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Warming effects on photosynthesis of subtropical tree species: a translocation experiment along an altitudinal gradient

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Ongoing climate warming induced by human activities may have great impacts on trees, yet it remains unresolved how subtropical tree species respond to rising temperature in the field. Here, we used downward translocation to investigate the effects of climate warming on leaf photosynthesis of six common tree species in subtropical China. During the experimental period between 2012 and 2014, the mean average photosynthetic rates (A_{sat}) under saturating light for *Schima superba*, *Machilus breviflora*, *Pinus massoniana* and *Ardisia lindleyana* in the warm site were 7%, 19%, 20% and 29% higher than those in the control site. In contrast, seasonal A_{sat} for *Castanopsis hystrix* in the warm site were lower compared to the control site. Changes in A_{sat} in response to translocation were mainly associated with those in leaf stomatal conductance (g_s) and photosynthetic capacity (RuBP carboxylation, RuBP regeneration capacity). Our results imply that climate warming could have potential impacts on species composition and community structure in subtropical forests.

Tropical and subtropical forests can provide crucial ecosystem services to natural systems and human kind (i.e., carbon sequestration^{1,2}, biodiversity conservation, climatic regulation). As a result of climate change, global mean temperatures are projected to increase 1–5 °C in the next 50–100 years³. Such warming may have deleterious consequences on subtropical and tropical forests since many tree species occur near the thermal optimum⁴. Over the last decade, field observational studies across subtropical and tropical forests have documented changes in forest structure and tree mortality which have been attributed to climate warming and severe droughts^{5–8}. However, the physiological mechanisms that govern such shifts are not well understood. It is well established that one of the primary physiological targets of warming is photosynthesis^{9,10} which acts as the fundamental basis for carbon accumulation, growth and biomass production of plants. Therefore, an improved understanding of the photosynthetic mechanisms underpinning plant response to climate warming in tropical and subtropical species may provide insights into shifts in forest community composition under future climates.

Photosynthesis is strongly affected by temperature. Photosynthesis usually increases as temperature rises when below the thermal optimum, while it may decline once the temperature exceeds the thermal optimum¹¹. The effects of temperature on photosynthesis can be divided into two primary driving mechanisms: stomatal effects (indirect) and biochemical effects (direct)¹². The increased leaf-to-air vapour pressure (VPD) associated with rising temperature has long been recognized as a major factor determining stomatal responses, thereby affecting plant carbon assimilation under warming^{12,13}. Additionally, biochemical processes are also temperature-dependent. Responses of biochemical processes in the mesophyll are characterized by the modulation of the rates of activity of photosynthetic enzymes and the electron transport chain¹⁴. The maximum rate of Rubisco (the main carboxylating enzyme of photosynthesis) carboxylation (V_{cmax}) and the maximum rate of photosynthetic electron transport (J_{max})⁹, are associated with the responses of photosynthesis^{15,16}.

Over recent decades, results from photosynthesis research among warming experiments are inconclusive: elevated temperature has been found to increase^{17–19}, decrease^{20–23} or no impacts^{24–27} on A_{sat} . While the

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photosynthetic responses of temperate plant species under climate warming are comparatively well studied^{17,28}, the responses of tropical tree species remain unexplored. Previous studies have found that species exposed to greater seasonal variation in temperature show greater in temperate optima. However, Cunningham and Read *et al.*²⁹ compared four temperate and four tropical species, and observed greater capacities for tropical species to acclimate higher temperature compared to temperate species. These inconsistent results require better understandings in to what degree tropical and subtropical species respond to increasing temperature^{12,30}.

Inferring how climate change affects plants has been typically relied on one of three approaches: experiments with warming facilities, historical comparisons, and space-for-time substitutions derived from sampling along environmental gradients³¹. Researchers who have investigated temperature effects have often studied seedlings in growth plots kept at constant temperatures^{32,33} and less is known about thermal responses in natural settings with temperature variability. Manipulative studies in the field remain a logistical challenge for global change scientists^{34,35}. To measure species across a wide range of temperatures in natural systems, field studies have made use of latitudinal and altitudinal gradients, as well as seasonal changes in temperature^{36,37}. While past research using growth chambers and temperature gradients has detected acclimation across wide temperature ranges, it is uncertain whether acclimation will also occur when plants are subjected to smaller temperature increases (1–2 °C).

Here, we conducted a transplant experiment in two sites along an altitudinal gradient. Using this approach, we assessed the effects of temperature on photosynthetic performance of co-existing species in subtropical China. We selected 6 subtropical tree species that are from different functional types (i.e., tree vs shrub, broadleaved vs coniferous tree) to determine photosynthetic responses to rising temperature. Our specific objectives are to address the following questions: 1) how does climate warming affect photosynthesis of different plant species in subtropical China? 2) What biochemical and stomatal mechanisms are involved in the changes in photosynthesis?

Results

Environmental variables. The monthly air and soil temperatures at the warm site were distinctly higher than those at the control site. Mean soil temperature was 0.93 °C higher in the warm site than in the control site (Fig. 1a, $p < 0.01$). Soil moisture and vapor pressure deficit (VPD) were significantly lower in the warm site than in the control site (Fig. 1a,c, $p < 0.01$). During the experimental period, mean of average, maximum and minimum monthly air temperature were 0.90 °C, 0.95 °C, and 1.29 °C higher in the warm site than those in the control site, respectively (Fig. 1b, $p < 0.01$).

Responses of A_{sat} . Over the entire experimental period, A_{sat} of the six tree species showed similar seasonal patterns (Fig. 2), with high A_{sat} occurred during the summer and low A_{sat} occurred during the winter. Downward translocation significantly increased annual mean average A_{sat} of *S. superba* (+7%), *P. massoniana* (+20%), *M. breviflora* (+19%), *A. lindleyana* (+29%) ($p < 0.05$ for all) (Tables 1 and 2); however, it decreased that of *C. hystrix* (–10%) ($p < 0.05$, Tables 1 and 2) and had no significant effect on *S. rehderianum*. The impact of measuring date on A_{sat} of all species was statistically significant ($p < 0.05$, Table 1), with greater A_{sat} in the warm-wet seasons and lower rates in the cool-dry ones (Table 2). Translocation treatment significantly increased mean A_{sat} for *S. superba* and *S. rehderianum* in wet-warm season (12% and 36%, respectively) but decreased it in cold-dry season (3% and 4%, respectively, not significant). By contrast, the magnitude of effects in A_{sat} for *P. massoniana* and *C. hystrix* was greater in cool-dry season (29% and –13%, respectively) compared to warm-wet season (8% and –8%, respectively) (Table 2).

The RE of A_{sat} was positively correlated with the increases in leaf temperature for *S. superba* (Fig. 3a, $R^2 = 0.44$), *M. breviflora* (Fig. 3c, $R^2 = 0.50$), *P. massoniana* (Fig. 3d, $R^2 = 0.49$), but negatively correlated with that for *C. hystrix* (Fig. 3b, $R^2 = 0.39$) and *A. lindleyana* (Fig. 3f, $R^2 = 0.65$). A_{sat} were negatively correlated with VpdL in wet season for all species except for *C. hystrix* (Fig. 4, $p < 0.01$ for all). The correlations between A_{sat} and VpdL in dry season were significant for *C. hystrix*, *P. massoniana*, and *A. lindleyana*. (Fig. 4, $p < 0.05$)

Responses of g_s . Consistent with A_{sat} , leaf g_s of the six species showed similar seasonal patterns (Fig. 5). Translocation significantly increased g_s of *S. rehderianum*, *P. massoniana*, and *A. lindleyana* ($p < 0.01$ for all) (Tables 1 and 2). For all tree species, there were significant relationship of A_{sat} with g_s in our study (Fig. 6, $p < 0.01$ for all). g_s were negatively correlated with VpdL in wet season for all species except for *C. hystrix* (Fig. 7, $p < 0.01$ for all). The correlations between g_s and VpdL in dry season were significantly for *C. hystrix*, *S. rehderianum*, *P. massoniana*, and *A. lindleyana*. (Fig. 7, $p < 0.05$)

Responses of V_{cmax} , J_{max} and TPU. The six species showed different responses of photosynthetic capacity to translocation (Fig. 8). Downward translocation significantly increased V_{cmax} , J_{max} and TPU of *S. superba*, *P. massoniana* and *M. breviflora* ($p < 0.05$); however, it significantly decreased those of *A. lindleyana* ($p < 0.05$) and had no significant effect on *C. hystrix* and *S. rehderianum* ($p > 0.05$).

Discussion

Our experimental set-up simulated climate warming by translocation of plant-soils to lower elevations. In our experiment, soil temperature and soil moisture at the lower elevation were significantly different from those in control site, resulting in a 0.90 °C and 0.93 °C increase in mean air and soil temperature, respectively (Fig. 1). Natural geographical gradients provide an excellent natural laboratory to investigate the potential effects of climate warming on terrestrial organisms³⁸. However, different plant ecotypes formed in their own habitats increase the difficulty of comparison and analysis. To overcome these problems accompanied by geographical gradients, we conduct transplant experiments to allow comparisons within the same ecotype. We are rarely aware of this combination application to studies specifically regarding forests, although this approach has been conducted in grass or shrub^{39,40}. Therefore, altitudinal gradients are still an underutilized resource in this respect. Although co-occurring changes in other environmental

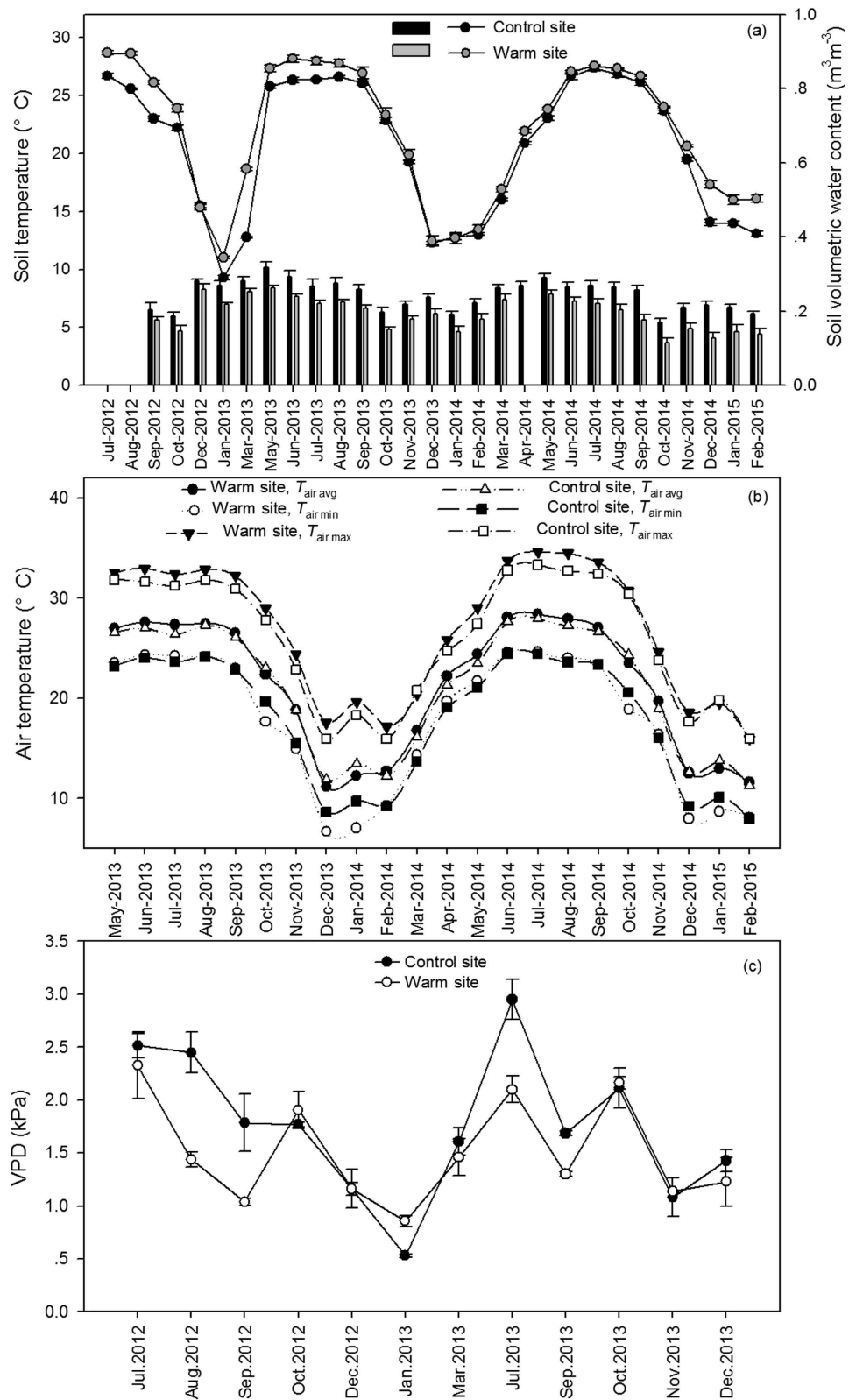


Figure 1. Monthly mean soil temperature (lines) and volumetric soil water content (bars) (a) monthly maximum, minimum and mean air temperature (b) monthly leaf-to-air vapor pressure deficit (c) VPD at the warm and control site.

factors might counteract the effect of elevated temperature on photosynthesis, moving on from these geographical effects to clearly inferring a temperature signal appears as an important step.

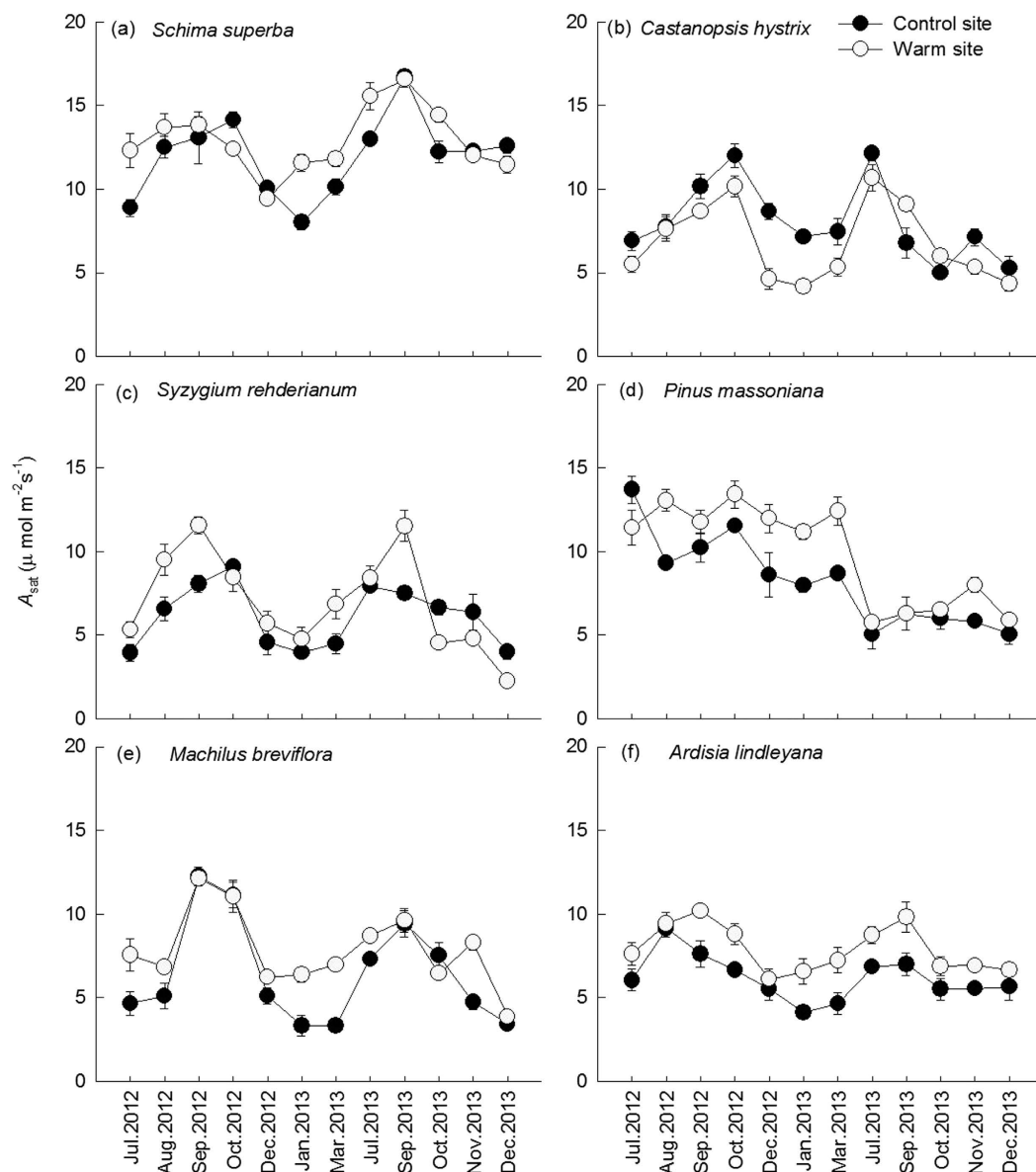


Figure 2. Seasonal dynamics of photosynthetic rate (A_{sat}) in *Schima superba* (a), *Castanopsis hystrix* (b), *Syzygium rehderianum* (c), *Pinus massoniana* (d), *Machilus breviflora* (e) and *Ardisia lindleyana* (f) grown in the warm and control site. Error bars are standard error (n = 3).

Effect	<i>Schima superba</i>	<i>Castanopsis hystrix</i>	<i>Syzygium rehderianum</i>	<i>Pinus massoniana</i>	<i>Machilus breviflora</i>	<i>Ardisia lindleyana</i>
A_{sat}						
Translocation	8.241**	3.224	2.655	42.091***	4.338*	22.103***
Time	15.554**	7.132***	9.749***	27.172***	5.807***	7.742***
Translocation*Time	3.299**	1.094	3.324**	10.790***	1.552	1.325
g_s						
Translocation	3.694	0.636	7.956**	10.731**	2.539	24.337***
Time	17.314***	9.112***	18.092***	56.615***	19.352***	11.784***
Translocation*Time	2.471*	2.785**	4.848***	7.617***	2.368*	3.655***

Table 1. Repeated-measures ANOVA of translocation, measuring time and their interactions on net photosynthetic rate (A_{sat}) and stomatal conductance (g_s). *Statistically significant at $p < 0.05$. **Statistically significant at $p < 0.01$. ***Statistically significant at $p < 0.001$.

Species	Site	A_{sat} ($\mu\text{mol m}^{-2}\text{s}^{-1}$)			g_s ($\text{mol m}^{-2}\text{s}^{-1}$)		
		Year	Wet season	Dry season	Year	Wet season	Dry season
<i>Schima superba</i>	Control site	12.34 ± 3.11	12.84 ± 1.24	12.31 ± 1.18	0.211 ± 0.005	0.254 ± 0.021	0.182 ± 0.003
	Warm site	13.26 ± 2.62	14.39 ± 0.75	11.90 ± 0.49	0.232 ± 0.006	0.315 ± 0.022	0.193 ± 0.004
<i>Castanopsis hystrix</i>	Control site	7.75 ± 0.73	8.75 ± 1.04	7.03 ± 0.98	0.163 ± 0.004	0.219 ± 0.008	0.120 ± 0.010
	Warm site	7.00 ± 0.62	8.68 ± 0.62	5.79 ± 0.67	0.171 ± 0.005	0.285 ± 0.010	0.104 ± 0.012
<i>Syzygium rehderianum</i>	Control site	6.10 ± 0.52	6.65 ± 0.82	5.58 ± 0.71	0.097 ± 0.013	0.130 ± 0.017	0.075 ± 0.007
	Warm site	6.78 ± 0.81	9.13 ± 0.87	5.10 ± 0.60	0.133 ± 0.013	0.224 ± 0.014	0.066 ± 0.008
<i>Pinus massoniana</i>	Control site	8.13 ± 0.80	8.92 ± 1.53	7.67 ± 0.84	0.201 ± 0.008	0.297 ± 0.021	0.133 ± 0.005
	Warm site	9.69 ± 0.87	8.97 ± 1.47	9.93 ± 1.14	0.248 ± 0.010	0.362 ± 0.025	0.167 ± 0.006
<i>Machilus breviflora</i>	Control site	6.46 ± 0.88	8.20 ± 1.45	5.22 ± 0.91	0.124 ± 0.004	0.195 ± 0.015	0.074 ± 0.008
	Warm site	7.23 ± 0.65	8.10 ± 1.15	6.61 ± 0.75	0.135 ± 0.004	0.209 ± 0.012	0.081 ± 0.008
<i>Ardisia lindleyana</i>	Control site	6.10 ± 0.42	7.12 ± 0.60	5.37 ± 0.41	0.113 ± 0.007	0.153 ± 0.011	0.084 ± 0.006
	Warm site	7.77 ± 0.62	9.60 ± 0.56	6.45 ± 0.62	0.182 ± 0.008	0.289 ± 0.014	0.105 ± 0.007

Table 2. Summary of net photosynthetic rate (A_{sat}) and stomatal conductance (g_s) at saturating light for species growth in the warm and control site. Data are means ± SE.

The overall objective of this study was to improve the limited understanding of temperature responses of photosynthesis in subtropical tree species. The effects of warming on tree or seedling photosynthesis have been previously studied, but the results have shown varying patterns^{17,21,25}. Furthermore, whether or not tropical and subtropical species are operating near thermal thresholds remained unclear. For example, Vårhammar *et al.*²¹ found negative effects of elevated temperature on tropical African trees. Several leaf and canopy measurements confirmed that gas exchange decreased with increased temperature⁴¹. Whereas to date, few temperature response assessments have focused on subtropical trees. In our study, we found significant greater A_{sat} in *S. superba*, *P. massoniana*, *M. breviflora* and *A. lindleyana* grown in warm site (Tables 1 and 2), with positive relationships between photosynthesis and changes in leaf temperature (Fig. 6). This finding was rejected to the hypothesis that subtropical tree species may be near a high temperature threshold^{42,43}. In contrast to those species, *C. hystrix* was found to have lower A_{sat} grown in warm site (Tables 1 and 2), indicating that the photosynthesis of these subtropical tree species have species-specific responses to rising temperatures. It is also consistent with findings of previous studies on temperate and boreal species^{44–47}. Seasonal responses of A_{sat} to warming for the two species (*S. superba* and *S. rehderianum*) were similar, but were different from other two species (*P. massoniana* and *C. hystrix*).

The response of photosynthesis under elevated temperature was usually associated with mesophyll (direct) effects or stomatal (indirect) effects¹². According to the models of Farquhar *et al.*⁹, the balance between V_{cmax} and J_{max} of ribulose-1,5-bisphosphate (RuBP) determines the temperature dependence of photosynthesis⁴⁸. In our study, *S. superba*, *P. massoniana* and *M. breviflora* which had higher A_{sat} at warm site also experienced increases in J_{max} and V_{cmax} (Fig. 8), indicating greater photosynthetic electron transport and Rubisco carboxylation⁹. This finding conforms to the models of Farquhar *et al.*⁹, and it is also consistent with previous experimental evidences^{49,50}. Leading models propose that the inhibition of A_{sat} at elevated temperature is a function of either declining capacity of RuBP activation, or Rubisco activase to maintain Rubisco in an active configuration^{51,52}. However, in our study, A_{sat} was decreased for *C. hystrix* in the warm site, yet the decreases of photosynthetic capacity were not statistically significant. For *A. lindleyana*, we found an enhancement in A_{sat} but a decrease in photosynthetic capacity (Fig. 8). This differs from previous studies in that V_{cmax} or J_{max} changed, inferring a shift in resource investment away from Rubisco. Bernacchi *et al.*⁵³ also found increased A_{sat} even though there was significant decrease in V_{cmax} . This may result from changes in mesophyll conductance (g_m), the transfer conductance of CO_2 from the intercellular air-space to the site of carboxylation within the chloroplast, has been shown to be a significant limitation to photosynthesis⁵⁴. In addition, *C. hystrix* had lower optimum temperatures for photosynthesis compared with warm-adapted species.

Stomatal regulation of the internal CO_2 concentration is well known for determining the temperature response of photosynthesis^{55–57}. In our experiment, enhancement or reduction of A_{sat} due to downward translocation were largely associated with the changes in g_s in all six species (Fig. 4), proposing g_s as a screening tool for gas exchange efficiency^{44,58}. The leaf to air vapour pressure difference (VPD) is the directly driving force of g_s ⁵⁹. Temperature and VPD are often merged together as a combined effect because the strong correlation between the two parameters^{60,61}. g_s is negatively correlated with VPD, which is supported by many previous studies^{62,63} as well as our experiment. VPD depends on the magnitude of changes in air temperature and humidity. VPD often increases as temperature rises if relative humidity remains constant or decreases^{60,61}. However, if relative humidity increases to a greater extent than temperature, VPD can exhibit declines. In our study, VPD was found lower in the warm site when compared to the control site (Fig. 1c), mainly due to the larger air relative humidity in the warm site. Therefore, lower VPD at the warm site can contribute to the increases in g_s and A_{sat} .

The inter-specific variation in responses of photosynthesis to elevated temperature also highlights the importance of considering leaf and life-history traits (i.e. succession stage, morphology traits, leaf nutrients) influencing the leaf energy balance when evaluating plant sensitivity to air temperature. For example, Cheesman *et al.*⁶⁴ have demonstrated negative effect of warming on growth in seedlings of tropical climax species than in those of pioneer species. Consistent with this, in our study, *C. hystrix* is one of the most important and dominant climax

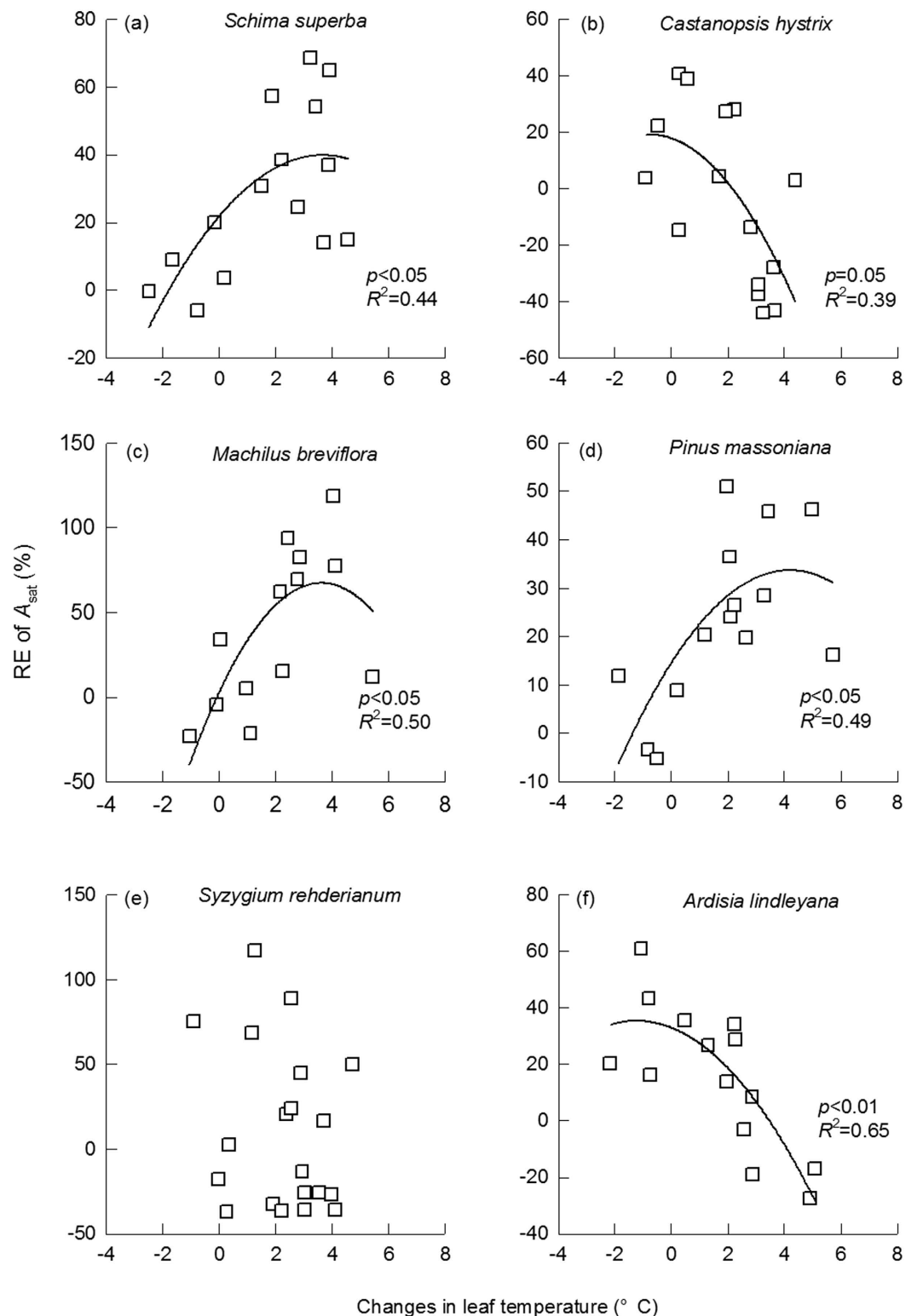


Figure 3. Correlations between relative effect (RE) of photosynthetic rate (A_{sat}) with changes in leaf temperature. Data were fitted using a nonlinear regression (solid line). The adjusted R^2 value and its significance for each fitting are shown.

species of the evergreen forests in subtropical China⁶⁵, photosynthesis of which was negatively affected by warming. The response of photosynthesis to warming might also be related to different leaf morphology traits. Higher specific leaf area associated with efficient light capture could have led to larger assimilation gains⁶⁶. Recently,

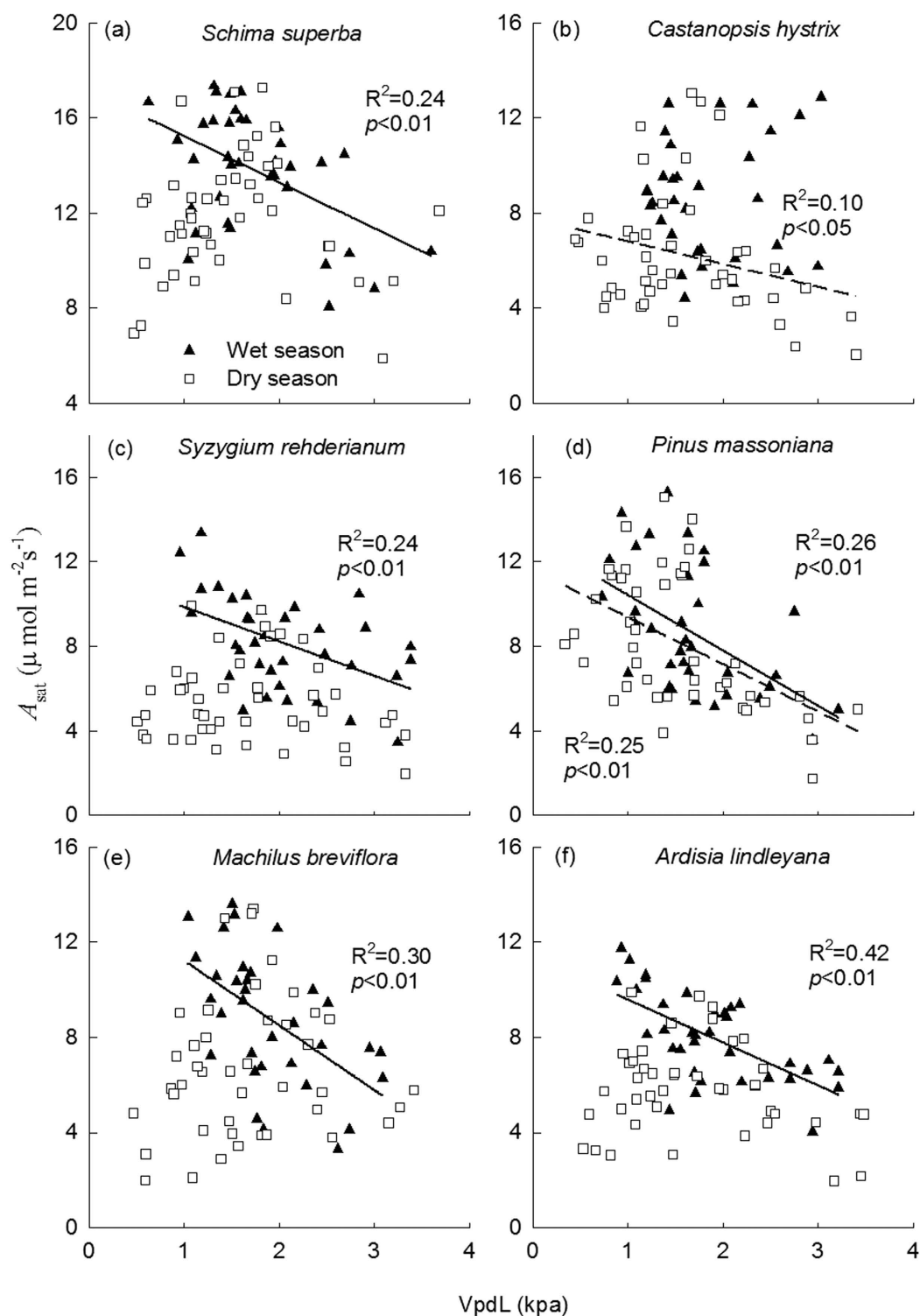


Figure 4. Correlations between photosynthetic rate (A_{sat}) with vapor pressure deficit (VpdL) in wet season (solid line) and dry season (dashed line), respectively. The adjusted R^2 value and its significance for each fitting are shown.

in connection with the studies of g_s under climate warming, response of stomatal anatomy and density are of special interest^{67,68}. Stomatal trait has a potential to set the limit for maximum g_s for gas diffusion⁶⁹. In the long term, changes in g_s can also be influenced by stomatal density, stomatal index and stomatal aperture⁷⁰. These results indicate that inter-specific variation in photosynthesis is often controlled by differences in leaf traits. More research is needed to better understand the possible tradeoffs of different strategies.

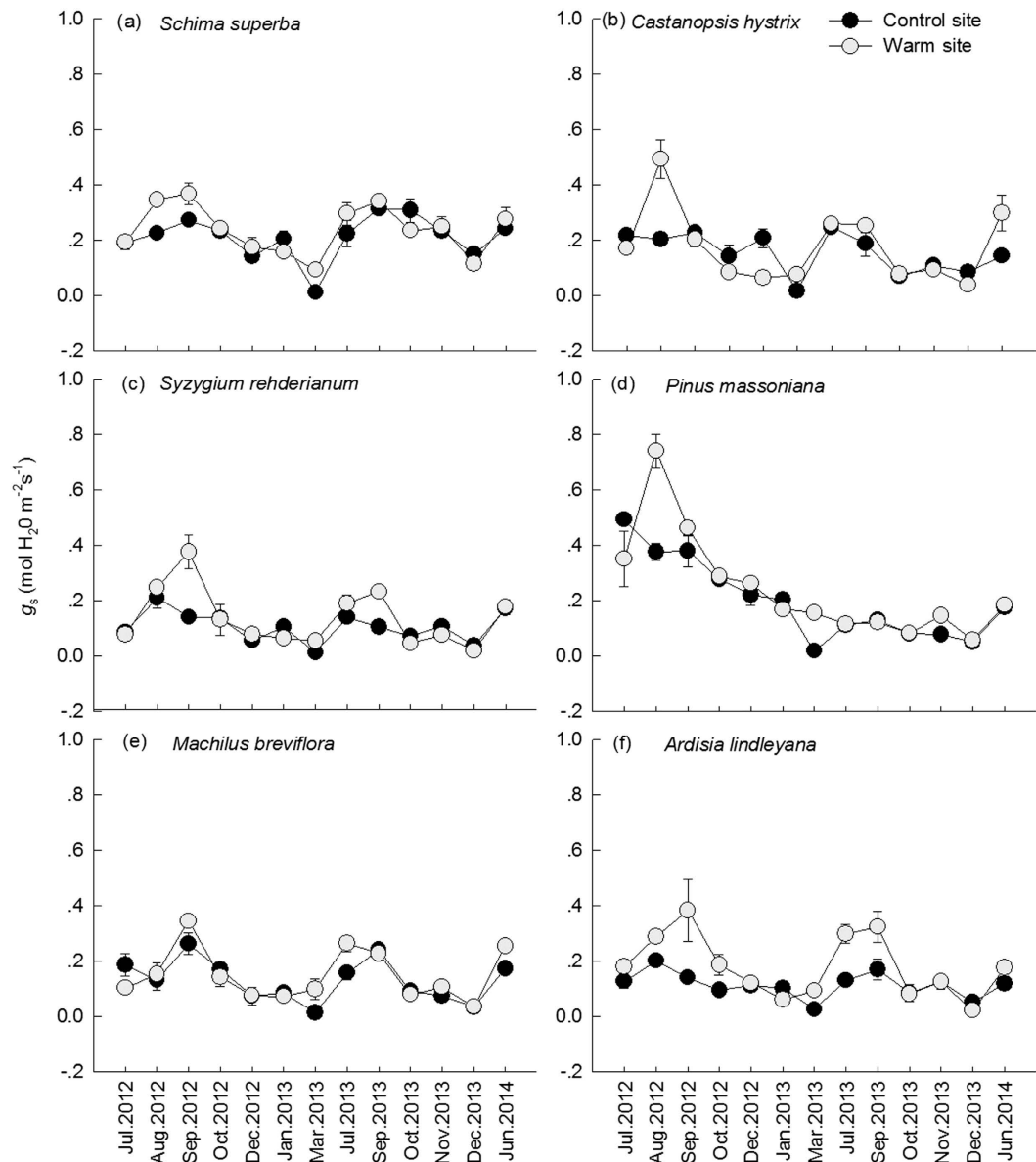


Figure 5. Seasonal dynamics of stomatal conductance (g_s) in *Schima superba* (a), *Castanopsis hystrix* (b), *Syzygium rehderianum* (c), *Pinus massoniana* (d), *Machilus breviflora* (e) and *Ardisia lindleyana* (f) grown in the warm site and control site. Error bars are standard error ($n = 3$).

In conclusion, the results demonstrated that translocation differentially affected photosynthesis of the dominant subtropical species and photosynthetic responses to translocation were season-dependent. The seasonal mean A_{sat} of *Schima superba*, *Machilus breviflora*, *Pinus massoniana* and *Ardisia lindleyana* in the warm site were significantly greater than those in the control site, but it was not affected for *Syzygium rehderianum* and declined for *Castanopsis hystrix*. The responses of A_{sat} were largely determined by g_s , which was sensitive to environmental variables (such as VPD). Amongst the six tree species, *Schima superba*, *Ardisia lindleyana*, and *Machilus breviflora* were more sensitive species to translocation. The specie-specific and season-dependent photosynthetic responses under translocation indicate that climatic warming could have potential impacts on species composition and community structure in subtropical forests in China.

Materials and Methods

Study site. The present study was conducted at the Dinghushan Biosphere Reserve, located in the central Guangdong Province in southern China (112°10'E, 23°10'N). This reserve lies between 10 and 1000 m above sea level (a.s.l.). This region is characterized by a typical south subtropical monsoon climate, with a mean annual temperature of 21 °C with the maximum and minimum monthly mean temperature being 28.0 °C in July and 12.6 °C in January, respectively. The mean annual precipitation of 1956 mm, of which nearly 80% falls in the warm-wet season (April-September) and 20% in the cold-dry season (October-March).

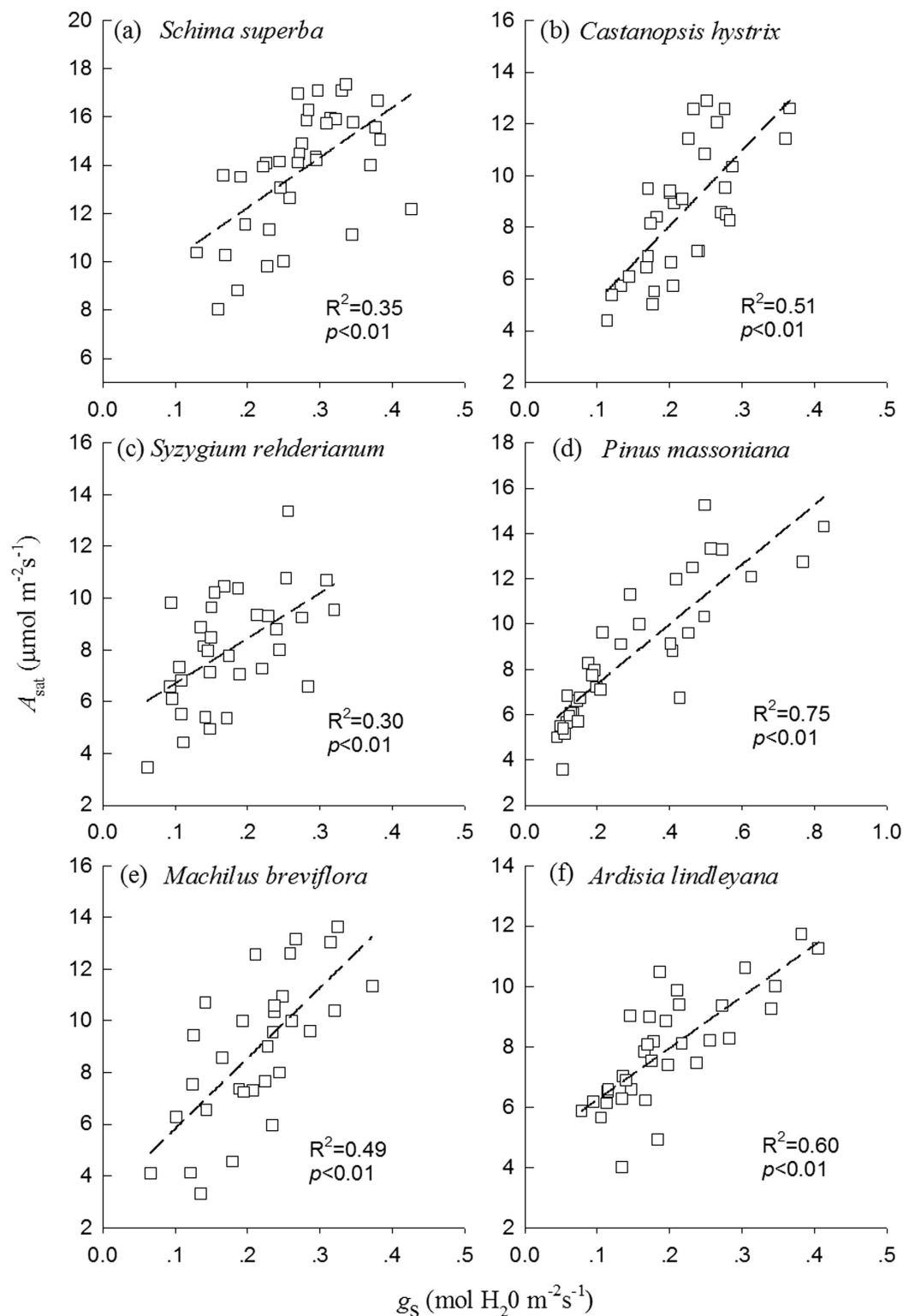


Figure 6. Correlations between photosynthetic rate (A_{sat}) and stomatal conductance (g_s) in *Schima superba*, *Castanopsis hystrix*, *Syzygium rehderianum*, *Pinus massoniana*, *Machilus breviflora* and *Ardisia lindleyana*.

Experimental design. We conducted a common garden translocation experiment at two sites (300 m and 30 m a.s.l.) (control and warm site) along an altitudinal gradient. Three 3×3 m plots were located in an open area at each site. Below-ground (0.8 m deep) in each plot was surrounded by concrete brick wall bonding with ceramic tile to prevent the lateral or vertical movement of water or element from the surrounding soils. There was one hole at the top and the bottom of the wall, respectively. In April 2012, soil and individual seedlings that was 1-year-old were collected from a coniferous and broadleaved mixed forest that near the control site. Three

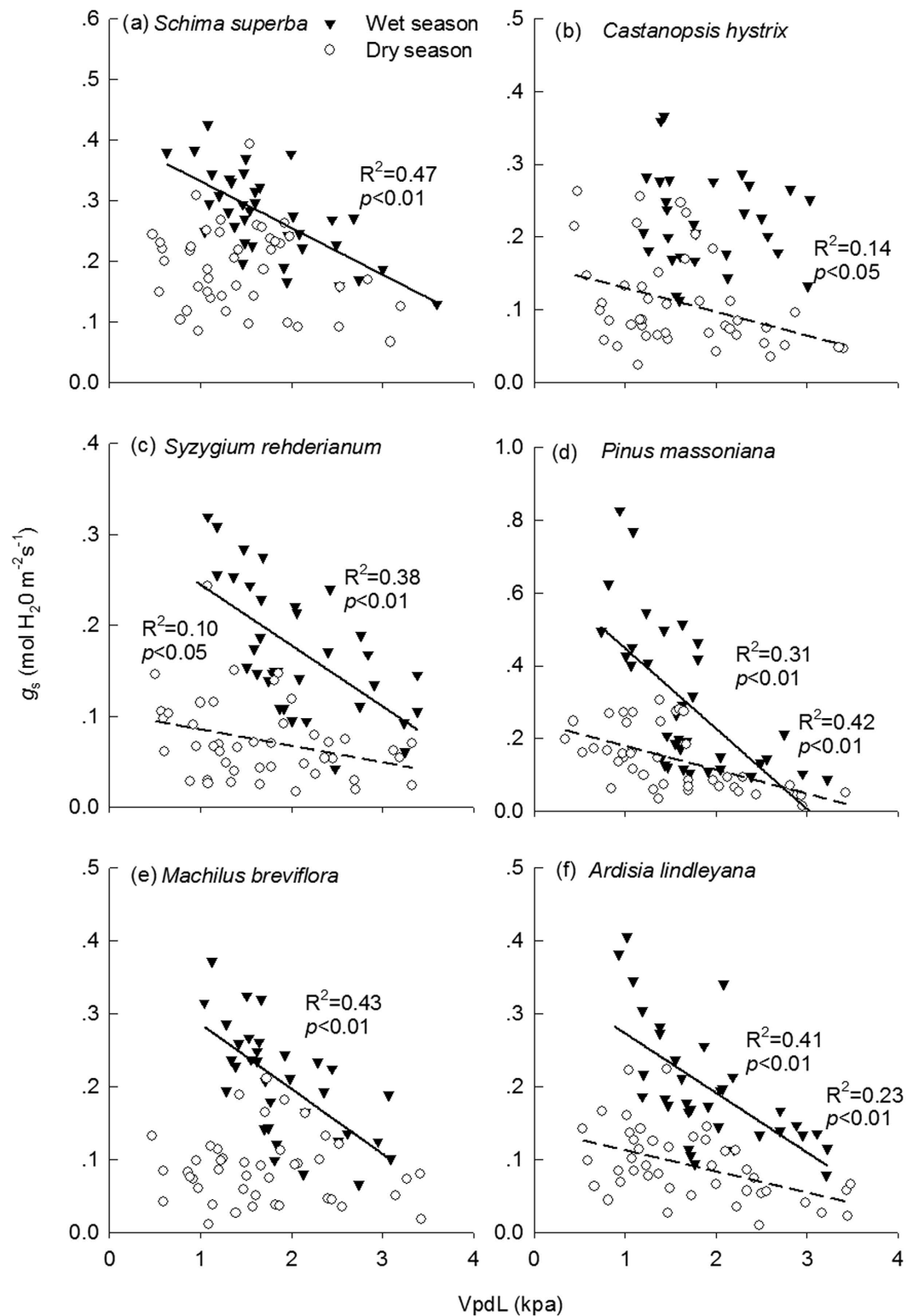


Figure 7. Correlations between stomatal conductance (g_s) with vapor pressure deficit (VpdL) in wet season (solid line) and dry season (dashed line), respectively. The adjusted R^2 value and its significance for each fitting are shown.

different layers of soils (0–20, 20–40 and 40–70 cm) were homogenized separately. Seedlings were stored in shade containers with soil from the collection sites. In May 2012, three different layers of soils were transferred into the plots correspondingly. The seedlings were transplanted into the plots in a randomized block design ($n = 6$ replicates per species).

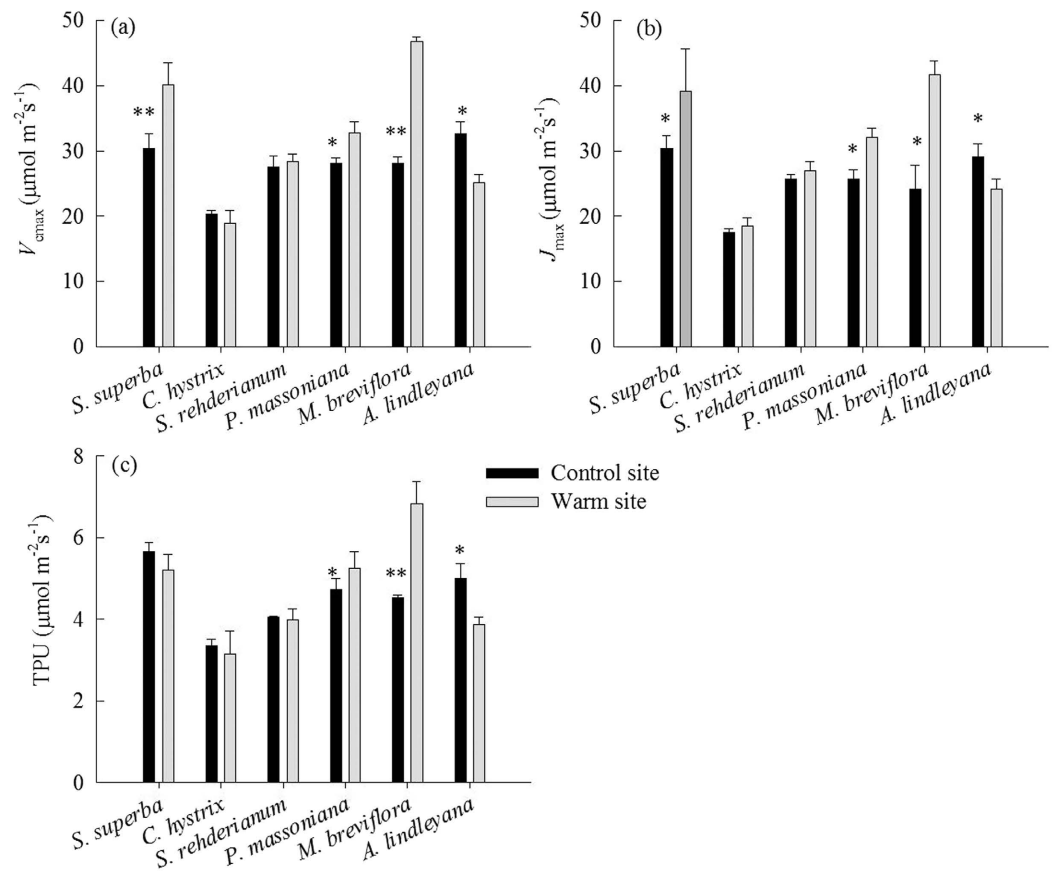


Figure 8. The maximum rate of maximum rate of photosynthetic carboxylation (a, V_{cmax}), the maximum rate of photosynthetic electron transport (b, J_{max}) and triose-phosphate utilization (c, TPU) for *Schima superba*, *Castanopsis hystrix*, *Syzygium rehderianum*, *Pinus massoniana*, *Machilus breviflora* and *Ardisia lindleyana* grown in the warm site and control site. Error bars are standard error ($n = 3$). Asterisks (*) and (**) indicate that there are significant differences at $p < 0.05$ and $p < 0.01$ between the warm site and the controls, respectively.

The six species included in this study were specifically selected due to their common occurrence and distribution range (existence in almost all regions along the altitudinal gradient) from mixed forest. They included *Schima superba* Gardn. et Champ, *Syzygium rehderianum* Merr. et Perry, *Machilus breviflora* (Benth.) Hemsl, *Pinus massoniana* Lamb., *Castanopsis hystrix* Hook. f. et Thomson ex A. DC, *Ardisia punctata* Lindl. All species were evergreen, ensuring that their leaves are exposed to the full seasonal changes of temperature. Coniferous and broadleaved species (*P. massoniana* vs other species) were chosen.

Environmental monitoring. A meteorological observation was installed in each experimental plot to monitor air temperature (HMP155A, Vaisala, Finland), soil profile temperatures (CT 109, Campbell, USA) and volumetric soil water content (CS616, Campbell, USA) at 10 cm depth. Each meteorological observation was connected with a data loggers (CR1000, Campbell, USA) to record data every 10 minutes since May 2013.

Gas exchange measurements. Photosynthetic seasonal patterns were measured on three newly developed and fully expanded leaves per species in each plot. Leaf net photosynthesis (A_{sat}) was measured using a portable open path gas exchange system (Li-6400; Li-Cor, Inc., Lin-coln, NE, USA) with red-blue light source (6400-02B). The photosynthetic photon flux density was maintained at $1200 \mu\text{mol m}^{-2}\text{s}^{-1}$ and $[\text{CO}_2]$ was maintained at $400 \mu\text{mol m}^{-2}\text{s}^{-1}$.

On 25–26 October 2013, A_{sat} to intercellular CO_2 ($A_{sat}-C_i$ curves) were measured at PPFD of $1200 \mu\text{mol m}^{-2}\text{s}^{-1}$, midday growth temperature (22°C or 23°C), relative humidity of 50–60% and leaf-to-air VPD between 1.0 and 2.0 kPa, by raising cuvette CO_2 in 9 steps (0, 50, 100, 200, 300, 400, 600, 800 and $1800 \mu\text{mol mol}^{-1}$). Analysis of $A_{sat}-C_i$ response curves involved calculation of parameters potentially limiting to photosynthesis: V_{cmax} (maximum rate of photosynthetic carboxylation, $\mu\text{mol m}^{-2}\text{s}^{-1}$), J_{max} (maximum rate of photosynthetic electron transport, $\mu\text{mol m}^{-2}\text{s}^{-1}$) and TPU (triose-phosphate utilization, $\mu\text{mol m}^{-2}\text{s}^{-1}$). This was achieved using Photosynthesis Assistant (v1.1, Dundee Scientific, Dundee, UK) which uses a biochemical model describing A_{sat} .

Data analysis. Repeated-measures ANOVA was used to investigate the effects of translocation on environmental factors, seasonal A_{sat} and g_s in each tree species over the experimental time. The differences in V_{cmax} , J_{max} ,

TPU between the control and warm sites were analyzed by t-test. As there were differences in A_{sat} and g_s between the six tree species, an effect-size index, relative effect (RE)^{57,58}, was used to estimate the responses of A_{sat} to translocation in the six tree species. RE is quantified by the ratio of the variable in the experimental group to the control group minus one. Relationships between A_{sat} , g_s and VPD were analyzed using linear regression analysis. Results were considered significant in all cases if $p < 0.05$. All analyses were performed in SPSS (SPSS 17.0 for windows, USA).

References

1. Yu, G. *et al.* High carbon dioxide uptake by subtropical forest ecosystems in the East Asian monsoon region. *Proc. Natl. Acad. Sci. USA* **111**, 4910–4915 (2014).
2. Yan, J. *et al.* Seasonal and inter-annual variations in net ecosystem exchange of two old-growth forests in southern China. *Agric. For. Meteorol.* **182**, 257–265 (2013).
3. Pachauri, R. K. *et al.* Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. (2014).
4. Way, D. A. & Oren, R. Differential responses to changes in growth temperature between trees from different functional groups and biomes: a review and synthesis of data. *Tree Physiol.* **30**, 669–688 (2010).
5. Zhou, G. *et al.* A climate change-induced threat to the ecological resilience of a subtropical monsoon evergreen broad-leaved forest in Southern China. *Glob. Change Biol.* **19**, 1197–1210 (2013).
6. McIntyre, P. J. *et al.* Twentieth-century shifts in forest structure in California: Denser forests, smaller trees, and increased dominance of oaks. *Proc. Natl. Acad. Sci. USA* **112**, 1458–1463 (2015).
7. Anderegg, W. R., Kane, J. M. & Anderegg, L. D. Consequences of widespread tree mortality triggered by drought and temperature stress. *Nat. Clim. Chang.* **3**, 30–36 (2013).
8. Zhou, G. *et al.* Substantial reorganization of China's tropical and subtropical forests: based on the permanent plots. *Glob. Change Biol.* **20**, 240–250 (2014).
9. Farquhar, G., von Caemmerer, S. v. & Berry, J. A biochemical model of photosynthetic CO₂ assimilation in leaves of C₃ species. *Planta* **149**, 78–90 (1980).
10. Norby, R. J. & Luo, Y. Evaluating ecosystem responses to rising atmospheric CO₂ and global warming in a multi-factor world. *New Phytol.* **162**, 281–293 (2004).
11. Way, D. A., Oren, R. & Kroner, Y. The space-time continuum: the effects of elevated CO₂ and temperature on trees and the importance of scaling. *Plant Cell Environ.* **38**, 991–1007 (2015).
12. Lloyd, J. & Farquhar, G. D. Effects of rising temperatures and CO₂ on the physiology of tropical forest trees. *Philos. Trans. R. Soc. B-Biol. Sci.* **363**, 1811–1817 (2008).
13. Shirke, P. A. & Pathre, U. V. Influence of leaf-to-air vapour pressure deficit (VPD) on the biochemistry and physiology of photosynthesis in *Prosopis juliflora*. *J. Exp. Bot.* **55**, 2111–2120 (2004).
14. Sage, R. F. & Kubien, D. S. The temperature response of C₃ and C₄ photosynthesis. *Plant Cell Environ.* **30**, 1086–1106 (2007).
15. Easlon, H. M., Carlisle, E., McKay, J. & Bloom, A. Does low stomatal conductance or photosynthetic capacity enhance growth at elevated CO₂ in *Arabidopsis thaliana*? *Plant Physiol.* **167**, 793–799 (2015).
16. Dusenge, M. E. *et al.* Photosynthetic capacity of tropical montane tree species in relation to leaf nutrients, successional strategy and growth temperature. *Oecologia* **177**, 1183–1194 (2015).
17. Sendall, K. M. *et al.* Acclimation of photosynthetic temperature optima of temperate and boreal tree species in response to experimental forest warming. *Glob. Change Biol.* **21**, 1342–1357 (2015).
18. Gimeno, T. E., Camarero, J. J., Granda, E., Pías, B. & Valladares, F. Enhanced growth of *Juniperus thurifera* under a warmer climate is explained by a positive carbon gain under cold and drought. *Tree Physiol.* **32**, 326–336 (2012).
19. Zhao, H., Li, Y., Zhang, X., Korpelainen, H. & Li, C. Sex-related and stage-dependent source-to-sink transition in *Populus cathayana* grown at elevated CO₂ and elevated temperature. *Tree Physiol.* **32**, 1325–1338 (2012).
20. Wertin, T. M., McGuire, M. A. & Teskey, R. O. Higher growth temperatures decreased net carbon assimilation and biomass accumulation of northern red oak seedlings near the southern limit of the species range. *Tree Physiol.* **31**, 1277–1288 (2011).
21. Vårhammar, A. *et al.* Photosynthetic temperature responses of tree species in Rwanda: evidence of pronounced negative effects of high temperature in montane rainforest climax species. *New Phytol.* **206**, 1000–1012 (2015).
22. Ruiz-Vera, U. M. *et al.* Global warming can negate the expected CO₂ stimulation in photosynthesis and productivity for soybean grown in the Midwestern United States. *Plant Physiol.* **162**, 410–423 (2013).
23. Darbah, J. N., Sharkey, T. D., Calfapietra, C. & Karnosky, D. F. Differential response of aspen and birch trees to heat stress under elevated carbon dioxide. *Environ. Pollut.* **158**, 1008–1014 (2010).
24. Bronson, D. R. & Gower, S. T. Ecosystem warming does not affect photosynthesis or aboveground autotrophic respiration for boreal black spruce. *Tree Physiol.* **30**, 441–449 (2010).
25. Streit, K., Siegwolf, R. T., Hagedorn, F., Schaub, M. & Buchmann, N. Lack of photosynthetic or stomatal regulation after 9 years of elevated [CO₂] and 4 years of soil warming in two conifer species at the alpine treeline. *Plant Cell Environ.* **37**, 315–326 (2014).
26. Stanton, D. E., Merlin, M., Bryant, G. & Ball, M. C. Water redistribution determines photosynthetic responses to warming and drying in two polar mosses. *Funct. Plant Biol.* **41**, 178–186 (2014).
27. Adachi, M. *et al.* Soil and water warming accelerates phenology and down-regulation of leaf photosynthesis of rice plants grown under free-air CO₂ enrichment (FACE). *Plant Cell Physiol.* **55**, 370–380 (2014).
28. Bagley, J. *et al.* The influence of photosynthetic acclimation to rising CO₂ and warmer temperatures on leaf and canopy photosynthesis models. *Glob. Biogeochem. Cycle* **29**, 194–206 (2015).
29. Cunningham, S. & Read, J. Comparison of temperate and tropical rainforest tree species: photosynthetic responses to growth temperature. *Oecologia* **133**, 112–119 (2002).
30. Doughty, C. E. & Goulden, M. L. Are tropical forests near a high temperature threshold? *J. Geophys. Res.* **113**, doi: 10.1029/2007jg000632 (2008).
31. Elmendorf, S. C. *et al.* Experiment, monitoring, and gradient methods used to infer climate change effects on plant communities yield consistent patterns. *Proc. Natl. Acad. Sci. USA* **112**, 448–452 (2015).
32. Duan, H. *et al.* Drought responses of two gymnosperm species with contrasting stomatal regulation strategies under elevated [CO₂] and temperature. *Tree Physiol.*, doi: 10.1093/treephys/tpv047 (2015).
33. Duan, H. *et al.* Elevated [CO₂] does not ameliorate the negative effects of elevated temperature on drought-induced mortality in *Eucalyptus radiata* seedlings. *Plant Cell Environ.* **37**, 1598–1613 (2014).
34. Rich, R. L. *et al.* Design and performance of combined infrared canopy and belowground warming in the B4WarmED (Boreal Forest Warming at an Ecotone in Danger) experiment. *Glob. Change Biol.* **21**, 2334–2348 (2015).
35. De Frenne, P. Innovative empirical approaches for inferring climate-warming impacts on plants in remote areas. *New Phytol.* **205**, 1015–1021 (2015).
36. Dillaway, D. N. & Kruger, E. L. Thermal acclimation of photosynthesis: a comparison of boreal and temperate tree species along a latitudinal transect. *Plant Cell Environ.* **33**, 888–899 (2010).

37. Han, Q. M., Kawasaki, T., Nakano, T. & Chiba, Y. Spatial and seasonal variability of temperature responses of biochemical photosynthesis parameters and leaf nitrogen content within a *Pinus densiflora* crown. *Tree Physiol.* **24**, 737–744 (2004).
38. Frenne, P. *et al.* Latitudinal gradients as natural laboratories to infer species' responses to temperature. *J. Ecol.* **101**, 784–795 (2013).
39. De Frenne, P. *et al.* Temperature effects on forest herbs assessed by warming and transplant experiments along a latitudinal gradient. *Glob. Change Biol.* **17**, 3240–3253 (2011).
40. Frei, E. R., Ghazoul, J., Matter, P., Heggli, M. & Pluess, A. R. Plant population differentiation and climate change: responses of grassland species along an elevational gradient. *Glob. Change Biol.* **20**, 441–455 (2014).
41. Doughty, C. E. & Goulden, M. L. Are tropical forests near a high temperature threshold? *J. Geophys. Res.* (2005–2012) **113** (2008).
42. Bowman, D. M., Williamson, G. J., Keenan, R. & Prior, L. D. A warmer world will reduce tree growth in evergreen broadleaf forests: evidence from Australian temperate and subtropical eucalypt forests. *Glob. Ecol. Biogeogr.* **23**, 925–934 (2014).
43. Clark, D. B., Clark, D. A. & Oberbauer, S. F. Annual wood production in a tropical rain forest in NE Costa Rica linked to climatic variation but not to increasing CO₂. *Glob. Change Biol.* **16**, 747–759 (2010).
44. Zhang, Y. F., Niu, S. L., Xu, W. H. & Han, Y. Species-specific response of photosynthesis to burning and nitrogen fertilization. *J. Integr. Plant Biol.* **50**, 565–574 (2008).
45. Xu, Z. *et al.* Effects of elevated CO₂, warming and precipitation change on plant growth, photosynthesis and peroxidation in dominant species from North China grassland. *Planta* **239**, 421–435 (2014).
46. Liang, J., Xia, J., Liu, L. & Wan, S. Global patterns of the responses of leaf-level photosynthesis and respiration in terrestrial plants to experimental warming. *J. Plant Ecol.* doi: 10.1093/jpe/rtt003 (2013).
47. Crous, K. Y. *et al.* Photosynthesis of temperate *Eucalyptus globulus* trees outside their native range has limited adjustment to elevated CO₂ and climate warming. *Glob. Change Biol.* **19**, 3790–3807 (2013).
48. Kattge, J. & Knorr, W. Temperature acclimation in a biochemical model of photosynthesis: a reanalysis of data from 36 species. *Plant Cell Environ.* **30**, 1176–1190 (2007).
49. Stinziano, J. R., Hüner, N. P. & Way, D. A. Warming delays autumn declines in photosynthetic capacity in a boreal conifer, Norway spruce (*Picea abies*). *Tree Physiol.* **35**, 1303–1313 (2015).
50. Ghannoum, O. *et al.* Photosynthetic responses of two eucalypts to industrial-age changes in atmospheric CO₂ and temperature. *Plant Cell Environ.* **33**, 1671–1681 (2010).
51. Haldimann, P. & Feller, U. Inhibition of photosynthesis by high temperature in oak (*Quercus pubescens* L.) leaves grown under natural conditions closely correlates with a reversible heat-dependent reduction of the activation state of ribulose-1,5-bisphosphate carboxylase/oxygenase. *Plant Cell Environ.* **27**, 1169–1183 (2004).
52. Sage, R. F., Way, D. A. & Kubien, D. S. Rubisco, Rubisco activase, and global climate change. *J. Exp. Bot.* **59**, 1581–1595 (2008).
53. Bernacchi, C. J., Morgan, P. B., Ort, D. R. & Long, S. P. The growth of soybean under free air [CO₂] enrichment (FACE) stimulates photosynthesis while decreasing *in vivo* Rubisco capacity. *Planta* **220**, 434–446 (2005).
54. Singaas, E., Ort, D. & Delucia, E. Elevated CO₂ effects on mesophyll conductance and its consequences for interpreting photosynthetic physiology. *Plant Cell Environ.* **27**, 41–50 (2004).
55. Wong, S., Cowan, I. & Farquhar, G. Stomatal conductance correlates with photosynthetic capacity. *Nature*. **282**, 424–426 (1979).
56. Kirschbaum, M. & Farquhar, G. Temperature dependence of whole-leaf photosynthesis in *Eucalyptus pauciflora* Sieb. ex Spreng. *Funct. Plant Biol.* **11**, 519–538 (1984).
57. Lin, Y.-S., Medlyn, B. E. & Ellsworth, D. S. Temperature responses of leaf net photosynthesis: the role of component processes. *Tree Physiol.* **32**, 219–231 (2012).
58. Rao, N. K. S., Bhatt, R. M. & Sadashiva, A. T. Tolerance to water stress in tomato cultivars. *Photosynthetica*. **38**, 465–467 (2000).
59. Farquhar, G. D. & Sharkey, T. D. Stomatal conductance and photosynthesis. *Annu. Rev. Plant Physiol. Plant Molec. Biol.* **33**, 317–345 (1982).
60. Medlyn, B. *et al.* Temperature response of parameters of a biochemically based model of photosynthesis. II. A review of experimental data. *Plant Cell Environ.* **25**, 1167–1179 (2002).
61. Duursma, R. A. *et al.* The peaked response of transpiration rate to vapour pressure deficit in field conditions can be explained by the temperature optimum of photosynthesis. *Agric. For. Meteorol.* **189**, 2–10 (2014).
62. Horton, J. L., Kolb, T. E. & Hart, S. C. Leaf gas exchange characteristics differ among Sonoran Desert riparian tree species. *Tree Physiol.* **21**, 233–241 (2001).
63. Barron-Gafford, G. A., Grieve, K. A. & Murthy, R. Leaf- and stand-level responses of a forested mesocosm to independent manipulations of temperature and vapor pressure deficit. *New Phytol.* **174**, 614–625 (2007).
64. Cheesman, A. W. & Winter, K. Growth response and acclimation of CO₂ exchange characteristics to elevated temperatures in tropical tree seedlings. *J. Exp. Bot.* **64**, 3817–3828 (2013).
65. Li, J., Ge, X., Cao, H. & Ye, W. Chloroplast DNA diversity in *Castanopsis hystrix* populations in south China. *For. Ecol. Manage.* **243**, 94–101 (2007).
66. Wang, J., Duan, B. & Zhang, Y. Effects of experimental warming on growth, biomass allocation, and needle chemistry of *Abies faxoniana* in even-aged monospecific stands. *Plant Ecol.* **213**, 47–55 (2012).
67. Xu, Z. & Zhou, G. Responses of leaf stomatal density to water status and its relationship with photosynthesis in a grass. *J. Exp. Bot.* **59**, 3317–3325 (2008).
68. Lake, J. A. & Woodward, F. I. Response of stomatal numbers to CO₂ and humidity: control by transpiration rate and abscisic acid. *New Phytol.* **179**, 397–404 (2008).
69. Luomala, E. M., Laitinen, K., Sutinen, S., Kellomaki, S. & Vapaavuori, E. Stomatal density, anatomy and nutrient concentrations of Scots pine needles are affected by elevated CO₂ and temperature. *Plant Cell Environ.* **28**, 733–749 (2005).
70. Ainsworth, E. A. & Rogers, A. The response of photosynthesis and stomatal conductance to rising [CO₂]: mechanisms and environmental interactions. *Plant Cell Environ.* **30**, 258–270 (2007).

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Author Contributions

J.X.L. and G.Y.Z. conceived and designed the experiments. Y.Y.L. performed the experiments. Y.Y.L., W.J.H. and H.L.D. analyzed the data and prepared figures and tables. Y.Y.L. wrote the main manuscript text and all authors reviewed the manuscript.

Additional Information

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