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Leaf traits from stomata to morphology are associated with climatic and edaphic variables for dominant tropical forest evergreen oaks

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Abstract

Aims Understanding variation and coordination of leaf traits at multiscales along elevational gradients can help predict the likely responses of dominant species to climate change. We seek to determine the extent to which variation in leaf stomatal, anatomical and morphological traits is associated with environmental factors, and whether ecological strategies of *Cyclobalanopsis* species shift with elevations.

Methods In a tropical forest landscape in Jianfengling, South China, we determined leaf traits related to stomata, anatomy and morphology of six evergreen oak species (*Cyclobalanopsis bambusaefolia*, *C. hui*, *C. patelliformis*, *C. fleuryi*, *C. tiaoloshanica* and *C. phanera*) along a long elevational gradient (400–1400 m above sea level).

Important Findings We found that stomatal density and stomatal pore index increased, whereas spongy mesophyll thickness to leaf thickness ratios decreased, significantly with elevation. The leaf area and leaf dry matter content increased and decreased, respectively, with elevation. Variations in stomatal, anatomical and morphological traits were mainly correlated to the mean annual temperature, mean annual sum precipitation and soil pH. At low and high elevations, the oak species exhibited strong stress tolerance combined with competition strategy, while they shifted toward more clearly the competitive strategy at intermediate elevations. And the changes in soil phosphorus concentration and soil pH along the elevation may drive the shift of ecological strategy. The results showed that the dominant oak species in tropical forests respond to environmental change by modulating traits at multiple levels, from that of the individual cell, through tissue and up to the whole leaf scale.

Keywords elevational gradient, ecological strategy, leaf traits, oak species, tropical forest

热带森林优势种青冈叶片气孔、解剖和形态性状与气候、土壤因子的关联

摘要：了解优势树种叶片多水平的功能性状沿海拔梯度的变化及其内在关联，有助于预测优势种应对气候变化的响应与适应。本文研究了青冈属树种叶片气孔、解剖和形态性状沿海拔梯度的变化及其与环境调控因子的关联，探究了其生态策略是否随海拔发生改变。在海南尖峰岭热带森林，沿海拔梯度(400–1400 m)采集了6种常绿青冈：竹叶青冈(*Cyclobalanopsis bambusaefolia*)、雷公青冈(*C. hui*)、托盘青冈(*C. patelliformis*)、饭甑青冈(*C. fleuryi*)、吊罗山青冈(*C. tiaoloshanica*)和亮叶青冈(*C. phanera*)叶片，用于气孔、解剖和形态性状的测定。研究结果表明，随海拔升高，青冈树种叶片气孔密度、气孔孔隙度指数和叶面积显著增加，但海绵组织厚度比和干物质含量则显着降低。叶片气孔、解剖和形态性状沿海拔梯度的变化主要受年均温、年降水量和土壤pH值调控。在低海拔和高海拔处，青冈属采取“耐受”和“竞争”策略，而在中海拔处，则是“竞争”策略。土壤磷含量和土壤pH值随海拔的变化可能是驱动其生态策略转变的主要原因。该结果揭示，热带森林优势树种青冈可通过从气孔细胞–组织解剖结构–叶片水平功能性状的改变来响应环境变化。

关键词：海拔梯度，生态策略，叶性状，青冈，热带森林

INTRODUCTION

Anthropogenic climate change is already well underway, as indicated by an average global temperate increase of >1.0 °C over the past century (Masson-Delmotte *et al.* 2018), and by widespread changes in precipitation regimes (e.g. Greve *et al.* 2014). Temperature increases and precipitation changes, in combination with soil properties, greatly affect plant distributions, community dynamics and even forest ecosystem functions (Aleixo *et al.* 2019; Bellard *et al.* 2012; Pérez-Ramos *et al.* 2017). Elevational gradients provide potentiality for understanding and predicting how plant species respond to future climate change (Matías *et al.* 2017; Sundqvist *et al.* 2013).

Plant leaf traits at the cell level (e.g. stomatal traits), tissue level (e.g. anatomical traits) and organ level (morphological traits) may be especially informative in relation to climate as they reflect aspects of carbon acquisition, water use and gaseous exchange (Baillie and Fleming 2020; Lambers and Poorter 1992; Liu *et al.* 2019). At the cellular level, stomata are the essential structures for the leaf to exchange gases with the atmosphere (Baillie and Fleming 2020; Lee and Bergmann 2019). The open–close behavior of stomata directly regulates the balance of carbon gain (photosynthesis) and water loss (transpiration) (Ainsworth and Rogers 2007). At the tissue level, the palisade mesophyll thickness (PT) is linked to light absorption and the leaf photosynthetic capacity (Terashima *et al.* 2011). In addition, thicker spongy mesophyll enhances the drought and cold resistance of plants in temperate forests thus adapt to low temperature and moisture (Liu *et al.* 2019). At the organ level, specific leaf area (SLA) is well known to positively associate with the plant’s relative growth rate (Lambers and Poorter 1992). A high leaf SLA represents a resource-acquisitive plant strategy, while a high leaf dry matter content (LDMC) represents a resource-conservative strategy (Lambers and Poorter 1992; Wigley *et al.* 2016). Understanding the variation and coordination of leaf traits at cell-, tissue- and organ scale can help predict the responses of plants to climate change. Up to date, there is little research concerning leaf traits at such multiple scales along elevational gradients where climatic and edaphic variables vary.

The C (competitive)–S (stress-tolerant)–R (ruderal) system (Grime 1977) describes plant strategies at different geographical scales (Pierce *et al.* 2013, 2017; Rosado *et al.* 2017). However, few studies have determined whether CSR strategies of common tree species in tropical forests change along elevational gradients. Despite the considerable advances in understanding the relationships between leaf traits and plant growth, survival, reproduction, distribution and ecosystem dynamics (Cyrille *et al.* 2007; Liu and Ma 2015), the relationships between leaf traits and ecological strategies have seldom been studied along elevational gradients; for the tropics, we are not aware of a single study investigating variation in CSR strategy of canopy trees over an elevation gradient. By determining how leaf traits and plant ecological strategies change with elevation, it should be possible to generate new insights and understanding of potential shifts in plant distribution, community structure and ecosystem function caused by climate change (Reich *et al.* 2003).

Tropical forests contribute more species, biomass, gross primary productivity and global carbon cycling than any other biome on Earth (Fauset *et al.* 2015; Pan *et al.* 2013). Therefore, assessing their likely functional and compositional short-medium term responses to climate change is especially critical. While these forests are extremely diverse, relatively small numbers of tree species can dominate the canopy as well as the key carbon functions of tropical ecosystems (Fauset *et al.* 2015), which may facilitate the prediction of the effects of climate change on ecosystem function. In Asian tropical and subtropical evergreen forests, evergreen oaks (*Cyclobalanopsis*, Fagaceae) are widely distributed and frequently dominant especially in montane systems. As this one genus plays a critical role in forest ecosystem function (Luo and Zhou 2001; Xu *et al.* 2015b), it merits particular attention for assessing how their functional traits respond to climatic change over time and space. Thus, the variation of leaf traits in *Cyclobalanopsis* species along elevational gradients may indicate the extent of potential responses to climate change. Here, we investigated leaf traits of six evergreen oak species over an elevational gradient (400–1400 m above sea level, a.s.l.) in Jianfengling National Nature Reserve in Hainan, China, and assessed the relationships between multiple leaf traits and environmental factors over the whole gradient. We aimed to test three hypotheses based on expectations from work elsewhere: (i) leaf traits of evergreen oaks exhibit adaptation along an elevational gradient, i.e. they show adaptive divergence, as documented for other oaks (Cavender-Bares and Ramírez-Valiente 2017; Ramírez-Valiente *et al.* 2015); (ii) water availability controls the variation and correlations of leaf traits, as documented for dominant Fagaceae species in subtropical forests (Liang *et al.* 2019) and (iii) the position within the ecological CSR triangle (competitor–stress-tolerator–ruderal) system, calculated based on leaf area (LA), LDMC and SLA, of these oaks shifts with elevations because plant strategies respond to habitat conditions by adjusting the balance between resource acquisition and conservation (Dayrell *et al.* 2018; Rosado *et al.* 2017). Testing these hypotheses should provide insights into predicting the responses and adaptations of tree species in tropical forests to climate change.

MATERIALS AND METHODS

Site description

Jianfengling National Nature Reserve (18°20′–18°57′ N, 108°41′–109°12′ E) is located in Hainan Province in south China and has a tropical monsoon climate with a distinct wet (from May to October) and dry season (from November to next April) (Zhou *et al.* 2009). Within the reserve, the mean annual sum precipitation (MAP) is 2450 mm, with 80% of which occurs from May to October (Xu *et al.* 2015a), and the mean annual temperature (MAT) is 24.5 °C, with a mean temperature of 19.4 °C in the coldest and 27.3 °C in the warmest month (Zhou *et al.* 2009). Mean values in those variables from 1970 to 2000 along the elevation can be found in Supplementary Table S1. Soils in the tropical rain forest are brick yellow loam and yellow loam with high concentrations of soil water and humus (Xu *et al.* 2015b).

There is a large and well-preserved tropical primary rain forest whose canopy is dominated by Fagaceae species including evergreen oaks with high importance values within this reserve (Fang *et al.* 2004). The range position of oak species along the gradient (Fig. 1) allows studying their responses to environmental changes in the view of leaf traits.

Field sampling and trait measurement

Leaves of dominant evergreen oaks were sampled from six *Cyclobalanopsis* species (*C. bambusaefolia*, *C. hui*, *C. patelliformis*, *C. fleuryi*, *C. tiaoloshanica* and *C. phanera*) in June 2019. The sampling was conducted at 22 sites from 400 to 1400 m a.s.l. At least 90 fully expanded and sun-exposed leaves were collected from each species (at least three individuals) per site and stored in ice bags. Soils at 0–200 mm depth below each tree were sampled at the same time. Detailed information on the sampling is provided in Supplementary Table S1.

In the laboratory, we measured leaf traits separately. We used an optical microscope (B302, Chongqing Optical Instrument Co., Ltd., Chongqing, China) to measure stomatal traits, including stomatal length (SL, μm), stomatal width (SW, μm) and stomatal density (SD, number mm^{-2}), and anatomical traits, including leaf thickness (LT, μm), PT (μm) and spongy mesophyll thickness (ST, μm). The stomatal pore index (SPI) was calculated as $\mathrm{SPI} = \mathrm{SD} \times \mathrm{SL}^2 \times 10^{-4}$ according to Sack *et al.* (2003). The palisade mesophyll thickness to leaf thickness ratio (PTR) and the spongy mesophyll thickness to leaf thickness ratio (STR) were calculated as PT to LT ratio and ST to LT ratio, respectively (Liang *et al.* 2019; Wang *et al.* 2016). SLA (mm^2 mg^{-1}) and LDMC (g g^{-1}) were calculated as follows:

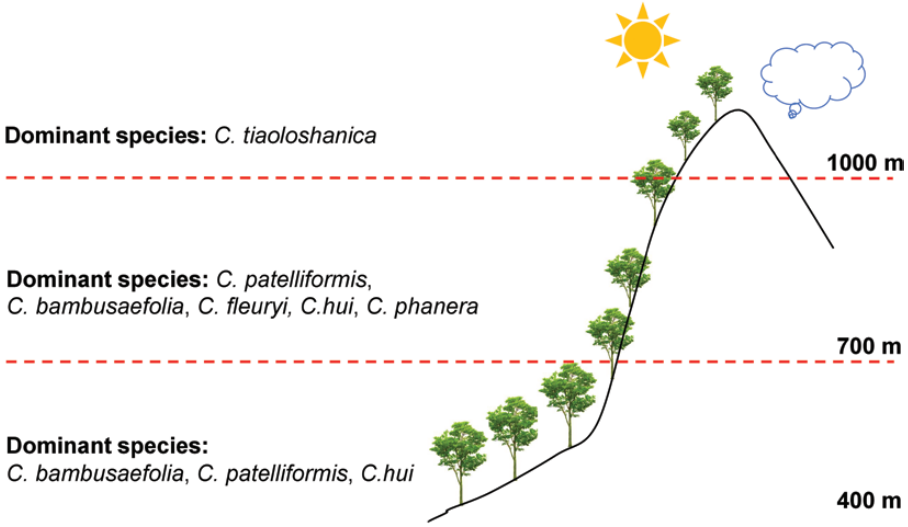

Figure 1: Distribution of the dominant species of *Cyclobalanopsis* along the elevational gradient within Jianfengling Natural Reserve. Data are from Jiang and Lu (1991).

$$\text{SLA} = \text{Leaf area/leaf dry weight } (\text{mm}^2 \cdot \text{mg}^{-1})$$

$$\text{LDMC} = \text{Leaf dry weight/leaf fresh weight } (\text{g} \cdot \text{g}^{-1})$$

Environmental factors

The content of soil organic matter (SOM), total nitrogen (SN) and total phosphorus (SP), and soil pH value (pH) were determined according to standard procedures (Liu 1996) after the samples were airdried and ground. Data on MAP and MAT of the 22 observation points along the elevational gradient from 1970 to 2000 were obtained from the WorldClim dataset (http://www.Worldclim.org). The aridity index (AI) was calculated as the ratio of precipitation to potential evapotranspiration at a given site (global drought geospatial database; http://www.cgiar-csi.org). Then we used the extract function in 'raster' R packages to extract the corresponding climate variables. Variation in SOM, SN, SP, pH, MAT, MAP and AI along the elevational gradient is presented in Supplementary Fig. S1.

Statistical analyses and C–S–R calculation

To make the relationships between leaf traits occur in a relatively uniform temperature, we divided our sites into low (400–700 m), intermediate (700–1000 m) and high (1000–1400 m) elevations, respectively, because temperature theoretically declines 0.6 °C when elevation increases 100 m (Wright *et al.* 2017). The interval of 300 m in elevation can lead to ~1.8 °C change in air temperature, which is above the average global warming of >1.0 °C over the past century (Masson-Delmotte *et al.* 2018), leading to possible variations in plant physiology and ecology including plant ecological strategies.

Prior to analyses, data except for PTR, STR and LDMC were $\log_{10}$-transformed to approximate normality. To eliminate the interference of plant species on the relationships between leaf traits and elevations, general linear-mixed effect models were employed with species as the random effects (Crawley 2007). Due to the higher correlation between MAP and AI, SOM and SN (Supplementary Fig. S2), we used principal component analysis to obtain new factors: MPA (factor score of MAP and AI) and SMN (factor score of SOM and SN). Then, an aggregated boosted tree analysis was performed to quantify the relative effects of the environmental variables on leaf traits using the 'gbmplus' package for boosting (De'ath 2007) in R v.3.6.3 software (R Development Core Team 2019). After obtaining these important variables, we use lmer function in 'lmerTest' R packages to select the variables significantly affecting leaf traits, with species as random effects and established the best-fitted models. And the correlations among leaf traits at different elevations were estimated by the idaFast function in 'pcalg' R package (Kalisch *et al.* 2012). Statistical analyses were performed using the R software platform (R Core Team 2019). Significance was set at $P < 0.05$.

The C–S–R strategies at the low-, intermediate- and high elevation were classified based on LA, SLA and LDMC values using the 'StrateFy' tool (Pierce *et al.* 2017). Plants with C (competitor) strategy are characterized by high SLA, low LDMC and generally grown at habitats with rich resources and less interference; plants with S (stress-tolerator) strategy are characterized by low SLA, high LDMC and usually found in environments with poor resources and great interference; plants with R (ruderal) strategy are characterized by low SLA and LDMC (Lambers and Poorter 1992; Wigley *et al.* 2016).

RESULTS

Effects of elevation and species on leaf traits

The variance of leaf traits was mainly explained by elevation (Supplementary Table S2). The SD, SPI and LA increased significantly and substantially with increasing elevation, while STR and LDMC declined with elevation ($P < 0.05$, Fig. 2a, b, g, h and j). Five other traits including SL, SW, LT, PTR and SLA did not change significantly along the elevational gradient (Fig. 2c–f and i). More specifically, the *C. bambusaefolia* tends to coexist with *C. hui*, *C. phanera*, *C. fleuryi* and *C. patelliformis* along the elevational gradient except for high elevation, whereas only *C. tiaoloshanica* inhabits the highest elevations (Fig. 2).

Correlations between leaf traits and environmental variables

Most of the measured leaf traits were significantly correlated with climate and soil variables along the elevational gradient (Table 1). Specifically, based on the aggregated boosted tree analysis that without considering the effects of species, MAT was the most important factor affecting the leaf traits except for SD, STR and LA (Fig. 3). MPA had great effects on SD and STR, while SP and MAP exhibited effects on LA (Fig. 3; Supplementary Table S3). Furthermore, according to the linear-mixed models (Table 1), both SD and SPI significantly increased with MPA ($P < 0.01$), while STR significantly decreased with MPA ($P < 0.01$). SL (positively), SW (positively), PTR (negatively) and LA (negatively) were significantly correlated with soil pH ($P < 0.05$). MAT was negatively related to LDMC ($P < 0.01$).

Correlations among leaf traits at different elevations

Correlations of leaf traits varied within and among stomatal, anatomical and morphological traits, but this covariation itself was elevation dependent (Fig. 4). Specifically, SLA was always correlated with other traits at different elevations. For instance, negatively correlated with LA ($r = -0.76$, $P < 0.05$) and LT ($r = -0.76$, $P < 0.05$, Fig. 4a) at low elevation, with SL at intermediate elevation ($r = -0.76$, $P < 0.05$, Fig. 4b) and with LT ($r = -0.96$, $P < 0.05$) and SPI ($r = -0.89$, $P < 0.05$, Fig. 4c) at high elevation. At intermediate elevation, LT was significantly correlated with SPI ($r = 0.79$, $P < 0.05$, Fig. 4b), and SL was positively correlated with SW ($r = 0.78$, $P < 0.05$, Fig. 4b). At high elevation, the negative correlations occurred in PTR and STR ($r = -0.91$, $P < 0.05$, Fig. 4c). Notably, the correlations within stomatal, anatomical and morphological traits at low elevation were more complex than those at intermediate and high elevation.

CSR strategies of *Cyclobalanopsis* based on LA, SLA and LDMC

Cyclobalanopsis species exhibited the greatest variation in CSR ecological strategies at intermediate elevations. Thus, while a competition + stress tolerance (CS) strategy dominated at low and high elevations (strong strategic convergence), both competition (C) and competition + stress tolerance (CS) strategies with a marked increase in competition scores dominated at intermediate elevations (Fig. 5; Supplementary Table S4). Specifically, as shown by the position of the individuals of *Cyclobalanopsis* species, *C. bambusaefolia* (low elevation) and *C. tiaoloshanica* (high elevation) occupied the nearest position toward S strategy in a narrow space, whereas *C. patelliformis* and *C. fleuryi* (both in intermediate elevation) occupied the position more toward C strategy and also in S strategy.

DISCUSSION

Leaf traits at cellular, tissue and organ level are sensitive to elevation

Determination of the ecophysiological patterns of leaf traits along elevational gradients helps to better understand and predict how plant species will respond to climate change (Blonder *et al.* 2017; Körner 2007). The significant increases in SD and SPI with elevation (Fig. 2a and b) in our study imply that evergreen oaks might maintain photosynthetic rates by increasing SD and SPI at high elevation, where soil nutrients and water availability are relatively high (Supplementary Fig. S1), due to their close relationships to stomatal conductance, carbon assimilation, water-use efficiency (Fusaro *et al.*

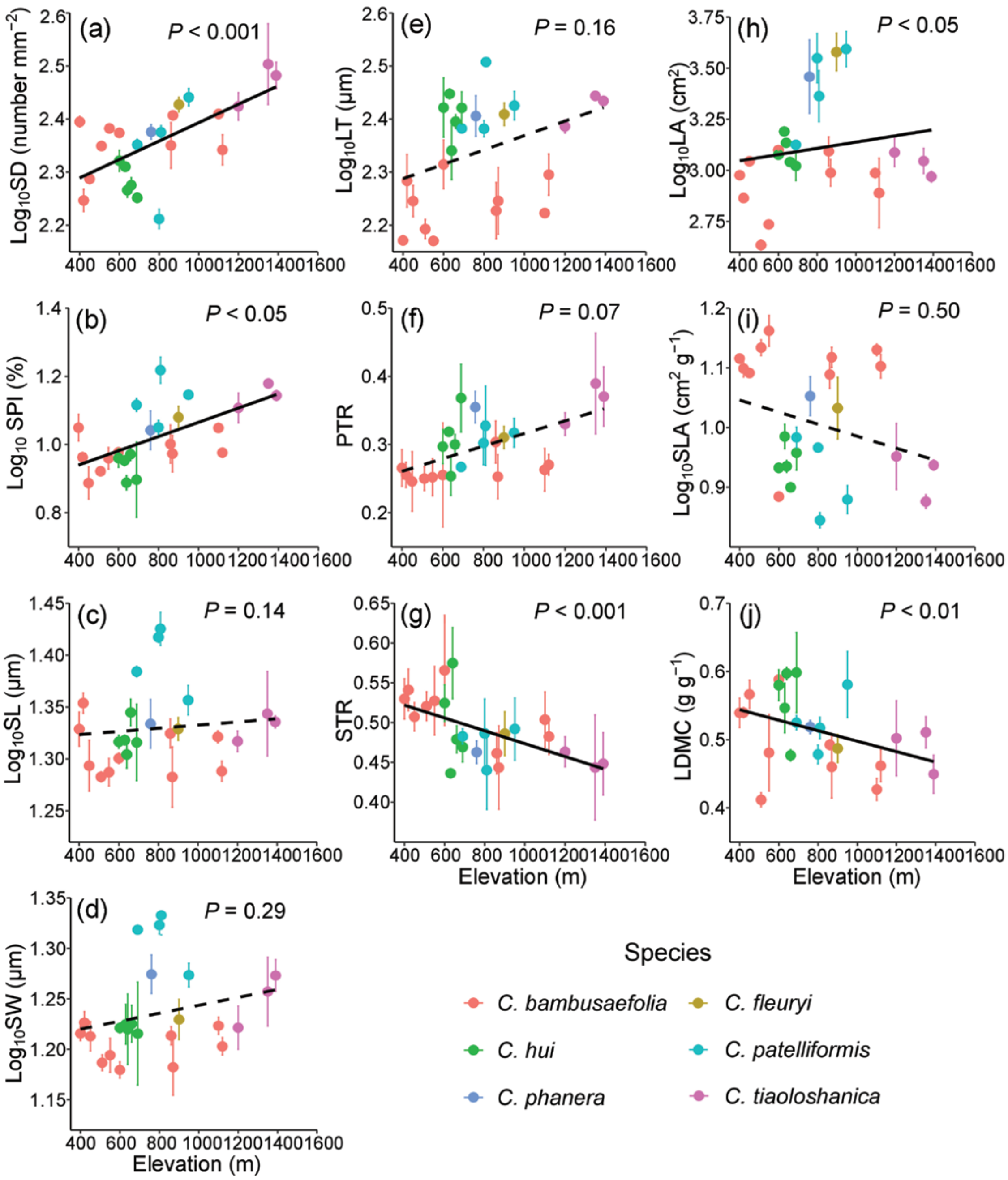

Figure 2: Variation in leaf traits of evergreen oak species (*Cyclobalanopsis*) along an elevational gradient revealed by general linear-mixed effect models. The six *Cyclobalanopsis* species are represented by different colors. Values for each species are means ± standard deviation. Solid and dashed lines show significant and non-significant correlations, respectively. (**a**) SD, stomatal density; (**b**) SPI, stomatal pore index; (**c**) SL, stomatal length; (**d**) SW, stomatal width; (**e**) LT, leaf thickness; (**f**) PTR, palisade mesophyll thickness to leaf thickness ratio; (**g**) STR, spongy mesophyll thickness to leaf thickness ratio; (**h**) LA, leaf area; (**i**) SLA, specific leaf area; (**j**) LDMC, leaf dry mass content.

2016; Liu *et al.* 2019; Tarin *et al.* 2020). For example, *C. tiaoloshanica* inhabits the highest elevation probably coinciding with the most stress-tolerant traits such as the largest SPI (Fig. 2b). When the CO_2 diffusion is reduced caused by low temperatures in high elevation, leaf stomatal conductance reduced and likely led to a large SPI (Liu *et al.* 2019).

The thickness of palisade and spongy mesophyll affects photosynthesis (Zhang *et al.* 2019; Zhu *et al.* 2019). The significant decreases in STR of evergreen oaks along the elevational gradient (Fig. 2g) may be one of the adaptive strategies on photosynthesis regulation. Specifically, the *C. bambusaefolia* tends to coexist with *C. hui*, *C. phanera*, *C. fleuryi* and

Table 1: Relationships between the measured leaf traits and environmental variables based on their relative important values in Supplementary Table S3

Trait	Category	Variable	Estimate	Standard error	*t* value	*P*
SD	Atmosphere	(Intercept)	2.366	0.019	122.98	<0.001
		MPA	0.038	0.009	4.11	**<0.001**
SPI	Atmosphere	(Intercept)	−0.525	2.724	−1.93	0.06
		MAT	4.675	2.024	2.31	**<0.05**
		MPA	0.067	0.020	3.28	**<0.01**
SL	Soil	(Intercept)	0.961	0.129	7.47	<0.001
		pH	0.502	0.171	2.93	**<0.001**
SW	Soil	(Intercept)	0.949	0.109	8.75	<0.001
		pH	0.397	0.144	2.76	**<0.001**
LT	Soil	(Intercept)	2.388	0.033	72.60	<0.001
		SMN	0.012	0.010	1.22	0.23
PTR	Soil	(Intercept)	0.732	0.192	3.81	<0.001
		pH	−0.555	0.256	−2.17	**<0.05**
STR	Atmosphere	(Intercept)	0.488	0.007	73.98	<0.001
		MPA	−0.019	0.006	−3.33	**<0.01**
LA	Soil	(Intercept)	4.697	0.696	6.75	<0.001
		pH	−1.967	0.921	−2.14	**<0.05**
SLA	Atmosphere + soil	(Intercept)	0.975	0.036	26.88	<0.001
		MPA	0.020	0.016	1.26	0.21
		SMN	−0.024	0.017	−1.47	0.15
LDMC	Atmosphere	(Intercept)	−2.980	1.039	−2.87	<0.01
		MAT	2.600	0.772	3.37	**<0.01**

Abbreviations: MAT = mean annual temperature; MPA = factor score of mean annual sum precipitation and aridity index; pH = soil pH value; SMN = factor score of soil organic matter and nitrogen concentration. The bold values represent the significant correlations ($P < 0.05$). All abbreviations of leaf traits can be found in Figure 2. All variables were standardized.

C. patelliformis with large variations of leaf anatomical traits along the elevational gradient except for high elevations, whereas *C. tiaoloshanica* inhabits the highest elevations with the smallest STR (Fig. 2g). This shows that *Cyclobanipsis* species acclimated to different habits via leaf anatomical traits, as documented before (He *et al.* 2018).

Morphological traits, such as LA, SLA and LDMC, reflect the leaf economic spectrum and plant adaptation (Wilson *et al.* 1999; Wright *et al.* 2004). The non-significant changes in SLA along the elevational gradient in our study (Fig. 2i) do not coincide with previous findings (Körner 1998). We assume that, although both soil nutrient limitation and low rainfall at high elevation, which differ from those in this study (Supplementary Fig. S1), may result in a low SLA (Körner 1998), decreasing temperature with increasing elevation counteracted, and hence SLA was not greatly affected by elevation in the studied tropical forest. As one of the conservative leaf traits, a high LDMC provides defense against insect pests or mechanical damage (Wright *et al.* 2005). We found that LDMC significantly decreased with increasing elevation (Fig. 2j), which suggests that the defense of *Cyclobalanopsis* species as related to LDMC decreased gradually.

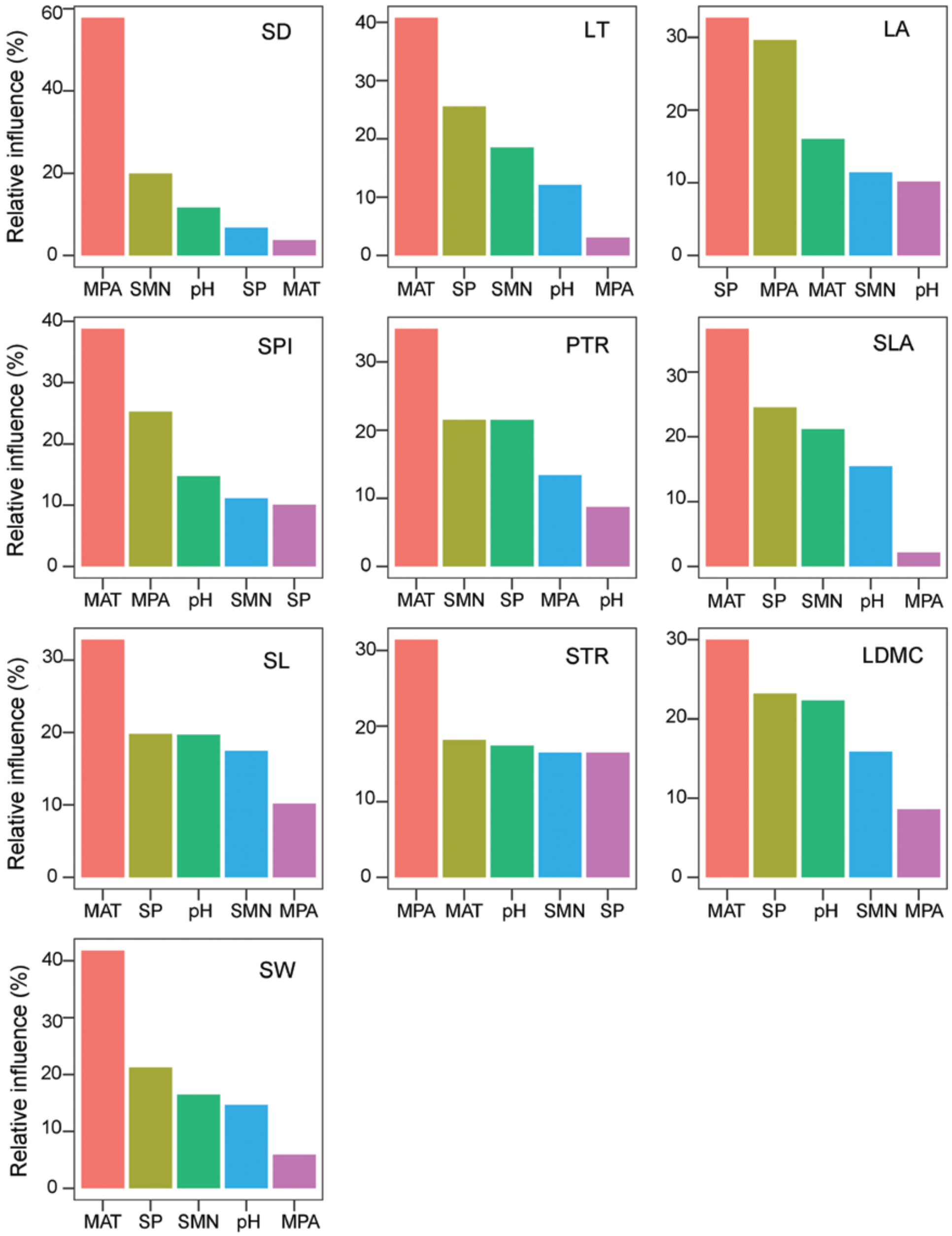

Figure 3: The relative influence of environmental factors on the measured leaf traits, identified by aggregated boosted tree (ABT) analysis. Abbreviations: MAT = mean annual temperature; MPA = factor score of mean annual sum precipitation and aridity index; pH = soil pH value; SMN = factor score of soil organic matter and nitrogen concentration; SP = soil phosphorus concentration. The corresponding leaf trait can be seen at the upper right corner of each figure. All abbreviations of leaf traits can be found in Figure 2.

Overall, the present findings support our hypothesis that along with an elevational gradient evergreen oaks exhibited adaptive divergence. The changes in stomatal and anatomical traits (relating to growth or resource acquisition) and morphological traits (relating to defense or conservation) with elevation suggests that *Cyclobalanopsis* species flexibly adjust their ecophysiology via altering leaf traits at the cellular, tissue and leaf levels to adapt or acclimate to future climate change. Research on stomatal, anatomical and morphological traits of evergreen oaks with elevation may provide insights into adaptive responses of tree species in tropical forests to climate change.

Climate variables and soil properties determine variation in leaf traits

Plant leaves in forests play a key role in energy capture for photosynthesis, and they are an important link between individuals and biogeochemical cycles

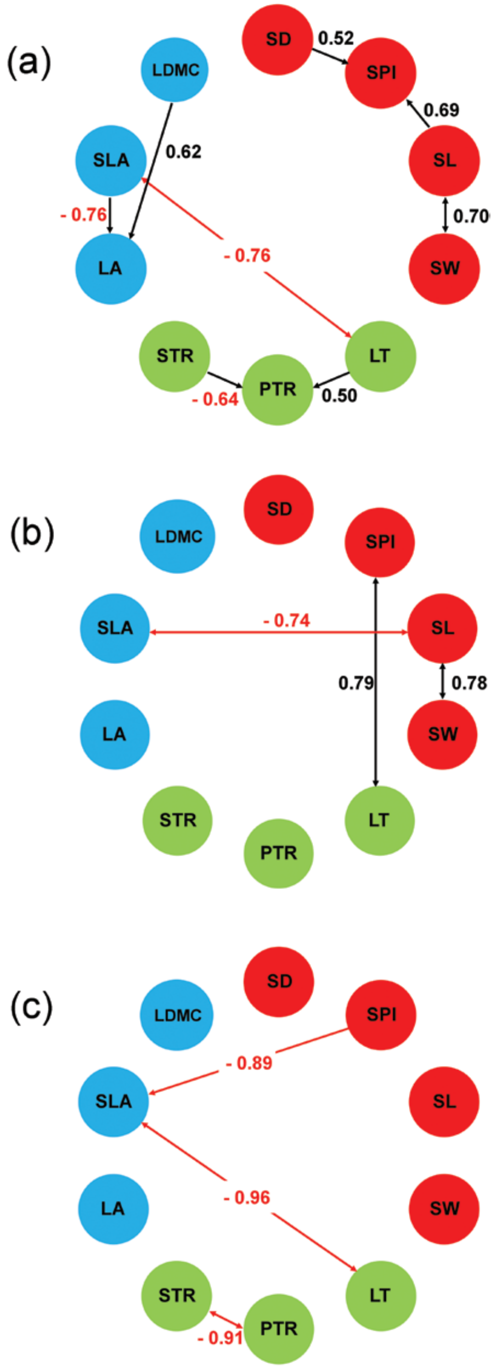

Figure 4: Correlations of leaf traits of *Cyclobalanopsis* species at (**a**) low elevation (400–700 m), (**b**) intermediate elevation (700–1000 m) and (**c**) high elevation (1000–1400 m). The traits in red, green and blue circles represent stomatal, anatomical and morphological traits, respectively. The solid lines linking two traits indicate significant correlations ($P < 0.05$, black for positive and red for negative correlations). The absence of a line between two traits indicates no significant correlation. All abbreviations of leaf traits can be found in Figure 2.

(Vitousek and Sanford 1986). In this study, the significant differences in climatic variables and soil properties along the elevational gradient (Supplementary Fig. S1) exhibited significant effects on stomatal traits with MPA (factor score of mean annual sum precipitation and aridity index) and soil pH being the main drivers (Table 1; Fig. 3). The results imply that changes in water availability and soil pH affect photosynthesis of *Cyclobanipsis* by altering stomatal traits, which was identified by the significant positive correlations between MPA and stomatal traits including SD and SPI, as well as soil pH and SL, SW (Table 1; Fig. 3). This is to be expected because drier and acidifier conditions tend to restrict photosynthesis by reducing leaf stomatal properties.

Variation in anatomical traits was mainly controlled by soil pH (PTR) and MPA (STR), whereas morphological traits, by soil pH (LA) and by MAT (LDMC) (Table 1; Fig. 3). The results indicate that climatic variables (MAT and MPA) and soil pH determined the response of cell-, tissue- and leaf-level traits of evergreen oaks, which was partly consistent with our second hypothesis and previous findings on other Fagaceae species that water availability determines the variation in leaf traits (Liang *et al.* 2019). At lower elevations, conditions tend to favor resource-acquisitive species (Raich and Schlesinger 1992). In contrast, at a higher elevation, harsh environmental conditions and lower resource availability will favor stress-tolerant species (Cairns 2013). Nevertheless, it is a challenge to understand the degree to which environmental filters affect plant functional traits (Read *et al.* 2014).

Elevation alter the correlations among leaf traits

Coordinated variation in leaf traits is generally considered to reflect plant adaptation (Ramírez-Valiente *et al.* 2019; Wright *et al.* 2004). At low elevation, leaf traits of *Cyclobalanopsis* species were closely correlated within stomatal, anatomical and morphological traits (Fig. 4a). At higher elevation, however, the correlations among leaf traits at the cell, tissue and organ levels became stronger (Fig. 4b and c). These notable changes with elevation suggest that evergreen oaks have flexible ecological trade-offs in different habitats. The results were consistent with some woody species in temperate forests that trade-offs among leaf traits can be affected by elevation (Yin *et al.* 2018). An understanding of leaf traits at the cell (stomata), tissue (anatomy) and organ (morphology) levels is useful for predicting alterations related to carbon and water cycling of the forest ecosystem under climate change (Tarin *et al.* 2020).

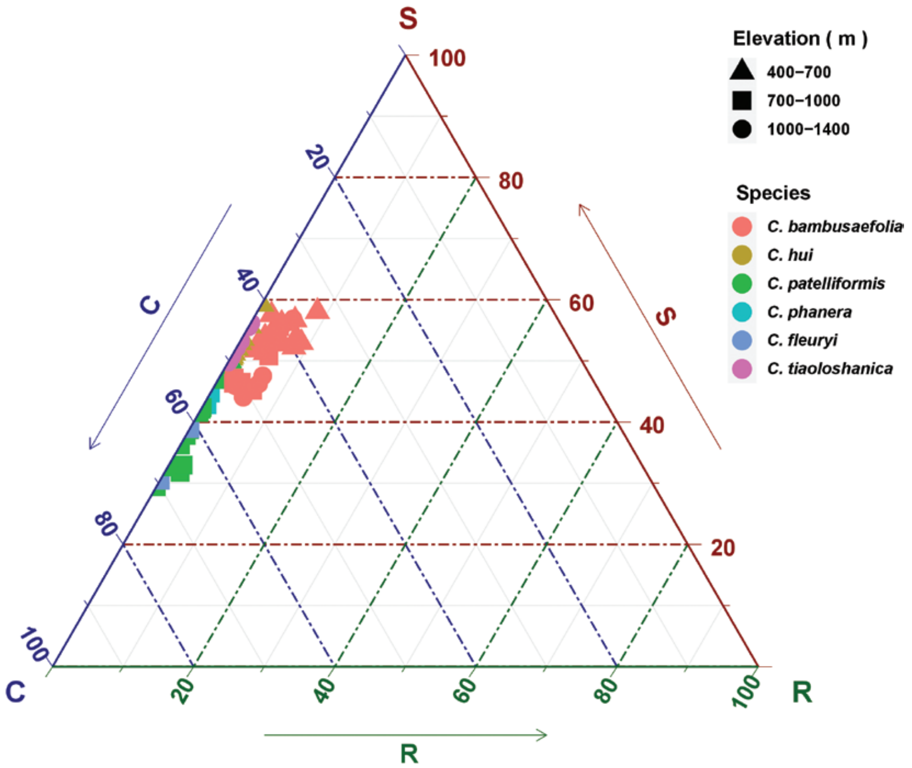

Figure 5: Triangular plot showing shifts in CSR (competition, stress tolerance, ruderal) strategies of *Cyclobalanopsis* species in a tropical forest of Jianfengling National Nature Reserve as affected by elevation. Shifts in the relative proportion of C-, S- and R strategies are indicated by the direction of arrows in the plot.

Cyclobalanopsis species likely adapt to environmental change

Key leaf traits like LA, SLA and LDMC are used to classified ecological strategies according to CSR theory (Grime 1977; Pierce *et al.* 2017). Assessed by the little variation of strategies at low and high elevations (Fig. 4), we suggest that *Cyclobalanopsis* species exhibiting convergent strategies are competitive but invest especially in stress tolerance with slow growth and resource conservation (Reich 2014). Specifically, the *C. bambusaefolia* which inhabited in low elevations, and *C. tiaoloshanica* that grown at high elevations distributed the position toward S strategy.

At intermediate elevations, though with generally relatively low levels of disturbance, some *Cyclobalanopsis* species such as *C. patelliformis* and *C. fleuryi* exhibited a more markedly C strategy, i.e. they allocated resources to growth and maximized resource acquisition, while others still exhibited CS strategies (Fig. 4). Taking the environmental variables along the elevational gradient into consideration, we infer the shifts of ecological strategies from stress tolerance (intermediate elevation) to competition (low and high elevations) are due to the significant changes in soil phosphorus content and soil pH value among the elevations (Supplementary Fig. S1). The results support the third hypothesis that *Cyclobalanopsis* species in tropical forests can shift their ecological strategies in specific habitats, suggesting an innate ability of oaks to respond to environmental change.

CONCLUSIONS

Analysis of leaf stomatal, anatomical and morphological traits of six *Cyclobalanopsis* species responding to an elevational gradient over a variable tropical forest landscape revealed that the stomatal (at cell level), anatomical (at tissue level) and morphological (at organ level) traits were sensitive to the variation in elevation. The temperature, precipitation and soil pH play important roles in driving variation of the cell-, tissue- and leaf-level traits of *Cyclobalanopsis* species. *Cyclobalanopsis* species exhibit flexible ecological strategies for that they shifting stress tolerance combined with competition strategy at low and high elevations toward more clearly the competitive strategy at intermediate elevations.

Research on a range of leaf traits along elevational gradients provides new insights into the potential response and adaptability of dominant tree species in tropical forests to future climate change.

Supplementary Material

Supplementary material is available at *Journal of Plant Ecology* online.

Table S1: Edaphic and climatic variables along the elevation gradient in a tropical forest within Jianfengling National Natural Reserve.

Table S2: The effects of elevation on leaf traits identified by general linear-mixed effect models, in which leaf traits as fixed effects (elevation) and species as random effects (species).

Table S3: The relative influence of environmental variables on the studied leaf traits of evergreen oaks based on aggregated boosted tree (ABT) analysis.

Table S4: The CSR classification of the evergreen oaks in a tropical forest within Jianfengling National Natural Reserve.

Figure S1: The variations of environmental variables along the elevation gradient in a tropical forest within Jiangfengling National Nature Reserve.

Figure S2: Principal component analysis (PCA) analysis of environmental factors along the elevation gradient in a tropical forest within Jiangfengling National Nature Reserve.

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Authors' Contribution

Y.W. and D.X. designed the study; Y.T., L.H., S.B., Z.J., Z.F., Y.L. and M.X. performed the experiments; Y.T., L.H., S.B. and Y.W. analyzed the data; Y.T. and Y.W. drafted the manuscript; Z.F., O.L., J.S., J.P. and L.H. revised the manuscript.

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