m responds to fluctuation of CO_2 concentration. Terashima *et al*. (2006) have confirmed that CO_2 permeable aquaporin, located in the plasma membrane and inner envelope of chloroplasts (Uehlein et al. 2008), could regulate the change of g_m.

The ^{13}C fractionation of CO_2 from air surrounding leaf to sub-stomatal cavity may be simply considered (Eqn. 6), whereas the fractionation induced by mesophyll conductance from sub-stomatic cavities to the site of carboxylation in the chloroplast cannot be neglected (Pons et al., 2009; Cano et al., 2014). As estimating the post-photosynthetic fractionation in leaf, carbon discrimination generated by mesophyll conductance must be subtracted from ^{13}C fractionation from the site of carboxylation to cytoplasm before sugars transportation, estimated from the difference between $\delta^{13}C_{WSC}$ ($\delta^{13}C$ of water soluble compounds by carbon isotopic method) and $\delta^{13}C_{WSC}$ ($\delta^{13}C$ modeled from gas exchange measurement), which was closely associated with g_m. Consequently, considering your constructive suggestions, g_m in our study was determined based on the Evans method, which can be adapted to recent assimilates (Pons et al. 2009). And then we can estimate the variation of g_m under SWC × [CO_2] treatments. Related data in Figures, methods, results, discussions and conclusion of g_m have been added in the revised manuscript (see Figure 6 and 8, Pages 6-7, Lines 210-258, Pages 8-9, Lines 302-348, Pages 11-12, Lines 401-451 and Page 12, lines 455-459 and 464-469 in the revised manuscript). Subsequently, it has been shown that mesophyll conductance and post-carboxylation fractionation both contribute to the ^{13}C fractionation from the site of carboxylation to cytoplasm (the difference between $\delta^{13}C_{WSC}$ and $\delta^{13}C_{obs}$), which is derived from ^{13}C fractionation following the carboxylation while photosynthate having not been transported to the twigs of plant in our study.

Added citations:

Brooks, A. and Farquhar, G. D.: Effect of temperature on the CO_2/O_2 specificity of ribulose-1,5-bisphosphate carboxylase/oxygenase and the rate of respiration in the light, Planta, 165, 397–406, 1985.

Cano, F. J., López, R., and Warren, C. R.: Implications of the mesophyll conductance to CO_2 for photosynthesis and water-use efficiency during long-term water stress and recovery in two contrasting Eucalyptus species, Plant Cell Environ., 37, 2470–2490, 2014.

Flexas, J., Diaz-Espejo, A., Galmés, J., Kaldenhoff, R., Medano, H., and Ribas-Carbo, M.: Rapid variations of mesophyll conductance in response to changes in CO_2 concentration around leaves, Plant Cell Environ., 30, 1284–1298, 2007.

Flexas, J., Ribas-Carbó, M., Diaz-Espejo, A., Galmés, J., and Medrano, H.: Mesophyll conductance to CO_2: current knowledge and future prospects, Plant Cell Environ., 31, 602–621, 2008.

Galmés, J., Medrano, H., and Flexas, J.: Photosynthetic limitations in response to water stress and recovery in Mediterranean plants with different growth forms, New Phytol., 175, 81–93. 2007.

Gillon, J. S., Griffiths, H.: The influence of (photo)respiration on carbon isotope discrimination in plants. Plant Cell Environ., 20, 1217–1230, 1997.

Guy, R. D., Fogel, M. L., and Berry, J. A.: Photosynthetic fractionation of the stable isotopes of oxygen and carbon, Plant Physiol., 101, 37–47, 1993.

Han, J. M., Meng, H. F., Wang, S. Y., Jiang, C. D., Liu, F., Zhang, W. F., and Zhang, Y. L.: Variability of mesophyll conductance and its relationship with water use efficiency in cotton leaves under drought pretreatment, J. Plant Physiol., 194, 61–71, 2016.

Igamberdiev, A. U., Mikkelsen, T. N., Ambus, P., Bauwe, H., and Lea, P. J.: Photorespiration contributes to stomatal regulation and carbon isotope fractionation: a study with barley, potato and Arabidopsis plants deficient in glycine decarboxylase, Photosynth. Res., 81, 139–152, 2004.

Lanigan, G. J., Betson, N., Griffiths, H., and Seibt, U.: Carbon isotope fractionation during photorespiration and carboxylation in Senecio, Plant Physiol., 148, 2013–2020, 2008.

Pons, T. L., Flexas, J., von Caemmerer, S., Evans, J. R., Genty, B., Ribas-Carbo, M., and Brugnoli, E.: Estimating mesophyll conductance to CO_2: methodology, potential errors, and recommendations, J. Exp. Bot., 8, 1–18, 2009.

Tazoe, Y., von Caemmerer, S., Estavillo, G. M., and Evans, J. R.: Using tunable diode laser spectroscopy to measure carbon isotope discrimination and mesophyll conductance to CO_2 diffusion dynamically at different CO_2 concentrations, Plant Cell Environ., 34, 580–591, 2011.

Terashima, I., Hanba, Y.T., Tazoe, Y., Vyas, P., and Yano, S.: Irradiance and phenotype: comparative eco-development of sun and shade leaves in relation to photosynthetic CO_2 diffusion, J. Exp. Bot., 57, 343–354, 2006.

Uehlein, N., Otto, B., Hanson, D. T., Fischer, M., McDowell, N., and Kaldenhoff, R.: Function of Nicotiana tabacum aquaporins as chloroplast gas pores challenges the concept of membrane CO_2 permeability, Plant Cell, 20, 648–657, 2008.

Technical corrections

*In its present form, the title may suggest that instantaneous water use efficiency is changing because of post-carboxylation fractionation, which is clearly not the case. Besides, after considering the role of mesophyll conductance, post-carboxylation fractionation should not play such a major role in the title. An alternative might be "The interaction of CO_2 concentrations and water stress in semi-arid areas causes diverging response in instantaneous water use efficiency and carbon isotope composition". This leaves open the possibility to discuss both post-photosynthetic fractionation and mesophyll conductance as potential causes for the observed divergence.

Response: We thank referee and greatly appreciate the thoughtful and constructive comments. Following your suggestions, the title was changed as "The interaction of CO_2 concentrations and water stress in semi-arid areas causes diverging response in instantaneous water use efficiency and carbon isotope composition" in the revised manuscript, which can more comprehensively discuss both post-carboxylation fractionation and mesophyll conductance as potential causes for the observed divergence.

*In the abstract, lines 11-14: it seems that several concepts are mixed together here, trying to summarize everything in one sentence, but the result is unclear. I would recommend to split the ideas in shorter lines, and to try to go step by step in the argumentation line of the abstract.

Response: Based on your constructive recommendation, we rewrote this part as (starting on Lines 10-14 in the abstract of revised manuscript):

"It is commonly surveyed that the ^{13}C fractionation derived from the CO_2 diffusion occurred from ambient air to sub-stomatal cavity, and little investigate the ^{13}C fractionation generated from the site of carboxylation to cytoplasm before sugars transportation outward the leaf, which may respond to the environmental conditions (i. e. CO_2 concentration and water stress) and their interactions".

*The number of replicates (saplings) per treatment is not given in the methods (however it is shown in the figures, n=32). Please add, and also specify the number of leaves measured/sampled per tree, number of gas-exchange measurements per leaf, etc.

Response: Considering your suggestions, we modified and specified the sampling and measuring process in gas-exchange measurements and the extractions of water soluble compound of leaves to read (starting on Page 3, Lines 114-116, Page 4, Lines 156-158 and Pages 4-5, Line 164-169, respectively):

"Saplings of two species that have similar ground diameters, heights, and growth statuses were selected. One sapling from two species was placed in one pot (22 cm in diameter and 22 cm in height)".

"Two saplings per specie were replicated per treatment (SWC× [CO_2]). For each sapling, four leaves were chosen and then four measurements were conducted on each leaf".

"Recently-expanded, eight sun leaves per sapling were selected and homogenized in liquid nitrogen since the gas-exchange measurements accomplished. For the extraction of the water-soluble compounds (WSCs) from the leaves (Gessler et al., 2004), 50 mg of ground leaves and 100 mg of PVPP (polyvinylpolypyrrolidone) were mixed and incubated in 1mL double demineralized water for 60 min at 5℃ in a centrifuge tube. Each leaf was replicated two times. Two saplings per specie were chosen for each orthogonal treatment".

*In line 263 an attempt to quantify the so-called 'post-carboxylation fractionation' is given, but the methodology used is not described. As it is written, the sentence "When comparing WUEge and WUEcp, the 13C-depletion" is misleading, since it is not WUE calculated by the two methods what is compared here, but observed and modelled d13C. I guess the value results from the difference between observed d13C and modelled d13C calculated from gas-exchange data, i.e. by reverting equations 3 and 4, however this is not explained in the methods.

Response: Thanks for your helpful comments. Consistent with your speculation and considering the effect of mesophyll conductance, the defined ‘post-carboxylation’ or ‘post-photosynthesis’ that can explain part of the ^{13}C fractionation from the site of carboxylation to cytoplasm before sugars transportation that is the difference between observed $\delta^{13}C$ of water soluble compounds from leaves and the modeled $\delta^{13}C$ calculated from gas-exchange, which in unmodified manuscript was not been explained in the methods, misleading that with the difference between WUE_{ge} and WUE_{cp}. Considering with your suggestions, we added the methodology of post-carboxylation in the revised manuscript.

“2.4.1 ^{13}C fractionation from the site of carboxylation to cytoplasm before sugars transportation” that reads (starting on Page 6, Lines 203-209):

“Then the ^{13}C fractionation from the site of carboxylation to cytoplasm before sugars transportation (total ^{13}C fractionation) can be estimated by the observed $\delta^{13}C$ of water soluble compounds from leaves ($\delta^{13}C_{WSC}$) and the modeled $\delta^{13}C$ calculated from gas-exchange ($\delta^{13}C_{model}$). The $\delta^{13}C_{model}$ is calculated from Δ_{model} from Eqn. (2). The Δ_{model} can be determined by Eqns. (3 and 4) as:

$$\Delta_{model} = (b-a)\left(1-\frac{1.6\Delta e \mathrm{WUE}_{ge}}{c_a}\right)+a \tag{7}$$

$$\delta^{13}C_{model} = \frac{c_a-\Delta_{model}}{1+\Delta_{model}} \tag{8}$$

$$\text{Total } ^{13}\text{C fractionation} = \delta^{13}C_{WSC} - \delta^{13}C_{model} \tag{9}$$”.

“3.4 ^{13}C fractionation from the site of carboxylation to cytoplasm before sugars transportation” has been modified as (starting on Pages 8, Lines 303-313):

“We evaluated the total ^{13}C fractionation from the site of carboxylation to cytoplasm by gas exchange and $\delta^{13}C$ of water-soluble compounds from leaf (Table 1), which can retrace ^{13}C fractionation before carboxylation transport to the twig. Comparing $\delta^{13}C_{WSC}$ with $\delta^{13}C_{model}$ from Eqns. (4, 7 and 8), total ^{13}C fractionation of *P. orientalis* ranged from 0.0328‰ to 0.0472‰, which was smaller than that of *Q. variabilis* (0.0384‰ to 0.0466‰). The total fractionations of *P. orientalis* were magnified with soil wetting especially that reached 35%–80% of FC from C_{400} to C_{800} (increased by 21.30%–42.04%). The total fractionation under C_{400} and C_{500} were amplified as SWC increased until 50%–60% of FC in *Q. variabilis*, whereas it was increased at 50%–80% of FC and decreased at 100% FC under C_{600} and C_{800}. Elevated [CO_2] enhanced the average total fractionation effect of *P. orientalis*, while those of *Q. variabilis* declined sharply from C_{600} to C_{800}. Total ^{13}C fractionation in *P. orientalis* increased faster than did those of *Q. variabilis* with increased soil moisture”.

“4.4 Post-carboxylation fractionation generated before photosynthate leaving leaves” was been improved as (starting on Page 11-12, Lines 441-444):

“When comparing $\delta^{13}C_{WSC}$ with $\delta^{13}C_{obs}$, total ^{13}C fractionation of *P. orientalis* ranged from 0.0328‰ to 0.0472‰, less than that of *Q. variabilis* (from 0.0384‰ to 0.0466‰). The post-carboxylation fractionation contributed 75.30%-98.9% of total ^{13}C fractionation, which was determined by subtracting the fractionation of mesophyll conductance from total ^{13}C fractionation”.

The conclusion of this manuscript need to be modified as (starting on Page 12, Lines 455-459 and 464-469):

“The influence of mesophyll conductance on the difference of ^{13}C fractionation between the sub-stomatic cavities and the ambient environment need to be considered, while testing the hypothesis that the post-carboxylation will contribute to the ^{13}C fractionation from the site of carboxylation to cytoplasm before sugars transportation".

"Mesophyll conductance and post-photosynthesis were manifested both contributing to the ^{13}C fractionation from the site of carboxylation to cytoplasm before sugars transportation determined by gas exchange and carbon isotopic measurements. Rising $[CO_2]$ and/or soil moistening generated increasing disparities between $\delta^{13}C_{WSC}$ and in $\delta^{13}C_{model}$ *P. orientalis*; nevertheless, the differences between $\delta^{13}C_{WSC}$ and $\delta^{13}C_{model}$ in *Q. variabilis* increased as $[CO_2]$ being less than 600 ppm and/or water stress was alleviated. Total ^{13}C fractionation in leaf was linearly dependent on g_s".

*Text in the legends of Figs. 2-5 could be larger. Since each panel is associated to one single species, they could be simplified by including the name of the species elsewhere in the figure, and using the symbols only for the CO2 levels. The symbols for a given CO2 level could be the same in all panels, regardless of the species (in this way, one legend would be enough for all the panels).

Response: Thanks for your constructive comments. Considering your suggestions, the legends of Figs. 2-5 were simplified in the revised manuscript. The symbols for CO_2 concentration of 400 ppm, 500 ppm, 600 ppm and 800 ppm were uniformly presented as C_{400}, C_{500}, C_{600} and C_{800} in sequence. One legend was shown in all panels of one Figure shown in Figs. 2-5 of revised manuscript. Furthermore, we have revised the captions of Figs. 2-5 and 7 shown in the revised manuscript, and then numbered and named the individual panels within a composite figure with a lower-case letter in the upper left hand corner of the graph and cite in the simplified caption.

*In Figure 6 I would use the symbols to indicate CO2 levels, as in the rest of figures. This would be useful to see whether the positive association between "fractionation" and gs is linked with CO2 or water availability.

Response: Thank you for suggestions about the graphic settings. According your consideration, we have redrawn the images of Figs. 7 and 8 in the revised manuscript, which could obviously illustrate the relationships between g_s/g_m and total ^{13}C fractionation. The legends of Figs. 7 and 8 were simplified. The symbols for CO_2 concentration of 400 ppm, 500 ppm, 600 ppm and 800 ppm were uniformly presented as C_{400}, C_{500}, C_{600} and C_{800} in sequence. Furthermore, the captions have been simplified to number the panels of the composite figure with a lower-case letter in the upper left hand corner of the graph and cite in the simplified caption.

Referee #2

General comments

*In the context of global warming derived from the rising CO_2 levels, severe drought conditions can be anticipated and are poised to change rapidly. Simultaneously, elevated CO_2 concentrations ($[CO_2]$) and more frequent droughts may also have interactive effects on physiological indexes and processes in plant. The carbon discrimination ($^{13}\Delta$) assimilated recently could more subtly provide timely feedback to environmental changes and their influences on diffusion via plant physiology and metabolic process within plants. Post-photosynthetic fractionation at the biochemical level is a well-documented phenomenon, which is caused by the difference in signatures between metabolites and intramolecullar position isotopic effects. Further, there is no clear consensus on the interpretation of $\delta^{13}C$ changes in response to the interaction of increasing CO_2 and soil-water stresses. This paper distinctly presents the interaction of CO_2 concentrations and water stress on the instantaneous water use efficiency and carbon isotope composition. The post-photosynthesis fractionation can explained the differences of the instantaneous water use efficiency measured by the gas-exchange method and the carbon isotopic composition from water-soluble compounds of leaves. The results of this study suggested that rising [CO_2] coupled with moistened soil generated increasing disparities of $\delta^{13}C$ between the water soluble compounds ($\delta^{13}C_{wsc}$) and estimated by gas-exchange observation ($\delta^{13}C_{obs}$) in two species. Thus, cautious descriptions of the magnitude and environmental dependence of apparent post-carboxylation fractionation are worth our attention in photosynthetic fractionation. The experiment is well-designed and the data is generally well presented. This manuscript is suitable and has a merit for publication in this journal, although some details on the methodology and statement on results require some improvements (in special comments).

Response: We thank and greatly appreciate the thoughtful and constructive comments. According your helpful suggestions, revisions for methodology and results have been made and the specific descriptions have been supplemented with the related contents.

Special comments

*In abstract, the author tried to state the carbon fractionation was generated from the carbon assimilation in the chloroplast to the sugars synthesized in the cytoplasm before photosynthetic products transportation outward the leaf. The vague concepts on Line 11-14 are stated. Separation of the long sentence into the shorter ones would be more beneficial for the readers to understand.

Response: We accept the referee's constructive suggestions and have rewritten the descriptions as (starting on Lines 10-14 in the abstract of revised manuscript):

"It is commonly surveyed that the ^{13}C fractionation derived from the CO_2 diffusion occurred from ambient air to sub-stomatal cavity, and little investigate the ^{13}C fractionation generated from the site of carboxylation to cytoplasm before sugars transportation outward the leaf, which may respond to the environmental conditions (i. e. CO_2 concentration and water stress) and their interactions".

*The replications of the measurements of gas-exchange and extractions of water-soluble compounds of leaves could not be found in the part of the materials and methods. Please specify the replications of leaves and trees measured in the gas-exchange and the number of leaves extracted the water-soluble compounds.

Response: As the referee's comments pointed out, we specified the sampling process in gas-exchange measurements and the extracted number for water soluble compound of leaves (starting on Page 3, Lines 114-116, Page 4, Lines 156-158 and Pages 4-5, Line 164-169, respectively):

"Saplings of two species that have similar ground diameters, heights, and growth statuses were selected. One sapling from two species was placed in one pot (22 cm in diameter and 22 cm in height)".

"Two saplings per specie were replicated per treatment (SWC× [CO_2]). For each sapling, four leaves were chosen and then four measurements were conducted on each leaf".

"Recently-expanded, eight sun leaves per sapling were selected and homogenized in liquid nitrogen since the gas-exchange measurements accomplished. For the extraction of the water-soluble compounds (WSCs) from the leaves (Gessler et al., 2004), 50 mg of ground leaves and 100 mg of PVPP (polyvinylpolypyrrolidone) were mixed and incubated in 1mL double demineralized water for 60 min at 5℃ in a centrifuge tube. Each leaf was replicated two times.

Two saplings per specie were chosen for each orthogonal treatment".

*There are the ^{13}C fractionation coefficients of two species involved in Tab. 1, which has not been defined in the introductions of methods. Please add and detail the definition of the ^{13}C fractionation coefficients in the materials and methods.

Response: Considering your advices combined with the first comments posted by the Professor Ferrio Diaz, we have redefined the '^{13}C fractionation coefficients' as the 'total ^{13}C fractionation' that represented the ^{13}C fractionation from the site of carboxylation to cytoplasm before sugars transportation outward leaves. The 'total ^{13}C fractionation' can be estimated by the observed δ^{13}C of water soluble compounds from leaves ($\delta^{13}C_{WSC}$) and the modeled δ^{13}C calculated from gas-exchange ($\delta^{13}C_{model}$). Further, the calculation of mesophyll conductance and its contribution to the total ^{13}C fractionation have been determined in the results and discussions (starting from Line 182 on Page 5 to Line 258 on Page 7):

"2.4.1 ^{13}C fractionation from the site of carboxylation to cytoplasm before sugars transportation

Based on the linear model developed by Farquhar and Sharkey (1982), the isotope discrimination, Δ, is calculated as:

$$\Delta = \left(\delta^{13}C_a - \delta^{13}C_{WSC}\right)/\left(1+\delta^{13}C_{WSC}\right) \quad (2)$$

where $\delta^{13}C_a$ is the isotope signature of ambient [CO_2] in chambers; $\delta^{13}C_{WSC}$ is the carbon isotopic composition of water soluble compounds extracted from leaves. The C_i:C_a is determined by:

$$C_i{:}C_a = (\Delta - a)/(b-a) \quad (3)$$

where C_i is the intercellular CO_2 concentration, and C_a is the ambient CO_2 concentration in chambers; a is the fractionation occurring CO_2 diffusion in still air (4‰) and b refers to the discrimination during CO_2 fixation by ribulose 1,5- bisphosphate carboxylase/oxygenase (Rubisco) and internal diffusion (27‰). Instantaneous water use efficiency by gas-exchange measurements (WUE_{ge}) is calculated as:

$$WUE_{ge} = P_n{:}T_r = (C_a - C_i)/1.6\Delta e \quad (4)$$

where 1.6 is the diffusion ratio of stomatal conductance to water vapor to CO_2 in chambers and Δe is the difference between e_{lf} and e_{atm} that represent the extra- and intra-cellular water vapor pressure, respectively:

$$\Delta e = e_{lf} - e_{atm} = 0.611 \times e^{17.502T/(240.97+T)} \times (1-RH) \quad (5)$$

where T and RH are the temperature and relative humidity on leaf surface, respectively. Combining Eqns. (2, 3 and 4), the instantaneous water use efficiency could be determined by the $\delta^{13}C_{WSC}$ of leaves, defined as WUE_{cp}:

$$WUE_{cp} = \frac{P_n}{T_r} = (1-\varphi)(C_a - C_i)/1.6\Delta e = C_a(1-\varphi)\left[\frac{b-\delta^{13}C_a+(b+1)\delta^{13}C_{WSC}}{(b-a)(1+\delta^{13}C_{WSC})}\right]/1.6\Delta e \quad (6)$$

where φ is the respiratory ratio of leaf carbohydrates to other organs at night (0.3).

Then the ^{13}C fractionation from the site of carboxylation to cytoplasm before sugars transportation (total ^{13}C fractionation) can be estimated by the observed δ^{13}C of water soluble compounds from leaves ($\delta^{13}C_{WSC}$) and the modeled δ^{13}C calculated from gas-exchange ($\delta^{13}C_{model}$). The $\delta^{13}C_{model}$ is calculated from Δ_{model} from Eqn. (2). The Δ_{model} can be determined by Eqns. (3 and 4) as:

$$\Delta_{model} = (b - a)\left(1 - \frac{1.6\Delta e\mathrm{WUE_{ge}}}{C_a}\right) + a \tag{7}$$

$$\delta^{13}\mathrm{C_{model}} = \frac{C_a - \Delta_{model}}{1 + \Delta_{model}} \tag{8}$$

$$\text{Total } ^{13}\mathrm{C} \text{ fractionation} = \delta^{13}\mathrm{C}_{WSC} - \delta^{13}\mathrm{C}_{model} \tag{9}$$

2.4.2 Methodology of calculating mesophyll conductance and estimating contribution of post-carboxylation fractionation

Actually, the carbon isotope discrimination is generated from the relative contribution of diffusion and carboxylation, reflected by the ratio of CO_2 concentration at the site of carboxylation (C_c) to that in the ambient environment surrounding plants (C_a). The carbon isotopic discrimination (Δ) could be presented as (Farquhar et al. 1982):

$$\Delta = a_b \frac{C_a - C_s}{C_a} + a\frac{C_s - C_i}{C_a} + (e_s + a_l)\frac{C_i - C_c}{C_a} + b\frac{C_c}{C_a} - \frac{\frac{eR_D}{k} + f\Gamma_*}{C_a} \tag{10}$$

Where C_a, C_s, C_i, and C_c indicate the CO_2 concentrations in the ambient environment, at the boundary layer of leaf, in the intercellular air spaces before entrancing into solution, and at the sites of carboxylation, respectively; a_b is the fractionation for the CO_2 diffusion at the boundary layer (2.9‰); e_s is the discrimination of CO_2 diffusion when CO_2 enters in solution (1.1‰, at 25 ℃); a_l is the fractionation derived from diffusion in the liquid phase (0.7‰); e and f are carbon discrimination derived in dark respiration (R_D) and photorespiration, respectively; k is the carboxylation efficiency, and Γ^* is the CO_2 compensation point in the absence of dark respiration (Brooks and Farquhar,1985).

When the gas in the cuvette could be well stirred during measurements of carbon isotopic discrimination and gas exchange, the diffusion in the boundary layer could be neglected and Equation 10 could be shown:

$$\Delta = a\frac{C_a - C_i}{C_a} + (e_s + a_l)\frac{C_i - C_c}{C_a} + b\frac{C_c}{C_a} - \frac{\frac{eR_D}{k} + f\Gamma_*}{C_a} \tag{11}$$

There was no agreement about the value of e, although recent measurements estimated it as 0-4‰. Value of f has been estimated ranging at 8-12‰ (Gillon and Griffiths, 1997; Igamberdiev et al., 2004; Lanigan et al., 2008). As the most direct factor, the value of b would influence the calculation for g_m, had been thought to be close to 30‰ in higher plants (Guy et al., 1993).

The difference of CO_2 concentration between the substomatal cavities and the chloroplast is omitted while diffusion discrimination related with dark-respiration and photorespiration is negligible, Equation 11 could be simplified as:

$$\Delta_i = a + (b - a)\frac{C_i}{C_a} \tag{12}$$

Equation 12 presents the linear relationship between carbon discrimination and C_i/C_a that is used normally in carbon isotopic fractionation. That underlines the subsequent comparison between the expected Δ (originated from gas-exchange, Δ_i, and those actually measured Δ_{obs}), that is the ^{13}C fractionation from mesophyll conductance, could evaluate the differences of CO_2 concentration between the intercellular air and the sites of carboxylation that generated by mesophyll resistance. Consequently, g_m can be estimated by performing the Δ_{obs} by isotope ratio mass spectrometry and expected Δ_i from C_i/C_a by gas exchange measurements.

Then the ^{13}C fractionation from mesophyll conductance is calculated by subtracting Δ_{obs} of Equation 11 from Δ_i (Equation 12):

$$\Delta_i - \Delta_{obs} = (b - e_s - a_l)\frac{C_i - C_c}{C_a} + \frac{\frac{eR_D}{k} + f\Gamma^*}{C_a} \quad (13)$$

and the P_n from the first Fick's law is presented by:

$$P_n = g_m(C_i - C_c) \quad (14)$$

Substitute Equation 14 into Equation 13 we obtain:

$$\Delta_i - \Delta_{obs} = (b - e_s - a_l)\frac{P_n}{g_m C_a} + \frac{\frac{eR_D}{k} + f\Gamma^*}{C_a} \quad (15)$$

$$g_m = \frac{(b - e_s - a_l)\frac{P_n}{C_a}}{(\Delta_i - \Delta_{obs}) - \frac{eR_D/k + f\Gamma^*}{C_a}} \quad (16)$$

In calculation of g_m, the respiratory and photorespiratory terms could be ignored or be given the specific constant values. Here, e and f are assumed to be zero or be cancelled out in the calculation of g_m.

Then Equation 16 can be transformed into:

$$g_m = \frac{(b - e_s - a_l)\frac{P_n}{C_a}}{\Delta_i - \Delta_{obs}} \quad (17)$$

Therefore, the contribution of post-carboxylation fractionation could be estimated by:

$$Contribution\ of\ post - \text{carboxylation}\ fractionation = \frac{(\text{Total } ^{13}\text{C fractionation} - \text{fractionation from mesophll conductance})}{\text{Total } ^{13}\text{C fractionation}} \times 100\% \quad (18)$$".

*In Line 202-232, the results of photosynthetic parameters were described one by one in detail. I would recommend stating the parameters with the same or similar trends all together. The physiological response of plants to the interactions of rising CO_2 and water stresses could be better presented.

Response: Thanks for your constructive comments. We have restated the photosynthetic parameters with the similar trends of CO_2 concentrations coupling the water stress (on Pages 7-8, Lines 261-272):

"Saplings of *P. orientalis* and *Q. variabilis* were exposed to the orthogonal treatments. When SWC increased, P_n, g_s and T_r in *P. orientalis* and *Q. variabilis* peaked at 70%–80% of FC or/and 100% FC (Fig. 2). The C_i in *P. orientalis* rose as SWC increased, while it peaked at 60%–70% of FC and declined thereafter with increased SWC in *Q. variabilis*. The capacity of carbon uptake and C_i were improved significantly by elevated [CO_2] at any given SWC for two species (p<0.05). Furthermore, greater increments of P_n in *P. orientalis* were found at 50%–70% of FC from C_{400} to C_{800}, which was at 35%–45% of FC in *Q. variabilis*. As the water stress was alleviated (at 70%–80% of FC and 100% FC), the reduction of g_s in *P. orientalis* was more pronounced with elevated [CO_2] at a given SWC (p<0.01). Nevertheless, g_s of *Q. variabilis* in C_{400}, C_{500}, and C_{600} was significantly higher than that in C_{800} at 50%–80% of FC (p<0.01). Coordinated with g_s, T_r of two species in

C_{400} and C_{500} was significantly higher than that in C_{600} and C_{800} except for 35%–60% of FC (p<0.01, Figs. 2g and 2h). Larger P_n, g_s, C_i and T_r of *Q. variabilis* was significantly presented than that of *P. orientalis* (p<0.01, Fig. 2)".

Response list to the editor's comments#

*P1, L13-14: the sentence, "Either its variation according to…" is awkward and should be rephrased;

Response: We appreciate your helpful comments. Based on your constructive recommendation, we have rewritten this part as (starting on Lines 10-14 in the abstract):

"It is commonly surveyed that the ^{13}C fractionation derived from the CO_2 diffusion occurred from ambient air to sub-stomatal cavity, and little investigate the ^{13}C fractionation generated from the site of carboxylation to cytoplasm before sugars transportation outward the leaf, which may respond to the environmental conditions (i. e. CO_2 concentration and water stress) and their interactions".

*P1, L22: "…of the two saplings…"; gives the impression that you examined two saplings only, when in fact many more than two were studied per species (e.g., see caption of Figure 2); please rephrased; if it is the case, please provide more detail;

Response: Thank you for careful suggestions. As you observed, there were more than two saplings repeated in each orthogonal treatment. To avoid the confusion, we have rephrased "…of the two saplings…" into "…of the two species…" in the whole article.

*P1, L23: "Field Capacity", no need to capitalize the first letter in each word;

Response: Thank you for suggestions on writing form. We have changed the first letters of "Field Capacity" with lowercase ones on Page 1, Line 22 and Page 4, Line 139.

*Many unnecessary uses of "the"; you may remove without loss of meaning (e.g., P1, L25, "…differed between the species." and P2, L70, "phloem transport, the remobilization…", P4, L131, "…the soil moisture sensors");

Response: Based on your suggestions, we will remove the unnecessary article "the" throughout the whole manuscript.

*P1, L30: "Further" should be "Furthermore";

Response: According to the context and your comments, we have corrected this grammatical error throughout the whole text.

*P1, L31: "…increased as CO2 concentration increased and water stress alleviated (…" can be simplified to "“…increased as CO2 concentration and water stress increased";

Response: Based on the first referee's comments that considering the effect of mesophyll conductance on the ^{13}C fractionation from the site of carboxylation to cytoplasm before sugars transportation (total ^{13}C fractionation), the defined 'post-carboxylation' or 'post-photosynthesis' that can explain part of total ^{13}C fractionation. The total ^{13}C fractionation is the difference between observed $\delta^{13}C$ of water soluble compounds from leaves and the modeled $\delta^{13}C$ calculated from gas-exchange, which has been misled with the difference between WUE_{ge} and WUE_{cp}. Consequently, following your suggestions, "Further, the differences between WUE_{ge} and WUE_{cp} of *Q. variabilis* increased as CO_2 concentration increased and water stress alleviated (0.0384‰–0.0466‰)" has been simplified as "Furthermore, differences between $\delta^{13}C_{WSC}$ and $\delta^{13}C_{obs}$ of *Q. variabilis* increased as CO_2 concentration and SWC increased (0.0384‰–0.0466‰)" on Page 1,

Lines 30-31 in the revised manuscript.
*P1, L33: “cautious descriptions” or “clear description”?
Response: We agree with your suggestion and have changed “cautious descriptions” to “clear description” on Page 1, Line 35 in the revised manuscript.
*P2, L43: “,but also will…” should be “,but will…”; “also” is not needed;
Response: Thanks for the suggestion about writing grammar. We have removed the “also” on Page 2, Lines 43-44 in the revised manuscript.
*P2, L49: “physiology” should be “physiological”
Response: We thank for your helpful corrections on grammatical errors and have corrected the spelling problems on Page 2, Line 49 in the revised manuscript.
*P2, L60: “considerably” is not needed; remove;
Response: We agree with your advice and have removed “considerably” on Page 2, Line 60 in the revised manuscript.
*P2, L61: “well” is not needed; please remove;
Response: We agree with your suggestion and have removed “well” on Page 2, Line 61 in the revised manuscript.
*P2, L70: “fractionations” can be made singular; remove the “s”;
Response: Based on your suggestion, we have changed “fractionations” to “fractionation” on Page 2, Line 70 in the revised manuscript.
*P3, L82: “…isotope studies…” should be “…isotopic studies…”;
Response: Thanks for your suggestion. We will use the adjective “isotopic” on Page 3, Line 88 in the revised manuscript.
*P3, L83: “…, and will help…” should be “…, which may help…”;
Response: According your helpful comments and the unrevised context, we will change “…, and will help…” into “…, which may help…” on Page 3 Line 89, which are much clearer and easier for readers.
*P3, L86: “…, which also can…” should be “…, which can also…”;
Response: Thank you for pointing out the mistake. We have checked and corrected the similar mistakes on Page 2, Line 51, Page 3, Line 92 and Page 10, Line 383 in the whole article.
*P3, L90: rephrase “…has not yet been observed” to “…has yet to be observed”;
Response: We agree with your suggestion and have changed the expression on Page 3, Line 97-98 in the revised manuscript.
*P3, L92: change “in” to “to”;
Response: Based on your helpful comments, we have changed “in” to “to” on Page 3, Line 100.
*P3, L106-107: indicate the number of samples/pots per species; throughout the manuscript you refer to examining two saplings; from your results it is clear that you studied more than two saplings; be more precise in describing the methods, so there is no confusion;
Response: Thanks for your constructive suggestions. On the basis of two different methods determining instantaneous water use efficiency and the related $\delta^{13}C$ involved, we will add the specific number of measurements, leaves, and saplings of each species in one orthogonal treatment for each method, which corresponds to the repeats n=32 in the results analysis as follows:
On Page 3, Lines 113-116: “Saplings of two species that have similar ground diameters, heights, and growth statuses were selected. One sapling from two species was placed in one pot (22 cm in

diameter and 22 cm in height). Undisturbed soil samples were collected from the field, sieved (with all particles >10 mm removed), and placed into the pots".

On Page 4, Lines 144-153: "While undergoing 20 groups of orthogonal treatments for [CO_2] × SWC, the saplings were ready for investigation. Due to one chamber only containing five plant-pots (per species) and one pot one SWC level under one CO_2 concentration, two saplings per specie in one orthogonal treatment were replicated for two periods, respectively. Each period per orthogonal treatment continued for 7 days. Pots were rearranged periodically to minimize non-uniform illumination. All orthogonal tests were formed as: elevated CO_2 concentration gradient for C_{400} (during June 2–9, June 12–19, June 21–28, and July 2–9, 2015, C_{400}), C_{500} (during July 11–18, July 22–29, August 4–11, and August 15–22, 2015, C_{500}), C_{600} (during June 2–9, June 12–19, June 21–28, and July 2–9, 2015, C_{600}), and C_{800} (during July 11–18, July 22–29, August 4–11, and August 15–22, 2015, C_{800}), combined with a soil-water gradient for 35%–45% of FC, 50%–60% of FC, 60%–70% of FC, and 70%–80% of FC and 100% FC (CK)".

On Page 4, Lines 156-158: "Two saplings per specie were replicated per treatment (SWC × [CO_2]). For each sapling, four leaves were chosen and then four measurements were conducted on each leaf".

On Pages 4-5, Lines 164-169: "Recently-expanded, eight sun leaves per sapling were selected and homogenized in liquid nitrogen since the gas-exchange measurements accomplished. For the extraction of the water-soluble compounds (WSCs) from the leaves (Gessler et al., 2004), 50 mg of ground leaves and 100 mg of PVPP (polyvinylpolypyrrolidone) were mixed and incubated in 1mL double demineralized water for 60 min at 5℃ in a centrifuge tube. Each leaf was replicated two times. Two saplings per specie were chosen for each orthogonal treatment".

*P3, L115 (and other places in the manuscript, e.g., P4, L161, P5, L182, and P6, L214): Never start a sentence with a symbol, a number, or an acronym. Please spell out each time when used at the start of a sentence; make changes throughout the manuscript.

Response: Based on your suggestions about the writing form, we have rephrased the sentences with the meaning unchanged on Page 4, Lines 125-126 "The central controlling system of the chambers (FH-230) can timely monitor and control the CO_2 concentration".

On Pages 4-5, Lines 165-168: "For the extraction of the water-soluble compounds (WSCs) from the leaves (Gessler et al., 2004), 50 mg of ground leaves and 100 mg of PVPP (polyvinylpolypyrrolidone) were mixed and incubated in 1mL double demineralized water for 60 min at 5℃ in a centrifuge tube".

On Page 5, Lines 190-192: "…; *a* is the discrimination dependent on a fraction factor (4‰) and *b* refers to the discrimination during CO_2 fixation by ribulose 1,5- bisphosphate carboxylase/oxygenase (Rubisco) and internal diffusion (30‰)".

On Pages 7-8, Lines 261-272: "Saplings of *P. orientalis* and *Q. variabilis* were exposed to the orthogonal treatments. When SWC increased, P_n, g_s and T_r in *P. orientalis* and *Q. variabilis* peaked at 70%–80% of FC or/and FC (Fig. 2). The C_i in *P. orientalis* rose as SWC increased, while it peaked at 60%–70% of FC and declined thereafter with increased SWC in *Q. variabilis*. The capacity of carbon uptake and C_i were elevated significantly by elevated [CO_2] at any given SWC for two species (p<0.05). Furthermore, greater increasing magnitudes of P_n in *P. orientalis* were found at 50%–70% of FC from C_{400} to C_{800}, which was at 35%–45% of FC in *Q. variabilis*. As the water stress was alleviated (at 70%–80% of FC and FC), the reduction of g_s in *P. orientalis* was more pronounced with elevated [CO_2] at a given SWC (p<0.01). Nevertheless, g_s of *Q.*

variabilis in C_{400}, C_{500}, and C_{600} was significantly higher than that in C_{800} at 50%–80% of FC (p<0.01). Coordinated with g_s, T_r of two species in C_{400} and C_{500} was significantly higher than that in C_{600} and C_{800} except for 35%–60% of FC (p<0.01, Figs. 2g and 2h). Larger P_n, g_s, C_i and T_r of *Q. variabilis* was significantly presented than that of *P. orientalis* (p<0.01, Fig. 2)".

*Redundancy throughout the manuscript should be removed (e.g., P4, L123-124, P5, L186, P5, L198-199, and other places in the manuscript); on P5, L186 you defined Pn and Tr (you also define the terms on P4); no need to do repeat;

Response: Based on your suggestions, we have removed the redundancy throughout the manuscript, which has been mentioned or defined as discussed before.

*P4, L127: "It consisted of the water…" should be "It consisted of a water…";

Response: Based on your suggestions, we have changed the "the" to "a" on Page 4, Line 133 and examined similar mistakes throughout the manuscript.

*P4, L130: "…specific soil water…", does this refer to the "…specific soil water content.." or something else? Please specify.

Response: We thank your suggestion and have specified this presentation as "…, target soil volumetric water content (SWC) could be set and monitored by soil moisture sensors" on Page 4, Lines 136-137 in the revised manuscript.

*P4, L131-132, L138: awkward phrasing, e.g., "the chamber" does not "determine"; please rephrase both sentences;

Response: Considering your comments, we have rephrased the sentence on Page 4, Lines 137-138:

"Since timely SWC could be sensed by the sensors, the automatic irrigation device can be regulated to water or stop watering the plants".

And the sentence on Page 4, Line 137 of unrevised manuscript is unnecessary to detail under the meaning unchanged and have been removed in the revised manuscript.

*P4, L140: what do you mean by "equilibrium circumstances"? Please rephrase;

Response: Thanks for your suggestions and we have rephrased this sentence on Page 4, Lines 144-145 in the revised manuscript as "While undergoing 20 groups of orthogonal treatments for $[CO_2]$ ×SWC, the saplings were ready for investigation".

*P4, L141: "investigation" or "sampling"?

Response: We have substituted the word "sampling" for "investigation" on Page 4, Line 145 of revised manuscript.

*P4, L144-146: not a proper sentence; please rephrase;

Response: Following your helpful comments, we have rephrased the sentence on Page 4, Lines 148-153 of revised manuscript:

"All orthogonal tests were formed as: elevated CO_2 concentration gradient for C_{400} (during June 2–9, June 12–19, June 21–28, and July 2–9, 2015, C_{400}), C_{500} (during July 11–18, July 22–29, August 4–11, and August 15–22, 2015, C_{500}), C_{600} (during June 2–9, June 12–19, June 21–28, and July 2–9, 2015, C_{600}), and C_{800} (during July 11–18, July 22–29, August 4–11, and August 15–22, 2015, C_{800}), combined with a soil-water gradient for 35%–45% of FC, 50%–60% of FC, 60%–70% of FC, and 70%–80% of FC and 100% FC (CK)".

*P4, L149-150: "7-day cultivation in the chambers."; no need to include "in the chambers", this is obvious;

Response: Thanks for pointing the inappropriate sentence, and we accepted the suggestion and removed "in the chambers" on Page 4, Line 156 of revised manuscript.

*P4, L148-155 (and other parts of the manuscript): it appears you use two different symbols for the same thing, (i.e., Tr and E for transpiration); please eliminate one of the symbols and replace with the one you decided to go with;

Response: Based on your helpful comments, we have checked the errors and uniformed the symbol for the same meaning throughout the whole article.

*P4, L164: "12000 xg", please specify;

Response: We made the mistake in writing the unit of centrifugal force under the high speed centrifugation and have rewritten it as "(12000 $\times g$ for 5 min, g represents one gravity)" on Page 5, Line 171 of revised manuscript.

*P5, 167: "analyzed in the mass…" should be "analyzed with a mass…";

Response: Thank you for pointing the improper use of preposition and we have corrected them on Page 5, Line 174 of revised manuscript according your suggestion.

*P5, L168 (and other places in the manuscript): "are" should be "were"; do not change verb tense within the same paragraph;

Response: Thanks for your helpful suggestions. Due to the expression of carbon isotope signatures and its related equation (Equation 1) have been defined and are commonly recognized, we will descript this part in a separate paragraph on page 5, lines175-180. Meanwhile, we have changed "was" into "is" to keep the same verb tense within the same paragraph on Page 5, line 184 in the revised manuscript.

*P5, L188: "intercellular" what?

Response: Thanks for your careful reminder. We have rephrased this part under its meaning unchanged on Page 5, Lines 194-196 of revised manuscript:

"…where 1.6 is the diffusion ratio of stomatal conductance to water vapor to CO_2 in the chamber and Δe is the difference between e_{lf} and e_{atm} that represent the extra- and intra-cellular water vapor pressure, respectively:…".

*P5, L186-195: sentence structure is awkward; please address;

Response: Considering your suggestions, we have modified and rephrased this part to read (starting on Pages 5-6, Lines 194-202):

"where 1.6 is the diffusion ratio of stomatal conductance to water vapor to CO_2 in the chamber and Δe is the difference between e_{lf} and e_{atm} that represent the extra- and intra-cellular water vapor pressure, respectively:

$$\Delta e = e_{lf} - e_{atm} = 0.611 \times e^{17.502\mathrm{T}/(240.97+\mathrm{T})} \times (1 - \mathrm{RH}) \qquad (5)$$

where T and RH are the temperature and relative humidity on leaf surface, respectively. Combining Eqns. (2, 3 and 4), the instantaneous water use efficiency could be determined by the $\delta^{13}C_{WSC}$ of leaves, defined as WUE_{cp}:

$$\mathrm{WUE_{cp}} = \frac{P_n}{T_r} = (1-\varphi)\,(C_a - C_i)/1.6\Delta e = C_a(1-\varphi)\left[\frac{b-\delta^{13}C_a+(b+1)\delta^{13}\mathrm{C}_{WSC}}{(b-a)(1+\delta^{13}\mathrm{C}_{WSC})}\right]/1.6\Delta e \qquad (6)$$

where φ is the respiratory ratio of leaf carbohydrates to other organs at night (0.3)".

*P5, L201 and P6, L212: "…70%-80% of FC and FC"; I'm not sure how to interpret this; please clarify;

Response: Thanks for your comments. On Page 7, Line 262 and 267 of revised manuscript "…70%-80% of FC and FC" is that the photosynthetic parameters of plants peaked at two SWC levels, 70%-80% of FC and 100% FC. We have rewritten the “FC (CK)” as “100% FC” to read throughout the revised manuscript.

*P6, L204: remove “magnitude of”;

Response: We accept your helpful suggestion and have removed the “magnitude of” on Page 7, Line 266 in the revised manuscript.

*P6, L217 (and other places in the manuscript): “maximums” should be “maxima”;

Response: Based on your comments, we have examined the similar errors and changed the “maximums” to “maxima” within the whole article.

*P6, L219: “elevated” or “increased”?

Response: Considering your helpful suggestion, we have changed the “elevated” to “increased” on Page 8, Line 287 and Page 9, Line 339 of revised manuscript.

*P6, L220 & L225 (second “was”) and P7, L259: remove “was”;

Response: Thanks for pointing out the redundancy “was” and we have removed the second “was” on Page 7, Lines 271-272 and Page 8, Lines 300-301 of revised manuscript.

*Clarify the sampling methodology; two saplings?

Response: Considering your helpful suggestion, we have presented the sampling methodologies of two methods, respectively.

On Page 3, Lines 113-116: “Saplings of two species that have similar ground diameters, heights, and growth statuses were selected. One sapling from two species was placed in one pot (22 cm in diameter and 22 cm in height). Undisturbed soil samples were collected from the field, sieved (with all particles >10 mm removed), and placed into the pots”.

On Page 4, Lines 144-153: “While undergoing 20 groups of orthogonal treatments for [CO_2] × SWC, the saplings were ready for investigation. Due to one chamber only containing five plant-pots (per species) and one pot one SWC level under one CO_2 concentration, two saplings per specie in one orthogonal treatment were replicated for two periods, respectively. Each period per orthogonal treatment continued for 7 days. Pots were rearranged periodically to minimize non-uniform illumination. All orthogonal tests were formed as: elevated CO_2 concentration gradient for C_{400} (during June 2–9, June 12–19, June 21–28, and July 2–9, 2015, C_{400}), C_{500} (during July 11–18, July 22–29, August 4–11, and August 15–22, 2015, C_{500}), C_{600} (during June 2–9, June 12–19, June 21–28, and July 2–9, 2015, C_{600}), and C_{800} (during July 11–18, July 22–29, August 4–11, and August 15–22, 2015, C_{800}), combined with a soil-water gradient for 35%–45% of FC, 50%–60% of FC, 60%–70% of FC, and 70%–80% of FC and 100% FC (CK)”.

On Page 4, Lines 156-158: “Two saplings per specie were replicated per treatment (SWC× [CO_2]). For each sapling, four leaves were chosen and then four measurements were conducted on each leaf”.

On Pages 4-5, Lines 164-169: “Recently-expanded, eight sun leaves per sapling were selected and homogenized in liquid nitrogen since the gas-exchange measurements accomplished. For the extraction of the water-soluble compounds (WSCs) from the leaves (Gessler et al., 2004), 50 mg of ground leaves and 100 mg of PVPP (polyvinylpolypyrrolidone) were mixed and incubated in 1mL double demineralized water for 60 min at 5℃ in a centrifuge tube. Each leaf was replicated two times. Two saplings per specie were chosen for each orthogonal treatment”.

*P6, L240: redundant (see P4); please remove;

Response: According your suggestion, we have removed the sentence starting on Page 8, Line 285 in the revised manuscript.

*P6, L243: change “reduced” to “decreased”;

Response: Thanks for your helpful comments. We have changed “reduced” to “decreased” on Page 8, Line 287 in the revised manuscript.

*P6, L244: remove “remarkably”;

Response: Thanks for your helpful comments. We have removed “remarkably” on Page 8, Line 287 in the revised manuscript.

*P6, L244-P7, 245: awkward structure; please revise;

Response: Considering your constructive suggestion, we have rephrased the sentence on Page 8, Lines 287-289 in the revised manuscript “Differing from variation in WUE_{ge} of *P. orientalis* with soil moistened, WUE_{ge} in *Q. variabilis* were improved slightly at 100% FC in C_{600} or C_{800} (Fig. 4b)”.

*P7, L246-247: rewrite “…orthogonal treatments; this was also observed in Q. variabilis.”;

Response: Based on your helpful comments, we have written the sentence on Page 8, Lines 289-290 in the revised manuscript “The maximum of WUE_{ge} thus occurred at 35%–45% of FC in C_{800} among all orthogonal treatments for *P. orientalis*; this was also observed in *Q. variabilis*”.

*P7, L249: “most saplings” is ambiguous; please be more specific;

Response: According your suggestion, we have specified the number of *P. orientalis* which have the greater WUE_{ge} than did *Q. variabilis* as on Page 8, Lines 292-293 of revised manuscript:
“Thirty-two saplings of *P. orientalis* had greater WUE_{ge} than did *Q. variabilis* between the same [CO_2] × SWC treatments (p<0.05)”.

*P7, L254-255: from “while the…” to the end of the sentence is awkward, please revise;

Response: Thanks for your helpful suggestion, we have rephrased the sentence on Page 8, Lines 295-297 of revised manuscript:
“As illustrated in Fig. 5a, WUE_{cp} of *P. orientalis* in C_{600} or C_{800} climbed up as water stress alleviated beyond 50%–60% of FC, as well as that in C_{400} or C_{500} while SWC exceeding 60%–70% of FC”.

*P7, L269 and 270: the word “coefficients” is non-descriptive; please elaborate as to which coefficients are being referred to?

Response: Considering your helpful suggestion and the first reviewer’s comments, we have added the mesophyll conductance together with post-carboxylation fractionation to explain the ^{13}C fractionation from the site of carboxylation to cytoplasm before sugars transportation, defined as “total ^{13}C fractionation”. The total ^{13}C fractionation in the revised manuscript is supposed to be consisted of the fractionations from mesophyll conductance and post-carboxylation. In the unrevised manuscript, the “coefficients” represented the fractionation from the site of carboxylation to cytoplasm before sugars transportation as “post-carboxylation fractionation” without considering the mesophyll conductance. Consequently, we have redefined the ^{13}C fractionation from the site of carboxylation to cytoplasm before sugars transportation as “total ^{13}C fractionation, which is composed by the fractionations from mesophyll conductance and post-carboxylation, and hence the “coefficients” in the previous version is equal to “total ^{13}C fractionation” throughout the whole revised manuscript.

*P7, L273, i.e., “Stoma are the …”: An obvious point; no need to state;

Response: Thanks for your suggestion. We have removed that sentence on Page 9, Line 342 of revised manuscript.

*P7, L283: “under any” or “irrespective of”?

Response: Thanks for your helpful proposal. We have substituted the "irrespective of" for "under any" on Page 9, Lines 353 in the revised manuscript.

*P7, L284-285: "maximal values… were generated successively…", not clear, please clarify;

Response: Considering your helpful suggestion, we have rephrased this sentence on Pages 9, Lines 354-355 in the revised manuscript:

"The decrease of g_s responding to the elevated [CO_2] could be mitigated by the coupling effects of soil wetting".

*P8, L296: remove the word "intensive";

Response: Considering your helpful suggestion, we have removed "intensive" on Page 10, Line 363 in the revised manuscript.

*P8, L306: "an evident" or "a clear"?

Response: Based on your helpful suggestion, we have changed the "an evident" to "a clear" and rephrased the sentence to read on Page 10, Lines 371-373 in the revised manuscript:

"…demonstrating that there was a clear irrigation maximum of SWC below which the plant could manage itself to adjust changing environment".

*P8, L308: "of" or "in"?

Response: Thanks for your suggestion. We have changed "of" to "in" on Page 10, Line 374 in the revised manuscript.

*P8, L311: "proves" or "suggests"?

Response: Based on your helpful suggestion, we have changed "proves" to "suggests" on Page 10, Line 377 in the revised manuscript.

*P9, L330-331: awkward, please revise

Response: Thanks for your helpful suggestion. Due to the revised manuscript will focus on discussing the causes of total ^{13}C fractionation that is composed by mesophyll conductance and post-carboxylation, we have removed the discussions about the $\delta^{13}C_{WSC}$ of two species under orthogonal treatments. So the sentence on Lines 330-331 of unrevised manuscript has been removed in the revised manuscript.

*P9, L334: "profoundly" or "greatly"?

Response: Thanks for your helpful suggestion. Due to the revised manuscript will focus on discussing the causes of total ^{13}C fractionation that is composed by mesophyll conductance and post-carboxylation, we have removed the discussions about the $\delta^{13}C_{WSC}$ of two species under orthogonal treatments. So the sentence on Lines 334 of unrevised manuscript has been removed in the revised manuscript.

*P9, L339: change to "followed by a reduction in Tr";

Response: Considering your helpful suggestion, we have changed "followed by the reduction of T_r" "followed by a reduction in T_r" on Page 10, Line 383 of revised manuscript.

*P9, L345: change to "than in conifers";

Response: Thanks for your helpful suggestion. We have changed "than is that of conifers" to "than in conifers" on Page 10, Line 388 of revised manuscript.

*P9, L350: awkward, please rephrase;

Response: Considering your helpful suggestion, we have rephrased the previous sentence as "The WUE_{ge} of *P. orientalis* and *Q. variabilis* was enhanced with soil drying, as presented by Parker and Pallardy (1991), DeLucia and Heckathorn (1989), Reich et al. (1989), and Leakey (2009)" on Page 10, Line 390-392 in the revised manuscript.

*P9, L354-355: not sure the significance of the sentence; please address;

Response: Based on your helpful suggestion, we have rephrased the sentence on Page 10, Lines 395-396 in the revised manuscript:

"Pons et al. (2009) reviewed that *Δ* of leaf soluble sugar is coupled with environmental dynamics over a period ranging from a few hours to 1–2 d".

*P9, L356: "synthetically"; not sure what this means in the context of the rest of the sentence; please revise;

Response: Considering your helpful suggestion, we have rephrased the previous sentence as "The WUE_{cp} of our materials could respond to $[CO_2]$ × SWC treatments over cultivated days, whereas WUE_{ge} is characterized as the instantaneous physiology of plants to conditions" on Page 10, Lines 396-398 of revised manuscript.

*P10, L394: remove the first "was";

Response: Thanks for your helpful suggestion. We have removed the first "was" in the sentence on Page 12, Line 463 of revised manuscript.

*P10, L410: "cautious descriptions" or "clear description";

Response: Considering your helpful suggestion, we have changed "cautious descriptions" to "clear description" on Page 12, Line 472 of revised manuscript.

*Figure captions of Fig. 2-6: captions can be simplified; identify individual graphs within a composite figure with a lower-case letter in the upper left hand corner of the graph and cite in the caption; e.g., for Fig. 6 "Regression between stomatal conductance and 13C fractionation coefficient of P. orientalis (a) and Q. variabilis (b) for four CO2 concentrations X five soil volumetric water contents (p=0.05, n=32)." Because the caption identifies the individual graphs according to species, there is no need to identify the species in the graph. Figure captions of Fig. 2-5 can be treated in a similar fashion.

Response: Thanks for your helpful suggestion. We have simplified and revised the captions of Figs. 2-5 and 7 shown in the revised manuscript, and have numbered and named the individual graphs within a composite figure with a lower-case letter in the upper left hand corner of the graph and cite in the simplified caption. Considering the first referee's comments about the supplement of mesophyll conductance in results and discussions, we have added Figure 6 that illustrates the mesophyll conductance of two species in orthogonal treatments, and Figure 8 that presents the regression between mesophyll conductance and total ^{13}C fractionation of two species under orthogonal treatments in the revised manuscript:

"**Figure 2.** Net photosynthetic rates (P_n, μmol m^{-2} s^{-1}, a and b), stomatal conductance (g_s, mol H_2O m^{-2} s^{-1}, c and d), intercellular CO_2 concentration (C_i, μmol CO_2 mol^{-1}, e and f), and transpiration rates (T_r, mmol H_2O m^{-2} s^{-1}, g and h) of *P. orientalis* and *Q. variabilis* for four CO_2 concentrations × five soil volumetric water contents. Means ± SDs, n = 32".

"**Figure 3.** Carbon isotope composition of water-soluble compounds ($\delta^{13}C_{WSC}$) extracted from leaves of *P. orientalis* (a) and *Q. variabilis* (b) for four CO_2 concentrations × five soil volumetric water contents. Means ± SDs, n = 32".

"**Figure 4.** Instantaneous water use efficiency through gas exchange measurements (WUE_{ge}) for leaves of *P. orientalis* (a) and *Q. variabilis* (b) for four CO_2 concentrations × five soil volumetric water contents. Means ± SDs, n = 32".

"**Figure 5.** Instantaneous water use efficiency estimated by $\delta^{13}C$ of water-soluble compounds (WUE_{cp}) from leaves of *P. orientalis* (a) and *Q. variabilis* (b) for four CO_2 concentrations × five soil volumetric water contents. Means ±SDs, n = 32".

"**Figure 6.** Mesophyll conductance of *P. orientalis* (a) and *Q. variabilis* (b) for four CO_2 concentrations ×five soil volumetric water contents. Means ±SDs, n = 32".

"**Figure 7.** Regression between stomatal conductance and total ^{13}C fractionation of *P. orientalis* (a) and *Q. variabilis* (b) for four CO_2 concentrations × five soil volumetric water contents (p=0.01, n = 32)".

"**Figure 8.** Regression between mesophyll conductance and total ^{13}C fractionation of *P. orientalis* (a) and *Q. variabilis* (b) for four CO_2 concentrations ×five soil volumetric water contents (p=0.01, n = 32)".

*Table 1: increase the font size of some of the text/numbers in the Table

Response: Considering your precious suggestions combining the comments given by the first two referees, Table 1 has been recreated to support the above-mentioned descriptions and analysis in the revised manuscript. In order to display the Table more clearly, we use the horizontal direction paper in the page layout and have increased the font size of the text/numbers in the revised Table. Correspondingly, we have added the results and discussions for the mesophyll conductance and the contribution of post-carboxylation fractionation on the total ^{13}C fractionation in the revised manuscript.