

## ***Interactive comment on “Differences in instantaneous water use efficiency derived from post-carboxylation fractionation respond to the interaction of CO<sub>2</sub> concentrations and water stress in semi-arid areas” by Na Zhao et al.***

**Na Zhao et al.**

yuxinxiao0707@126.com

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Response to referee's comments

Thanks for your thoughtful and constructive comments that provide scientific guidance for our writing and future research. We have fully considered your suggestions in the revised manuscript (marked in red color).

General comments

In the context of global warming derived from the rising CO<sub>2</sub> levels, severe drought

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conditions can be anticipated and are poised to change rapidly. Simultaneously, elevated CO<sub>2</sub> concentrations ([CO<sub>2</sub>]) and more frequent droughts may also have interactive effects on physiological indexes and processes in plant. The carbon discrimination ( $\delta^{13}\Delta$ ) 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 intramolecular position isotopic effects. Further, there is no clear consensus on the interpretation of  $\delta^{13}\text{C}$  changes in response to the interaction of increasing CO<sub>2</sub> and soil-water stresses. This paper distinctly presents the interaction of CO<sub>2</sub> 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<sub>2</sub>] coupled with moistened soil generated increasing disparities of  $\delta^{13}\text{C}$  between the water soluble compounds ( $\delta^{13}\text{C}_{\text{wsc}}$ ) and estimated by gas-exchange observation ( $\delta^{13}\text{C}_{\text{obs}}$ ) 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

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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):

"It is commonly surveyed that the  $^{13}\text{C}$  fractionation derived from the  $\text{CO}_2$  diffusion occurred from ambient air to stomatal sub-cavity, and little investigate the  $^{13}\text{C}$  fractionation generated from the site of carboxylation to cytoplasm before sugars transportation outward the leaf, which may respond to the environmental conditions (i. e.  $\text{CO}_2$  concentration and water stress) and their interactive effects".

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 4, Line 158-159 and on Page 4-5, Line 165-167, respectively):

"Two saplings per specie were replicated per treatment ( $[\text{CO}_2] \times$  water stress). For each sapling, four leaves were chosen and then four measurements were conducted on each leaf" on Page 4, Line 161-162.

"Recently-expanded, eight sun leaves per sapling were selected and frozen immediately in liquid nitrogen since the gas-exchange measurements accomplished. Two saplings per specie were chosen for each treatment" on Page 5, Line 168-170.

There are the  $^{13}\text{C}$  fractionation coefficients of two species involved in Tab. 1, which has

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not been defined in the introductions of methods. Please add and detail the definition of the  $^{13}\text{C}$  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 ' $^{13}\text{C}$  fractionation coefficients' as the 'total  $^{13}\text{C}$  fractionation' that represented the  $^{13}\text{C}$  fractionation from the site of carboxylation to cytoplasm before sugars transportation outward leaves. The 'total  $^{13}\text{C}$  fractionation' can be estimated by the observed  $\delta^{13}\text{C}$  of water soluble compounds from leaves ( $\delta^{13}\text{C}_{\text{WSC}}$ ) and the modeled  $\delta^{13}\text{C}$  calculated from gas-exchange ( $\delta^{13}\text{C}_{\text{model}}$ ). Further, the calculation of mesophyll conductance and its contribution to the total  $^{13}\text{C}$  fractionation have been determined in the results and discussions (starting from Line 183 on Page 5 to Line 258 on Page 7):

"2.4.1  $^{13}\text{C}$  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 factor,  $\Delta$ , was calculated as:

$$\Delta = \left( \frac{(^{13}\text{C})_{\text{a}} - (^{13}\text{C})_{\text{P}}}{(1 + (^{13}\text{C})_{\text{P}})} \right) \quad (2)$$

where  $(^{13}\text{C})_{\text{a}}$  is the isotope signature of ambient  $[\text{CO}_2]$  in the chamber;  $(^{13}\text{C})_{\text{P}}$  is the  $(^{13}\text{C})_{\text{C}} / (^{12}\text{C})_{\text{C}}$  of the water-soluble compounds extracted from foliage. The  $\text{C}_i:\text{C}_a$  is determined by:

$$\text{C}_i:\text{C}_a = \frac{(\Delta - a)}{(b - a)} \quad (3)$$

where  $\text{C}_i$  is the intercellular  $\text{CO}_2$  concentration, and  $\text{C}_a$  is the ambient  $\text{CO}_2$  concentration in the chamber;  $a$  is the discrimination dependent on a fraction factor (4‰.  $b$  is the discrimination during  $\text{CO}_2$  fixation by ribulose 1,5- biphosphate carboxylase/oxygenase (Rubisco) and internal diffusion (30‰. Instantaneous water use efficiency by gas-exchange measurements ( $\text{WUE}_{\text{ge}}$ ) is calculated as:  $\text{WUE}_{\text{ge}} = \text{P}_n:\text{T}_r = ((\text{C}_a - \text{C}_i)) / 1.6\Delta e$  (4) where  $\text{P}_n$  is the net carbon assim-

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ilation,  $T_r$  is the molar rate of transpiration, and 1.6 is the diffusion ratio of stomatal conductance to water vapor to CO<sub>2</sub> in the chamber.  $\Delta e$  is the difference in water vapor pressure between the intracellular in leaves and ambient air, which may be calculated as:

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

where  $e_{lf}$  and  $e_{atm}$  represent the extra- and intra-cellular water vapor pressure, respectively.  $T$  and  $RH$  is temperature and relative humidity on leaf surface. The instantaneous water use efficiency could be determined by the  $\delta^{13}C_{WSC}$  of leaves of two species, defined as  $WUE_{cp}$ :

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

$\varphi$  is the ratio between carbohydrates consumed during respiration of the leaves and that of other organs at night (0.3).  $\delta^{13}C_{WSC}$  is the carbon isotopic composition of water soluble compounds extracted from leaves.

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  $\delta^{13}C_{model}$  can be calculated from  $\Delta_{model}$  from Eqn. (2). The  $\Delta_{model}$  can be determined by Eqns. (3 and 4) as:

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

$$\delta^{13}C_{model} = \frac{C_a - \Delta_{model}}{1 + \Delta_{model}} \quad (8)$$

$$\text{Total } (^{13}C) \text{ fractionation} = \delta^{13}C_{WSC} - \delta^{13}C_{model} \quad (9)$$

#### 2.4.2 Methodology of calculating mesophyll conductance

Actually, the carbon isotope discrimination is generated from the relative contribution of diffusion and carboxylation, reflected by the ratio of CO<sub>2</sub> concentration at the site

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of carboxylation ( $C_c$ ) to that in the ambient environment surrounding plants ( $C_a$ ). The carbon isotopic discrimination ( $\Delta$ ) could be presented as (Farquhar et al. 1982):

$$\Delta = a_b \frac{C_a - C_s}{C_a} + a \frac{C_s - C_i}{C_a} + (e_s + a_l) \frac{C_i - C_c}{C_a} + b \frac{C_c}{C_a} - \frac{(e_{RD}) / (k + \Gamma^*)}{C_a} \quad (10)$$

where  $C_a, C_s, C_i$ , and  $C_c$  indicate the CO<sub>2</sub> 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<sub>2</sub> diffusion at the boundary layer (2.9‰;  $a$  is the fractionation occurring CO<sub>2</sub> diffusion in still air (4‰;  $e_s$  is the discrimination of CO<sub>2</sub> diffusion when CO<sub>2</sub> enters in solution (1.1‰ at 25 °C);  $a_l$  is the fractionation derived from diffusion in the liquid phase (0.7‰;  $b$  is the carboxylation discrimination in C<sub>3</sub> plants (27‰;  $e$  and  $f$  are carbon discrimination derived in dark respiration (RD) and photorespiration, respectively.  $k$  is the carboxylation efficiency, and  $\Gamma^*$  is the CO<sub>2</sub> 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 7 could be shown:

$$\Delta = a \frac{C_a - C_i}{C_a} + (e_s + a_l) \frac{C_i - C_c}{C_a} + b \frac{C_c}{C_a} - \frac{(e_{RD}) / (k + \Gamma^*)}{C_a} \quad (11)$$

There is 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  $b$  will influence the calculation for  $g_m$ , has been thought to be close to 30‰ in higher plants (Guy et al., 1993).

The difference of CO<sub>2</sub> concentration between the substomatal cavities and the chloroplast is omitted while diffusion discrimination related with dark-respiration and photorespiration is also negligible, the Equation 8 could be simplified as:

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$$\Delta_i = a + (b-a) C_i/C_a \quad (12)$$

Equation 12 presents the linear relationship between carbon discrimination and  $C_i/C_a$  that used normally in carbon isotopic fractionation. That underlined the subsequent comparison between the expected  $\Delta$  (originated from gas-exchange,  $\Delta_{i,exp}$ ) and those actually measured ( $\Delta_{i,obs}$ ), which could evaluate the magnitude of differences of CO<sub>2</sub> concentration between the intercellular air and the sites of carboxylation that generated by mesophyll resistance. Consequently,  $g_m$  can be estimated by performing the  $\Delta_{i,obs}$  by isotope ratio mass spectrometry and expected  $\Delta_{i,exp}$  from  $C_i/C_a$  by gas exchange measurements.

Then subtract  $\Delta_{i,obs}$  of Equation 11 from  $\Delta_{i,exp}$  calculated by Equation 12:

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

and the net assimilation rate ( $A_n$ ) from the first Fick's law is presented by:

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

Substitute Equation 14 into Equation 13 we obtain:

$$\Delta_{i,exp} - \Delta_{i,obs} = (b - e_s - a_l) A_n / (g_m C_a) + ((eR_D)/k + f\Gamma^*)/C_a \quad (15)$$

$$g_m = ((b - e_s - a_l) A_n / C_a) / ((\Delta_{i,exp} - \Delta_{i,obs}) - ((eR_D)/k + f\Gamma^*)/C_a) \quad (16)$$

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:

$$g_m = ((b - e_s - a_l) A_n / C_a) / (\Delta_{i,exp} - \Delta_{i,obs}) \quad (17)$$

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<sub>2</sub> and

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water stresses could be better presented.

Response: Thanks for your constructive comments. We have restated the photosynthetic parameters with the similar trends of CO<sub>2</sub> concentrations coupling the water stress (on Page 7, Lines 261-272):

“*P. orientalis* and *Q. variabilis* saplings 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<sub>2</sub>] at any given SWC for two species ( $p < 0.05$ ). Further, greater increasing magnitudes of  $P_n$  in *P. orientalis* were found at 50%–70% of FC from C400 to C800, 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<sub>2</sub>] at a given SWC ( $p < 0.01$ ). Nevertheless,  $g_s$  of *Q. variabilis* in C400, C500, and C600 was significantly higher than that in C800 at 50%–80% of FC ( $p < 0.01$ ). Coordinated with  $g_s$ ,  $T_r$  of two species in C400 and C500 was significantly higher than that in C600 and C800 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)”.

Please also note the supplement to this comment:

<http://www.biogeosciences-discuss.net/bg-2016-372/bg-2016-372-AC2-supplement.zip>

Interactive comment on Biogeosciences Discuss., doi:10.5194/bg-2016-372, 2016.

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