***Pak. J. Bot.*, 44(3): 939-948, 2012.**

EFFECTS OF SLOPE ASPECTS AND STAND AGE ON THE PHOTOSYNTHETIC AND PHYSIOLOGICAL CHARACTERISTICS OF THE BLACK LOCUST (*ROBINIA PSEUDOACACIA* L.) ON THE LOESS PLATEAU

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Abstract

To investigate the effects of slope aspects and stand age on the photosynthetic and physiological characteristics of black locusts on the Loess Plateau, the responses of photosynthesis and physiology to light radiation and CO_2 concentration between juvenile (6-year-old) and mature (18-year-old) black locusts located on the sunny (southeast-facing) and shady (northwest-facing) slopes were compared and analyzed. Mature black locusts on the shady slopes present significantly higher A_{max}, V_{cmax}, AQY, ACE, R_d, R_p, Rubisco, LSP but lower J_{max}/V_{cmax}, LCP, CSP than those on the sunny slopes, and the responses of E and WUE to PAR and $[C_i]$ show considerable discrepancies at different slope aspects, which is mainly due to the variations in the microclimatic conditions between the sunny and shady slope aspects. Juvenile black locusts have significantly greater A_{max}, J_{max}, J_{max}/V_{cmax}, R_d, LCP, CCP, CSP, E but lower AQY, WUE compared to mature trees, which is likely associated with age-related differences in physiological activities. It is concluded that the mature and juvenile black locusts perform various effective acclimations of photosynthetic physiology to different slope conditions on the Loess Plateau.

Introduction

The Loess Plateau is located in the middle of the Yellow River basin of China, which has experienced serious soil erosion and degradation problems (He *et al.*, 2006; Li *et al.*, 2006). Accordingly, a lot of campaigns for vegetation restoration have been launched to prevent soil erosion and degradation (Cao *et al.*, 2009). On the Loess Plateau, black locust (*Robinia pseudoacacia* L.) as the pioneer tree species has been widely planted for its adaptability and aggressive growth (Xu *et al.*, 2009). Moreover, black locust can well improve soil fertility (Gillespie & Pope, 1990) and fix nitrogen (Feldhake, 2001). Currently, black locust plantations on the Loess Plateau play an important part in vegetation restoration and ecosystem regeneration (Xu *et al.*, 2009), which are not only effective shelters against soil erosion and degradation, but also probably to be large carbon sinks through photosynthesis processes. As a result, information of photosynthetic and physiological characteristics for black locusts on the Loess Plateau is sorely needed.

Plant photosynthetic and physiological performances are complex processes influenced by various factors. Slope aspect is an important factor due to its micro-environmental factors (such as solar radiation, temperature, and soil moisture) greatly affecting plant photosynthesis under field conditions (Johnson *et al.*, 2004; Kayama *et al.*, 2009). Despite the acknowledged importance of slope aspect for plant dynamics and performance in arid and semiarid areas, and the increasing body of studies describing plant responses to this factor, few studies so far have evaluated the effect of slope aspect on the photosynthetic physiology of plants in this region. Microclimate may vary markedly at different slope aspects and in turn have great influences on plant photosynthesis (Hasler, 1982). In addition, small scale variations in climate can have strong effects on physiological variables related to carbon fixation and water use of several woody species (e.g. Flexas *et al.*, 2001; Balaguer *et al.*, 2002), affecting plant productivity and species distribution in arid/semiarid areas. On the Loess Plateau black locusts play critical roles in fixing atmospheric CO_2 via photosynthesis. However, little report is available on how slope aspects affect the photosynthetic and physiological characteristics of black locusts.

Plant photosynthetic and physiological characteristics are affected not only by environmental factors, but also by plant endogenous factors. Although the underlying mechanisms of stand age effects on plant photosynthesis are not well understood, plant age is a key factor which impacts plant photosynthetic physiology (Bond, 2000; Munne-Bosch, 2007, 2008). In general, foliar photosynthetic capacities decrease with increasing tree age and size (Day *et al.*, 2001; Greenwood *et al.*, 2008; Merilo *et al.*, 2009). Exceptional findings, however, indicate that tree physiology varies as trees mature (Hanson *et al.*, 1994; DeSoyza *et al.*, 1996; Qi *et al.*, 2012). Nevertheless, previous studies on photosynthetic physiology in the black locust focused on seedlings (Mebrahtu *et al.*, 1993; Feng *et al.*, 2004; Wang *et al.*, 2007). On the Loess Plateau black locust trees were planted at different times in the last few decades, but there is little information on photosynthetic and physiological status in these different-aged black locust plantations.

The aims of the present study were (i) to compare the influence of slope aspects on the photosynthetic and physiological characteristics of mature black locusts, and (ii) to analyze the differences of photosynthetic and physiological status in juvenile versus mature black locusts on the Loess Plateau. The analysis of effects of both slope aspects and stand age on the photosynthetic physiology of black locusts may be of great importance to improve our knowledge about the functioning of these plantations on the Loess Plateau, which can provide us with insights into understanding of photosynthetic acclimation for these communities to different micro-environments.

Materials and Methods

Site description and sample plots: The study area is located in the Maliantan watershed of Yongshou County in Shaanxi province on the Loess Plateau of China (34°48′N, 108°07′E; altitude 1196 m above sea level). The area has undergone serious deforestation and then revegetation over the past few decades. The annual mean air temperature is 10.8°C and the annual mean precipitation is 601.6 mm, with 53% of annual precipitation happening between July and September. The annual average potential evaporation is 807.4 mm. The soil is typical loess with ca. organic matter content 1%, available N 44.2 μg g^{-1}, and available K 189.1 μg g^{-1} (Zheng *et al.*, 2011).

In the study area six mature and three juvenile plantations of black locust (*Robinia pseudoacacia* L.) with the age of 18 and 6 years, respectively, were chosen. They were located on three parallel ridges with the distance to each other of approximately 200 meters. On each ridge one mature plantation is located on the upper part of a sunny, southeast-facing slope and the other on the upper part of a shady, northwest-facing slope. On each ridge the juvenile plantation is also located on the upper part of the shady slope. A sample plot (20 m × 20 m) was randomly selected in each plantation. The diameter at breast height of each tree in each sample plot was measured using a caliper and the height was determined using a height gauge. The light radiation and air temperature were automatically recorded by the portable gas exchange system (Li-Cor 6400; Li-Cor Inc., Lincoln, NE, USA). The daily mean light radiation and air temperature at canopy level were estimated by averaging data across all times of day. The soil moisture in top 10 cm soil was measured by the gravimetric method (Wang *et al.*, 2009). The characteristics of these plantations are given in Table 1. In each sample plot nine average sample trees were selected to measure photosynthetic and physiological parameters.

Table 1. Characteristics of black locust (*Robinia pseudoacacia*) plantations. The diameter at breast height of each tree in a sample plot of each plantation was measured using a caliper and the height was determined using a height gauge. The data for diameters and heights are means ± SE (n=9). For each parameter, the values followed by different letters indicate significant difference.

Ridge	1			2			3		
Plantation	**I**	**II**	**III**	**IV**	**V**	**VI**	**VII**	**VIII**	**IX**
Slope aspect	sunny	shady	shady	sunny	shady	shady	sunny	shady	shady
Stand age (a)	18	18	6	18	18	6	18	18	6
Light radiation (μmol photon m^{-2} s^{-1})	1024	880	879	1022	884	882	1028	887	876
Air temperature at canopy level (°C)	31.4	28.1	28.6	30.2	28.4	28.7	32.5	28.2	28.6
Soil moisture in top 10 cm soil (%)	11.8	16.5	17	11.7	16.8	17	11.9	16.3	17.1
Diameter (cm)	8.6±0.4a	6.8±0.3b	4.0±0.2c	9.3±0.4a	6.2±0.3b	4.7±0.2c	7.8±0.4a	7.3±0.4b	3.6±0.1c
Height (m)	10.2±0.6a	8.5±0.4b	4.5±0.3c	11.1±0.6a	8.2±0.4b	5.2±0.3c	9.6±0.5a	9.0±0.5b	4.3±0.2c
Stand density (tree numbers ha^{-1})	1695	1770	1755	1650	1815	1725	1740	1710	1800

Field measurements

Photosynthesis and physiology response curve measurements: A scaffold was used to access the canopy for intact measurements of leaf photosynthesis. The mature and healthy leaves on the south branch at the middle layer of the tree canopy were selected to determine the response of net CO_2 assimilation rate (A_n), transpiration rate (E), and water use efficiency (WUE=A_n/E) to photosynthetic active radiation (PAR) and intercellular CO_2 concentration (C_i). All the response curves were measured *in situ* in leaves by using the portable gas exchange system. Because black locusts' biological clock can significantly influence the stomatal movements and photosynthetic enzyme activities, the photosynthetic light response (A/PAR) and CO_2 response (A/C_i) curves, and the physiological light response (E/PAR, WUE/PAR) and CO_2 response (E/C_i, WUE/C_i) curves were measured from 8: 30 to 11: 30 during August, 2008 when the black locust reaches its maximum annual growth rate (Zou, 1986; Feldhake, 2001). During the maximum growth rate period, leaves are fully expanded and mature, and the photosynthetic acclimation to micro-climate is more stable and evident compared to other growth stages. A/PAR, E/PAR, and WUE/PAR curves were constructed by taking measurements at 13 PAR values, ranging from 0 to 1900 μmol m^{-2} s^{-1}. At the same time, the ambient CO_2 concentration was stabilized at 360±2 μmol mol^{-1} through the CO_2 buffer bottle, with the atmospheric relative humidity at 41±1%. A/C_i, E/C_i, WUE/C_i curves were constructed by taking measurements at 16 CO_2 concentrations, ranging from 50 to 1900 μmol mol^{-1}. In these measurements, the leaf surfaces were exposed to the 6400-02B LED light source at controlled PAR (1000 μmol m^{-2} s^{-1}).

Model descriptions: To date, the non-rectangular hyperbolic models have been largely applied (Thornley, 1998; Meir *et al.*, 2007) to predict photosynthetic response process of plant, and the parameters of these models have good biological significances. Accordingly, non-rectangular hyperbolic models to simulate photosynthesis response curves of black locusts were chosen in this study.

Simulations of A/PAR curves: The relationship between A_n and PAR can be fitted by the non-rectangular hyperbolic model below (Herrick & Thomas, 1999):

$$A_n = \frac{AQY \times PAR + A_{\max} - \sqrt{(AQY \times PAR + A_{\max})^2 - 4 \times AQY \times PAR \times \theta \times A_{\max}}}{2 \times \theta} - R_d \quad (1)$$

where A_n is the net CO_2 assimilation rate, PAR is the photosynthetic active radiation, AQY is the apparent quantum yield, A_{max} is the maximum photosynthetic rate, θ is the convexity, R_d is the dark respiration rate. Since the response of A_n to PAR was linear when PAR is below 200 µmol m^{-2} s^{-1} (Yuzei & Liliana, 1999), AQY was calculated from the initial slopes by performing a linear regression of the data obtained under PAR values between 0 and 200 µmol m^{-2} s^{-1} (Liao *et al.*, 2005). R_d and light compensation point (LCP) could be obtained by fitting between the linear regression and A/PAR curve (Yang *et al.*, 2008). Light saturation point (LSP) was estimated according to the trends of A/PAR curves (Zhang & Xu, 2000).

Simulations of A/C_i curves: The relation between A_n and C_i of black locust leaves was fitted by the model of Farquhar and von Caemmerer (1982):

$$A_n = \frac{ACE \times C_i + J_{\max} - \sqrt{(ACE \times C_i + J_{\max})^2 - 4 \times ACE \times C_i \times \theta \times J_{\max}}}{2 \times \theta} - R_p \quad (2)$$

where C_i is the intercellular CO_2 concentration, ACE is the apparent carboxylation efficiency, J_{max} is the maximum electron transport rate, and R_p is the light respiration rate. ACE was calculated from the initial slopes by linear regression using C_i concentrations below 200 µmol mol^{-1} (Farquhar & Sharky, 1982). R_P and CO_2 compensation point (CCP) could be obtained by fitting the linear regression to A/C_i curve (Yang *et al.*, 2008). CO_2 saturation point (CSP) was estimated according to the trends of A/C_i curves (Zhang & Xu, 2000).

The maximum carboxylation efficiency (V_{cmax}) was estimated from the A/C_i curve at $C_i \leq 200$ µmol mol^{-1}, and it was assumed that, below this level, assimilation of CO_2 was limited solely by the amount, activity and kinetic properties of Rubisco (ribulose-1, 5-bisphosphate carboxylose) at low C_i concentration (Han & Chiba, 2009), so A_n under the condition of limited carboxylation could be expressed as (Farquhar *et al.*, 1980):

$$A_n = V_{c\max} \times \frac{C_i - CCP}{C_i + k_c \times [1 + (o_i / k_o)]} - R_p \quad (3)$$

where K_c is the Rubisco Michaelis constant of the carboxylation reaction at 25°C (460 µPa). K_o is the Rubisco Michaelis constant of the oxidation at 25 °C (330 mPa). O_i is the oxygen partial pressure at the chloroplast carboxylation part (210 mPa).

V_{cmax} reached maximal carboxylation rate when Rubisco content was saturated, and it had a positive correlation with the quantity and activity of Rubisco (Farquhar *et al.*, 1980), thus the Rubisco content could be estimated by the following equation (Hymus *et al.*, 2002):

$$\text{Rubisco content} = V_{c\max} / (8 \times k_{cat}) \quad (4)$$

where eight is the number of Rubisco active site. K_{cat} is the carboxylation capacity of each active site (3.3).

Simulations of E/PAR, WUE/PAR, E/C_i, and WUE/C_i curve: The E/PAR, WUE/PAR, E/C_i, and WUE/C_i curves were simulated by using the curve estimation module of the software program SPSS (version 13, SPSS Inc., Chicago, USA) and subsequently selected and optimized according to the correlation coefficients.

Statistical analysis: To determine the effects of slope aspects and stand age on photosynthetic and physiological parameters of black locusts, the experiment was designed by using the mature trees located on the upper part of the shady slopes to compare either with the mature trees on the upper part of the sunny slopes (slope aspect effect) or with the juvenile trees on the upper part of the shady slopes (age effect). Analysis of variance (ANOVA) was conducted to test the effects of slope aspects and stand age on the photosynthetic parameters of black locusts by using the SPSS software. Significant differences and correlations between parameter means were tested and indicated by asterisks. *: $p \leq 0.05$, **: $p \leq 0.01$, ***: $p \leq 0.001$.

Results

Effects of slope aspects on photosynthetic and physiological characteristics: To gain information on the effects of slope aspects on the photosynthetic characteristics, data from gas exchange measurements in black locusts were compared (Fig. 1; Table 2). The photosynthetic light response (A/PAR) curves of black locusts were illustrated in Fig. 1a. In the beginning, the net CO_2 assimilation rate (A_n) increases rapidly under the photosynthetic active radiation (PAR) values between 0 and 200 µmol m^{-2} s^{-1}. At this stage, PAR is the main limiting factor to photosynthesis. Subsequently, the A_n increases slowly with the increasing PAR and even decreases slightly, which is probably because of the light supersaturation phenomenon of A_n, making the leaves not adequately absorb and utilize the high-intensity lights attributed to the limit of the enzymatic reaction. The responses of A_n to $[C_i]$ in black locusts on the sunny and shady slopes are shown in Fig. 1b. The primary source of biochemical limitation to A_n depends generally on the $[C_i]$. Measured and simulated A_n respond asymptotically to increase in C_i in all data sets with an initial steep slope at subambient $[C_i]$, where A_n is generally limited by Rubisco activity, and then the A/C_i curves exhibit a gradual saturation as C_i increases above ambient $[C_i]$ and approaches the Rubisco-regeneration limited part of the A/C_i curve. This response of A_n to $[C_i]$ is expected because increased $[CO_2]$ provides more substrate for carboxylation and overcomes the competitive inhibition of Rubisco enzyme by oxygen.

Although the photosynthesis response curves in black locusts on the sunny and shady slopes have similar trends, aspect changes affect the gas exchange of trees. For the A/PAR curves, the measured and simulated A_{ns} of black locusts on the sunny slopes are lower than those on the shady slopes under the PAR values between 800 and 1900 µmol m^{-2} s^{-1} (Fig. 1a). For the A/C_i curves, as $[C_i]$ is below 600 µmol mol^{-1}, the A_{ns} on the sunny slopes are lower compared to counterparts on the shady slopes, but the opposite is true when $[C_i]$ is approximately from 800 to 1600 µmol mol^{-1} (Fig. 1b). To further understand the effects of slope aspects on photosynthetic characteristics in black locusts, the photosynthetic parameters of mature trees on sunny and shady slopes were compared by using the variance analysis (Table 2). The AQY, ACE, A_{max}, V_{cmax}, Rubisco, R_d, R_p, and LSP in mature black locusts on the shady slopes are significantly higher than those on the sunny slopes. However, the J_{max}/V_{cmax}, LCP, and CSP in mature trees on the shady slopes are markedly lower compared to counterparts on the sunny slopes. In addition, black locusts on the shady slopes have slightly lower J_{maxs} and CCPs relative to those on the sunny slopes.

Table 2. Results of variance analysis on the apparent quantum yield (AQY), maximum photosynthetic rate (A_{max}), dark respiration rate (R_d), apparent carboxylation efficiency (ACE), maximum electron transport rate (J_{max}), light respiration rate (R_p), maximum carboxylation efficiency (V_{cmax}), ratio of J_{max} to V_{cmax} (J_{max} / V_{cmax}), rubisco content (Rubisco), light compensation point (LCP), light saturation point (LSP), CO_2 compensation point (CCP), and CO_2 saturation point (CSP) under different comparison conditions in black locust plantations. The mean values ± SE (n=27).

Photosynthetic parameter	Sunny and mature	Shady and mature	Shady and juvenile	slope aspect	stand age
AQY (µmol CO_2 µmol^{-1} photon flux density)	0.051 ± 0.004	0.065 ± 0.003	0.055 ± 0.003	*	*
A_{max} (µmol m^{-2} s^{-1})	11.04 ± 0.64	13.77 ± 0.80	17.46 ± 1.01	*	*
R_d (µmol m^{-2} s^{-1})	0.34 ± 0.06	0.65 ± 0.06	1.75 ± 0.12	**	***
ACE (µmol m^{-2} s^{-1})	0.050 ± 0.003	0.087 ± 0.006	0.077 ± 0.004	**	NS
J_{max} (µmol m^{-2} s^{-1})	35.33 ± 2.04	29.51 ± 1.70	50.00 ± 2.89	NS	**
R_p (µmol m^{-2} s^{-1})	2.92 ± 0.15	5.28 ± 0.33	5.50 ± 0.26	**	NS
V_{cmax} (µmol m^{-2} s^{-1})	15.00 ± 0.82	22.21 ± 1.32	26.89 ± 1.30	**	NS
J_{max}/V_{cmax}	2.36 ± 0.14	1.33 ± 0.07	2.04 ± 0.13	**	**
Rubisco (µmol m^{-2})	0.57 ± 0.03	0.84 ± 0.05	1.02 ± 0.05	**	NS
LCP (µmol m^{-2} s^{-1})	18.35 ± 1.23	8.10 ± 0.87	41.84 ± 2.28	***	***
LSP (µmol m^{-2} s^{-1})	778 ± 27	911 ± 20	859 ± 23	***	NS
CCP (µmol mol^{-1})	60.80 ± 3.52	58.25 ± 3.16	71.12 ± 3.53	NS	***
CSP (µmol mol^{-1})	948 ± 15	779 ± 21	915 ± 21	***	***

Significant differences were examined and indicated by asterisks.*: $p \le 0.05$, **: $p \le 0.01$, ***: $p \le 0.001$.

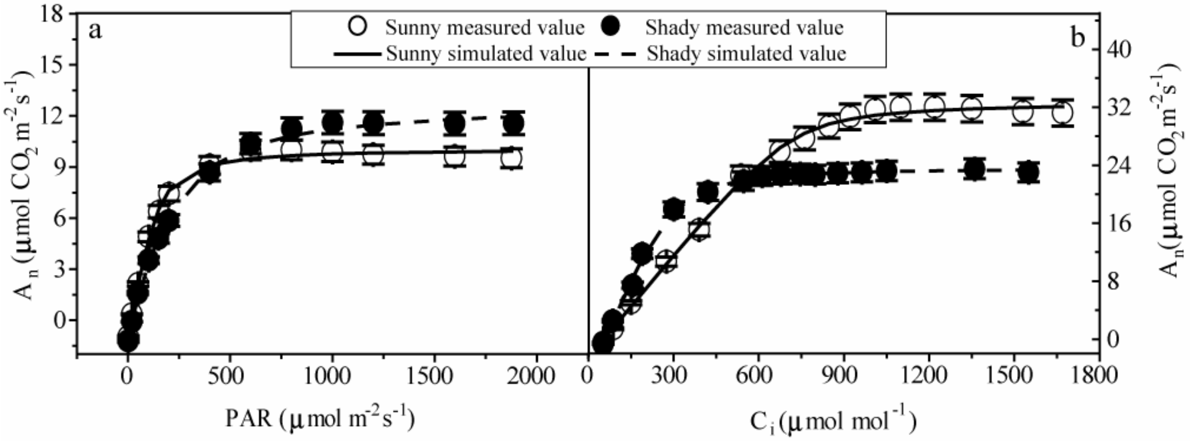

Fig. 1. Measured and simulated values of responses of net CO_2 assimilation rate (A_n) to photosynthetic active radiation (PAR) and intercellular CO_2 concentration (C_i) of mature black locust trees on the sunny and shady slopes. The mean values ± SE (n=27).

To estimate the physiological parameters of black locusts, the E/PAR, E/C_i, WUE/PAR, and WUE/C_i curves of mature trees on the sunny and shady slopes were analyzed (Fig. 2). The transpiration rates increase rapidly with increasing PAR values at the beginning and then keep stable (Fig. 2a). In contrast, the Es decrease quickly with enhancive [C_i] firstly but increase slowly later (Fig. 2b). The measured and simulated Es on the sunny slopes are higher than those on the shady slopes as $0 \leq PAR \leq 200$ (μmol m^{-2} s^{-1}) and then are markedly lower compared to the counterparts on the shady slopes (Fig. 2a), while the responses of E to [C_i] in black locusts on the sunny slopes are greater than those on the shady slopes except values under the [C_i] is below 200 μmol mol^{-1} (Fig. 2b). The responses of water use efficiencies to both PAR and [C_i] for black locusts look like the parabolic shapes (Fig. 2c, d), which is mainly because the increase rate of A_n is higher than the value of E under the low PAR value and [C_i] but lower compared to that of E above the LSP and CSP. Most simulated and measured WUEs response to the PAR on the sunny slopes are greater than counterparts on the shady slopes, while which response to the [C_i] on the sunny slopes are lower relative to values on the shady slopes (Fig. 2c, d).

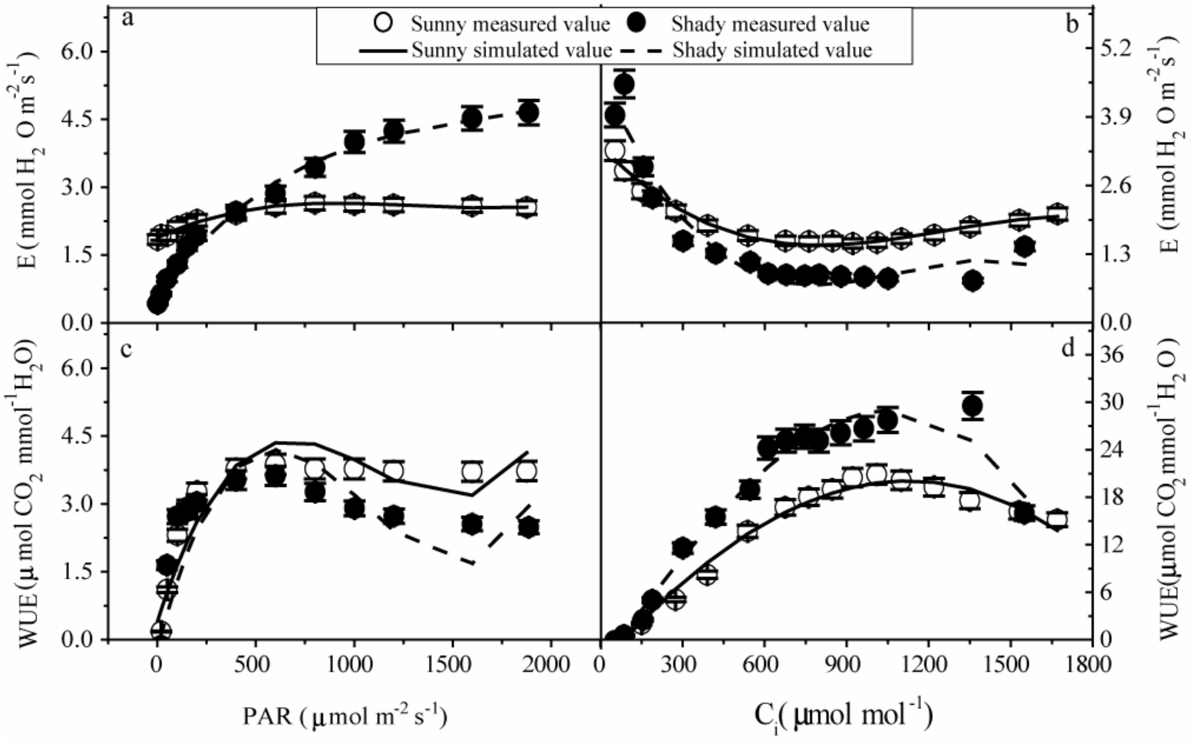

Fig. 2. Measured and simulated values of responses of transpiration rate (E) and water use efficiency (WUE) to photosynthetic active radiation (PAR) and intercellular CO_2 concentration (C_i) of mature black locust trees on the sunny and shady slopes. The mean values ± SE (n=27).

Effects of stand age on photosynthetic and physiological characteristics: As already mentioned in the introduction, stand age is a key factor impacting the photosynthetic characteristics. To obtain information on how stand age affects the photosynthetic parameters, data recorded from gas exchange measurements in mature and juvenile black locust trees on shady slopes were analyzed (Fig. 3; Table 2). Although the trends of photosynthesis response curves show similar patterns in mature and juvenile trees, stand age has a great influence on each parameter of photosynthesis response curves. The measured and simulated A_{ns} of juvenile black locusts response to PAR and [C_i] are higher than those of mature trees at approximately 800 µmol m^{-2} s^{-1} and 600 µmol mol^{-1} onwards, respectively (Fig. 3a, b). The comparison results of photosynthetic parameters of mature and juvenile black locusts are presented in Table 2. The juvenile black locusts have significant higher A_{max}, J_{max}, J_{max}/V_{cmax}, R_d, LCP, CCP, and CSP values than the mature trees. Moreover, the V_{cmax}, Rubisco, and R_p of juvenile black locusts are slightly greater compared to those of mature trees. Nevertheless, the mature black locust trees have pronouncedly greater AQY and slightly higher ACE and LSP compared to the juvenile ones.

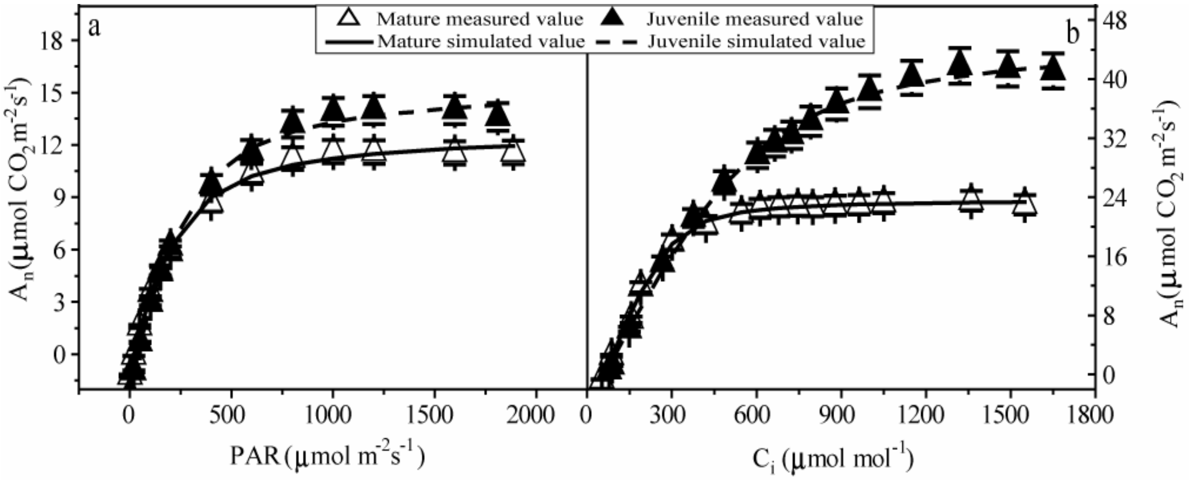

Fig. 3. Measured and simulated values of responses of net CO_2 assimilation rate (A_n) to photosynthetic active radiation (PAR) and intercellular CO_2 concentration (C_i) of mature and juvenile black locust trees on the shady slopes. The mean values ± SE (n=27).

The responses of E and WUE on PAR and [C_i] show that E/PAR, E/C_i, WUE/PAR, and WUE/C_i curves vary markedly in mature and juvenile trees located on the shady slopes (Fig. 4). The measured and simulated Es of juvenile black locusts are considerably higher than those of mature trees at all measuring PAR and [C_i] points (Fig. 4a, b). The WUEs of juvenile trees, however, are lower compared to values of mature trees at most measuring PAR points, and similar results exist in WUE/C_i curves (Fig. 4c, d). Moreover, the simulated equations for E/PAR, E/C_i, WUE/PAR, and WUE/C_i curves of mature and juvenile black locusts on sunny and shady slopes are illustrated in Table 3, which indicate that the coefficients of each physiology response curve show great variations either between the mature black locusts on sunny and shady slopes or between the mature and juvenile trees on the shady slopes (Table 3).

Table 3. Simulated equations of transpiration rate (E) and water use efficiency (WUE) response to photosynthetic active radiation (PAR) and intercellular CO_2 concentration (C_i) of black locust trees on the Loess Plateau.

Plantation	Curve	Simulated equation	R^2
Sunny and mature	E/PAR	$E=1.88+2.04\times10^{-3}\ PAR-1.72\times10^{-6}\ PAR^2+4.40\times10^{-10}\ PAR^3$	0.986^{***}
	E/C_i	$E=3.36-5.62\times10^{-3}\ C_i+5.15\times10^{-6}\ C_i^2-1.36\times10^{-9}\ C_i^3$	0.981^{***}
	WUE/PAR	$WUE=0.40+1.38\times10^{-2}\ PAR-1.47\times10^{-5}\ PAR^2+4.48\times10^{-9}\ PAR^3$	0.871^{***}
	WUE/C_i	$WUE=-3.21+3.91\times10^{-2}\ C_i-1.42\times10^{-5}\ C_i^2+1.80\times10^{-9}\ C_i^3$	0.984^{***}
Shady and mature	E/PAR	$E=0.66+5.68\times10^{-3}\ PAR-3.06\times10^{-6}\ PAR^2+6.24\times10^{-10}\ PAR^3$	0.989^{***}
	E/C_i	$E=4.73-1.26\times10^{-2}\ C_i+1.25\times10^{-5}\ C_i^2-3.81\times10^{-9}\ C_i^3$	0.933^{***}
	WUE/PAR	$WUE=-0.21+1.69\times10^{-2}\ PAR-1.96\times10^{-5}\ PAR^2+6.11\times10^{-9}\ PAR^3$	0.650^{*}
	WUE/C_i	$WUE=-3.47+4.90\times10^{-2}\ C_i-5.98\times10^{-6}\ C_i^2-1.07\times10^{-8}\ C_i^3$	0.972^{***}
Shady and juvenile	E/PAR	$E=2.01+9.94\times10^{-3}\ PAR-5.93\times10^{-6}\ PAR^2+1.20\times10^{-9}\ PAR^3$	0.984^{***}
	E/C_i	$E=8.43-1.83\times10^{-2}\ C_i+1.77\times10^{-5}\ C_i^2-4.59\times10^{-9}\ C_i^3$	0.978^{***}
	WUE/PAR	$WUE=-0.50+1.03\times10^{-2}\ PAR-1.14\times10^{-5}\ PAR^2+3.58\times10^{-9}\ PAR^3$	0.871^{***}
	WUE/C_i	$WUE=-3.78+3.84\times10^{-2}\ C_i-2.76\times10^{-5}\ C_i^2+4.91\times10^{-9}\ C_i^3$	0.947^{***}

Significant correlations were tested and indicated by asterisks. *: $p\leq0.05$, **: $p\leq0.01$, ***: $p\leq0.001$

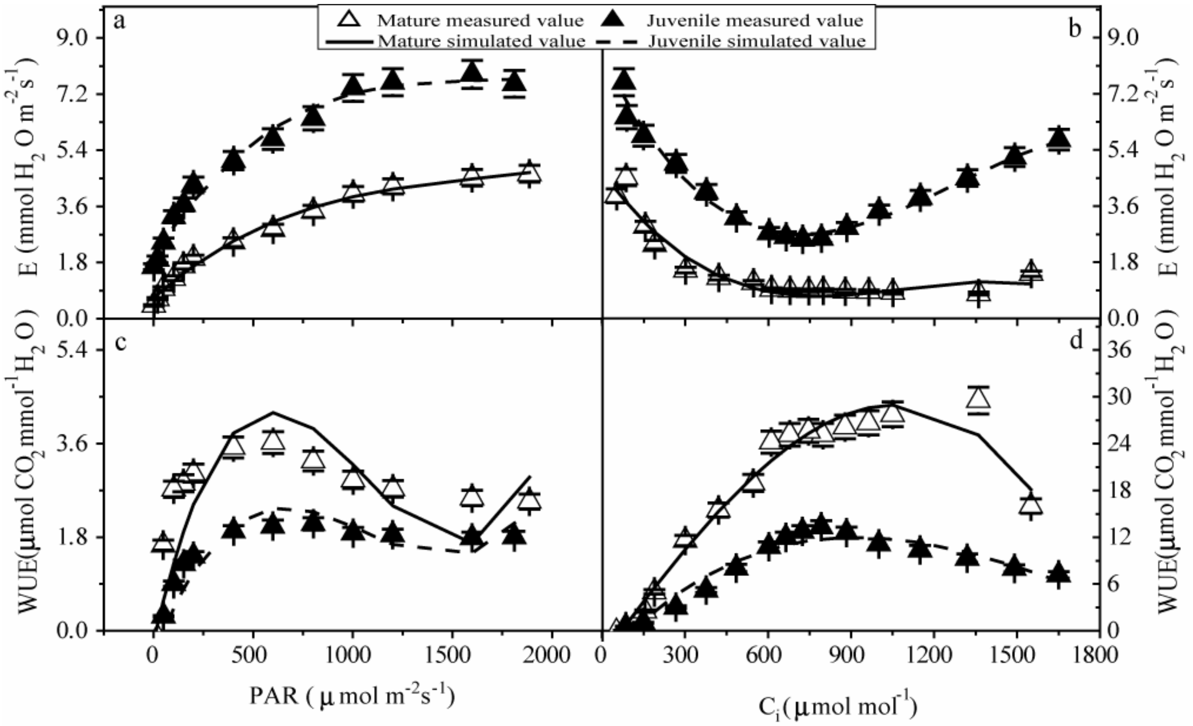

Fig. 4. Measured and simulated values of responses of transpiration rate (E) and water use efficiency (WUE) to photosynthetic active radiation (PAR) and intercellular CO_2 concentration (C_i) of mature and juvenile black locust trees on the shady slopes. The mean values ± SE (n=27).

Discussion

Since light and CO_2 is the energy and material source of plant photosynthesis, respectively, the study on photosynthetic physiology response to light and CO_2 is a critical basis of the carbon cycle. Plant photosynthetic physiology is highly sensitive to environmental and endogenous factors, especially under field conditions. In present study, slope aspect as an important environmental condition was selected to evaluate its effect on photosynthetic and physiological characteristics of mature black locusts on the Loess Plateau. In addition, stand age as one of the critical endogenous factor of plants and its influence on the photosynthetic and physiological status for black locusts on the shady slopes was also assessed. To our knowledge, little research has addressed the question of the effects of these factors on photosynthesis in juvenile and mature black locust plantations, and thus this study may help to better understand the photosynthetic and physiological responses and acclimations to different microclimates for juvenile and mature black locusts planted at different slope aspects on the Loess Plateau.

Slope aspect: The Loess Plateau is a typical arid/semiarid area, and one of the factors that may strongly influence plant photosynthesis on the Loess Plateau is slope aspect (Zheng *et al.*, 2011). In this study, the microclimatic conditions (light radiation, air temperature, and soil moisture) of different slope aspects show considerable discrepancies (Table 1). Based on the data in this study, the differences in light radiation, air temperature, and soil moisture on sunny and shady slopes most likely lead to variations in the values of the photosynthetic and physiological parameters of mature black locust trees (Figs. 1, 2; Tables 2, 3).

An earlier study reported that the photosynthesis of pine seedlings (*Pinus Montana*) on a shady, north-facing slope was lower compared to that on the sunny, east-facing slope, which is attributed to less light radiation, cooler air temperature, and lower VPD at the shady slope in comparison with those at the sunny slope (Hasler, 1982). In contrast, recent studies indicated that the photosynthetic capacities of plants on the north-facing slope is significantly higher than those of the south-facing slope, which is probably due to the fact that soil nutrient concentrations and moisture contents are greater on the north-facing slope relative to those on the south-facing slope (e. g. Running *et al.*, 1987; Kayama *et al.*, 2009). In present study, on the one hand, the finding that mature black locusts on shady slopes demonstrate significantly higher A_{maxs} is likely because of their roots going deeper distributions and absorbing more nutrients due to the larger soil moisture content of the shady slopes (Table 1).

On the other hand, black locusts on the sunny slopes show pronouncedly lower V_{cmaxs} and Rubisco contents than those on the shady slopes, which may be due to the lower Rubisco activities of the former, and this is unfavourable for Calvin cycle (Bruggemann *et al.*, 1992; Du *et al.*, 1999) and in turn leading to the decrease of photosynthetic capacities of mature trees on the sunny slopes. Black locusts on the shady slopes have greater A_{maxs} and V_{cmaxs}, however, they present slightly lower

J_{maxs} than counterparts on the sunny slopes (Table 2). Previous studies reported that plants grown at low light radiation for a long time had lesser contents of electron transfer components and photosynthetic enzymes compared to those grown at high light radiation (Xu & Shen, 1998), which could cause the J_{max} decrease on the shady slope (Liao *et al.*, 2005). Their deduction may explain and support our results.

The AQY and ACE in mature black locusts on the shady slopes are markedly higher relative to those on the sunny slopes, which indicates that black locusts on the shady slopes have stronger capacities to absorb and utilize low light radiation and CO_2 concentration. The observation that the LSP of black locusts on the sunny slopes is lower in comparison with that on the shady slopes is probably because the trees can decrease the LSP value to protect their photosynthetic apparatus from injury when they expose themselves to the hard light condition for long times on the sunny slopes. Black locusts on the sunny slopes have the higher CSP, which proves that their photosynthesis is not easily inhibited under the high CO_2 concentration. On the shady slopes black locusts present the higher LSP, AQY and Rd but lower LCP compared to the values on the sunny slopes, which is likely due to the relatively better soil water conditions on the shady slopes, and similar result about these parameters has also been recorded in sweet sorghum (Xie *et al.*, 2010). In addition, the greater response of E to PAR and lower response of E to $[C_i]$ in black locusts on the shady slopes lead to the lower WUE response to PAR and higher WUE response to $[C_i]$ of them (Fig. 2). Overall, these findings demonstrate that photosynthetic and physiological acclimations to light regimes and CO_2 concentrations play important roles for black locusts' growth and survival on the sunny and shady slopes with different microclimatic conditions.

Stand age: Forest stand age plays a critical role in physiological dynamics (Ewers *et al.*, 2005; Thompson & Rothstein, 2009). Although the black locust is a deciduous species and the leaves of juvenile and mature trees have similar annual growth progress, our results indicate that the juvenile and mature black locusts on the Loess Plateau have significantly different photosynthetic and physiological status (Figs. 3, 4; Tables 2, 3).

Juvenile black locusts show the markedly higher A_{max} compared to that of mature trees, which is probably because the juvenile black locust is under the rapid growth and development period and has to introduce more organisms via photosynthesis to suffice itself requirement. Relative to juvenile trees in other researches, decreases in photosynthetic capacities were also found in mature trees (Day *et al.*, 2001; Myung *et al.*, 2007). Previous studies indicated that the longer distance between shoot apices and roots in mature trees could decrease the efficient transport of water, nutrients, carbohydrates, etc. between them (Greenwood *et al.*, 2008; Abdul-Hamid & Mencuccini, 2009). Thus, the drop in the photosynthetic capacity with increasing stand age is likely associated with the increasing diameters and heights of black locusts (Table 1). Moreover, the nutrient status of the leaves of mature trees may also affect the vigor for photosynthesis (Munne-Bosch, 2007, 2008). There is evidence that accumulation of live biomass (such as wood) can immobilise nitrogen in trees (Luo & Polle, 2009), making nitrogen less available for new leaf growth (Merilo *et al.*, 2009). Since the nitrogen content of leaves is closely correlated with the photosynthetic capacity (Liberloo *et al.*, 2007), reduced nitrogen contents in the leaves of mature trees lead to a lower photosynthetic capacity (Bond, 2000; Feng *et al.*, 2004).

In current study the higher R_d, R_p, and E of juvenile black locusts may be due to their more vigorous physiological activities, and the greater CSP of juvenile trees indicates their better growth adaptabilities to high CO_2 concentration compared to those of mature trees. In addition, mature black locusts show the significantly lower J_{max} and slightly lower V_{cmax}, Rubisco content relative to juvenile trees (Table 2). This is mainly because for mature trees, the components of photosynthetic units, such as light-harvesting complexes (LHC), D1 proteins, coupling factors (CF1) and photosynthetic pigments start degradation in chloroplasts and leaf nitrogen concentration has been reduced, which in turn impact the absorption and transformation of light energy (Bruggemann *et al.*, 1992; Guo *et al.*, 2009; Ashraf & Ashraf, 2012).

Nevertheless, in this study the findings that mature black locusts have the greater AQY, ACE, LSP and lower LCP (Table 2) indicate that, relative to the juvenile black locusts, the mature trees on shady slopes have developed more effective and sensible photosynthetic systems, which can catch more light resources through the larger light acclimation range. Moreover, the mature black locusts have higher WUE responses to both PAR and $[C_i]$ (Fig. 4), which may have contributed to the variation and stabilization of the photosynthetic apparatus beneficial to plants grown especially in water-limited environments (Liao *et al.*, 2005).

In summary, mature black locusts on the shady slopes present significantly higher A_{max}, V_{cmax}, AQY, ACE, R_d, R_p, Rubisco, LSP and lower J_{max}/V_{cmax}, LCP, CSP than those on the sunny slopes, while J_{max} and CCP of mature trees change little on sunny and shady slopes, but the responses of E and WUE to PAR and $[C_i]$ show numerous discrepancies at different slope aspects. This is mainly due to the variations in the microclimatic conditions between the sunny and shady slope aspects. Juvenile black locusts have significantly greater A_{max}, J_{max}, J_{max}/V_{cmax}, R_d, LCP, CCP, CSP, E and lower AQY, WUE compared to mature trees, however, there is no markedly difference of ACE, R_p, V_{cmax}, Rubisco, LSP between juvenile and mature black locusts. This is likely associated with age-related differences in physiological activities. To better survive and grow, mature and juvenile black locusts perform various effective acclimations of photosynthetic physiology to local conditions at different slopes on the Loess Plateau.

Acknowledgements

This work was supported by the program of Eleventh Five-year Plan of National Science and Technology in China by a grant to Z.Z. (grant No. 2006BAD03A1207). The authors are grateful to Prof. Guang-Zhe Liu for English corrections and Dr. Jun Cui for help during the manuscript preparation.

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(Received for publication 7 July 2010)