The point-by-point response to the reviews

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).

The list of all relevant changes made in the manuscript

Here are the relevant changes made in the manuscript.

(1) Page 1 lines 1-2: The title has been changed as "The strategies of water-carbon regulation of plants in a subtropical primary forest on Karst soils in China".

(2) Page 2 lines 31-33: We have rephrased the starting sentence of Abstract.

(3) Page 2 lines 40-48: The results in Abstract have been split into short sentences according to reviewer #2's comment.

(4) Page 3 lines 57-63 and line 67-70: We have rephrased the starting paragraph of Instruction to highlighting the importance of trade off between water loss and carbon gain and its implication.

(5) Page 3 lines 63-67: The key characteristics of Karst have been rephrased.

(6) Page 3 line 79: "indeed" changed to "however".

(7) Page 4 line 90: "within leaf" changed to "within a leaf".

(8) Page 4 lines 113-114: "(29 trees, 11 trees/shrubs, 11 shrubs, 4 grasses, 5 vines, and 3 ferns)" changed to "(Tree (n=29), Tree/Shrub (n=11), Shrub (n=11), Grass (n=11), Vine (n=5), and Fern (n=3))".

(9) Page 5 lines 120-121: added ", and to understanding the patterns of strategies of carbon-water regulation of Karst plants. "

(10) Page 5 lines 132, 133, 134 and 137: "was" changed to "is".

(11) Page 5 line 141: "(Wang et al., in review)" changed to " (Wang et al., 2018)".

(12) Page 6 lines 144-152: The criteria of the species selection have been added.

(13) Page 6 lines 154-172: Details of leaf sampling and measurements of the CO2 response curve have been added.

(14) Page 7 line 174: "2.3 Response curve analyses" has been added as a new section in "Materials and Methods".

(15) Page 7 lines 182-198: The explanation and justification of the chosen methodology for measuring have been moved from "Discussion" Section to "Materials and Methods" Section.

(16) Page 7 line 200: "2.3" changed to "2.4".

(17) Page 8 line 208: "Besides stomata, mesophyll is another barrier for CO_2 inside the leaf." changed to "Mesophyll is the barrier for CO_2 inside the leaf.".

(18) Page 9 line 257: "8" changed to "9".

(19) Page 9 line 259: "9" changed to "10".

(20) Page 9 line 261: "10" changed to "11".

(21) Page 10 lines 267-283: More detail and justification in the statistical analysis section have been added.

(22) Page 11 line 312: "Eqs. (8), (9) and (10)" changed to " Eqs. (9), (10) and (11)".

(23) Page 11 line 312: "contributions" changed to " limitations".

(24) Page 11 line 314: ", respectively, " changed to " , respectively.".

(25) Page 11 line 315 to page 12 line 325: we have reanalyzed our data, and revised corresponding results.

(26) Page 12 line 339: "4.2 Co-variation in gs, gm and Vcmax in regulating A " changed to "4.1 Co-variation in gs, gm and Vcmax in regulating A ".

(27) Page 12 line 340 to page 15 line 412: we have reanalyzed our data, and revised the corresponding results and discussions in Section “4.1 Co-variation in g_s, g_m and V_{cmax} in regulating A”.

(28) Page 15 line 415: "maintain high photosynthetic efficiency " changed to "relative high A ".

(29) Page 15 line 418: "normalized gs by A (Gt =gs/A) (P<0.001) (Fig. 2c)" changed to " normalized gt by A (Gt =gt/A) (P<0.001) (Fig. 2c)".

(30) Page 15 lines 424-425: "highly-efficient " changed to "highly efficient".

(31) Page 15 lines 427-428: "g_m and A " changed to "g_m, V_{cmax} and A ".

(32) Page 15 line 430: "4.3 Co-variation of g_s, g_m and V_{cmax} in regulating iWUE" changed to "4.2 Co-variation of g_s, g_m and V_{cmax} in regulating iWUE".

(33) Page 17 line 489 to page 18 line 499: "Conclusions" section has been rephrased.

(34) Page 18 line 513: "6 Reference" changed to "6 References".

(35) Page 24 lines 701-706: Two reference article have been added.

(36) Page 24 lines 722-724: The reference article information has been changed.

(37) Figures 1-7 have been re-drew.

The strategies of water-carbon regulation of plants in a subtropical primary forest on Karst soils in China

Jing Wang[1,2,3]; Xuefa Wen[1,2*], Xinyu Zhang[1,2*], Shenggong Li[1,2]

1 Key Laboratory of Ecosystem Network Observation and Modeling, Institute of Geographic Sciences and Natural Resources Research, Chinese Academy of Sciences, Beijing 100101, China

2 College of Resources and Environment, University of Chinese Academy of Sciences, Beijing 100190, China

3 School of Life Sciences, Beijing Normal University, Beijing 100875, China

*Correspondence: Xuefa Wen (Email: wenxf@igsnrr.ac.cn. Phone +86-010-64889272) and Xinyu Zhang (zhangxy@igsnrr.ac.cn. Phone +86-10-64889679)

Abstract:

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. Understanding of the impact of CO_2 diffusion and maximum carboxylase activity of Rubisco (V_{cmax}) on the light-saturated net photosynthesis (A) and intrinsic water use efficiency (iWUE) can provide insight into physiological strategies of water-carbon regulation of coexisting plant species used in adaptation to Karst environments at the leaf scale. We selected 63 dominant species (across 6 life forms) in a subtropical Karst primary forest in southwestern China, measured their CO_2 response curves, and calculated the corresponding stomatal conductance to CO_2 (g_s), mesophyll conductance to CO_2 (g_m), and V_{cmax}. 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. These results indicated that Karst plants maintained relatively high A and low iWUE through the co-variation of g_s, g_m, and V_{cmax} as adaptation to Karst environment.

Key words: iWUE; mesophyll conductance; stomatal conductance; Karst critical zone; V_{cmax}

1 Introduction

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.

Based on Fick's first law, A has been shown to be limited only by leaf stomatal conductance to CO_2 ($g_s = g_{sw}/1.6$) and V_{cmax} (Flexas et al., 2012; Buckley and Warren, 2014); originally, mesophyll conductance to CO_2 (g_m) was proposed to be infinite, i.e. CO_2 concentration in chloroplast (C_c) was equal to the CO_2 concentration in intercellular air space (C_i). However, g_m varies greatly among species (Warren and Adams, 2006; Flexas et al., 2013). Recent studies have confirmed that A was constrained jointly by g_s, g_m, and V_{cmax}, and their relative contribution to A was species-dependent and site-specific (Carriqui et al., 2015; Tosens et al., 2016; Galmes et al., 2017; Peguero-Pina et al., 2017a; Peguero-Pina et al., 2017b; Veromann-Jurgenson et al., 2017).

Variation in iWUE (=A/g_{sw}) depends on the relative changes in A (g_s, g_m, V_{cmax}) and g_{sw} (g_{sw}=1.6g_s) (Flexas et al., 2013; Gago et al., 2014). Theoretical relationships between iWUE and g_s, g_m, and V_{cmax} have been deduced using two approaches. Based on Fick's first law of CO_2 diffusion, Flexas et al. (2013) deduced that iWUE was a function of g_m/g_s and CO_2 gradients (C_a-C_c) within a leaf. On the other hand, combining Fick's first law of CO_2 diffusion and Farquhar biochemical model (Farquhar and Sharkey, 1982), Flexas et al. (2016) deduced that iWUE was a function of V_{cmax}/g_s, C_c, CO_2 compensation point of photosynthesis (Γ^*), and the effective Michaelis–Menten constant of Rubisco for CO_2 (K_m). Until now, most previous studies focused on the role of CO_2 diffusion in limiting iWUE, and suggested that iWUE was negatively related to g_s, and positively related to g_m/g_s (Flexas et al., 2013). Gago et al. (2014) used a meta-analysis with 239 species, and were the first to confirm that iWUE was positively related to V_{cmax}/g_s. Although both g_m/g_s and V_{cmax}/g_s were positively correlated with iWUE, there was only a weak correlation between g_m/g_s and V_{cmax}/g_s, which indicates that iWUE can be improved by increasing V_{cmax} or g_m (proportionally higher than g_s), not both (Gago et al., 2014).

It is noteworthy that Flexas et al. (2016) and Gago et al. (2014) found that most of the previous work on constraints of g_s, g_m, and V_{cmax} on A were conducted in crops or saplings, and only a few studies were in natural ecosystems. For example, g_m was the main factor limiting A in two Antarctic vascular grasses (Saez et al., 2017), and in 35 Australian sclerophylls (Niinemets et al., 2009b) in different habitats. The A of two closely-related Mediterranean *Abies* species growing in two different habitats was mainly constrained by g_m in one, and by g_s in the other habitat (Peguero-Pina et al., 2012). Beyond that, it still remains unknown how g_s, g_m, and V_{cmax} regulate A and iWUE across species in natural ecosystems.

In this study, we selected 63 dominant plant species, including six life forms (Tree (n=29), Tree/Shrub (n=11), Shrub (n=11), Grass (n=11), Vine (n=5), and Fern (n=3)), from a subtropical primary forest in the Karst critical zone of southwestern China, and measured their *A* and CO_2 response curves. g_m was calculated using the curve-fitting method (Ethier and Livingston, 2004). The obtained g_m was used to transform the A-C_i into A-C_c response curves, and then to calculate the *A* and V_{cmax}. Our objective was to determine and distinguish the limitations of CO_2 diffusion (g_s and g_m) and V_{cmax} on *A* and iWUE in different life forms in this Karst primary forest, and to understanding the patterns of strategies of carbon-water regulation of Karst plants.

2 Materials and Methods

2.1 Site information

This study was conducted in a subtropical primary forest (26°14′48″N, 105°45′51″E; elevation, 1460 m), located in the Karst CZ of southwestern China. This region has a typical subtropical monsoon climate, with a mean annual precipitation of 1255 mm, and mean annual air temperature of 15.1 ℃ (Zeng et al., 2016). The soils are characterized by a high ratio of exposed rock, shallow and nonhomogeneous soil cover, and complex underground drainage networks, e.g. grooves, channels and depressions (Chen et al., 2010; Zhang et al., 2011; Wen et al., 2016). Soils and soil water are easily leached into underground drainage networks. Soil texture is silt-clay loam, and soil PH is 6.80 ± 0.16 (Chang et al., 2018). The total nitrogen and phosphorus content in soil is 7.30 ± 0.66 and 1.18 ± 0.35 g Kg^{-1}, respectively, which was similar with that of non-Karst CZs (Wang et al., in review). However, the soil quantities (16.04~61.89 Kg m^{-2}) and nitrogen and phosphorus storage (12.04 and 1.68 t hm^{-2}) is much lower than that of non-Karst CZs, due to the thin and heterogeneous soil layer (He et al., 2008; Jobbagy et al., 2000; Lu et al., 2010; Li et al., 2008). The typical vegetation type is mixed evergreen and broadleaf deciduous primary forest, dominated by *Itea yunnanensis* Franch, *Carpinus pubescens* Burk., and *Lithocarpus confinis* Huang, etc. (Wang et al., 2018).

2.2 Leaf gas-exchange measurements

In July and August 2016, 63 species (Table S1) were selected for measurements of the *A* and 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), 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.

Details of leaf sampling and measurements of the CO_2 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 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^{o}C for 30 min. Fully-expanded and mature leaves were induced for 30 minutes at a saturating light density (1500 μmol m^{-2} s^{-1}). CO_2 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.

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. Leaf area, thickness (LT) and dry mass were measured after the CO_2 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).

2.3 Response curve analyses

A and the corresponding g_{sw} ($g_s = g_{sw}/1.6$), C_a, and C_i were extracted from the CO_2 response curve under saturating light (1500 μmol m^{-2} s^{-1}) conditions, with CO_2 concentration inside the cuvette set to 400 μmol mol^{-1} (Domingues et al., 2010). V_{cmax} was estimated by fitting A-C_c curves (Ethier and Livingston, 2004). The obtained values of g_m were used to transform the A-C_i into A-C_c response curves as $C_c = C_i - A/g_m$.

Three methods are most commonly used for g_m estimation. Those methods have been reviewed by Warren (2006) and Pons et al. (2009). Briefly, g_m 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; Laisk 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).

g_m 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_i is a prerequisite for estimating g_m 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_c and the initial slope of A-C_c curves were positive, suggesting that the measured g_m was reliable (Warren, 2006).

2.4 Theory of trade-off between carbon and water at leaf scale

The exchange of H_2O and CO_2 between the leaf and the atmosphere is regulated by stomata (Gago et al., 2014). According to Fick's first law of diffusion, A and g_s are related as:

$$A = g_s(C_a\text{-}C_i) \qquad (1)$$

where A is the photosynthetic rate (μmol CO_2 m^{-2} s^{-1}); C_a is the ambient CO_2 concentration (μmol mol^{-1}); C_i is the intercellular CO_2 concentration (μmol mol^{-1}).

Mesophyll is the barrier for CO_2 inside the leaf. A and mesophyll conductance to CO_2 (g_m) are related as:

$$A = g_m(C_i\text{-}C_c) \qquad (2)$$

where C_c is the CO_2 concentration at the sites of carboxylation (μmol mol^{-1}). C_c not only depends on CO_2 supply by g_m, but also on CO_2 demand (the maximum carboxylase activity of Rubisco, V_{cmax}).

(1) The relationship between iWUE and g_m/g_s

iWUE is a function of CO_2 diffusion conductances (e.g. g_s and g_m) and leaf CO_2 concentration gradients. We can express A as the product of the total CO_2 diffusion conductance (g_t) from ambient air to chloroplasts, and the corresponding CO_2 concentration gradients by combining Eq. (1) and (2) (Flexas et al., 2013):

$$A = g_t\left[(C_a\text{-}C_i)+(C_i\text{-}C_c)\right] \qquad (3)$$

where $g_t = 1/(1/g_s+1/g_m)$. This equation demonstrates that CO_2 concentration gradients in leaves are constrained by stomatal and mesophyll resistance to CO_2. Therefore, iWUE can be expressed as：

$$\frac{A}{g_{sw}} = \frac{1}{1.6}\left(\frac{g_m/g_s}{1+g_m/g_s}\right)\left[(C_a\text{-}C_i)+(C_i\text{-}C_c)\right] \qquad (4)$$

Eq. (4) means that iWUE is positively related to g_m/g_s, but not to g_m itself (Warren and Adams, 2006; Flexas et al., 2013; Buckley and Warren, 2014; Cano et al., 2014).

(2) The relationship between iWUE and V_{cmax}/g_s

When Fick's first law and the Farquhar biochemical model (Farquhar and Sharkey, 1982) are combined, iWUE is also a function of V_{cmax}. Based on the Farquhar biochemical model (Farquhar and Sharkey, 1982), when A is limited by Rubisco, it can be expressed by the following equation (Sharkey et al., 2007):

$$A = \frac{V_{\mathrm{cmax}}(C_c - \Gamma^*)}{(C_c + K_m)} - R_d \qquad (5)$$

where Γ^* is the CO_2 compensation point of photosynthesis in the absence of non-photorespiratory respiration in light (R_d), and K_m is the effective Michaelis–Menten constant of Rubisco for CO_2. Combining Eq. (1) and (5) (Flexas et al., 2016), we obtain:

$$\frac{V_{\mathrm{cmax}}}{g_s} = \frac{(C_c + K_m)(C_a - C_i)(A + R_d)}{(C_c - \Gamma^*)A} \qquad (6)$$

Because R_d is much smaller than A in actively photosynthesizing leaves, V_{cmax}/g_s can be approximated as:

$$\frac{V_{\mathrm{cmax}}}{g_s} \approx \frac{(C_c + K_m)(C_a - C_i)}{(C_c - \Gamma^*)} = \frac{(C_c + K_m)}{(C_c - \Gamma^*)} \frac{A}{g_s} \qquad (7)$$

Consequently, iWUE can be expressed as:

$$\frac{A}{g_{sw}} = \frac{1}{1.6} \frac{V_{\mathrm{cmax}}}{g_s} \frac{(C_c - \Gamma^*)}{(C_c + K_m)} \qquad (8)$$

2.5 Statistical analysis

(1) Quantitative analysis of limitations on *A*

The relative contribution of g_s (l_s), g_m (l_m) and V_{cmax} (l_b) to A can be separated by a quantitative limitation model introduced by Jones (Jones, 1985) and further developed by Grassi & Magnani (2005). The sum of l_s, l_m, and l_b is 1. l_s, l_m and l_b can be calculated as:

$$l_s = \frac{g_t / g_s \bullet \partial A / \partial C_c}{g_t + \partial A / \partial C_c} \qquad (9)$$

$$l_m = \frac{g_t / g_m \bullet \partial A / \partial C_c}{g_t + \partial A / \partial C_c} \qquad (10)$$

$$l_b = \frac{g_t}{g_t + \partial A / \partial C_c} \qquad (11)$$

where $\partial A/\partial C_c$ was calculated as the slope of A-C_c response curves over a C_c range of 50–100 μmol mol^{-1}. l_s, l_m and l_b have no units. A is co-limited by the three factors when $l_s \approx 0.3$, $l_m \approx 0.3$ and $l_b \approx 0.4$ (Galmes, J. et al., 2017).

(2) Data 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 $\log_e$-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 $p < 0.05$.

3 Results

3.1 Interrelation among g_s, g_m, g_t, and V_{cmax}

CO_2 concentration gradients in leaf were controlled by CO_2 diffusion conductance and V_{cmax}. Fig. 1 shows the relationship between CO_2 gradients (C_a-C_i, C_i-C_c and C_a-C_c) in leaf and the corresponding CO_2 diffusion conductance (g_s, g_m and g_t) (Fig. 1a-c), and between C_a-C_c and V_{cmax} (Fig. 1d). CO_2 concentration gradients (C_a-C_i,

C_i-C_c and C_a-C_c) were significantly negatively associated with the corresponding CO_2 diffusion conductance (g_s, g_m and g_t) (P<0.001). V_{cmax} was positively associated with C_a-C_c (P<0.001).

g_s, g_m, and g_t were significantly positively related to each other (P<0.001) (Fig. S1). The contribution of g_m to leaf CO_2 gradient was similar to that of g_s. The contribution of g_s (57.51–155.13 μmol mol^{-1}) to C_a-C_c (98.50–282.94 μmol mol^{-1}) varied from 28% to 86%, and the contribution of g_m (18.15–179.36 μmol mol^{-1}) to C_a-C_c varied from 14% to 72%. But the variation range of g_m (0.02 –0.69 mol CO_2 m^{-2} s^{-1}) was 4.5 times that of g_s (0.05–0.38 mol CO_2 m^{-2} s^{-1}).

No relationship was found between the CO_2 diffusion conductance (g_s, g_m, and g_t) and V_{cmax} (Fig. S2). However, after normalization of g_s, g_m, g_t, and V_{cmax} for A (normalized parameters are hereafter called G_S=g_S/A, G_m=g_m/A, G_t=g_t/A, and V=V_{cmax}/A), V was significantly positively correlated with G_m and G_t (P<0.001) (Fig. 2b and c), and was slightly positively correlated with G_s (P<0.05) (Fig. 2a), which represented the trade-off between CO_2 supply and demand.

3.2 Contribution of g_s, g_m and V_{cmax} to A

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±0.12) was significantly larger than l_b (0.34±0.14), and l_s (0.28±0.07) (P<0.05).

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).

3.3 Effect of g_s, g_m and V_{cmax} on iWUE

iWUE varied from 29.52 to 88.92 μmol CO_2 mol^{-1} H_2O. In theory, iWUE is regulated by g_s (g_{sw}=1.6g_s), g_m, and V_{cmax}. However, a simple correlation analysis showed that iWUE was negatively related to g_s (Fig. 6b), and not related to A (Fig. 6a), g_m (Fig. 6c), and V_{cmax} (Fig. 6d).

A correlation analysis was used to test how g_m/g_s and V_{cmax}/g_s affected iWUE. The results showed that iWUE was positively correlated with g_m/g_s (Fig. 7a) and V_{cmax}/g_s (Fig. 7b). However, there was no significant relationship between g_m/g_s and V_{cmax}/g_s. iWUE was regulated by co-variation between g_s, g_m and V_{cmax}.

4 Discussion

4.1 Co-variation in g_s, g_m and V_{cmax} in regulating A

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).

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).

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., 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 explored.

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).

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}.

The trade-off between CO_2 supply (g_s and g_m) 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_{cmax} as a proxy for the carboxylation capacity of Rubisco, and the normalized V_{cmax} by *A* ($V=V_{cmax}/A$) was significantly negatively correlated with the normalized g_t by *A* ($G_t = g_t/A$) ($P<0.001$) (Fig. 2c), indicating that the trade-off between CO_2 supply and demand also existed among different species in the same ecosystems. For genus *Limonium* (flowering plants) (Galmes et al., 2017), g_t was significantly positively related to Rubisco carboxylase specific activity, and significantly negatively related to Rubisco specificity factor to CO_2. In case of Antarctic vascular (Saez et al., 2017) and Mediterranean plants (Flexas et al., 2014), *A* was mainly limited by low g_m, but it could be partially counterbalanced by a highly efficient Rubisco through high specificity for CO_2. This highlights the importance of the trade-off between CO_2 supply 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.

4.2 Co-variation of g_s, g_m and V_{cmax} in regulating iWUE

Compared with the global dataset under well-watered conditions (19.27-171.88 μmol CO_2 mol^{-1} H_2O) (Flexas et al., 2016), iWUE (52.85±13.08 μmol CO_2 mol^{-1} H_2O) was somewhat lower in this study. iWUE varied from 29.53 to 88.91 μmol CO_2 mol^{-1} H_2O, 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±13.21 μmol CO_2 mol^{-1} H_2O (Chen et al., 2015), while that of 6 evergreen and 6 deciduous Trees was 66.7±4.9 and 49.7±2.0

μmol CO_2 mol^{-1} H_2O, respectively (Fu et al., 2012). The results demonstrated that Karst plants use a diverse strategies of carbon-water regulation to adopt to the harsh Karst environment.

Coexisting species have a diversity strategies of carbon-water regulation, ranging from 'profligate/opportunistic' to 'conservative, that means their ecophysiological niche are separate (Moreno-Gutierrez et al., 2012; Nie et al., 2014; Prentice et al., 2014). Species with high g_s, and low iWUE were defined to have 'profligate/opportunistic' water use strategy, and species with low g_s and high iWUE were defined to exhibit 'conservative' water use strategy (Moreno-Gutierrez et al., 2012). In consistent with previous study (Moreno-Gutierrez et al., 2012), 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.

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 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).

iWUE is regulated by the co-variation of g_s, g_m, and V_{cmax}. In theory, water loss is regulated by g_s only, while carbon gain (A) was regulated by g_s, g_m, and V_{cmax} (Fig. 3) (Lawson and and Blatt, 2014). However, iWUE in this study was negatively related to g_s (R^2=0.30), negatively related to V_{cmax} (R^2=0.09), and not related to A, g_m (Fig. 6).

CO_2 diffusion and Farquhar biochemical model indicated that iWUE is affected by g_m/g_s and V_{cmax}/g_s (Gago et al., 2014; Flexas et al., 2016). There was a hyperbolic dependency of iWUE on g_m/g_s due to the roles of g_s and g_m in C_i and C_c, and of C_c in A (Flexas et al., 2016). In meta-analyses, both Gago et al. (2014) and Flexas et al. (2016) found that iWUE was significantly positively related to g_m/g_s and V_{cmax}/g_s. The results of this study are consistent with the meta-analyses (Fig. 7), demonstrating that plant species with relatively high g_m/g_s or V_{cmax}/g_s had relatively high iWUE. The relationship between iWUE and V_{cmax}/g_s (R^2=0.50) was stronger than the relationship between iWUE and g_m/g_s (R^2=0.20), demonstrating iWUE was mainly regulated by V_{cmax}/g_s. The reason maybe that iWUE was correlated to g_s and V_{cmax}, and g_s was positive related to g_m.

However, plants cannot simultaneously have high g_m/g_s and high V_{cmax}/g_s. Similarly to the study of Gago et al. (2014), we found no relationship between g_m/g_s and V_{cmax}/g_s. Gago et al. (2014) thought that the poor relationship between g_m/g_s and V_{cmax}/g_s indicated that the iWUE may be improved by g_m/g_s or V_{cmax}/g_s separately; if both of them were simultaneously improved, the enhanced effect on iWUE could be anticipated. In addition, Flexas et al. (2016) showed in a simulation that the increase in iWUE caused by overinvestment in photosynthetic capacity would progressively lead to inefficiency in the trade-off between carbon gain and water use, causing an imbalance between CO_2 supply and demand.

5 Conclusions

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.

Acknowledgements

This study was supported by the National Natural Science Foundation of China [41571130043, 31470500, and 41671257].

Author contributions

JW, XFW. and XYZ planed and designed the research. JW performed experiments and analyzed data. JW prepared the manuscript with contributions from all co-authors.

Competing interests.

The authors declare that they have no conflict of interest.

6 References

Bongi, G. and Loreto, F.: Gas-exchange properties of salt-stressed olive (Olea-Europra L) leaves, Plant Physiol., 90, 1408-1416, 1989.

Buckley, T.N. and Warren, C.R.: The role of mesophyll conductance in the economics of nitrogen and water use in photosynthesis, Photosynthesis Res., 119, 77-88, 2014.

Chang, J.J., Zhu, J.X., Xu, L., Su, H.X., Gao, Y., Cai, X.L., Peng, T., Wen, X.F., Zhang, J.J., He, N.P.: Rational land-use types in the karst regions of China: Insights from soil organic matter composition and stability, Catena, 160, 345-353, 2018.

Cano, F.J., Lopez, R. and Warren, C.R.: Implications of the mesophyll conductance to CO2 for photosynthesis and water-use efficiency during long-term water stress and recovery in two contrasting Eucalyptus species, Plant Cell Environ., 37, 2470-2490, 2014.

Carriqui, M., Cabrera, H.M., Conesa, M.A., Coopman, R.E., Douthe, C., Gago, J., Galle, A., Galmes, J., Ribas-Carbo, M., Tomas, M. and Flexas, J.: Diffusional limitations explain the lower photosynthetic capacity of ferns as compared with angiosperms in a common garden study, Plant Cell Environ., 38, 448-460, 2015.

Chen, H., Zhang, W., Wang, K. and Fu, W.: Soil moisture dynamics under different land uses on karst hillslope in northwest Guangxi, China, Environ. Earth Sci., 61, 1105-1111, 2010.

Chen, P. and Zhou, Y.: Soil nutrient capacity and forest tree sustainability in plateau Karst region. Earth and Enviroment, 45, 32-37, 2017. (In Chinese)

Chen, Y.J., Cao, K.F., Schnitzer, S.A., Fan, Z.X., Zhang, J.L. and Bongers, F.: Water-use advantage for lianas over trees in tropical seasonal forests, New Phytol., 205, 128-136, 2015.

Dimarco, G., Manes, F., Tricoli, D. and Vitale, E.: Fluorescence Parameters Measured Concurrently with Net Photosynthesis to Investigate Chloroplastic CO_2 Concentration in Leaves of Quercus ilex L, J. Plant Physiol., 136, 538-543, 1990.

Domingues, T.F., Meir, P., Feldpausch, T.R., Saiz, G., Veenendaal, E.M., Schrodt, F., Bird, M., Djagbletey, G., Hien, F., Compaore, H., Diallo, A., Grace, J. and Lloyd, J.: Co-limitation of photosynthetic capacity by nitrogen and phosphorus in West Africa woodlands, Plant Cell Environ., 33, 959-980, 2010.

Epron, D., Godard, D., Cornic, G. and Genty, B.: Limitation of net CO_2 assimilation rate by internal resistances to CO_2 transfer in the leaves of two tree species (Fagus sylvatica L. and Castanea sativa Mill), Plant Cell Environ., 18, 43-51, 1995.

Ethier, G.J. and Livingston, N.J.: On the need to incorporate sensitivity to CO_2 transfer conductance into the Farquhar-von Caemmerer-Berry leaf photosynthesis model, Plant Cell Environ., 27, 137-153, 2004.

Evans, J.R.: Nitrogen and photosynthesis in the flag leaf of Wheat (Triticum aestivum L.), Plant Physiol., 72, 297-302, 1983.

Evans, J.R. and Voncaemmerer, S.: Carbon dioxide diffusion inside leaves, Plant Physiol., 110, 339-346, 1996.

Farquhar, G.D. and Sharkey, T.D.: Stomatal conductance and photosynthesis, Annu. Rev. Plant Physiol. Plant Mol. Biol., 33, 317-345, 1982.

Flanagan, L.B. and Farquhar, G.D.: Variation in the carbon and oxygen isotope composition of plant biomass and its relationship to water-use efficiency at the leaf- and ecosystem-scales in a northern Great Plains grassland, Plant Cell Environ., 37, 425-438, 2014.

Flexas, J., Barbour, M.M., Brendel, O., Cabrera, H.M., Carriqui, M., Diaz-Espejo, A., Douthe, C., Dreyer, E., Ferrio, J.P., Gago, J., Galle, A., Galmes, J., Kodama, N., Medrano, H., Niinemets, U., Peguero-Pina, J.J., Pou, A., Ribas-Carbo, M., Tomas, M., Tosens, T. and Warren, C.R.: Mesophyll diffusion conductance to CO_2: An unappreciated central player in photosynthesis, Plant Sci., 193, 70-84, 2012.

Flexas, J., Niinemets, U., Galle, A., Barbour, M.M., Centritto, M., Diaz-Espejo, A., Douthe, C., Galmes, J., Ribas-Carbo, M., Rodriguez, P., Rossello, F., Soolanayakanahally, R., Tomas, M., Wright, I.J., Farquhar, G.D. and Medrano, H.: Diffusional conductances to CO_2 as a target for increasing photosynthesis and photosynthetic water-use efficiency, Photosynthesis Res., 117, 45-59, 2013.

Flexas, J., Diaz-Espejo, A., Gago, J., Galle, A., Galmes, J., Gulias, J. and Medrano, H.: Photosynthetic limitations in Mediterranean plants: A review, Environ. Exp. Bot., 103, 12-23, 2014.

Flexas, J., Diaz-Espejo, A., Conesa, M.A., Coopman, R.E., Douthe, C., Gago, J., Galle, A., Galmes, J., Medrano, H., Ribas-Carbo, M., Tomas, M. and Niinemets, U.: Mesophyll conductance to CO_2 and Rubisco as targets for improving intrinsic water use efficiency in C-3 plants, Plant Cell Environ., 39, 965-982, 2016.

Fu, P.-L., Jiang, Y.-J., Wang, A.-Y., Brodribb, T.J., Zhang, J.-L., Zhu, S.-D. and Cao, K.-F.: Stem hydraulic traits and leaf water-stress tolerance are co-ordinated with the leaf phenology of angiosperm trees in an Asian tropical dry karst forest, Ann. Bot., 110, 189-199, 2012.

Gago, J., Douthe, C., Florez-Sarasa, I., Escalona, J.M., Galmes, J., Fernie, A.R., Flexas, J. and Medrano, H.: Opportunities for improving leaf water use efficiency under climate change conditions, Plant Sci., 226, 108-119, 2014.

Galmes, J., Angel Conesa, M., Manuel Ochogavia, J., Alejandro Perdomo, J., Francis, D.M., Ribas-Carbo, M., Save, R., Flexas, J., Medrano, H. and Cifre, J.: Physiological and morphological adaptations in relation to water use efficiency in Mediterranean accessions of Solanum lycopersicum, Plant Cell Environ., 34, 245-260, 2011.

Galmes, J., Molins, A., Flexas, J. and Conesa, M.A.: Coordination between leaf CO2 diffusion and Rubisco properties allows maximizing photosynthetic efficiency in Limonium species, Plant Cell Environ., 40, 2081-2094, 2017.

Giuliani, R., Koteyeva, N., Voznesenskaya, E., Evans, M.A., Cousins, A.B. and Edwards, G.E.: Coordination of leaf photosynthesis, transpiration, and structural traits in rice and wild relatives (Genus Oryza), Plant Physiol., 162, 1632-1651, 2013.

Grassi, G. and Magnani, F.: Stomatal, mesophyll conductance and biochemical limitations to photosynthesis as affected by drought and leaf ontogeny in ash and oak trees, Plant Cell Environ., 28, 834-849, 2005.

Gusewell, S.: N : P ratios in terrestrial plants: variation and functional significance, New Phytol., 164, 243-266, 2004.

Harley, P.C., Loreto, F., Dimarco, G. and Sharkey, T.D.: Theoretical considerations when estimating the mesophyll conductance to CO_2 flux by analysis of the response of photosynthesis to CO_2, Plant Physiol., 98, 1429-1436, 1992.

He, N.P., Yu, Q., Wu, L., Wang, Y.S. and Han, X.G.: Carbon and nitrogen store and storage potential as affected by land-use in a Leymus chinensis grassland of northern China. Soil Biol. Biochem. 40, 2952-2959, 2008.

Jobbagy, E. G. and Jackson, R. B.: The vertical distribution of soil organic carbon and its relation to climate and vegetation. Ecol. Appl. 10, 423-436, 2000.

Jones, H.G.: Partitioning stomatal and non-stomatal limitations to photosynthesis, Plant Cell Environ., 8, 95-104, 1985.

Laisk, A., Eichelmann, H., Oja, V., Rasulov, B., Padu, E., Bichele, I., Pettai, H. and Kull, O.: Adjustment of leaf photosynthesis to shade in a natural canopy: rate parameters, Plant Cell Environ., 28, 375-388, 2005.

Lawson, T. and Blatt, M.R.: Stomatal Size, Speed, and Responsiveness Impact on Photosynthesis and Water Use Efficiency, Plant Physiol., 164, 1556-1570, 2014.

Li, Z.P., Han, F. X., Su, Y., Zhang, T.L., Sun, B., Monts, D.L., and Plodinec, M.J.: Assessment of soil organic and carbonate carbon storage in China. Geoderma 138, 119-126, 2007.

Liu, C.C., Liu, Y.G., Guo, K., Fan, D.Y., Yu, L.F. and Yang, R.: Exploitation of patchy soil water resources by the clonal vine Ficus tikoua in karst habitats of southwestern China, Acta Physiol. Plant., 33, 93-102, 2011.

Long, S.P. and Bernacchi, C.J.: Gas exchange measurements, what can they tell us about the underlying limitations to photosynthesis? Procedures and sources of error, J. Exp. Bot., 54, 2393-2401, 2003.

Loreto, F., Harley, P.C., Dimarco, G. and Sharkey, T.D.: Estimation of mesophyll conductance to CO_2 flux by three different methods, Plant Physiol., 98, 1437-1443, 1992.

Lu, X.T., Yin, J.X., Jepsen, M.R. and Tang, J.W.: Ecosystem carbon storage and partitioning in a tropical seasonal forest in Southwestern China. For. Ecol. Manage. 260, 1798-1803, 2010.

Moreno-Gutierrez, C., Dawson, T.E., Nicolas, E. and Querejeta, J.I.: Isotopes reveal contrasting water use strategies among coexisting plant species in a Mediterranean ecosystem, New Phytol., 196, 489-496, 2012.

Muir, C.D., Conesa, M.A., Roldan, E.J., Molins, A. and Galmes, J.: Weak coordination between leaf structure and function among closely related tomato species, New Phytol., 213, 1642-1653, 2017.

Nie, Y.P., Chen, H.S., Wang, K.L. and Ding, Y.L.: Seasonal variations in leaf $\delta^{13}C$ values: implications for different water-use strategies among species growing on continuous dolomite outcrops in subtropical China, Acta Physiol. Plant., 36, 2571-2579, 2014.

Niinemets, U., Diaz-Espejo, A., Flexas, J., Galmes, J. and Warren, C.R.: Role of mesophyll diffusion conductance in constraining potential photosynthetic productivity in the field, J. Exp. Bot., 60, 2249-2270, 2009a.

Niinemets, U., Wright, I.J. and Evans, J.R.: Leaf mesophyll diffusion conductance in 35 Australian sclerophylls covering a broad range of foliage structural and physiological variation, J. Exp. Bot., 60, 2433-2449, 2009b.

Olsovska, K., Kovar, M., Brestic, M., Zivcak, M., Slamka, P. and Shao, H.B.: Genotypically identifying Wheat mesophyll conductance regulation under progressive drought stress, Front. Plant Sci., DOI 10.3389/fpls.2016.01111, 2016.

Peguero-Pina, J.J., Flexas, J., Galmes, J., Niinemets, U., Sancho-Knapik, D., Barredo, G., Villarroya, D. and Gil-Pelegrin, E.: Leaf anatomical properties in relation to differences in mesophyll conductance to CO_2 and photosynthesis in two related Mediterranean Abies species, Plant Cell Environ., 35, 2121-2129, 2012.

Peguero-Pina, J.J., Siso, S., Flexas, J., Galmes, J., Garcia-Nogales, A., Niinemets, U., Sancho-Knapik, D., Saz, M.A. and Gil-Pelegrin, E.: Cell-level anatomical characteristics explain high mesophyll conductance and photosynthetic capacity in sclerophyllous Mediterranean oaks, New Phytol., 214, 585-596, 2017a.

Peguero-Pina, J.J., Siso, S., Flexas, J., Galmes, J., Niinemets, U., Sancho-Knapik, D. and Gil-Pelegrin, E.: Coordinated modifications in mesophyll conductance, photosynthetic potentials and leaf nitrogen contribute to explain the large variation in foliage net assimilation rates across Quercus ilex provenances, Tree Physiol., 37, 1084-1094, 2017b.

Perdomo, J.A., Capo-Bauca, S., Carmo-Silva, E. and Galmes, J.: Rubisco and Rubisco Activase Play an Important Role in the Biochemical Limitations of photosynthesis in rice, wheat, and maize under high temperature and water deficit, Front. Plant Sci., DOI: 10.3389/fpls.2017.00490, 2017.

Pons, T.L., Flexas, J., Von Caemmerer, S., Evans, J.R., Genty, B., Ribas-Carbo, M. and Brugnoli, E.: Estimating mesophyll conductance to CO_2: methodology, potential errors, and recommendations, J. Exp. Bot., 60, 2217-2234, 2009.

Prentice, I.C., Dong, N., Gleason, S.M., Maire, V. and Wright, I.J.: Balancing the costs of carbon gain and water transport: testing a new theoretical framework for plant functional ecology, Ecol. Lett., 17, 82-91, 2014.

Saez, P.L., Bravo, L.A., Cavieres, L.A., Vallejos, V., Sanhueza, C., Font-Carrascosa, M., Gil-Pelegrin, E., Peguero-Pina, J.J. and Galmes, J.: Photosynthetic limitations in two Antarctic vascular plants: importance of leaf anatomical traits and Rubisco kinetic parameters, J. Exp. Bot., 68, 2871-2883, 2017.

Sharkey, T.D., Vassey, T.L., Vanderveer, P.J. and Vierstra, R.D.: Carbon metabolism enzymes and photosynthesis in transgenic tobacco (Nicotiana tabacum L.) having excess phytochrome, Planta, 185, 287-296, 1991.

Sharkey, T.D., Bernacchi, C.J., Farquhar, G.D. and Singsaas, E.L.: Fitting photosynthetic carbon dioxide response curves for C3 leaves, Plant Cell Environ., 30, 1035-1040, 2007.

Sharkey, T.D.: Mesophyll conductance: constraint on carbon acquisition by C3 plants, Plant Cell Environ., 35, 1881-1883, 2012.

Sun, Y., Gu, L.H., Dickinson, R.E., Pallardy, S.G., Baker, J., Cao, Y.H., Damatta, F.M., Dong, X.J., Ellsworth, D., Van Goethem, D., Jensen, A.M., Law, B.E., Loos, R., Martins, S.C.V., Norby, R.J., Warren, J., Weston, D. and Winter, K.: Asymmetrical effects of mesophyll conductance on fundamental photosynthetic parameters and their relationships estimated from leaf gas exchange measurements, Plant Cell Environ., 37, 978-994, 2014.

Sullivan, P. L., Wymore, A., McDowell, B., and co-authors: New Opportunities for Critical Zone Science, Report of 2017 Arlington CZO All Hands Meeting white booklet: Discuss new opportunities for CZ Science, http://www.czen.org/sites/default/files/CZO_2017_White_Booklet_20171015a.pdf, 2017.

Terashima, I., Araya, T., Miyazawa, S., Sone, K.and Yano, S.: Construction and maintenance of the optimal photosynthetic systems of the leaf, herbaceous plant and tree: an eco-developmental treatise, Ann. Bot., 95, 507-519, 2005.

Terashima, I., Hanba, Y.T., Tazoe, Y., Vyas, P.and Yano, S.: Irradiance and phenotype: comparative eco-development of sun and shade leaves in relation to photosynthetic CO_2 diffusion, J. Exp. Bot., 57, 343-354, 2006.

Terashima, I., Hanba, Y.T., Tholen, D. and Niinemets, U.: Leaf Functional Anatomy in Relation to Photosynthesis, Plant Physiol., 155, 108-116, 2011.

Tomas, M., Flexas, J., Copolovici, L., Galmes, J., Hallik, L., Medrano, H., Ribas-Carbo, M., Tosens, T., Vislap, V. and Niinemets, U.: Importance of leaf anatomy in determining mesophyll diffusion conductance to CO_2 across species: quantitative limitations and scaling up by models, J. Exp. Bot., 64, 2269-2281, 2013.

Tosens, T., Nishida, K., Gago, J., Coopman, R.E., Cabrera, H.M., Carriqui, M., Laanisto, L., Morales, L., Nadal, M., Rojas, R., Talts, E., Tomas, M., Hanba, Y., Niinemets, U. and Flexas, J.: The photosynthetic capacity in 35 ferns and fern allies: mesophyll CO_2 diffusion as a key trait, New Phytol., 209, 1576-1590, 2016.

Veromann-Jurgenson, L.L., Tosens, T., Laanisto, L. and Niinemets, U.: Extremely thick cell walls and low mesophyll conductance: welcome to the world of ancient living!, J. Exp. Bot., 68, 1639-1653, 2017.

Wang, J., Wen,X.F., Zhang, X.Y., Li, S.G., and Zhang, D.Y.: Magnesium enhances the photosynthetic capacity of a subtropical primary forest in the Karst critical zone，SCI REP-UK, DOI:10.1038/s41598-018-25839-1, 2018.

Warren, C.: Estimating the internal conductance to CO_2 movement, Funct. Plant Biol., 33, 431-442, 2006.

Warren, C.R. and Adams, M.A.: Internal conductance does not scale with photosynthetic capacity: implications for carbon isotope discrimination and the economics of water and nitrogen use in photosynthesis, Plant Cell Environ., 29, 192-201, 2006.

Wen, L., Li, D.J., Yang, L.Q., Luo, P., Chen, H., Xiao, K.C., Song, T.Q., Zhang, W., He, X.Y., Chen, H.S. and Wang, K.L.: Rapid recuperation of soil nitrogen following agricultural abandonment in a karst area, southwest China, Biogeochemistry, 129, 341-354, 2016.

Zeng, C., Liu, Z.H., Zhao, M. and Yang, R.: Hydrologically-driven variations in the karst-related carbon sink fluxes: Insights from high-resolution monitoring of three karst catchments in Southwest China, Journal of Hydrology, 533, 74-90, 2016.

Zhang, X.B., Bai, X.Y. and He, X.B.: Soil creeping in the weathering crust of carbonate rocks and underground soil losses in the karst mountain areas of southwest china, Carbonates and Evaporites, 26, 149-153, 2011.

Figures

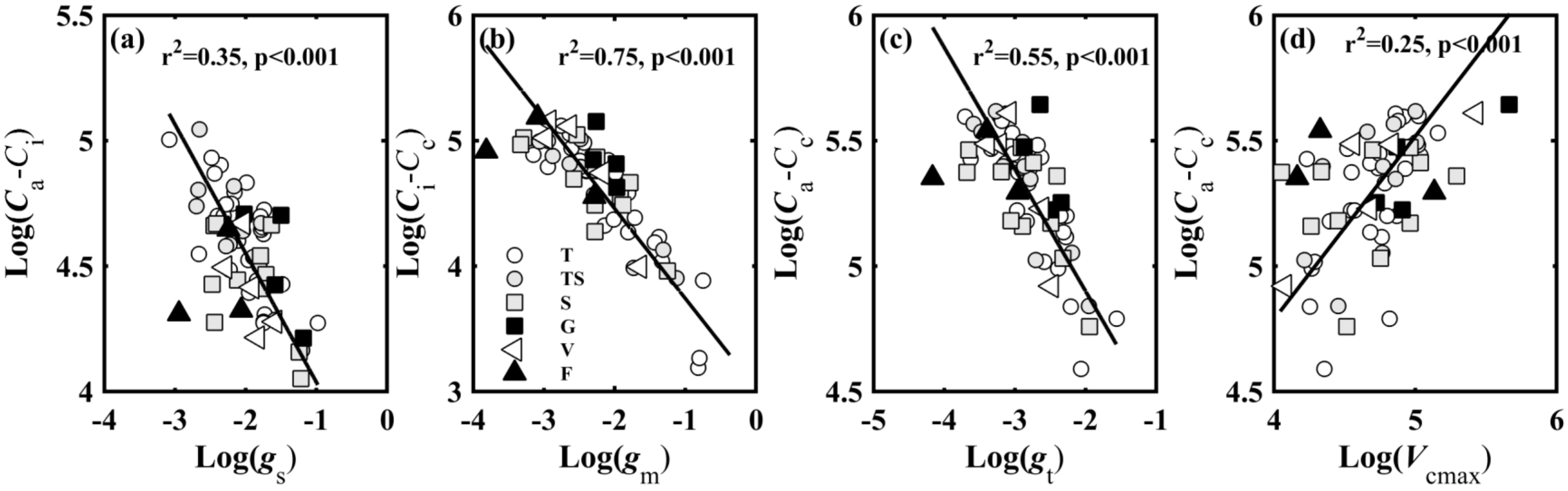

Figure 1. Relationships between (a) CO_2 gradient between ambient air and intercellular air space (C_a-C_i, μmol mol^{-1}) and stomatal conductance to CO_2 (g_s, mol CO_2 m^{-2} s^{-1}); (b) CO_2 gradient between intercellular air space and chloroplasts (C_i-C_c, μmol mol^{-1}) and mesophyll conductance to CO_2 (g_m, mol CO_2 m^{-2} s^{-1}); (c) CO_2 concentration gradient between ambient air and chloroplasts (C_a-C_c, μmol mol^{-1}) and total conductance to CO_2 (g_t, mol CO_2 m^{-2} s^{-1}); and (d) C_a-C_c and the maximum carboxylase activity of Rubisco (V_{cmax}, μmol CO_2 m^{-2} s^{-1}). Lines refer to regression line for 63 species. T, TS, S, G, V, and F represent Tree, Tree/Shrub, Shrub, Grass, Vine, and Fern, respectively.

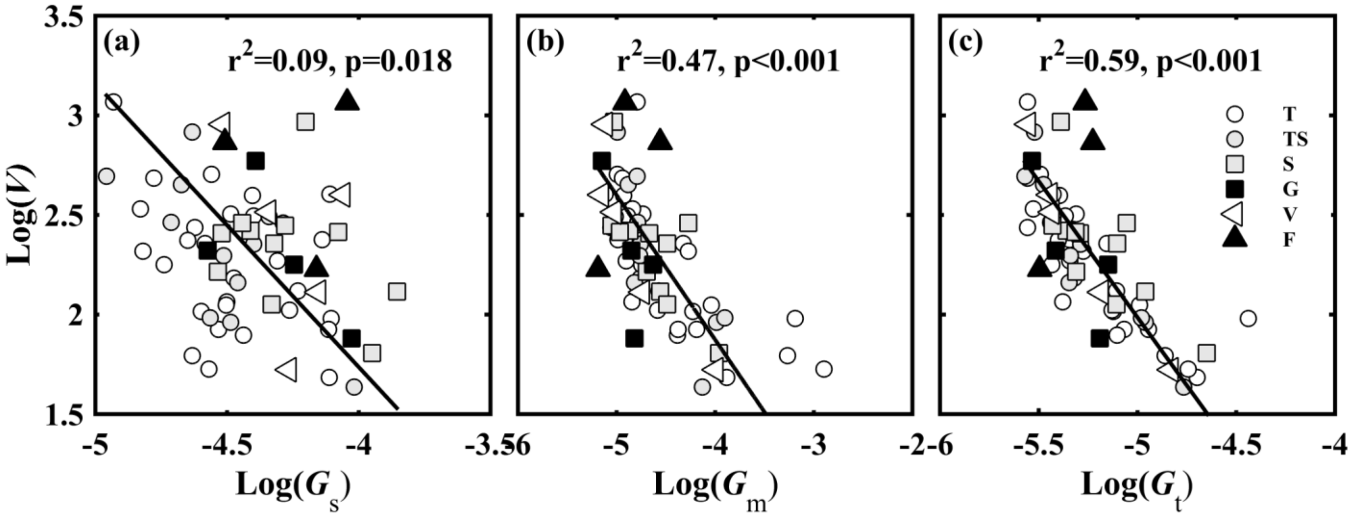

Figure 2. Relationships between (a) V and G_s; (b) V and G_m; and (c) V and G_t. V is the ratio of photosynthetic capacity (V_{cmax}) to light-saturated net photosynthesis (A, μmol CO_2 m^{-2} s^{-1}); G_s is the ratio of stomatal conductance to CO_2 (g_s, mol CO_2 m^{-2} s^{-1}) to A; G_m is the ratio of mesophyll conductance to CO_2 (g_m, mol CO_2 m^{-2} s^{-1}) to A; G_t is the ratio of total conductance to CO_2 (g_t, mol CO_2 m^{-2} s^{-1}) to A. Lines refer to regression line for 63 species. T, TS, S, G, V, and F represent Tree, Tree/Shrub, Shrub, Grass, Vine, and Fern, respectively.

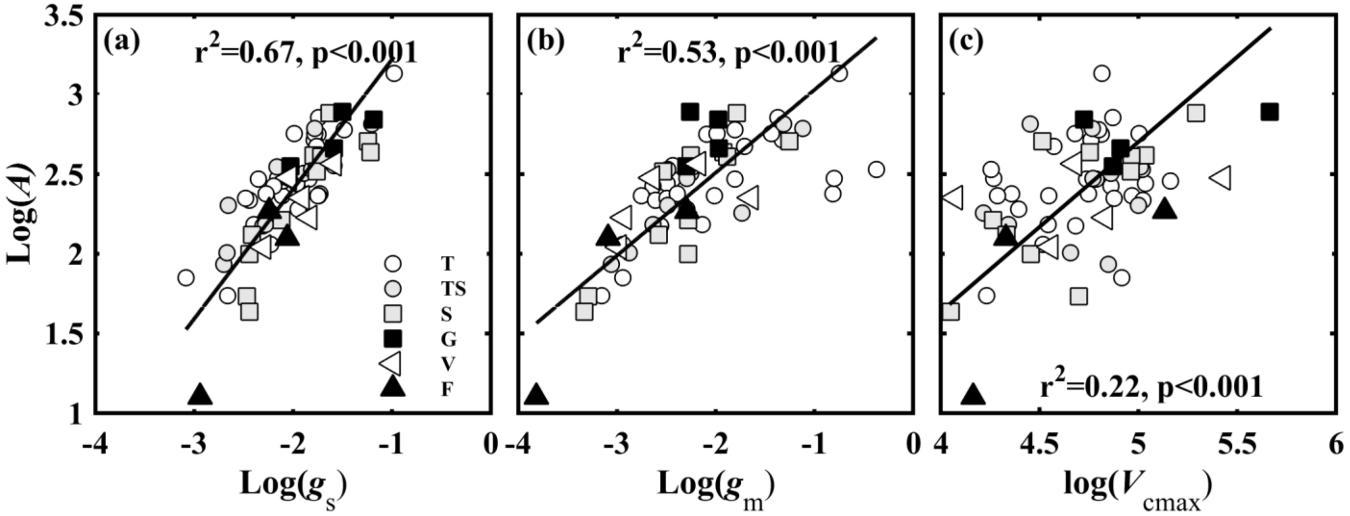

Figure 3. Relationships between light-saturated net photosynthesis (A, µmol CO$_2$ m^{-2} s^{-1}) and (a) stomatal conductance to CO$_2$ (g_s, mol CO$_2$ m^{-2} s^{-1}); (b) mesophyll conductance to CO$_2$ (g_m, mol CO$_2$ m^{-2} s^{-1}); and (c) the maximum carboxylase activity of Rubisco (V_{cmax}, µmol CO$_2$ m^{-2} s^{-1}). Lines refer to regression line for 63 species. T, TS, S, G, V, and F represent Tree, Tree/Shrub, Shrub, Grass, Vine, and Fern, respectively.

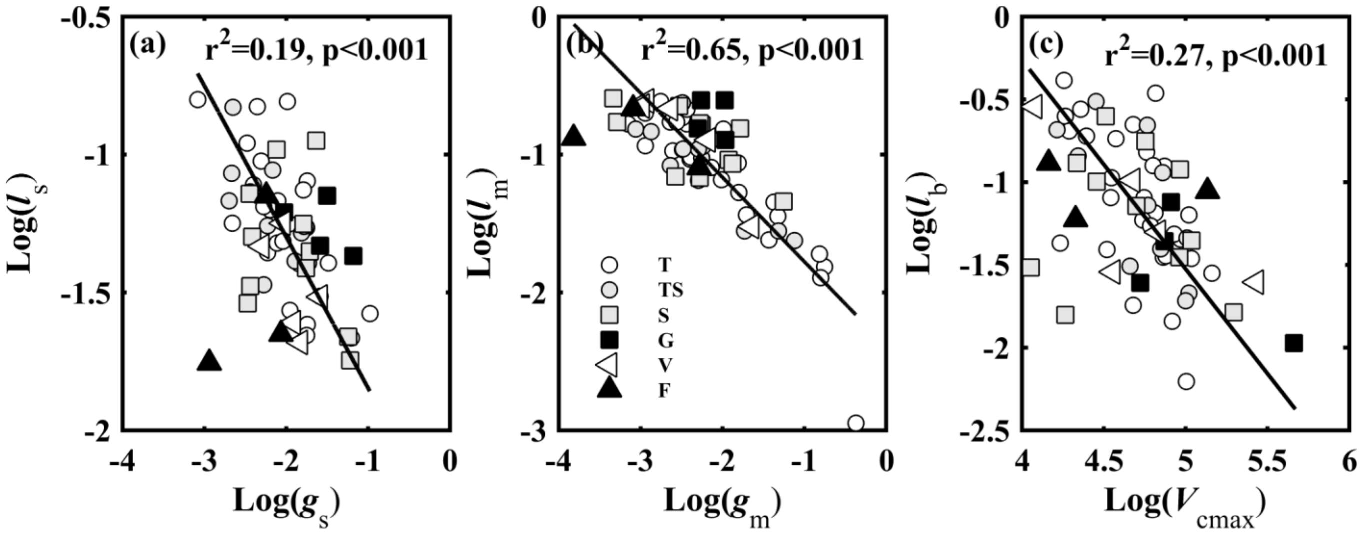

Figure 4. Relationships between (a) stomatal conductance to CO_2 (g_s, mol CO_2 m^{-2} s^{-1}) and l_s (g_s limitation on light-saturated net photosynthesis (*A*)); (b) mesophyll conductance to CO_2 (g_m, mol CO_2 m^{-2} s^{-1}) and l_m (g_m limitation on *A*); and (c) the maximum carboxylase activity of Rubisco (V_{cmax}, μmol CO_2 m^{-2} s^{-1}) and l_b (V_{cmax} limitation on *A*). Lines refer to regression line for 63 species. T, TS, S, G, V, and F represent Tree, Tree/Shrub, Shrub, Grass, Vine, and Fern, respectively.

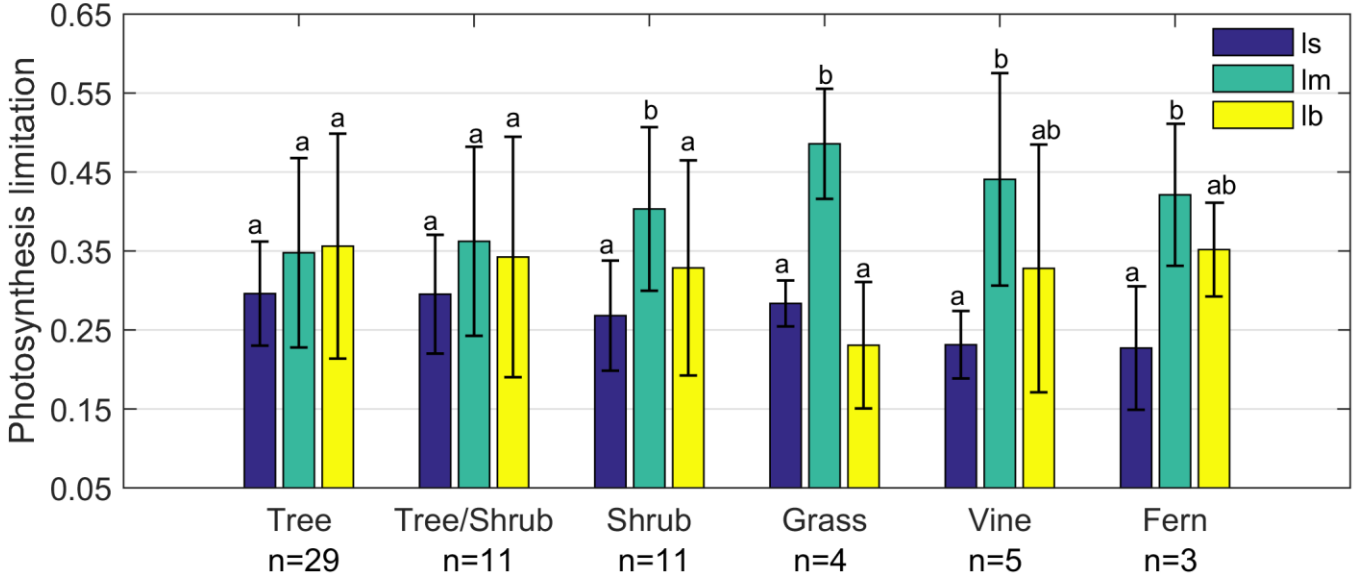

Figure 5. Limitation to light-saturated net photosynthesis (A) in six life forms by stomatal conductance to CO_2 (l_s), mesophyll conductance to CO_2 (l_m), and the maximum carboxylase activity of Rubisco (l_b). Error bars denominate standard deviation (1σ).

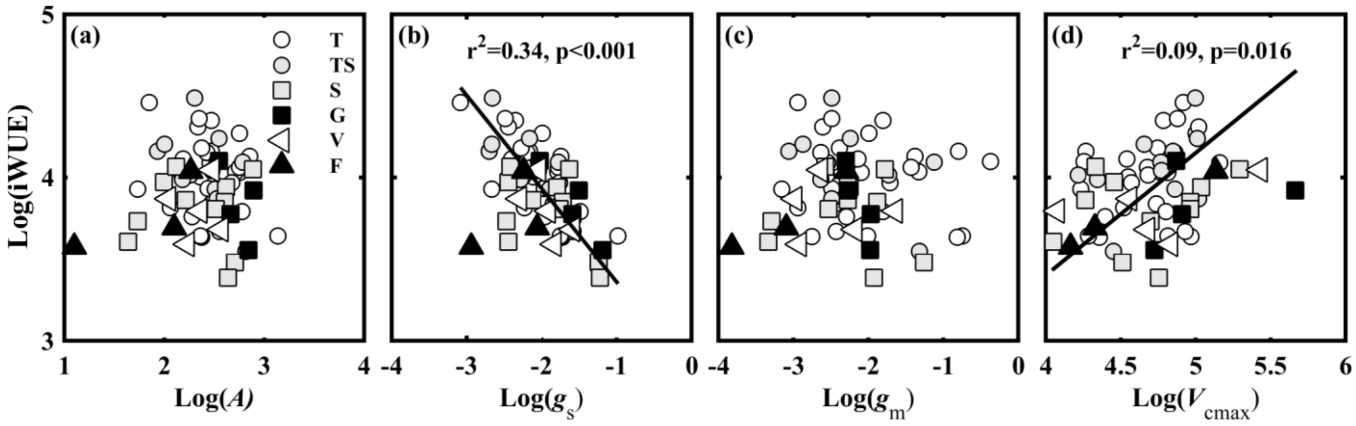

Figure 6. Relationships between the observed intrinsic water use efficiency (iWUE, μmol CO_2 mol^{-1} H_2O) and (a) light-saturated net photosynthesis (A, μmol CO_2 m^{-2} s^{-1}); (b) stomatal conductance to CO_2 (g_s, mol CO_2 m^{-2} s^{-1}); (c) mesophyll conductance to CO_2 (g_m, mol CO_2 m^{-2} s^{-1}) and (d) the maximum carboxylase activity of Rubisco (V_{cmax}, μmol CO_2 m^{-2} s^{-1}). Lines refer to regression line for 63 species. T, TS, S, G, V, and F represent Tree, Tree/Shrub, Shrub, Grass, Vine, and Fern, respectively.

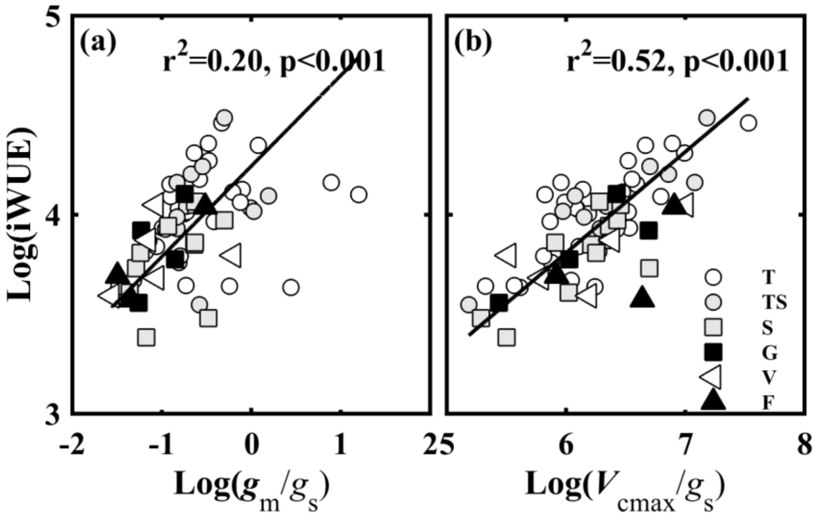

Figure 7. The relationships of the intrinsic water use efficiency (iWUE, μmol CO$_2$ mol^{-1} H$_2$O) and (a) the ratio of mesophyll conductance to CO$_2$ (g_m) to (g_s) ($g_\mathrm{m}/g_\mathrm{s}$) and (b) the ratio of the maximum carboxylase activity of Rubisco (V_cmax) to gs ($V_\mathrm{cmax}/g_\mathrm{s}$). Lines refer to regression line for 63 species. T, TS, S, G, V, and F represent Tree, Tree/Shrub, Shrub, Grass, Vine, and Fern, respectively.