ESTIMATION OF LEAF AREA, LEAF MASS AND SPECIFIC LEAF AREA FOR TREES OF DIFFERENT LIFE-FORMS IN A KARST FOREST BASED ON LINEAR MIXED-EFFECTS MODELS

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Abstract. Leaves act as an important bridge between plants and the external environment. Many studies have been conducted to establish more accurate and efficient models for predicting values of leaf traits. However, the model based on tree species is not representative enough for tropical and subtropical forests with abundant tree species and complex structures. Additionally, ordinary models are generally insufficient to describe the spatial and temporal changes of leaves because of the variations between trees. Based on linear mixed-effects models (LMM), we estimated the leaf area (LA), leaf mass (LM), and specific leaf area (SLA) for tree species of four life forms in a karst primary forest. Our results suggested that LMM were reasonable and accurate in fitting and predicting LA and LM for different life forms in different seasons. The most accurate predictions were obtained while using the product of leaf length and leaf width. Specifically, LMM performed better ($R^2 = 0.92$ to 0.99 and AIC = 118.5 to 4306.76 for leaf area; $R^2 = 0.88$ to 0.94 and AIC = -4389.5 to -969.1 for leaf mass) than the models only considered the fixed effects ($R^2 = 0.89$ to 0.99 and AIC = 119.1 to 4464.79 for leaf area; $R^2 = 0.79$ to 0.87 and AIC = -3179.7 to -940.2 for leaf mass). The mean absolute error percent values were 0.9%–14.4% for leaf area and 1.1% to 17.1% for leaf mass for four life forms. Considering the accuracy of the models and the sampling effort, the optimal number of sample leaves for SLA estimation was about 60–80.
Keywords: *leaf structure parameters, seasonal variations, horizontal directions of canopy, random effects, non-destructive measurement*

Introduction

Leaves are important for plant growth, biomass, and nutrient conversion and form the basis for the functioning of terrestrial ecosystems (Reich et al., 1992; Kikuzawa and Lechowicz, 2011). As the important leaf morphological traits, leaf area (LA) and leaf mass (LM) estimate the leaf area index, closely relating to the plant photosynthetic efficiency, growth, and productivity (Milla and Reich, 2007; Weraduwage et al., 2015), and strongly indicate climate change and matter cycle interactions (Chen, 2017). Specific leaf area (SLA), the ratio of LM and LA, is an indicator of ecophysiological characteristics such as relative growth rate, photosynthetic capacity, and leaf longevity (Wright and Westoby, 2002; Anderson et al., 2020). Therefore, an accurate estimation of the LA, LM, and SLA can better elucidate the importance of the leaves for the efficient functioning of the forest ecosystem.

The most popular method for measuring broadleaf LA is sampling and using a scanner to scan the leaves, taking images with a fixed camera, or other such digital instruments (Peksen, 2007). Then, LA was calculated by ImageJ software (Gao et al., 2022; Yang et al., 2021), Blackspot leaf area calculator (Varma and Osuri, 2013;

Basnett and Devy. 2021), or Photoshop (Kostadinov and Moteva, 2014; Liang et al., 2010). LM is usually determined by sampling the leaves, drying (in an oven), and weighing (Dwyer et al., 2014; Freschet et al., 2015). These direct methods involve destructive plant sampling, and multiple measurements on the same leaf cannot be conducted (Suárez Salazar et al., 2018). However, due to their ability to measure leaf parameters relatively accurately (Kostadinov and Moteva, 2014), they are often widely used as the basic data for modeling (Wang et al., 2019; Liu et al., 2017) and canopy structure parameters estimating, such as leaf area index (Ern et al., 2020; Liu et al., 2015). Some non-destructive alternative direct methods that can be used to measure leaf area involves using portable scanning planimeters (Lu et al., 2004), portable area meter (Santiago and Wright, 2007; Olivas et al., 2013), RGB-D sensor (Yau et al., 2021), etc. However, these tools are usually expensive and complex to conduct basic studies (Adji et al., 2021). Additionally, model methods (Pompelli et al., 2012; Serdar et al., 2006; Keramatlou et al., 2015) have been developed to estimate leaf parameters because of their merits of being non-destructive, efficient, and highly accurate. Theoretically, they estimate LA/LM by establishing mathematical models between LA/LM and one or more leaf structural parameters (e.g., length or width) (Tondjo et al., 2015; Meng et al., 2015; Cai et al., 2017). In most studies, analysis is usually performed using ordinary least squares models. However, data for modeling is usually recorded from multiple time points (i.e., longitudinal data) or multiple locations (i.e., horizontal data). There is a temporal or spatial autocorrelation in longitudinal or horizontal data (Zhang et al., 2009). For example, there are differences between plots and trees due to the geographic location, site condition, and environmental factors. In such situations, ordinary least squares methods generate certain predictive biases because they rarely consider the correlation of those data and cannot reflect individual differences (Cantoni et al., 2021). Thus, ordinary models are insufficient to describe the spatial and temporal changes in leaves (Zhang et al., 2009). Moreover, leaf traits of some species vary with seasonal changes and present significant spatial variability within canopies (Weiskittel et al., 2008; Nouvellon et al., 2010). Most of the previous studies did not consider the effects of the above two factors when constructing the models. Therefore, improving the accuracy of model estimation is an important problem that needs to be solved urgently.

Compared to traditional regression models, linear mixed-effects models contain fixed and random effects and have the advantages of incorporating diversity data, which can be a better fit and explain the potential effects of random variables that help effectively reveal the sources, such as variations in time and space (Tao, 2002). Recently, linear mixed-effects models have been widely used in forestry research. For example, Cysneiros et al. (2020) modeled the tree height-diameter relationships using linear mixed-effects models in the Atlantic Forest and confirmed the effect of the local environment on the height–diameter relationship of trees. Qi et al. (2020) applied a linear mixed model to estimate the forest biomass of Guizhou province, which solved spatial autocorrelation of the forest biomass caused by the neighborhood space regions. Zheng et al. (2021) collected tree ring growth data from 128 sites for 21 high altitude tree species and used linear mixed-effects models to quantify the best explanatory climate variables of tree growth and the spatio-temporal pattern of climate sensitivity. Besides accounting for trees as random effects, Prats et al. (2019) studied the influence of dry season on Quercus suber L. leaf traits in the Iberian Peninsula, and Liu et al. (2017) estimated the LA and LM of five deciduous broad-leaved trees in the Xiaoxing'an Mountains, both of which confirmed the validity of linear mixed-effects

models. However, the applications of linear mixed-effects models for estimating leaf structure parameters are few, in general, and even fewer for estimating the parameters in karst forests of the subtropics, where tree species richness is high, and the microenvironment is heterogeneous.

This study aimed to construct linear mixed-effects models using leaf structure parameters to non-destructively and efficiently estimate LA, LM, and SLA of trees in karst primary forests. Karst primary forests have many tree species and complex structures, and hence, the model based on tree species is not representative. Life forms encompass the long-term performance in life and appearance of plants which respond and adapt to variations in environmental conditions (Jiang et al., 1999). In other words, life forms are a combination of plant structure and growth dynamics (Molles, 2000). Plants with similar life forms show convergence to adapt to the environment, which creates differences in the characteristics of the leaf structure among plants (Kenzo et al., 2016). In a karst primary forest, the stratification and life forms of tree species are obvious (Zhu, 1997). To accurately, effectively, and quickly predict the dynamic changes of LA and LM of leaves in the karst primary forest, we classified tree species based on different life forms. Therefore, taking individual trees as the random effect, we constructed linear mixed-effects models of LA and LM of four life forms using leaf size, season, and crown canopy direction as the independent variables. The aims of this study were as follows: (1) to evaluate leaf trait variations of different life forms during the growing periods and horizontal directions in the canopy (HDC); (2) to select the optimal variable and test whether growing periods and HDC have a significant effect on the development of the linear mixed-effects models for predicting LA or LM; (3) to establish linear mixed-effects models of LA and LM and evaluate the forecast accuracy of these models; (4) to determine the feasibility of predicting SLA using LA and LM prediction models.

Materials and methods

Site description

The study was conducted in the Maolan National Natural Reserve (25°09′ 20″-25°20′ 50″N, 107°52′ 10″-108°05′ 04″E) in Libo County of Guizhou, a southwest province in China. The region belongs to a central subtropical monsoon humid climate with an annual average temperature of 15.3 °C (5.2 °C in January, 23.5 °C in July), annual precipitation of 1752.5 mm, and annual relative humidity of 83%. The altitude is 430~1078.6 m with bare ground rocks and shallow soil, and the rock exposed rate is up to 90%. The vegetation is a subtropical evergreen and deciduous broad-leaved mixed forest with a stable ecosystem and an estimated 87% forest coverage. The mean annual relative humidity (RH) and precipitation are 83% and 1,320.5 mm, respectively. The reserve is rich in species and has high biodiversity.

Experimental design

We randomly selected 24 representative tree species in this area and classified them into evergreen trees, deciduous trees, evergreen shrubs, and deciduous shrubs according to their life forms. There were eight evergreen tree species with DBH (diameter at breast height) ranging from 3.4 cm to 7.9 cm, nine deciduous tree species with DBH ranging between 2.1 and 7.4 cm, six evergreen shrubs species with the DBH ranging

from 1.5 to 2.1 cm, and one deciduous shrub with a DBH of 3.5 cm (*Table 1*). This dataset represented the traits of the leaves of the major tree species in the region. The trees we selected in the analysis were not inclined to larger ones. For one thing, the study area has complex terrain and high rock coverage, which is full of high risk and makes it difficult to acquire and measure leaf samples of large trees. For another thing, trees grew slowly, and small trees accounted high proportion in this study area with the harsh environment (Zhu, 1997). Based on the accuracy and sample size, the leaves of each sample tree were selected from different HDC (east, west, south, and north) and different seasons (January, April, July, and October), i.e., at least 10 sample leaves were collected in each season and each HDC, and 3460 sample leaves were obtained in total. January, April, July, and October represent the major phenological period of all broadleaf leaves, i.e., growth, lush, aging, and fall, respectively. First, we cut off the handles of the collected leaves and numbered each leaf. Then, the length and width of the leaf were measured with a ruler (with a precision of 0.1 cm). The length (L) was defined as the straight distance from the tip of the leaf to the base of the petiole, and the width (W) was the widest point perpendicular to the longitudinal axis of the leaf (Liu et al., 2017). The thickness (T) was measured by a Vernier caliper (with a precision of 0.01 mm) at the upper, middle, and lower parts of the leaf, and the final value of the thickness of each leaf was the average of three measurements. The LA was obtained by scanning the leaf using the Epson Perfection V19 image scanner (China, 300 dpi resolution). Next, we imported the images of the leaves obtained from the scanner to Photoshop 7.0 and calculated the actual area of each leaf from the proportion of the pixels of each leaf to that of the A4 paper. Finally, we dried the leaves in a 65 °C oven for 72 h and weighed each leaf to obtain the LM (precision of 0.001 g) as the actual leaf quality. The statistical characteristics of the structural parameters of the leaves are shown in *Table 1*.

Selection of the optimal independent variable

Regression models were based on linear functions of LA and power functions of LM. Specifically, leaf structure parameters such as leaf length (L), leaf width (W), leaf thickness (T), the product of leaf length and leaf width (LW), and the product of leaf length, leaf width, and leaf thickness (LWT) were used to predict LA and LM of the tree species. The statistical criteria for optimal independent variable selection were based on the lowest Akaike Information Code (AIC) value of each tree species in each life form. The two empirical models offer relatively equal support and cannot be distinguished from one another, when the AIC value difference between the first and second optimal models is less than 2.0. Then, the optimal predictive model selected is based on higher values of the coefficient of determination (R^2) (Burnham and Anderson, 2002).

Construction of linear mixed-effects models

Linear mixed-effects models of LA and LM for tree species of each life form were constructed based on the optimal independent variable selected in the previous step. Data were randomly selected (75%) from the tree species in each life form for model fitting, and 25% of the data were used for model validation. Before constructing the linear mixed-effects models, basic models were constructed as follows:

The linear model of the LA is presented in the form of *Equation 1*:

$$y = bx + a \tag{Eq.1}$$

The nonlinear model of LM (power model) was (*Eq. 2*):

$$y = mx^n \tag{Eq.2}$$

To construct a linear mixed-effects model, we first transformed *Equation 2* into linear models (*Eq. 3*) as follows:

$$\ln(y) = \ln(m) + n\ln(x) \tag{Eq.3}$$

Here, y represents either the LA or the LM; b and n are coefficients; x is an independent variable (e.g., length, width); a and $\ln(m)$ are the intercept. Seasons (i.e., June, July, and September) and HDC (i.e., West, South, and North) were treated as categorical variables.

Table 1. *Basic statistical characteristics of leaf structural parameters for tree species of four life forms in a karst forest (SD: standard deviation)*

Life forms	DBH (cm)	Number of leaf samples	Mean length (cm)	Mean width (cm)	Mean thickness (mm)	Tree species
Evergreen trees n = 1256	3.4	162	2.6 (3.3)	1.2 (1.1)	0.061 (0.11)	*Boniodendron minus*
	6.8	151	9.5 (2.4)	4.1 (0.9)	0.107 (0.11)	*Cinnamomum burmanni*
	7.9	154	11.0 (2.4)	3.7 (0.9)	0.085 (0.11)	*Cyclobalanopsis glauca*
	5.4	150	10.5 (2.4)	3.0 (0.9)	0.107 (0.11)	*Machilus rehderi*
	3.7	148	10.3 (2.4)	2.4 (0.9)	0.054 (0.11)	*Euonymus dielsianus*
	3.6	176	9.9 (2.3)	3.5 (0.9)	0.088 (0.13)	*Pittosporum tenuifolium*
	4.2	164	8.7 (2.4)	3.6 (0.9)	0.072 (0.16)	*Viburnum propinquum*
	6.3	151	10.9 (2.4)	3.5 (1.0)	0.054 (0.18)	*Acer cinnamomifolium*
Deciduous trees n = 1063	3.6	131	14.1 (3.7)	8.5 (2.4)	0.092 (0.15)	*Ficus hirtavahl*
	4.9	133	8.0 (3.4)	3.3 (2.1)	0.122 (0.16)	*Platycarya longipes*
	4.7	118	8.2 (3.4)	3.7 (2.1)	0.125 (0.16)	*Carpinus kweichowensis*
	7.4	99	13.0 (3.6)	6.7 (2.3)	0.075 (0.17)	*Bridelia minutiflora*
	2.1	119	6.7 (3.2)	2.9 (2.1)	0.039 (0.17)	*Clausena dunniana*
	3.8	121	9.6 (3.4)	3.9 (2.2)	0.130 (0.18)	*Diospyros kaki*
	5.2	125	8.0 (2.8)	7.0 (2.0)	0.119 (0.19)	*Schoepfia chinensis*
	5.4	82	8.5 (2.8)	4.5 (1.7)	0.091 (0.20)	*Celtis tetrandra*
	4.7	135	8.1 (3.1)	3.3 (1.9)	0.092 (0.21)	*Sapium rotundifolium*
Evergreen shrubs n = 964	1.6	140	4.6 (2.7)	2.3 (0.8)	0.079 (0.11)	*Murraya exotica*
	1.5	150	7.4 (2.5)	3.0 (0.7)	0.075 (0.11)	*Mahonia fortunei*
	2.1	157	7.5 (2.7)	2.6 (0.8)	0.095 (0.13)	*Distylium myricoides*
	1.7	164	8.2 (2.8)	3.1 (0.8)	0.124 (0.14)	*Lindera communis*
	1.9	183	6.8 (3.2)	3.4 (0.8)	0.114 (0.14)	*Tirpitzia sinensis*
	1.8	170	11.7 (3.6)	3.7 (0.9)	0.047 (0.15)	*Mallotus philippensis*
Deciduous shrubs n = 177	3.5	177	4.8 (1.3)	2.0 (0.5)	0.079 (0.09)	*Nandina domestice*

Then, linear mixed-effects models were constructed according to the theory of Littell et al. (2006) as follows:

Leaf-area linear model (*Eq. 4*):

$$y = bx + a + \theta + \varphi \tag{Eq.4}$$

Leaf-mass linear model (*Eq. 5*):

$$\ln(y) = n\ln(x) + \ln(m) + \theta + \varphi \tag{Eq.5}$$

where θ represents seasonal and canopy horizontal categorical variables; φ (as a random effect) represents tree tags to prevent potential autocorrelation among leaves of the same tree.

Therefore, taking individual trees as the random effect, we constructed linear mixed-effects models of LA and LM for four life forms using leaf size (continuous variable), season (categorical variables), and crown canopy direction (categorical variables) as the independent variables. Moreover, the conditional coefficients of determination (R_m^2) and marginal coefficients of determination (R_c^2) were used to determine how much of the variation was explained by fixed factors (season, canopy direction, and leaf size), as well as by both fixed and random factors (season, canopy direction, leaf size, and individual tree).

Validation of linear mixed-effects models

The remaining 25% of the total observed data were used to evaluate the performance of the prediction models. Then, the actual LA or LM values of the tree species of each life form were taken as a reference, and the mean absolute error (MAE) (*Eq. 6*) and the mean absolute error percent (MAE%) (*Eq. 7*) were calculated for evaluation.

$$\text{MAE} = \sum_{i=1}^{n} \left| \frac{y_i - \hat{y}_i}{n} \right| \tag{Eq.6}$$

$$\text{MAE\%} = \frac{1}{n} \sum_{i=1}^{n} \left| \frac{y_i - \hat{y}_i}{y_i} \right| \times 100\% \tag{Eq.7}$$

Here, y_i and $\hat{y}_i$ represent the actual SLA or LM values for the ith evaluation, and n is the number of samples.

Prediction of SLA by the regression models of LA and LM

The linear mixed-effects models of LA and LM were used to predict SLA for the tree species of each life form, and the parameters MAE and MAE% were used to assess the effectiveness of these models in predicting SLA. Additionally, to accurately and quickly predict the optimum number of leaves required for SLA per life form, all data were used to construct models, and the relationship between the difference (the mean actual SLA and the predicted SLA) and the sample size of each life form was analyzed. The formula used is as follows (*Eq. 8*):

$$\text{Difference}_n\,(\%) = \left|\frac{\text{actual SLA}\bar{n} - \text{predicted SLA}\bar{n}}{\text{actual SLA}\bar{n}}\right| \times 100\% \qquad \text{(Eq.8)}$$

Here, Difference$_n$ represents the difference between the average actual SLA and the predicted SLA when the sample size is n.

Statistical analysis

One-way analysis of variance (ANOVA) and multiple comparisons of least significant difference (LSD) were used to analyze the differences of LA or LM (at a significance level of $\alpha = 0.05$) between different seasons and different HDC. R_m^2 and AIC$_m$ explained by fixed factors, and R_c^2 and AIC$_c$ explained by both the fixed and random factors of the linear mixed-effects models were calculated. Models were constructed in SPSS22, and the figures were plotted in Origin2018 and Excel2010.

Results

Seasonal changes of LA and LM

The (mean) LA and (mean) LM for trees of four life forms showed significant differences across the four seasons but no significant differences across the four HDC (*Fig. 1*).
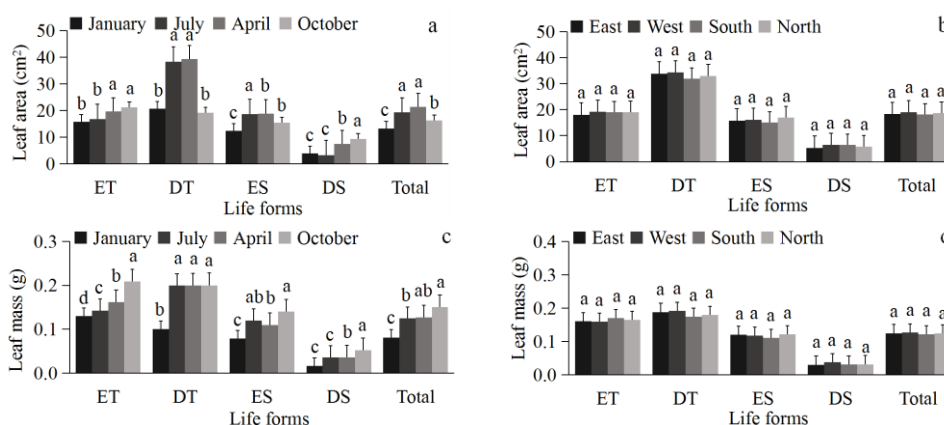

Figure 1. *The LA and LM variations in different seasons (January, April, July, and October) and HDC (east, west, south, and north) for trees of four life forms. Different lowercase letters indicate significant differences at the 0.05 significance level between LA/LM in the seasonal or canopy horizontal categories for tree species; error bars are represented by the standard errors. ER: evergreen trees, DT: deciduous trees, ES: evergreen shrubs, DS: deciduous shrubs*

Linear mixed-effects models of LA and LM

As shown in *Tables 2* and *3*, LW was the optimal dependent variable for predicting the LA and LM, based on the linear mixed-effects models. Besides, seasonal variations significantly affected the models constructed for all the tree species in the different life forms. However, the HDC had a significant effect ($P < 0.05$) in the regression model for the LA of evergreen trees and deciduous trees and did not significantly affect the LA and LM of the other life forms. The R_m^2 values of the models for LA and LM of the tree species ranged from 0.89 to 0.99 and 0.79 to 0.87, respectively. The R_c^2 values of the

models for LA and LM ranged from 0.92 to 0.99 and 0.88 to 0.94, respectively. The AIC_m values of the models for LA and LM of all the tree species ranged from 119.1 to 4464.79, and -3179.7 to -940.2, respectively. The AIC_c values of the models for LA and LM ranged from 118.5 to 4306.76 and -4389.5 to -969.1, respectively. Thus, when both fixed and random factors were considered, R^2 was significantly higher and AIC was significantly lower. This indicated that the mixed-effects models could explain the variance of LA and LM of trees in the karst forest by more than 92% and 88%, respectively. Therefore, the mixed-effects models can better predict the LA and LM of trees than only considering the fixed-effects models in karst forests.

Table 2. *Linear mixed-effects model for predicting LA (cm^2) using leaf structural parameters for trees of four life forms, examined in a karst forest*

Life forms	Variable	Estimate	SE	t value	R_m^2	R_c^2	AIC_m	AIC_c
Evergreen trees n = 949	Intercept	0.6713	0.2567	2.62**	0.89	0.92	4321.9	4306.8
	Mouth (January)	0.0258	0.2448	0.11NS				
	Mouth (April)	0.6323	0.2067	3.06**				
	Mouth (July)	0.8058	0.2030	3.97***				
	Direction (east)	-0.4991	0.2142	-2.33*				
	Direction (west)	-0.1496	0.2158	-0.69NS				
	Direction (north)	-0.6350	0.2159	-2.94**				
	LW cm^2	0.6133	0.0050	122.67***				
Deciduous trees n = 800	Intercept	-1.9086	0.5353	-3.57***	0.93	0.97	4464.8	4149.5
	Mouth (January)	1.8356	0.5666	3.24**				
	Mouth (April)	1.3224	0.5295	2.50*				
	Mouth (July)	2.1084	0.5273	3.99***				
	Direction (east)	0.2325	0.3934	0.59***				
	Direction (west)	0.5967	0.3954	1.51NS				
	Direction (north)	0.3139	0.3954	0.79NS				
	LW cm^2	0.6706	0.0031	210.75***				
Evergreen shrubs n = 737	Intercept	0.3315	0.1919	1.73#	0.96	0.96	2876.0	2793.3
	Mouth (January)	-0.4491	0.2044	-2.20*				
	Mouth (April)	0.0643	0.1670	0.39NS				
	Mouth (July)	-0.2567	0.1655	-1.55NS				
	Direction (east)	0.2273	0.1748	1.30NS				
	Direction (west)	-0.0046	0.1750	-0.03NS				
	Direction (north)	0.0680	0.1765	0.39NS				
	LW cm^2	0.6519	0.0044	146.45***				
Deciduous shrubs n = 137	Intercept	0.4599	0.1935	2.38**	0.99	0.99	119.1	118.4
	Mouth (January)	-0.2683	0.1355	-1.98*				
	Mouth (April)	-0.3464	0.1449	-2.39*				
	Mouth (July)	0.1716	0.0922	1.86#				
	Direction (east)	-0.0246	0.0826	-0.30NS				
	Direction (west)	0.0848	0.0831	1.02NS				
	Direction (north)	0.1507	0.0834	1.81#				
	LW cm^2	0.5699	0.0117	48.53***				

LW is the product of length and width; L and W is the product of length and width, R_m^2 represents the variance explained by the fixed factor, and R_c^2 represents the variance explained by the fixed and random factors. AIC_m represents the Akaike information criterion of fixed effect, AIC_c represents the Akaike information criterion of fixed effect and random effect. NS represents no significant; # represents significance at the 0.1 level; * represents significance at the 0.05 level; ** represents significance at the 0.01 level and *** represents significance at the 0.001 level

Table 3. *Linear mixed-effects model for predicting LM (g) using leaf structural parameters for trees of four life forms examined in a karst forest*

Life forms	Variable	Estimate	SE	t value	R_m^2	R_c^2	AIC_m	AIC_c
Evergreen trees n = 949	Intercept	0.0166	0.0047	3.51***	0.79	0.88	-3179.6	-4389.5
	Month (January)	-0.0356	0.0045	-7.87***				
	Month (April)	-0.0237	0.0038	-6.20***				
	Month (July)	-0.0229	0.0037	-6.12***				
	Direction (east)	-0.0021	0.0039	-0.55NS				
	Direction (west)	-0.0017	0.0039	-0.43NS				
	Direction (north)	-0.00518	0.0039	-1.30NS				
	LWcm^2	0.0057	0.0001	61.73***				
Deciduous trees n = 800	Intercept	0.0669	0.0125	5.32***	0.86	0.91	-1467.1	-1744.8
	Month (January)	-0.0718	0.0133	-5.39***				
	Month (April)	-0.0691	0.0124	-5.55***				
	Month (July)	-0.055	0.0124	-4.44***				
	Direction (east)	0.0052	0.0092	0.57NS				
	Direction (west)	0.0121	0.0093	1.31NS				
	Direction (north)	0.0023	0.0093	0.25NS				
	LWcm^2	0.0034	0.0001	46.67***				
Evergreen shrubs n = 737	Intercept	0.0558	0.0066	8.38***	0.87	0.93	-2017.5	-2770.1
	Month (January)	-0.0563	0.007	-7.94***				
	Month (April)	-0.0327	0.0057	-5.65***				
	Month (July)	-0.034	0.0057	-5.93***				
	Direction (east)	0.0025	0.006	0.42NS				
	Direction (west)	0.0018	0.006	0.31NS				
	Direction (north)	0.0007	0.0061	0.12NS				
	LWcm^2	0.0035	0.0001	23.13***				
Deciduous shrubs n = 137	Intercept	-0.0032	0.003	-1.05NS	0.87	0.94	-940.2	-969.1
	Month (January)	-0.0037	0.0021	-1.72NS				
	Month (April)	0.0035	0.0023	1.55NS				
	Month (July)	0.0036	0.0014	2.45*				
	Direction (east)	0.0014	0.0013	1.07NS				
	Direction (west)	0.0017	0.0013	1.33NS				
	Direction (north)	0.0011	0.0013	0.89NS				
	LWcm^2	0.0034	0.0001	18.66***				

Validation of the linear mixed-effects model

The linear mixed-effects models were used to calculate the predicted values of LA and LM, and the relationships between the predicted values and the actual values were obtained for trees of four life forms (*Fig. 2*). Significantly reliable relationships (P < 0.01) between the actual and predicted LA were obtained for trees of four life forms. The R^2 value ranged from 0.97 to 0.99 and from 0.84 to 0.98 for LA and LM, respectively. The mean MAE% of the LA from the linear mixed-effects models for four seasons was found to be 0.9%-14.4%, the mean MAE of LA ranged from 0.01 cm^2 to 1.45 cm^2. The mean MAE% of LM ranged from 1.1% to 17.1%, and the mean MAE of LM was between 0.01 g and 0.83 g (*Table 4*).
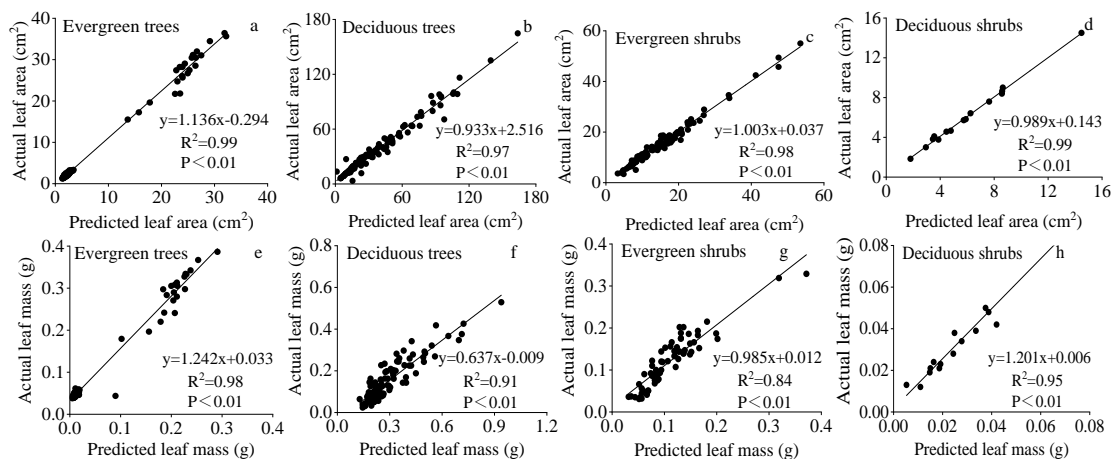
Figure 2. *The relationship between predicted and actual LA and LM from linear mixed-effects models for trees of four life forms. The datasets consisted of sample leaves (25%) for constructing models in all categories of season and HDC*

Table 4. *Validation of the linear mixed-effects models of LA (cm^2) and LM (g) for trees of four life forms, examined based on the different categories of season and HDC*

Month	Leaf traits	Evergreen trees		Deciduous trees		Evergreen shrubs		Deciduous shrubs	
		MAE	MAE%	MAE	MAE%	MAE	MAE%	MAE	MAE%
January	Leaf area	1.06	1.4	0.17	3.2	0.28	2.6	0.01	8.9
	Leaf mass	0.26	2.7	0.83	17.1	0.02	2.8	0.02	9.2
April	Leaf area	1.37	1.7	0.95	5.8	0.18	1.7	0.07	7.2
	Leaf mass	0.02	4.7	0.09	16.6	0.01	3.4	0.01	9.2
July	Leaf area	0.94	1.1	1.45	6.4	0.15	1.2	0.03	5.8
	Leaf mass	0.02	4.7	0.09	14.4	0.01	3.0	0.01	8.4
October	Leaf area	0.76	0.9	0.21	4.10	0.36	2.1	0.11	2.9
	Leaf mass	0.01	4.8	0.03	11.9	0.02	1.1	0.01	9.2

MAE: mean absolute error; MAE%: mean absolute error percent

SLA prediction

MAE% of SLA predicted from the linear mixed-effects models of LA and LM ranged from 15.6% (deciduous trees) to 19.4% (evergreen shrubs), and MAE of SLA ranged from 18.96 cm^2/g to 26.82 cm^2/g for tree species in all life forms (*Table 5*). The difference between the mean of actual and predicted SLA and the standard error (SE) of actual SLA showed a similar trend for variation (*Fig. 3*), i.e., the differences decreased with an increase in the sample size. We can see that the value of difference remained stable when the leaf sample size was 80 for evergreen trees, 60 for deciduous trees, and 70 for both evergreen shrubs and deciduous shrubs. Considering the accuracy of the models and the sampling effort, we determined that the optimum number of leaves for SLA estimation was 70 for evergreen trees (*Fig. 3a*), 60 for deciduous trees (*Fig. 3b*), 80 for evergreen shrubs (*Fig. 3c*), and 70 for deciduous shrubs (*Fig. 3d*).

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Table 5. *Comparison of actual SLA (cm²/g) and predicted SLA for trees of four life forms*

Life forms (sample size)	Actual SLA	Predicted SLA	MAE	MAE%
Evergreen trees (307)	128.10	114.90	18.96	15.6
Deciduous trees (263)	211.49	198.41	22.54	17.5
Evergreen shrubs (227)	151.47	144.65	26.82	19.4
Deciduous shrub (40)	185.79	178.50	21.56	16.8
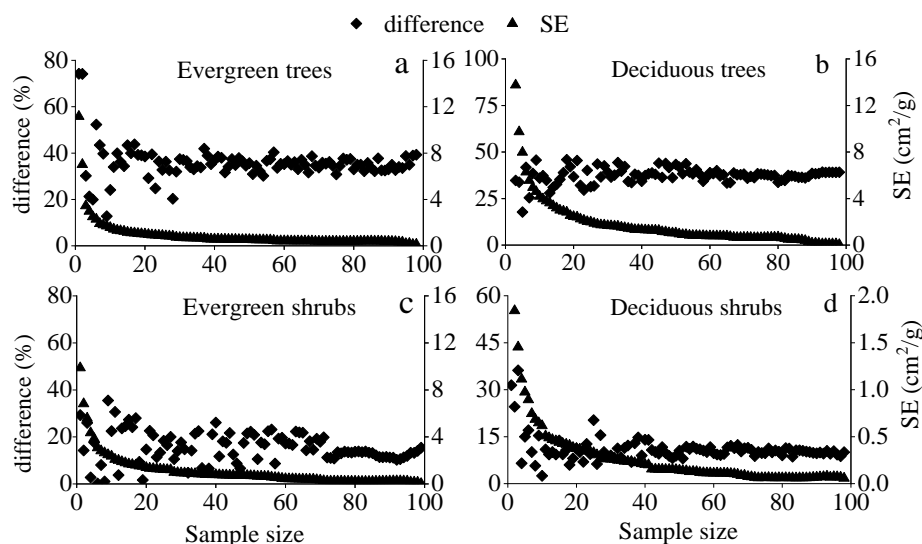
Figure 3. *Differences between the mean actual SLA and the predicted SLA as a function of sample size; SE: standard error of actual SLA*

Discussion

Optimum variable

Linear mixed-effects models were constructed in our study for estimating LA and LM in a karst primary forest. According to the results, LW was the optimal estimation variable for estimating LA when considering only the length or the width. Other studies had similar conclusions (Demirsoy et al., 2004; Pompelli et al., 2012; Montelatto et al., 2020). Especially, LW has been accepted as the optimal independent variable for predicting LA in different growing periods (Liu et al., 2017) and even in different life-history stages (Wang et al., 2019). We found similar results for different growing periods and for different life forms (*Table 3*). Moreover, LW was also the optimal estimation variable, rather than LWT or other variables in constructing the linear mixed-effects models of LM in our study. Generally, the total increase in LM is the sum of the increase in the mass due to the increase of LA and leaf thickness (Weraduwage et al., 2015), which indicates that the optimal independent variables for predicting LM are more diverse (Wang et al., 2019). Liu et al. (2017) found that for leaves with thickness greater than 0.1 mm, LWT was more suitable for linear mixed-effects models than LW, and for leaves with thickness lesser than 0.1 mm, LW was more suitable for linear mixed effects models than LWT. Similarly, Wang et al. (2019) predicted the LA and LM for broad-leaved trees of two life forms in northeastern China and found that LWT can better predict LM when L:W is greater than 1.5, and either LW or LWT can predict

LM for a certain period when L:W is lesser than 1.5. These studies confirmed that LM is more sensitive to the environment than LA (Wang et al., 2019). Bell (1991) reported that the leaf size of most species results from cell multiplication in some defined meristem zones, which further influences the leaf shape. During growth, the length/width ratio of leaves remains constant (Liu et al., 2017). Thus, LM is affected by factors such as leaf length-to-width ratio (Wang et al., 2019) and leaf thickness (Liu et al., 2017). In our study, 8 of 24 tree species had average leaf thickness greater than 0.1 mm, including *Cinnamomum burmanni*, *Machilus rehderi*, *Carpinus kweichowensis*, *Bridelia minutiflora*, *Diospyros kaki*, *Schoepfia chinensis*, *Lindera communis* and *Tirpitzia sinensis* (*Fig. 1*). The other 16 tree species had less than 0.1 mm leaf thickness and L:W less than 1.5. The tree species with small and thin leaves comprised nearly 80% of all tree species. Therefore, leaf shape (LW) plays a significant role in predicting the LM of trees in karst primary forests.

Linear mixed-effects models

The linear mixed-effects models for estimating LA and LM for trees of four forms in karst forests were significantly better than the models that only considered the fixed effects, which illustrated that random effects caused by differences of leaves between individual/tree species should be taken into account. For karst region with abundant tree species and complex structures, modeling by different life forms considered differences between tree species and solved the problem of underrepresentation of relying only on species to build models. Actually, the linear mixed model considered fixed effects and random effects for model construction and predicted the variance of the dependent variables by establishing the design matrix of random-effects (Cantoni et al., 2021). Meanwhile, it indicated the covariance structures of the random-effects covariance matrix and the model residual covariance matrix (Tao, 2002; Littell et al., 2006), and thus, eliminated the biased estimation caused by differences among individual trees. Hence, only considering the fix effects was not the optimal parameter prediction approach.

The season has an important effect on leaf growth and is a non-negligible variable for both LA and LM model estimation. Many studies have concluded that seasonal effects on leaf growth and development (Cai et al., 2017; Liu et al., 2017; Wang et al., 2019). However, fewer reports have estimated the LM of deciduous leaves than the LA. At the beginning of leaf expansion, LA and LM are small, and most of the resources are required for photosynthesis to increase LA, which results in lower organic content and lower LM. With the increase in temperature at the beginning of the rainy season, the number of fence cells increases, and dry matter accumulates, producing an additional cell layer and increasing the leaf thickness. Finally, during the leaf shedding period, the size and dry matter accumulation of the leaves stabilizes (Lambers et al., 2008; Delagrange, 2011). Thus, the relationship between leaf length, leaf width, and leaf thickness with LA and LM differ across seasons.

Except for the LA of evergreen and deciduous trees, canopy horizontal positions showed no significant effect on the LA and LM in the linear mixed effects models (*Tables 2* and *3*). This could be related to the competition for important resources in the canopy horizontal positions. Normally, the canopy structure regulates light intensity, temperature, water, and other environmental factors in the forest by absorbing, transmitting, and scattering the photosynthetic radiation, leading to a significantly different microenvironment in the forest (Green et al., 2001; Roel et al., 2021). The

microenvironment and microtopography in karst forests affect the non-uniform distribution of light at different canopy horizontal positions, which give rise to irregular differences among the trees at the same canopy horizontal position because of high heterogeneity. This is similar to the effect of the light distribution at the vertical positions of canopy layers (Liu et al., 2017), which showed that the sensibility of LA and LM to light distribution is different. We did not consider vertical positions of canopy layers because of the specificity of the study region, i.e., high rock coverage and considerable heterogeneity (Zhu, 1997), which increased the difficulty of sampling at different canopy vertical locations.

Additionally, previous studies usually constructed models of LA and LM based on tree species (Athokpam et al., 2014; Tondjo et al., 2015), and some studies have also shown that the types of empirical models for predicting LA and LM varied with species (Tondjo et al., 2015; Cai et al., 2017). However, because our study area has a high diversity of tree species, the representation of the models based on individuals from a small number of tree species was insufficient. Plants can reveal the structural characteristics of the community and the mechanism of adaptation to environmental gradients (Jiang et al., 1999; Yakimov et al., 2020). Several studies have shown that plant life forms are related to leaf traits (Wang et al., 2017; Cheng et al., 2021), although there are phylogenetic constraints on them (Grubb et al., 1975; Kenzo et al., 2016). Evergreen species may have thick and robust leaves to extend longevity in less productive habitats, such as the dark understory, whereas deciduous species may have thinner leaves with high nitrogen content and a high photosynthetic ability. These leaves might favor sun-exposed conditions, such as the canopy, to maximize carbon gain over the short periods favorable for photosynthesis (Chabot and Hicks, 1982; Niinemets et al., 2015). Karst primary forests show stratification of life forms (Zhu, 1997). Therefore, we classified the tree species into four groups based on life forms and then constructed linear mixed-effects models of these four life forms. Though few studies have constructed models for the LA and LM in plants of different life forms across growing periods, the forecast accuracy ranged from 1.9%-10.1%, 7.7%-15.1%, and 11.6%-20.6% for LA, LM, and SLA, which demonstrated that the models were effective and robust.

Moreover, our results suggested that it is feasible to predict SLA through the linear mixed-effects models of LA and LM since the predicted SLA did not significantly differ from the actual SLA. The maximum mean MAE% was 20.6% for evergreen bush, and the minimum mean MAE% was 11.6% for deciduous trees (*Table 5*). These results were similar to those obtained in other studies (Liu et al., 2017), which confirmed that the linear mixed-effects model of LA and LM is a fast and efficient method. It is also a new way to estimate SLA values of broad-leaved tree species accurately. Additionally, while considering the accuracy of the estimated model and the effort of leaf sampling, the optimum sample size of leaves from different tree species for SLA estimation was estimated to be at least 60–80 (*Fig. 3*). Compared to other studies (i.e., Liu et al., 2017), the values of the differences in *Figure 3* fluctuated considerably more with the sample size before stabilizing, and the optimal number of leaves required was greater. This might be related to the high heterogeneity in the microtopography and the microenvironment of the study area, as well as the richness in the composition of the species (Zhu, 1997; Qi et al., 2021), which simultaneously increased within-group differences and the sample size.

Conclusion

In our study, linear mixed-effects performed better than the models only considered fixed effects, demonstrating they were non-destructive, fast, and reliable methods for predicting the LA and LM for trees of four life forms in a karst forest. The LW was the best predictive variable. The sampling season was an important factor affecting leaf growth and development and thus was the key variable in models' construction. The sample size for SLA estimation should be about 60–80 leaves for different life forms. In conclusion, our study provided a deep understanding of individual trees adapting to the environment in a karst primary forest and supplied a reference for the efficient and accurate determination of LA and LM for trees of different forms in species-rich forests.

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