



Growth and photosynthetic responses of *Picea asperata* seedlings to enhanced ultraviolet-B and to nitrogen supply

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Ultraviolet-B (UV-B) radiation and nitrogen are expected to increase simultaneously with future changes in global climate. In this study, growth and photosynthetic responses of *Picea asperata* seedlings to enhanced UV-B and to nitrogen supply were studied. The experimental design included two levels of UV-B treatments (ambient UV-B, 11.02 kJ m⁻² d⁻¹; enhanced UV-B, 14.33 kJ m⁻² d⁻¹) and two nitrogen levels (0; 20 g N m⁻²) to determine whether nitrogen can alleviate the negative impacts of enhanced UV-B on seedling growth and photosynthesis. Enhanced UV-B significantly inhibited plant growth and impaired net photosynthetic rate, stomatal conductance, transpiration rate, the light-saturated assimilation rate, assimilation capacity, light compensation point, dark respiration rate, apparent quantum yield, photosynthetic pigments and maximum quantum yield of photosynthesis of *P. asperata* seedlings, whereas minimal fluorescence and intercellular CO₂ concentration increased by enhanced UV-B. On the other hand, nitrogen supply improved the photosynthetic performance and plant growth, but only under ambient UV-B. In fact, nitrogen supply could not alleviate the photosynthetic impairments in *P. asperata* seedlings exposed to enhanced UV-B radiation.

Key words: growth, gas exchange, nitrogen, *Picea asperata*, photosynthesis, UV-B

Crescimento e respostas fotossintéticas de plântulas de *Picea asperata* ao aumento da radiação ultravioleta-B e ao suprimento de nitrogênio. Presume-se que a radiação ultravioleta-B (UV-B) e nitrogênio aumentem simultaneamente com as mudanças climáticas globais futuras. Neste estudo, avaliaram-se o crescimento e respostas fotossintéticas de plântulas de *Picea asperata* ao aumento da radiação ultravioleta-B e ao suprimento de nitrogênio. Utilizaram-se dois tratamentos de UV-B (UV-B ambiente, 11.02 kJ m⁻² d⁻¹; e UV-B suplementar, 14.33 kJ m⁻² d⁻¹) e duas doses de N (0 e 20 g N m⁻²), a fim de se determinar se o suprimento de N poderia atenuar os impactos negativos do aumento da radiação UV-B sobre o crescimento e a fotossíntese. O aumento da radiação UV-B inibiu o crescimento e fez reduzir a taxa de fotossíntese líquida, a condutância estomática, a taxa transpiratória, a taxa de fotossíntese saturada à luz, a irradiância de compensação, a taxa de respiração escura, o rendimento quântico aparente, a concentração de pigmentos fotossintéticos e o rendimento quântico potencial das plântulas de *P. asperata*, enquanto a fluorescência mínima e a concentração intercelular de CO₂ aumentaram com o aumento da radiação UV-B. Por outro lado, o suprimento de N afetou positivamente o desempenho fotossintético e o crescimento, porém apenas sob níveis ambientes de radiação UV-B. Com efeito, N não esteve associado à proteção da maquinaria fotossintética das plântulas de *P. asperata* expostas à radiação UV-B suplementar.

Palavras-chave: crescimento, fotossíntese, nitrogênio, *Picea asperata*, radiação ultravioleta, trocas gasosas

Abbreviations: *A* – net photosynthetic rate; *A/C_i* – assimilate capacity; *A_{max}* – light-saturated assimilation rate; Car – carotenoids; Chl – chlorophyll; *C_i* – intercellular CO₂ concentration; *E* – transpiration rate; *F₀* – minimal fluorescence; *F_m* – maximal fluorescence; *F_v/F_m* – potential quantum yield of photosystem II; LCP – light compensation point; N – nitrogen; PPFD – photosynthetic photon flux density; *R_d* – dark respiration rate; UV – ultraviolet; WUE – water-use efficiency; α – apparent quantum yield

INTRODUCTION

Atmospheric ozone (O_3) remains depleted and the annual average ozone loss is approximately 3 % globally (Executive Summary, 2003). Researches have shown that enhanced ultraviolet-B (UV-B) reaching the surface of the earth has very many adverse impacts on plants (Jordan, 2002; Yao and Liu, 2006). Photosynthetic processes of plants are very important for plant growth. Musil et al. (2003) reported that photosynthesis of legume was unaffected by enhanced UV-B, whereas Correia et al. (2005) reported that photosynthesis of maize was inhibited by enhanced UV-B. The depression of photosynthesis induced by enhanced UV-B includes direct and indirect causes: direct depression is from the down-regulation of photosynthetic genes, photomodification of chloroplast thylakoid membranes (Strid et al., 1994), damage to photosystem II (Bornman, 1989) and the inhibition of photosynthetic enzymes (Murthy and Rajagopal, 1995); the indirect effects are linked to altered stomatal function, photosynthetic pigments, leaf and canopy morphology (Teramura and Sullivan, 1994).

Plants usually grow under a combination of stresses related to the present changes of global climate, such as temperature, precipitation, atmospheric CO_2 enrichment, O_3 and nitrogen deposition. Nitrogen is the mineral nutrient needed in largest amounts by plants and it is usually also the limiting factor for plant growth in terrestrial ecosystems (Vitousek and Howarth, 1991). At the same time, nitrogen is also an important constituent of the photosynthetic apparatus (Correia et al., 2005). Maximum photosynthetic capacity is strongly regulated by leaf nitrogen concentration (Field and Mooney, 1986). In contrast to UV-B radiation, nitrogen supply improved growth and net photosynthesis of plants (Nakaji et al., 2001).

UV-B radiation and nitrogen nutrition are expected to increase simultaneously with future changes in global climate. Nitrogen can affect UV-B response in plant photosynthesis (Pinto et al., 1999; Correia et al., 2005). Previous studies have mainly focused on crop and herb plants, though forests account for over two-thirds of global net primary productivity, compared to about 11 % for agricultural land (Barnes et al., 1998). Unfortunately, only a few papers have reported the combined effects of nitrogen nutrition and enhanced UV-B radiation on woody plants (especially coniferous tree species).

Picea asperata is a key species in the southeast of the Qinghai-Tibetan Plateau of China and presently widely used in reforestation programs (Liu, 2002). This paper describes the short-term influence of enhanced UV-B radiation and nitrogen supply on growth and photosynthesis of *P. asperata* seedlings under semi-controlled conditions. On the basis of previous studies in other species, we hypothesized that nitrogen supply would reduce the photosynthetic sensitivity of *P. asperata* to UV-B. Our major goal was, therefore, to achieve a better understanding of the responses of a woody plant species to both enhanced UV-B and to nitrogen supply.

MATERIAL AND METHODS

Plant material and experiment design: The experiment was conducted in open semi-field conditions from April 15 to October 15, 2005 at the Maoxian Ecological Station of the Chinese Academy of Sciences (31°41'N, 103°53'E, 1820 m asl), Sichuan province, China. Four-year-old *P. asperata* seedlings were obtained from a local nursery. The plant height, basal diameter and whole-plant fresh weight at the beginning of the experiment were 15.38 ± 0.48 cm, 6.52 ± 0.35 mm, and 7.52 ± 0.43 g, respectively. Seedlings were transplanted (one seedling per pot) into plastic pots (25 cm diameter and 35 cm depth) and grown under a 12 h photoperiod with a diurnal average photosynthetic photon flux density (PPFD) of $1200 \mu\text{mol m}^{-2} \text{s}^{-1}$. The substrate used for growing the seedlings was a sieved topsoil from a spruce-forest. In a preliminary experiment, the plastic pots did not affect growth of seedling roots during a 2-year growth period.

The experiment consisted of four treatments: (1) ambient UV-B without extra nitrogen supply (control, C); (2) ambient UV-B with extra nitrogen supply (N); (3) enhanced UV-B without extra nitrogen supply (UV-B); (4) enhanced UV-B with extra nitrogen supply (UV-B+N)). Each treatment consisted of three blocks and each block was composed of 10 pots. The pots within blocks were rotated approximately every 20 d.

UV-B treatments and nitrogen treatments: Supplementary UV-B was supplied by UV-B fluorescent lamps (Beijing Electronic Resource Institute, Beijing, China) mounted in metal frames with minimum shading.

The distance from the lamps to the top of plant apex was 100 cm and kept constant throughout the experiment. In ambient UV-B frames, UV-B from the lamps was excluded by wrapping the tubes with 0.125 mm polyester film (Chenguang Research Institute of Chemical Industry, Chengdu, China), which transmits UV-A. In enhanced UV-B frames, lamps were wrapped with 0.10 mm cellulose diacetate film, which transmits both UV-B and UV-A. Vertical polyester curtains were placed between the frames in order to prevent the UV-B radiation from reaching the control seedlings (De La Rose et al., 2003). Films were replaced every week. The lamps were changed at monthly intervals. The spectral irradiance from the lamps was determined with a spectroradiometer (model 742, Optronics, Orlando, USA). The spectral irradiance was evaluated according to the generalized plant action spectrum (Caldwell, 1971) and normalized at 300 nm to obtain the effective radiation ($UV-B_{BE}$). The supplemental $UV-B_{BE}$ dose was $3.31 \text{ kJ m}^{-2} \text{ d}^{-1}$ (a 30% difference in ambient $UV-B_{BE}$) in addition to the effective $11.02 \text{ kJ m}^{-2} \text{ d}^{-1}$ $UV-B_{BE}$ (ambient $UV-B_{BE}$) from the sky. All pots also received natural solar radiation. Seedlings were irradiated for 8 h daily centered on the solar noon.

Nitrogen was added as a 9.5 mM NH_4NO_3 solution (300 mL) to the potted soil every 3 d. The treatment without extra nitrogen supply was watered with 300 mL water at the same interval. The amount of nitrogen added to the soil was equivalent to 20 g N m^{-2} on the basis of the soil surface area. Nitrogen supply was based on other similar studies (Nakaji et al., 2001; Bowden et al., 2004).

Growth parameters: Six randomly selected seedlings from each treatment were harvested at the end of the experiment. Seedlings were divided into needle, root and stem. Roots were rinsed free of soil. All the organs were dried at 80°C for one week and weighed.

Pigment analysis: Needles after photosynthetic parameters and chlorophyll (Chl) fluorescence measurements were taken for pigment analyses. Needles were ground in 80% acetone for determination of Chl and carotenoids (Car) according to Lichtenthaler (1987).

Gas exchange: Net photosynthetic rate (A , $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), transpiration rate (E , $\text{mmol m}^{-2} \text{ s}^{-1}$), stomatal conductance (g_s , $\text{mmol m}^{-2} \text{ s}^{-1}$) and intercellular CO_2 concentration (C_i , $\mu\text{mol mol}^{-1}$) of the fully expanded

needles from six plants in each treatment were measured with a portable photosynthesis system (LI-6400, Li-Cor, Lincoln, USA) at a saturating photosynthetic photon flux density (PPFD) of $1000 \pm 50 \mu\text{mol m}^{-2} \text{ s}^{-1}$. The saturating PPFD was determined from a light response curve (between 0 and $1500 \mu\text{mol m}^{-2} \text{ s}^{-1}$), provided by a LED module and the steady-state rate of photosynthesis was calculated. The CO_2 concentration during measurements was maintained between 360 and $400 \mu\text{mol mol}^{-1}$ air, and leaf temperature was maintained at $25 \pm 1.5^\circ\text{C}$. Needle carboxylation capacity was expressed as the ratio of A to C_i (A/C_i ; Blum, 1990) and needle water-use efficiency (WUE) was calculated as the ratio of A to E . Data from two replicate measurements were averaged for each plant.

Photosynthetic light response curves of needles were determined as follows. The temperature inside the leaf was set to 25°C and the CO_2 was set to $400 \mu\text{mol mol}^{-1}$ air. The PPFD was gradually increased from 0 to $1500 \mu\text{mol m}^{-2} \text{ s}^{-1}$ (0, 20, 50, 80, 100, 200, 400, 600, 800, 1000, 1200, 1500), allowing a 3 min interval at each light intensity to achieve steady-state photosynthesis. The photosynthetic light-response curves were fit using the Michaelis-Menten equation (Zhang et al., 2006).

Chlorophyll fluorescence: Chlorophyll fluorescence was measured in dark-adapted (15 min) needles (the same needles used for gas-exchange measurements) with a portable fluorometer (PAM-2100, Walz, Effeltrich, Germany). The maximal fluorescence (F_m) with all PSII reaction centers closed was determined using a 0.8 s saturating pulse of $8000 \mu\text{mol m}^{-2} \text{ s}^{-1}$. The minimal fluorescence (F_0) was measured with weak light which did not induce significant variable fluorescence. The variable fluorescence ($F_v = F_m - F_0$) and the maximum quantum efficiency of PSII (F_v/F_m) were then calculated.

Statistical analysis: Analyses were performed using the Software Statistical Package for the Social Science (SPSS) version 11.0. Homogeneity of variance was tested using the Levene test prior to analysis. A two-way analysis of variance was used to determine the main effects of UV-B radiation, nitrogen supply and their interactions on growth and photosynthetic characteristics. Individual treatment means were compared using Duncan's test to determine whether they were significantly different at the 0.05 probability level.

RESULTS

Growth parameters: Enhanced UV-B significantly decreased plant height and total biomass of *P. asperata* seedlings (Table 1), whereas basal diameter and the ratio of root / shoot were not affected by enhanced UV-B. On the other hand, nitrogen supply significantly increased height of *P. asperata* seedlings, but basal diameter and total biomass were affected by nitrogen supply only under ambient UV-B. Significant interactive effects of UV-B \times nitrogen were also detected for plant height and basal diameter.

Gas exchange: Enhanced UV-B markedly reduced A , g_s , E , and A/C_i of *P. asperata* seedlings, and increased C_i significantly (Figure 1), whereas WUE was not affected by enhanced UV-B. Nitrogen supply, per se, significantly enhanced g_s , whilst E , C_i and WUE were unresponsive to nitrogen supply; in contrast, A and A/C_i in plants grown at ambient UV-B were increased with extra nitrogen. Prominent UV-B \times nitrogen interactions were observed for A and A/C_i (Table 2).

Photosynthetic light response curves: Photosynthetic capabilities of the needles of *P. asperata* seedlings were compared by measuring several parameters (Table 3) derived from photosynthetic light-response curves. Enhanced UV-B induced a decrease in the light-saturated assimilation rate (A_{max}), the light compensation point (LCP), dark respiration rate (R_d) and apparent quantum yield (α) of needles. On the other hand, nitrogen supply caused a significant increase in A_{max} under ambient UV-B, but induced a significant decrease in A_{max} under

enhanced UV-B. Nitrogen supply also markedly increased LCP, whereas R_d and α were not affected by nitrogen supply. Significant interactive effects of UV-B \times nitrogen were also detected for A_{max} , LCP and α .

Chlorophyll fluorescence: Enhanced UV-B significantly increased F_0 and reduced F_v/F_m in needles of *P. asperata* seedlings (Table 4). On the other hand, nitrogen supply reduced F_0 under both ambient UV-B and enhanced UV-B, whereas nitrogen supply caused an increase in F_v/F_m only under ambient UV-B. F_m was not affected by enhanced UV-B and nitrogen. The interaction between UV-B and nitrogen was also evident for F_0 and F_v/F_m .

Photosynthetic pigments: Enhanced UV-B markedly reduced Chl *a*, Chl *b*, Chl (*a* + *b*) and Car concentrations (Table 5). On the other hand, Chl *a*, Chl *b* and Chl (*a* + *b*) concentrations of plants grown at ambient UV-B were increased by nitrogen supply, whereas nitrogen supply had no influence on Chl pigment under enhanced UV-B. Car concentration was increased by nitrogen supply. A parallel change in Chl *a* and Chl *b* resulted in no significant change in Chl *a/b* ratio under enhanced UV-B or nitrogen supply. Significant interactive effects of UV-B and nitrogen were also detected for Chl *a* content ($P = 0.001$).

DISCUSSION

The decreases of Chl (*a* + *b*) and Car concentrations we found were also observed in other plant species under extra UV-B (e.g., Casati et al., 2001; Correia et al., 2005). The decrease of total Chl concentration might be due to the decreases in Car concentration because Car may

Table 1. The effects of enhanced UV-B and nitrogen supply on growth parameters of *P. asperata* seedlings. Seedlings were grown under ambient UV-B without extra nitrogen supply (control, C); ambient UV-B with extra nitrogen supply (N); enhanced UV-B without extra nitrogen supply (UV-B); and enhanced UV-B with extra nitrogen supply (UV-B+N). Values are the mean \pm SE of six replicates. The values in the same row with different letters are significantly different from each other ($P < 0.05$). *, **, *** indicate significant difference among treatments at $P < 0.05$, $P < 0.01$, $P < 0.001$, respectively. ns = not significant ($P > 0.05$). N \times UV-B interaction effects are also shown.

Growth parameters	Treatments						
	C	N	UV-B	UV-B+N	N	UV-B	N \times UV-B
Plant height (cm)	18.36 \pm 0.06 b	24.10 \pm 0.28 a	16.46 \pm 0.46 c	19.53 \pm 0.46 b	***	***	**
Basal diameter (mm)	7.08 \pm 0.26 b	8.48 \pm 0.15 a	7.62 \pm 0.36 ab	7.29 \pm 0.51 ab	ns	ns	*
Total biomass (g)	20.80 \pm 0.27 b	24.32 \pm 0.71 a	16.41 \pm 1.30 c	17.22 \pm 1.02 c	*	***	ns
Root / shoot ratio	0.86 \pm 0.10 a	0.91 \pm 0.09 a	0.80 \pm 0.04 a	0.82 \pm 0.08 a	ns	ns	ns

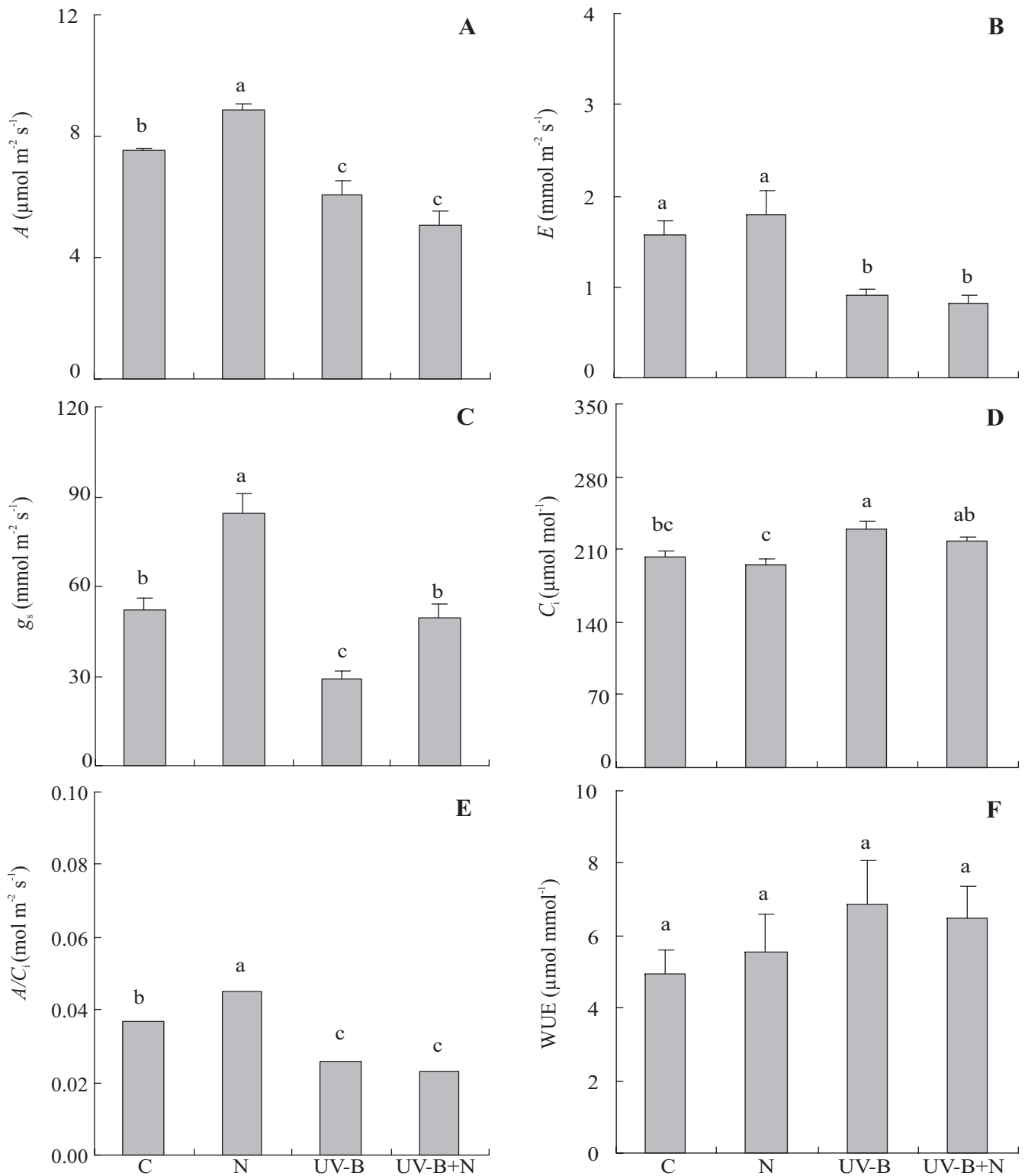


Figure 1. Net photosynthetic rate, A (**A**), transpiration rate, E (**B**), stomatal conductance to water vapour, g_s (**C**), intercellular CO_2 concentration, C_i (**D**), assimilate capacity, A/C_i (**E**) and water-use efficiency, WUE (**F**) of *P. asperata* seedlings affected by enhanced UV-B and nitrogen supply. The bars with different letters are significantly different from each other ($P < 0.05$). Values are means of six replicates \pm SE.

Table 2. The effects of UV-B, nitrogen supply and their interaction by analysis of variance (ANOVA) for photosynthetic parameters of *P. asperata* seedlings. Values are the mean \pm SE of six replicates. Symbols as in abbreviation list. See further details in Table 1.

	<i>A</i>		<i>E</i>		g_s		C_i		A/C_i		WUE	
	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
Between-subjects												
N	0.32	ns	0.12	ns	32.56	***	2.56	ns	2.70	ns	0.02	ns
UV-B	51.83	***	24.89	***	38.34	***	15.70	***	103.56	***	2.40	ns
N \times UV-B	9.65	*	0.84	ns	1.75	ns	0.08	ns	12.22	**	0.28	ns

Table 3. Photosynthetic variables and fitted parameters for light response curves of net photosynthetic rate of *P. asperata* seedlings. Values are the mean \pm SE of six replicates. Symbols as in abbreviation list. See further details in Table 1.

Variables	Treatments						
	C	N	UV-B	UV-B+N	N	UV-B	N \times UV-B
A_{max} ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)	9.56 \pm 0.18 b	10.84 \pm 0.54 a	7.97 \pm 0.44 c	5.74 \pm 0.20 d	ns	***	**
LCP ($\mu\text{mol m}^{-2} \text{ s}^{-1}$)	16.06 \pm 0.08 b	18.32 \pm 1.07 a	11.65 \pm 0.26 c	16.33 \pm 0.32 b	***	ns	***
R_d ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)	0.90 \pm 0.05 a	1.06 \pm 0.13 a	0.59 \pm 0.03 b	0.60 \pm 0.10 b	ns	***	ns
α ($\mu\text{mol CO}_2 \text{ mol}^{-1}$)	0.0617 \pm 0.00 a	0.0717 \pm 0.00 a	0.0452 \pm 0.00 b	0.0383 \pm 0.01 b	ns	***	**

Table 4. The effects of enhanced UV-B and nitrogen supply on chlorophyll fluorescence variables of *P. asperata* seedlings. Values are the mean \pm SE of six replicates. Symbols as in abbreviation list. See further details in Table 1.

Variables	Treatments						
	C	N	UV-B	UV-B+N	N	UV-B	N \times UV-B
F_0	0.25 \pm 0.01 b	0.18 \pm 0.01 c	0.29 \pm 0.01 a	0.25 \pm 0.00 b	***	***	*
F_m	1.06 \pm 0.03 a	1.28 \pm 0.14a	0.98 \pm 0.11 a	0.87 \pm 0.20 a	ns	ns	ns
F_v/F_m	0.76 \pm 0.00 b	0.86 \pm 0.03 a	0.70 \pm 0.02 c	0.71 \pm 0.00 c	*	***	*

protect chlorophylls from photooxidative destruction (Singh, 1996). On the other hand, the decrease in Car may be directly associated with the damage caused by UV-B radiation.

Since nitrogen is closely related to photosynthetic pigment content and activity of Rubisco, nitrogen nutrition strongly affects the processes of photosynthesis and related gas exchange (Li et al., 2004). Overall, A , A/C_i and photosynthetic pigments responded positively to the nitrogen supply under ambient UV-B, but not under extra UV-B radiation, i.e. the harmful effects of enhanced UV-B on photosynthetic pigments were not alleviated by the nitrogen supply.

Changes in Chl fluorescence parameters may indicate the absorption and transmission of light energy, the conversion processes of physiological condition and its

photosynthetic effect in plants. The F_v/F_m ratio, which is believed to be a good measure of the photosynthetic efficiency (Hanelt et al., 1995), is often used as a stress indicator of the photosynthetic apparatus and describes the potential yield of photochemical reactions (Björkman and Demmig, 1987). The decrease in F_v/F_m ratio under enhanced UV-B suggests that *P. asperata* seedlings were under stress, which may be associated with decreased efficiency of energy transfer from the antenna to the reaction centres and/or inactivation of PSII reaction centres (Briantais et al., 1986). Similar results have been reported elsewhere (Bjerke et al., 2005). The decrease of F_v/F_m might impair photosynthesis, and ultimately plant growth and development. It should be stressed that nitrogen supply enhanced F_v/F_m under ambient UV-B, as also noted by Guo et al. (2005) in larch seedlings, but not

Table 5. The effects of enhanced UV-B and nitrogen supply on the concentration (on a fresh weight basis) of photosynthetic pigments of *P. asperata* seedlings. Values are the mean \pm SE of six replicates. See further details in Table 1.

Pigments	Treatments						
	C	N	UV-B	UV-B+N	N	UV-B	N \times UV-B
Chl <i>a</i> (g kg ⁻¹)	0.35 \pm 0.01 b	0.43 \pm 0.00 a	0.28 \pm 0.01 c	0.30 \pm 0.00 c	***	***	***
Chl <i>b</i> (g kg ⁻¹)	0.10 \pm 0.00 b	0.12 \pm 0.00 a	0.07 \pm 0.01 c	0.08 \pm 0.01 c	*	***	ns
Chl <i>a/b</i>	3.50 \pm 0.38 a	3.58 \pm 0.31 a	4.00 \pm 0.49 a	3.75 \pm 0.24 a	ns	ns	ns
Chl (<i>a + b</i>) (g kg ⁻¹)	0.45 \pm 0.02 b	0.55 \pm 0.00 a	0.35 \pm 0.05 c	0.37 \pm 0.00 c	*	***	ns
Car (g kg ⁻¹)	0.08 \pm 0.00 b	0.10 \pm 0.00 a	0.04 \pm 0.00 d	0.06 \pm 0.00 c	***	***	ns

under extra UV-B, which further suggests that nitrogen supply was unable to alleviate the harmful effects of UV-B on the photochemical reactions.

In summary, this paper illustrated that enhanced UV-B significantly affected the growth and photosynthesis of *P. asperata* seedlings. On the other hand, nitrogen supply was more favorable for the growth and photosynthesis of *P. asperata* seedlings under ambient UV-B, whereas nitrogen supply could not reduce the photosynthetic impairments in *P. asperata* seedlings exposed to extra UV-B radiation.

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