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Responses of photosynthetic parameters to drought in subtropical forest ecosystem of China

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Lei Zhou¹, Shaoqiang Wang¹, Yonggang Chi², Qingkang Li¹, Kun Huang¹ & Quanzhou Yu¹

The mechanism underlying the effect of drought on the photosynthetic traits of leaves in forest ecosystems in subtropical regions is unclear. In this study, three limiting processes (stomatal, mesophyll and biochemical limitations) that control the photosynthetic capacity and three resource use efficiencies (intrinsic water use efficiency (iWUE), nitrogen use efficiency (NUE) and light use efficiency (LUE)), which were characterized as the interactions between photosynthesis and environmental resources, were estimated in two species (*Schima superba* and *Pinus massoniana*) under drought conditions. A quantitative limitation analysis demonstrated that the drought-induced limitation of photosynthesis in *Schima superba* was primarily due to stomatal limitation, whereas for *Pinus massoniana*, both stomatal and non-stomatal limitations generally exhibited similar magnitudes. Although the mesophyll limitation represented only 1% of the total limitation in *Schima superba*, it accounted for 24% of the total limitations for *Pinus massoniana*. Furthermore, a positive relationship between the LUE and NUE and a marginally negative relationship or trade-off between the NUE and iWUE were observed in the control plots. However, drought disrupted the relationships between the resource use efficiencies. Our findings may have important implications for reducing the uncertainties in model simulations and advancing the understanding of the interactions between ecosystem functions and climate change.

Water deficit is the primary factor that limits ecosystem productivity in most terrestrial biomes¹. The physiological responses of trees to drought (i.e., carbon uptake) are directly related to vegetation growth², ecosystem productivity^{3,4}, frequency of fires^{5,6} and tree mortality^{7,8}. The subtropical region experiences frequent seasonal droughts⁹ that result in declines in terrestrial carbon sequestration¹⁰. However, the mechanism underlying the effects of drought on the carbon uptake of subtropical ecosystems at the leaf level remains unclear¹¹.

The carbon uptake of forest ecosystems is driven by leaf photosynthesis, the responses of which to drought are mediated by three physiological processes. First, stomatal closure is recognized as the main driver of the photosynthetic response to water stress by limiting CO₂ diffusion from the atmosphere to the substomatal cavities to slow photosynthesis^{12,13}. Second, the mesophyll conductance (g_m) may rapidly decrease, thereby limiting CO₂ diffusion from the substomatal cavities to the chloroplast stroma during water stress^{14,15}. Finally, photosynthesis may be limited by biochemical processes in long-lasting, severe droughts, resulting in decreased photosynthetic enzyme activity (i.e., the maximum rate of Rubisco carboxylation, V_{max}), ribulose-1,5-bisphosphate (RuBP) regeneration capacity (i.e., the maximum rate of photosynthetic electron transport, J_{max}) and triose-phosphate utilization (TPU)^{16–18}. As a result, drought stress directly influences CO₂ diffusion and/or the biochemical process of photosynthesis, which in turn limits the net CO₂ assimilation rate (A_n). For example, Maseda and Fernandez (2006) found that the rapid closure of stomata during water stress resulted in a decline in transpiration and the A_n ¹⁹. Increasing evidence has shown that mesophyll conductance is finite²⁰ and plays an important role in limiting the photosynthetic capacity¹². Additionally, drought-stressed plants exhibit significant reductions in V_{max} , J_{max} and TPU relative to plants with sufficient water²¹, indicating that biochemical processes dramatically inhibit photosynthesis during long-term severe droughts. These apparent discrepancies may arise from the fact that photosynthesis induced

¹Key Laboratory of Ecosystem Network Observation and Modelling, Institute of Geographic Sciences and Natural Resources Research, Chinese Academy of Sciences, Beijing 100101, China. ²State Key Laboratory of Vegetation and Environmental Change, Institute of Botany, Chinese Academy of Sciences, Beijing 100093, China. Correspondence and requests for materials should be addressed to S.W. (email: sqwang@igsnrr.ac.cn)

Treatments	SWC (g g ⁻¹)	<i>Schima superba</i>				<i>Pinus massoniana</i>			
		SLA (m ² kg ⁻¹)	C _{area} (g C m ⁻²)	N _{area} (g N m ⁻²)	Leaf C/N ratio (g g ⁻¹)	SLA (m ² kg ⁻¹)	C _{area} (g C m ⁻²)	N _{area} (g N m ⁻²)	Leaf C/N ratio (g g ⁻¹)
Control	0.21 ± 0.01	10.81 ± 0.58	47.01 ± 2.19	1.67 ± 0.09	28.27 ± 0.62	6.88 ± 0.24	75.84 ± 3.10	2.25 ± 0.09	33.86 ± 1.03
Drought	0.13 ± 0.01	10.85 ± 0.32	46.27 ± 1.35	1.54 ± 0.04	30.08 ± 0.57	7.52 ± 0.41	70.50 ± 4.68	2.10 ± 0.13	33.90 ± 1.37
<i>p</i> value	0.000	0.959	0.777	0.191	0.044	0.262	0.351	0.351	0.982

Table 1. Soil water content and leaf traits of *Schima superba* and *Pinus massoniana* grown in control and drought plots. Note: The drought effects on the soil water content (SWC), specific leaf area (SLA), C concentration (C_{area}), N concentration (N_{area}) and Leaf C/N ratio were analyzed for *Schima superba* and *Pinus massoniana* using an independent sample T-test. Significant values ($P < 0.05$) are shown bold (Mean ± SE, N=12).

by drought stress is not limited by a single process. Instead, the combined effect of the stomatal, mesophyll, and biochemical limitations simultaneously regulates the decrease in photosynthesis in response to water stress^{21–23}. However, a quantitative limitation analysis is needed to separate the three physiological processes in subtropical climatic zones^{12,22,24}.

Drought not only decreases the leaf photosynthetic rate but also regulates the interaction of plant carbon uptake and environmental resources, which is termed the resource use efficiency. Three resource use efficiencies (water use efficiency (WUE), carbon gain at the expense of water loss; nitrogen use efficiency (NUE), carbon gain per nitrogen content; and light use efficiency (LUE), carbon gain per available light quantum flux density) are important functional parameters that intimately couple the uptake of carbon with the major growth limiting factors (water, nitrogen and light). In general, the leaf-level WUE has been reported to increase during soil water stress^{25,26}, which suggests that stomata closure in response to H₂O flux is more sensitive than the response to carbon flux²⁷. Apart from increasing the WUE, stomatal closure during drought stress has an effect on photosynthesis but no effect on leaf nitrogen, leading to a decline in the NUE^{26,27}. During drought stress periods, the LUE generally decreases with increasing drought intensity; indeed, no change was observed in the electron transport rate under mild and moderate water stress²⁸, or the electron transport rate declined to a lesser extent than the net CO₂ assimilation rate²⁹. The changes in single resource use efficiency induced by drought have been well documented^{25,26,28}, but the trade-off among the multiple resource use efficiencies of plants requires investigation.

Based on a recent integration of eddy covariance observations, subtropical forests in the East Asian region exhibit a high carbon dioxide uptake rate (362 g C m⁻² year⁻¹) compared with Asian tropical and temperate forests³⁰. A model simulation indicated that drought caused the net exchange of carbon in the subtropical forests in Southern China to decrease by 63% and 47% in 2003 and 2004, respectively³¹. Despite the ecological importance of this region, the carbon uptake response of subtropical forests to drought is poorly constrained. To address this knowledge gap, a rainfall exclusion experiment was established in 2010 using two gradients of soil moisture content and three replicates. The three limiting processes (i.e., stomatal limitation, mesophyll limitation and biochemical limitation) that control photosynthesis and the three resource use efficiencies (iWUE, NUE and LUE) that represent the interaction of photosynthesis and environmental resources were estimated after a 3-year drought. We focused on (1) testing the sensitivity of the photosynthetic characteristics of *Schima superba* and *Pinus massoniana* in subtropical regions of China during an experimental drought and (2) determining the changes in the iWUE, NUE and LUE in response to water stress and identifying whether a trade-off existed among resource use efficiencies.

Results

The response of soil water content and leaf chemical characteristics to drought. The experimental drought significantly reduced the soil water content by 38% ($t = 9.840$; $P < 0.0001$) (Table 1). In general, the leaf traits (i.e., SLA, C concentration, N concentration and C/N ratio) for each species were not affected by the drought based on an independent sample T-test, except that a significant difference in the C/N ratio for *Schima superba* ($P = 0.044$) occurred between the control and drought plots (Table 1).

The response of the carbon assimilation process to drought. Based on an independent sample T-test, significant effects of drought on the A_n of *Schima superba* ($t = 3.080$, $P = 0.005$) and *Pinus massoniana* ($t = 3.769$, $P = 0.001$) were observed, which showed significant reductions during drought treatment (Fig. 1a,e). However, no significant effects of soil moisture on the R_d were observed (both $P > 0.05$) for either species (Fig. 1b,f). For *Schima superba* and *Pinus massoniana*, the responses of the A_g to the soil water treatments were significant ($t = 3.134$, $P = 0.005$ and $t = 3.867$, $P = 0.001$) and resulted in significant decreases in the drought plots compared with the control plots (Fig. 1c,g). Although there were no significant differences in R_d/A_g between drought treatments for each species, a general increasing trend from the control to the drought plots was indicated (Fig. 1d,h). No significant differences in drought resistance for A_n , R_d , A_g , and R_d/A_g were observed between *Schima superba* and *Pinus massoniana* (Fig. 1i–l).

The response of the CO₂ diffusion process to drought. Drought produced a 42% decrease in the g_s ($t = 2.709$, $P = 0.013$) for *Schima superba* (Fig. 2a), whereas the effect of drought on the g_s for *Pinus massoniana* was not significantly different between the control and drought plots ($P > 0.05$) (Fig. 2d). The responses of the g_m to drought were not significantly different for either species, based on an independent sample T-test (both $P > 0.05$) (Fig. 2b,e). However, significant decreases in the g_{tot} were observed (*Schima superba*: $t = 2.618$, $P = 0.016$; *Pinus massoniana*: $t = 3.583$, $P = 0.002$) in the drought plots relative to the control plots (Fig. 2c,f). The drought

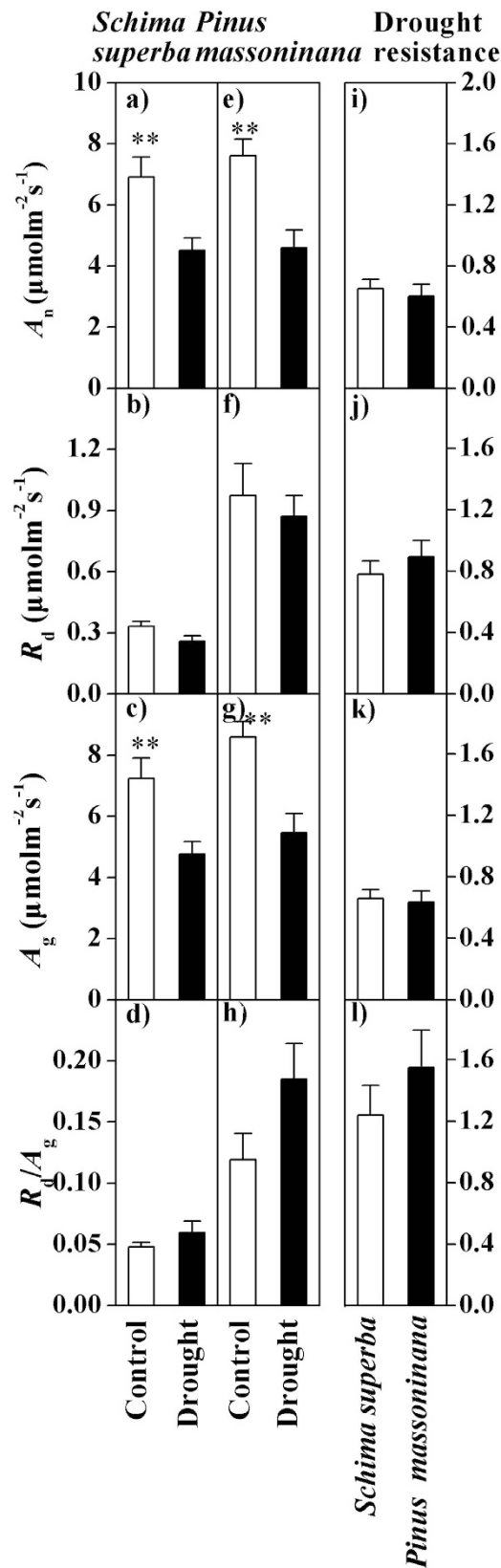


Figure 1. The effect of drought on the carbon assimilation process for the two species. (a,e) The A_n (net CO_2 assimilation rate, $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), (b,f) R_d (day respiration, $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), (c,g) A_g (gross CO_2 assimilation, $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) and (d,h) ratio of R_d and A_g in the control and drought plots for *Schima superba* (a–d) and *Pinus massoniana* (e–h) are shown. The drought resistance of (i) A_n , (j) R_d , (k) A_g and (l) R_d/A_g in *Schima superba* and *Pinus massoniana* is indicated. ANOVA: * $P < 0.05$; ** $P < 0.01$; and *** $P < 0.001$.

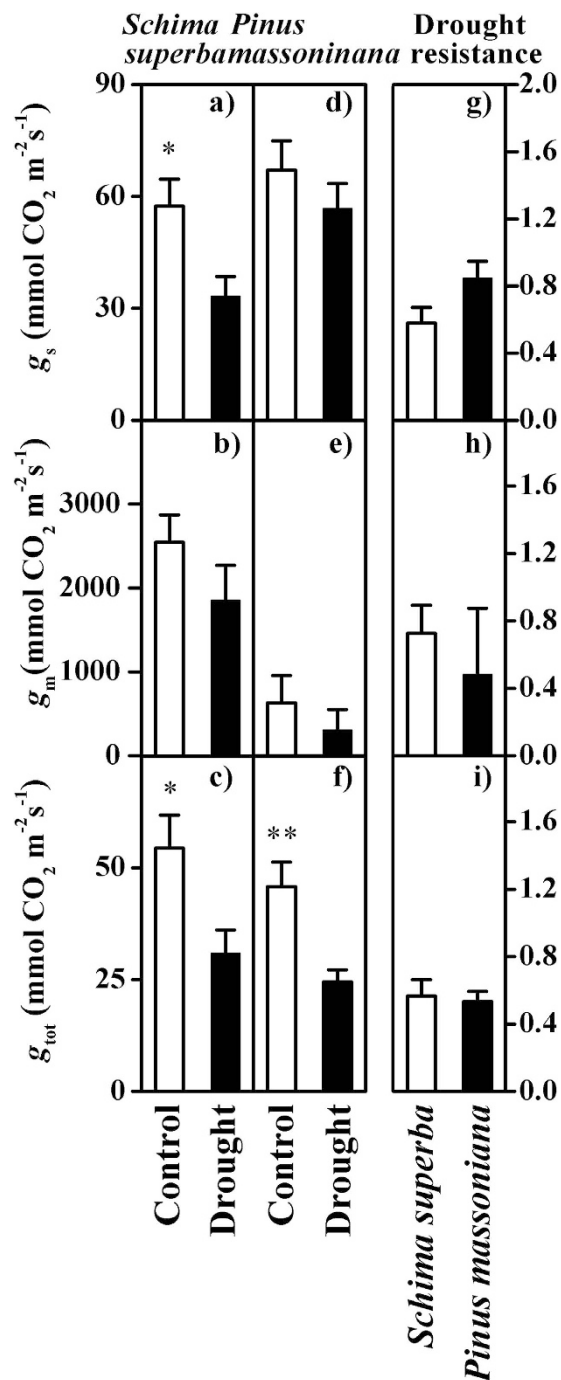


Figure 2. The effect of drought on the CO₂ diffusion process for the two species. (a,d) The g_s (stomatal conductance, mmol CO₂ m⁻² s⁻¹), (b,e) g_m (mesophyll conductance, mmol CO₂ m⁻² s⁻¹) and (c,f) g_{tot} (the total conductance, mmol CO₂ m⁻² s⁻¹) in the control and drought plots of *Schima superba* (a–c) and *Pinus massoniana* (d–f) are shown. The drought resistance of (g) g_s , (h) g_m and (i) g_{tot} in *Schima superba* and *Pinus massoniana* is indicated. ANOVA: * $P < 0.05$; ** $P < 0.01$; and *** $P < 0.001$.

resistance of *Pinus massoniana* with regard to the g_s appeared to be considerably higher than that of *Schima superba* ($P = 0.065$), whereas no significant differences in drought resistance related to the g_m and g_{tot} were found between the species (Fig. 2g–i).

The response of biochemical processes to drought. The effects of drought on V_{cmax} and J_{max} were not significantly different between the control and drought plots for *Schima superba* and *Pinus massoniana* (all $P > 0.05$, Figure S1a–b, e–f). For *Schima superba*, a significant increasing trend in J_{max}/V_{cmax} ($t = -2.229$, $P = 0.036$) was observed from the control to the drought plots, whereas no significant effect of drought on J_{max}/V_{cmax} was observed

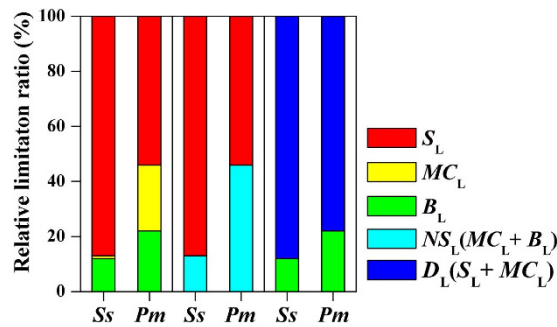


Figure 3. The quantitative limitation of A_n (net CO_2 assimilation rate, $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) for *Schima superba* (Ss) and *Pinus massoniana* (Pm) during drought stress. The stomatal limitation (S_L , red area), mesophyll conductance limitation (MC_L , yellow area) and biochemical limitation (B_L , green area) are shown. The total diffusional limitation ($D_L = S_L + MC_L$, blue area) and the non-stomatal limitation ($NS_L = MC_L + B_L$, sky blue area) are also indicated.

for *Pinus massoniana* ($P > 0.05$). No significant differences in drought resistance were observed for V_{cmax} , J_{max} , TPU and $J_{\text{max}}/V_{\text{cmax}}$ between *Schima superba* and *Pinus massoniana* (Figure S11–l).

Quantitative limitation analysis. For *Schima superba*, the values of S_L , MC_L and B_L accounted for 87%, 1% and 12% of the limitations, respectively (Fig. 3). The contributions of the stomatal (S_L) and non-stomatal limitations ($NS_L = MC_L + B_L$) represented approximately seven-eighths and one-eighth of the total limitation, respectively. The role of the total diffusional limitation ($D_L = S_L + MC_L$) was more important than that of the biochemical (B_L) limitation.

For *Pinus massoniana*, the S_L , MC_L and B_L values were equal to 54%, 24%, and 22% of the limitations, respectively. The stomatal (S_L) and non-stomatal limitations (NS_L) generally showed a similar magnitude. The contributions of the total diffusional limitation (D_L) and biochemical limitation (B_L) represented approximately two-thirds and one-third of the total limitation, respectively.

Resource use efficiency. A general increasing trend in iWUE from the control to the drought plots was observed for *Schima superba* ($P = 0.056$), whereas significant declines in the iWUE due to the drought treatments ($P = 0.021$) were observed for *Pinus massoniana* (Fig. 4a,d). The effects of drought on the NUE of *Schima superba* and *Pinus massoniana* exhibited significant reductions from the control to drought plots (Fig. 4b,e). No significant differences were observed in the LUE for *Schima superba* and *Pinus massoniana* during drought treatments (all $P > 0.05$, Fig. 4c,f). The drought resistance of the iWUE exhibited significant differences between species, whereas no significant differences in drought resistance related to the LUE and NUE were observed between species (Fig. 4g–i).

To determine the trade-off in resource use efficiency, simple linear regressions of LUE vs. NUE and NUE vs. iWUE were performed (Fig. 5). A significant positive correlation was found between the LUE and NUE in the control plots ($y = 7.29x - 0.06$, $R^2 = 0.57$, $P < 0.0001$). However, poor correlations between the LUE and NUE were found for all species in the drought plots ($R^2 = 0.06$, $P = 0.237$). As a result, the regression slopes of the LUE and NUE were different between the control and drought treatments ($P = 0.034$). A marginally negative relationship between the NUE and iWUE was observed for all species in the control plots ($y = -0.06x + 0.09$, $R^2 = 0.12$, $P = 0.092$), whereas the correlations between the NUE and iWUE were weak ($R^2 = 0.02$, $P = 0.481$) in the drought plots. However, no significant difference was observed in the regression slopes of the NUE and iWUE for the control and drought treatments ($P = 0.179$).

Discussion

Quantitative limitation analysis of photosynthesis in response to drought. As expected, drought stress significantly decreased the leaf photosynthesis of the dominate species (*Schima superba* and *Pinus massoniana*) in subtropical forests. Our study reported that an approximately 37% decrease in the A_n in the drought plots was related to a decrease of approximately 38% in the SWC. The pattern of decreasing A_n with drought was similar to the pattern observed in forests under field conditions^{24,32,33}. However, the degree to which drought affected the A_n did not significantly vary between *Schima superba* and *Pinus massoniana* (Fig. 1i). Two independent experiments on mesic and xeric species from diverse hydroclimates in Australia and Europe indicated interspecific differences in the drought response²³. The velocity of the photosynthetic changes in response to water stress imposition were faster in evergreen forests than in semi-deciduous forests, although the declines in photosynthetic rate were similar in magnitude³⁴. Although water stress is known to reduce the photosynthetic rate, the processes responsible for the key limitations are still a matter of debate³⁵. Previous studies have demonstrated that the photosynthetic reaction to water stress is dominated by only one of the three physiological processes (stomatal conductance, mesophyll conductance and biochemical processes)^{36–39}. Increasing evidence had shown that the combined effect of the stomatal, mesophyll, and biochemical limitations simultaneously regulates the decline in photosynthesis in response to drought. Thus, a quantitative limitation analysis of the changes in the photosynthetic rate in response to water stress was necessary^{12,40,41}. Our quantitative limitation analysis demonstrated that the drought-induced limitation of photosynthesis in *Schima superba* was mainly due to the stomatal limitation (87%), whereas the stomatal (S_L ,

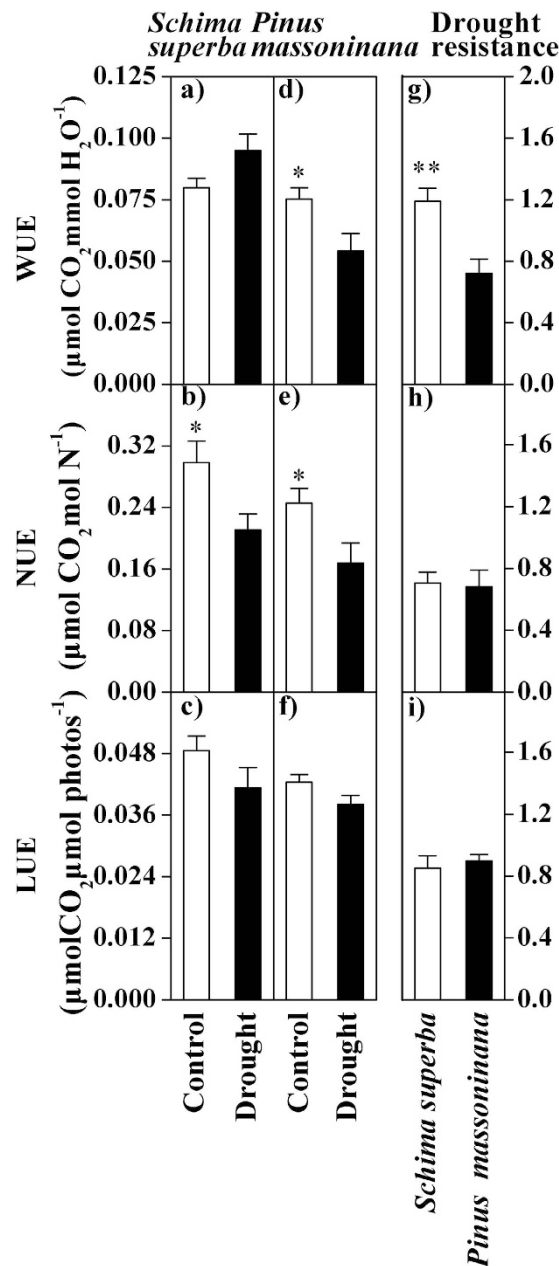


Figure 4. The effect of drought on the resource use efficiency for the two species. (a,d) The iWUE (the water use efficiency, $\mu\text{mol CO}_2 \text{ mmol H}_2\text{O}^{-1}$), (b,e) NUE (the nitrogen use efficiency, $\mu\text{mol CO}_2 \text{ mol N}^{-1}$) and (c,f) LUE (the light use efficiency, $\mu\text{mol CO}_2 \mu\text{mol photons}^{-1}$) in the control and drought plots of *Schima superba* (a–c) and *Pinus massoniana* (d–f) are shown. The drought resistance of the (g) iWUE, (h) NUE, and (i) LUE in *Schima superba* and *Pinus massoniana* is indicated. ANOVA: * $P < 0.05$; ** $P < 0.01$; and *** $P < 0.001$.

54%) and non-stomatal limitations (NS_L , 46%) for *Pinus massoniana* generally showed similar magnitudes. A study of temperate deciduous forests reported a maximum value of 50% for the S_L during drought stress³⁹. A study of tropical evergreen forests (*Campsiandra laurifolia*, *Symmeria paniculata*, *Acosmium nitens* and *Eschweilera tenuifolia*) indicated that the S_L accounted for 30–39% of the limitations in the dry season (March 2004)⁴². Therefore, the magnitude of the photosynthetic reaction to water stress for the three types of limitations varied between species.

Mesophyll conductance is typically absent in gas exchange measurements, which are assumed to be infinite⁴³. However, studies have demonstrated that changes in mesophyll conductance in response to stress and limit photosynthesis are an important physiological process^{12,44}. For a variety of climate zones and species, the MC_L is responsible for approximately 14–30% of the limitations^{12,22–24}. Similarly, Grassi and Magnani (2005) found a maximum value of 14% for the MC_L for an ash forest under seasonal drought¹². Another rainfall exclusion experiment in a *Quercus ilex* forest showed a maximum value of 20% for the MC_L ²². Our quantitative limitation analysis showed that the MC_L was responsible for only 1% of the total limitation of *Schima superba*, although this limitation accounted

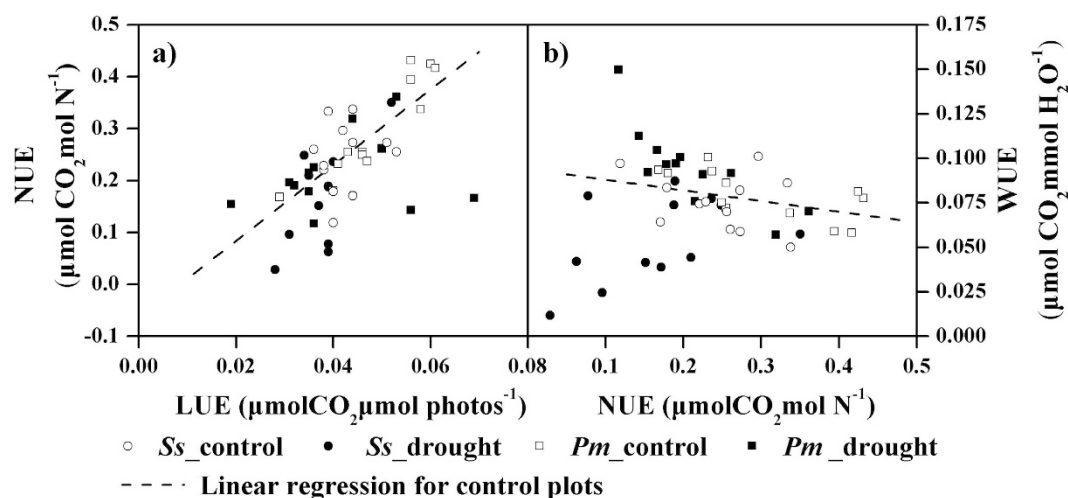


Figure 5. The relationships between the LUE (light use efficiency, $\mu\text{mol CO}_2 \mu\text{mol Photons}^{-1}$) and NUE (nitrogen use efficiency, $\mu\text{mol CO}_2 \text{ mol N}^{-1}$) (a) or the NUE and iWUE (water use efficiency, $\mu\text{mol CO}_2 \text{ mmol H}_2\text{O}^{-1}$) (b) in the control and drought plots. Ss and Pm represent *Schima superba* and *Pinus massoniana*, respectively. The dotted line indicates the linear regression for the control plots.

for 24% of the total limitation for *Pinus massoniana*. Therefore, it is important to include mesophyll conductance into any detailed study of the gas exchange response to drought and the processed model.

An explanation for the discrepancies in the MC_L between both species in the subtropical region may be related to the different phylogenetic clades (gymnosperms vs. angiosperms)⁴⁵. The SLA was reported to strongly separate gymnosperms from angiosperms based on 305 North American woody species that spanned boreal to subtropical climates⁴⁶. The g_m can be influenced by changes in leaf anatomical characteristics, such as the thickness of leaf/mesophyll cell walls/chloroplasts and the stomata density^{47–49}. Gymnosperms have a lower SLA value⁵⁰, lower mesophyll porosity, thicker mesophyll cell wall⁵¹, and lower g_m ^{51,52} than angiosperms. In our study, the gymnosperm species (*Pinus massoniana*) had lower SLA and g_m values than angiosperms, which might contribute to the high MC_L in evergreen conifers.

The balance between $J_{\max}$ and $V_{\max}$. The $J_{\max}$ and $V_{\max}$ relationship represents the resource allocation between the two photosynthetic cycles: electron transport and the Calvin cycle⁵³. In the biochemically-based photosynthesis model, $V_{\max}$ was scaled to $J_{\max}$ based on the hypothesis that the average $J_{\max}/V_{\max}$ ratio was 2.1⁵⁴. However, some studies have demonstrated that the $J_{\max}$ to $V_{\max}$ ratio is not constant but varies with temperature⁵⁵, leaf nitrogen⁵⁶, and species⁵⁷. The underlying processes responsible for the changes in the $J_{\max}/V_{\max}$ ratio due to water stress are still a matter of debate. We found that the $J_{\max}/V_{\max}$ ratio varied considerably among drought treatments in *Schima superba* (Fig. S1d), which was consistent with previous studies^{24,58}. The hypothesis that droughts modify the balance between RuBP carboxylation and regeneration was supported by our study (i.e., the resource allocation between the two photosynthetic cycles (the Calvin cycle and electron transport) was changed). Current ecosystem models are less capable of accounting for climate extremes (warming, nitrogen deposition or drought), which attributes to model calibration using data collected under standard climate conditions⁵⁹. The findings from manipulation experiments could reduce the uncertainties of model parameter estimates and the predictions made by the models.

The trade-off between resource use efficiencies (iWUE, NUE and LUE). In the present study, we found a significant positive correlation between the LUE and NUE for all species in the control plots (Fig. 5a). Generally, the plants tended to obtain the maximum NUE with an increase in the LUE⁶⁰. However, a poor correlation was observed between the LUE and NUE in the drought plots, indicating that drought stress might have weakened the relationship between the LUE and NUE in these plots (Fig. 5a). The slopes of the regression lines (LUE vs. NUE) from the control and drought plots were significantly different ($P = 0.034$). Furthermore, the NUE but not the LUE was significantly decreased in the drought treatments for each species (Fig. 4b,c,e,f). These results suggested that the responses of the LUE and NUE to drought were uncoupled. The use of resources (light and nitrogen) for plants may also be influenced by other factors, such as water stress.

Additionally, a marginally negative correlation was observed between the NUE and iWUE in the control plots for the two species studied here, which provided evidence for the existence of a trade-off between the uses of resources. An increase in the NUE together with a decrease in the iWUE indicated that compromises existed between the iWUE and NUE. This finding was consistent with previous studies of a large number of plants species^{61–63}. An increase in the efficiency of the use of one resource can lead to a reduction in the efficiency of use of another resource⁶⁰, suggesting that maximization of resource use efficiency depends on the most limited resources³³. This trade-off may help enforce the species distribution across moisture and nutrient gradients⁶².



Figure 6. Rainfall exclusion experiments at the QYZ station: (a) Control plot and (b) Drought plot. Photo credit: Lei Zhou.

Methods

Experimental site. The experimental site was located in the Qianyanzhou (QYZ) Forest Experimental Station (26°44'N, 115°03'E) in Jiangxi Province of South China and belongs to Chinaflux. The mean annual air temperature is approximately 17.9 °C, and the highest and lowest daily temperatures recorded are 39.5 and −5.8 °C, respectively⁶⁴. The annual average precipitation is 1,489 mm and mainly occurs from March to June (52% of total). The annual air temperature and annual precipitation in 2012 were 18.5 °C and 1741 mm, respectively. These characteristics are typical of the prevailing subtropical monsoon climate. The soil mainly consists of the red soil type formed principally from red sandstone, sand gravel or mudstone and river alluvial deposits. The main forest types include Masson's pine (*Pinus massoniana*), slash pine (*Pinus elliottii*), Chinese fir (*Cunninghamia lanceolata*), and Schima (*Schima superba*). *Pinus massoniana* and *Schima superba* are the pioneer species and the dominant species in this region, respectively. Both species were mixed as conifer-broadleaf forests, with a tree density of stems of 700 ha^{−1}.

Rainfall exclusion experiment. The rainfall exclusion experiment was initiated in January 2010. Rainfall was withheld for the entire year with no changes in other meteorological variables (Fig. 6). We used transparent polyester film placed at a height of 2 m on the trees to partially exclude throughfall drops onto the floor and allow light penetration. Trenches were dug around the perimeters of the plots to reduce the lateral inflow of water from the surrounding forest into the plots. Three rainfall exclusion plots (12 m × 12 m) were used. Three control plots were established in the same environment. An average of three *Schima superba* and five *Pinus massoniana* were growing in each plot. The height and diameter at breast height for each species in the control and drought plots are listed in Table S1. The plots were established in the W middle of the forests, and we attempted to select trees in the middle of the plots.

CO₂ response curve. We measured the foliar gas exchange of two species (*Schima superba* and *Pinus massoniana*) using a portable photosynthesis system (LI-6400, LI-COR Inc., USA) during the growing season (early September) of 2012 (Figure S2). Measurements were performed with two portable photosynthesis systems on clear sunny days between 8:00 a.m. to 2:00 p.m. Twelve individuals (4 individuals per plot) for each species were measured in each treatment (control and drought plots). All measurements were performed on fully expanded leaves with no signs of senescence or immaturity. Branches with sun-facing leaves were excised from the middle of the crown with the help of a lopper (3 m) affixed to a bamboo shoot (approximately 10 m) (Figure S3) and then immediately stored in a bottle with fresh water. The gas exchange measurements were conducted soon after branch excision. The time period from branch excision to the completion of the measurement was typically less than 30 min. Typical A_n/C_i curves (A_n versus the calculated intercellular CO₂ concentrations, C_i) were measured at the ambient CO₂ concentration (C_a) (ranging from 50 to 1400 μmol mol^{−1}). The C_a was lowered stepwise from 400 to 50 μmol mol^{−1} and then increased again from 50 to 1400 μmol mol^{−1}, with a total of 10 points. Photosynthesis was induced for 10 min at the saturating photosynthetically active photon flux density (PPFD) (1500 μmol photons m^{−2} s^{−1}) at a given leaf temperature (25 °C). The CO₂ concentrations in the cuvette were controlled using an injector system (LI-6400-01, LI-COR Inc.) that used a CO₂ mixer and compressed CO₂ cartridges. The PPFD was provided by the red/blue LED light source built into the foliar cuvette (LI-6400-02B, LI-COR Inc.) and was calibrated against an internal photodiode. The average value of the air temperature on the measurement days was 24.1 °C. The leaf temperature in the cuvette, which was controlled by the thermoelectric cooling elements of the Li-6400, was 25 °C. The cuvette was sealed with plasticine to prevent leakage. We placed twelve needles of *Pinus massoniana* side by side into a 2 × 3 cm sealed cuvette. The cuvette was sealed with plasticine to prevent leakage^{65,66}.

A_n/C_i curves were fitted to estimate the V_{cmax} , J_{max} , TPU and g_m using spreadsheet-based software by minimizing the root mean square error (RMSE) of each curve⁶⁷. The g_s (mmol H₂O m^{−2} s^{−1}) was initially measured with the Li-6400. Due to the differences in diffusion coefficients between water vapor and CO₂, the stomatal conductance to H₂O was 1.6-fold higher than the stomatal conductance to CO₂⁶⁸. To achieve consistency with the g_m , we converted the $g_{s,w}$ to $g_{s,c}$. The A_n measured at the 400 μmol mol^{−1} CO₂ concentration and 25 °C leaf temperature from each A_n/C_i curve was used to track the differences between treatments for each species. The total conductance (g_{tot}) was calculated from the sum of the g_s and g_m .

Light response curve. The light response curves of *Schima superba* and *Pinus massoniana* were measured using the LI-6400 after each A_n/C_i curve measurement. The PPFD was sequentially lowered from 1800 to 0 $\mu\text{mol m}^{-2} \text{s}^{-1}$, with a total of 14 points. During each measurement, the CO_2 concentration was maintained at 400 $\mu\text{mol mol}^{-1}$, and the leaf temperature was maintained at 25 °C.

A non-rectangular hyperbola model⁶⁹ solved for its negative root was used to describe the light response curves. In our study, the leaf maximum apparent quantum yield of CO_2 uptake (AQY , $\mu\text{mol CO}_2 \text{ m}^{-2} \text{s}^{-1}$) and R_d ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{s}^{-1}$) were derived from the light response curve. The gross CO_2 assimilation (A_g) was calculated by adding R_d to A_n .

Soil water content and leaf chemical characteristics. The gravimetric soil water content (SWC) was measured at the depth of 0–20 cm in the field. Soil samples from each plot were placed into aluminum boxes and then dried in an oven at 106 °C for 24 h. The SWC in this study was expressed as follows:

$$\text{SWC} (\text{gg}^{-1}) = (W_1 - W_2) / W_2 \quad (1)$$

where W_1 is the sample fresh weight, and W_2 is the sample dry weight. Finally, eight soil moisture contents were averaged to represent the water content of each plot.

The foliage covered in the cuvette during the gas exchange measurements was used to measure the leaf C and N concentrations (C_{area} and N_{area}) with respect to area. Foliage was removed from branches after the gas exchange measurements, and then the area was measured with an area meter (LI-3100, Li-Cor Inc.). Foliage samples were dried at 65 °C for 48 h, and the leaf characteristics were measured with a CN analyzer. The leaf C/N ratio with respect to area was calculated using the leaf C and N concentrations. The specific leaf area (SLA) was calculated based on the measurements of the leaf area and dry mass.

Data analyses. The relative limitation to assimilation imposed by the stomatal conductance (S_L), mesophyll conductance (MC_L) and biochemical processes (B_L) were separated using the approach¹² proposed by Grassi and Magnani (2005). This approach makes it possible to compare relative limitations to assimilation, which partitions photosynthetic limitations into components related to stomatal conductance, mesophyll conductance, and leaf biochemical characteristics. The non-stomatal limitation (NS_L) was defined as the sum of the contribution due to the mesophyll conductance and leaf biochemistry ($NS_L = MC_L + B_L$). The total diffuse limitation (D_L) was the sum of the stomatal and mesophyll conductance components ($D_L = S_L + MC_L$).

The leaf-level intrinsic WUE (iWUE, $\mu\text{mol CO}_2 \text{ mmol H}_2\text{O}^{-1}$) was expressed as the ratio of the net CO_2 assimilation rate versus the stomatal conductance. The NUE ($\mu\text{mol CO}_2 \text{ mol N}^{-1}$) for leaf photosynthesis was defined as the ratio of the net photosynthesis rate to the leaf nitrogen content. The AQY derived from the light response curve was the proxy for the leaf-level LUE ($\mu\text{mol CO}_2 \mu\text{mol Photons}^{-1}$)⁷⁰ in this study.

The drought resistance in this study was expressed as the ratio of the variables in drought plots to those in control plots^{71,72} (i.e., variable_drought/variable_control). Values closer to 1 imply greater drought resistance.

We performed independent sample T-tests with a 95% confidence level to examine the differences in each variable among treatments. Previously, the homogeneity of variables was evaluated with Levene's test ($P < 0.05$). If the homogeneity test failed, the variables were log-transformed or sin-transformed prior to analysis. Regression models were used to determine the relationship between different resource use efficiencies. The general linear model (GLM) was used to test the significance of the slopes of the linear regression among resource use efficiencies. All statistical analyses were performed using SPSS Version 14.0 (SPSS Inc. Chicago, IL, USA).

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Author Contributions

L.Z., Y.G.C. and Q.K.L. conceived and designed the experiment. L.Z., S.Q.W. and Y.G.C. analyzed the data and wrote the paper. L.Z., Y.G.C., K.H. and Q.Z.Y. performed the field investigation and sample analyses and contributed to the draft of the manuscript.

Additional Information

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