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

Dear Editor,

We deeply appreciate you for giving us an opportunity to revise our manuscript. Here are the point-to-point responses (responses in upright Roman) to the 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".

Response to Associate Editor comments

Here are the point-to-point responses to the comments (original queries in Italic).

1)Please rephrase the starting sentence by highlighting the importance of trade off between water loss and carbon gain and its implication. And the key characteristics of Karst can be briefly introduced.

Response: Thanks a lot for your comment and suggestion. In response, we have rephrased the starting sentence of Abstract as "Coexisting plant species in a Karst ecosystem may use diversity strategies of trade off between carbon gain and water loss to adopt to the low soil nutrient and water availability conditions." (see Page 2 lines 31-33).

Meanwhile, we have rephrased the first paragraph in Section "Introduction" as "Diversity strategies of trade off between carbon gain and water loss are critical for the survival of coexisting plant species. In order to adapt to the harsh environment, coexisting plant species develop distinct patterns of strategies of carbon-water regulation (light-saturated net photosynthesis (*A*) and intrinsic water use efficiency

(iWUE)) (Sullivan et al., 2017). iWUE is the ratio of *A* to stomatal conductance to H_2O (g_{sw}) (Moreno-Gutierrez et al., 2012). Plants with high iWUE are better able to adapt to the nutrient- and water-limited environment (Flexas et al., 2016). Due to the greater hydraulic erosion and complex underground drainage network (Nie et al., 2014; Chen et al., 2015), Karst soils cannot retain enough nutrients and water for plant growth even though precipitation is high (1000-2000 mm) (Liu et al., 2011; Fu et al., 2012; Chen et al., 2015). Understanding of the impact of CO_2 diffusion and maximum carboxylase activity of Rubisco (V_{cmax}) on *A* and iWUE in Karst plants can provide insight into physiological strategies of water-carbon regulation of plants used in adaptation to Karst environments at the leaf scale. Until now, variability in *A* and iWUE has been reported only in 13 co-occurring trees and 12 vines (Chen et al., 2015), and 12 co-occurring tree species (Fu et al., 2012) in two tropical Karst forests in southwestern China.". (see Page 3 lines 57-73).

2) In addition the manuscript needs to be carefully checked for some typos.

Response: Thanks a lot for your comment. We have carefully checked and corrected the typos.

Response to reviewer#1

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. (see Page 7 lines 182-198).

(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 have added more details about leaf sampling and measurements in Section "Materials and Methods". Such as, we have added the method of how to collect and prepare the leaves before CO_2 response curves measurements. (see Page 6 lines 154-162). Meanwhile, we have described the method and conditions of CO_2 response curves measurements in more detail. (see page 6 lines 164-168).

Secondly, we clarified that g_m was estimated by the 'curve-fitting' method in this study (see page 7 line191). As the fluorescence was not measured in this study, the Harley method cannot be used to calculate g_m. Details about why we choose the 'curve-fitting' method to calculate g_m, and the data valid confirmation have been added. (see page 7 lines 191-198).

(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 revised the Section "2.5 Statistical analysis" in two aspects. Firstly, we have moved the Section "2.4 Quantitative analysis of limitations on *A*" to Section "2.5 Statistical analysis". (see page 9 line 251 to page 10 line 265). Secondly, we have added more details about the

statistical analysis in Section "2.5 Statistical analysis" (see Section "(2) Data analysis", page 10 line3 267-283). Such as, we have added the data analysis method. (see page 10 lines 268-269). We have added the bivariate linear regressions method. (see page 10 lines 269-271). We have added what method used to compare the difference of linear regressions. (see page 10 lines 273-279).

(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.

Response: Thank you for your comment and suggestion. In response, we have reanalyzed 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 corresponding results in Section "4.1 Co-variation in g_s, g_m and V_{cmax} in regulating A". (see page 12 line 343 to page 13 line 354). In brief, 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). 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, 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 corresponding results in Section "4.1 Co-variation in g_s, g_m and V_{cmax} in regulating A". (see page 13 lines 356 -363). In brief, 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 possible 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 LT (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).

(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_2 supply (g_s and g_m) and demand (V_{cmax}) regulated A, indicating that species maintain a relatively high A through co-varing 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." . (see page 17 line 489 to page 18 line 499).

(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 gm 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_2 (l_s), mesophyll conductance to CO_2 (l_m), and the maximum

carboxylase activity of Rubisco (l_b). Error bars denominate standard deviation.”. (see page 31 lines 788-789).

Technical comments

(7) Line 31: grammatical error, should be “plants’”

Response: This sentence has been deleted.

(8) Line 38: delete first “and”

Response: Deleted. Thank you. (see page 2 line 38).

(9) Line 38: add “their” between “measured” and “CO2”

Response: Change has been made. Thank you. (see page 2 line 38).

(10)Line 38: … calculated “the” corresponding…

Response: Change has been made. Thank you. (see page 2 line 39).

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

Response: This change has been made. (see page 3 line 79).

(12) Line 84: within “a” leaf.

Response: Change has been made. Thank you. (see page 4 line 90).

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

Response: Deleted. Thank you. (see page 5 line 116).

(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. (see page 5 lines 132-133).

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

Response: Change has been made. (see page 5 line 137).

(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)." (see page 6 lines 171-172).

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

Response: Deleted. Thank you. (See page 7 line 177).

(18) Line 153: delete "The"

Response: Deleted. Thank you. (See page 7 line 191).

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

Response: Change has been made. (See page 7 line 202).

(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_2 inside the leaf. ”. (See page 8 line 208).

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

Response: This changed have been made. Thank you. (See page 9 line 257).

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

Response: Deleted. (See page 11 line 310).

(23) Line 256: delete “The”

Response: Deleted. (See page 11 line 313).

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

Response: Change has been made. Thank you. (See page 11 line 314).

(25) Line 269: delete “The”

Response: Deleted. (See page 11 line 315).

(26) Line 271: delete “The”

Response: Deleted. (See page 11 line 315).

(27) Line 272: Change to “Grasses”

Response: Change has been made. Thank you. (See page 12 line 342).

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

Response: Change has been made. (See page 12 line 323).

(29) Line 276: delete "The"

Response: Deleted. (See page 12 line 328).

(30) Line 284: delete "The"

Response: Deleted. (See page 12 line 336).

(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). " (See page 7 lines 188-189).

(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 g_m in constraining A was variable, and depended on leaf structural traits, only LMA, LT, and LD were analyzed in this study." (See page 13 lines 365-366).

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

Response: Thank you for your suggestion and comment. This mistake has been corrected: "cell wall thickness of mesophyll". (See page 13 lines 372-373).

(34) Line 402: "highly efficient"

Response: This change has been made. (See page 15 lines 424-425).

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

Response: Deleted. (See page 15 line 433).

(36)Line 415: "lose" not "loss"

Response: Corrected. Thank you. (See page 16 line 456).

(37) Lines 416-417 "The results …": unnecessary sentence, delete

Response: Deleted.

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

Response: Added. Thank you. (See page 16 line 438).

(39) Line 424: delete "The"

Response: This change has been made. (See page 16 line 461).

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

Response: This change has been made. (See page 16 line 461).

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

This change has been made. (See page 16 line 466).

(42) Line 448: …inefficiency in "the" trade-off

Response: This change has been made. (See page 17 line 485).

(43) Line 452: "low nutrient"

Response: This change has been made. (See page 16 lines 451).

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

Response: This change has been made. (See page 17 line 489).

(45)Line 462: ...forms in "the" field

Response: This change has been made. (See page 17 line 490).

(46) Line 463: ... used "a" diverse

Response: Change has been made, thank you. (See page 17 line 491).

(47) Line 464: ... maintain "a" relatively

Response: This change has been made. (See page 17 line 493).

(48) Line 465: ... used "the"

Response: Thank you for your suggestion and comment. Chang has been made. (see page 17 line 495).

(49)Line 483: "References"

Response: Change has been made. (See page 18 line 513).

Response to reviewer#2

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. On one 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 g_m in CO_2 diffusion and V_{cmax}”, and merged it with “4.2 Co-variation in g_s, g_m and V_{cmax} in regulating A ”. Such as, the explanation and justification of the chosen methodology for measuring and calculating g_m have been moved to Section “Materials and Methods” according to Reviewer#1's comment. (see Page 7 lines 182-198). Paragraphs “Uncertainties introduced by ignoring g_m.” have been deleted. We have revised, corrected and re-organized the paragraph “Large variability in g_m”, and merged it with “4.2 Co-variation in g_s, g_m and V_{cmax} in regulating A ”. (see page 13 lines 365-377).

Secondly, we have revised the title of Section “4.2 Co-variation in g_s, g_m and V_{cmax} in regulating A” as “4.1 Co-variation in g_s, g_m and V_{cmax} in regulating A ”. And we have re-analyzed our data, and rephrased the Section “4.1 Co-variation in g_s, g_m and V_{cmax} in regulating A ” to discuss about the limitation of different components on A and iWUE. (see page 13 line 339 to page 15 line 412). In brief, Karst 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. A was regulated by the co-variation of g_s and g_m. The strong investment in supportive structures was the main reason for the limitation of g_m on A. The wide variation range of l_b (0.11-0.68) highlighted the importance role of V_{cmax} in regulating A. The trade-off between CO_2 supply (g_s and g_m) and demand (carboxylation capacity of Rubisco) can help maintain relative high A.

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 Section "4.2 Co-variation in g_s, g_m and V_{cmax} in regulating iWUE ". (see page 15 line 430 to page 17 line 486). In brief, coexisting plant species growing in the Karst ecosystem had a diversity water use strategies. However, Karst plants tended to lose more water to gain more carbon, i.e. Karst plants used 'profligate/opportunistic' water use strategy to adopt to the low nutrient availability and water stress conditions. iWUE was correlated to g_s, V_{cmax}, g_m/g_s and V_{cmax}/g_s.

(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 CO2 diffusion and Vcmax 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". (see page 6 lines 145-152). In brief, the species sampled were selected according to their abundance in the study site. They are the main component of this forest. To distinguish the strategies of water-carbon regulation of plants among

different life forms, those species were grouped into 6 life forms, including Tree, Tree/Shrub, Shrub, Grass, Vine, and Fern. “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. Meanwhile, we have added how to collect and sample leaves. (see page 6 lines 154-162). For example, Branches exposed to the sun were excised from the upper part of the crown or aboveground portion, 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”. (see page 10 lines 268-279).

Thirdly, all of data of six life forms were separately presented in Figure 1-4, 6,7 and Figure S1,S2, S4-S6 (See Supplement). 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 gs sensitivity for those species ïj §: : :..

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 to sample and prepare the leaves before CO_2 response curves measurements. (see page 6 lines 154-162). In brief, branches exposed to the sun were excised from the upper part of the crown or above the ground, and immediately re-cut under water to maintain xylem water continuity. Back into the laboratory, branches were kept at 25^{o}C for 30 min. Fully-expanded and mature leaves were induced for 30 minutes at a saturating light density. CO_2 response curves measurements were performed when A and g_s was stable. However, the height of targeted individuals did not measured.

Meanwhile, we have described the method and conditions of CO_2 response curves measurements in more detail. (see page 6 lines 164-172). In brief, the CO_2 response curves were measured with 11 CO_2 concentration gradients in chamber following the procedural guidelines described by Longand Bernacchi (2003). The photosynthetic photon flux density was 1500 μmol m^{-2} s^{-1}. The leaf temperature was 25 ℃, controlled by the block temperature. The humidity in the leaf chamber was maintained at ambient condition.

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." . (see page 10 lines 281-283).

Meanwhile, we have re-drew figure 5 and revised corresponding results in the Section "3.2 Contribution of g_s, g_m and V_{cmax} to A". (see page 11 line 314 to page 12 line 325). In brief, the contributions by g_s, g_m, and V_{cmax} to limiting A were different for each species (Fig. S3). Overall, l_m was significantly larger than l_b, and l_s ($P<0.05$). 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). Meanwhile, we have revised the corresponding results and discussions in Section "4.1 Co-variation in g_s, g_m and V_{cmax} in regulating A ". (see page 12 line 340 to page 13 line 354).

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 ". (see page 13 line 365 to page 14 line 389). In brief, No significant difference of LMA, LT, and LD was found among life forms ($P<0.05$). 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).

(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*. Thus, we revised the corresponding section in "4.1 Co-variation in g_s, g_m and V_{cmax} in regulating *A* ". (see page 12 line 340 to page 13 line 354). In brief, No significant difference of LMA, LT, and LD was found among life forms ($P<0.05$). 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).

(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: "g_s is responsible for CO_2 exchange between atmosphere and leaf, and regulate the CO_2 fixation (*A*) and water loss (Lawsonand Blatt, 2014). The variability of g_s was controlled by stomatal anatomy, i.e. stomata density and size, and mesophyll demands for CO_2 (Lawsonand 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_2 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)." (see page 14 lines 391-403).

(7) Line 384-389: I don't think you have enough evidences to state "there was a trend of increasing lm 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 l_m and leaf N:P (P=0.66). We corrected this mistake, and rephrased this paragraph : " 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_2 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}." . (see page 14 line 405 to page 15 line 412).

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

Response: Rephrased as: "The results showed that g_s and g_m varied about 7.6- and 34.5-fold, respectively, and g_s was positively related to g_m. The contribution of g_m to leaf CO_2 gradient was similar to that of g_s. g_s/A, g_m/A and g_t/A was negative related to V_{cmax}/A. The relative limitations of g_s (l_s), g_m (l_m) and V_{cmax} (l_b) to A for the whole group (combined 6 life forms) were significantly different from each other ($P<0.05$). l_m was the largest (0.38 ± 0.12), followed by l_b (0.34 ± 0.14) and l_s (0.28 ± 0.07). No significant difference was found between l_s, l_m, and l_b for Trees and Tree/shrubs, while l_m was the largest, followed by l_b and l_s for Shrubs, Grasses, Viens and Ferns ($P<0.05$). iWUE varied about 3-fold (from 29.52 to 88.92 μmol CO_2 mol^{-1} H_2O)

across all species, and was significantly correlated with g_s, V_{cmax}, g_m/g_s, and V_{cmax}/g_s." (see page 3 lines 40-50).