

# Sensitivity of leaf physiognomy to climate: applications to habitat-scaled and species-based climate proxy

Fei XU<sup>1,3</sup>, Renqing WANG<sup>2,3</sup> and Weihua GUO<sup>2,3\*</sup>

<sup>1</sup> College of Life Sciences, Shandong Normal University, 250014 Jinan, P. R. China

<sup>2</sup> Institute of Ecology and Biodiversity, College of Life Sciences, Shandong University, 250100 Jinan, P. R. China

<sup>3</sup> Shandong Provincial Engineering and Technology Research Center for Vegetation Ecology, Shandong University, 250100 Jinan, P. R. China

Received: 14 February 2013

Accepted after revision: 6 December 2013

Leaf physiognomy is climate-sensitive and used for quantitative climate reconstruction by applying leaf-climate correlations. Most studies have focused on site-level means of species sets in large-scale environments. The sensitivity of leaf physiognomy to habitat microclimate within species is poorly known, which limits our understanding of leaf-climate relationships and applications to climate proxies for forest monitoring. An experiment was performed in the present study to investigate the responses of leaf size, shape, and venation pattern in the seedlings of *Quercus acutissima* to different gradients of water and light availability. Multiple linear regressions and a contour extraction method were developed and their ability to predict microclimate was assessed by using variables derived from leaf physiognomy. The trends of leaf morphological variations along the gradients share a general resource acquisition and conservation enhancement pattern. The synergy of leaf size, shape, and venation pattern optimized the tradeoff relationship between investment and return of restricted resources. The water-induced plasticity of leaves was lower compared to light-induced plasticity, which resulted in the predictive methods' poor ability to estimate water availability compared to their ability to estimate light availability. The contour extraction method was more precise, especially for combined predictions of extreme environments because multiple linear regressions exhibited overestimation and underestimation at lower and higher gradients, respectively. The present study demonstrated that intraspecific variations of leaf physiognomy can provide a functional link to habitat, and climate proxies based on these relationships may contribute useful information towards forest management.

**Key words:** climate proxies, contour extraction method, leaf physiognomy, multiple linear regression, water and light availability.

This open-access article is distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, printing, distributing, transmitting and reproduction in any medium, provided the original author and source are appropriately cited. Full text and supplementary material (if any) is available on [www.jbr.gr](http://www.jbr.gr)

## INTRODUCTION

Plants have evolved to exist in conditions that are rarely ideal for normal maintenance and may be at the survival limit. In response, plants can adapt to avoid and overcome stress by using various rapid or slow responding mechanisms, such as leaf movement and phenotypic plasticity (Kato *et al.*, 2003; Shepherd &

Griffiths, 2006; Xu *et al.*, 2009a). As the fundamental energy unit of plants, leaves are considered as a nexus between plants and environments, which can have important ecological implications for species survival, growth, and distribution. Recently, general scaling relationships between leaf traits and climate have been the subject of interest. A worldwide “economic” spectrum of correlated leaf traits (that can provide a link between the various environmental factors and leaf

\* Corresponding author: e-mail: [guo\\_wh@yahoo.com](mailto:guo_wh@yahoo.com)

functions) has been identified (Niinemets, 2001; Westoby *et al.*, 2002; Westoby & Wright, 2003; Wright *et al.*, 2004, 2007; Shipley *et al.*, 2006a) and widely used from functional individuals to communities and ecosystems (Garnier *et al.*, 2004; Shipley *et al.*, 2006b; Westoby & Wright, 2006). In addition, this spectrum of traits has become the proxy for reconstructing paleoclimates or predicting future climates (Royer *et al.*, 2005; Whitfield, 2006).

Leaf morphological characteristics are a useful visual guide for constructing relationships between different plants as well as between plants and their environments (Navas & Garnier, 2002; Roche *et al.*, 2004). Leaf physiognomy can serve as an excellent tool for botanical and ecological studies (Traiser *et al.*, 2005). Plant species with widely varying leaf shapes, sizes and venation co-occur in vegetation. The significance of leaf variations for species niche differentiation is still not entirely understood, whereas the differences in leaf physiognomy have been the subject of extensive research (Niinemets *et al.*, 2007b). The morphological traits of leaves are often used in taxonomy. However, attention needs to be given to the problem of the reliability of leaf morphological characteristics, which are dependent on environmental conditions (Viscosi *et al.*, 2009). The development of digital image processing and analysis technology has improved the ability to recognize and conduct geometric measurements of leaf morphology in the field (Du *et al.*, 2007), which has led to the increased number of studies on the sensitivity of leaf physiognomy to climate.

Environmental sensitivity of leaf physiognomy has led to an upsurge in developing the technique for climate proxies. The most common leaf physiognomic methods are leaf-margin analysis and leaf-area analysis, both of which are based on a single variable, namely, the percentage of untoothed species at a site and site-mean leaf size, respectively (Peppe *et al.*, 2011). The Climate-Leaf Analysis Multivariate Program, which uses additional categorical leaf states, was developed to obtain more accurate climate estimates compared to the results of univariate approaches (Wolfe, 1995). However, errors and biases were also found in problems related to character definitions, states, and analysis methods in the predictive framework. An ameliorative digital leaf physiognomy, which uses continuous variables to replace the discrete ones, is considerably more accurate because it uses stricter character definitions (Huff *et al.*, 2003). Multiple linear regression models are the preferred multivariate analysis methods for simple application (Royer *et al.*, 2005;

Peppe *et al.*, 2011). Other complex analyses based on computer algorithms have also been applied (Meziane & Shipley, 2001; Blonder *et al.*, 2011). These previous studies were usually based on the averages of several species, i.e. site-based analysis (Greenwood, 2005; Royer *et al.*, 2009). In such case, the climate was predicted at large scales, while predictions at local scale have not been conducted yet. Therefore, species-based analysis of leaf physiognomy may reveal new insights concerning its relation with microclimate. A contour method, which is usually used in geography, can display the continuous variations of multi-parameters and account for small changes of leaf characteristics according to microclimate. In the present study, we originally developed a contour extraction method to construct the visual relationships between leaf physiognomy and microclimate.

The oak species of genus *Quercus* L. generally exhibit large plasticity in leaf morphology and have been commonly used for the analysis of leaf-climate relationships (Sisó *et al.*, 2001; Quero *et al.*, 2006; Royer *et al.*, 2008; Viscosi *et al.*, 2009; Zhu *et al.*, 2012). *Quercus acutissima* Carr. is one of the most widespread oak species in north China and is the dominant deciduous broadleaved species in the study area. The growth of *Q. acutissima* can be mainly affected by restrictions caused by habitat conditions. Increasingly different environmental conditions result in an increasingly different phenotypic plasticity of the whole plant and leaf traits (Xu *et al.*, 2008). In addition, many *Q. acutissima* forests exhibit patch distribution because of anthropogenic disturbance. Other strong competitors, such as *Robinia pseudoacacia* L., are gradually invading and changing native habitats, which lead to the decline in seedling recruitment and decrease in growth rates through aggressive capture of light and water resources (Xu *et al.*, 2010). The adaptive mechanisms may be investigated by analyzing the leaf morphology of *Q. acutissima* and the function of environmental indication in response to diverse water and light conditions, which are conducive for guiding vegetation maintenance and restoration.

We designed an experiment with controlled water and light conditions to investigate responses of leaf size, shape, and venation pattern in the seedlings of *Q. acutissima* to different gradients of water and light availability. In previous studies, we have found that variations of leaf physiognomy in the nature can be simulated artificially and there are interspecific differences between coexisted species (Xu *et al.* 2008, 2009b).

On this basis, the objectives of this study were to (1) investigate the role of intraspecific variation in leaf-climate relationships, (2) develop microclimate prediction models by using variables derived from leaf physiognomy, and (3) assess the potential application of models as microclimate proxies.

## MATERIALS AND METHODS

### *Study site*

The study was conducted at the Fanggan Research Station of Shandong University, Shandong Province, China (36°26'N, 117°27'E). The site is characterized by a warm temperate monsoon climate, with a mean annual temperature of  $13 \pm 1^\circ\text{C}$  and a mean annual precipitation of  $700 \pm 25$  mm, which occurs mostly during the summer. The soil of the area is a cinnamon-type, and the parent material is limestone (Xu et al., 2008). Sawtooth oak (*Q. acutissima*) and black locust (*R. pseudoacacia*) coexist and form mixed forests in this area. The canopy has a dominant layer which reaches 14 m, and the lower limits of the crown are at about 9 m. The leaf area index can reach  $\sim 5.12$  when the trees are flourishing in August (Xu et al., 2009b).

### *Plant materials*

One-year-old seedlings of *Q. acutissima* were used as experimental materials in this study. Acorns of *Q. acutissima* were collected from a hill near the research station in early spring and planted in plastic pots (32 × 29 cm in height × diameter, one acorn in each pot). The soil was a 64:22:14 (v/v/v) mixture of humic soil, sand, and loam, with a saturated water content of 36% by mass, the largest volumetric water content of 28%, and 68% porosity. The pH was 4.4, and the major chemical components included 88.4 g organic matter, 3.7 g total nitrogen and 42.3 mg available phosphorus per kilogram. All the pots were regularly irrigated and subjected to weed control before the beginning of the experiment.

### *Experimental design*

The seedlings were submitted to controlled experiments from July to September. A factorial experiment of two factors (water and light) of four and three levels, respectively, was designed. Water was withheld from the drought groups until the soil moisture reached  $\sim 50\%$  (W2), 30% (W3), and 10% (W4) of field capacity, whereas the well-watered groups received daily irrigation to maintain soil water content between

70% and 80% (W1) of field capacity. All the pots were moved into a rain-out shelter to avoid precipitation disturbance. The soil water content was controlled by gravimetric probe and the pots were weighed daily to maintain the four different water contents. Top irrigation evenly supplemented the water lost via transpiration and evaporation.

The light-control treatment was conducted in shade shelters covered by plastic films or woven black nylon nets. The frame of the shelter was  $5.0 \times 2.5 \times 3.0$  m (length × width × height). The microclimate was monitored daily by using a micro-quantum sensor and a temperature sensor of Mini-PAM (Walz GmbH, Effeltrich, Germany). The average Photosynthetic Active Radiation (PAR) measured from 07:00 to 16:00 was  $544 \pm 71$ ,  $361 \pm 17$ , and  $56 \pm 6.7 \mu\text{mol m}^{-2} \text{s}^{-1}$  in the open field (L1), under plastic films (L2), and nylon nets (L3), respectively. The light transmission ratio was  $\sim 66\%$  and 10% under the stress condition compared with the control. Analysis of variance (ANOVA) found no significant difference ( $p = 0.534$ ) in air temperature between the three light gradients ( $32.8 \pm 0.46$ ,  $32.4 \pm 0.40$  and  $32.1 \pm 0.39^\circ\text{C}$ ).

The Relative Water Content (RWC) and Relative Light Intensity (RLI) were used as the standards to quantify the gradients of water and light availability. RWC was calculated by averaging the diurnal soil water content of the sampled seedlings. The average light intensity of the sampled seedlings was obtained by measuring PAR in four directions where the seedlings were located. RLI was then calculated by dividing the average light intensity with the maximal value. Ten pots were randomly assigned to each water and light availability treatment. The gradients of W2 and W4 were eliminated from the high (L1) and low (L3) gradients of light availability considering the heavy management and measurement workload. After a two-month treatment period, 15 mature leaves from three seedlings per treatment were taken for morphological measurements.

### *Morphological measurements*

The leaf area was measured by using a CI-203 laser area meter (CID Inc., Washington, USA). The linear measurement was taken by using a digital caliper. Leaf dry mass was measured after oven drying at  $80^\circ\text{C}$  for 48 hrs. The detailed information of the morphological parameter measurements and definitions are shown in Figure 1 and Table 1.

TABLE 1. Leaf morphological parameters and their definitions. The two capital letters in the definition demonstrate the linear distance between the corresponding two points presented in Figure 1

Variable	Definition
LA (cm <sup>2</sup> )	Leaf area
LDM (g)	Leaf dry mass
SLA (cm <sup>2</sup> g <sup>-1</sup> )	Specific leaf area (ratio of leaf area to leaf dry mass)
LL (cm)	Leaf length = AB
LW (cm)	Leaf width = FG
LPL (cm)	Leaf petiole length = BC
LE	Leaf elongation (ratio of leaf length to leaf width)
LL/LPL	Leaf length to petiole length ratio
LWD	Leaf widest division = AD/BD
LBD	Leaf bulgy division = AE/BE
NLT (ea)	Number of leaf teeth
MDV (cm)	Mean distance between veins = $2AB / (NLT + 1)$

*Statistical analysis*

Two-way ANOVA with type III sums of squares was used to test the interactive effects of environmental factors on leaf physiognomy. Pearson’s correlations between leaf morphological traits and climate parameters were calculated, and Ordinary Least Square (OLS) regression lines were fitted to predict the habitat information by using variables that were considered to represent the primary responses. The multicollinearity influence could be ignored without using elimination methods (e.g., stepwise or ridge regression) because focus was given only to the predictive power of the functions (Mela & Kopalle, 2002; Dormann *et al.*, 2013). The jackknife-type approach was used to evaluate the accuracy of the regression functions to build a group of virtual data as the test set, and the absolute residuals were used as the predictive criterion. The level of response to the variation of each factor (water and light) was estimated by using the  $PI_{RWC}$  and  $PI_{RLI}$  indices, respectively, which ranged from 0 to 1. The index of plasticity (PI) was calculated as the difference between the maximum and the minimum mean values divided by the maximum mean value (Valladares *et al.*, 2000b). The changes of leaf morphological variables in the different gradients of water and light availability were illustrated in three-dimensional surface and contour plots. The smoothing cubic spline was employed to trace the variability of the leaf variables (Schimek, 2000), and contour extraction was carried out by adjusting the step and mi-

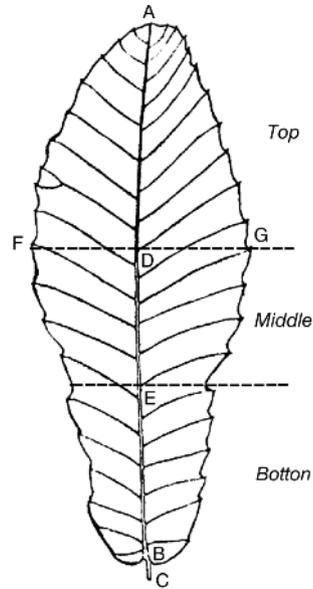


FIG. 1. Illustrated diagram of leaf morphology measurements in *Q. acutissima*. The dashed lines perpendicular to the midrib indicate the widest (above) and bulgy (below) part of the leaf lamina. The two lines are determined by the angles between the midrib and the edge of the lamina. The positions where the angle is the smallest and the largest represent the widest and bulgy part of the leaf lamina, respectively. The leaf is divided into three fractions (top, middle, and bottom) by these two lines. The capital letters along the lines represent the positions located at the midrib or the edge of the lamina (see Table 1 for more details).

nor number for values of the Z-axis (data of leaf morphology). The intersections of contours from different leaf variables, which are considered to provide information about the habitat, were easily fixed by graphmerged and data-drawn application. All statistical analyses were performed by using the SPSS 13.0 software package (SPSS Inc., Chicago, USA). Plots were drawn by using the Statistica 6.0 software (StatSoft Inc., Oklahoma, USA).

RESULTS

LA significantly increased at increasing water and light availability (Fig. 2A, Table 2). The range of the changes was broader with sufficient resources than with seedlings under serious stress. Similar to LA, variables LDM, LL, and LW were positively correlated with water and light availability, respectively (Fig. 2B, D, E). SLA showed a positive correlation with water availability, but was negatively correlated with light availability. The variations were nearly linear in the surface plot (Fig. 2C). Although LPL was significantly correlated with water and light availability, the trend was not consistent with environmental gradients be-

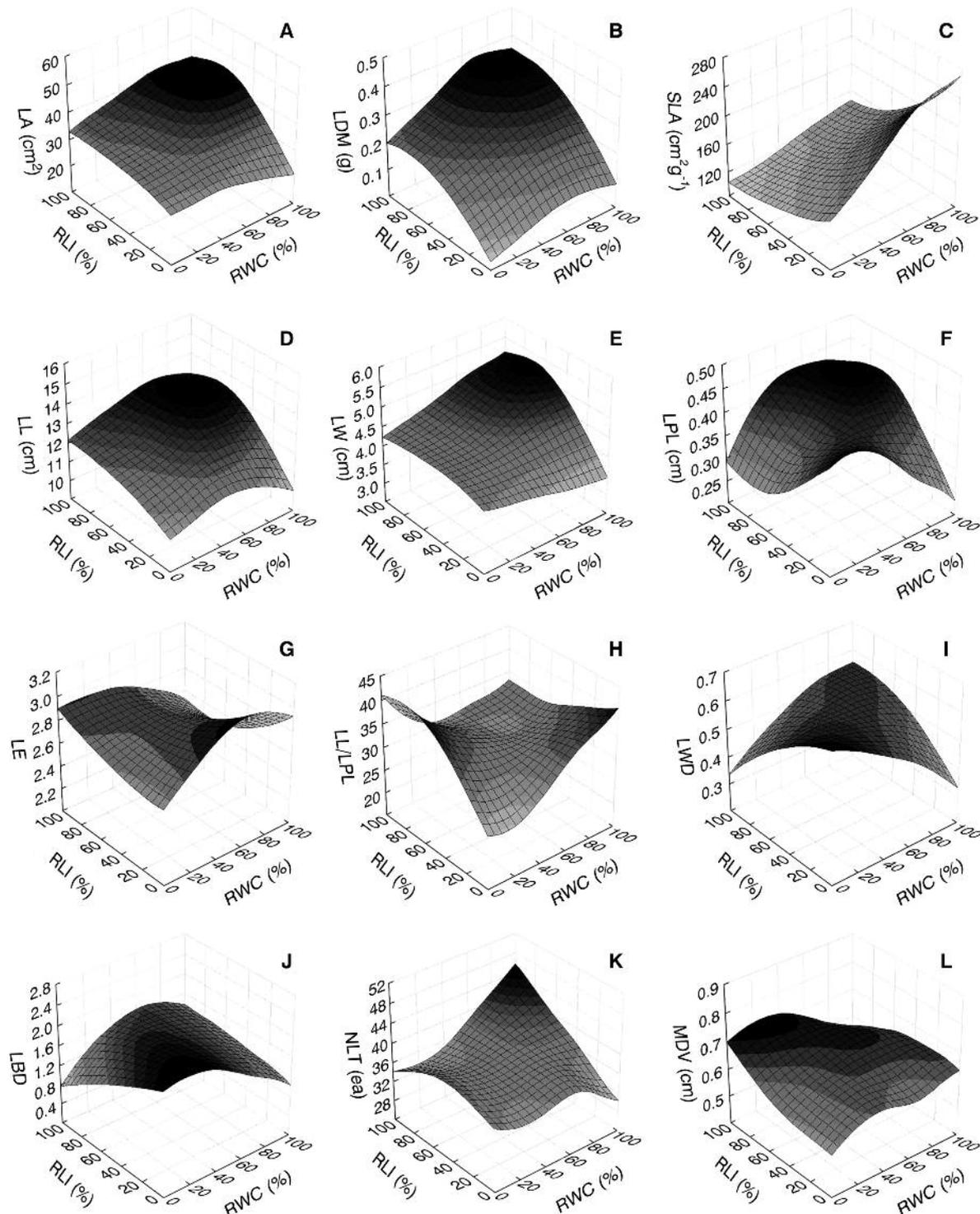


FIG. 2. Three-dimensional surface plots for LA (A), LDM (B), SLA (C), LL (D), LW (E), LPL (F), LE (G), LL/LPL (H), LWD (I), LBD (J), NLT (K) and MDV (L) of *Q. acutissima* in the different gradients of water and light availability. The gray-scale maps indicate that the darker chroma corresponds to the higher value of leaf morphological variable. The spline function is employed in the curved surface fitting and  $n = 120$  for each variable contained climate information.

TABLE 2. Pearson's correlation coefficients between *Q. acutissima* leaf morphological traits and climate parameters (N = 120 for each leaf trait, and n = 24 for each factor of environments with five replications). Significance level: <sup>ns</sup> $p > 0.05$ , \* $p \leq 0.05$ , \*\* $p \leq 0.01$ , \*\*\* $p \leq 0.001$

Variable	LA	LDM	SLA	LL	LW	LPL	LE	LL/LPL	LWD	LBD	NLT	MDV
LDM	0.76 <sup>***</sup>											
SLA	-0.31 <sup>***</sup>	-0.43 <sup>***</sup>										
LL	0.91 <sup>***</sup>	0.73 <sup>***</sup>	-0.21 <sup>*</sup>									
LW	0.95 <sup>***</sup>	0.73 <sup>***</sup>	-0.38 <sup>***</sup>	0.75 <sup>***</sup>								
LPL	0.43 <sup>***</sup>	0.32 <sup>***</sup>	-0.13 <sup>ns</sup>	0.44 <sup>***</sup>	0.37 <sup>***</sup>							
LE	-0.36 <sup>***</sup>	-0.26 <sup>**</sup>	0.33 <sup>***</sup>	0.03 <sup>ns</sup>	-0.63 <sup>***</sup>	-0.05 <sup>ns</sup>						
LL/LPL	0.11 <sup>ns</sup>	0.11 <sup>ns</sup>	0.01 <sup>ns</sup>	0.16 <sup>ns</sup>	0.07 <sup>ns</sup>	-0.80 <sup>***</sup>	0.07 <sup>ns</sup>					
LWD	0.02 <sup>ns</sup>	0.12 <sup>ns</sup>	0.00 <sup>ns</sup>	-0.05 <sup>ns</sup>	0.09 <sup>ns</sup>	0.03 <sup>ns</sup>	-0.22 <sup>*</sup>	-0.06 <sup>ns</sup>				
LBD	-0.05 <sup>ns</sup>	-0.06 <sup>ns</sup>	-0.02 <sup>ns</sup>	-0.18 <sup>*</sup>	0.05 <sup>ns</sup>	-0.08 <sup>ns</sup>	-0.29 <sup>**</sup>	-0.03 <sup>ns</sup>	0.66 <sup>***</sup>			
NLT	0.17 <sup>ns</sup>	0.23 <sup>*</sup>	0.02 <sup>ns</sup>	0.31 <sup>***</sup>	0.06 <sup>ns</sup>	0.02 <sup>ns</sup>	0.25 <sup>**</sup>	0.18 <sup>*</sup>	0.16 <sup>ns</sup>	-0.05 <sup>ns</sup>		
MDV	0.51 <sup>***</sup>	0.31 <sup>***</sup>	-0.19 <sup>*</sup>	0.46 <sup>***</sup>	0.48 <sup>***</sup>	0.32 <sup>***</sup>	-0.17 <sup>ns</sup>	-0.06 <sup>ns</sup>	-0.22 <sup>*</sup>	-0.13 <sup>ns</sup>	-0.69 <sup>***</sup>	
RWC	0.24 <sup>**</sup>	0.28 <sup>**</sup>	0.40 <sup>***</sup>	0.24 <sup>**</sup>	0.22 <sup>*</sup>	0.27 <sup>**</sup>	-0.04 <sup>ns</sup>	-0.15 <sup>ns</sup>	-0.01 <sup>ns</sup>	-0.14 <sup>ns</sup>	0.20 <sup>*</sup>	-0.02 <sup>ns</sup>
RLI	0.42 <sup>***</sup>	0.71 <sup>***</sup>	-0.72 <sup>***</sup>	0.38 <sup>***</sup>	0.43 <sup>***</sup>	0.23 <sup>*</sup>	-0.20 <sup>*</sup>	-0.03 <sup>ns</sup>	-0.16 <sup>ns</sup>	-0.26 <sup>**</sup>	0.08 <sup>ns</sup>	0.21 <sup>*</sup>

cause of a high value under combined drought and shade conditions (Fig. 2F).

LE and LL/LPL were found to increase in shade and decrease in sunlight with increasing water availability, whereas LWD and LBD showed inverse trends (Fig. 2G-J). Conflicting variations also occurred in the gradients of light availability. LE and LL/LPL increased in drought and decreased under well-watered conditions, whereas LWD and LBD decreased in drought and increased in well-watered conditions with increasing light availability. These results caused either weak or zero correlations for these four variables with water and light availability (Table 2).

NLT only showed significant correlation with water availability (Table 2). Water scarcity resulted in fewer leaf teeth. NLT increased along the gradient of light availability with abundant irrigation. On the contrary, NLT decreased in high irradiance when the drought stress was a burden (Fig. 2K). MDV only showed significant correlation with light availability and mainly increased with increasing light availability except when the water resources were sufficient. The maximum values of MDV appeared when high irradiance and serious drought were simultaneously imposed (Fig. 2L).

The correlation coefficients of leaf traits to RWC were smaller than those of RLI, except for LPL, LL/LPL, and NLT, whose water-induced responses were higher and under the diagonal of the bivariate diagram (Fig. 3). A high variation existed in the degree of response to light *versus* water. The response to light had a mean value of 0.17 (range 0.03-0.54), while the response to water had a mean value of 0.12 (range 0.02-0.30).

The overall determination coefficient of multiple linear regression was larger for RLI than that for RWC (Table 3). The mean values of absolute residuals were 9.20 for RWC and 6.43 for RLI, which indicated a 95% confidence interval on the RWC prediction wider than that of RLI (Fig. 4). The predicted values were significantly overestimated and significantly underestimated in the lower left and upper right of the scatter plots, respectively, for both RWC and RLI predictions by *t*-tests ( $p < 0.05$ ).

Actually, the leaf traits did not show absolutely linear correlations with RWC and RLI, especially in the prediction of the bivariate of dependence. The effects of linear and spline functions fitted on the leaf traits to the environmental gradients in contour plots for the case of LDM are shown in Figure 5. LDM was used as an example because it was significantly corre-

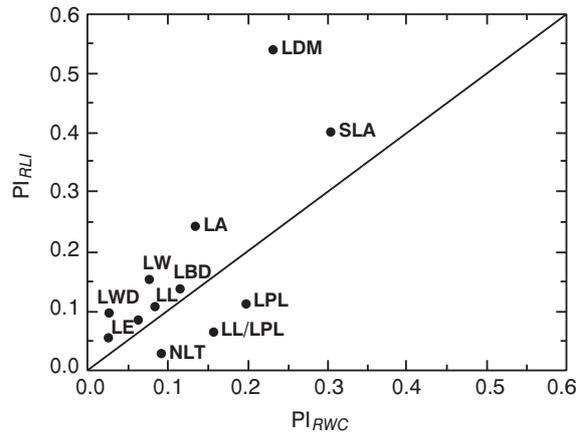


FIG. 3. Bivariate diagram of the comparative plasticity indices to RWC ( $PI_{RWC}$ ) versus the plasticity indices to RLI ( $PI_{RLI}$ ) for leaf morphological traits of *Q. acutissima*. PI was calculated as (maximum value – minimum value) / maximum value.

lated with both RWC and RLI (Table 2). The contours fitted by the linear functions showed a disordered trend of LDM variations in the different gradients of water and light availability. For example, the larger LDM (0.34 g) in W1L2 was located on the lower contour when compared to that (0.28 g) in W3L1 (Fig. 5A). The contours fitted by the spline functions resolved the problem (Fig. 5B). The differences between the linear and spline contours can explain the biased predictions in the multiple linear regressions. A higher probability of overestimating RWC and RLI exists

TABLE 3. Multiple linear regression functions for predicting RWC and RLI of *Q. acutissima* from leaf physiognomy. Ordinary least squares (OLS) regressions were used by setting climate as the dependent variable and leaf traits as the independent variables. The leaf parameters chosen in the functions show significant correlations with RWC and RLI according to the analysis in Table 2. The significance level of each variable: <sup>ns</sup> $p > 0.05$ , \* $p \leq 0.05$ , \*\* $p \leq 0.01$ , \*\*\* $p \leq 0.001$

Variables	Coefficient for RWC	Coefficient for RLI
Intercept	-154.91*	-150.99*
LA (cm <sup>2</sup> )	-2.56 <sup>ns</sup>	-1.75 <sup>ns</sup>
LDM (g)	115.50***	239.98***
SLA (cm <sup>2</sup> g <sup>-1</sup> )	0.33***	-0.36***
LL (cm)	2.67 <sup>ns</sup>	-20.15*
LW (cm)	29.23 <sup>ns</sup>	65.04*
LPL (cm)	76.12**	25.75*
LE	–	90.42*
LBD	–	-23.45***
NLT (ea)	0.55*	–
MDV (cm)	–	0.57 <sup>ns</sup>
$r^2$	0.49***	0.81***

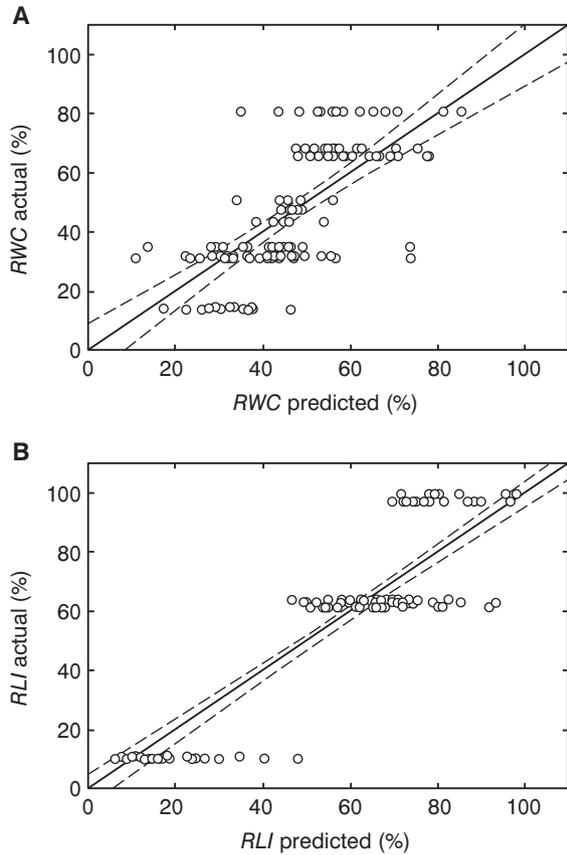


FIG. 4. Actual and predicted values of RWC (A) and RLI (B) from the multiple linear regressions shown in Table 3. Dashed lines indicate 95% confidence intervals.

when both were at the lower gradients because the linear contour was above the spline contour with the same values in the shadowed area (Fig. 5C). The area outside the shadowed area indicated an underestimation that frequently occurs at the higher gradients.

We applied the contours of SLA and NLT, which are widely used as important leaf traits functionally linked to climate, in the contour extraction method to gauge the accuracy of predictions (Fig. 6 and online supplementary material Fig. S1). The intersections directly showed the environmental information in the plots. The mean values of absolute residuals were 3.45 for RWC and 2.50 for RLI. Furthermore, a paired *t*-test indicated that the values of the residuals were significantly smaller in the contour extraction method than in the multiple linear regressions for RWC ( $p = 0.014$ ) and RLI ( $p = 0.038$ ), respectively.

## DISCUSSION

### Sensitivity of leaf size and shape to climate

In the current research, leaf size was obviously restricted by the shortage of water and light as shown by

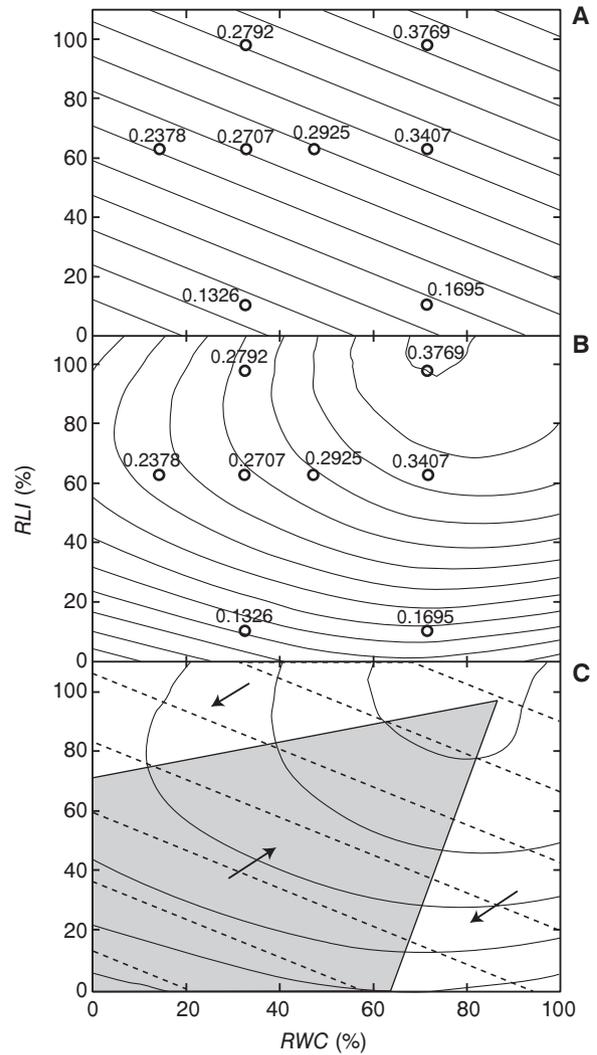


FIG. 5. Contour plots for LDM of *Q. acutissima* in the different gradients of water and light availability. The linear (A) and spline (B) functions are employed to test the effect of fitted curve. The scatters are the locations of LDM in every treatment, and the means are listed as the labels. When overlapped, these two contour plot lines with the same values intersected at two points (C). The diagram can be divided into two parts by sketching the intersections together. The arrow in the shadowed area indicates the average values of RWC and RLI that should be overestimated by using the linear function instead of the spline function. The arrows in the white area contrast with the shadowed area.

LA and LDM. Although leaf size variation in *Q. acutissima* can be partly linked to allometric factors, the ecological strategy with respect to environmental stress has an important role (Xu et al., 2008, 2009b). Variations in leaf size along the climatic gradients may result from higher water demand and overheating of larger leaves because of photosynthesis-transpiration compromise and heat dissipation. The trend of selecting relatively small leaves may be caused by o-

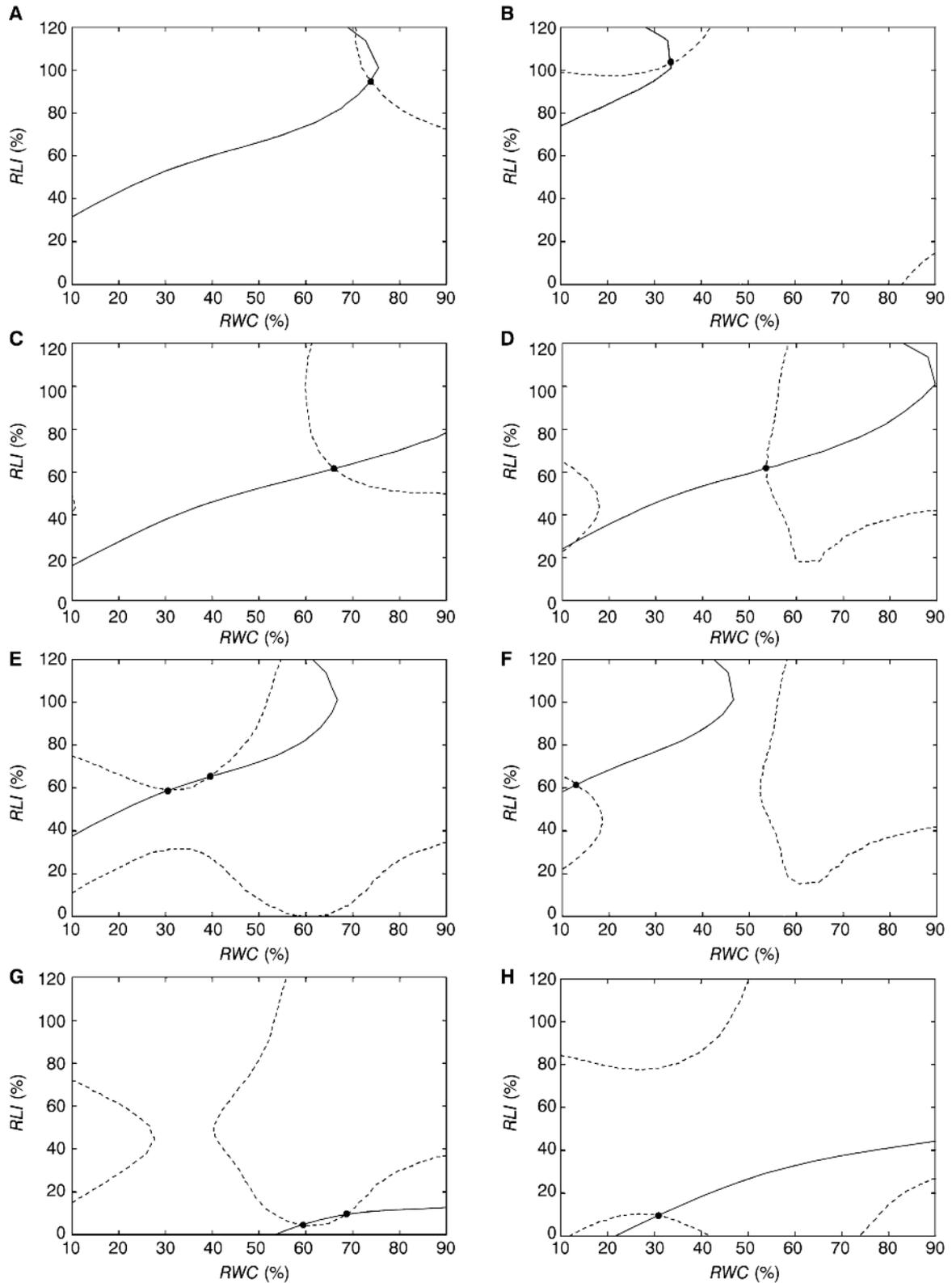


FIG. 6. Validation experiment for the RWC and RLI of *Q. acutissima* in the treatment of W1L1 (A), W3L1 (B), W1L2 (C), W2L2 (D), W3L2 (E), W4L2 (F), W1L3 (G) and W3L3 (H) by using the contour extraction method. Solid and dashed lines refer to the contours of SLA and NLT, respectively. The intersections are established by the tool of drawing data. When two intersections appear in a plot, the mean value is used as a substitute.

verall resource limitation in stressful environments, which makes the construction of large leaves with extensive vascular and cell-wall fractions overly expensive and will reduce the investment in LDM (Niinemets *et al.*, 2007a). Furthermore, smaller leaves have an advantage in minimizing self-shading (Falster & Westoby, 2003).

An integrated variation of LA and LDM can be expressed by SLA. This functional trait of leaves is important in connection with other traits and the climate (Wright *et al.*, 2004). To achieve different ameliorative aims under resource stress, the variations along the gradients of water and light availability exhibited divergence. Lower values in water-limited environments tend to correspond to relatively high investments in leaf defenses and long leaf lifespan. Shade-tolerant leaves have remarkably larger SLA, which is an accommodation to decrease self-shading of chloroplasts in the abaxial surface of leaves (Quero *et al.*, 2006). Another advantage is that the construction and maintenance costs of the production of leaves with more symplastic component in shade are reduced (Lusk *et al.*, 2008).

LE, calculated as LL divided by LW, characterizes the overall slenderness of the leaves (Niinemets *et al.*, 2007b), and often changes with leaf size because of the effect of allometry. However, plasticity to environmental conditions also occurs (Tsialtas & Maslaris, 2007; Xu *et al.*, 2009b). Leaves became longer instead of wider in the open field with the decrease of water availability. This correlation most likely allows the leaves to reduce transpiration by reducing the size of the boundary layer and to shed heat better in warm habitats. Leaves were also narrower in the shade and moist conditions, which is thought to be an adaptation to decrease self-shading and curliness of leaves with high SLA.

Leaf shape was not only represented by the integrated changes of leaf major and minor axes, but the distribution of leaf area fractions (partitioned by LWD and LBD) in the present research also includes additional information. When LE increased, lamina areas located close to the leaf apex decreased, which represents the narrow part of the leaf increasing in proportion to the whole leaf lamina. The cooperation between LE and leaf area fractions was also displayed in the case of the formation of wider leaves, which are capable to maximize their areas for light capture.

### *Synergy between leaf venation pattern and leaf dimension*

In the present study, the changes of LPL were consistent with leaf size, which provides an available proxy for leaf size from petiole dimensions when the leaf blade was disfeatured (Jordan, 2011). The elongation of petioles will achieve optimal leaf display to deal with the shade stress. The increases in the relative distance of LA from the stem by longer petioles can reduce the between-row shading. Furthermore, a leaf with a narrow blade can reduce both between-row and within-row shadings (Takenaka, 1994). Leaf petiole and shape alternatively contributed to larger light capture in this research, shown by the similar changes in LE and LL/LPL, as plants had a tradeoff between the need for increasing interception areas and support structures. Increasing the investment in petioles requires the synthesis of more xylogens and limits the biomass invested into functional leaf activity. Longer petioles also have a disadvantage which leads to the bending of the shaded leaf in a moist habitat (Pickup *et al.*, 2005).

Large leaves require disproportionately more mass allocated to petioles and veins for mechanical support (Niklas *et al.*, 2007), especially for thin leaves. A shade-tolerant leaf shape, whose centroid is far from the leaf base, requires higher vein density to withstand the increased bending moment. Also, closer spacing of veins (decreased MDV) results in both higher water fluxes and carbon assimilation rates because of shorter path lengths between veins and stomata (Brodrribb *et al.*, 2007; Brodrribb & Field, 2010). However, investments in support will transform the invested biomass into functional leaf activity. This inverse relationship is likely because of the displacement of the lamina tissue by non-photosynthetic venation tissue (Poorter *et al.*, 2006). Xeromorphic leaves eliminated the dependence of veins because of the self-support of lamina, which can rely on lamina cells (non-specific support) in addition to vasculature (Niinemets, 2001).

Characteristics of leaf teeth are functional traits to reflect a tradeoff between carbon uptake and water loss. The relationship between leaf teeth and enhanced sap flow may help explain why leaf teeth are more absent in drought environments where the water cost associated with teeth may be more important (Peppe *et al.*, 2011). This pulse in gas-exchange activity may be also adaptive in a shade condition because it may extend the season of potential growth (Royer & Wilf, 2006). In contrast, tooth-driven pulse in sap flow is

more muted for thin leaves in wet conditions, because the potential benefit is outweighed by the diminishing proportion of functional leaf tissues to support structures. The present study highlights water availability as an important factor that affects NLT. Variations of the leaf-margin state in light availability may also be a likely effect of water availability because shade stress causes less water consumption.

#### *Application to predictions*

The study results are consistent with the previous findings, which showed that seedlings of deciduous species are more tolerant to shade but not necessarily to drought (Quero *et al.*, 2006; Sanz-Pérez & Castro-Diez, 2010; Zhu *et al.*, 2012). The adaptive mechanisms are mainly to lessen shade stress, even under serious drought. Also, the correlation coefficients and plasticity indices are larger under light availability compared to water availability. The fact that neither multiple linear regression nor contour extraction is better in estimating RWC than RLI indicates that leaf traits respond less to water than to light availability. This trend was also found in models predicting large-scale precipitation (Jacobs, 2002; Peppe *et al.*, 2011).

The parameters taken into account ensure the accuracy of multiple linear regressions. The inclusion of certain leaf morphological variables (e.g. variables of leaf-margin state), which are significantly correlated with the predicted climate, will improve the predictive power (Royer *et al.*, 2012). Although some leaf traits exhibit multi-collinearity with each other, this multi-collinearity may not be a problem for the prediction. The models can achieve accurate predictions when the overall determination coefficients  $r^2$  are large enough, because the combined linear relationships of explanatory variables will continue to apply in all cases (Mela & Kopalle, 2002). The sample and amount of independent variables will also affect the coefficients of each variable and the constants of the whole regression functions to cause the instability of the predictive system. Even if the slopes of the regressions that include the diverse variables show no statistical difference, the probability of misestimating may increase toward the ends, which reflects an overestimation to the lower end and underestimation to the upper end in the present study. This bias is caused by the rigid characteristics of the linear model, which can not be adjusted with the values of non-linear variables.

The contour extraction method in the present research had some advantages in climate prediction over multiple linear regressions. First, the characteristics of our prediction system are flexible for using spline curves. Therefore, a good foundation for predictive accuracy is established. Second, more alternatives are available in choosing leaf traits for prediction. Any two or more leaf traits that are not perfectly collinear can locate the climate information. The contour extraction method is suitable for application to dead or fossil leaves, because some leaf traits (e.g. SLA) cannot be reliably reconstructed (Royer *et al.*, 2007). Third, the bi-variables of environmental gradients can be synchronously estimated, which means that the variability of leaf traits should consider the effects of double environmental factors at the same time. However, the effects are discrete in multiple linear regressions because of one function for each environmental factor prediction. The ignored factor will introduce an error in estimation of another factor, because at a given value of one factor, the leaf traits will also vary significantly as a result of the effect of the other factors (Peppe *et al.*, 2011). This may be another reason why the accuracy of multiple linear regressions is lower than that of contour extraction method. However, the contour extraction method has been recognized to have its own shortcomings in prediction. The value intervals for the test set are limited because the contours are drawn based on the sample set. Any leaf whose values of traits are beyond the range of the models will have no contour to extract. Therefore, this method does not fit for leaves derived from different regions or growth phases as well as those abnormally derived. Furthermore, the method cannot perform batch processing as one value of leaf trait corresponds to one contour to extract.

#### *Implications and future research*

Sensitivity of leaf physiognomy to climate established a foundation for constructing and improving leaf-climate analysis methods for microclimate prediction. The oak species investigated in the present study share a general trend of leaf traits that can be suited to a tradeoff demand for resources as well as part of a conservative resource-use strategy (Valladares *et al.*, 2000a; Xu *et al.*, 2010). The response of the leaves can even occur due to the effect of climate in the current-year growing season, which serves as a significant extension for dynamic prediction by investigating the leaves in different temporal and spatial scales.

The contour extraction method in the present study is more precise compared with the multiple linear regressions, especially for the combined predictions of extreme environments. However, the diversity of the sampled leaves may result in the invalidity of this method. Therefore, the allometric relationships of leaf traits must be established to improve the applicability of the contour extraction method (Niklas *et al.*, 2009). In addition, other environmental factors (e.g. air temperature, soil fertility, wind) may affect the leaf morphological plasticity in the field (Royer *et al.*, 2008). Regional differences and genetic variability will also introduce errors to leaf-climate relationships (Hameed *et al.*, 2012). Further extensions, coupled with more detailed experimental measurements of the traits and modified relationships between leaf and climate, will enable leaf physiognomy to become a robust proxy to macro- and microclimate.

#### ACKNOWLEDGMENTS

The present work was financially supported by the National Natural Science Foundation of China (Nos. 31200143 and 31270374). We are grateful to Yinghua Wei and Yuanzu Xu for building the experimental equipment and to Weihong Xu and Yue Yu for the measurement assistance.

#### REFERENCES

- Blonder B, Violle C, Bentley LP, Enquist BJ, 2011. Venation networks and the origin of the leaf economics spectrum. *Ecology Letters*, 14: 91-100.
- Brodribb TJ, Field TS, 2010. Leaf hydraulic evolution led a surge in leaf photosynthetic capacity during early angiosperm diversification. *Ecology Letters*, 13: 175-183.
- Brodribb TJ, Feild TS, Jordan GJ, 2007. Leaf maximum photosynthetic rate and venation are linked by hydraulics. *Plant Physiology*, 144: 1890-1898.
- Dormann CF, Elith J, Bacher S, Buchmann C, Carl G, Carré G, Marquéz JRG, Gruber B, Lafourcade B, Leitão PJ, *et al.*, 2013. Collinearity: a review of methods to deal with it and a simulation study evaluating their performance. *Ecography*, 36: 27-46.
- Du JX, Wang XF, Zhang GJ, 2007. Leaf shape based plant species recognition. *Applied Mathematics and Computation*, 185: 883-893.
- Falster DS, Westoby M, 2003. Leaf size and angle vary widely across species: what consequences for light interception? *New Phytologist*, 158: 509-525.
- Garnier E, Cortez J, Billès G, Navas ML, Roumet C, Debussche M, Laurent G, Blanchard A, Aubry D, Bellmann A, *et al.*, 2004. Plant functional markers capture ecosystem properties during secondary succession. *Ecology*, 85: 2630-2637.
- Greenwood DR, 2005. Leaf form and the reconstruction of past climates. *New Phytologist*, 166: 355-357.
- Hameed M, Batool S, Naz N, Nawaz T, Ashraf M, 2012. Leaf structural modifications for drought tolerance in some differentially adapted ecotypes of blue panic (*Panicum antidotale* Retz.). *Acta Physiologiae Plantarum*, 34: 1479-1491.
- Huff PM, Wilf P, Azumah EJ, 2003. Digital future for paleoclimate estimation from fossil leaves? Preliminary results. *Palaios*, 18: 266-274.
- Jacobs BF, 2002. Estimation of low-latitude paleoclimates using fossil angiosperm leaves: examples from the Miocene Tugen Hills, Kenya. *Paleobiology*, 28: 399-421.
- Jordan GJ, 2011. A critical framework for the assessment of biological palaeoproxies: predicting past climate and levels of atmospheric CO<sub>2</sub> from fossil leaves. *New Phytologist*, 192: 29-44.
- Kato E, Nagano H, Yamamura S, Ueda M, 2003. Synthetic inhibitor of leaf-closure that reveals the biological importance of leaf-movement for the survival of leguminous plants. *Tetrahedron*, 59: 5909-5917.
- Lusk CH, Reich PB, Montgomery RA, Ackerly DD, Cavender-Bares J, 2008. Why are evergreen leaves so contrary about shade? *Trends in Ecology and Evolution*, 23: 299-303.
- Mela CF, Kopalle PK, 2002. The impact of collinearity on regression analysis: the asymmetric effect of negative and positive correlations. *Applied Economics*, 34: 667-677.
- Meziane D, Shipley B, 2001. Direct and indirect relationships between specific leaf area, leaf nitrogen and leaf gas exchange. Effects of irradiance and nutrient supply. *Annals of Botany*, 88: 915-927.
- Navas ML, Garnier E, 2002. Plasticity of whole plant and leaf traits in *Rubia peregrina* in response to light, nutrient and water availability. *Acta Oecologica*, 23: 375-383.
- Niinemets Ü, 2001. Global-scale climatic controls of leaf dry mass per area, density, and thickness in trees and shrubs. *Ecology*, 82: 453-469.
- Niinemets Ü, Portsmouth A, Tena D, Tobias M, Matesanz S, Valladares F, 2007a. Do we underestimate the importance of leaf size in plant economics? Disproportional scaling of support costs within the spectrum of leaf physiognomy. *Annals of Botany*, 100: 283-303.
- Niinemets Ü, Portsmouth A, Tobias M, 2007b. Leaf shape and venation pattern alter the support investments within leaf lamina in temperate species: a neglected source of leaf physiological differentiation? *Functional Ecology*, 21: 28-40.
- Niklas KJ, Cobb ED, Niinemets Ü, Reich PB, Sellin A, Shipley B, Wright IJ, 2007. "Diminishing returns" in the scaling of functional leaf traits across and within species groups. *Proceedings of the National Academy of Sciences of the United States of America*, 104: 8891-8896.

- Niklas KJ, Cobb ED, Spatz HC, 2009. Predicting the allometry of leaf surface area and dry mass. *American Journal of Botany*, 96: 531-536.
- Peppe DJ, Royer DL, Cariglino B, Oliver SY, Newman S, Leight E, Enikolopov G, Fernandez-Burgos M, Herrera F, Adams JM, et al., 2011. Sensitivity of leaf size and shape to climate: global patterns and paleoclimatic applications. *New Phytologist*, 190: 724-739.
- Pickup M, Westoby M, Basden A, 2005. Dry mass costs of deploying leaf area in relation to leaf size. *Functional Ecology*, 19: 88-97.
- Poorter H, Pepin S, Rijkers T, de Jong Y, Evans JR, Körner C, 2006. Construction costs, chemical composition and payback time of high- and low-irradiance leaves. *Journal of Experimental Botany*, 57: 355-371.
- Quero JL, Villar R, Marañón T, Zamora R, 2006. Interactions of drought and shade effects on seedlings of four *Quercus* species: physiological and structural leaf responses. *New Phytologist*, 170: 819-834.
- Roche P, Díaz-Burlinson N, Gachet S, 2004. Congruency analysis of species ranking based on leaf traits: which traits are the more reliable? *Plant Ecology*, 174: 37-48.
- Royer DL, Wilf P, 2006. Why do toothed leaves correlate with cold climates? Gas exchange at leaf margins provides new insights into a classic paleotemperature proxy. *International Journal of Plant Sciences*, 167: 11-18.
- Royer DL, Wilf P, Janesko DA, Kowalski EA, Dilcher DL, 2005. Correlations of climate and plant ecology to leaf size and shape: potential proxies for the fossil record. *American Journal of Botany*, 92: 1141-1151.
- Royer DL, Sack L, Wilf P, Lusk CH, Jordan GJ, Niinemets Ü, Wright IJ, Westoby M, Cariglino B, Coley PD, et al., 2007. Fossil leaf economics quantified: calibration, Eocene case study, and implications. *Paleobiology*, 33: 574-589.
- Royer DL, McElwain JC, Adams JM, Wilf P, 2008. Sensitivity of leaf size and shape to climate within *Acer rubrum* and *Quercus kelloggii*. *New Phytologist*, 179: 808-817.
- Royer DL, Kooyman RM, Little SA, Wilf P, 2009. Ecology of leaf teeth: a multi-site analysis from an Australian subtropical rainforest. *American Journal of Botany*, 96: 738-750.
- Royer DL, Peppe DJ, Wheeler EA, Niinemets Ü, 2012. Roles of climate and functional traits in controlling toothed vs. untoothed leaf margins. *American Journal of Botany*, 99: 915-922.
- Sanz-Pérez V, Castro-Díez P, 2010. Summer water stress and shade alter bud size and budburst date in three mediterranean *Quercus* species. *Trees*, 24: 89-97.
- Schimke MG, 2000. Estimation and inference in partially linear models with smoothing splines. *Journal of Statistical Planning and Inference*, 91: 525-540.
- Shepherd T, Griffiths DW, 2006. The effects of stress on plant cuticular waxes. *New Phytologist*, 171: 469-499.
- Shipley B, Lechowicz MJ, Wright IJ, Reich PB, 2006a. Fundamental trade-offs generating the worldwide leaf economics spectrum. *Ecology*, 87: 535-541.
- Shipley B, Vile D, Garnier É, 2006b. From plant traits to plant communities: a statistical mechanistic approach to biodiversity. *Science*, 314: 812-814.
- Sisó S, Camarero JJ, Gil-Pelegrín E, 2001. Relationship between hydraulic resistance and leaf morphology in broad-leaf *Quercus* species: a new interpretation of leaf lobation. *Trees*, 15: 341-345.
- Takenaka A, 1994. Effects of leaf blade narrowness and petiole length on the light capture efficiency of a shoot. *Ecological Research*, 9: 109-114.
- Traiser C, Klotz S, Uhl D, Mosbrugger V, 2005. Environmental signals from leaves – a physiognomic analysis of European vegetation. *New Phytologist*, 166: 465-484.
- Tsialtas JT, Maslaris N, 2007. Leaf shape and its relationship with leaf area index in a sugar beet (*Beta vulgaris* L.) cultivar. *Photosynthetica*, 45: 527-532.
- Valladares F, Martínez-Ferri E, Balaguer L, Pérez-Corona E, Manrique E, 2000a. Low leaf-level response to light and nutrients in Mediterranean evergreen oaks: a conservative resource-use strategy? *New Phytologist*, 148: 79-91.
- Valladares F, Wright SJ, Lasso E, Kitajima K, Pearcy RW, 2000b. Plastic phenotypic response to light of 16 congeneric shrubs from a Panamanian rainforest. *Ecology*, 81: 1925-1936.
- Viscosi V, Lepais O, Gerber S, Fortini P, 2009. Leaf morphological analyses in four European oak species (*Quercus*) and their hybrids: a comparison of traditional and geometric morphometric methods. *Plant Biosystems*, 143: 564-574.
- Westoby M, Wright IJ, 2003. The leaf size – twig size spectrum and its relationship to other important spectra of variation among species. *Oecologia*, 135: 621-628.
- Westoby M, Wright IJ, 2006. Land-plant ecology on the basis of functional traits. *Trends in Ecology and Evolution*, 21: 261-268.
- Westoby M, Falster DS, Moles AT, Vesk PA, Wright IJ, 2002. Plant ecological strategies: some leading dimensions of variation between species. *Annual Review of Ecology and Systematics*, 33: 125-159.
- Whitfield J, 2006. The cost of leafing. *Nature*, 444: 539-541.
- Wolfe JA, 1995. Paleoclimatic estimates from Tertiary leaf assemblages. *Annual Review of Earth and Planetary Sciences*, 23: 119-142.
- Wright IJ, Reich PB, Westoby M, Ackerly DD, Baruch Z, Bongers F, Cavender-Bares J, Chapin T, Cornelissen JHC, Diemer M, et al. 2004. The worldwide leaf economics spectrum. *Nature*, 428: 821-827.
- Wright IJ, Ackerly DD, Bongers F, Harms KE, Ibarra-Manriquez G, Martinez-Ramos M, Mazer SJ, Muller-Landau HC, Paz H, Pitman NCA, et al., 2007. Relationships among ecologically important dimensions of plant trait variation in seven neotropical forests. *Annals of*

- Botany*, 99: 1003-1015.
- Xu F, Guo W, Xu W, Wang R, 2008. Habitat effects on leaf morphological plasticity in *Quercus acutissima*. *Acta Biologica Cracoviensia Series Botanica*, 50: 19-26.
- Xu F, Guo W, Wang R, Xu W, Du N, Wang Y, 2009a. Leaf movement and photosynthetic plasticity of black locust (*Robinia pseudoacacia*) alleviate stress under different light and water conditions. *Acta Physiologiae Plantarum*, 31: 553-563.
- Xu F, Guo W, Xu W, Wei Y, Wang R, 2009b. Leaf morphology correlates with water and light availability: what consequences for simple and compound leaves? *Progress in Natural Science*, 19: 1789-1798.
- Xu F, Guo W, Xu W, Wang R, 2010. Effects of light intensity on growth and photosynthesis of seedlings of *Quercus acutissima* and *Robinia pseudoacacia*. *Acta Ecologica Sinica*, 30: 3098-3107.
- Zhu Y, Kang H, Xie Q, Wang Z, Yin S, Liu C, 2012. Pattern of leaf vein density and climate relationship of *Quercus variabilis* populations remains unchanged with environmental changes. *Trees*, 26: 597-607.