

# ***Interactive comment on* “Trade-offs between water loss and carbon gain in a subtropical primary forest on Karst soils in China” by Jing Wang et al.**

**Jing Wang et al.**

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Dear Reviewer,

We would like to thank you for the thoughtful and valuable comments and suggestions on our manuscript entitled “Trade-offs between water loss and carbon gain in a subtropical primary forest on Karst soils in China” (bg-2018-44). We have carefully revised our manuscript to take account of your comments and suggestions. Please find below our responses (upright Roman) to comments (original queries in *Italic*). Meanwhile, we have rephrased our manuscript title as “The strategies of water-carbon regulation of plants in a subtropical primary forest on Karst soils in China”. The line numbers mentioned here refer to our original manuscript. The changed figures and tables are

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presented in the Appendix (listed at the end of the “Response to reviewer”).

Specific comments (1) I feel the explanation and justification of the chosen methodology for measuring and calculating mesophyll conductance should be in the Materials and Methods section, not in the discussion. It takes away from your actual results.

Response: Thank you for your suggestion. This section have been moved to Section “Materials and Methods” according to your suggestion.

(2) Although an “in review” article is cited in the materials and methods, I think this is not an acceptable description of methodology (line 140). This should be written out in detail as I cannot access the information from there. I would like to have more details about leaf sampling and measurements. What were the temperature and humidity chosen for the measurements? How were the leaves collected? Did you collect leaves or twigs which you then cut under water or did you collect separate leaves which you measured in the field? Did you measure fluorescence? Could you calculate your results with the Harley method as well? It is common nowadays to confirm your results with a second method as all methods have some constraints.

Response: Thank you for your suggestions. In response, we have revised the Section “Materials and Methods” in two aspects. Firstly, we added more details about leaf sampling and measurements in Section “Materials and Methods”. Such as, we have added the method of how were the leaves collected and prepared before CO<sub>2</sub> response curves measurements “Branches exposed to the sun were excised from the upper part of the crown (Trees, Tree/Shrubs, Shrubs and Vines) or aboveground portion (Grasses, Ferns), and immediately re-cut under water to maintain xylem water continuity. Back into the laboratory, branches and aboveground portions were kept at 25°C for 30 min. Fully-expanded and mature leaves were induced for 30 minutes at a saturating light density (1500  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ). CO<sub>2</sub> response curves measurements were performed when A and g<sub>s</sub> was stable. Three leaves per species were collected and measured. A total of 189 leaves were collected from adult individuals of 63 species.”

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We have described the method and conditions of CO<sub>2</sub> response curves measurements in more detail as: “The CO<sub>2</sub> response curves were measured with 11 CO<sub>2</sub> concentration gradients in chamber following the procedural guidelines described by Longand Bernacchi (2003). The photosynthetic photon flux density was 1500  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . The leaf temperature was 25°C, controlled by the block temperature. The humidity in the leaf chamber was maintained at ambient condition. Leaf area, thickness (LT) and dry mass were measured after the CO<sub>2</sub> response measurements. Leaf mass per area (LMA) was calculated by dividing the corresponding dry mass by leaf area. And leaf density (LD) was calculated by dividing the corresponding LMA by LT. More details were described in Wang et al. (2018).”

Secondly, we clarified that gm was estimated by the ‘curve-fitting’ method in this study. As the fluorescence was not measured in this study, the Harley method cannot be used to calculate gm. Details about why we choose the ‘curve-fitting’ method to calculate gm, and the data valid confirmation have been added “Three methods are most commonly used for gm estimation. Those methods have been reviewed by Warren (2006) and Pons et al. (2009). Briefly, gm can be calculated by the stable isotope method (Evans, 1983; Sharkey et al., 1991; Loreto et al., 1992), J method (Bongi and Loreto, 1989; Dimarco et al., 1990; Harley et al., 1992; Epron et al., 1995; Laik et al., 2005), and ‘curve-fitting’ method (Ethier and Livingston, 2004; Sharkey et al., 2007). All of these methods are based on gas exchange measurements (Pons et al., 2009), and some common assumptions (Warren, 2006). Thus, the accuracy of each method is to some extent unknown (Warren, 2006). gm was estimated by the ‘curve-fitting’ method in this study. Although the ‘curve-fitting’ method is less precise than the stable isotope method, the ‘curve-fitting’ method is much more readily available and has been used for several decades (Warren, 2006; Sharkey, 2012). Accurate measurements of A and C<sub>i</sub> is a prerequisite for estimating gm using the ‘curve-fitting’ method (Pons et al., 2009). Warren (2006) pointed out that highly-accurate measurements need small leaf area and low flow rates. We confirmed that the calculated C<sub>c</sub> and the initial slope of A-C<sub>c</sub> curves were positive, suggesting that the measured gm was reliable (Warren,

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2006). ”

(3) I would also like to see more detail and justification in the statistical analysis section of the materials and methods

Response: Thank you for your comment. In response, we have moved the Section “2.4 Quantitative analysis of limitations on A” to Section “2.5 Statistical analysis” as the first section. Meanwhile, we have added more data analysis details in Section “2.5 Statistical analysis” as the second section. Such as, we have added the data analysis method “Data were analyzed either as a whole group (six life forms combined) or by individual life forms.”. We have added the bivariate linear regressions method “The bivariate linear regressions of leaf gas exchange parameters were performed using the standardized major axis (SMA) regression fits, and all of the data were made on loge-transformed data (Table S2).”. We have added what method was used to compare the difference of linear regressions “To test for the differences among life forms, SMA regression fits were used to compare the slope of regression lines which significant relationships had already been obtained. Note that Grass, Vine and Fern were not considered due to the small sample size. A similar trend was obtained, and no significant difference was found between life forms although significant relationships were not obtained for some bivariate linear regressions. Accordingly, six life forms were grouped together to analyze the strategy of water-carbon regulation of plants in the whole text. ”. We have added what method was used to compare the difference of the relative limitations of gs, gm and Vcmax to A. “ The difference of the contribution of gs, gm and Vcmax to A among life forms or as a whole group were performed using one-way ANOVA and Duncan multiple comparison. The probability of significance was defined at  $p < 0.05$ . ”

(4) In the results, you bring out that gs was better correlated with A, but lm was more limiting. This would be important to discuss in detail in the discussion. This is an extremely important result.

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Response: Thank you for your comment and suggestion. In response, we have re-analyzed our data, and revised Section “4.1 Co-variation in  $g_s$ ,  $g_m$  and  $V_{cmax}$  in regulating A”. Firstly, we analyzed the relationships between  $CO_2$  diffusion conductance ( $g_s$  and  $g_m$ ) and  $V_{cmax}$ , compared the relative limitations of  $g_s$ ,  $g_m$  and  $V_{cmax}$  to A, and analyzed the relationships between the limitation factors and the corresponding relative limitations. Consequently, we have revised the paragraph in Section “4.1 Co-variation in  $g_s$ ,  $g_m$  and  $V_{cmax}$  in regulating A” “The A was constrained by  $g_s$ ,  $g_m$ , and  $V_{cmax}$  acting together, however, variability in the relative contribution of these three factors depended on species and habitats (Tosens et al., 2016; Galmes et al., 2017; Peguero-Pina et al., 2017a; Veromann-Jurgenson et al., 2017). .... In addition, 20 of the 63 species were mainly limited by  $V_{cmax}$  ( $l_b > 0.4$ , with the largest value of 0.68). (lines 340-351)” to “A was constrained by  $g_s$ ,  $g_m$ , and  $V_{cmax}$  acting together, however, variability in the relative contribution of these three factors depended on species and habitats (Tosens et al., 2016; Galmes et al., 2017; Peguero-Pina et al., 2017a; Veromann-Jurgenson et al., 2017). A was significantly correlated with  $g_s$ ,  $g_m$ , and  $V_{cmax}$  (Fig.3a-c).  $g_s$  was positively related to  $g_m$  (Fig.S1c), while no relationship was found between the  $CO_2$  diffusion conductance ( $g_s$  and  $g_m$ ) and  $V_{cmax}$  (Fig. S2). The relative limitations of  $g_s$ ,  $g_m$ , and  $V_{cmax}$  were separated by a quantitative limitation model (Jones, 1985; Grassi & Magnani, 2005). The results showed that  $l_s$ ,  $l_m$  and  $l_b$  of 63 species varied in a large range (Fig. S3), indicating plants have a diverse strategies to co-ordinate the  $CO_2$  diffusion ( $g_s$  and  $g_m$ ) and  $V_{cmax}$  to maintain relative high A. The order of factors limitations to A was  $l_m > l_b > l_s$  ( $P < 0.05$ ) (Fig.S3). Furthermore, we tested the relationship between the relative limitations and the corresponding limitation factors. The results showed that  $l_s$ ,  $l_m$ , and  $l_b$  were negatively associated with  $g_s$ ,  $g_m$ , and  $V_{cmax}$ , respectively (Fig. 4). And the relationship was stronger for  $g_m$ -  $l_m$  ( $r^2=0.65$ ) than  $V_{cmax}$ -  $l_b$  ( $r^2=0.27$ ) and  $g_s$ -  $l_s$  ( $r^2=0.19$ ). ”

Secondly, we have discussed two possible reasons of the results in Section “4.1 Co-variation in  $g_s$ ,  $g_m$  and  $V_{cmax}$  in regulating A”. “ $g_s$  was better correlated with A, while the results showed that A was more limited by  $g_m$ . That could be explained by two pos-

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sible reasons. Firstly, compare to the linear relationship between  $A$  and  $g_s$ , a nonlinear trend has been found between  $A$  and  $g_m$  when  $g_m > 0.4$  (Fig. 3a, b). Secondly, leaf structure plays an important role in regulating  $g_m$  and  $V_{cmax}$ , consequently, in determining  $A$  (Veromann-Jurgenson et al., 2017). Negative relationships between  $A/LMA$  and  $LT$  ( $r^2=0.16$ ,  $p=0.002$ ), and  $A/LMA$  and  $LD$  ( $r^2=0.3$ ,  $p<0.001$ ) have been observed (Fig. S4c,d), while  $A$  was not correlated to  $LT$  and  $LD$  (Fig. S4a,b).

The importance of  $g_m$  in constraining  $A$  was variable, and depended on leaf structural traits, only  $LMA$ ,  $LT$ , and  $LD$  were analyzed in this study. Large variability in  $g_m$  has been shown both between and within species with different life forms and habits (Gago et al., 2014; Flexas et al., 2016). Variability in  $g_m$  in this study is similar to that in global datasets (Gago et al., 2014; Flexas et al., 2016). There was no significant difference among life forms ( $P>0.05$ ). Previous studies have confirmed that  $LMA$  (Tomas et al., 2013), thickness of leaf cell wall (Peguero-Pina et al., 2017b), liquid phase of mesophyll (Veromann-Jurgenson et al., 2017), cell wall thickness of mesophyll (Terashima et al., 2011; Tosens et al., 2016), and surface area of mesophyll and chloroplast exposed to intercellular space (Veromann-Jurgenson et al., 2017) were the main limitations for  $g_m$ . The wide variability of  $g_m$  between different species and life forms in the same ecosystem seems to be related to the diversity of leaf anatomical traits.

No significant difference of  $LMA$ ,  $LT$ , and  $LD$  was found among life forms ( $P<0.05$ ). The negative correlation of  $g_m$  (Terashima et al., 2005) or  $g_m/LMA$  (Niinemets et al., 2009; Veromann-Jurgenson et al., 2017) with  $LMA$  have been reported. In this study, there was a significant relationship between  $g_m/LMA$  with  $LMA$  ( $P<0.01$ ), however, no relationship was found between  $g_m$  with  $LMA$ .  $g_m/LMA$  was significantly negative related to  $LD$  ( $p<0.01$ ) (Fig. S5c), and weak negative related to  $LT$  ( $p=0.06$ ) (Fig. S5d), demonstrating that the negative role of cell wall thickness on  $g_m$  (Terashima et al., 2006; Niinemets et al., 2009). The strong investment in supportive structures was the main reason for the limitation of  $g_m$  on  $A$  (Veromann-Jurgenson et al., 2017). However, it is still unknown how leaf anatomical traits affect  $g_m$  and  $A$ , and this should be further

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explored.

$g_s$  is responsible for CO<sub>2</sub> exchange between atmosphere and leaf, and regulate the CO<sub>2</sub> fixation (A) and water loss (Lawson and Blatt, 2014). The variability of  $g_s$  was controlled by stomatal anatomy, i.e. stomata density and size, and mesophyll demands for CO<sub>2</sub> (Lawson and Blatt, 2014). However, the stomatal anatomy was not analyzed in this study. We only focused on how the relationship between  $g_s$  and  $g_m$  regulate A. Positive relationship between  $g_s$  and  $g_m$  has been observed (Flexas et al., 2013). For example, the restricted CO<sub>2</sub> diffusion from the ambient air to chloroplast is the main reason for a decreased A under water stress conditions due to both the stomatal and mesophyll limitations (Olsovska et al., 2016).  $g_s$  was significantly positive related to  $g_m$  for 63 species ( $P < 0.001$ , Fig. S1) in this study, and no difference of the slopes of regression lines between  $g_s$  and  $g_m$  was found among life forms, demonstrating that A was regulated by the co-variation of  $g_s$  and  $g_m$ . However, the variability of  $g_m$  and  $l_m$  was larger than  $g_s$  and  $l_s$ , respectively (Fig.1 and Fig.S3).

The wide variation range of  $l_b$  (0.11-0.68) highlighted the importance role of  $V_{cmax}$  in regulating A.  $V_{cmax}$  was used to represent the CO<sub>2</sub> demand in photosynthetic process in this study. The relative contribution of  $V_{cmax}$  to A not only depends on  $C_a - C_c$ , but also on leaf nutrient levels. Positive relationship was found between  $C_a - C_c$  and  $V_{cmax}$  (Fig. 1d). And the  $V_{cmax}/LMA$  was co-regulated by leaf N, P and Mg content (Jing et al. 2018). In addition,  $V_{cmax}/LMA$  was negatively related to LT ( $p < 0.05$ ) (Fig. S6c) and LD ( $p < 0.05$ ) (Fig. S6d), while  $V_{cmax}$  was not correlated to LT and LD (Fig. S6a,b), demonstrating that leaf structure plays an important role in regulating  $V_{cmax}$ .”

(5) The conclusions are a bit flat, I would like to see the paragraph rephrased so it is a bit more exciting.

Response: Thank you for your comment. The Section “Conclusions” has been rephrased as: “This study provides information of limitations of A and  $iWUE$  by  $g_s$ ,  $g_m$ , and  $V_{cmax}$  in 63 species across 6 life forms in the field. The results showed that

plants growing in Karst CZs used a diverse strategies of carbon-water regulation, but no difference was found among life forms. The co-variation of CO<sub>2</sub> supply ( $g_s$  and  $g_m$ ) and demand ( $V_{cmax}$ ) regulated A, indicating that species maintain relative high A through co-varying their leaf anatomical structure and  $V_{cmax}$ .  $iWUE$  was relatively low, but ranged widely, indicating that plants used the 'profligate/opportunistic' water use strategy to maintain the survival, growth, and structure of the community.  $iWUE$  was regulated by  $g_s$ ,  $V_{cmax}$ ,  $g_m/g_s$  and  $V_{cmax}/g_s$ , indicating that species with high  $g_m/g_s$  or  $V_{cmax}/g_s$  will have to be much more competitive to response to the ongoing rapid warming and drought in the Karst CZs."

(6) Figure 5 needs an explanation about the whiskers: are they SEs or SDs? If they are SEs, I do not find it likely that  $g_m$  was indeed the most important limiter in vies and ferns, but only grasses.

Response: Thank you for your suggestion and comment. We clarified that whickers in Figure 5 was standard deviation. The Figure 5 legend rephrased as: "Figure 5. Limitation to light-saturated net photosynthesis (A) in six life forms by stomatal conductance to CO<sub>2</sub> ( $l_s$ ), mesophyll conductance to CO<sub>2</sub> ( $l_m$ ), and the maximum carboxylase activity of Rubisco ( $l_b$ ). Error bars denominate standard deviation."

Technical comments

(7) Line 31: grammatical error, should be "plants"

Response: Corrected. Thank you.

(8) Line 38: delete first "and"

Response: Deleted. Thank you.

(9) Line 38: add "their" between "measured" and "CO<sub>2</sub>"

Response: Change has been made. Thank you.

(10)Line 38: . . . calculated "the" corresponding. . .

Response: Change has been made. Thank you.

(11) Line 73: replace “indeed” with “however”

Response: This change has been made.

(12) Line 84: within “a” leaf.

Response: Change has been made. Thank you.

(13) Line 110: delete “The”. Sentences should not be started with an article before an abbreviation. This is bad style.

Response: Deleted. Thank you.

(14) Lines 125 and 126: this sentence should be in the present if the soil conditions are unlikely to radically change in a short period of time.

Response: Change has been made.

(15) Line 130: same comment as the previous, should be in the present if this does not change rapidly.

Response: Change has been made.

(16) Line 140: You cannot use “were” if the article you are citing is still in review. This is chronologically incoherent.

Response: Thank you for your suggestion and comment. The cited article has been accepted by Scientific Reports”. And this sentence has been rephrased as “More details were described in Wang et al. (2018).”

(17) Line 148: the citation is doubles, delete one

Response: Deleted. Thank you. (See page 7 line 174)

(18) Line 153: delete “The”

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Response: Deleted. Thank you.

(19) Line 161: no need to redefine abbreviations in each section – once is enough

Response: Change has been made.

(20) Line 166: this sentence needs to be rephrased. Stomata are not a barrier inside the leaf, like this sentence seems to claim.

Response: Thank you for your suggestion and comment. Rephrased as: “Mesophyll is the barrier for CO<sub>2</sub> inside the leaf. ”

(21) Line 214: last equation was 8, this should be 9

Response: This changed have been made. Thank you.

(22)Line 253: both implies 2 variables: delete “both of”

Response: Deleted.

(23) Line 256: delete “The”

Response: Deleted.

(24) Line 257: move “respectively” to the end of the sentence

Response: Change has been made. Thank you.

(25) Line 269: delete “The”

Response: Deleted.

(26) Line 271: delete “The”

Response: Deleted.

(27) Line 272: Change to “Grasses”

Response: Change has been made. Thank you.

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(28) Line 273: Change to “Accordingly, grasses”

Response: Change has been made.

(29) Line 276: delete “The”

Response: Deleted.

(30) Line 284: delete “The”

Response: Deleted.

(31) Line 295: Recent work has compared Harley, Ethier and the anatomical models finding good correlations, so I would not write largely unknown, rather “to some extent”

Response: Rephrased as: “Thus, the accuracy of each method is to some extent unknown (Warren, 2006).”

(32) Line 353: this sentence should be rephrased, leads to the impression that you also did ultrastructural sampling

Response: Thank you for your suggestion and comment. Rephrased as: “The importance of gm in constraining A was variable, and depended on leaf structural traits, only LMA, LT, and LD were analyzed in this study.”

(33) Lines 368-374: chloroplasts do not have cell walls, the sentences need to be rephrased

Response: Thank you for your suggestion and comment. This mistake has been corrected.

(34) Line 402: “highly efficient”

Response: This change has been made.

(35) Line 411: delete the first “in this study”

Response: Deleted.

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(36)Line 415: “lose” not “loss”

Response: Corrected. Thank you.

(37) Lines 416-417 “The results ...”: unnecessary sentence, delete

Response: Deleted.

(38)Line 422: full stop missing from the end

Response: Added. Thank you.

(39) Line 424: delete “The”

Response: This change has been made.

(40) Lines 424-425 stating with “In theory”: should be in the present

Response: This change has been made.

(41) Line 433: This sentence should be in the present

This change has been made.

(42) Line 448: ...inefficiency in “the” trade-off

Response: This change has been made.

(43) Line 452: “low nutrient”

Response: This change has been made.

(44) Line 461: *iWUE* is not in italic in any other place

Response: This change has been made.

(45)Line 462: ...forms in “the” field

Response: This change has been made.

(46) Line 463: ... used “a” diverse

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Response: Change has been made, thank you.

(47) Line 464: ... maintain “a” relatively

Response: This change has been made.

(48) Line 465: ... used “the”

Response: Thank you for your suggestion and comment. Change has been made.

(49) Line 483: “References”

Response: Change has been made.

Please also note the supplement to this comment:

<https://www.biogeosciences-discuss.net/bg-2018-44/bg-2018-44-AC1-supplement.pdf>

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