

## ***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.***

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Response to reviews of manuscript “Trade-offs between water loss and carbon gain in a subtropical primary forest on Karst soils in China” bg-2018-44

Response to reviewer#2

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,

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

General comments:

(1) The author use “Trade-offs between water loss and carbon gain” in the title, however, the whole-text actually talk about the limitation of different components on A and iWUE.

Response: Thanks a lot for your comment. We response to this comment from two aspects. One on hand, 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”.

On the other hand, we have revised the Section “Discussion”. Firstly, we have re-organized and revised Section “4.1 The role of gm in CO<sub>2</sub> diffusion and V<sub>cmax</sub>”, and merged it with “4.2 Co-variation in g<sub>s</sub>, g<sub>m</sub> and V<sub>cmax</sub> in regulating A”. Such as, we have moved two paragraphs “Three methods are most commonly used for g<sub>m</sub> estimation. .... 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 largely unknown (Warren, 2006) (Lines 288-295). The g<sub>m</sub> was estimated by the ‘curve-fitting’ method in this study. ... 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 g<sub>m</sub> was reliable (Warren, 2006). (Lines 297-304)” to Section “Methods and Materials”.

We have deleted two paragraphs “Large uncertainties can be introduced by ignoring g<sub>m</sub>. ....  $\delta^{13}\text{C}_{\text{gm}}$  represented the carbon isotope discrimination when g<sub>m</sub> was finite, and  $\delta^{13}\text{C}_{\text{gs}}$  represented the carbon isotope discrimination when g<sub>m</sub> was infinite (Lines 319-328). On the other hand, ignoring g<sub>m</sub> would underestimate V<sub>cmax</sub> up to 75% (Sun et al., 2014). .... Furthermore, the leaf barrier to CO<sub>2</sub> caused by g<sub>m</sub> has

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not been represented in the global carbon cycles, leading to an overestimation of CO<sub>2</sub> supply for carboxylation and an underestimation of the response of photosynthesis to atmospheric CO<sub>2</sub> (Sun et al., 2014) (Lines 330-337).”.

We have revised the paragraph “Large variability in gm has been shown both between and within species with different leaf forms and habits (Gago et al., 2014; Flexas et al., 2016). .... Hence, the wide variability of gm between different species and life forms in the same ecosystem seems to be related to the diversity in leaf anatomical traits. (Lines 306-317)” to “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. Large variability in gm has been shown both between and within species with different life forms and habits (Gago et al., 2014; Flexas et al., 2016). Variability in gm in this study is similar to that in global datasets (Gago et al., 2014; Flexas et al., 2016). There was no significantly 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 gm. The wide variability of gm between different species and life forms in the same ecosystem seems to be related to the diversity of leaf anatomical traits.”. And we have merged this paragraph with “4.2 Co-variation in gs, gm and Vcmax in regulating A ”.

Secondly, we revised the title of Section “4.2 Co-variation in gs, gm and Vcmax in regulating A” as “4.1 Co-variation in gs, gm and Vcmax in regulating A ”. And we have re-analyzed our data, and revised the paragraph “The A was constrained by gs, gm, and Vcmax 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 Vcmax ( $lb>0.4$ , with the largest value of 0.68).

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(Lines 340-351) ” to “A was constrained by gs, gm, and Vcmax 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 gs, gm, and Vcmax (Fig.3a-c). gs was positively related to gm (Fig.S1c), while no relationship was found between the CO<sub>2</sub> diffusion conductance (gs and gm) and Vcmax (Fig. S2). The relative limitations of gs, gm, and Vcmax were separated by a quantitative limitation model (Jones, 1985; Grassi & Magnani, 2005). The results showed that ls, lm and lb of 63 species varied in a large range (Fig. S3), indicating plants have a diverse strategies to co-ordinate the CO<sub>2</sub> diffusion (gs and gm) and Vcmax to maintain relative high A. The order of factors limitations to A was  $lm>lb>ls$  ( $P<0.05$ ) (Fig.S3). Furthermore, we tested the relationship between the relative limitations and the corresponding limitation factors. The results showed that ls, lm, and lb were negatively associated with gs, gm, and Vcmax, respectively (Fig. 4). And the relationship was stronger for gm- lm ( $r^2=0.65$ ) than Vcmax- lb ( $r^2=0.27$ ) and gs- ls ( $r^2=0.19$ ).

gs was better correlated with A, while the results showed that A was more limited by gm. That could be explained by two possible reasons. Firstly, compare to the linear relationship between A and gs, a nonlinear trend has been found between A and gm when  $gm>0.4$  (Fig. 3a, b). Secondly, leaf structure plays an important role in regulating gm and Vcmax, 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).”

We have tested the difference of LMA, leaf thickness (LT) and leaf density (LD) among life forms, no significantly different have been found. And then we tested the roles of leaf structure (LT and LD) on A, gm, and Vcmax. The results showed that leaf structure plays important role in regulating A, gm, and Vcmax. Consequently, we revised discussions about the carbon fixation (A) strategies of plants (Lines 353-406) as

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“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. Large variability in gm has been shown both between and within species with different life forms and habits (Gago et al., 2014; Flexas et al., 2016). Variability in gm 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 gm. The wide variability of gm 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 gm (Terashima et al., 2005) or gm/LMA (Niinemets et al., 2009; Veromann-Jurgenson et al., 2017) with LMA have been reported. In this study, there was a significant relationship between gm/LMA with LMA ( $P < 0.01$ ), however, no relationship was found between gm with LMA. gm/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 gm (Terashima et al., 2006; Niinemets et al., 2009). The strong investment in supportive structures was the main reason for the limitation of gm on A (Veromann-Jurgenson et al., 2017). However, it is still unknown how leaf anatomical traits affect gm and A, and this should be further explored.

gs 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 gs 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 gs and gm regulate A.

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Positive relationship between gs and gm 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 (Olsovská et al., 2016). gs was significantly positive related to gm for 63 species ( $P < 0.001$ , Fig. S1) in this study, and no difference of the slopes of regression lines between gs and gm was found among life forms, demonstrating that A was regulated by the co-variation of gs and gm. However, the variability of gm and lm was larger than gs and ls, respectively (Fig. 1 and Fig. S3).

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

The trade-off between CO<sub>2</sub> supply (gs and gm) and demand (carboxylation capacity of Rubisco) can help maintain relative high A (Galmes et al., 2017; Saez et al., 2017). In this study, we used V<sub>cmax</sub> as a proxy for the carboxylation capacity of Rubisco, and the normalized V<sub>cmax</sub> by A ( $V = V_{cmax}/A$ ) was significantly negatively correlated with the normalized gt by A ( $Gt = gt/A$ ) ( $P < 0.001$ ) (Fig. 2c), indicating that the trade-off between CO<sub>2</sub> supply and demand also existed among different species in the same ecosystems. For genus *Limonium* (flowering plants) (Galmes et al., 2017), gt was significantly positively related to Rubisco carboxylase specific activity, and significantly negatively related to Rubisco specificity factor to CO<sub>2</sub>. In case of Antarctic vascular (Saez et al., 2017) and Mediterranean plants (Flexas et al., 2014), A was mainly limited by low gm, but it could be partially counterbalanced by a highly efficient Rubisco through high specificity for CO<sub>2</sub>. This highlights the importance of the trade-off between CO<sub>2</sub> supply

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and demand in plant adaptation to Karst environment. However, it is still unknown how leaf anatomical traits affect  $g_m$ ,  $V_{cmax}$  and  $A$ , and this should be further explored. ”

Thirdly, we have revised the title of Section “4.3 Co-variation in  $g_s$ ,  $g_m$  and  $V_{cmax}$  in regulating  $iWUE$ ” as “4.2 Co-variation in  $g_s$ ,  $g_m$  and  $V_{cmax}$  in regulating  $iWUE$ ”. To emphasize the diverse carbon-water regulation strategies of plants in Karst CZs, and highlighted the role of trade-off between carbon gain and water loss, we have revised the paragraph “Compared with the global dataset under well-watered conditions (19.27-171.88  $\mu\text{mol CO}_2 \text{ mol}^{-1} \text{ H}_2\text{O}$ ) (Flexas et al., 2016), the  $iWUE$  (29.52-88.92  $\mu\text{mol CO}_2 \text{ mol}^{-1} \text{ H}_2\text{O}$ ) in this study was somewhat lower in this study....The average  $iWUE$  of 12 vines and 13 trees in the Karst tropical primary forest was  $41.23 \pm 13.21 \mu\text{mol CO}_2 \text{ mol}^{-1} \text{ H}_2\text{O}$  (Chen et al., 2015), while that of 6 evergreen and 6 deciduous trees was  $66.7 \pm 4.9$  and  $49.7 \pm 2.0 \mu\text{mol CO}_2 \text{ mol}^{-1} \text{ H}_2\text{O}$ , respectively (Fu et al., 2012). (Lines 409-422)” to “Compared with the global dataset under well-watered conditions (19.27-171.88  $\mu\text{mol CO}_2 \text{ mol}^{-1} \text{ H}_2\text{O}$ ) (Flexas et al., 2016),  $iWUE$  ( $52.85 \pm 13.08 \mu\text{mol CO}_2 \text{ mol}^{-1} \text{ H}_2\text{O}$ ) was somewhat lower in this study.  $iWUE$  varied from 29.53 to 88.91  $\mu\text{mol CO}_2 \text{ mol}^{-1} \text{ H}_2\text{O}$ , and the variability of  $iWUE$  was larger than in the Karst tropical primary forest (Fu et al., 2012; Chen et al., 2015). The average  $iWUE$  of 12 Vines and 13 Trees in the Karst tropical primary forest was  $41.23 \pm 13.21 \mu\text{mol CO}_2 \text{ mol}^{-1} \text{ H}_2\text{O}$  (Chen et al., 2015), while that of 6 evergreen and 6 deciduous Trees was  $66.7 \pm 4.9$  and  $49.7 \pm 2.0 \mu\text{mol CO}_2 \text{ mol}^{-1} \text{ H}_2\text{O}$ , respectively (Fu et al., 2012). The results demonstrated that Karst plants use a diverse strategies of carbon-water regulation to adapt to the harsh Karst environment.

The trade-off between carbon gain and water loss is one of important strategies of carbon-water regulation of plants, and was exist among species and life forms (Prentice et al., 2014). Prentice et al. (2014) studied the trade-off between carbon gain and water loss of woody species in contrasting climates, and found that species in hot and wet regions tend to lose more water in order to fix more carbon (high  $g_s/A$ , low  $V_{cmax\_Ci}/A$ ), and vice versa. Although Karst soils cannot contain enough water for

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plant growth, the trade-off between carbon gain and water loss (high  $g_s/A$  and low  $V_{cmax\_Ci}/A$ ) were similar to the shown for plants growing in hot and wet regions (Prentice et al., 2014). ”

(2) In the method section: The species covered wide range of functional groups, including 6 life forms. What the criteria of the species selection? Because the leaf habit (evergreen or deciduous), the shade or light-demanding behaviors also will affect the strategy of plant carbon-water regulation. For example, does fern grow in the canopy or understory, how you can put them together when analyze the data? More important, the main objective of this paper was to determine and distinguish the limitations of  $\text{CO}_2$  diffusion and  $V_{cmax}$  on  $A$  and  $iWUE$  in different life forms Karst forest, however, you combine all species together for most analysis, actually we donot know what's the difference between different life forms in Figs 1-4, 6,7. I Believe most land plant will behave in similar way to adapt to the environmental factor no matter where they grow, the interesting things is to what extent by different plants. For example, Based on Fig 5, we could not see any difference among the groups. So, I suggest the author should separate into 6 groups to see the differences of regression lines among groups for all the figures, and compare the difference among the life forms using proper statistical method.

Response: Thank you for your comments and suggestions. We response to revised the manuscript from three aspects according to your comments and suggestions. Firstly, we have added our criteria of the species selection in Section “2.2 Leaf gas-exchange measurements” “In July and August 2016, 63 species (Table S1) were selected for measurements of the  $A$  and  $\text{CO}_2$  response curves. The species sampled were selected according to their abundance in the study site. They are the main component of this forest, including 55 woody species (46 deciduous and 10 evergreen species) and 5 herb species. To distinguish the strategies of water-carbon regulation of plants among different life forms, those species were grouped into 6 life forms, including (1) Tree ( $n=29$ ), (2) Tree/Shrub ( $n=11$ ), (3) Shrub ( $n=11$ ), (4) Grass ( $n=11$ ), (5) Vine ( $n=5$ ),

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and (6) Fern (n=3). "Tree/Shrub" is a kind of low wood plant between Tree and Shrub. Fern grow in understory. Vine climb up to the shrub canopy to get light. " We have added how were the leaves collected "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. "

Secondly, we have re-analyzed our data either as a whole group (six life forms combined) or by individual life forms, and the difference between different life forms was tested using the standardized major axis (SMA) regression fits. The results showed that no significantly difference between life forms. Thus six life forms were grouped together to analyze the strategy of water-carbon regulation of plants in the whole text. The statistical method and results have been added in Section "2.5 Statistical analysis" "Data were analyzed either as a whole group (six life forms combined) or by individual life forms. 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).

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.

The difference of relative limitation of  $g_s$ ,  $g_m$  and  $V_{cmax}$  to  $A$  for life forms or as a whole group were performed using one-way ANOVA and Duncan multiple comparison. The probability of significance was defined at  $p < 0.05$ . "

Thirdly, all of data of six life forms were separately presented in Figure 1-4, 6,7 and

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Figure S1,S2, S4-S6 (See Appendix). Only the regression line for 63 species were presented in figures.

(3) lines 139-140, because the A-Ci curve is the key data of this paper, author should describe in detail how this measurement was done rather than just cite other submitted papers. For example, you should introduce the height of your targeted individuals? how you can measure the sun-exposed leaf for canopy trees and climbing plants: : :.?did you measure in situ or cut down, if the latter, for A-Ci curve you normally need ca. 30 min, how you can avoid the effects of cutting on stomatal conductance because some species are very sensitive, do you have some information on the  $g_s$  sensitivity for those species? : :..

Response: Thank you for your suggestions. In response, we have 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 CO2 response curves measurements "Details of leaf sampling and measurements of the CO2 response curve were briefly described as follows. Branches exposed to the sun were excised from the upper part of the crown (Trees, Tree/Shrubs, Shrubs and Vines) or above the ground (Grasses, Ferns), and immediately re-cut under water to maintain xylem water continuity. Back into the laboratory, branches were kept at 25°C for 30 min. Fully-expanded and mature leaves were induced for 30 minutes at a saturating light density ( $1500 \mu\text{mol m}^{-2} \text{s}^{-1}$ ). CO2 response curves measurements were performed when  $A$  and  $g_s$  was stable. Three leaves per species were collected and measured. A total of 189 leaves were collected from adult individuals of 63 species." However, the height of targeted individuals did not measured.

We have described the method and conditions of CO2 response curves measurements in more detail as: "The CO2 response curves were measured with 11 CO2 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

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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).”

Specific comments:

(4) Line 267-269: There is no statistic tests of the differences of the results in figure 5, so it is not proper to give the statements in line 309-310. Figure 5 can't give any information that is about LMA. Please use data to demonstrate the relationship between LMA and other parameters instead of qualitative description.

Response: Thank you for your comments and suggestions. We response to the comments and suggestions from two aspects. Firstly, we have analyzed the data of figure 5 using statistical method, and revised the corresponding Sections. Such as, we have added statistical method used to test the difference of the results in figure 5 in Section “2.5 Statistical analysis” “The difference of relative limitation of  $g_s$ ,  $g_m$  and  $V_{cmax}$  to A for life forms or as a whole group were performed using one-way ANOVA and Duncan multiple comparison. The probability of significance was defined at  $p < 0.05$ .”.

We have drew figure 5 and revised the Section “3.2 Contribution of  $g_s$ ,  $g_m$  and  $V_{cmax}$  to A” as “The variation in A was attributed to variation in  $g_s$ ,  $g_m$ ,  $g_t$ , and  $V_{cmax}$ . A was positively correlated with  $g_s$  (Fig. 3a),  $g_m$  (Fig. 3b), and  $V_{cmax}$  (Fig. 3c). We used the quantitative limitation model (Eqs. (9), (10) and (11)) to separate  $g_s$  ( $l_s$ ),  $g_m$  ( $l_m$ ), and  $V_{cmax}$  ( $l_b$ ) limitations to A.  $l_s$ ,  $l_m$ , and  $l_b$  were negatively associated with  $g_s$ ,  $g_m$ , and  $V_{cmax}$ , respectively (Fig. 4). The contributions by  $g_s$ ,  $g_m$ , and  $V_{cmax}$  to limiting A were different for each species (Fig. S3).  $l_s$  varied 2.6-fold ( from 0.17 to 0.45),  $l_m$  varied 10.5-fold ( from 0.05 to 0.55), and  $l_b$  varied 6.2-fold ( from 0.11 to 0.68) across species. Overall,  $l_m$  ( $0.38 \pm 0.12$ ) was significantly larger than  $l_b$  ( $0.34 \pm 0.14$ ), and  $l_s$  ( $0.28 \pm 0.07$ ) ( $P < 0.05$ ).

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To further understand how A was limited by  $g_s$ ,  $g_m$ , and  $V_{cmax}$  among life forms, we grouped the 63 species into 6 life forms: Tree, Tree/Shrub, Shrub, Grass, Vine, and Fern. The results showed that there was no significantly difference between  $l_s$ ,  $l_m$  and  $l_b$  for Trees and Tree/shrubs.  $l_m$  of Shrubs and Grasses was significantly higher than that of  $l_s$  and  $l_b$  ( $P < 0.05$ ).  $l_m$  of Vines and Ferns was significantly higher than that of  $l_s$  ( $P < 0.05$ ) (Fig. 5). ”. We have revised the Section “4.1 Co-variation in  $g_s$ ,  $g_m$  and  $V_{cmax}$  in regulating A ”. Please also see the response to reviewer #1.

Secondly, we have tested the difference of LMA across life forms using one-way ANOVA and Duncan multiple comparison. The results showed that no difference of LMA was found among life forms. Consequently, lines 309-310 have been removed. We have tested the role of leaf structure (leaf thickness (LT) and leaf density (LD)) in A,  $g_m$  and  $V_{cmax}$ , and rephrased the Section “4.1 Co-variation in  $g_s$ ,  $g_m$  and  $V_{cmax}$  in regulating A ”. Please also see the response to reviewer #1.

(5) Line 372: Species with low LMA may have thick cell walls in mesophyll and chloroplast.

Response: Thank you for your suggestion. We have tested the difference of LMA across life forms using one-way ANOVA and Duncan multiple comparison. The results showed that no difference of LMA was found among life forms. Meanwhile, We have tested the role of leaf structure (leaf thickness (LT) and leaf density (LD)) in A,  $g_m$  and  $V_{cmax}$ . The results showed that leaf structure plays important role in regulating  $g_m$  and  $V_{cmax}$ , consequently, in determining A. Consequently, we revised the corresponding section in “4.1 Co-variation in  $g_s$ ,  $g_m$  and  $V_{cmax}$  in regulating A ” as “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 significantly difference among life forms ( $P > 0.05$ ). Previous studies have confirmed that LMA (Tomas et al.,

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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 gm. The wide variability of gm 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 gm (Terashima et al., 2005) or gm/LMA (Niinemets et al., 2009; Veromann-Jurgenson et al., 2017) with LMA have been reported. In this study, there was a significant relationship between gm/LMA with LMA ( $P < 0.01$ ), however, no relationship was found between gm with LMA. gm/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 gm (Terashima et al., 2006; Niinemets et al., 2009). The strong investment in supportive structures was the main reason for the limitation of gm on A (Veromann-Jurgenson et al., 2017). However, it is still unknown how leaf anatomical traits affect gm and A, and this should be further explored. ”

(6) Line 381-382: In your results, gs and gm are positively correlated, why did you conclude gm is a compensate for reductions in gs? Did you observe an increasing of gm when gs decreased.

Response: Thank you for your comment. We corrected this mistake, and we rephrased this paragraph as: “gs 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 gs 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 gs and gm regulate A. Positive relationship between gs and gm has been observed (Flexas et al., 2013). For example. the restricted CO<sub>2</sub> diffusion from the

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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). gs was significantly positive related to gm for 63 species ( $P < 0.001$ , Fig. S1) in this study, and no difference of the slopes of regression lines between gs and gm was found among life forms, demonstrating that A was regulated by the co-variation of gs and gm. However, the variability of gm and Im was larger than gs and Is, respectively (Fig.1 and Fig.S3).”

(7) Line 384-389: I don't think you have enough evidences to state “there was a trend of increasing Im with increasing leaf N:P”, unless you add this part of research in your draft.

Response: Thank you for your comment. There was no significant statistical relationship between Im and leaf N:P ( $P = 0.66$ ). We corrected this mistake, and rephrased this paragraph : “ The wide variation range of lb (0.11-0.68) highlighted the importance role of Vcmax in regulating A. Vcmax was used to represent the CO<sub>2</sub> demand in photosynthetic process in this study. The relative contribution of Vcmax to A not only depends on Ca-Cc, but also on leaf nutrient levels. Positive relationship was found between Ca-Cc and Vcmax (Fig. 1d). And the Vcmax/LMA was co-regulated by leaf N, P and Mg content (Jing et al. 2018). In addition, Vcmax/LMA was negatively related to LT ( $p < 0.05$ ) (Fig. S6c) and LD ( $p < 0.05$ ) (Fig. S6d), while Vcmax was not correlated to LT and LD (Fig. S6a,b), demonstrating that leaf structure plays an important role in regulating Vcmax.”

(8) Awful sentences, Lines 39-35, should split into short sentences

Response: Rephrased as: “The results showed that (1) gs and gm varied about 7.6- and 34.5-fold, respectively, and gs was positively related to gm. The contribution of gm to leaf CO<sub>2</sub> gradient was similar to that of gs. The gs/A, gm/A and gt/A was negative related to Vcmax/A. (2) the relative limitations of gs (Is), gm (Im) and Vcmax (lb) to A for the whole group (combined 6 life forms) were significantly different from

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each other ( $P < 0.05$ ).  $I_m$  was the largest ( $0.38 \pm 0.12$ ), followed by  $I_b$  ( $0.34 \pm 0.14$ ) and  $I_s$  ( $0.28 \pm 0.07$ ). No significant difference was found between  $I_s$ ,  $I_m$ , and  $I_b$  for Trees and Tree/shrubs, while  $I_m$  was the largest, followed by  $I_b$  and  $I_s$  for Shrubs, Grasses, Viens and Ferns ( $P < 0.05$ ). (3)  $iWUE$  varied about 3-fold (from 29.52 to 88.92  $\mu\text{mol CO}_2 \text{ mol}^{-1} \text{ H}_2\text{O}$ ) across all species, and was significantly correlated with  $g_s$ ,  $V_{cmax}$ ,  $g_m/g_s$ , and  $V_{cmax}/g_s$ .”

Please also note the supplement to this comment:

<https://www.biogeosciences-discuss.net/bg-2018-44/bg-2018-44-AC2-supplement.pdf>

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Interactive comment on Biogeosciences Discuss., <https://doi.org/10.5194/bg-2018-44>, 2018.