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EFFECTS OF IRRADIANCE ON GROWTH AND MORPHOPHYSIOLOGY IN *CATALPA BUNGEI* PLANTLETS

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Abstract

With the aim of examining the effect of irradiance on growth and morphophysiology in *Catalpa bungei*, and its acclimation strategy to different shade intensity treatments, a pot experiment was conducted to determine the growth, morphological, and physiological parameters of *C. bungei* clone 008-1 plantlets under high (HI), medium (MI), and low (LI) irradiance levels, i.e. at approximately 80, 50, and 30% of full sunlight, respectively. Irradiance provoked significant and varied changes in phenotypic plasticity index (PPI) values for growth (ca. 0.44), morphology (ca. 0.37), and physiology (ca. 0.28). Shade treatments (MI and HI) reduced growth, biomass yield, crown leaf area per plant, expansion rate of newly emerged leaves and their size at the end of expansion (length, width, and area), specific leaf weight, net photosynthetic rate, stomatal conductance, intercellular CO_2 concentration, transpiration rate, instantaneous water use efficiency, and $\delta^{13}C$ values, whereas specific leaf area (SLA), leaf chlorophyll concentration, and leaf nitrogen (N) concentration were greatly decreased. MI did not affect maximum quantum yield of PSII (Fv/Fm) values; however, LI induced a significant decrease in Fv/Fm. Shaded plantlets generally had higher or similar non-structural carbohydrate (NSC) concentrations than HI plantlets. Overall, growth traits linearly decreased with decreasing irradiance, but morphological and physiological traits exhibited different dynamic trends. Our results showed that *C. bungei* coped with shade through morphophysiological adjustments, *i.e.* increasing SLA, leaf chlorophyll content, and NSC reserves; however, shade (ca. 30–50% full sunlight) still induced a significant reduction in plantlet growth due to photosynthesis restriction (MI: stomatal closure; LI: stomatal closure and lower photosystem II efficiency) and changes in NSC allocation strategy, preferring to maintain metabolism and survival rather than growth investment.

Key words: Irradiance, Growth, Physiology, *Catalpa bungei*.

Introduction

Irradiance plays an important role in determining plant growth and metabolism. Irradiance is widely known to cause significant variation in plant growth, biomass allocation and accumulation, leaf morphology, structure, and physiology (Yeh and Atherton, 1999; Avramov *et al*., 2007; Zheng *et al*., 2009; Moraes *et al*., 2010; Jarcuska *et al*., 2011; Hallik *et al.*, 2012; Chmura *et al*., 2017; Wang *et al.*, 2017; Díaz-Barradas *et al.*, 2018). Morphological responses (*e.g.* length, width, and area) are readily observed in leaves under changing environmental factors (*e.g.* light and water), especially in newly emerged leaves; these responses generally alter their expansion process as extrinsic factors change (Hieke *et al*., 2002; Zhu *et al.*, 2005; Murphy *et al*., 2012). In previous studies, physiological measures such as photosynthesis rate, stomatal conductance (Cond), transpiration rate (Tr), photosynthetic pigment content, and chlorophyll fluorescence were commonly measured to determine physiological adaptations in response to changing irradiance (Zheng *et al*., 2009; Hallik *et al*., 2012; Yao *et al*., 2014). Recently, researchers have begun to focus on plant water use efficiency (WUE) and carbohydrate metabolism in response to irradiance (Ahemd *et al*., 2016; Maguire & Kobe, 2016; Mcausland *et al.*, 2016; Piper & Fajardo, 2016). WUE is generally studied at two levels: instantaneous (WUEi) and long-term (WUEl). WUEi has been widely used to assess irradiance effects on physiology (Ahemd *et al*., 2016; Mcausland *et al*., 2016); however, WUEl has seldom been applied in this context. Leaf carbon isotope composition ($\delta^{13}C$) is a stable index that reliably estimates leaf WUEl (Farquhar *et al*., 1989) and photosynthetic capacity (Flanagan & Farquhar, 2014); it has been shown to be significantly influenced by irradiance (Berry *et al*., 1997). Carbohydrates represent the main photosynthate reserves, and comprise structural and non-structural carbohydrates (NSCs) (Luo *et al*., 2006). In general, NSC reserves in plants, predominantly soluble sugars and starches, are used for cell growth and maintenance, including respiratory metabolism and osmotic adjustment (McDowell, 2011), and plant NSC reserves are often consumed to maintain metabolism and defences when subjected to environmental stressors such as low light, drought, and low temperature. Many studies have used NSC concentration as a useful physiological index that reflects environmentally adaptive strategies (Myers & Kitajima, 2007; Poorter & Kitajima, 2007). Therefore, changes in plant NSC reserves can provide data that allow accurate assessment of carbohydrate storage, supply, and consumption in plants under different light conditions.

Catalpa bungei, native to China, is grown widely in temperate regions of China due to its economic benefits and beauty; several recent studies have reported its physiological characteristics and growth in response to water stress and nitrogen (N) application (Qiu *et al*., 2016; Wu *et al*., 2017; Zheng *et al*., 2017). As a result, water and fertiliser management strategies for this species are well developed in cultivation and afforestation practices, and contribute greatly to increased productivity. However, density control approaches (*e.g.* thinning and pruning) suitable for C. bungei plantation have become a serious technological bottleneck to increased productivity, due to poor understanding of its adaptation to changing light conditions. Hence, new research on irradiance effects on the growth and morphophysiology of *C. bungei* is needed. Wu *et al*., (2017) examined the effects of irradiance on the growth, mature leaf morphology, WUEi, photosynthetic capacity, photosynthetic pigment content, and chlorophyll fluorescence of *C. bungei* clone 9-1, and a few similar studies focusing on other clones have been conducted. The expansion process of a single newly emerged leaf, WUEl, and NSC reserves have proven to be effective indicators of the morphological responses of *C. bungei* to irradiance, yet have rarely been applied in this context.

In this study, we conducted a pot experiment to determine the growth, morphological, and physiological parameters of *C. bungei* clone 008-1 plantlets under different irradiance levels. We then applied these data to analyse growth and morphophysiology responses to changing irradiance. Our objective was to examine the effect of irradiance on the growth and morphophysiology of *C. bungei*, evaluate its acclimation strategies for different shade intensity levels, and provide a basic structure for future density control strategies for *C. bungei* plantations.

Material and Methods

Plant material and growth conditions: *C. bungei* clone 008-1 plantlets were obtained from Luoyang City, Henan Province, China, and transplanted into flower pots in early March 2015. The flower pots measured 30 cm (top diameter) × 30 cm (bottom diameter) × 45 cm (height). Plastic pellets were placed at the bottom of each flower pot to reduce water and soil loss, and the empty space was filled with potting soil. Soil field capacity (FC) and bulk density (BD) were determined using the core cutter method, and soil chemical properties were measured following the methods described by Lu (2000). Soil FC was 31.95% (in volume), BD was 1.04 g cm^{-3}, pH was 6.86, organic matter content was 64.70 g kg^{-1}, total N content was 2.30 g kg^{-1}, total phosphorus (P) content was 0.80 g kg^{-1}, total potassium (K) content was 18.14 g kg^{-1}, available N content was 178.94 mg kg^{-1}, available P content was 25.46 mg kg^{-1}, and available K content was 179.08 mg kg^{-1}. The plantlets were planted and allowed to acclimate for 3 months in a plastic film greenhouse measuring 60.0 m × 8.0 m × 1.6 m, with an arch height of 3.0 m, arch space of 1.0 m, and total area of 480.0 m^2. During acclimation, plantlets were abundantly watered daily and protected against insects and disease. The study site was located at the Xiaolongshan Forestry Science and Technology Research Institution, Tianshui, Gansu Province (34º29' N, 105º48' E, 1160 m a.s.l.), which is in a temperate zone within a semi-humid monsoon climatic region. The average annual rainfall and evaporation capacity at the site are 600–800 mm and 1,290 mm, respectively. The average annual temperature is 11°C, and the frost-free period lasts ca. 180 days. During the experiment, the daily average temperature in the greenhouse ranged from 20 to 38°C, and daily average humidity was 40–65%. Before treatment, the average stem height (SH) and basal diameter of the plantlets were 0.49 m and 8.63 mm, respectively. An explanation of symbols and abbreviations is provided in Table 1.

Experimental design: On 1 June, 2015, 30 pots of biennial *C. bungei* plantlets were transplanted into three fixed-light environments in a greenhouse to create low (LI), medium (MI), and high (HI) irradiance treatments, which received ca. 30, 50, and 80% of full sunlight, with 10 plantlets in each treatment. The actual light environments in all treatments were measured as shown in Fig. 1. The LI and MI treatments were produced using black shade nets with different light transmittance values, and the HI treatment was wide open (*i.e.* the light transmittance of the greenhouse was ca. 80% of full sunlight). During the experimental period (early June to early September), abundant irrigation (> 80% FC) and N fertilisation (2 g N $month^{-1}$ in early June, early July, and early August 2015) was performed to satisfy the water and fertiliser requirements of healthy plantlets.

Growth and morphological determination: The SH and ground diameter (GD) were measured at 15-day intervals from the start of light treatments (1 June, 2015). GD was determined approximately 0.5 cm above ground level using digital callipers. SH and GD were recorded and crown leaf area per plant (CLA) was measured using an LI-3100 leaf area meter (LI-COR Inc., Lincoln, NE, USA) at the end of the experimental period (1 September, 2015). Plantlets were then harvested and oven dried at 75°C for 72 h to calculate biomass yield (BY; including root, stem, and leaf; Singh & Singh 2006). To examine possible changes in leaf emergence in association with changes in irradiance, we determined single-leaf morphological parameters. On 1 July and 1 August, one newly emerged leaf (area > 1 cm^2) per plant was selected to determine its length (LL, from the leaf base to the tip; Fig. 2), maximum width (LW; Fig. 2), and area (LA) at 3-day intervals during the duration of leaf expansion (DLE). If there was no change in LL or LW between two consecutive observations, leaf expansion was considered complete (Zhu *et al*., 2005). LL, LW, and LA at the end of leaf expansion were recorded as mature single-leaf morphological parameters; the mean single-leaf expansion rate (MLER) was calculated as MLER = LA/DLE.

Table 1. Symbols and abbreviations used in this study. PSII: photosystem II; SPAD: Soil and Plant Analysis Development.

Abbreviation	Description	Precision
$\delta^{13}C$	Carbon isotope composition	0.01‰
BY	Total biomass yield	0.01 g
Ci	Intercellular CO_2 concentration	0.01 μmol CO_2 mol^{-1}
CLA	Crown leaf area per plant	0.01 m^2 $plant^{-1}$
Cond	Stomatal conductance	0.01 mol H_2O m^{-2} s^{-1}
DLE	Duration of leaf expansion	1 d
Fv/Fm	Maximum quantum yield of PSII	/
GD	Ground diameter	0.01 mm
LA	Single newly emerged leaf area	0.01 cm^2
LL	Single newly emerged leaf length	0.1 cm
LN	Leaf nitrogen concentration	0.01 g kg^{-1}
LW	Single newly emerged leaf width	0.1 cm
MLER	Mean single leaf expansion rate	0.01 cm^2 d^{-1}
NSC	Non-structural carbohydrate	/
$[NSC]_{root}$	NSC concentration in root	0.01 mg g^{-1}
$[NSC]_{stem}$	NSC concentration in stem	0.01 mg g^{-1}
$[NSC]_{leaf}$	NSC concentration in leaf	0.01 mg g^{-1}
Pn	Net photosynthetic rate	0.01 μmol CO_2 m^{-2} s^{-1}
PPFD	Photosynthetic photon flux density	1 μmol m^{-2} s^{-1}
PPI	Phenotypic plasticity index	/
SH	Stem height	0.01 m
SLA	Specific leaf area	0.01 cm^2 g^{-1}
SLW	Specific leaf weight	0.01 g m^{-2}
SPAD	SPAD-502 leaf chlorophyll metre readings	/
Tr	Transpiration rate	0.01 mmol H_2O m^{-2} s^{-1}
WUE	Water use efficiency	/
WUEi	Instantaneous WUE	0.01 μmol CO_2 $mmol^{-1}$ H_2O
WUEl	Long-term WUE	/

NSC measurements: Following BY measurements, samples were crushed, sieved through a 100-mesh screen, stored in sample bags, and prepared for NSC measurements. NSC concentrations in roots ($[NSC]_{root}$), stems ($[NSC]_{stem}$), and leaves ($[NSC]_{leaf}$) were calculated as the sum of total soluble sugar concentration ([TSS]) and starch concentration ([Starch]). [TSS] and [Starch] were measured using the anthrone–sulphuric acid colourimetric method (Zou, 1995) and expressed as mg g^{-1} dry matter.

Gas exchange parameter measurements: Net photosynthetic rate (Pn), Cond, intercellular CO_2 concentration (Ci), and Tr were determined monthly (mid-June, mid-July, and mid-August 2015) in the morning (09:00–11:00) on the fourth fully expanded leaf (from the apex) of each plant. Pn values were recorded using a portable photosynthesis system (LI-6400; LI-COR Inc.). To obtain stable measurements and simulate actual external environmental conditions, in accordance with actual light conditions under different irradiance treatments, photosynthetic photon flux density (PPFD) at the leaf surface was set at 1,000 μmol m^{-2} s^{-1} (HI), 600 μmol m^{-2} s^{-1} (MI), or 300 μmol m^{-2} s^{-1} (LI), and the temperature at the leaf surface was set at 35°C, with 42.3% relative humidity and a reference carbon dioxide (CO_2) concentration of 403.74 μmol mol^{-1}. WUEi was calculated as WUEi = Pn/Tr.

Leaf maximum quantum yield of photosystem II (PSII) (Fv/Fm) and soil and plant analysis development (SPAD) measurements: Leaf SPAD and Fv/Fm values were respectively determined monthly (mid-June, mid-July, and mid-August 2015) using a portable chlorophyll meter (SPAD-502; Minolta Camera Co. Ltd., Tokyo, Japan) and a portable chlorophyll fluorometer (MINI-PAM; Walz, Effeltrich, Germany) on the fourth and fifth fully expanded leaves from the apex.

Leaf N concentration and $\delta^{13}C$ measurements: Leaf samples were collected in mid-June, mid-July, and mid-August 2015. Each sample comprised 3–5 whole fresh leaves (fourth and fifth fully expanded leaves from the apex) from each plant. After washing, samples were oven dried at 75°C for 72 h (Singh and Singh, 2006), collected in numbered sample bags, crushed, and sieved through a 100-mesh screen. These samples were used to measure leaf N concentration, which was determined following the method described by Lu (2000). Only leaf samples collected in mid-August 2015 were used to measure leaf $\delta^{13}C$ values. As described by Bidartondo *et al*., (2004), Yang *et al*., (2012), and Qiu *et al*., (2016), leaf $\delta^{13}C$ values were determined in a stable isotope laboratory at the Chinese Academy of Forestry (Beijing, China); the overall analytical precision was ± 0.1‰.

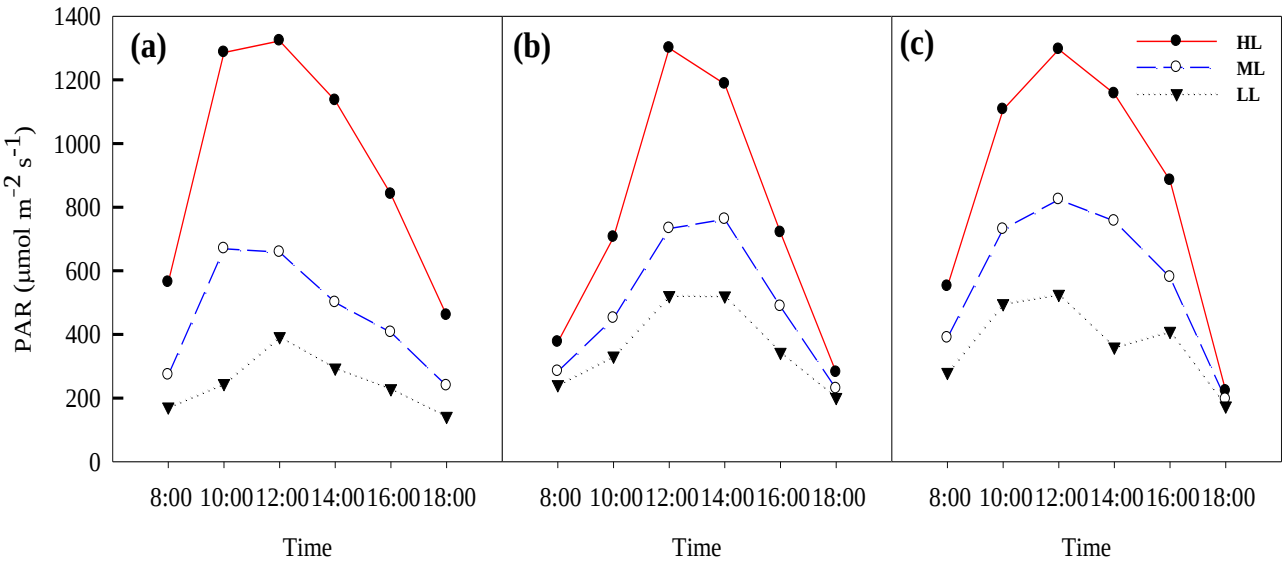

Fig. 1. Diurnal variation in photosynthetically active radiation (PAR) at the leaf level under three irradiance treatments: high (HI), medium (MI), and low (LI), at ca. 80, 50, and 30% of full sunlight, respectively. a, b, and c represent June, July, and August, respectively.

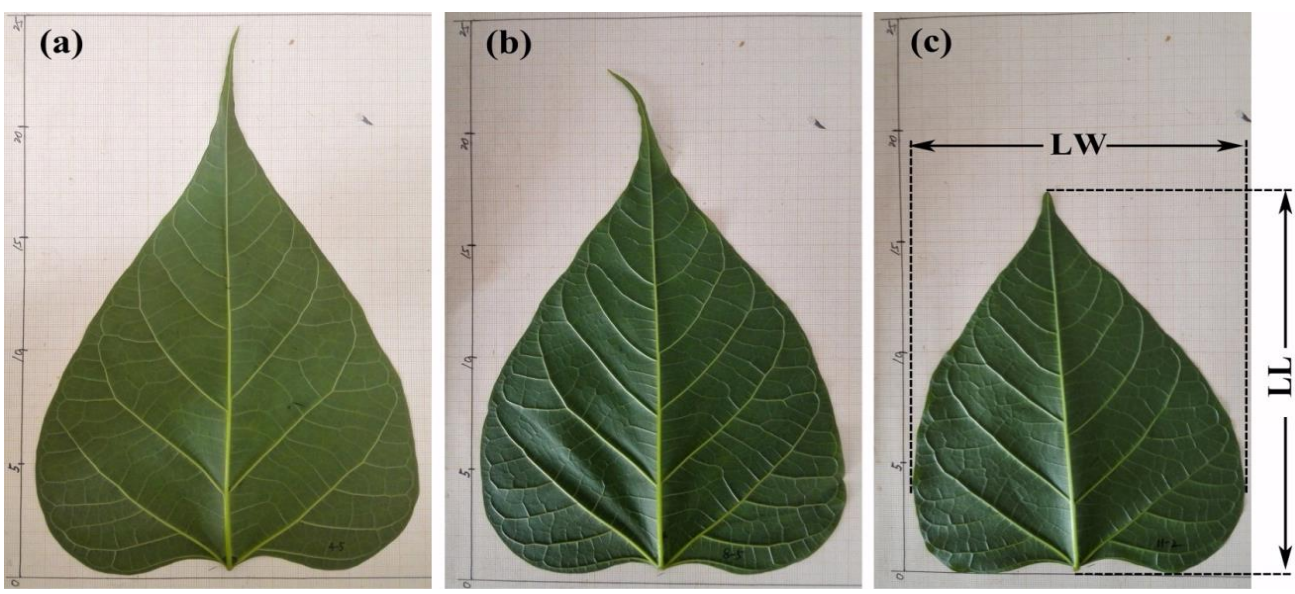

Fig. 2. Morphology of single mature leaves under HI (a), MI (b), and LI (c) treatments. LL: leaf length; LW: leaf width. Images captured in July 2015.

Statistical analyses

Effects of irradiance on plant traits were evaluated using a Kruskal–Wallis test followed by pairwise multiple comparisons. Bivariate relationships among plant traits were calculated using Pearson's correlation. The phenotypic plasticity index [PPI = |(max – min)/max|, where max and min represent mean maximum and minimum values for each plant trait, respectively] was calculated separately for each morphological and physiological parameter (Valladares *et al.*, 2000). Data represent means ± standard deviation (SD). All statistical analyses were conducted using SPSS software (ver. 20.0; SPSS Inc., Chicago, IL, USA). Figures were constructed using SigmaPlot software (ver. 10.0; Systat Software, Inc., Richmond, CA, USA).

Results

Growth and leaf morphological response: Monthly dynamics of specific leaf weight (SLW) (Fig. 3a), specific leaf area (SLA) (Fig. 3b), and single newly expanded leaf morphological parameters (LL, LW, and LA; Fig. 4) demonstrated that these traits all changed dramatically as the growth period progressed; therefore, we calculated their mean values to represent leaf morphological responses to irradiance. As shown in Fig. 4, irradiance did not affect DLE in July and August (DLE = 21 d). Kruskal–Wallis results showed that growth and morphological parameters were all significantly different between irradiance treatments (Table 2). SH (Fig. 5a), GD (Fig. 5b), BY (Fig. 5c), CLA (Fig. 5d), and SLW (Fig. 5e) decreased significantly with decreasing irradiance; SLA (Fig. 3) increased significantly with decreasing irradiance;

LL (Figs. 2, 5g), LW (Fig. 5h), LA (Figs. 2, 5i), and MLER (Fig. 5j) initially decreased (MI vs. HI) and then remained constant (LI vs. MI) as irradiance decreased.

Physiological response: Gas exchange parameters Fv/Fm, LN, and SPAD all exhibited continuous change during the experimental period (Figs. 3c–j). The physiological parameters all responded significantly to irradiance treatments (Table 2), and their responses varied (Fig. 6). Pn (Fig. 6a), Cond (Fig. 6b), and Ci (Fig. 6c) decreased significantly with decreasing irradiance. Tr (Fig. 6d), WUEi (Fig. 6e), and $\delta^{13}C$ (Fig. 6f) initially decreased (MI vs. HI) and then remained constant (LI vs. MI) as irradiance decreased. Fv/Fm (Fig. 4g) initially remained constant (MI vs. HI) and then decreased (LI vs. MI) with decreasing irradiance. LN (Fig. 4h) initially increased (MI vs. HI) and then remained constant (LI vs. MI) with decreasing irradiance. MI had similar SPAD values to HI, but lower values than LI (Fig. 6i). MI had $[NSC]_{leaf}$ levels that were higher than HI (Fig. 4j), but similar to LI. $[NSC]_{stem}$ (Fig. 4k) and $[NSC]_{root}$ (Fig. 6l) generally initially increased (MI vs. HI) and then remained constant (LI vs. MI) as irradiance decreased.

Phenotypic plasticity: PPI values of the examined traits are listed in Table 2. There were strong and weak responses to irradiance among all variables analysed (ranging from 0.11 to 0.69), and the order of the average PPI for each trait group was as follows: growth (0.44) > morphology (0.37) > physiology (0.28). Within the growth trait group, BY (0.58) and GD (0.46) had relatively higher PPI than SH (0.24). Among the morphological parameters, LL (0.24) and LW (0.14) had lower PPI than the other parameters (ranging from 0.32 to 0.63). There was a high degree of variation in the phenotypic plasticity of physiological parameters responding to irradiance, with PPI values of gas exchange parameters (*i.e*. Pn, Cond, Tr, and WUEi; > 0.3) being higher than those of Ci, $\delta^{13}C$, Fv/Fm, SPAD, LN, $[NSC]_{leaf}$, $[NSC]_{stem}$, and $[NSC]_{root}$ (< 0.3).

Relationships among plant traits: There were diverse correlations among the growth and morphophysiological parameters (Table 3). In general, there were significant positive correlations among parameters for growth, morphology (except SLA), gas exchange, $\delta^{13}C$, and Fv/Fm; however, these were all significantly negatively correlated with SLA, LN, and SPAD. In addition, there were significant positive correlations among [NSC] in different organs; however, they showed different correlations with the remaining parameters. For example, $[NSC]_{leaf}$ generally presented no significant correlation with other parameters (except Ci and Fv/Fm); $[NSC]_{stem}$ was generally significantly negatively correlated with parameters for growth, morphology (except SLA), gas exchange (except Ci), $\delta^{13}C$, and Fv/Fm, but significantly positively correlated with SLA, LN, and SPAD, and was non-significantly correlated with Ci; $[NSC]_{root}$ generally exhibited significant negative correlations with parameters for growth, morphology (except SLA), gas exchange, $\delta^{13}C$, and Fv/Fm, but was positively correlated with SLA, LN, and SPAD.

Table 2. Results of Kruskal–Wallis tests of irradiance effects and phenotypic plasticity indices (PPIs) of plant traits.

Group	Trait	Measurement interval	Calculation	*p* value	PPI
Growth	SH	15 d	End of experiment	< 0.001	0.26
	GD	15 d	End of experiment	< 0.001	0.46
	BY	End of experiment	End of experiment	< 0.001	0.58
	Mean	/	/	/	0.44
Morphology	CLA	End of experiment		< 0.001	0.63
	SLW	June, July, August	(June + July + August)/3	< 0.001	0.43
	SLA	June, July, August	(June + July + August)/3	< 0.001	0.43
	LL	July, August	(July + August)/2	< 0.001	0.24
	LW	July, August	(July + August)/2	< 0.001	0.14
	LA	July, August	(July + August)/2	< 0.001	0.36
	MLER	July, August	(July + August)/2	< 0.001	0.32
	Mean	/	/	/	0.37
Physiology	Pn	June, July, August	(June + July + August)/3	< 0.001	0.69
	Cond	June, July, August	(June + July + August)/3	< 0.001	0.41
	Ci	June, July, August	(June + July + August)/3	< 0.001	0.16
	Tr	June, July, August	(June + July + August)/3	< 0.001	0.50
	WUEi	June, July, August	(June + July + August)/3	< 0.001	0.33
	δ13C	August	August	< 0.001	0.11
	Fv/Fm	June, July, August	(June + July + August)/3	< 0.001	0.22
	LN	June, July, August	(June + July + August)/3	< 0.001	0.17
	SPAD	June, July, August	(June + July + August)/3	< 0.001	0.05
	$[NSC]_{root}$	End of experiment	End of experiment	< 0.001	0.12
	$[NSC]_{stem}$	End of experiment	End of experiment	< 0.001	0.28
	$[NSC]_{leaf}$	End of experiment	End of experiment	< 0.001	0.29
	Mean	/	/	/	0.28

Mean: mean PPI values for each trait group. LL, LW, LA, and MLER values were recorded at the end of leaf expansion. Abbreviations are defined in Table 1

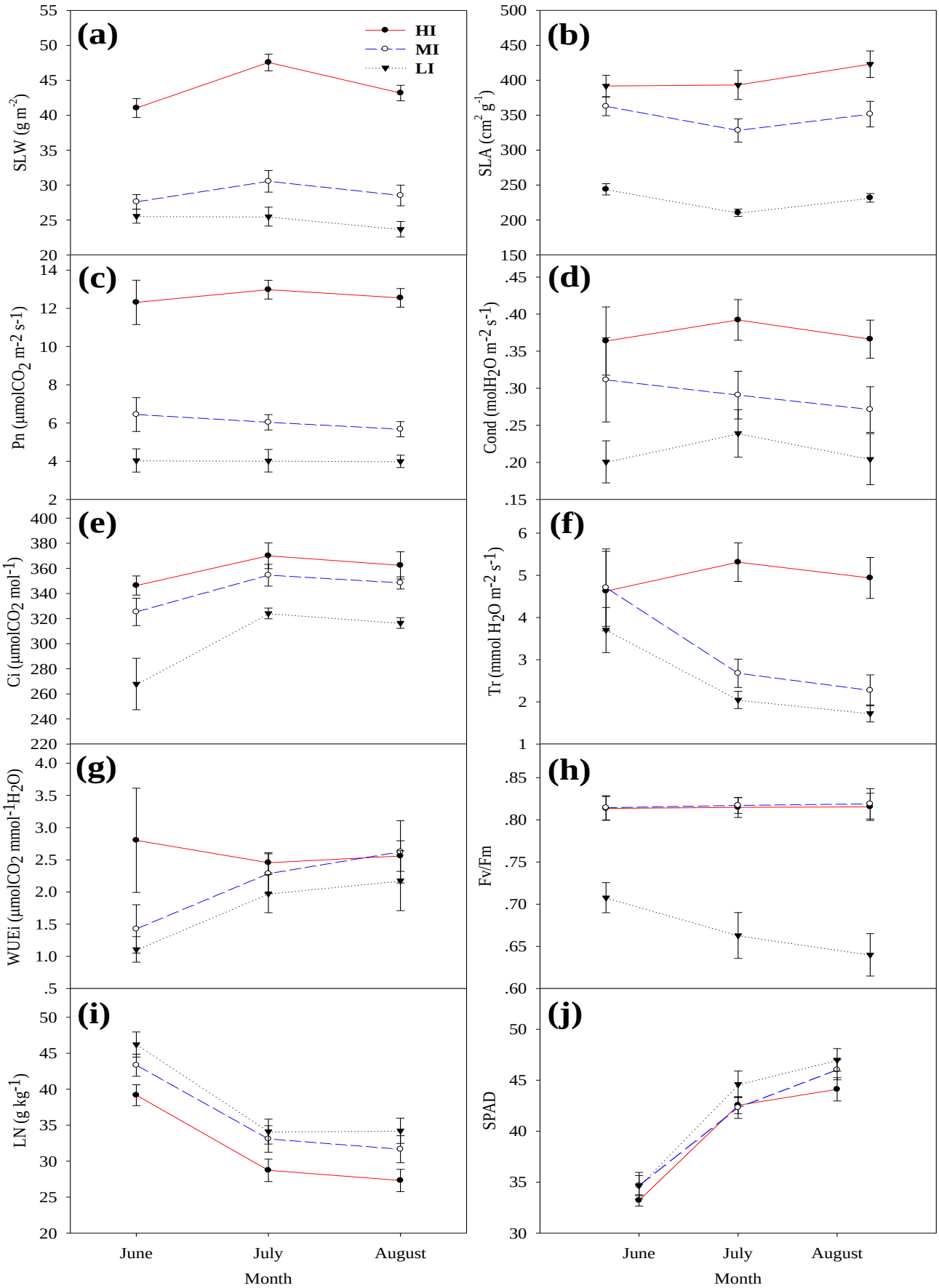

Fig. 3 Monthly dynamics of specific leaf weight (SLW) (a), specific leaf area (SLA) (b), net photosynthetic rate (Pn) (c), stomatal conductance (Cond) (d), intercellular CO_2 concentration (Ci) (e), transpiration rate (Tr) (f), instantaneous water use efficiency (WUEi) (g), maximum quantum yield of PSII (Fv/Fm) (h), leaf nitrogen concentration (LN) (i), and Soil and Plant Analysis Development (SPAD) (j) under different irradiance treatments. Data represent means ± standard deviation (SD). Abbreviations are defined in Table 1.

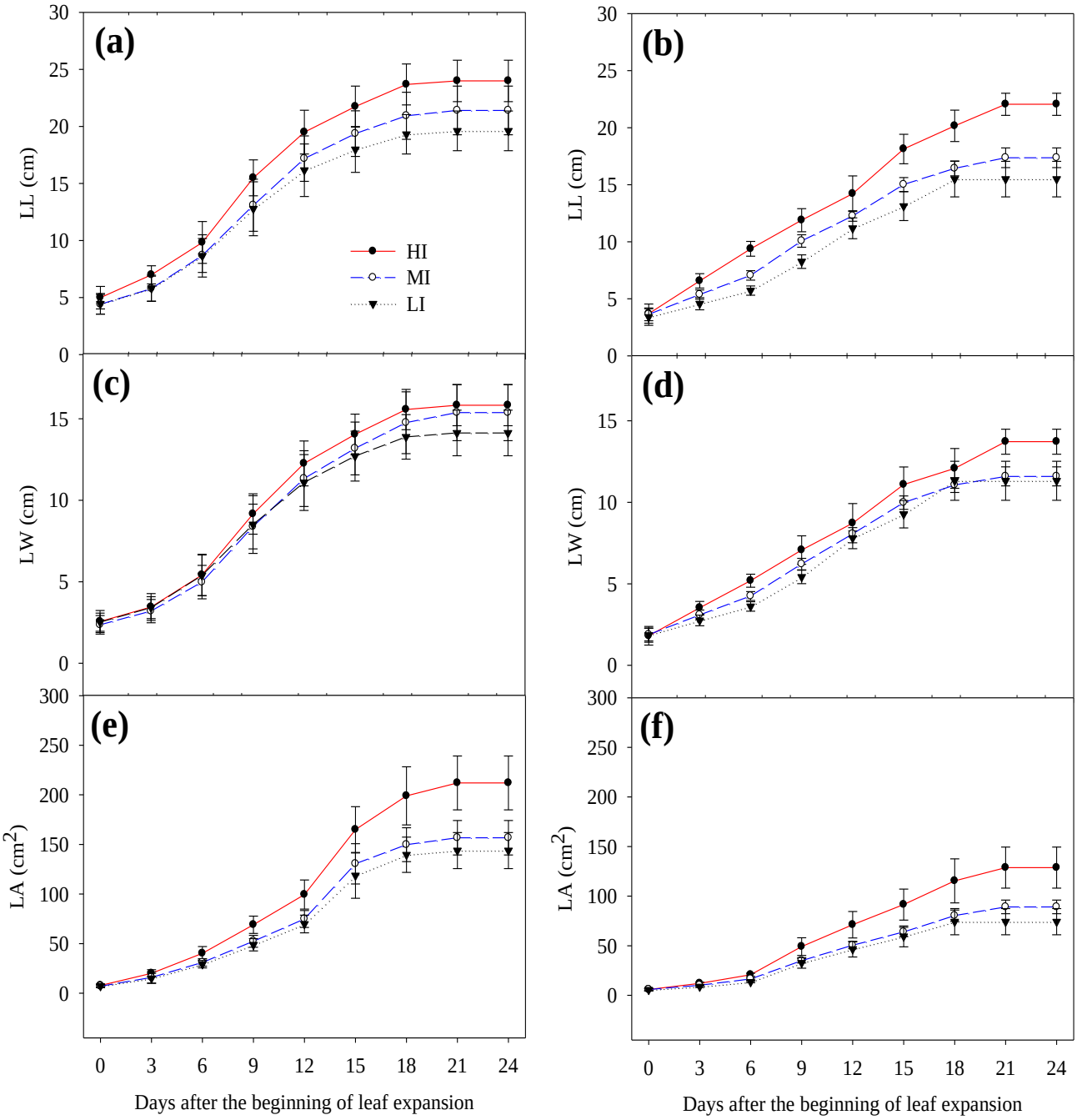

Fig. 4. Temporal dynamics of leaf length (LL) (a), maximum leaf width (LW) (c), and LA (e) in July and LL (b), LW (d), and leaf area (LA) (f) in August during leaf expansion under different irradiance treatments. Data represent means ± SD. Abbreviations are defined in Table 1.

Discussion

Our results showed that there were significant differences among PPI values for trait groups in *C. bungei* plantlets in response to irradiance (*i.e.* growth: 0.44; morphology: 0.37; physiology: 0.28), which can be mainly attributed to asynchronous adjustment (Hallik *et al.*, 2012). For example, when morphological adjustments are relatively slow and occur after leaf maturation, major structural rearrangements may not be possible due to rigidified cell walls (Yamashita *et al.*, 2002). Relatively slow physiological adjustments have been shown to result mainly from lower PPI values for leaf N, chlorophyll content, and NSC reserves in seedlings rather than gas exchange parameters (Oguchi *et al.*, 2005, 2006); our results are consistent with these findings, such that qualitative adjustments in leaf photosynthetic capacity occurred more rapidly in response to irradiance. Overall, growth and photosynthetic capacity, represented by leaf area (LA and CLA), MLER, SLW, and SLA, responded more rapidly to irradiance than the other morphological traits, whereas most physiological traits (except gas exchange parameters) exhibited a narrower range of responses.

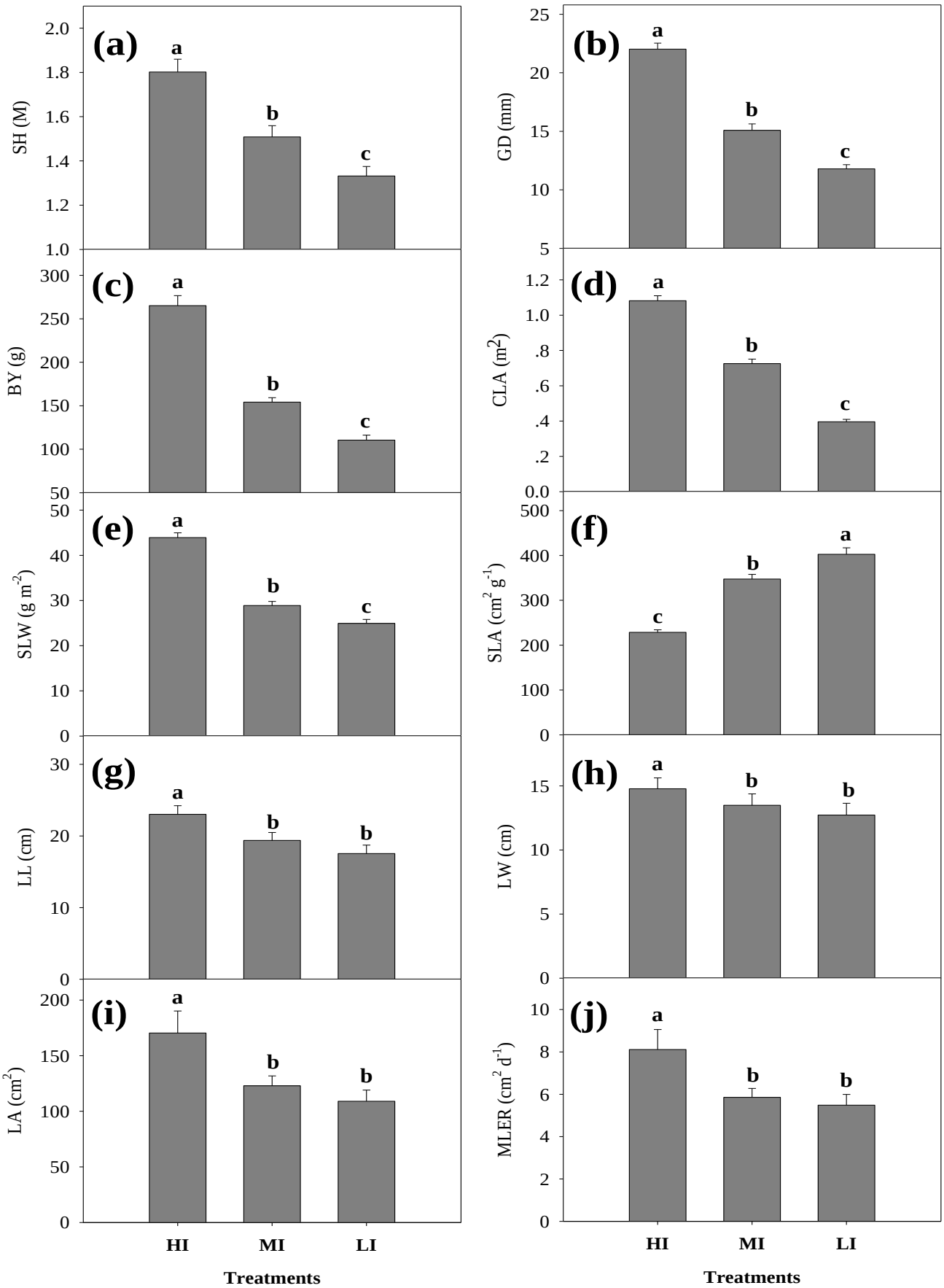

Fig. 5. Responses of stem height (SH) (a), ground diameter (GD) (b), biomass yield (BY) (c), crown leaf area per plant (CLA) (d), SLW (e), SLA (f), LL (g), LW (h), LA (i), and mean single-leaf expansion rate (MLER) (j) to irradiance treatments. Parameters calculations are shown in Table 2. Differences between treatments were evaluated by Kruskal–Wallis tests followed by pairwise multiple comparisons; different lowercase letters indicate significant differences ($p \leq 0.05$). Abbreviations are defined in Table 1.

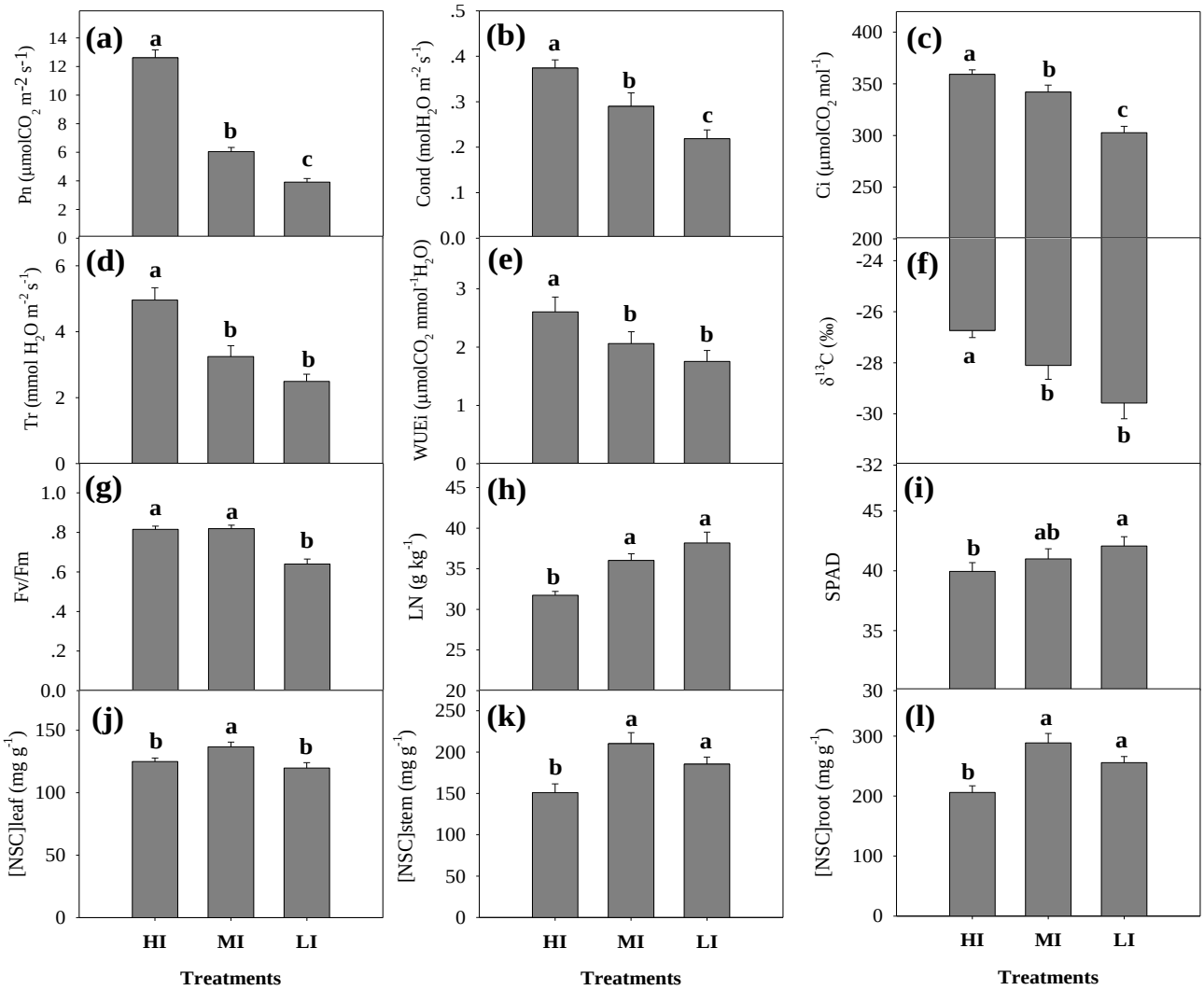

Fig. 6. Responses of Pn (a), Cond (b), Ci (c), Tr (d), WUEi (e), leaf carbon isotope composition ($\delta^{13}C$) (f), Fv/Fm (g), LN (h), SPAD (i), NSC concentration in leaves ($[NSC]_{leaf}$) (j), NSC concentration in stems ($[NSC]_{stem}$) (k), and NSC concentration in roots ($[NSC]_{root}$) (l) to irradiance treatments. Parameter calculations are provided in Table 2. Differences between treatments were evaluated using Kruskal–Wallis tests followed by pairwise multiple comparisons; different lowercase letters indicate significant differences ($p \leq 0.05$).

In several studies, plants exhibited varied ecophysiological responses to cope with shade (Dai *et al*., 2009; Díaz-Barradas *et al*., 2018). Dai *et al*., (2009) reported that shade limited light interception and carbon assimilation, and led to decreased plant growth; similarly, we also observed lower growth levels (represented by SH, GD, and BY) in plantlets grown in shade, which we attribute to the loss of photosynthetic capacity (positive relationship shown in Table 3) and carbohydrate metabolism and allocation strategy. It is widely accepted that environmental stress-induced variability in leaf photosynthesis can be mediated by stomatal closure (stomatal limitation) and caused by non-stomatal limitations (Ni & Pallardy, 1992; Broeckx *et al*., 2014). In the current study, we observed that light shade (MI) induced a decrease in light interception and stomatal limitation (Cond and Ci both decreased with decreasing irradiance; SPAD and Fv/Fm maintained relatively high levels; Fig. 6) and a decrease in Pn. In contrast, extreme shade (LI) induced a greater loss of photosynthetic capacity due to a combined mechanism of stomatal and nonstomatal limitations, as suggested by the decreases in Cond, Ci, and Fv/Fm; in particular, lower Fv/Fm values suggest that leaves that developed in severe shade exhibited lower PSII photochemical efficiency (Fig. 6g), caused by the response of plants to an imbalance between energy absorbed and utilized through photosynthesis when there is an increased employment of photoprotective energy dissipation (Huner *et al*., 1998). Numerous studies have demonstrated that leaves grown in shade are generally characterised by lower photosynthetic capacity, lower N content per unit leaf area, higher pigment content per unit leaf dry mass, and higher SLA (Niinemets, 2007; 2010; Dai *et al*., 2009; Hallik *et al*., 2012). In the present study, plantlets grown in shade (MI and LI) also exhibited smaller leaves (CLA and LA), slower MLER, higher pigment content, lower photosynthetic capacity (SLW and Pn), and greater SLA. Leaves developed in shade produce enlarged light-harvesting structures and increased chlorophyll content, thus capturing more photons and enhancing light interception (Hallik *et al*., 2012). Hallik *et al*., (2012) suggested that dry-mass-based N content was unrelated to, or increased, with decreasing irradiance, and it appears that leaf N content responses to irradiance are species-

dependent. Notably, our data indicate that shade induced higher leaf N concentration, which might be explained by N allocation. Cruz (1997) previously found that N was preferentially allocated to laminae under reduced irradiance, with more N allocated to improve leaf chlorophyll synthesis and thus promote photosynthetic capacity.

In contrast, Myers & Kitajima (2007) reported that carbohydrate storage could enhance plant shade and stress tolerance; our data also showed that plantlets grown in shade (MI and LI) generally exhibited higher or similar NSC concentrations in all organs than HI plantlets, suggesting that they maintained greater NSC reserves for physiological metabolism and defence when subjected to insufficient photosynthate supply. Based on the disparate responses of growth and NSC reserves, we speculated that plantlets allocate resources to survival (increasing NSC reserves for metabolism and defence) rather than growth (reduction of carbon investment for growth). Consistent with a previous observation that tree survival time was determined by a carbon utilisation strategy during drought (Doughty *et al*., 2015), a carbon utilisation strategy could explain how plantlets grown under long-term severe shade can survive. Barbaroux *et al*., (2003) also reported that the allocation of carbohydrate reserves to roots and shoots varies among tree species, and is determined by NSC allocation and storage strategy. Differences in NSC reserves in response to irradiance between organs were detected in our study; inconsistent with roots and stems, leaves developed in LI could not maintain similar levels of NSC reserves to leaves developed in MI, and this result may be due to the NSC translocation strategy. We suggest that LI plantlets subjected to serious NSC supply deficiency tended to allocate more NSC to stems and roots to guarantee absorption and transport.

We measured WUEi and WUEl ($\delta^{13}C$) in plantlets according to their responses to irradiance, and observed that these parameters exhibited a significant positive relationship, and similar responses, to irradiance, *i.e*. WUEi and WUEl losses were induced by shade (MI and LI), as previously reported by Berry *et al*., (1997), providing evidence that shade induced a reduction in WUE. This result could be explained by differences in the degree of impact of irradiance on Pn and Tr; shade induced a greater reduction in carbon assimilation (Pn) than water consumption (Tr).

As was previously observed in clone 9-1 (Wu *et al*., 2017), we found that shade greatly decreased growth, BY, leaf area (LA and CLA), and photosynthetic capacity; in contrast, shade increased SLA and chlorophyll content (SPAD) in clone 008-1, further demonstrating that *C. bungei* is an intolerant tree species: ca. 30–50% shade induced a significant loss of growth. Thus, we recommend that an appropriate initial stand density be determined in afforestation planning, and that accurate thinning should be conducted to control stand density. Wu *et al*., (2017) observed that the growth and morphology of clone 9-1 exhibited a narrow response to irradiance (PPI: 0.20–0.23). However, our data indicate that the growth and morphology of clone 008-1 showed greater responses to irradiance (PPI: 0.28–0.44), further demonstrating that clone 008-1 is more susceptible to light limitation. Future studies should explore differences in shade tolerance and irradiance responses between more *C. bungei* clones.

Table 3. Pearson's correlation coefficients for growth and morphophysiological parameters. Abbreviations are defined in Table 1. Parameter calculations are provided in Table 2.

Variable	GD	BY	CLA	SLW	SLA	LL	LW	LA	MER	Pn	Cond	Ci	Tr	WUEi	$\delta^{13}C$	Fv/Fm	LN	SPAD	$[NSC]_{leaf}$	$[NSC]_{stem}$	$[NSC]_{root}$
SH	0.963**	0.953**	0.954**	0.941**	−0.952**	0.877**	0.719**	0.861**	0.829**	0.949**	0.908**	0.867**	0.912**	0.827**	0.848**	0.728**	−0.904**	−0.737**	0.150	−0.619**	−0.654**
GD		0.985**	0.972**	0.981**	−0.984**	0.902**	0.709**	0.893**	0.866**	0.985**	0.935**	0.880**	0.944**	0.867**	0.902**	0.723**	−0.945**	−0.701**	0.096	−0.665**	−0.701**
BY			0.969**	0.986**	−0.985**	0.879**	0.680**	0.870**	0.845**	0.991**	0.918**	0.867**	0.964**	0.845**	0.890**	0.689**	−0.942**	−0.736**	0.039	−0.680**	−0.714**
CLA				0.949**	−0.973**	0.879**	0.682**	0.853**	0.813**	0.958**	0.951**	0.944**	0.949**	0.842**	0.924**	0.826**	−0.934**	−0.764**	0.253	−0.542**	−0.582**
SLW					−0.992**	0.883**	0.677**	0.893**	0.876**	0.988**	0.910**	0.829**	0.944**	0.857**	0.875**	0.628**	−0.926**	−0.728**	0.003	−0.729**	−0.772**
SLA						−0.879**	−0.670**	−0.883**	−0.856**	−0.982**	−0.933**	−0.879**	−0.946**	−0.867**	−0.902**	−0.702**	0.923**	0.759**	−0.113	0.658**	0.705**
LL							0.916**	0.961**	0.949**	0.879**	0.858**	0.800**	0.855**	0.746**	0.802**	0.680**	−0.887**	−0.660**	0.039	−0.636**	−0.581**
LW								0.871**	0.868**	0.687**	0.677**	0.620**	0.666**	0.563**	0.606**	0.538**	−0.725**	−0.526**	−0.039	−0.496**	−0.413*
LA									0.996**	0.879**	0.835**	0.743**	0.858**	0.730**	0.775**	0.601**	−0.841**	−0.630**	−0.006	−0.703**	−0.661**
MER										0.859**	0.799**	0.689**	0.835**	0.701**	0.737**	0.534**	−0.816**	−0.594**	−0.078	−0.737**	−0.689**
Pn											0.906**	0.848**	0.962**	0.866**	0.882**	0.664**	−0.938**	−0.709**	0.009	−0.707**	−0.747**
Cond												0.902**	0.914**	0.742**	0.861**	0.760**	−0.865**	−0.722**	0.238	−0.499**	−0.567**
Ci													0.852**	0.799**	0.905**	0.910**	−0.845**	−0.767**	0.444*	−0.354	−0.385*
Tr														0.729**	0.865**	0.694**	−0.907**	−0.695**	0.054	−0.650**	−0.669**
WUEi															0.807**	0.645**	−0.816**	−0.685**	0.132	−0.556**	−0.616**
$\delta^{13}C$																0.796**	−0.919**	−0.627**	0.301	−0.466**	−0.518**
Fv/Fm																	−0.703**	−0.611**	0.665**	−0.099	−0.096
LN																		0.625**	−0.064	0.638**	0.651**
SPAD																			−0.197	0.427*	0.419*
$[NSC]_{leaf}$																				0.480**	0.476**
$[NSC]_{stem}$																					0.865**

* and ** indicate $p \leq 0.05$ and $p \leq 0.01$, respectively; $n = 30$

Conclusions

Various irradiance treatments induced significant and different changes in the growth (PPI = 0.44), morphology (PPI = 0.37), and physiology (PPI = 0.28) of *C. bungei* plantlets. Shade (MI and LI) reduced growth, BY, leaf area (CLA), newly emerged leaf expansion rate (MLER), size at the end of expansion (LL, LW, and LA), SLW, Pn, Cond, CI, Tr, and WUE (WUEi and $\delta^{13}C$), and greatly increased SLA, LN, and SPAD. MI and HI had similar Fv/Fm values, but LI values were significantly lower. Shaded plantlets generally exhibited higher or similar NSC concentrations in all organs than HI plantlets. Generally, as irradiance decreased, all growth traits decreased linearly, but morphological and physiological traits exhibited varying dynamics. Overall, irradiance was an important driving factor controlling growth, morphology, and physiology in *C. bungei*. *C. bungei* could cope with shade through morphophysiological adjustments, *i.e*. increasing SLA, leaf chlorophyll concentration, and NSC reserves (especially in stems and roots); however, shade (ca. 30–50% full sunlight) still induced significant decreases in plantlet growth due to photosynthesis restriction (MI: stomatal closure; LI: stomatal closure and lower PSII efficiency) and a change in NSC allocation strategy (maintenance metabolism and survival rather than growth investment). This study provides a basis for effective density control planning in *C. bungei* plantations.

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References

Ahemd, H.A., A.A. Al-Faraj and A.M. Abdel-Ghany. 2016. Shading greenhouses to improve the microclimate, energy and water saving in hot regions: a review. *Sci. Hort*., 201: 36-45.

Avramov, S., D. Pemac and B. Tucić. 2007. Phenotypic plasticity in response to an irradiance gradient in *Iris Pumila*: adaptive value and evolutionary constraints. *Plant Ecol*., 190: 275-290.

Barbaroux, C., N. Bréda and E. Dufrêne. 2003. Distribution of above-ground and below-ground carbohydrate reserves in adult trees of two contrasting broad-leaved species (*Quercus petraea* and *Fagus sylvatica*). *New Phytol*., 157: 605-615.

Berry, S.C., G.T. Varney and L.B. Flanagan.1997. Leaf $\delta^{13}C$ in *Pinus resinosa* trees and understory plants: variation associated with light and CO_2 gradients. *Oecologia*, 109: 499-506.

Bidartondo, M.I., B. Burghardt, G. Gebauer, T.D. Bruns and D.J. Read. 2004. Changing partners in the dark: isotopic and molecular evidence of ectomycorrhizal liaisons between forest orchids and trees. *P. Roy. Soc. B–Biol. Sci*., 271: 1799-1806.

Broeckx, L.S., R. Fichot, M.S. Verlinden and R. Ceulemans. 2014. Seasonal variations in photosynthesis, intrinsic water-use efficiency and stable isotope composition of poplar leaves in a short-rotation plantation. *Tree Physiol*., 34: 701-715.

Chmura, D.J., J. Modrzyński, P. Chmielarz and M.G. Tjoelker. 2017. Plasticity in seedling morphology, biomass allocation and physiology among ten temperate tree species in response to shade is related to shade tolerance and not leaf habit. *Plant Biol.*, 19: 172-182.

Cruz, P. 1997. Effect of shade on the carbon and nitrogen allocation in a perennial tropical grass, *Dichanthium aristatum*. *J. Exp. Bot.*, 48: 15-23.

Dai, Y.J., Z.G. Shen, L. Ying, L.L. Wang, D. Hannaway and H.F. Lu. 2009. Effects of shade treatments on the photosynthetic capacity, chlorophyll fluorescence, and chlorophyll content of *Tetrastigma hemsleyanum* Diels et Gilg. *Environ. Exp. Bot.*, 65: 177-182.

Díaz-Barradas, M.C., M. Zunzunegui, L. Alvarez-Cansino, M.P. Esquivias, J. Valera and H. Rodríguez. 2018. How do mediterranean shrub species cope with shade? ecophysiological response to different light intensities. *Plant Biol*., 20: 296-306.

Doughty, C.E., D.B. Metcalfe, C.A.J. Girardin, F.F. Amézquita, D.G. Cabrera, W.H. Huasco, J.E. Silva-Espejo, A. Araujo-Murakami, M.C. da Costa and W. Rocha. 2015. Drought impact on forest carbon dynamics and fluxes in Amazonia. *Nature*, 519: 78-82.

Farquhar, G.D., J.R. Ehleringer and K.T. Hubick. 1989. Carbon isotope discrimination and photosynthesis. *Ann. Rev. Plant Physiol. Plant Mol. Biol*., 40: 503-537.

Flanagan, L.B. and G.D. Farquhar. 2014. Variation in the carbon and oxygen isotope composition of plant biomass and its relationship to water-use efficiency at the leaf- and ecosystem-scales in a northern Great Plains grassland. *Plant Cell Environ*., 37: 425-438.

Hallik, L., U. Niinemets and O. Kull. 2012. Photosynthetic acclimation to light in woody and herbaceous species: a comparison of leaf structure, pigment content and chlorophyll fluorescence characteristics measured in the field. *Plant Biol*., 14: 88-99.

Hieke, S., C.M. Menzel and P. Lüdders. 2002. Effects of light availability on leaf gas exchange and expansion in lychee (*Litchi chinensis*). *Tree Physiol.*, 22: 1249-1256.

Huner, N.P.A., G. Öquist and F. Sarhan. 1998. Energy balance and acclimation to light and cold. *Trends Plant Sci*., 3: 224-230.

Jarcuska, B. and M. Barna. 2011. Plasticity in above-ground biomass allocation in *Fagus sylvatica* L. saplings in response to light availability. *Ann. For. Res*. 54: 151-160.

Lu, R.K. 2000. Analytical methods of soil agricultural chemistry. Beijing: China Agricultural Science and Technology Press, pp 12-18, 106, 146-194 (in Chinese).

Luo, Z.B., C. Calfapietra, M. Liberloo, G. Scarascia-Mugnozza and A. Polle. 2006. Carbon partitioning to mobile and structural fractions in poplar wood under elevated CO_2 (EUROFACE) and N fertilization. *Global Change Biol.*, 12: 272-283.

Maguire, A.J and R.K. Kobe. 2016. Drought and shade deplete nonstructural carbohydrate reserves in seedlings of five temperate tree species. *Ecol. Evol.*, 5: 5711-5721.

Mcausland, L., S. Vialet-Chabrand, P. Davey, N.R. Baker, O. Brendel and T. Lawson. 2016. Effects of kinetics of light-induced stomatal responses on photosynthesis and water-use efficiency. *New Phytol*., 211: 1209-1220.

McDowell, N.G. 2011. Mechanisms linking drought, hydraulics, carbon metabolism, and vegetation mortality. *Plant Physiol*., 155: 1051-1059.

Moraes, G.A.B.K., A.R.M. Chaves, S.C.V. Martins, R.S. Barros and F.M. Damatta. 2010. Why is it better to produce coffee seedlings in full sunlight than in the shade? a morphophysiological approach. *Photosynthetica*, 48: 199-207.

Murphy, M.R.C., G.J. Jordan and T.J. Brodribb. 2012. Differential leaf expansion can enable hydraulic acclimation to sun and shade. *Plant Cell Environ*., 35: 1407-1418.

Myers, J.A. and K. Kitajima. 2007. Carbohydrate storage enhances seedling shade and stress tolerance in a neotropical forest. *J. Ecol.*, 95: 383-395.

Ni, B.R. and S.G. Pallardy. 1992. Stomatal and nonstomatal limitations to net photosynthesis in seedlings of woody angiosperms. *Plant Physiol*., 99: 1502-1508.

Niinemets, Ü. 2007. Photosynthesis and resource distribution through plant canopies. *Plant Cell Environ*., 30: 1052-1071.

Niinemets, Ü. 2010. A review of light interception in plant stands from leaf to canopy in different plant functional types and in species with varying shade tolerance. *Ecol. Res.*, 25: 693-714.

Oguchi, R., K. Hikosaka and T. Hirose. 2005. Leaf anatomy as a constraint for photosynthetic acclimation: differential responses in leaf anatomy to increasing growth irradiance among three deciduous trees. *Plant Cell Environ*., 28: 916-927.

Oguchi, R., K. Hikosaka, T. Hiura and T. Hirose. 2006. Leaf anatomy and light acclimation in woody seedlings after gap formation in a cool-temperate deciduous forest. *Oecologia*, 149: 571-582.

Piper, F.I. and A. Fajardo. 2016. Carbon dynamics of *Acer pseudoplatanus* seedlings under drought and complete darkness. *Tree Physiol.*, 36: 1400-1408.

Poorter, L. and K. Kitajima. 2007. Carbohydrate storage and light requirements of tropical moist and dry forest tree species. *Ecol*., 88: 1000-1011.

Qiu, Q., Q. He, J.H. Wang, Y. Su, J.W. Ma, J.W. Wu and J.Y. Li. 2016. Interactions between soil water and fertilizer on leaf photosynthesis and $\delta^{13}C$ of *Catalpa bungei* seedlings. *Dendrobiology*, 76: 25-34.

Singh, B. and G. Singh. 2006. Effects of controlled irrigation on water potential, nitrogen uptake and biomass production in *Dalbergia sissoo* seedlings. *Environ. Exp. Bot.*, 55: 209-219.

Valladares, F., E. Martinez-Ferri, L. Balaguer, E. Perez-Corona and E. Manrique. 2000. Low leaf-level response to light and nutrients in mediterranean evergreen oaks: a conservative resource-use strategy?. *New Phytol*., 148: 79-91.

Wang, J., H.J. Huang., S. Jia, X.M. Zhong., F.H. Li., K.Y. Zhang and Z.S. Shi. 2017. Photosynthesis and chlorophyll fluorescence reaction to different shade stresses of weak light sensitive maize. *Pak. J. Bot.*, 49: 1681-1688.

Wu, J.W., J.Y. Li, Y. Su, Q. He, J.H. Wang, Q. Qiu and J.W. Ma. 2017. A morphophysiological analysis of the effects of drought and shade on *Catalpa bungei* plantlets. *Acta Physiol. Plant*., 39: 80. https://doi.org/ 10.1007/s11738-017-2380-2.

Yamashita, N., N. Koike and A. Ishida. 2002. Leaf ontogenetic dependence of light acclimation in invasive and native subtropical trees of different successional status. *Plant Cell Environ.*, 25: 1341-1356.

Yang, Y., X. Yi, M. Peng and Y. Zhou. 2012. Stable carbon and nitrogen isotope signatures of root-holoparasitic *Cynomorium songaricum* and its hosts at the Tibetan plateau and the surrounding Gobi desert in China. *Isot. Environ. Healt. S.*, 48: 483-493.

Yao, Z., C. Xu, Y. Chai and H. We. 2014. Effect of light intensities on the photosynthetic characteristics of *Abies holophylla* seedlings from different provenances. *Ann. For. Res.*, 57: 181-192.

Yeh, D.M. and J.G. Atherton. 1999. Effects of irradiance on growth and flowering in the shade plant, cineraria. *Ann. App. Biol.*, 134: 329-334.

Zheng, H.F., X. Zhang, W.J. Ma, J. Song, S.U. Rahman, J.H. Wang and Y. Zhang. 2017. Morphological and physiological responses to cyclic drought in two contrasting genotypes of *Catalpa bungei*. *Environ. Exp. Bot*., 138: 77-87.

Zheng, Y.L., Y.L. Feng, W.X. Liu and Z.Y. Liao. 2009. Growth, biomass allocation, morphology, and photosynthesis of invasive *Eupatorium adenophorum* and its native congeners grown at four irradiances. *Plant Ecol*., 203: 263-271.

Zhu, X.B., Y.M. Liu and S.C. Sun. 2005. Leaf expansion of the dominant woody species of three deciduous oak forests in Nanjing, east China. *Acta Phytoecologica Sinica*, 29: 128-136 (in Chinese).

Zou, Q. 1995. Guidance of plant physiological and biochemical experiments. China Agriculture Press, Beijing, pp 53-55 (in Chinese).

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