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Responses of four succession tree species in low subtropics to enhanced UV-B radiation in the field

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Abstract

The tested tree species included pioneer species *Acacia mangium*, early succession stage species *Schima superba*, mesophyte intermediate-succession species *Machilus chinensis*, and shade-tolerant plant or late-succession species *Cryptocarya concinna* which occur in the lower subtropical forest community. A comparison with the current ambient level of UV-B radiation (UV-B) showed the leaf net photosynthetic rate (P_N), transpiration rate (E), and stomatal conductance (g_s) of the four species ranged from significantly decreased to no significant change. Additionally, the thickness of palisade and mesophyll in leaves of four tree species were decreased sharply by enhanced UV-B. The thickness of spongy parenchyma in leaves was also decreased except for *M. chinensis*. UV-B increased the leaf width of *A. mangium* but its leaf length, leaf thickness, and dry mass per unit area were not affected. Significantly increased stomata width was observed in *A. mangium* leaf epidermis in response to UV-B. Significantly decreased stomata width and significantly increased stomata density of leaf abaxial epidermis in *M. chinensis* were also observed. The stomata density of abaxial epidermis of *C. concinna* was remarkably increased by enhanced UV-B. The height and branch biomass of *A. mangium* and the height of *S. superba* were reduced visibly by enhanced UV-B. The four plant species could be classified into three groups of UV-B sensitiveness by hierarchical cluster analysis. *A. mangium* was sensitive to enhanced UV-B, while *C. concinna* showed more tolerance.

Additional key words: *Acacia*; anatomical structure; *Cryptocarya*; epidermal morphology; growth; *Machilus*; net photosynthetic rate; respiration rate; *Schima*; stomatal conductance; transpiration rate.

Introduction

Since atmospheric ozone (O_3) is the principal absorber of UV-B radiation (UV-B, 280–320 nm), changes in ozone concentration will strongly affect UV-B level on earth's surface. According to the global climate model, the content of O_3 in the stratosphere will continue to fall before severe declines occur in the years 2010–2019 in the northern hemisphere (Shindell *et al*. 1998). Recovery of stratosphere ozone to early 1980s levels will happen after 2050 (Shindell *et al.* 1998). Consequently, many biomes will experience abnormally high UV-B for a considerable time. UV-B can induce a wide range of responses in plants, including decreased rates of CO_2 assimilation and plant growth (Teramura and Sullivan 1994, Schnitzler *et al.* 1997), reduction in seedling biomass and height, decreased leaf expansion and increased leaf thickness and width, increased stomatal frequency, and changed local biomass allocation (Ormrod *et al.* 1997, Schumaker *et al.* 1997, Caldwell *et al*. 1998, Urban *et al*. 2006). Although the effects of enhanced UV-B have been extensively studied in higher plants, there remains a paucity of information on the mechanism of UV-B effects on plant growth and succession. In low

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Abbreviations: AQY – apparent quantum yield; CI – compensation irradiance; E – transpiration rate; g_s – stomatal conductance; P_{max} – maximum photosynthetic rate; P_N – net photosynthetic rate; PAR – photosynthetically active radiation; PFD – photon flux density; R_D – dark respiration rate; SEM – scanning electron microscope; SI – saturation irradiance; T_{air} – air temperature; UV-B – ultraviolet radiation (280–320 nm); WUE – water use efficiency.
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subtropical region, tree species reaction on exposure to UV-B (Sun *et al.* 2002, Zhao *et al.* 2004) and UV-B involved in plant succession were studied only rarely. We tested the physiological process for the four succession stages of dominant tree species to enhanced UV-B *via* artificial simulation. The pioneer species *Acacia mangium*, an early-succession species *Schima superba*, an intermediate-succession species *Machilus chinensis*, and a late-succession species *Cryptocarya concinna* grow in a lower subtropical forest community. These species are commonly used for re-vegetation of subtropical areas; hence they were treated by enhanced UV-B to see their reaction. We hypothesize that these four succession plants show measurable differences in growth, and physiological and structural changes when exposed to enhanced UV-B in the open field. The main objective of this work was to study the changes in plant community potentially affected by enhanced UV-B.

Materials and methods

Tree species selection and UV-B treatment: Two-year-old trees of the four species (*A. mangium*, *S. superba*, *M. chinensis,* and *C. concinna*) were planted in plastic pots (33-cm diameter, 38-cm depth) containing soil (about 13±1 kg) separately in an exposed open field. Each pot contained one plant that was subjected to enhanced UV-B *in situ* at the South China Botanical Garden, Guangzhou, China (23°1′N, 113°2′E). Plants were watered in the morning to maintain soil water near field capacity (12–18 % water content). The experiments were carried out from May 2004 to August 2005.

The system of UV-B consisted of 36 lamp frames, which were arranged in 3 groups with one for high UV-B dose treatment (H), one for low UV-B dose treatment (L), and one for an ambient control (C). Each treatment contained the four species with 96 individuals (24 plants per species). H was C+30 % UV-B (+7.24 kJ m^{-2} d^{-1}) and L was C+20 % UV-B (+4.83 kJ m^{-2} d^{-1}). Each group consisted of a 2×3 m of lighting boards containing 12 fluorescent lamps (*UV-B-313*, Donghua, China). The C group was set-up with identical un-energized lamps. The spectral irradiance was weighted with the generalized plant response action spectrum (Caldwell 1971) and normalized at 300 nm to obtain biologically effective UV-B_{BE}. The ambient UV-B_{BE} level was 0.838 J m^{-2} s^{-1} in the midday under cloudless conditions in mid-August under 0.13 mm cellulose diacetate film measured with a spectroradiometer (*RS312* UV-B meter, UK). H and L groups' lamps were covered with 0.13-mm cellulose diacetate filters (absorb radiation <290 nm; no supplemental UV-A was supplied beyond that emitted by the lamps) and once per week adjusted to ensure the distance between the lamps and plant canopy controlling the dose of supplemental UV-B_{BE}. H and L groups were maintained at a constant 30 and 20 % increase in UV-B_{BE} in daylight, which corresponded to 15 and 10 % stratospheric ozone reduction in Guangzhou China, respectively. The enhanced UV-B_{BE} doses are comparable to the expected change in UV-B_{BE} between 2000 and 2030 at this region. Because of material weathering, the cellulose diacetate film was replaced every 14–28 d. The UV-B treatment system was turned on from 09:00 to 17:00 h in daylight. If rain occurred the system was suspended.

Gas exchange: After the plants were treated for 60 and 160 d (Table 1), three fully expanded leaves *in situ* from three individual plants per species per treatment were selected randomly to assess diurnal gas exchange characteristics. Measurements were conducted every 1 h between 07:00 and 17:00 h local time for three consecutive clear days in the field. The leaves fully exposed to sunlight were sampled to minimize the variation in radiant energy interception between leaves. The same leaves were used for assessment throughout the day. Net photosynthetic rates (P_N) and stomatal conductance (g_s) were assessed simultaneously using an infrared gas analyzer, *Li-6400* Portable Photosynthetic System (*Li-COR*, USA) in the field. This system also recorded air temperature (T_{air}), photosynthetically active radiation (PAR), and transpiration rate (E). From the above measurements we calculated the mean day values and leaf water use efficiency (WUE) as P_N/E.

P_N-PFD response curves were measured on three leaves *in situ* from three individual plants per species per treatment (one leaf per plant per species). In each observation, a leaf was placed in the chamber and allowed to acclimate to a maximum PFD of 1 500 μmol(photon) m^{-2} s^{-1} for 20 min. Then, gas exchange was measured as PFD was decreased stepwise to 0 μmol(photon) m^{-2} s^{-1}. Sample CO_2 concentration was set at 400 μmol mol^{-1} with a CO_2 mixer, and leaf temperature at 25 ºC at a flow of 500 μmol s^{-1}.

For each replicate, compensation irradiance (CI, x-axis intercept) was calculated from a linear regression equation, using the first five data points above 0 μmol(photon) m^{-2} s^{-1} of the response curve. The entire irradiance response curves were fitted using the modified Von Bertalanffy equation (Horton and Neufeld 1998): $P_N = R_D + P_{max} (1 - e^{-k\,PFD})$ where k is apparent quantum yield and PFD is photon flux density.

Leaf anatomy: Portion of leaf tissues from three leaves of three different plants per species per treatment were collected (36 plants sampled). Samples were dissected and fixed in 2.5 % glutaraldehyde in 0.1 M phosphate buffer, pH 7.0 overnight at 4 °C. The specimens were then rinsed, post-fixed in 2 % osmium tetroxide, dehydrated in series to 100 % ethanol, and then freeze dried

Table 1. Effects of enhanced UV-B radiation on photosynthetic parameters in four succession tree species (first column; *A. mangium = Am, S. superba = Ss, M. chinensis = Mc, C. concina = Cc*) after 60 or 160 d treatment (2nd column) by ambient and enhanced levels of UV-B radiation (3rd column; C = control, L = low, H = high). Apparent quantum yield, AQY [mmol mol^{-1}], net and maximum photosynthetic rates, P_N and P_{max} [μmol m^{-2} s^{-1}], dark respiration rate, R_D [μmol m^{-2} s^{-1}], saturation (SI) and compensation, CI irradiance [μmol m^{-2} s^{-1}], transpiration rate, E [mmol m^{-2} s^{-1}], stomatal conductance, g_s [μmol m^{-2} s^{-1}], and water use efficiency WUE [μmol $mmol^{-1}$]. Means±SE. *Different capital letters* for the same species indicate a significant difference between different enhanced UV-B treatments for 60 d at $p<0.05$; *different minuscule letters* for the same species indicate a significant difference between different enhanced UV-B radiation treatments for 160 d at $p<0.05$, according to LSD test (AQY, P_{max}, R_D, SI, and CI, $n = 3$ leaves, one leaf per plant per species; P_N, E, g_s, and WUE: 3 leaves of different tree with 3 d of measurement). L and H represent the enhancement of UV-B by 20 % (+4.83 kJ m^{-2} d^{-1}) and 30 % (7.24 kJ m^{-2} d^{-1}), respectively.

			AQY	P_{max}	R_D	SI	CI	P_N	E	g_s	WUE
Am	60	C	1.9±0.2^{A}	21.30±0.85^{A}	0.44±0.41^{A}	1100±34^{A}	19.5±3.1^{A}	9.30±0.52^{A}	2.63±0.19^{A}	0.170±0.020^{A}	4.03±0.42^{A}
		L	2.5±0.4^{A}	17.60±0.74^{B}	0.98±0.54^{A}	900±32^{B}	18.0±2.3^{A}	8.06±0.46^{B}	2.40±0.17^{AB}	0.140±0.015^{AB}	3.92±0.42^{A}
		H	3.3±0.3^{B}	15.06±0.33^{C}	1.07±0.29^{A}	880±31^{B}	18.2±2.8^{A}	7.46±0.37^{B}	2.32±0.15^{B}	0.130±0.018^{B}	3.57±0.51^{A}
	160	C	2.6±0.2^{ab}	18.34±0.43^{a}	0.98±0.39^{a}	1100±30^{a}	20.0±4.1^{a}	10.3±0.31^{a}	2.97±0.12^{a}	0.231±0.022^{a}	3.81±0.96^{a}
		L	2.2±0.4^{a}	17.08±0.87^{a}	0.74±0.67^{a}	1100±33^{a}	18.6±4.7^{a}	9.40±0.23^{b}	2.92±0.11^{b}	0.212±0.019^{b}	3.92±0.84^{a}
		H	3.0±0.4^{b}	12.90±0.51^{b}	0.80±0.44^{a}	900±35^{b}	19.4±5.2^{a}	8.60± 0.19^{c}	2.74±0.12^{b}	0.209±0.014^{b}	3.63±0.88^{a}
Ss	60	C	3.6±0.2^{A}	15.34±0.20^{A}	0.90±0.17^{A}	800±34^{A}	6.8±2.1^{A}	6.27±0.22^{A}	1.29±0.17^{A}	0.075±0.021^{A}	4.86±0.42^{A}
		L	4.9±0.6^{B}	10.53±0.46^{B}	0.52±0.42^{A}	680±31^{B}	8.4±1.8^{A}	5.45±0.23^{B}	1.06±0.15^{AB}	0.060±0.018^{A}	5.19±0.47^{A}
		H	5.7±0.6^{B}	9.38±0.34^{C}	0.53±0.33^{A}	640±29^{B}	9.2±2.2^{A}	5.20±0.19^{B}	0.96±0.14^{B}	0.062±0.016^{A}	5.58±0.51^{A}
	160	C	4.6±0.5^{a}	11.74±0.38^{a}	0.65±0.38^{a}	820±35^{a}	11.9±2.7^{a}	7.13±0.22^{a}	1.42±0.11^{a}	0.082±0.021^{a}	5.03±0.31^{a}
		L	6.3±1.1^{b}	8.86±0.61^{b}	0.55±0.61^{a}	680±37^{b}	11.5±3.1^{a}	6.62±0.19^{b}	1.33±0.13^{ab}	0.072±0.018^{a}	5.08±0.42^{a}
		H	5.8±0.6^{ab}	8.27±0.34^{b}	0.65±0.35^{a}	680±32^{b}	16.8±4.2^{a}	5.97±0.17^{c}	1.18±0.15^{b}	0.072±0.013^{a}	5.16±0.45^{a}
Mc	60	C	4.5±0.3^{A}	8.51±0.17^{A}	0.72±0.16^{A}	600±36^{A}	10.3±2.9^{A}	6.06±0.31^{A}	1.93±0.21^{A}	0.110±0.021^{A}	3.68±0.41^{A}
		L	4.0±0.3^{AB}	8.35±0.17^{A}	0.56±0.15^{AB}	600±43^{A}	13.9±3.4^{A}	5.90±0.35^{A}	1.83±0.23^{A}	0.101±0.024^{A}	3.64±0.46^{A}
		H	3.6±0.2^{B}	7.93±0.16^{B}	0.33±0.14^{B}	600±49^{A}	14.7±2.7^{A}	5.71±0.41^{A}	1.79±0.31^{A}	0.105±0.019^{A}	3.74±0.48^{A}
	160	C	3.4±0.6^{a}	6.56±0.28^{a}	0.39±0.27^{a}	580±31^{a}	9.2±2.6^{a}	6.58±0.32^{a}	2.20±0.23^{a}	0.126±0.031^{a}	3.56±0.38^{a}
		L	4.9±1.4^{ab}	6.66±0.61^{a}	0.11±0.62^{a}	560±42^{a}	7.6±3.1^{a}	6.59±0.37^{ab}	2.09±0.24^{a}	0.113±0.025^{a}	3.67±0.42^{a}
		H	5.7±0.6^{b}	7.03±0.26^{a}	0.42±0.26^{a}	560±29^{a}	8.2±4.3^{a}	5.84±0.41^{b}	2.01±0.29^{a}	0.106±0.023^{a}	3.04±0.47^{a}
Cc	60	C	6.0±0.9^{A}	7.24±0.41^{A}	0.15±0.38^{A}	640±29^{A}	8.6±2.3^{A}	3.42±0.25^{A}	1.08±0.19^{A}	0.042±0.014^{A}	4.01±0.44^{A}
		L	5.9±0.9^{A}	6.75±0.40^{A}	0.15±0.38^{A}	640±34^{A}	5.0±3.2^{A}	3.39±0.28^{A}	1.08±0.18^{A}	0.041±0.012^{A}	3.79±0.47^{A}
		H	6.4±0.8^{A}	6.92±0.34^{A}	0.18±0.32^{A}	640±41^{A}	6.5±2.8^{A}	3.37±0.34^{A}	1.09±0.16^{A}	0.044±0.015^{A}	3.25±0.51^{A}
	160	C	3.8±0.6^{a}	5.90±0.25^{a}	0.46±0.26^{a}	620±32^{a}	8.4±2.6^{a}	3.86±0.21^{a}	1.13±0.17^{a}	0.050±0.013^{a}	4.12±1.66^{a}
		L	5.0±0.9^{ab}	6.12±0.39^{a}	0.39±0.38^{a}	620±46^{a}	7.1±3.5^{a}	3.85±0.26^{a}	1.16±0.16^{a}	0.050±0.015^{a}	4.02±1.51^{a}
		H	6.3±1.0^{b}	6.15±0.32^{a}	0.36±0.31^{a}	620±40^{a}	11.6±4.2^{a}	3.85±0.29^{a}	1.26±0.19^{a}	0.050±0.019^{a}	3.34±0.79^{a}

(device *JFD-310*). Before being mounted on metal stubs, they were sputter-coated with palladium using *JFC-1600* auto fine coater. Specimens were examined with SEM (*JSM-6360LV*, USA) at an accelerating voltage of 15 kV. Images were recorded on a digital camera. Stomata counts were made at random in 30 visual sections at both the adaxial and abaxial epidermes under the SEM. Final numbers were presented as stomata density [mm^{-2}]. Stomata length and width were measured randomly in 20 stomata of the same specimen under the SEM with its attached measurement file.

To study the internal anatomy, portion of leaf tissue was fixed (from the same leaves selected for SEM), osmicated, dehydrated in an ethanol series as for SEM, and then infiltrated and embedded in Spurr's epoxy resin. Samples were sectioned on a *Leica Ultracut E* ultramicrotome. Semi-thin 1 μm sections were obtained and stained with toluidine blue. Images of the sections and leaf tissue palisade, mesophyll, and total leaf thicknesses were measured with a light microscope (*Carl Zeiss Axiovert 200*) and *Axiovision 4.2* version program connected to a digital camera.

Characteristics of plants: Plant height, stem diameter (10 cm above the ground), leaf area, and canopy size were measured at the end of the gas exchange experiment. Eleven plants per species per treatment were randomly selected for final biomass measurement. Plants were harvested and partitioned into leaf, stem (main stem and lateral branches), and root components. All biomass components were dried at 70 ºC to a constant mass and then weighed. Shoot (sum of stem and leaf dry mass) to root ratio was calculated for each plant. 33 leaves randomly selected from 11 harvested plants were measured with a leaf area meter *Li-Cor Li-3000*, and they were also measured for leaf length and width. Leaf dry mass per unit of leaf area [g m^{-2}] was calculated.

Statistical analysis was performed using the statistical package *SPSS 11.5*. One-way ANOVA was performed for all measured data. Means for each sample were treated as replicates within species. Fisher's protected LSD was applied to detect significant differences between treatments at $p<0.05$ or $p<0.10$. Hierarchical cluster analysis was performed using the *SPSS* statistical package with squared Euclidean distance method.

Results

Response of photosynthetic parameters: The P_N–PFD response curves under the UV-B treatment (Fig. 1) showed that plants at different succession stages responded to irradiance in a different way. Under low irradiance there were lower quantum yields for *A. mangium* and *S. superba* than for *M. chinensis* and *C. concinna* subjected to UV-B radiation. P_N of *M. chinensis* and *C. concinna* reached the P_{max} value more quickly as the irradiance increased. The P_{max} of *A. mangium* and *S. superba* was significantly decreased by enhanced UV-B and were aggravated by high dose UV-B and longer treatment time. However, the P_{max} values of *M. chinensis* and *C. concinna* were not affected significantly. The scale of reduction of P_{max} from high to low irradiance was *A. mangium*>*S. superba*>*C. concinna*>*M. chinensis* (Table 1). After a 160-d treatment the apparent quantum yields (AQY) of the four tree species were significantly increased. The increase in AQY was most significant in *M. chinensis* and *C. concinna*, followed by *S. superba* and finally by *A. mangium*.

Hence *M. chinensis* and *C. concinna* showed some degree of plasticity and adaptation to the negative

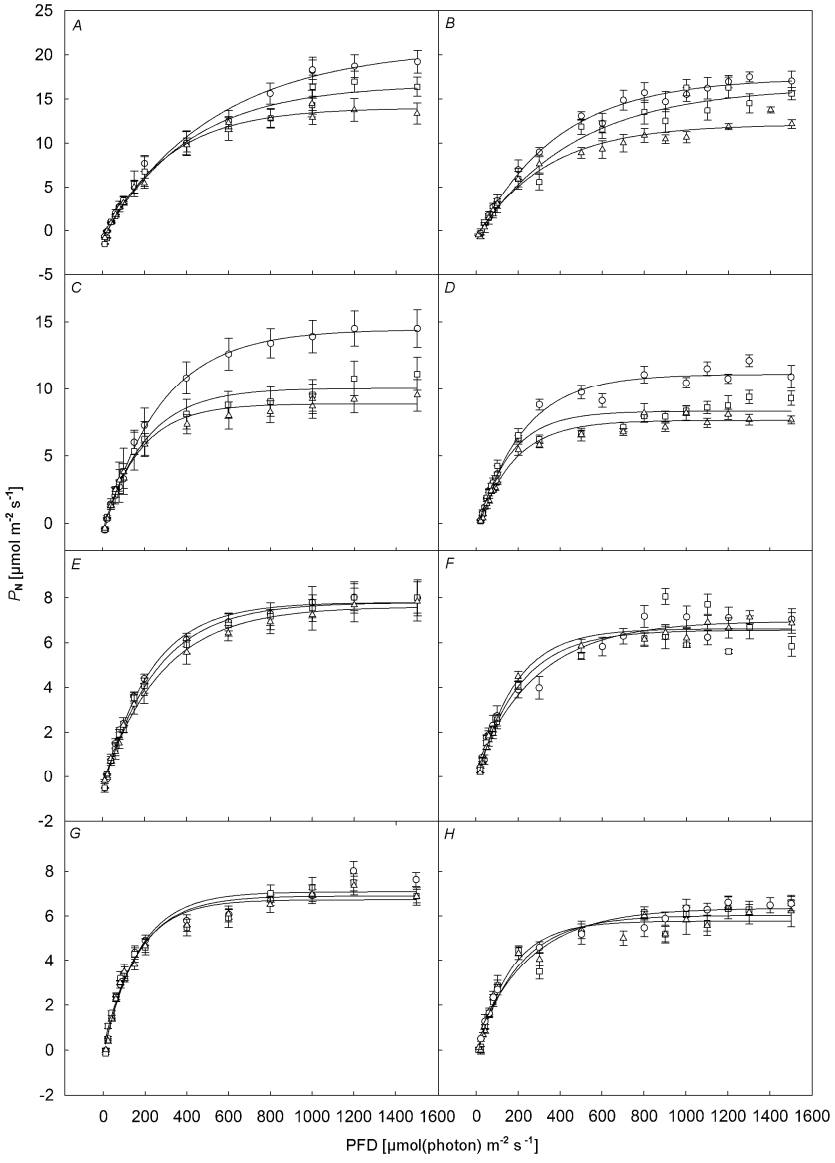

Fig. 1. Photon flux density (PFD) response curves of net photosynthetic rate (P_N) of four succession tree species after 60- and 160-d treatments by enhanced UV-B radiation. Means at different PFD; *bars* represent standard error (n = 3 leaves, one leaf per plant per species). The lines represent the exponential models. ○, □, Δ indicate C, L, and H treatments, respectively. L and H represent the enhanced UV-B by 20 % (+4.83 kJ m^{-2} d^{-1}) and 30 % (+7.24 kJ m^{-2} d^{-1}), respectively; C is the ambient UV-B radiation. *A*, *C*, *E*, and *G* are the enhanced UV-B radiation after 60 d treatment; *B*, *D*, *F*, and *H* are the enhanced UV-B radiation after 160 d treatment. *A*, *B*: *A. mangium, C, D*: *S. superba, E, F*: *M. chinensis, G, H*: *C. concinna*.

influence of enhanced UV-B through adjusting the photon energy utilization efficiency. Saturation irradiances (SI) of *A. mangium* and *S. superba* were decreased by enhanced UV-B, but those of *M. chinensis* and *C. concinna* were not; whilst the dark respiration rate (R_D) and compensation irradiance (CI) of the four species were not affected by enhanced UV-B. Thus the responses were very different in the four tested tree species: *M. chinensis* and *C. concinna* were more adaptive to enhanced UV-B than *A. mangium* and *S. superba.*

Gas exchange (Table 1): After 60-d exposure to UV-B, the daily average P_N was significantly decreased in *A. mangium* (13.2 and 19.4 % in L and H groups compared to C, respectively), and *S. superba* (13.1 and 17.1 %, respectively). When UV-B exposure time was longer than 160 d, the P_N of *A. mangium* and *S. superba* was also inhibited to some degree, however, this inhibition was less than the responses after 60-d treatment. The P_N of *M. chinensis* was not affected by UV-B in 60-d treatment, but after 160-d treatment the P_N was significantly inhibited. *C. concinna* was not affected both in 60-d and 160-d UV-B treatments. The photosynthetic responses of the pioneer species to enhanced UV-B were more significant than those of the species in intermediate- and late-succession stage.

After the 60-d treatment by enhanced UV-B, the *E* of *S. superba* in the H group was significantly reduced, but in the L group was not affected. After 160-d treatment, the *E* of *A. mangium* both in L and H groups was significantly reduced, and the *E* of *S. superba* was the same as in the 60-d treatment. The *E* values of *M. chinensis* and *C. concinna* were not affected by enhanced UV-B. Enhanced UV-B reduced g_s of *A. mangium,* however, the g_s values of *S. superba, M. chinensis,* and *C. concinna* were not affected. Similarly the WUE was not affected in the four species.

Leaf and growth characteristics (Table 2): Compared with the C plants, leaf width of *A. mangium* increased after L treatment by 8.6 and 11.7 % and after H treatment by 13.7 and 18.3 % (60-d and 160-d), respectively.

Table 2. Effects of enhanced UV-B radiation on growth of four succession tree species after 60 d and 160 d treatments by ambient and enhanced levels of UV-B radiation. *Different capital letters* for the same species indicate a significant difference between different treatments at $p<0.05$, at enhanced UV-B radiation treatment of 60 d. *Different minuscule letters* for same species indicate a significant difference between different treatments at $p<0.05$ at enhanced UV-B radiation treatment of 160 d. *significant difference to control (C) at $p<0.1$ for the same species. L and H represent the enhanced UV-B radiation levels by 20 % (+ 4.83 kJ m^{-2} d^{-1}) and 30 % (+ 7.24 kJ m^{-2} d^{-1}) treatments, respectively; C is the ambient UV-B radiation. Diameter, plant height, canopy (n = 11 plants); leaf length, leaf width, leaf dry mass per unit area (n = 33 leaves). For explanation of columns see Table 1. Means±SE.

			Diameter [cm]	Plant height [cm]	Canopy [cm×cm]	Leaf length [cm]	Leaf width [cm]	Leaf length/width	Leaf dry mass [g m^{-2}]
Am	60	C	1.29±0.14	137.8±14.4^A	64.4×64.9	17.8±2.6	5.8±0.9	3.08	181.2±14.2
		L	1.28±0.10	131.0±11.1^B	60.9×63.2	17.9±2.0	6.3±0.9*	2.88	182.1±21.6
		H	1.26±0.09	131.6±11.1^B	61.5×63.4	17.9±1.8	6.6±1.4*	2.84	183.5±31.2
	160	C	1.87±0.32	199.1±28.2^a	84.4×81.4	18.5±2.7	6.0±0.9	3.08	181.5±14.2
		L	1.80±0.29	185.8±15.8^b	82.6×83.1	18.8±2.1	6.7±0.9*	2.84	184.6±21.9
		H	1.76±0.15	181.4±19.8^b	78.6×81.9	19.3±1.9	7.1±1.4*	2.83	185.2±10.2
Ss	60	C	0.65±0.13	59. 6±6.7	35.9×35.6	11.7±1.6	3.4±0.4	3.46	108.6±9.0
		L	0.61±0.13	55.0±7.5*	36.8×36.1	11.8±1.6	3.5±0.4	3.39	109.8±18.2
		H	0.61±0.13	54.6±7.5*	37.2×33.4	11.7±1.2	3.6±0.5	3.33	110.7±7.8
	160	C	0.90±0.21	85.4±12.8	45.1×45.1	12.0±1.6	3.6±0.5	3.35	111.6±9.2
		L	0.85±0.22	76.1±15.3*	41.0×43.0	12.0±1.6	3.6±0.5	3.31	115.7±19.2
		H	0.84±0.22	76.7±15.4*	42.2×41.1	11.8±1.2	3.7±0.5	3.21	113.3±8.0
Mc	60	C	0.96±0.09	69.4±7.4	43.0×40.5	11.8±1.6	2.8±0.4	4.31	146.9±11.6
		L	0.98±0.09	68.6±7.2	42.2×40.8	11.5±1.3	2.8±0.4	4.20	145.1±10.2
		H	0.97±0.09	68.4±15.8	43.6×42.2	11.8±1.0	2.9±0.4	4.20	148.7±21.3
	160	C	1.37±0.18	101.0±12.3	58.9×59.7	12.0±1.7	2.8±0.5	4.27	148.4±11.8
		L	1.33±0.11	100.5±17.0	60.1×57.2	11.8±1.4	2.7±0.4	4.37	150.8±10.6
		H	1.40±0.18	105.8±15.6	58.3×58.8	12.1±1.1	2.9±0.4	4.30	149.4±21.4
Cc	60	C	0.96±0.15	72.0±10.4	42.3×43.2	7.5±1.1	2.7±0.2	2.82	130.2±13.2
		L	0.94±0.12	74.8±10.4	44.5×44.9	7.6±1.0	2.8±0.4	2.78	137.9±25.5
		H	0.94±0.09	72.6±10.0	43.5×42.7	7.6±0.9	2.8±0.3	2.79	136.7±23.6
	160	C	1.32±0.18	106.4±14.8	56.3×57.8	7.6±1.1	2.7±0.2	2.82	133.4±21.5
		L	1.30±0.12	112.4±13.6	58.0×57.3	7.7±1.0	2.7±0.4	2.93	139.5±52.1
		H	1.31±0.09	112.5±12.7	58.8×55.9	7.9±0.9	2.8±0.3	2.87	133.1±23.0

Enhanced UV-B reduced the ratio of leaf length to width because of increased leaf width. No significant differences in leaf width in the other three species were found. There were also no differences in leaf length and dry mass per unit area among the four species.

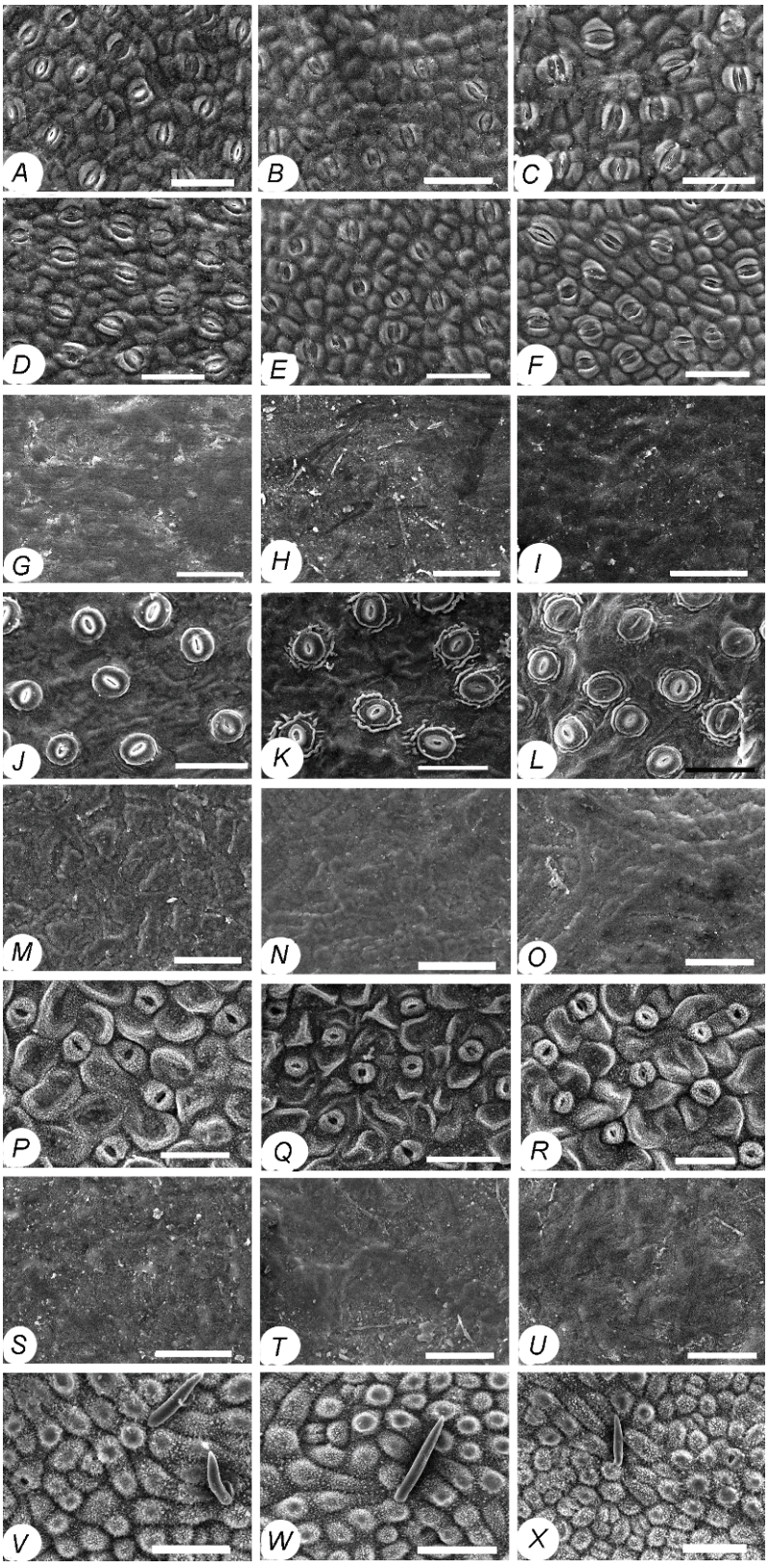

Fig. 2. Scanning electron micrographs of leaf epidermis for four succession species after 160 d treatment on ambient and enhanced levels of UV-B radiation. *A*−*F*: *A. mangium, G*−*L*: *S. superba, M*−*R*: *M. chinensis, S*−*X*: *C. concinna*. *A*−*C*, *G*−*I*, *M*−*O*, *S*−*U* show adaxial epidermis; *D*−*F*, *J*−*L*, *P*−*R*, *V*−*X* show abaxial epidermis. *Bar* = 50 μm. *A*, *D*, *G*, *J*, *M*, *P*, *S*, and *V* are the control treatment (C); *B*, *E*, *H*, *K*, *N*, *Q*, *T*, and *W* are the treatment of ambient+20 % (+4.83 kJ m^{-2} d^{-1}) UV-B (L); *C*, *F*, *I*, *L*, *O*, *R*, *U*, and *X* are the treatment of ambient+30 % (+7.24 kJ m^{-2} d^{-1}) UV-B (H).

A significant reduction in plant height was observed in *A. mangium* and *S. superba* after UV-B exposure. For the 60-d treatment, the reduction rates of plant height in L and H groups compared with C plants were 4.9 and 4.5 % for *A. mangium*, and 7.7 and 8.3 % for *S. superba*; the reductions were 6.7 and 8.9 % for *A. mangium* and 10.9 and 10.2 % for *S. superba* under the 160-d UV-B treatment, respectively. No significant differences in height in *M. chinensis* and *C. concinna* were observed. Tree canopy size and stem diameter of plants exposed to enhanced UV-B compared with C did not differ in the four species.

Biomass and its allocation: Biomass accumulation is a reliable indicator of plant sensitivity to UV-B. The biomass of *A. mangium* was significantly affected by UV-B. After the 60-d treatment, stem biomass of *A. mangium* was significantly reduced by 3.4 and 3.9 % in the L and H groups, respectively, and by 2.9 % in H group after 160-d UV-B exposure (Table 3). However, there were no significant differences between L and C groups after the 160-d UV-B treatment. The leaf and root biomasses and total biomass of *A. mangium* decreased over time compared with the C plants, but the differences were not significant. There were no significant differences in biomass of the leaf, stem, and root in the other three species. UV-B changed the biomass allocation in *A. mangium* reducing the stem biomass. However, in other three species the biomass allocation was not affected by the exposure to UV-B.

Epidermal morphology and anatomy (Figs. 2 and 3): After enhanced UV-B treatment for 160 d, the stomata densities in C, L, and H groups of *A. mangium* were 441, 442, 444 mm^{-2}, respectively, but the means were not significantly different. Their stomata densities of adaxial and abaxial epidermes were also not significantly different. The stomata densities in abaxial epidermes of *M. chinensis* and *C. concinna* were significantly increased by 14.0 and 33.7 % in L group and by 16.8 and 33.7 % in H group, respectively. However, there was no significant difference between L and H groups (Table 4).

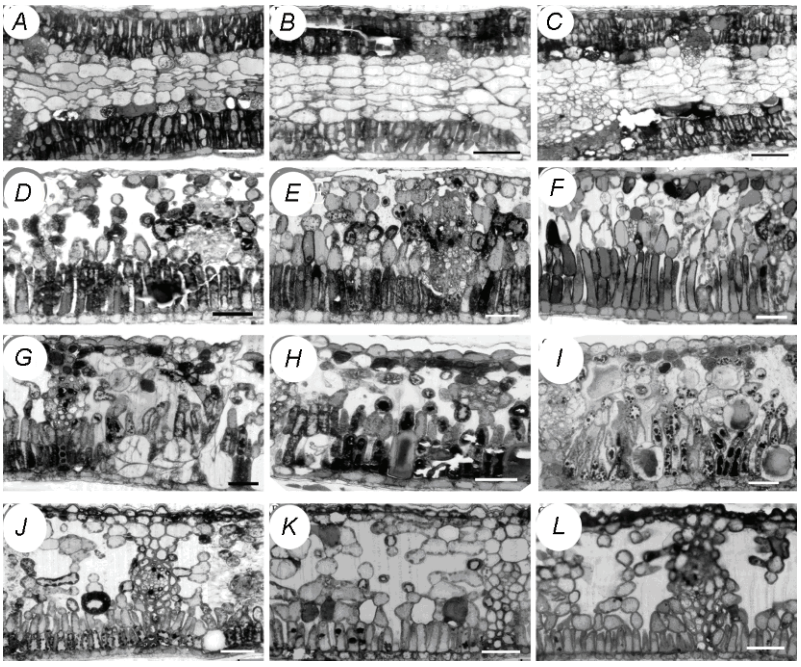

Fig. 3. Leaf anatomical structure on light micrographs of four succession tree species after 160-d treatment by ambient and enhanced UV-B radiation. *A*−*C*: *A. mangium, D*−*F*: *S. superba, G*−*I*: *M. chinensis, J*−*L*: *C. concinna. Bar* = 50 μm. *A*, *D*, *G*, and *J* are control treatment (C); *B*, *E*, *H*, and *K* are the treatments by ambient +20 % (+4.83 kJ m^{-2} d^{-1}) enhanced UV-B (L); *C*, *F*, *I*, and *L* are the treatment of ambient +30 % (+7.24 kJ m^{-2} d^{-1}) UV-B (H).

After the 160-d enhanced UV-B treatment, except for the thickness of mesophyll in *M. chinensis,* the thickness of palisade, mesophyll, and total leaf in L and H groups of *A. mangium, S. superba, C. concinna*, and *M. chinensis* were significantly reduced in comparison with the C group. However, these parameters in L and H groups were not significantly different. The mesophyll of *M. chinensis* was not affected. Thus the leaf tissues of the four species of different succession stages were perhaps injured by enhanced UV-B radiation. *M. chinensis* may possess a different protection mechanism which could effectively screen UV-B radiation.

Hierarchical cluster analysis: Based on the results of homogeneity of variance test in the measured indexes, we chose the cluster indexes which were significantly different between treatments in each species after 160-d enhanced UV-B treatment. The cluster indexes were AQY, P_{max}, SI, P_N, E, g_s, plant height, leaf width, biomass of branch, stomata width, stomata density, palisade thickness, mesophyll thickness, and total leaf thickness. All four plant species could be classified into three groups of UV-B sensitiveness by hierarchical cluster analysis (Fig. 4). Pioneer tree species *A. mangium* was more

Table 3. Effects of enhanced UV-B radiation on biomass and biomass allocation of four succession tree species after 60- and 160-d treatment. *Different capital letters* for the same species indicate a significant difference between different treatments by enhanced UV-B radiation treatment of 60 d at $p<0.05$. *Different minuscule letters* for the same species indicate a significant difference between different treatments at the $p<0.05$ at enhanced UV-B radiation treatment of 160 d. *significant difference for the same species to control (C) at $p<0.1$. $n = 11$ plants. L and H represent the enhanced UV-B radiation by 20 % (+4.83 kJ $m^{-2} d^{-1}$) and 30 % (+7.24 kJ $m^{-2} d^{-1}$) treatments, respectively; C is the ambient UV-B radiation. Means±SE. For other explanations see Table 1.

			Biomass of leaf [g]	stem [g]	root [g]	total [g]	Shoot/root
Aa	60	C	78.5±5.0	77.5±4.2	27.4±4.0	183.4±9.1	5.79±0.75
		L	76.3±5.5	74.9±3.9*	27.8±2.7	178.9±8.7	5.49±0.55
		H	77.8±4.2	74.5±2.6*	27.7±3.8	179.9±6.5	5.60±0.84
	160	C	203.8±12.9	200.2±7.1	67.8±9.8	471.9±19.9	6.06±0.84
		L	198.1±14.4	195.3±8.8*	67.7±4.8	461.0±20.0	5.84±0.52
		H	199.8±10.7	194.4±6.2*	65.9±8.2	460.1±16.4	6.07±0.80
Ss	60	C	19.2±2.9	15.6±1.5	24.9±1.5	59.8±4.8	1.40±0.14
		L	18.5±2.1	15.3±2.0	24.3±2.0	58.2±2.6	1.40±0.17
		H	18.8±3.4	15.8±1.7	24.3±2.1	58.9±4.6	1.43±0.19
	160	C	54.9±2.6	54.0±2.5	56.5±3.2	165.4±4.7	1.94±0.13
		L	54.6±2.4	53.7±4.1	54.6±3.4	162.9±4.2	1.99±0.18
		H	54.1±2.2	52.7±3.9	55.6±4.8	162.3±8.0	1.93±0.17
Mc	60	C	25.4±2.4	25.1±1.3	29.1±3.4	79.6±5.12	1.75±0.22
		L	25.6±2.4	24.6±2.3	28.5±2.4	78.8±4.9	1.77±0.17
		H	25.2±1.8	24.8±2.7	27.9±3.0	77.8±4.3	1.82±0.27
	160	C	66.1±4.7	68.4±3.6	73.7±6.6	208.3±9.3	1.84±0.17
		L	66.1±6.1	66.8±6.4	72.9±6.0	205.9±12.9	1.83±0.18
		H	65.3±4.7	66.4±7.5	74.4±6.8	206.1±11.8	1.78±0.23
Cc	60	C	25.0±3.0	22.1±2.1	28.9±6.4	76.0±6.0	1.70±0.40
		L	23.7±1.8	22.4±1.2	29.2±2.5	75.4±3.0	1.59±0.14
		H	24.3±4.2	21.8±1.9	28.7±4.8	74.8±9.0	1.62±0.20
	160	C	66.1±8.0	57.7±3.5	75.5±10.5	199.3±11.1	1.67±0.30
		L	64.1±4.9	55.6±3.3	75.7±7.1	195.4±8.6	1.59±0.15
		H	63.7±8.0	55.6±4.9	75.7±8.9	194.9±15.8	1.59±0.19

sensitive to enhanced UV-B than late-succession species *C. concinna*. The order of sensitivity to enhanced UV-B was *A. mangium*>*S. superba*≥*M. chinensis*>*C. concinna*. The cluster result was basically consistent with the actual test result.

Discussion

Pioneer species are accustomed to early habitat. On the contrary, late-succession species are acclimated to a mesophyte habitat (Lambers *et al.* 1998). Shade-tolerant plants would progressively replace the lower shade-tolerant plants in the intermediate- and late-succession stages. From the photosynthetic parameters, plants responded to UV-B in different way at different succession stages. Thus *A. mangium* showed a large reduction of P_{max} after UV-B treatment, reaching 25.5–29.3 %, and P_{max} of *S. superba* also declined by 16.3–26.0 %. High dose UV-B treatment and prolonged treatment time aggravated this reduction of P_{max} whilst the decrease of P_{max} of *M. chinensis* and *C. concinna* was not significant. Treated with enhanced UV-B radiation, SI of *A. mangium* and *S. superba* obviously dropped while in *M. chinensis* and *C. concinna* it changed only slightly. However, the AQY of the four species markedly increased. This shows that the intermediate- and late-succession species have some ability to acclimate to UV-B by adjusting energy utilization efficiency. The adaptation of pioneer species to UV-B was weak and vulnerable which is a significant finding for the future biomass composition of regeneration areas.

Sensitive species of plants and herbs responding to enhanced UV-B are usually characterized by changes in chlorophyll content, decreased CO_2 assimilation (Bornman 1989), and lower E and g_s (Tevini *et al.* 1991). We found that photosynthetic capacity of *A. mangium* was decreased significantly by enhanced UV-B treatment. Meanwhile its g_s and E also decreased obviously. P_N and E of *S. superba* dropped significantly, but no significant changes were found in g_s. The P_N, E, and g_s of

Table 4. Leaf stomatal and mesophyll characteristics of four succession tree species after 160-d treatment by ambient and enhanced UV-B radiation. *Different minuscule letters* for the same species indicate a significant difference between different treatments at $p<0.05$. *significant difference for the same species to control (C) at $p<0.1$. L and H represent the enhanced UV-B radiation by 20 % (+4.83 kJ m^{-2} d^{-1}) and 30 % (+7.24 kJ m^{-2} d^{-1}) treatments, respectively; C is the ambient UV-B. Stomata length, stomata width (n = 20), stomata density (n = 30), palisade thickness, mesophyll thickness, total leaf thickness (n = 11). Means±SE. For other explanations see Table 1.

		Stoma length [μm]	width [μm]	density [mm^{-2}]	Palisade thickness [μm]	Mesophyll thickness [μm]	Leaf thickness [μm]
Am	C	22±2	24±2^{a}	441±51	53±3^{a}	139±5^{a}	212±6^{a}
	L	22±1	25±2^{a*}	442±50	48±3^{b}	129±4^{b}	197±5^{b}
	H	23±2	26±2^{b}	444±59	46±4^{b}	128±3^{b}	194±1^{b}
Ss	C	28±1	24±2	309±55	73±8^{a}	128±5^{a}	223±5^{a}
	L	28±2	25±2	303±35	68±8^{a*}	113±3^{b}	203±5^{b}
	H	28±2	24±1	299±28	68±3^{a*}	113±5^{b}	207±6^{b}
Mc	C	21±2	23±3^{a}	214±36^{a}	105±12^{a}	108±6	244±7^{a}
	L	21±1	21±3	244±44^{b}	94±2^{b}	107±3	232±5^{b}
	H	21±2	21±2^{b}	250±39^{b}	93±6^{b}	107±4	231±9^{b}
Cc	C	17±1	14±1	193±51^{a}	43±4^{a}	157±6^{a}	223±5^{a}
	L	17±2	14±1	258±50^{b}	35±1^{b}	119±4^{b}	177±5^{b}
	H	16±1	14±1	258±61^{b}	37±2^{b}	118±5^{b}	178±2^{b}

M. chinensis did not show significant changes, while the P_N of *C. concinna* showed significant increase only in the extended time, but its g_s and E were not significantly reduced. Also *M. chinensis* and *C. concinna* did not respond to enhanced UV-B, which may be explained by a protective mechanism that effectively reduces the effects of UV-B.

After UV-B treatment, the width of *A. mangium* leaf significantly increased. However, the changes in leaf length, leaf area, and dry mass were not obvious; these characteristics in the other three species showed noticeable changes (Table 2). UV-B also led to significant reduction in height of *A. mangium* and *S. superba* plants. At the same time, stem biomass of *A. mangium* was significantly reduced. But enhanced UV-B did not affect the biomass of stems, roots, leaves, and total biomass, root/crown ratio, or stem and crown growth of *S. superba*. The measurements of plant biomass allocation showed that although enhanced UV-B treatment did not affect the total biomass of *A. mangium*, it reduced plant height and increased leaf width significantly; this led to increased leaf area. Hence the plant may allocate more nutrition from the stems to the leaves to stimulate growth. The result may be reduced stem biomass and increased leaf biomass. Similarly, this can explain the reduced growth in height of *S. superba*. Growth and biomass of *M. chinensis* and *C. concinna* were not affected by enhanced UV-B. Thus the intermediate- and late-succession species may have a better mechanism to adapt to UV-B. Sullivan *et al*. (1996) investigated *Liquidambar styraciflua* affected by enhanced UV-B in a two-year field study: the total biomass was not affected by enhanced UV-B, but leaf physiology, plant growth, and carbon allocation were impacted. UV-B also reduced the height and biomass, leaf length, thickness, and width of conifer trees (Sullivan and Teramura 1994, Caldwell *et al.* 1998, Nagel *et al.* 1998, Laakso *et al.* 2000). Comparison of growth and biomass of sun tree species *Pterospermum heterophyllum, Sapium discolor*, and *Albizzia lebbeck* with the shade tolerant tree species *Psychotria rubra* in low subtropics also showed that sun trees species are affected most (Chen *et al.* 2000). We conclude that pioneer tree species were more sensitive to UV-B than the intermediate- and late-succession ones (more shade-tolerant species).

Significantly increased stomata width was observed in *A. mangium* leaf epidermis in response to UV-B, while significantly decreased stomata width and significantly increased stomata density of leaf abaxial epidermis were observed in *M. chinensis*. The stomata density of abaxial epidermis of *C. concinna* was remarkably increased by enhanced UV-B, while it was not influenced in *S. superba.* No great differences were found between *S. superba* and *C. concinna* with respect to stomata width and length. Additionally, the thickness of palisade and mesophyll in leaves of the four tree species were decreased sharply by enhanced UV-B, and the thickness of their leaf spongy parenchyma was also decreased, except for *M. chinensis*. A question remains unanswered, whether or not the different morphological responses to enhanced UV-B among species were related to their succession stage?

In summary, UV-B had different impacts on plant growth and morphological characteristics of the species

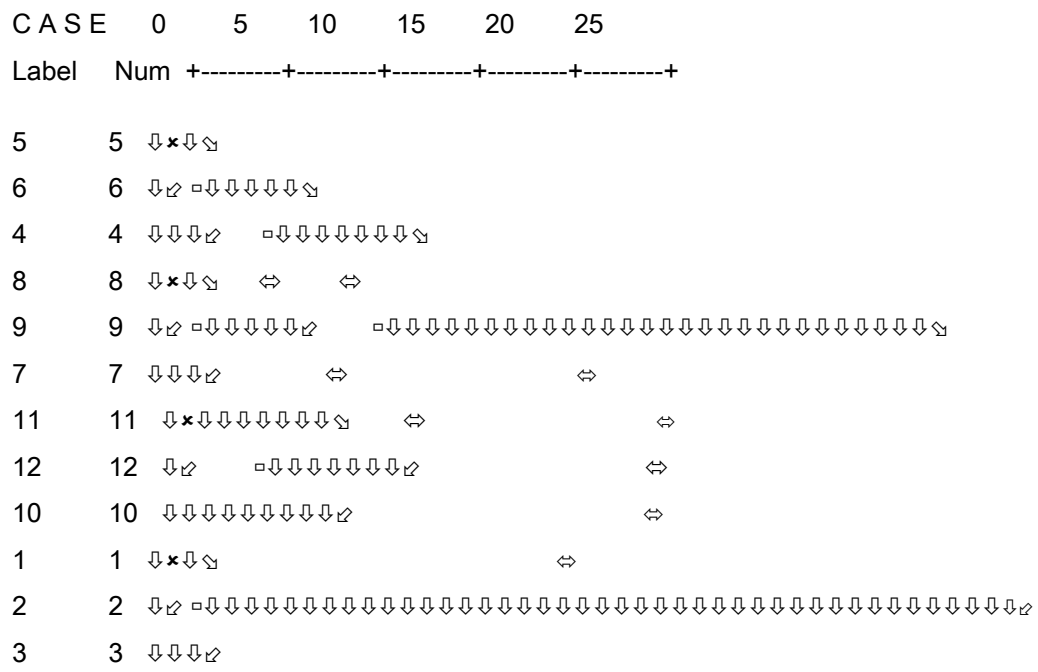

Fig. 4. Dendrogram of four succession tree species after 160-d treatment by ambient and enhanced UV-B radiation rescaled according to distance cluster combinations. 1−3: *A. mangium* (1: C, 2: L, and 3: H UV-B treatments, respectively), 4−6: *S. superba* (4: C, 5: L, and 6: H UV-B treatments, respectively), 7−9: *M. chinensis* (7: C, 8: L, and 9: H UV-B treatments, respectively), 10−12: *C. concinna* (10: C, 11: L, and 12: H UV-B treatments, respectively).

studied. Pioneer tree species were affected more severely than late-succession species (more shade-tolerant species). From the hierarchical cluster analysis, the sensitivity order to enhanced UV-B was ranked as *A. mangium*>*S. superba*≥*M. chinensis*>*C. concinna.* In communities, there is a great difference in the amount of UV-B in different positions of plant canopy. Taking *A. mangium* as an example, the significant increase in leaf width resulted in expansion of leaf area. Meanwhile, the significant decrease in tree height would change the uneven distribution of blade leaf, and even further affect the forest canopy structure. A change in *A. mangium* canopy structure will weaken the competitiveness of the individual in the community. Hence, as a global scale change factor, enhanced UV-B could induce sensitive species to demise or degradation, however, tolerant trees species have more adaptability and can compete for more resources to become dominant in ecosystems. So the sensitive species may accelerate degradation by enhanced UV-B, while tolerant species can keep dominant in ecosystems. Thus, enhanced UV-B may play an important role in plant community succession.

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