

Response list to the Editor's comments

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Title: **Differences in instantaneous water use efficiency derived from post-carboxylation fractionation respond to the interaction of CO₂ concentrations and water stress in semi-arid areas**

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Dear Editor,

Thanks for your thoughtful and constructive comments that provide scientific guidance for our writing and future research. We commissioned the LetPub Company (belonging to ACCDON (US) that is the professional editorial team) to provide professional editing help in rewriting the manuscript. We have been carefully considering your suggestions and revising the manuscript in the revised manuscript (marked in red color) accordingly. In addition to the following issues, we have corrected other mistakes with grammar and expression in the revised manuscript (marked in red color). The following below in blue are our point-to-point responses for your questions and comments. We are appreciated for your kind help on writing.

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

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Comments to the Author:

*Thank you for the re-submission of your manuscript. The three referees were unanimous in their support of the scientific content of the paper. I, however, have considerable difficulty in reaching a final decision regarding the publication of the paper, because of the quality of the writing. I would suggest that you get professional editing help in rewriting the manuscript. A lot of the problems I identify are associated with grammar and ways of expression. I will give you opportunity to fix these problems. I would like to see the revised manuscript before making a final decision. To help you with fixing some of these problems, I provide some guidance.

Response: Thank you for the careful review and constructive comments. We apologize for any inconvenience that we bring you for my carelessness in writing. Based on your helpful suggestions and professional editing help from Letpub Company, revisions throughout the whole article have been made and the results have been improved and supplemented with the related contents.

Title: ~~The~~ Interaction of CO₂ concentrations and water stress in semi-arid ~~areas-plants~~ causes diverging response in instantaneous water use efficiency and carbon isotope composition

Response: We appreciate your helpful comments. Based on your constructive recommendation, the title was changed as "Interaction of CO₂ concentrations and water stress in semi-arid plants causes diverging response in instantaneous water use efficiency and carbon isotope composition" on Lines 11-16, Page 1 in the revised manuscript.

L14-17: Therefore, saplings of two typical plant species found growing in semi-arid areas of Northern China of similar growing status—*Platycladus orientalis* and *Quercus variabilis*—were selected and cultivated in growth chambers with orthogonal treatments (four CO₂ concentrations [CO₂] × five soil volumetric water contents (SWC)).

Response: Considering your suggestions, we have modified and rephrased this part to read (starting on Page 1, Lines 16-19):

“Therefore, saplings of two typical plant species (*Platycladus orientalis* and *Quercus variabilis*) from semi-arid areas of Northern China were selected and cultivated in growth chambers with orthogonal treatments (four CO₂ concentrations [CO₂] × five soil volumetric water contents (SWC))”.

L23-24: Differences in instantaneous water use efficiency (iWUE) according to distinct environmental changes differed between the two species.

Response: Thank you for pointing the ambiguous use of preposition and we have corrected them on Lines 25-26, Page 1 of revised manuscript:

“Instantaneous water use efficiency (iWUE) according to environmental changes, differed between the two species”.

L24-28: The WUE_{ge} in *P. orientalis* was significantly greater than that in *Q. variabilis*, while an opposite trend was observed when comparing WUE_{cp} between the two species. Total ¹³C fractionation at the site of carboxylation to cytoplasm before sugar export (total ¹³C fractionation) was clearly species-specific, as demonstrated in the interaction of [CO₂] and SWC.

Response: Considering your helpful suggestion, we have rewritten this part on Lines 26-29, Page 1 of the revised manuscript:

“The WUE_{ge} in *P. orientalis* was significantly greater than that in *Q. variabilis*, while an opposite trend was observed when comparing WUE_{cp} between the two species. Total ¹³C fractionation at the site of carboxylation to cytoplasm before sugar export (total ¹³C fractionation) was species-specific, as demonstrated in the interaction of [CO₂] and SWC”.

L28-30: Rising [CO₂] coupled with moistened soil generated increasing disparities in δ¹³C between the water-soluble compounds (δ¹³C_{WSC}) and estimates based on gas-exchange observations (δ¹³C_{obs}) in *P. orientalis*, ranging between 0.0328‰–0.0472‰.

Response: We agree with your suggestion and have revised this sentence on Page 1, Lines 29-32 in the revised manuscript.

L34-37: Total ¹³C fractionation was linearly dependent on *g*_s, indicating post-carboxylation fractionation could be attributed to environmental variation. Thus, clear description of magnitude and environmental dependence of apparent post-carboxylation fractionation is worth our attention when addressing photosynthetic fractionation.

Response: According your helpful comments, we have revised this part as “Total ¹³C fractionation was linearly dependent on stomatal conductance, indicating post-carboxylation fractionation could be attributed to environmental variation. The magnitude and environmental dependence of apparent post-carboxylation fractionation is worth our attention when addressing photosynthetic fractionation” on Lines 35-38, Page 1 in the revised manuscript.

Due to redundant expression of 'Since the onset of industrial revolution', we changed that into 'Since the industrial revolution' on Line 42 Page 2 in the revised manuscript.

L42: 'together with' to '**culminating in**'

Response: Based on your suggestion, we have substituted 'together with' into 'culminating in' on Line 43, Page 2 in revised manuscript.

L43: 'low water availability' to '**dryness**'

Response: We have replaced 'low water availability' into 'dryness' on Line 43 Page 2 in the revised manuscript.

We have replaced 'trigger an ongoing' into 'exacerbate the' on Line 44 Page 2 in the revised manuscript.

On Lines 45-46, Page 2 of revised manuscript, '...not only lead to fluctuations in global patterns of precipitation, but will amplify drought in arid regions, and lead to more frequent extreme drought events in humid regions' has been rewritten as 'increase fluctuations in global precipitation patterns, but will probably amplify drought frequency in arid regions, and lead to more frequent extreme events in humid regions'.

On Lines 47-49, Page 2 of revised manuscript, the sentence were rewritten as 'mean $\delta^{13}\text{C}$ of atmospheric CO_2 is currently being depleted by 0.02‰ – 0.03‰ year⁻¹

(CU-INSTAAR/NOAACMDL network for atmospheric CO_2 ; <http://www.esrl.noaa.gov/gmd/>).

We have simplified the expression as 'The current carbon isotopic composition may respond to' on Line 50, Page 2 of revised manuscript.

L50 'environmental changes and their influences' to '**environmental change and their influence**'

Response: Based on your suggestion, we have changed the 'changes...influences' into 'change...influence' on Line 50, Page 2 of revised manuscript.

L51: 'While the depletion' to '**While depletion**'

Response: According your advice, we removed 'the' before the noun on Line 52, Page 2 of revised manuscript.

We changed the '...has been shown...' into 'is occurring' on Line 52, Page 2 of revised manuscript.

L52: 'itself might also affect the $\delta^{13}\text{C}$ of plant organs' to '**itself may affect $\delta^{13}\text{C}$ of plant organs**'

Response: Considering your helpful suggestions, we have changed '...itself might also affect the $\delta^{13}\text{C}$ of plant organs' into '...may affect $\delta^{13}\text{C}$ of plant organs' on Line 53, Page 2 of revised manuscript.

L53: 'climatic change' to '**changes in climate**'

Response: Based on your consideration, we have rewritten this sentence as 'in turn, are responding physiologically to changes in climate (Gessler et al., 2014)' on Lines 53-54, Page 2 of revised manuscript.

L55: 'Discrimination against' to '**Discrimination of**'

L57-58: '**even the mesophyll conductance derived from the difference of CO_2 concentrations between intercellular site and chloroplast (Farquhar et al., 1982; Cano et al., 2014)**' the addition of this segment of text does not fit well with the preceding text, please rewrite

Response: Thanks for your helpful advices. We rewrote this sentence as 'Discrimination of ^{13}C in leaves relies mainly on environmental factors that affect the ratio of intercellular to ambient CO_2

concentration (C_i/C_a). Rubisco activities and the mesophyll conductance derived from the difference of CO_2 concentrations between intercellular sites and chloroplasts are also involved (Farquhar et al., 1982; Cano et al., 2014)' on Lines 56-59, Page 2 of revised manuscript.

We have changed 'As changes' into 'Changes', '...', they are expected to be recorded differentially in the $\delta^{13}\text{C}$ of water-soluble compounds ($\delta^{13}\text{C}_{\text{WSC}}$) of the different plant organs' into '... and they will be recorded differentially in the $\delta^{13}\text{C}$ of water-soluble compounds ($\delta^{13}\text{C}_{\text{WSC}}$) in different plant organs', and '... has been described and reviewed elsewhere' into 'has been reviewed elsewhere' on Lines 59-63, Page 2 of revised manuscript.

We have rearranged '...', which determines' as '...that determine' on Lines 64-65, Page 2 of revised manuscript. And '...', defined as...' was rewritten as 'These are defined as ...' on Lines 65-67, Page 2 of revised manuscript.

L67: change 'the carbon discriminations that follow' to '**the carbon discrimination that follows**'

Response: Based on your suggestions, we have changed 'the carbon discriminations that follow' into 'the carbon discrimination that follows' on Lines 67-68, Page 2 of revised manuscript.

We have rewritten '...', what should also be considered' as '...', another consideration...' on Line 76, Page 2 of revised manuscript.

L77: misspelt Farquhar's name, please fix

Response: I am very sorry for my careless in spelling and have corrected the spelling mistake as ... 'Farquhar's ...' on Line 77, Page 2 of revised manuscript.

We have simplified 'Indeed, difference between gas-exchange derived values and online measurements of $\delta^{13}\text{C}$ has been widely used to ...' as 'Differences between gas-exchange derived values and online measurements of $\delta^{13}\text{C}$ have often been used to ...' on Lines 78-79, Page 2 of revised manuscript.

L82; 'for the differences from' to '**for the differences in the**'

Response: Considering your advice, we changed 'for the differences from' to 'for the differences in the' on Line 82, Page 3 of revised manuscript.

We changed 'whereas' to 'but' on Line 83, Page 3 of revised manuscript.

Changed 'of' to 'between', 'or/and' to 'and/or' on Lines 85-86, Page 3 of revised manuscript.

L87: change 'magnitude of these carbon fractionations are related to environmental variation have not yet been investigated.' to '**magnitude of carbon fractionation is related to environmental variation that has yet to be fully investigated.**'

Response: We accept your helpful suggestion and changed as 'The degree to magnitude of carbon fractionation is related to environmental variation that has yet to be fully investigated' on Lines 86-87, Page 3 of revised manuscript.

We have simplified the sentence as 'The simultaneous isotopic analysis of leaves is a recent refinement in isotopic studies that allows determination of the temporal variation in isotopic fractionation (Rinne et al., 2016). This will aid the accurate recording of environmental conditions' on Lines 88-89, Page 3 of revised manuscript, and changed 'and are defined as...' to 'and these are termed ...' on Line 90, Page 3 of revised manuscript.

L94-95: 'However, there is a dispute whether the fractionation stemmed...' to '**However, there is disagreement whether fractionation stemming...**'

Response: Thanks for your helpful suggestions and we have rewritten as 'However, there is disagreement whether fractionation caused by post-carboxylation and/or mesophyll resistance can alter the stable signatures of leaf carbon and thence influence instantaneous water use efficiency

(iWUE)' on Lines 93-95, Page 3 of revised manuscript.

L97-99: awkward, please rewrite

Response: Based on your advice, we have rewritten this part as 'In addition, the manner in which iWUE derived from these isotopic fractionations responds to environmental factors, such as elevated [CO₂] and/or soil water gradients, is unknown' on Lines 93-95, Page 3 of revised manuscript.

We rewrote the description on materials as '...in sapling leaves of two tree species, *Platycladus orientalis* (L.) Franco and *Quercus variabilis* Bl., native to semi-arid areas of China' on Lines 98-100, Page 3 of revised manuscript. We have translated the long sentence to several short sentences as 'We also conduct gas exchange measurements in controlled environment growth chambers (FH-230, Taiwan Hipoint Corporation, Kaohsiung City, Taiwan)' and 'One goal is to differentiate the ¹³C fractionation from the site of carboxylation to cytoplasm prior to sugars transportation in *P. orientalis* and *Q. variabilis*, that is the total ¹³C fractionation, determined from the δ¹³C of water-soluble compounds and gas-exchange measurements. The other is to discuss the potential causes for the observed divergence, estimate contributions of post-photosynthesis and mesophyll conductance on these differences, and describe how carbon isotopic fractionations respond to the interactive effects of elevated [CO₂] and water stress' on Lines 100-107, Page 3 of revised manuscript.

L103: at the first mention of the growth chamber (use the full citation that you provide on L120-121)

Response: We accept your suggestion and have supplied the full citation as '(FH-230, Taiwan Hipoint Corporation, Kaohsiung City, Taiwan)' on Line 101, Page 3 of revised manuscript.

We have rewritten the introduction of study region and the process of transplantation for potted saplings as follows: '*P. orientalis* and *Q. variabilis* saplings, selected as experimental material, were obtained from the Capital Circle forest ecosystem station, a part of Chinese Forest Ecosystem Research Network (CFERN), 40°03'45"N, 116°5'45"E in Beijing, China. This region is forested by *P. orientalis* and *Q. variabilis*. We chose saplings with similar basal diameters, heights, and growth classes. Each sapling was placed into an individual pot (22 cm diam. × 22 cm high). Undisturbed soil samples were collected from the field, sieved (with particles >10 mm removed), and placed into the pots. The soil bulk density in the pots was maintained at 1.337–1.447 g•cm⁻³. After a 30 d transplant recovery period, the saplings were placed into growth chambers for orthogonal cultivation' on Lines 110-117, Page 3 of revised manuscript.

L122-123: 'daytime temperature in chambers was set to 25 ± 0.5°C from 07:00 to 17:00, and the night-time temperature was 18 ± 0.5°C from 17:00 to 07:00' to 'daytime and nighttime temperatures in the chambers was set to 25 ± 0.5°C from 07:00 to 17:00 and 18 ± 0.5°C from 17:00 to 07:00'

Response: We accept your helpful suggestion and revised this part on Lines 120-121, Page 3 of revised manuscript.

Omit L 131 & 132.

Response: We accept your helpful suggestion and omitted the sentences.

L141-144: can this be simplified?

Response: According your consideration, we made a table that presents the orthogonal treatments on Lines 134-141, Page 4 and Page 25 of revised manuscript.

L148-154: can this also be simplified? Can you put this detail and the detail above in a table?

Response: According your consideration, we made a table that presents the orthogonal treatments involved in revised manuscript, as follows on Page 25 of revised manuscript:

Table 1. Orthogonal treatments of *P. orientalis* and *Q. variabilis* for four CO₂ concentrations × five soil volumetric water contents.

<i>P. orientalis</i>	Repeats (cultivated period)	B ₁	B ₂	B ₃	B ₄	B ₅
A ₁	R ₁ :June 2–9	A ₁ B ₁ R ₁	A ₁ B ₂ R ₁	A ₁ B ₃ R ₁	A ₁ B ₄ R ₁	A ₁ B ₅ R ₁
	R ₂ :June 12–19	A ₁ B ₁ R ₂	A ₁ B ₂ R ₂	A ₁ B ₃ R ₂	A ₁ B ₄ R ₂	A ₁ B ₅ R ₂
A ₂	R ₁ :July 11–18	A ₂ B ₁ R ₁	A ₂ B ₂ R ₁	A ₂ B ₃ R ₁	A ₂ B ₄ R ₁	A ₂ B ₅ R ₁
	R ₂ :July 22–29	A ₂ B ₁ R ₂	A ₂ B ₂ R ₂	A ₂ B ₃ R ₂	A ₂ B ₄ R ₂	A ₂ B ₅ R ₂
A ₃	R ₁ :June 2–9	A ₃ B ₁ R ₁	A ₃ B ₂ R ₁	A ₃ B ₃ R ₁	A ₃ B ₄ R ₁	A ₃ B ₅ R ₁
	R ₂ :June 12–19	A ₃ B ₁ R ₂	A ₃ B ₂ R ₂	A ₃ B ₃ R ₂	A ₃ B ₄ R ₂	A ₃ B ₅ R ₂
A ₄	R ₁ :July 11–18	A ₄ B ₁ R ₁	A ₄ B ₂ R ₁	A ₄ B ₃ R ₁	A ₄ B ₄ R ₁	A ₄ B ₅ R ₁
	R ₂ :July 22–29	A ₄ B ₁ R ₂	A ₄ B ₂ R ₂	A ₄ B ₃ R ₂	A ₄ B ₄ R ₂	A ₄ B ₅ R ₂
<i>Q. variabilis</i>	Repeats (cultivated period)	B ₁	B ₂	B ₃	B ₄	B ₅
A ₁	P ₁ :June 21–28	A ₁ B ₁ P ₁	A ₁ B ₂ P ₁	A ₁ B ₃ P ₁	A ₁ B ₄ P ₁	A ₁ B ₅ P ₁
	P ₂ :July 2–9	A ₁ B ₁ P ₂	A ₁ B ₂ P ₂	A ₁ B ₃ P ₂	A ₁ B ₄ P ₂	A ₁ B ₅ P ₂
A ₂	P ₁ :August 4–11	A ₂ B ₁ P ₁	A ₂ B ₂ P ₁	A ₂ B ₃ P ₁	A ₂ B ₄ P ₁	A ₂ B ₅ P ₁
	P ₂ :August 15–22	A ₂ B ₁ P ₂	A ₂ B ₂ P ₂	A ₂ B ₃ P ₂	A ₂ B ₄ P ₂	A ₂ B ₅ P ₂
A ₃	P ₁ :June 21–28	A ₃ B ₁ P ₁	A ₃ B ₂ P ₁	A ₃ B ₃ P ₁	A ₃ B ₄ P ₁	A ₃ B ₅ P ₁
	P ₂ :July 2–9	A ₃ B ₁ P ₂	A ₃ B ₂ P ₂	A ₃ B ₃ P ₂	A ₃ B ₄ P ₂	A ₃ B ₅ P ₂
A ₄	P ₁ :August 4–11	A ₄ B ₁ P ₁	A ₄ B ₂ P ₁	A ₄ B ₃ P ₁	A ₄ B ₄ P ₁	A ₄ B ₅ P ₁
	P ₂ :August 15–22	A ₄ B ₁ P ₂	A ₄ B ₂ P ₂	A ₄ B ₃ P ₂	A ₄ B ₄ P ₂	A ₄ B ₅ P ₂

L165-166: this needs revising

Response: Based on your suggestion, we have rewritten this part as follows ‘Eight recently-expanded sun leaves were selected per sapling and homogenized in liquid nitrogen after gas-exchange measurements were finished’ on Lines 152-153, Page 4 of revise manuscript.

L179: second Rsample needs to be change to Rstandard

Response: Thanks for your helpful advice, we have corrected the mistake in presentation as ‘*R_{standard}*’ on Line 165, Page 4 of revised manuscript.

We omitted the redundancy sentence on Line 246, Page 7 in revised manuscript.

Throughout the manuscript: usage of CO₂ concentration, sometimes you use [CO₂] and other times you spell it out; try to be consistent; since you introduced [CO₂] why not continue to use it? The labels on some of the Figures are simply too small; please fix

Response: Thanks your helpful suggestion. We have checked the usage of CO₂ concentration and changed it to ‘[CO₂]’ through the whole paper. We have used enlarged the font of labels in Figures 2-8.