

# MORPHOLOGICAL AND ANATOMICAL CHANGES OF PHOEBE BOURNEI IN DIFFERENT AGE CLASSES GROWN IN NATURAL FOREST UNDER DIFFERENT LIGHT ENVIRONMENTS

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**Abstract.** Little is known about the morphological structure and photosynthetic physiology of *Phoebe bournei* (*P. bournei*) of different age classes in different light environments in a natural forest. There are four light intensity levels under the natural forest of *P. bournei* in Taijiang county, Guizhou Province, China, with 10% of the total light being weak light (WL), 30-40% being medium low light (LL), 50-60% being medium light (ML) and 80-100% being high light (HL) respectively. Our results are as follows: (1) Under high light (HL), the occurrence of younger seedlings and saplings is less frequent, especially *P. bournei* of age I was not observed in HL light intensity. (2) In the same age class and different light intensity classes: The leaf area of the same age class increases gradually with decreasing light intensity, except for the first age class; The specific leaf area (SLA), chlorophyll a (Chl a) and chlorophyll b (Chl b) increase gradually with decreasing light intensity; The thickness of palisade tissue and leaves decreases with decreasing light intensity; (3) In the same light intensity class and different age level classes. Palisade tissue thickness, spongy tissue thickness and leaf thickness gradually increases with the increase of age grade; Chl a and Chl b decreased with increasing light age. With the current study of the change of morphological and photosynthetic physiological characteristics of *P. bournei* of different ages with the light environment, we aim at providing theoretical basis and technical guidance for the management and protection of *P. bournei* natural forests and the formulation of technical measures for plantation cultivation.

**Keywords:** *Phoebe bournei*, different age levels, light environment, morphology, leaf anatomical structure

## Introduction

At different stages of plant development, the light demand of plants differs (Lan et al., 2017). The degree of the shade tolerance of shade-resistant plants is different, and it declines with the increase of individual development. Some scholars pointed out that the light demand of *Pinus koraiensis* varies at different stages of development (Amahowe et al., 2016; Lusk and Jorgensen, 2013; Zhang et al., 2003). At the seedling and young tree stages, *P. koraiensis* exhibits a certain degree of shade tolerance, and the light demand rises with its age. In addition, a study of Messier and Nikinmaa (2016) showed that the differences decline in crown morphology and distribution ratio of *Acer saccharum*,

beech and *Betula alleghanensis* with medium shade tolerance gradually decline as individuals develop.

*Phoebe bournei* (Hemsl.) is a rare tree species with special national secondary protection in China, mainly distributed in southeast Asia, and southeast and southwest of China. *P. bournei* is a unique precious timber and ornamental forest tree species in China. In the natural forest of *P. bournei*, the seeds germinate and grow into seedlings under weak light conditions. The adaptability of *P. bournei* at different ages to the light environment directly affects its natural regeneration and the stability of the community. In order to promote the regeneration and population stability of *P. bournei* natural forest, the photosynthetic physiology and morphological structure of well-protected natural community of *P. bournei* in Denglu village, Taijiang, Guizhou Province, China were analyzed in this study. In addition, the morphological and photosynthetic physiological characteristics of the seedlings in a fluctuating light environment were investigated for the management and protection of the natural forest. In the natural forest of *P. bournei*, the research results of light environment morphology and photosynthetic physiological characteristics of *P. bournei* at different ages are aimed at providing theoretical basis and technical guidance for *P. bournei*'s natural forest protection and plantation cultivation.

## Methods

### *Experimental site*

The experiment was conducted at Denglu Village (23°38'N, 108°21'E), Taijiang County, Qiandongnan Miao and Dong Autonomous Prefecture, southwest China's Guizhou Province. The highest altitude is 1980 m, the lowest altitude is 455 m, and the average altitude is 717.5 m. Located at the north foot of Leigong Mountain, the main peak of Miao age, the south bank of southeast Guizhou, and the middle of low mountains and hills in eastern Guizhou, the site enjoys annual temperature of 16.5 °C, an annual rainfall of 1800 mm and frost-free period of 320 d.

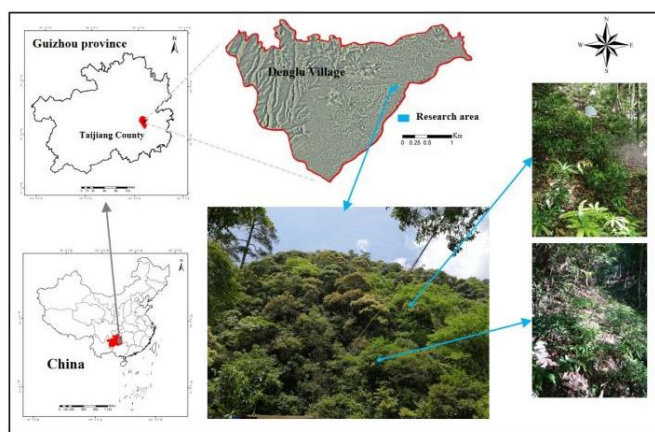
At the experimental site (Fig. 1), *P. bournei* (the number of research sites is 746) forms the main tree layer, and the relevant tree species include *Choerospondias axillaris* (the number of research sites is 6), *Cyclobalanopsis glauca* (the number of research sites is 4), *Liquidambar formosana* (the number of research sites is 3) and *Acer fabri* (the number of research sites is 4). The shrub layer mainly consists of a small number of shrubs such as *Camellia polyodonta*, *Eurya kueichowensis* and *Aidiacochin chinensis*. The herb layer of the forest is dominated by *Spider brake*, *Dicranopteris dichotoma* and other herbaceous plants.

### *Research methods*

#### *Sample plot setting*

The sample plots with similar slope (35°), soil (mountainous yellow soil in the south) and water conditions were selected in the natural forest of *P. bournei* to minimize the differences caused by site conditions. According to the methods proposed by Zang et al. (2009) and Zheng et al. (2020), quadrats were established to investigate plant properties and photosynthetic physiology of natural forests. A 40 m×10 m sample plot was selected, and the plot was divided into 25 small quadrats (4 m×4 m). Each small quadrat was marked with PVC pipes at the four corners and the center point. The sample plot

grew under 100% of full light on the bare ground at noon on a sunny day in April. The light intensity in the quadrats was measured by LightSout Light Sensor (Spectrum Technologies Inc., Dallas-Fort Worth, Texas, USA). Besides, the photosynthetic active radiation value of each small quadrat marked with PVC pipes at the 5 positions was measured and averaged. According to the results, the light intensity of each quadrat fell into four levels, that is, below 10% of the full light ( $<100 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ) is defined as weak light (WL) intensity, 30-40% of the full light ( $300\text{-}400 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ) is defined as low light (LL) intensity, 50-60% of the full light ( $500\text{-}600 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ) is defined as medium light (ML) intensity, and 80-100% of the full light ( $800\text{-}1000 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ) is defined as high light (HL) intensity.



**Figure 1.** Study Site map of *P. bournei*

### Age classification

Age classification of *P. bournei* in the sample plot: The sample plot was treated in two ways according to the age structure of seedlings (He et al., 2008; Wu et al., 2001). In the actual sampling, the size of the sampled populations grown under the same light intensity should be consistent, and the height or chest diameter of *P. bournei* at the same age class should also be basically the same (Table 1).

**Table 1.** Age classification of *P. bournei* in natural forest

Age class	I	II	III	IV	V	VI
Tree height (H) or DBH (D) (cm)	H = 1-33	H = 33-100	H > 100	D = 2.5-7.5	D = 7.5-22.5	D > 22.5

### Measurement parameters

#### (1) Leaf morphology

In April 2018, 8 mature and healthy leaves on the current year's branches were selected to measure chlorophyll (Chl), leaf area and other indicators. Besides, the undamaged blade was selected, and the leaf area was measured with LI-3000 instrument (LI-COR, USA). Next, the blade was killed at 105 °C for half an hour. After drying at

60 °C for 48 h, the dry weight was weighed, and the specific leaf area (SLA) was calculated using *Equation 1*:

$$SLA = \text{leaf area} / \text{blade dry weight} \quad (\text{Eq.1})$$

## (2) Leaf anatomy

The upper mature leaves of *P. bournei* with different treatments were sampled and cut along both sides of the main vein, measuring 0.2 cm×0.2 cm. After that, the samples were fixed in the field with FAA fixing solution (glacial acetic acid: formaldehyde: 70% ethanol = 90: 5: 5), and 5 mL of glycerol was added to prevent volatilization. Then the obtained mixture was fixed in a refrigerator at 4 °C for more than 24 h. Later, the samples were prepared into paraffin-embedded sections using the conventional paraffin section method (Catoni et al., 2015), and cut into slices with a thickness of 8-10 μm using Leica RM2335 Rotary Microtome, followed by gradient dehydration with alcohol, staining with Safranin O/Fast Green for 10 min, and sealing with neutral gum. Next, the slices were observed and photographed under Leica DM3000 Microscope (Leica Corporation, Germany). Subsequently, Nikon Eclipse software (Nikon's Digital Sight SD-Fi2, Nikon Corporation, Japan) was used to measure the leaf thickness, palisade tissue thickness and spongy tissue thickness of 9 slices in 5 randomly selected fields of view each. Finally, the average value was taken and standard error of the mean was calculated. Relevant indicators were calculated according to *Equation 2*:

$$\text{Palisade / spongy tissue thickness ratio} = \text{palisade tissue thickness} / \text{spongy tissue thickness} \quad (\text{Eq.2})$$

## (3) Chl content

In each treatment, the upper healthy and mature leaves of 5 young *P. bournei* trees were sampled using the method specified in the study of Sukran et al. (1998), immediately after which the samples were put into an ice box and taken back to the laboratory. In the laboratory, the samples were washed, wiped and weighed. Afterwards, 0.1 g of the samples were cut and put into a glass test tube. Subsequently, the samples were soaked in the tube added with 5 mL of 100% acetone, with the mouth sealed, in the dark environment for 36 h until they turned white. Later, the supernatant was collected, and the absorbance at 662 nm, 645 nm and 470 nm was calculated with an UV756CRT ultraviolet visible spectrophotometer (Shanghai Youke Instrument Co., Ltd., China) based on *Equations 3–6*:

$$\text{Chl } a = 11.75A_{662} - 2.350A_{645} \quad (\text{Eq.3})$$

$$\text{Chl } b = 18.61A_{645} - 3.960A_{662} \quad (\text{Eq.4})$$

$$\text{Car} = 1000A_{470} - 2.270Ca - 81.4Cb / 227 \quad (\text{Eq.5})$$

$$\text{Chl}(a+b) = \text{Chl } a + \text{Chl } b \quad (\text{Eq.6})$$

where Chl a is chlorophyll a, Chl b is chlorophyll b, Car is carotenoid, and Chl (a + b) is the total amount of chlorophyll.

#### (4) Photosynthetic rate

The *P. bournei* leaves were sampled and detected on 3 sunny days from 10:00 to 10:30 a.m. For *P. bournei* at age classes IV, V and VI, an in vitro branch bottle was utilized to insert clean water, and *P. bournei* leaf samples were cut again at about 3 cm from the end of the cut of the branch underwater. At the same time, most of the leaves on the branch were removed to reduce the water loss of the in vitro branch (Hughes, 2021). Three in vitro branches were used for each treatment, and a total of nine functional leaves with mature crown were selected for the branches, with three leaves each. The light intensity was detected by LI-6400XT portable photosynthesis instrument (LI-COR Inc., Lincoln, NE, USA) with a fast leaf fan based on a closed-circuit system under the conditions of a light intensity set at  $800 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ , an airflow rate controlled at  $500 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ , leaf temperature of  $25^\circ\text{C}$ , humidity of 75%, and  $\text{CO}_2$  concentration of  $400 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  (original  $\text{CO}_2$  small steel cylinder as  $\text{CO}_2$  source). It should be ensured that in vitro branches could complete the determination of photosynthetic parameters (Tang and Wang, 2011; Hughes, 2021), net photosynthetic rate ( $P_n$ ), intercellular  $\text{CO}_2$  concentration ( $C_i$ ), stomatal conductance ( $G_s$ ), transpiration rate ( $Tr$ ) and other parameters within half an hour.

#### (5) Data processing

Statistical analysis was tested using a One-Way analysis of variance (ANOVA) by the means and standard errors at least three replicates, and used to assess significant differences by Tukey's test ( $p < 0.05$ ) with SPSS 18.0 (Chicago, IL, Armonk, NY, USA). The charts and tables were made with Origin 19.0 and Excel software, respectively.

## Results

### *Changes of leaf morphology with age and light environment*

According to the results of this study, *P. bournei* leaves were not distributed at age classes III, IV, V and VI under WL intensity, not distributed at age classes V and VI under LL intensity, not distributed at age class VI under ML intensity, and almost not distributed at age classes I and II under HL intensity.

The leaf area at the same age class varied with the change of light intensity, and it showed statistically significant differences among different light intensity treatment groups ( $p < 0.05$ ) (Table 2; Fig. 2). The leaf area was increased gradually with the decrease of light intensity at the same age class (II-VI), while it was decreased with the decrease of light intensity at age class I. Under the same light intensity, the leaf area under WL, LL and ML intensities rose with the increase of the age class. Under LL intensity, the leaf area was the smallest at age class I and the largest at age class IV. Under ML intensity, the smallest leaf area appeared at age class I, and the largest leaf area appeared at age class V. Under HL intensity, the leaf area changed with the increase of the age class, and rose firstly and then dropped with the increase of the intensity. Moreover, the leaf area reached the maximum at age class V and reached the minimum at age class VI. The largest leaf area appeared at age class IV under LL intensity, while the smallest leaf area appeared at age class I under WL intensity, and the maximum leaf area was 11.48 times of the minimum leaf area.

At the same age class, the SLA showed a certain regularity with the change of the light intensity, and the differences among different light intensity treatment groups were

statistically significant ( $p < 0.05$ ) (Table 2; Fig. 1). The SLA of *P. bournei* at the same age class was increased with the decrease of the light intensity. It was the smallest at age class II and the largest at age class I under WL intensity. Under LL intensity, the SLA was the smallest at age class IV and the largest at age class I. Under ML intensity, the SLA was the smallest at age class V and the largest at age class I. Under HL intensity, the SLA was the smallest at age class VI and the largest at age class III. The SLA reached the maximum at age class I under WL intensity, while the minimum SLA was detected at age class VI under HL intensity, and the maximum SLA was 1.83 times of the minimum SLA.



**Figure 2.** Leaf morphology of *P. bournei* at different age levels. Note: I, II, III, IV, V and VI represent different grades, and WL represents low light intensity, LL represents medium-low light intensity, ML represents medium light intensity, HL represents highlight intensity

**Table 2.** Effects of different light intensities on leaf area and SLA of *P. bournei*

	Age class	Light intensity level			
		LL	MLL	ML	HL
Leaf area (cm <sup>2</sup> )	I	3.39 ± 0.240Bc	5.10 ± 0.295Ca	4.42 ± 0.185Cb	—
	II	21.12 ± 0.366Aa	17.35 ± 0.143Bb	14.90 ± 0.670Bc	—
	III	—	21.13 ± 0.354Ba	19.55 ± 0.336Bb	15.06 ± 0.541Bc
	IV	—	38.90 ± 1.778Aa	23.99 ± 1.410Ab	15.51 ± 0.555Bc
	V	—	—	26.24 ± 0.638Aa	22.27 ± 0.587Ab
	VI	—	—	—	14.89 ± 0.752B
Specific leaf area SLA (cm <sup>2</sup> .g <sup>-1</sup> )	I	134.31 ± 2.67Aa	126.53 ± 0.49Ab	101.35 ± 0.32Ac	—
	II	131.48 ± 0.55Ba	128.32 ± 0.35Bb	109.67 ± 0.45Ac	—
	III	—	125.67 ± 0.68Ca	113.53 ± 0.46Bb	107.16 ± 0.50Ac
	IV	—	114.44 ± 0.87Da	96.11 ± 0.97BCb	84.44 ± 0.31Bc
	V	—	—	89.58 ± 0.15Ca	80.93 ± 0.86Bb
	VI	—	—	—	73.47 ± 0.40C

The data are mean ± standard deviation. Different lowercase letters mean significant difference in the same row ( $p < 0.05$ ), and different uppercase letters mean significant difference in the same column ( $p < 0.05$ ). “—” indicates that *P. bournei* plants are not distributed. The same below

### Changes of leaf anatomical structure of *P. bournei* with age and light environment

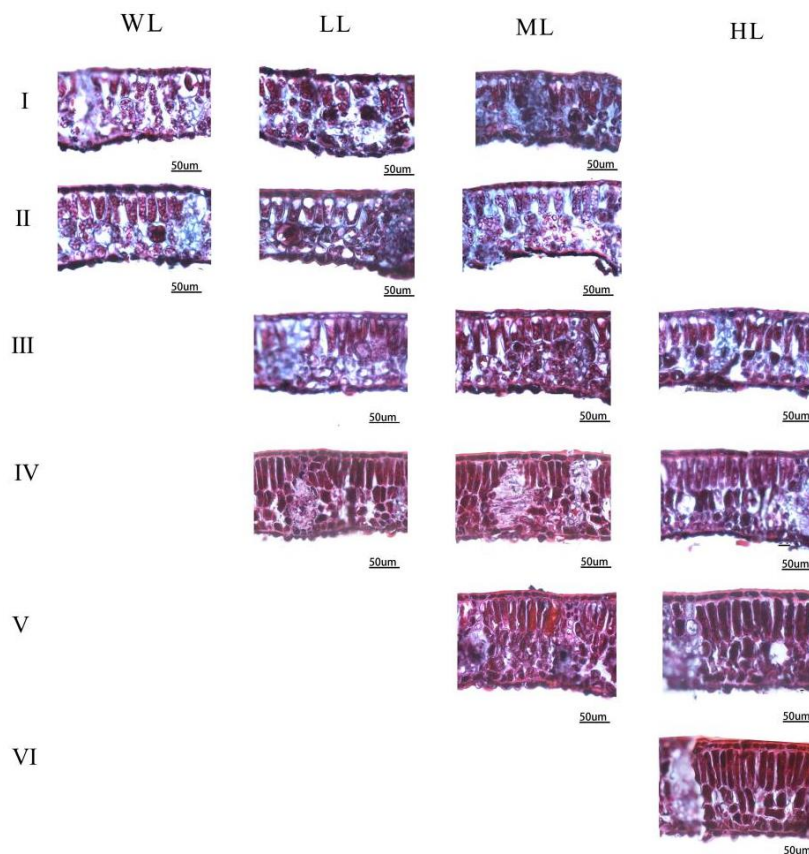
The palisade tissue thickness showed a certain regularity with the change of the light intensity at the same age class (Table 3; Fig. 3). Specifically, the palisade tissue thickness declined with the decrease of light intensity at the same age class, and showed an increasing trend with the increase of age class. It was found that the differences in the palisade tissue thickness among different light intensity treatment groups ( $p < 0.05$ ), except for the difference between age class II and III under LL density and that between age class IV and V under HL intensity. The palisade tissue thickness was the smallest at age class I and the largest at age class II under WL intensity, the smallest at age class I and the largest at age class IV under LL light intensity, the smallest at age class I and the largest at age class V under ML intensity, and the smallest at age class III and the largest at age class VI under HL light intensity level. Furthermore, the maximum palisade tissue thickness appeared at age class VI under HL intensity, whereas minimum palisade tissue thickness fell at age class I under LL intensity, and the maximum palisade tissue thickness was 2.38 times of the minimum palisade tissue thickness.

Spongy tissue thickness also exhibited regularity with the change of light intensity at the same age class (Table 3; Fig. 2). The spongy tissue thickness was decreased with the decrease of light intensity at the same age class, and statistically significant differences were not detected between ML and HL intensity treatments at age classes III and V, but detected among other different light intensity treatment groups ( $p < 0.05$ ). In addition, the spongy tissue thickness was increased with the increase of age, under ML intensity (at age classes II and III) and HL intensity (at age classes IV, V and VI) except for that under LL intensity (at age classes I, II and III), displaying statistically significant differences ( $p < 0.05$ ). The spongy tissue thickness was the smallest at age



class I and the largest at age class II under WL intensity, the smallest at age class I and the largest at age class IV under LL intensity, the smallest at age class I and the largest at age class V under ML intensity, the smallest at age class III and the largest at age class VI under HL intensity. Moreover, the spongy tissue thickness peaked at age class VI under HL intensity, which was 1.34 times that of the minimum spongy tissue thickness appearing at age class I under WL intensity.

The thickness of leaf blades at the same age class showed a certain regularity with the change of light intensity (Table 3; Fig. 2). More specifically, the blade thickness dropped with the decrease of light intensity. The differences in the blade thickness between LL and ML intensity groups at age classes I and II were statistically significant ( $p < 0.05$ ), and those among other light intensity treatment groups were also statistically significant ( $p < 0.05$ ). Besides, the blade thickness rose with the upgrade of the age class at the same light intensity, showing statistically significant differences ( $p < 0.05$ ). The blade thickness was the smallest at age class I and the largest at age class II under WL intensity, the smallest at age class I and the largest at age class IV under LL intensity, the smallest at age class I and the largest at age class V under ML intensity, the smallest at age class III and the largest at age class VI under HL intensity. Furthermore, the blade thickness reached the maximum at age class VI under HL intensity which was 1.83 times that of the minimum blade thickness appearing at age class I under WL intensity.



**Figure 3.** Cross section of *P. bournei* leaves under different light intensities. Note: I, II, III, IV, V and VI represent different grades, and WL represents low light intensity, LL represents medium-low light intensity, ML represents medium light intensity, HL represents highlight intensity



**Table 3.** Effects of different light intensity on leaf anatomical structure of *P. bournei*

	Age class	Light intensity level			
		WL	LL	ML	HL
Palisade tissue (µm)	I	29.57 ± 0.43Bc	34.95 ± 0.50Cb	40.95 ± 0.41Ea	—
	II	35.39 ± 0.65Ac	38.93 ± 0.28Bb	45.35 ± 0.39Da	—
	III	—	39.78 ± 0.74Bb	48.21 ± 0.29Ca	49.94 ± 0.19Ca
	IV	—	45.16 ± 1.19Ac	57.05 ± 0.19Bb	65.16 ± 4.89Ba
	V	—	—	64.30 ± 2.69Aa	65.42 ± 1.31Ba
	VI	—	—	—	70.31 ± 0.91A
Sponge tissue (µm)	I	45.73 ± 1.05Ba	44.15 ± 0.63Ca	45.22 ± 0.56Db	—
	II	50.59 ± 0.79Ab	54.33 ± 0.69Ba	49.26 ± 0.95Cb	—
	III	—	54.47 ± 1.05Ba	52.53 ± 0.91Cb	54.20 ± 0.20Ba
	IV	—	60.96 ± 0.79Aa	57.33 ± 0.28Bb	56.06 ± 0.7Aa
	V	—	—	61.40 ± 0.88Aa	60.61 ± 0.57Aa
	VI	—	—	—	61.25 ± 0.37Ac
Blade thickness (µm)	I	88.9 ± 0.33Bb	90.28 ± 0.32Ca	101.06 ± 0.22Ea	—
	II	102.09 ± 4.69Ab	113.13 ± 1.19Ba	111.78 ± 3.81Da	—
	III	—	115.38 ± 0.24Bc	124.59 ± 0.82Cb	134.21 ± 0.50Da
	IV	—	134.43 ± 1.88Ac	140.37 ± 0.79Bb	148.33 ± 0.24Ca
	V	—	—	147.66 ± 5.64Ab	156.87 ± 0.92Ba
	VI	—	—	—	162.41 ± 0.50A

### Changes of photosynthetic pigments in *P. bournei* leaves with age and light environment

The content of photosynthetic pigment was statistically significantly different among different light intensity treatment groups of *P. bournei* ( $p < 0.05$ ) (Table 4). The Chl a declined with the increase of the light intensity at the same age class. The Chl a of the leaves of *P. bournei* was decreased with the increase of the age class. It was found that the lowest content of Chl ( $1.51 \text{ mg} \cdot \text{g}^{-1}$ ) appeared at age class VI under HL intensity, while the highest content ( $3.82 \text{ mg} \cdot \text{g}^{-1}$ ) appeared at age class I under WL intensity.

Identical to the variation trend of Chl a, the Chl b of *P. bournei* at all age classes was decreased gradually with the increase of the light intensity. The comparison of Chl b among different age classes under the same light intensity, the content of Chl b was the lowest ( $0.70 \text{ mg} \cdot \text{g}^{-1}$ ) at age class VI under HL intensity, and the highest ( $1.88 \text{ mg} \cdot \text{g}^{-1}$ ) at age class I under WL intensity. In addition, under the same light intensity, with the increase of the age class, the content of Chl b rose under WL intensity, declined under ML intensity, and first rose and then dropped under HL intensity.

In addition, at the same age class, the content of Car was first increased and then decreased with the increase of the light intensity at age classes I, II and III, and it rose with the increase of light intensity at other age classes. Besides, the content of Car was increased first and then decreased with the increase of the light intensity at age class IV, and it was elevated with the increase of the light intensity at other classes. Under the same light intensity, the Car content rose with the increase of the age class under WL and HL intensities, while it declined with the increase of the age class under LL and ML intensities. At different light intensities and different age classes, the highest Car content ( $1.21 \text{ mg} \cdot \text{g}^{-1}$ ) appeared at age class VI under HL intensity, which was 1.7 times of the lowest Car content ( $0.71 \text{ mg} \cdot \text{g}^{-1}$ ) under WL intensity.

**Table 4.** Effects of different light intensities on the content of Chl and Car in *P. bournei*

	Age class	Light intensity level			
		WL	LL	ML	HL
Chlorophyll a (Chl a) (mg·g <sup>-1</sup> )	I	3.82 ± 0.03Aa	2.59 ± 0.01Bb	2.23 ± 0.03Ac	—
	II	3.07 ± 0.02Ba	2.57 ± 0.01Bb	2.04 ± 0.15Bc	—
	III	—	2.49 ± 0.04Aa	1.93 ± 0.10Cb	1.81 ± 0.01Ac
	IV	—	2.36 ± 0.01Ca	1.64 ± 0.02Db	1.55 ± 0.12Bb
	V	—	—	1.60 ± 0.21Da	1.52 ± 0.09Bb
	VI	—	—	—	1.51 ± 0.02B
Chlorophyll b (Chl b) (mg·g <sup>-1</sup> )	I	1.88 ± 0.02Aa	1.27 ± 0.01Ab	1.16 ± 0.01Ab	—
	II	1.50 ± 0.01Ba	1.21 ± 0.02Bb	1.11 ± 0.01Bc	—
	III	—	1.14 ± 0.07Ca	0.94 ± 0.01Cb	0.84 ± 0.03Ab
	IV	—	1.11 ± 0.03Da	0.83 ± 0.11Db	0.76 ± 0.02Bb
	V	—	—	0.74 ± 0.01Ea	0.71 ± 0.01Cb
	VI	—	—	—	0.70 ± 0.01C
Carotenoids (car) (mg·g <sup>-1</sup> )	I	0.71 ± 0.02Bc	1.13 ± 0.01Aa	1.08 ± 0.01Ab	—
	II	1.15 ± 0.01Aa	1.09 ± 0.02Bb	1.03 ± 0.02Ac	—
	III	—	0.77 ± 0.02Cc	0.91 ± 0.08Ba	0.84 ± 0.12Cb
	IV	—	0.56 ± 0.03Dc	0.89 ± 0.01Bb	0.91 ± 0.01Ba
	V	—	—	0.86 ± 0.03Cb	0.98 ± 0.02Aa
	VI	—	—	—	1.21 ± 0.21A

### Changes of photosynthetic rate of *P. bournei* leaves with age and light environment

As shown in Table 5, except for ML and HL intensities, the Pn in the *P. bournei* leaves of age classes I, II, III and IV rose first and then dropped with the increase of the light intensity. Under the same light intensity, except for WL, the Pn was increased first and then decreased with the increase of age, and it reached the highest level at age classes II, III and V, and then declined slightly. At different age classes and different light intensities, the Pn was the lowest (0.16 μmol·m<sup>-2</sup>·s<sup>-1</sup>) at age class I and the highest (3.28 μmol·m<sup>-2</sup>·s<sup>-1</sup>) at age class V.

At different light intensities and the same age class, except for ML and HL intensities, the intercellular Ci in the *P. bournei* leaves of age classes I, II, III and IV dropped first and then rose with the increase of the light intensity. Under the same light intensity, except for WL intensity, the Ci was increased first and then decreased with the increase of the age class of *P. bournei*.

At the same age class, the Gs was increased first and then decreased with the increase of the light intensity at age class I and declined at age classes I-IV. Under the same light intensity, the Gs was elevated first and then reduced with the increase of the age class except for age class II.

At the same age class, the Tr was raised first and then reduced with the increase of the light intensity at age class I and decreased at age classes I-IV. Under the same light intensity, the Tr was increased first and then decreased with the increase of the age class except for age class II.

**Table 5.** Effects of different light intensities on photosynthetic rate of *P. bournei*

	Age class	Light intensity level			
		WL	LL	ML	HL
Net photosynthetic rate (Pn) ( $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ )	I	0.16 ± 0.016Bc	1.09 ± 0.038Ba	0.44 ± 0.038Bb	—
	II	0.36 ± 0.014Ab	1.40 ± 0.077Aa	0.48 ± 0.036Bb	—
	III	—	0.74 ± 0.020Cc	1.76 ± 0.026Aa	1.12 ± 0.022Cb
	IV	—	0.41 ± 0.109Dc	1.97 ± 0.036Aa	1.24 ± 0.020Cb
	V	—	—	1.86 ± 0.049Ab	3.28 ± 0.034Aa
	VI	—	—	—	2.77 ± 0.037B
Intercellular CO <sub>2</sub> concentration (Ci) ( $\mu\text{mol}\cdot\text{mol}^{-1}$ )	I	363.63 ± 6.548Aa	361.11 ± 6.913Aa	369.78 ± 4.435Aa	—
	II	367.30 ± 0.450Aa	366.0 ± 6.013Aa	368.87 ± 7.502Aa	—
	III	—	346.11 ± 1.340Ba	345.58 ± 6.913Bc	352.08 ± 0.700Ab
	IV	—	363.57 ± 3.317Ab	323.05 ± 6.913Cc	351.96 ± 0.518Aa
	V	—	—	338.71 ± 6.913Ca	294.90 ± 0.680Bb
	VI	—	—	—	298.29 ± 2.975B
Stomatal conductance (Gs) ( $\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ )	I	0.03 ± 0.004Bb	0.04 ± 0.001Ca	0.03 ± 0.001Db	—
	II	0.04 ± 0.001Ab	0.06 ± 0.001Aa	0.03 ± 0.002Dc	—
	III	—	0.05 ± 0.001Bb	0.06 ± 0.001Ca	0.05 ± 0.001Cb
	IV	—	0.05 ± 0.001Bb	0.09 ± 0.001Aa	0.05 ± 0.001Cc
	V	—	—	0.08 ± 0.001Bb	0.12 ± 0.060Aa
	VI	—	—	—	0.09 ± 0.012B
Transpiration rate (Tr) ( $\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ )	I	0.52 ± 0.029Ba	0.61 ± 0.002Da	0.50 ± 0.002Da	—
	II	0.67 ± 0.002Aa	0.76 ± 0.002Aa	0.62 ± 0.002Ca	—
	III	—	0.68 ± 0.006Ba	0.79 ± 0.001Bb	0.70 ± 0.019Bb
	IV	—	0.63 ± 0.002Cb	1.26 ± 0.004Ab	1.06 ± 0.003Aa
	V	—	—	1.21 ± 0.001Ab	1.30 ± 0.002Aa
	VI	—	—	—	1.21 ± 0.166A

## Discussion

Judging from the seedling height, *P. bournei* at age class III is about 4-5 years old. In the natural forest of *P. bournei*, *P. bournei* above age class III (100 cm) is not distributed at 10% light intensity. When it is below age class III, *P. bournei* seedlings are mainly distributed in the ML and LL environment (Han et al., 2021). In this research center, the leaf distribution under 30-40% of the full light began at age class III, under 50-60% of the full light began at age classes III-V, and under 80-100% began at age classes III-VI. With the increase of the light intensity, the proportion of older plants was increased, while that of younger plants was decreased.

### *Adaptive changes of leaf morphological structure of P. bournei at different age classes to light intensity*

In an WL environment, the leaf area of plants will be increased to intercept more light energy to meet the growth needs under WL intensity (Yin and Shen, 2016; Jia et al., 2019). In this study, the leaf area of *P. bournei* of each age class except for age class I gradually declined with the weakening of light intensity, indicating that under WL and LL intensities, the adaptability of *P. bournei* to the WL environment can be promoted

by increasing the leaf area to capture more light quanta. However, the weaker the light is, the smaller the leaf area is. The possible reason is that at the seedling stage, *P. bournei* is still in the process of adapting to the outside world, and the light intensity can meet its photosynthetic physiological needs. In the WL environment, the increase of the SLA in plant leaves means that the leaves are thinner, which is conducive to intercepting more transmitted and scattered light, enhancing competitiveness and improving shade tolerance (Puglielli et al., 2015). The results of this study also revealed that the SLA of *P. bournei* leaves was also increased in the WL environment. This law is consistent with the conclusion of previous studies. *P. bournei* changes to a condition conducive to its own development in order to survive under the WL which also meets the growth needs for more light. Forest plants show extraordinary ability to adjust their morphological and physiological characteristics to adapt to changing light conditions, showing a wide range of phenotypic plasticity (Giertych et al., 2015; Yan et al., 2021). The adaptive changing rules of the leaf area of *P. bournei* at different age classes to the fluctuating light environment are different. Under WL, LL and ML intensities, the leaf area of *P. bournei* was increased with the increase of the age class, which indicated that the leaf area needed to be expanded to obtain more light energy. However, the largest leaf area appeared at age class V under HL intensity, and it was reduced probably owing to the water and nutrient supply of the tree. The specific reasons need to be further studied.

#### ***Adaptive changes of anatomical structure of P. bournei leaves at different age classes to light intensity***

The plasticity of leaf structure has become an important index to measure the adaptability of plants to light environment (Paludan et al., 2022). The transmittance can be regulated by adjusting the thickness of leaves to adapt to the WL environment (Cao et al., 2018).

In this study, the thickness of leaves, palisade tissue and spongy tissue of *P. bournei* were smaller under WL, LL and ML intensities, and the thickness of spongy tissue was larger than that of palisade tissue, which was consistent with that of white clover studied by Yang et al. (2015) in the WL environment. Besides, under HL intensity, the thickness of palisade tissue was larger than that of spongy tissue and that of the leaves under HL intensity. The thinning of leaves under WL intensity indicated that the leaf structure could be adjusted by changing the light intensity, so as to adapt to the fluctuating light conditions and develop in the direction of favorable for its own growth and development. When the light is strong, the thickening of palisade tissue can avoid the burn of the leaf flesh by strong light. With the decrease of the light intensity, the blade becomes thinner, which can improve the utilization of transmission and scattering light.

The adaptative changes to different light environments occur in the thickness of leaves, palisade tissue and spongy tissue of *P. bournei*. The morphological differences of trees under different light intensities are mainly caused by the environment, and have nothing to do with plant origin (heredity). However, whether trees adjust the environment actively or passively (Yin and Shen, 2016). During the sampling process, it was found that there were a large number of seedlings in the LL environment, but the seedlings were scattered or not distributed in the HL environment, especially for those at age classes I and II, probably because the leaves of the seedlings were thin, the palisade tissue was less, and the effect of the seedlings on the protection against the strong light was not strong.

### ***Adaptive changes of photosynthetic physiological parameters of *P. bournei* leaves at different age classes to light intensity***

Photosynthesis is essential for photosynthesis and growth and development of plants (Yang et al., 2019). During photosynthesis, leaves are very sensitive to environmental changes. Only under the most suitable environmental conditions can plant photosynthetic rate reach the highest level (Hang et al., 2018). The same plant has different adaptabilities under different light environments (Larrue et al., 2014), and its light-demand ability is constantly adapting to changes. The photosynthetic rate of young trees was the highest under LL intensity, that of older trees was the highest under ML intensity, and that of trees under HL intensity reached the maximum, which showed that with the increase of the age class of *P. bournei*, the photosynthetic rate was increased and the physiological function was enhanced. The Pn of *P. bournei* at five age classes was increased with the light intensity, which was consistent with the increasing trend of the leaf area at different age classes under the same light intensity (HL). The results of this study were similar to those of Zang et al. (2009).

With the increase of age, the Chl content of *P. bournei* was decreased under the same light intensity, that is, the Chl content of *P. bournei* was the highest under WL intensity. The reason is that the seedlings growing under WL intensity for a long time can obtain more light by synthesizing more Chl and increasing the leaf area, so as to ensure their normal growth. Chl a mainly absorbs red light waves, while Chl b mainly absorbs blue violet light waves, maintaining the energy balance between the optical system I and II (Wang et al., 2021). In the natural forest, the blue light/red light rises with the decrease of light transmittance (Zheng et al., 2020). Therefore, in order to improve the absorption capacity of light, Chl a in the canopy of *P. bournei* has more red light waves and relatively reduced blue light waves under the canopy. In addition, the seedlings of *P. bournei* can obtain more light energy by increasing Chl b absorption of blue violet light and Chl a absorption of red light.

The Car has two functions, namely, light capture and light damage defense. When light is insufficient, its main function is to assist Chl to capture light energy. When light is sufficient, it is mainly used for heat dissipation and light oxidation reduction (Ghanbary et al., 2017). The increase of Car in the seedlings of *P. bournei* under WL intensity is conducive to assisting Chl to capture more light energy, while the increase of Car under HL intensity is mainly used for heat dissipation. At the same time, appropriately increasing the light intensity can increase the Car content in *P. bournei* at age class III, which can effectively protect the growth and development of young *P. bournei* trees.

### **Conclusion**

The lower the age class, the stronger the adaptability under WL intensity. With the increase of age class, the light demand is also increasing. Therefore, *P. bournei* is a tree species with shade tolerance at the seedling stage, slight shade tolerance in young trees and slight light preference in adult trees.

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