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Interactive comment on "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" *by* Na Zhao et al.

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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 CO2 levels, severe drought

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conditions can be anticipated and are poised to change rapidly. Simultaneously, elevated CO2 concentrations ([CO2]) and more frequent droughts may also have interactive effects on physiological indexes and processes in plant. The carbon discrimination (13Δ) 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 δ13C changes in response to the interaction of increasing CO2 and soil-water stresses. This paper distinctly presents the interaction of CO2 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 [CO2] coupled with moistened soil generated increasing disparities of δ13C between the water soluble compounds (δ13Cwsc) and estimated by gas-exchange observation (δ13Cobs) 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):

"It is commonly surveyed that the 13C fractionation derived from the CO2 diffusion occurred from ambient air to stomatal sub-cavity, and little investigate the 13C fractionation generated from the site of carboxylation to cytoplasm before sugars transportation outward the leaf, which may respond to the environmental conditions (i. e. CO2 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 ([CO2] $\times$ 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 13C 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 13C 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 '13C fractionation coefficients' as the 'total 13C fractionation' that represented the 13C fractionation from the site of carboxylation to cytoplasm before sugars transportation outward leaves. The 'total 13C fractionation' can be estimated by the observed δ13C of water soluble compounds from leaves (δ13CWSC) and the modeled δ13C calculated from gas-exchange (δ13Cmodel). Further, the calculation of mesophyll conductance and its contribution to the total 13C 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 13C 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, Δ, was calculated as:

Δ=(((_ˆ13)C_a- (_ˆ13)C_P))/((1+(_ˆ13)C_P)) (2)

where (_ˆ13)C_a is the isotope signature of ambient [CO2] in the chamber; (_ˆ13)C_P is the (_ˆ13)CâĹű (_ˆ12)C of the water-soluble compounds extracted from foliage. The Ci:Ca is determined by:

C_i:C_a=((Δ-a))/((b-a)) (3)

where Ci is the intercellular CO2 concentration, and Ca is the ambient CO2 concentration in the chamber; a is the discrimination dependent on a fraction factor (4‰. b is the discrimination during CO2 fixation by ribulose 1,5- bisphosphate carboxylase/oxygenase (Rubisco) and internal diffusion (30‰. Instantaneous water use efficiency by gas-exchange measurements (WUE_ge) is calculated as: WUE_ge=P_n:T_r=((C_a-C_i))/1.6Δe (4) whereãĂŰ PãĂŮ_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 CO2 in the chamber. Δe is the difference in water vapor pressure between the intracellular in leaves and ambient air, which may be calculated as:

Δe=e_lf-e_atm=0.611$\times$eˆ(17.502T/((240.97+T)))$\times$(1-RH) (5)

where elf and eatm 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 δ13CWSC of leaves of two species, defined as WUEcp:

WUE_cp=(ãĂŰ PãĂŮ_n/T_r =(1-φ)ãĂŰ((C_a-C_i))/1.6Δe=CãĂŮ_a (1-φ)[(b-δˆ13 C_a+(b+1) δˆ13 C_WSC)/(b-a)(1+δˆ13 C_WSC)])/1.6Δe (6)

φ is the ratio between carbohydrates consumed during respiration of the leaves and that of other organs at night (0.3). δˆ13 C_WSC is the carbon isotopic composition of water soluble compounds extracted from leaves.

Then the 13C fractionation from the site of carboxylation to cytoplasm before sugars transportation (total 13C fractionation) can be estimated by the observed δ13C of water soluble compounds from leaves (δ13CWSC) and the modeled δ13C calculated from gas-exchange (δ13Cmodel). The δ13Cmodel can be calculated from Δ_model from Eqn. (2). The Δ_model can be determined by Eqns. (3 and 4) as:

Δ_model=(b-a)(1-(1.6ΔeWUE_ge)/C_a)+a (7)

δˆ13 C_model=(C_a-Δ_model)/(1+Δ_model) (8)

Total (_ˆ13)C fractionation=δˆ13 C_WSC-δˆ13 C_model (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 CO2 concentration at the site

of carboxylation (Cc) to that in the ambient environment surrounding plants (Ca). The carbon isotopic discrimination (Δ) could be presented as (Farquhar et al. 1982):

Δ=a_b (C_a-C_s)/C_a +a (C_s-C_i)/C_a +(e_s+a_l) (C_i-C_c)/C_a +b C_c/C_a -((eR_D)/k+fΓ_*)/C_a (10)

where C_a,C_s,C_i, and C_c indicate the CO2 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 CO2 diffusion at the boundary layer (2.9‰; a is the fractionation occurring CO2 diffusion in still air (4‰; e_s is the discrimination of CO2 diffusion when CO2 enters in solution (1.1‰ at 25 âĎČ); a_l is the fractionation derived from diffusion in the liquid phase (0.7‰; b is the carboxylation discrimination in C3 plants (27‰; e and f are carbon discrimination derived in dark respiration (RD) and photorespiration, respectively. k is the carboxylation efficiency, and Γ^* is the CO2 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:

Δ=a (C_a-C_i)/C_a +(e_s+a_l) (C_i-C_c)/C_a +b C_c/C_a -((eR_D)/k+fΓ_*)/C_a (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 gm, has been thought to be close to 30‰ in higher plants (Guy et al., 1993).

The difference of CO2 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:

Δ_i=a+(b-a) C_i/C_a (12)

Equation 12 presents the linear relationship between carbon discrimination and Ci/Ca that used normally in carbon isotopic fractionation. That underlined the subsequent comparison between the expected Δ (originated from gas-exchange,ãĂŰ ΔãĂŮ_i) and those actually measuredãĂŰ (ΔãĂŮ_obs), which could evaluate the magnitude of differences of CO2 concentration between the intercellular air and the sites of carboxylation that generated by mesophyll resistance. Consequently, gm can be estimated by performing the Δ_obs by isotope ratio mass spectrometry and expected Δ_i from Ci/Ca by gas exchange measurements.

Then subtract Δ_obs of Equation 11 from Δ_i calculated by Equation 12:

Δ_i-Δ_obs=(b-e_s-a_l) (C_i-C_c)/C_a +((eR_D)/k+fΓ*)/C_a (13)

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

A_n=g_m (C_i-C_c) (14)

Substitute Equation 14 into Equation 13 we obtain:

Δ_i-Δ_obs=(b-e_s-a_l) A_n/(g_m C_a)+((eR_D)/k+fΓ*)/C_a (15)

g_m=((b-e_s-a_l) A_n/C_a)/((Δ_i-Δ_obs)-(ãĂŰeRãĂŮ_D/k+fΓ*)/C_a) (16)

In calculation of gm, 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 gm.

Then Equation 16 can be transformed into:

g_m=((b-e_s-a_l) A_n/C_a)/(Δ_i-Δ_obs) (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 CO2 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 CO2 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, Pn, gs and Tr in P. orientalis and Q. variabilis peaked at 70%–80% of FC or/and FC (Fig. 2). The Ci 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 Ci were elevated significantly by elevated [CO2] at any given SWC for two species ($p<0.05$). Further, greater increasing magnitudes of Pn 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 gs in P. orientalis was more pronounced with elevated [CO2] at a given SWC ($p<0.01$). Nevertheless, gs 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 gs, Tr 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 Pn, gs, Ci and Tr of Q. variabilis was significantly presented than that of P. orientalis ($p<0.01$, Fig. 2)".

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