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

Response to reviewer

Dear Reviewer,

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

Specific comments

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

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

(2) Although an “in review” article is cited in the materials and methods, I think this is not an acceptable description of methodology (line 140). This should be written out in detail as I cannot access the information from there. I would like to have more details about leaf sampling and measurements. What were the temperature and humidity chosen for the measurements? How were the leaves collected? Did you collect leaves or twigs which you then cut under water or did you collect separate

leaves which you measured in the field? Did you measure fluorescence? Could you calculate your results with the Harley method as well? It is common nowadays to confirm your results with a second method as all methods have some constraints.

Response: Thank you for your suggestions. In response, we have revised the Section "Materials and Methods" in two aspects. Firstly, we added more details about leaf sampling and measurements in Section "Materials and Methods". Such as, we have added the method of how were the leaves collected and prepared before CO_2 response curves measurements "Branches exposed to the sun were excised from the upper part of the crown (Trees, Tree/Shrubs, Shrubs and Vines) or aboveground portion (Grasses, Ferns), and immediately re-cut under water to maintain xylem water continuity. Back into the laboratory, branches and aboveground portions were kept at 25^{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."

We have described the method and conditions of CO_2 response curves measurements in more detail as: "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)."

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

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

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

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

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 paragraph in Section “4.1 Co-variation in g_s, g_m and V_{cmax} in regulating A” “The A was constrained by g_s, g_m, and V_{cmax} acting together, however, variability in the relative contribution of these three factors depended on species and habitats (Tosens et al., 2016; Galmes et al., 2017; Peguero-Pina et al., 2017a; Veromann-Jurgenson et al., 2017). .... In addition, 20 of the 63 species were mainly limited by V_{cmax} (l_b>0.4, with the largest value of 0.68). (lines 340-351)” to “A was constrained by g_s, g_m, and V_{cmax} acting together, however, variability in the relative contribution of these three factors depended on species and habitats (Tosens et al., 2016; Galmes et al., 2017;

Peguero-Pina et al., 2017a; Veromann-Jurgenson et al., 2017). A was significantly correlated with g_s, g_m, and V_{cmax} (Fig.3a-c). g_s was positively related to g_m (Fig.S1c), while no relationship was found between the CO_2 diffusion conductance (g_s and g_m) and V_{cmax} (Fig. S2). The relative limitations of g_s, g_m, and V_{cmax} were separated by a quantitative limitation model (Jones, 1985; Grassi & Magnani, 2005). The results showed that l_s, l_m and l_b of 63 species varied in a large range (Fig. S3), indicating plants have a diverse strategies to co-ordinate the CO_2 diffusion (g_s and g_m) and V_{cmax} to maintain relative high A. The order of factors limitations to A was $l_m > l_b > l_s$ ($P<0.05$) (Fig.S3). Furthermore, we tested the relationship between the relative limitations and the corresponding limitation factors. The results showed that l_s, l_m, and l_b were negatively associated with g_s, g_m, and V_{cmax}, respectively (Fig. 4). And the relationship was stronger for g_m- l_m ($r^2=0.65$) than V_{cmax}- l_b ($r^2=0.27$) and g_s- l_s ($r^2=0.19$). ”

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

(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 relative 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."

(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.".

Technical comments

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

Response: Corrected. Thank you.

(8) Line 38: delete first "and"

Response: Deleted. Thank you.

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

Response: Change has been made. Thank you.

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

Response: Change has been made. Thank you.

(11) Line 73: replace "indeed" with "however"

Response: This change has been made.

(12) Line 84: within "a" leaf.

Response: Change has been made. Thank you.

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

Response: Deleted. Thank you.

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

Response: Change has been made.

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

Response: Change has been made.

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

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

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

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

(18) Line 153: delete "The"

Response: Deleted. Thank you.

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

Response: Change has been made.

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

Response: Thank you for your suggestion and comment. Rephrased as: "Mesophyll is the barrier for CO_2 inside the leaf. "

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

Response: This changed have been made. Thank you.

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

Response: Deleted.

(23) Line 256: delete "The"

Response: Deleted.

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

Response: Change has been made. Thank you.

(25) Line 269: delete "The"

Response: Deleted.

(26) Line 271: delete "The"

Response: Deleted.

(27) Line 272: Change to "Grasses"

Response: Change has been made. Thank you.

(28) Line 273: Change to "Accordingly, grasses"

Response: Change has been made.

(29) Line 276: delete "The"

Response: Deleted.

(30) Line 284: delete "The"

Response: Deleted.

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

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

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

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

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

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

(34) Line 402: "highly efficient"

Response: This change has been made.

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

Response: Deleted.

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

Response: Corrected. Thank you.

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

Response: Deleted.

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

Response: Added. Thank you.

(39) Line 424: delete "The"

Response: This change has been made.

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

Response: This change has been made.

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

This change has been made.

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

Response: This change has been made.

(43) Line 452: "low nutrient"

Response: This change has been made.

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

Response: This change has been made.

(45)Line 462: …forms in "the" field

Response: This change has been made.

(46) Line 463: … used "a" diverse

Response: Change has been made, thank you.

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

Response: This change has been made.

(48) Line 465: … used "the"

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

(49)Line 483: "References"

Response: Change has been made.

Appendix

1 Figures

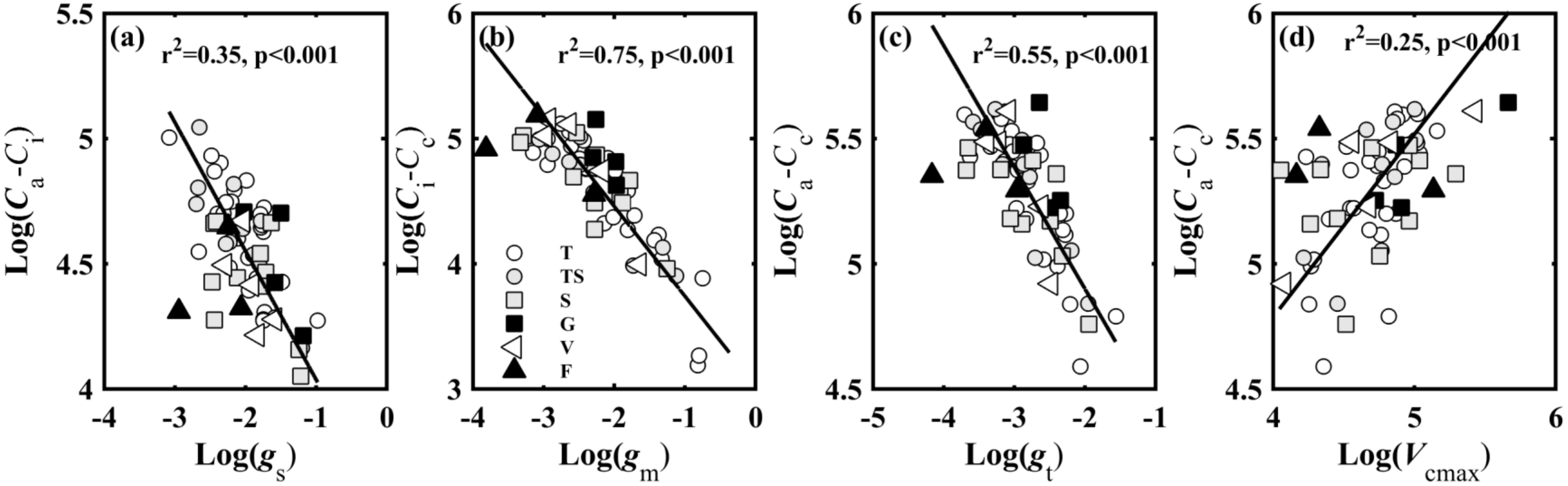

Figure 1. Relationships between (a) CO_2 gradient between ambient air and intercellular air space (C_a-C_i, μmol mol^{-1}) and stomatal conductance to CO_2 (g_s, mol CO_2 m^{-2} s^{-1}); (b) CO_2 gradient between intercellular air space and chloroplasts (C_i-C_c, μmol mol^{-1}) and mesophyll conductance 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.

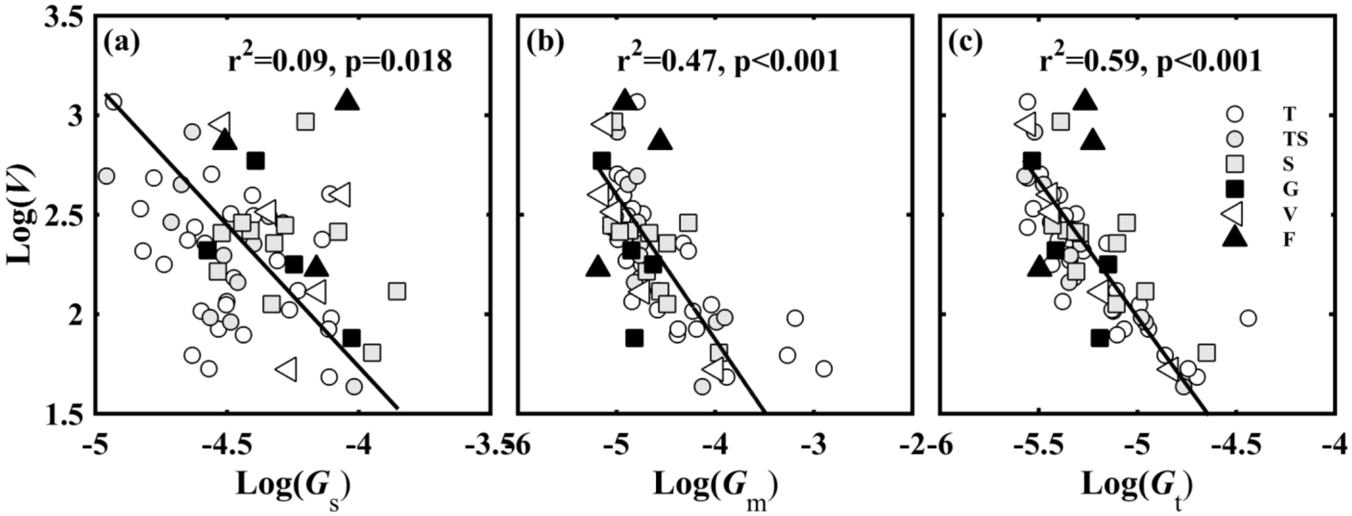

Figure 2. Relationships between (a) *V* and G_s; (b) *V* and G_m; and (c) *V* and G_t. *V* is the ratio of photosynthetic capacity (V_{cmax}) to light-saturated net photosynthesis (*A*, μmol CO_2 m^{-2} s^{-1}); G_s is the ratio of stomatal conductance to CO_2 (g_s, mol CO_2 m^{-2} s^{-1}) to *A*; G_m 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.

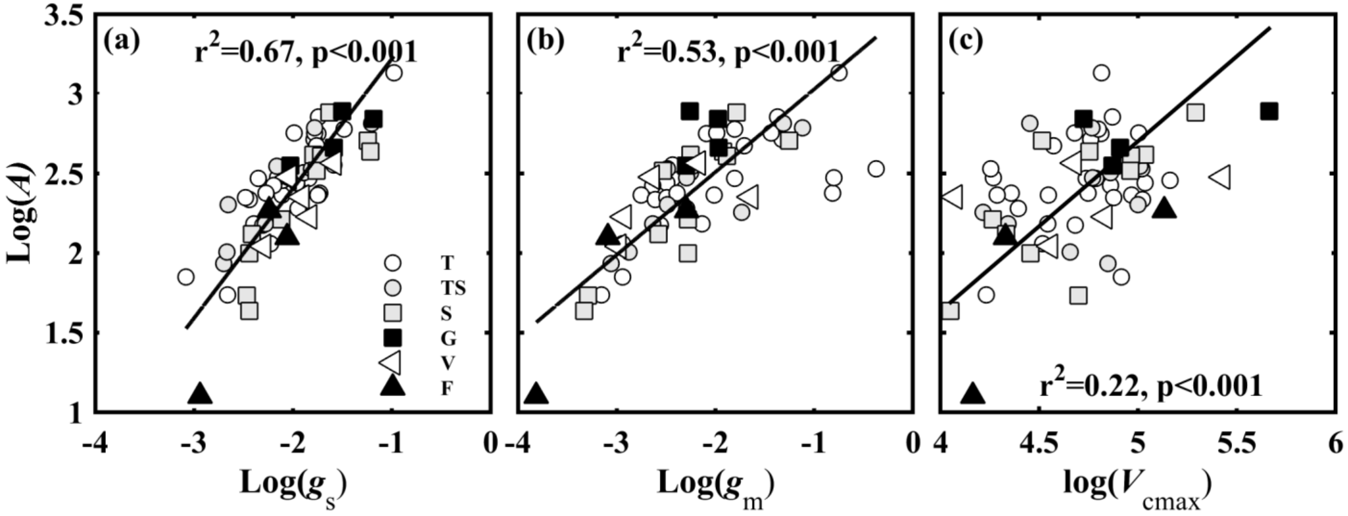

Figure 3. Relationships between light-saturated net photosynthesis (A, μmol CO_2 m^{-2} s^{-1}) and (a) stomatal conductance to CO_2 (g_s, mol CO_2 m^{-2} s^{-1}); (b) mesophyll conductance to CO_2 (g_m, mol CO_2 m^{-2} s^{-1}); and (c) the maximum carboxylase activity of Rubisco (V_{cmax}, μmol CO_2 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.

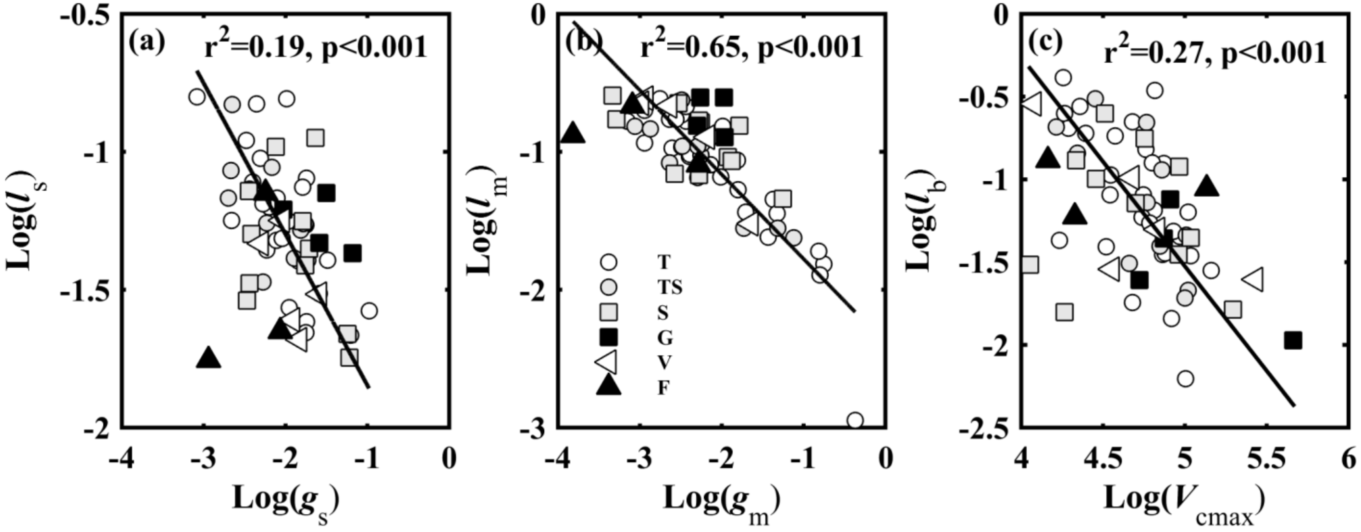

Figure 4. Relationships between (a) stomatal conductance to CO_2 (g_s, mol CO_2 m^{-2} s^{-1}) and l_s (g_s limitation on light-saturated net photosynthesis (A)); (b) mesophyll conductance to CO_2 (g_m, mol CO_2 m^{-2} s^{-1}) and l_m (g_m limitation on A); and (c) the maximum carboxylase activity of 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.

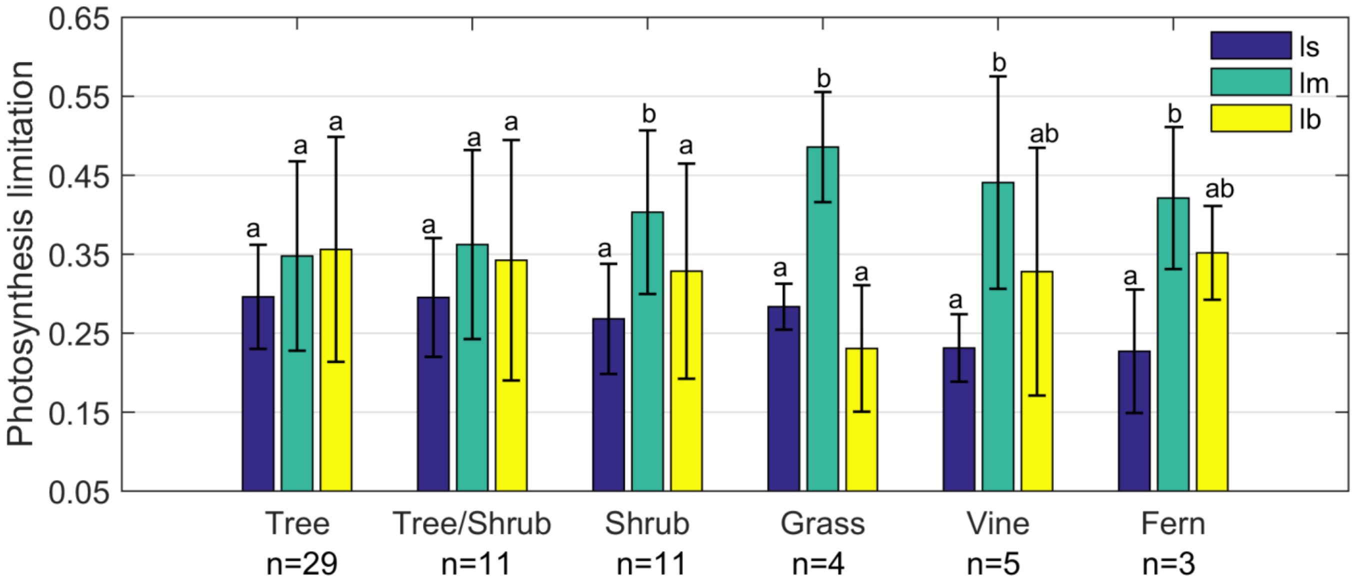

Figure 5. Limitation to light-saturated net photosynthesis (*A*) in six life forms by stomatal conductance to CO_2 (l_s), mesophyll conductance to CO_2 (l_m), and the maximum carboxylase activity of Rubisco (l_b). Error bars denominate standard deviation (1σ).

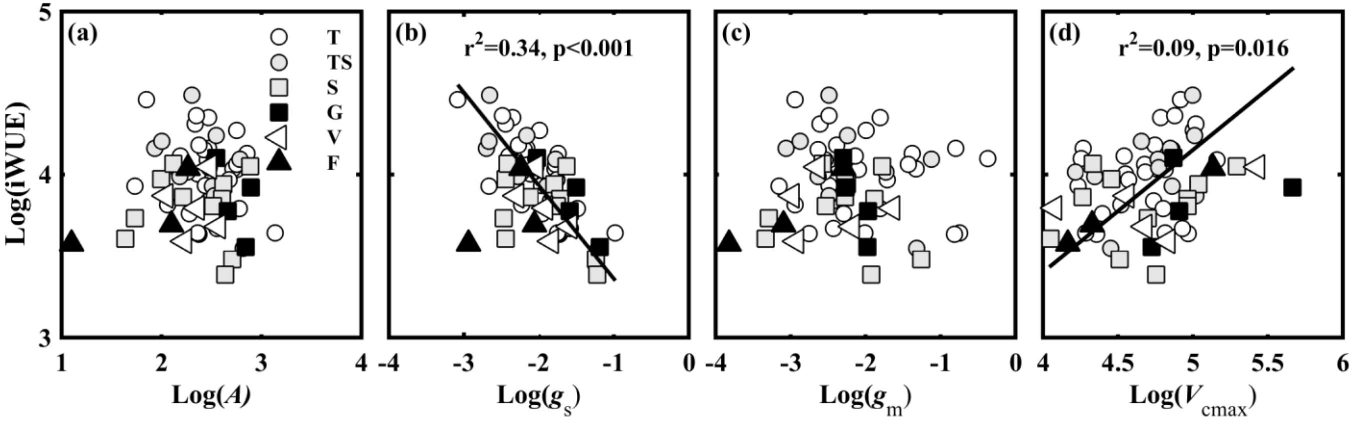

Figure 6. Relationships between the observed intrinsic water use efficiency (iWUE, μmol CO_2 mol^{-1} H_2O) and (a) light-saturated net photosynthesis (*A*, μmol CO_2 m^{-2} s^{-1}); (b) stomatal conductance to CO_2 (g_s, mol CO_2 m^{-2} s^{-1}); (c) mesophyll conductance to CO_2 (g_m, mol CO_2 m^{-2} 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.

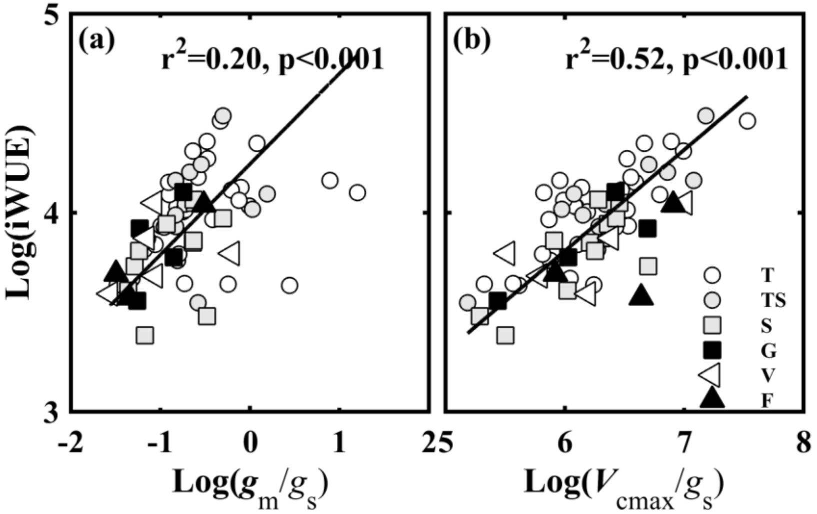

Figure 7. The relationships of the intrinsic water use efficiency (iWUE, μmol CO_2 mol^{-1} H_2O) and (a) the ratio of mesophyll conductance to CO_2 (g_m) to (g_s) (g_m/g_s) and (b) the ratio of the maximum carboxylase activity of Rubisco (V_{cmax}) to gs (V_{cmax}/g_s). Lines refer to regression line for 63 species. T, TS, S, G, V, and F represent Tree, Tree/Shrub, Shrub, Grass, Vine, and Fern, respectively.

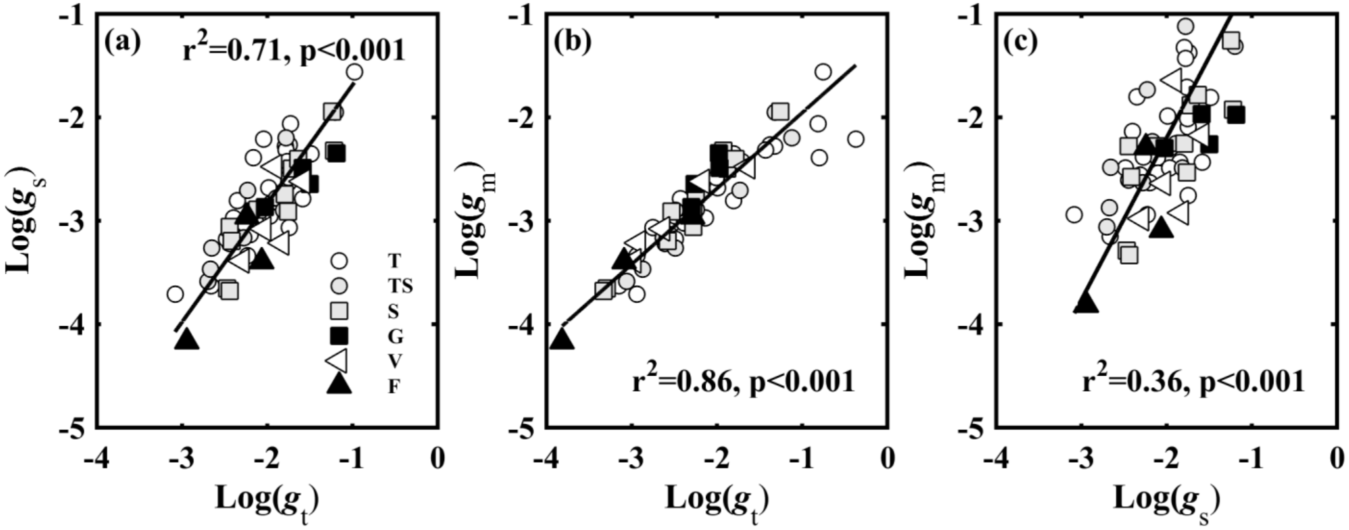

Figure S1 Relationship between (a) stomatal conductance to CO_2 (g_s, mol CO_2 m^{-2} s^{-1}) and total conductance to CO_2 (g_t, mol CO_2 m^{-2} s^{-1}); (b) mesophyll conductance to CO_2 (g_m, mol CO_2 m^{-2} s^{-1}) and gt; and (c) g_s and g_m. Lines refer to regression line for 63 species. T, TS, S, G, V, and F represent Tree, Tree/Shrub, Shrub, Grass, Vine, and Fern, respectively.

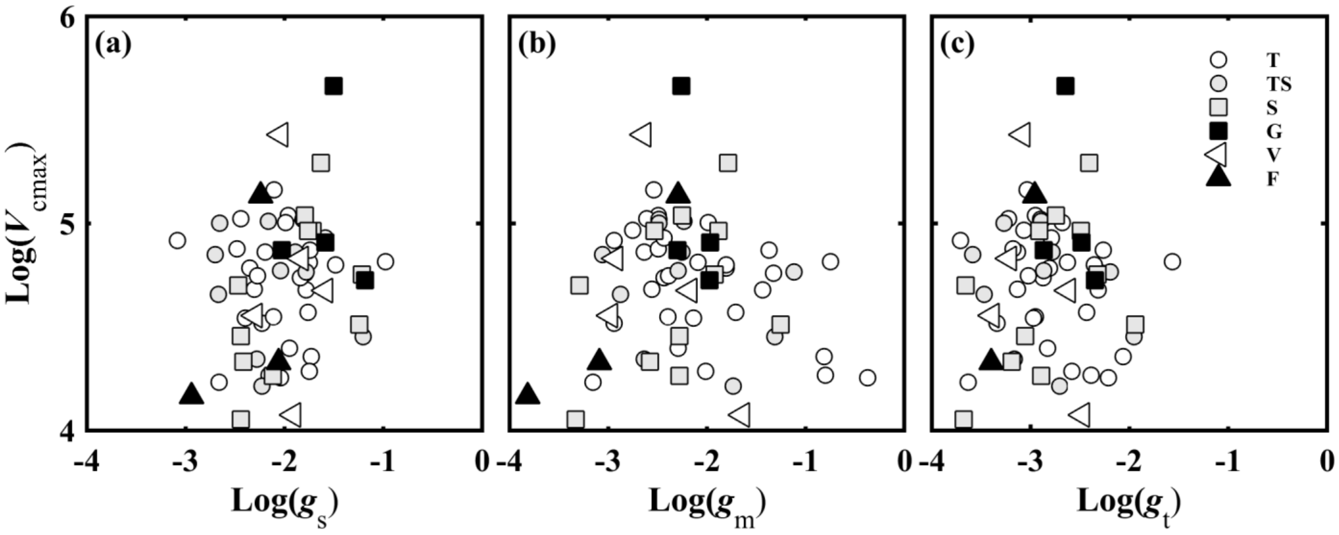

Figure S2 Relationship between (a) stomatal conductance to CO_2 (g_s, mol CO_2 m^{-2} s^{-1}) and the maximum carboxylase activity of Rubisco (V_{cmax}, μmol CO_2 m^{-2} s^{-1}); (b) mesophyll conductance to CO_2 (g_m, mol CO_2 m^{-2} s^{-1}) and V_{cmax}; and (c) total conductance to CO_2 (g_t) and V_{cmax}. 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.

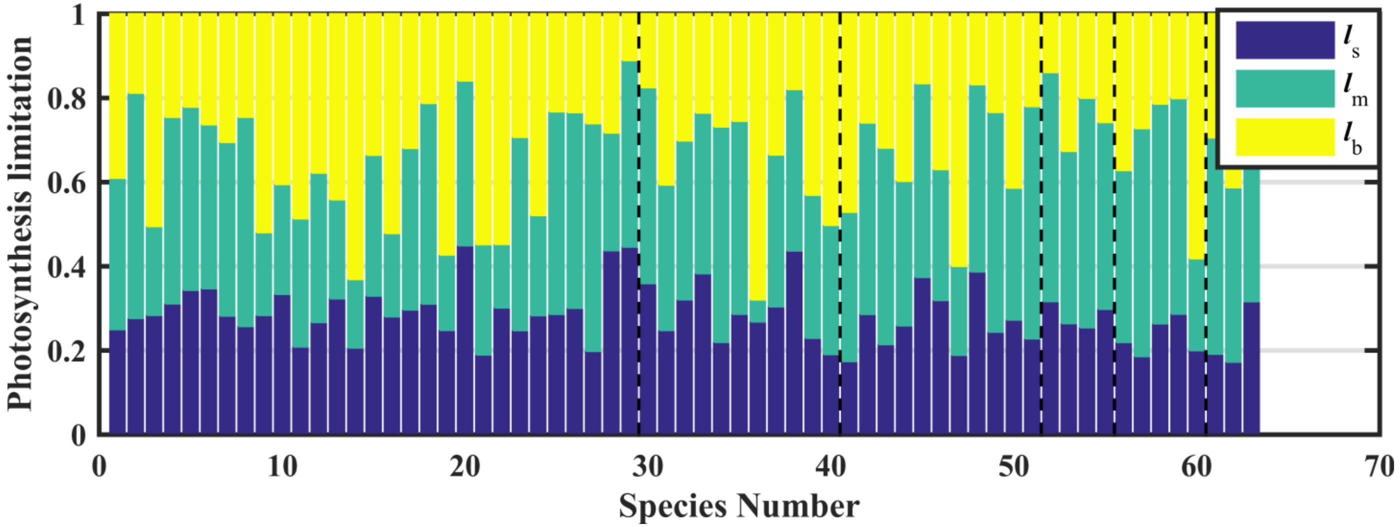

Figure S3 The limitation of (a) stomatal conductance to CO_2 (g_s) on photosynthesis rate (A) (l_s), (b) mesophyll conductance to CO_2 (g_m) on A (l_m) and (c) the maximum carboxylase activity of Rubisco (V_{cmax}) on A (l_b).

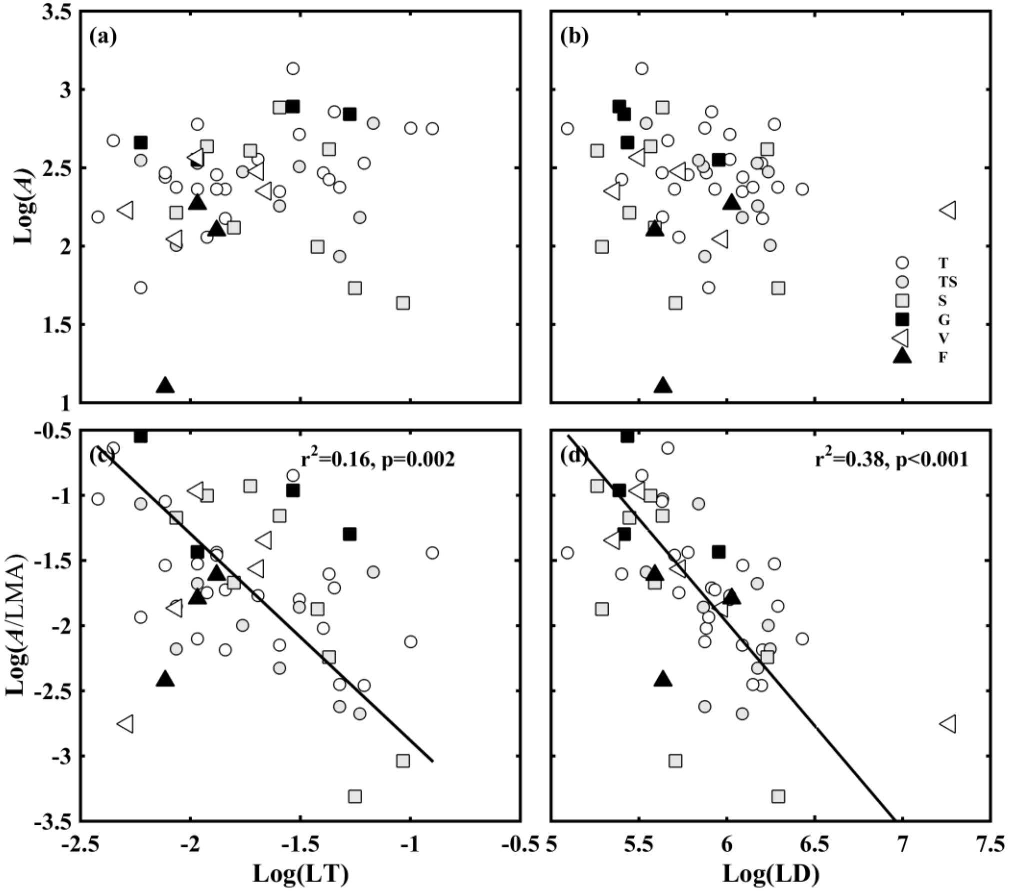

Figure S4 Relationship between (a) light-saturated net photosynthesis (*A*) and the leaf thickness (LT); (b) *A* and he leaf density (LD); (c) the ratio of *A* to leaf mass per area (LMA) (*A*/LMA); and (d) *A*/LMA and LD. Lines refer to regression line for 63 species. T, TS, S, G, V, and F represent Tree, Tree/Shrub, Shrub, Grass, Vine, and Fern, respectively.

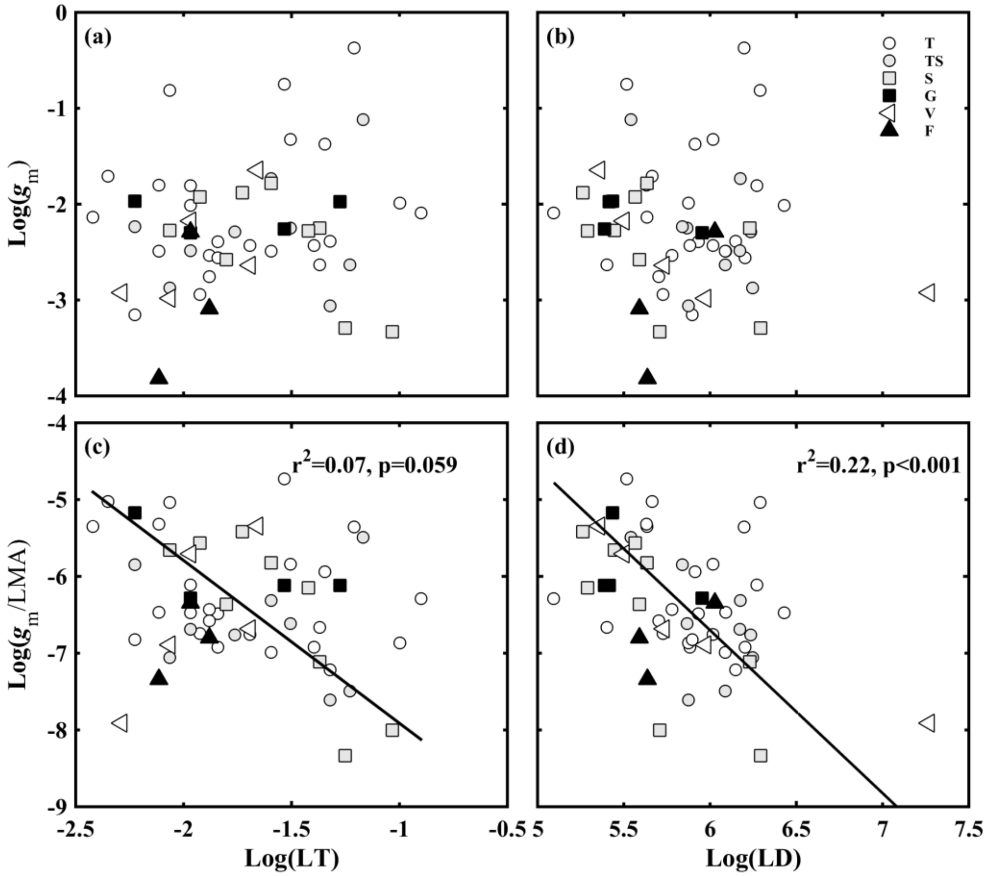

Figure S5 Relationship between (a) the mesophyll conductance to CO_2 (g_m) and the leaf thickness (LT); (b) g_m and he leaf density (LD); (c) the ratio of g_m to leaf mass per area (LMA) (g_m/LMA); and (d) g_m/LMA and LD. Lines refer to regression line for 63 species. T, TS, S, G, V, and F represent Tree, Tree/Shrub, Shrub, Grass, Vine, and Fern, respectively.

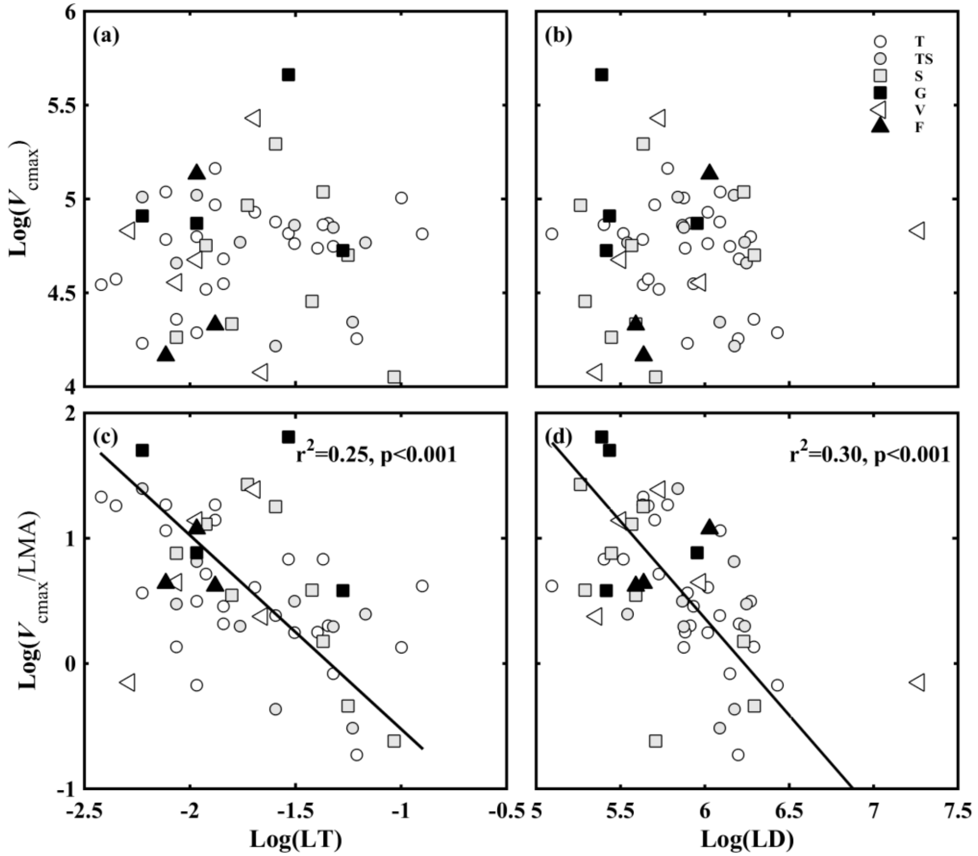

Figure S6 Relationship between (a) the maximum carboxylase activity of Rubisco (V_{cmax}) and the leaf thickness (LT); (b) V_{cmax} and he leaf density (LD); (c) the ratio of V_{cmax} to leaf mass per area (LMA) (V_{cmax}/LMA); and (d) V_{cmax}/LMA and LD. Lines refer to regression line for 63 species. T, TS, S, G, V, and F represent Tree, Tree/Shrub, Shrub, Grass, Vine, and Fern, respectively.

2 Tables

Table S1 Details information about the 63 species in the subtropical primary forest in Southwest China.

Species	Plant family	Life form		
Broussonetia papyifera (Linn.) L'Hert. ex Vent.	Moraceae	Tree	Deciduous	Woody
Machilus microcarpa Hemsl.	Lauraceae	Tree	Evergreen	Woody
Melia azedarach L.	Meliaceae	Tree	Deciduous	Woody
Populus ×canadensis Moench.	Salicaceae	Tree	Deciduous	Woody
Camptotheca acuminata Decne.	Nyssaceae	Tree	Deciduous	Woody
Cinnamomum bodinieri Levl.	Lauraceae	Tree	Evergreen	Woody
Catalpa ovata G. Don	Bignoniaceae	Tree	Deciduous	Woody
Toona sinensis (A. Juss.) Roem.	Meliaceae	Tree	Deciduous	Woody
Sapium sebiferum (Linn.) Roxb.	Euphorbiaceae	Tree	Deciduous	Woody
Cladrastis platycarpa (Maxim.) Makino	Leguminosae	Tree	Deciduous	Woody
Ulmus pumila L.	Ulmaceae	Tree	Deciduous	Woody
Ilex macrocarpa Oliv.	Aquifoliaceae	Tree	Deciduous	Woody
Vitex canescens Kurz	Verbenaceae	Tree	Deciduous	Woody
Eriobotrya japonica (Thunb.) Lindl.	Rosaceae	Tree	Evergreen	Woody
Morus alba L.	Moraceae	Tree	Deciduous	Woody
Prunus salicina Lindl.	Rosaceae	Tree	Deciduous	Woody
Eucommia ulmoides Oliver	Eucommiaceae	Tree	Deciduous	Woody
Platycarya strobilacea Sieb. et Zucc.	Juglandaceae	Tree	Deciduous	Woody
Kalopanax septemlobus (Thunb.) Koidz.	Araliaceae	Tree	Deciduous	Woody
Zanthoxylum armatum DC.	Rutaceae	Tree	Deciduous	Woody
Pyrus calleryana	Rosaceae	Tree	Deciduous	Woody
Amygdalus persica L. var.	Rosaceae	Tree	Deciduous	Woody

Euonymus meaackii Rupr.	Celastraceae	Tree	Deciduous	Woody
Zanthoxylum ovalifolium Wight	Rutaceae	Tree	Deciduous	Woody
Cerasus scopulorum (Koehne) Yu et Li	Rosaceae	Tree	Deciduous	Woody
Carpinus pubescens Burk.	Betulaceae	Tree	Deciduous	Woody
Lithocarpus confinis Huang	Fagaceae	Tree	Evergreen	Woody
Celtis sinensis Pers.	Ulmaceae	Tree	Deciduous	Woody
Diospyros kaki Thunb. var. silvestris Makino	Ebenaceae	Tree	Deciduous	Woody
Ligustrum lucidum Ait.	Oleaceae	Tree/Shrub	Deciduous	Woody
Rhamnus leptophylla Schneid.	Rhamnaceae	Tree/Shrub	Deciduous	Woody
Lindera communis Hemsl.	Lauraceae	Tree/Shrub	Evergreen	Woody
Itea yunnanensis Franch	Saxifragaceae	Tree/Shrub	Evergreen	Woody
Pittosporum brevicalyx (Oliv.) Gagnep	Pittosporaceae	Tree/Shrub	Evergreen	Woody
Litsea rubescens Lec.	Lauraceae	Tree/Shrub	Deciduous	Woody
Rhus chinensis Mill.	Anacardiaceae	Tree/Shrub	Deciduous	Woody
Alangium chinense (Lour.) Harms	Alangiaceae	Tree/Shrub	Deciduous	Woody
Evodia rutaecarpa (Juss.) Benth.	Rutaceae	Tree/Shrub	Deciduous	Woody
Machilus cavaleriei Levl.	Lauraceae	Tree/Shrub	Evergreen	Woody
Debregeasia longifolia (Burm. f.) Wedd.	Urticaceae	Tree/Shrub	Deciduous	Woody
Ziziphus jujuba Mill. var. spinosa (Bunge) Hu ex H. F. Chow	Rhamnaceae	Shrub	Deciduous	Woody
Rubus inopertus (Diels) Focke	Rosaceae	Shrub	Deciduous	Woody
Coriaria nepalensis Wall.	Coriariaceae	Shrub	Deciduous	Woody
Celastrus orbiculatus Thunb.	Celastraceae	Shrub	Deciduous	Woody
Wikstroemia scytophylla Diels	Thymelaeaceae	Shrub	Deciduous	Woody
Viburnum foetidum Wall. var. ceanothoides (C. H. Wright) Hand.-Mazz.	Caprifoliaceae	Shrub	Deciduous	Woody
Hedera nepalensis K. Koch var. sinensis (Tobl.) Rehd.	Araliaceae	Shrub	Deciduous	Woody
Rubus parvifolius L.	Rosaceae	Shrub	Deciduous	Woody

Rosa roxbunghii	Rosaceae	Shrub	Deciduous	Woody
Mallotus repandus (Willd.) Muell. Arg.	Euphorbiaceae	Shrub	Deciduous	Woody
Mahonia bealei (Fort.) Carr.	Berberidaceae	Shrub	Evergreen	Woody
Fallopia multiflora (Thunb.) Harald.	Polygonaceae	Grass		Herb
Conyza canadensis (L.) Cronq.	Compositae	Grass		Herb
Ipomoea batatas (L.) Lam.	Convolvulaceae	Grass		Herb
Senecio scandens Buch.-Ham. ex D. Don	Compositae	Grass		Herb
Vitis piasezkii Maxim.	Vitaceae	Vien	Deciduous	Woody
Clematis urophylla Franch.	Ranunculaceae	Vien	Deciduous	Woody
Bauhinia glauca (Wall. ex Benth.) Benth.	Leguminosae	Vien	Evergreen	Woody
Caesalpinia decapetala (Roth) Alston	Leguminosae	Vien	Deciduous	Woody
Paederia scandens (Lour.) Merr.	Rubiaceae	Vien		Herb
Cyclosorus parasiticus (L.) Farwell.	Thelypteridaceae	Fern		
Cyrtomium fortunei J. Sm.	Dryopteridaceae	Fern		
Pteris vittata L.	Pteridaceae	Fern		

Table S2 Coefficients of determination of linear regressions of fig. 1-4 and fig.6-7.

		Fig.1		Fig.2		Fig.3		Fig.4		Fig.6		Fig.7	
Subgraph	Life form	R^2	P	R^2	P	R^2	P	R^2	P	R^2	P	R^2	P
a	Total	0.35	0.000	0.09	0.018	0.67	0.000	0.19	0.000	0.00	0.922	0.20	0.000
	Tree	0.49	0.000	0.14	0.048	0.67	0.000	0.42	0.000	0.03	0.401	0.11	0.083
	Tree/Shrub	0.70	0.001	0.49	0.016	0.79	0.000	0.57	0.007	0.24	0.126	0.07	0.438
	Shrub	0.29	0.085	0.10	0.350	0.78	0.000	0.11	0.314	0.00	1.000	0.20	0.173
b	Total	0.75	0.000	0.47	0.000	0.53	0.000	0.65	0.000	0.34	0.000	0.52	0.000
	Tree	0.85	0.000	0.53	0.000	0.42	0.000	0.80	0.000	0.49	0.000	0.58	0.000
	Tree/Shrub	0.84	0.000	0.67	0.002	0.68	0.002	0.78	0.000	0.70	0.001	0.78	0.000
	Shrub	0.60	0.005	0.50	0.015	0.75	0.001	0.42	0.031	0.22	0.142	0.56	0.008
c	Total	0.55	0.000	0.59	0.000	0.76	0.000	0.38	0.000	0.00	0.934		
	Tree	0.68	0.000	0.67	0.000	0.70	0.000	0.63	0.000	0.01	0.549		
	Tree/Shrub	0.79	0.000	0.88	0.000	0.83	0.000	0.67	0.002	0.21	0.162		
	Shrub	0.50	0.014	0.55	0.009	0.84	0.000	0.23	0.138	0.01	0.771		
d	Total	0.25	0.000			0.22	0.000	0.27	0.000	0.09	0.016		
	Tree	0.36	0.001			0.09	0.121	0.34	0.001	0.08	0.133		
	Tree/Shrub	0.40	0.038			0.02	0.714	0.52	0.013	0.19	0.180		
	Shrub	0.04	0.552			0.53	0.011	0.01	0.734	0.06	0.471		