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RESEARCH ARTICLE

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Effects of enhanced ultraviolet-B radiation on water use efficiency, stomatal conductance, leaf nitrogen content and morphological characteristics of *Spiraea pubescens* in a warm-temperate deciduous broad-leaved forest

Abstract *Spiraea pubescens*, a common shrub in the warm-temperate deciduous forest zone which is distributed in the Dongling Mountain area of Beijing, was exposed to ambient and enhanced ultraviolet-B (UV-B, 280–320 nm) radiation by artificially supplying a daily dose of 9.4 kJ/m^2 for three growing seasons, a level that simulated a 17% depletion in stratospheric ozone. The objective of this study was to explore the effects of long-term UV-B enhancement on stomatal conductance, leaf tissue $\delta^{13}C$, leaf water content, and leaf area. Particular attention was paid to the effects of UV-B radiation on water use efficiency (WUE) and leaf total nitrogen content. Enhanced UV-B radiation significantly reduced leaf area (50.1%) but increased leaf total nitrogen content (102%). These changes were associated with a decrease in stomatal conductance (16.1%) and intercellular CO_2 concentration/air CO_2 concentration (C_i/C_a) (4.0%), and an increase in leaf tissue $\delta^{13}C$ (20.5‰), leaf water content (3.1%), specific leaf weight (SLW) (5.2%) and WUE (4.1%). The effects of UV-B on the plant were greatly affected by the water content of the deep soil (30–40 cm). During the dry season, differences in the stomatal conductance, $\delta^{13}C$, and WUE between the control and UV-B treated shrubs were very small; whereas, differences became much greater when soil water stress disappeared. Furthermore, the effects of UV-B became much less significant as the treatment period progressed over the three growing seasons. Correlation analysis showed that enhanced UV-B radiation decreased the strength of the correlation between soil water content and leaf water content, $\delta^{13}C$, C_i/C_a, stomatal conductance, with the exception of WUE that had a significant correlation coefficient with soil water content. These results suggest that WUE would become more sensitive to soil water variation due to UV-B radiation. Based on this experiment, it was found that enhanced UV-B radiation had much more significant effects on morphological traits and growth of *S. pubescens* than hydro-physiological characteristics.

Keywords warm-temperate deciduous broad-leaved forest, *Spiraea pubescens*, UV-B radiation, $\delta^{13}C$, water use efficiency, nutrition content

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1 Introduction

As the major ultraviolet radiation filter in the stratospheric air, the ozone is now depleted at a rapid rate in the past century due to the massive use of cloric and fluoric hydrocarbons and excessive release of waste gas into the air[1,2], which enhances the ultraviolet-B (UV-B) (280–320 nm) radiation that penetrates through the ozone layer and reaches the surface of the earth. The UV-B radiation will influence the growth of terrestrial plants and even a slight increase in the UV-B amount will have far-reaching impacts on a large number of species (Holmes, 1997). Many conventions have been agreed on among world communities including the *United Nations Framework Convention on Climate Change* and the *Treaty of Ozone Layer Protection*, suggesting that the impacts of global changes on the terrestrial species and human beings could not be overlooked any more. Nevertheless, the long-term ozone changes in the future and subsequent UV-B enhancement due

[1] World Meteorological Organization (1999). Scientific Assessment of Ozone Depletion: 1998. Report No. 22. WMO
[2] World Meteorological Organization (1999). Scientific Assessment of Ozone Depletion: 1998. Report No. 44. WMO, Geneva

to the difference in implementing these conventions among developing and developed countries as well as the interaction between UV-B and other climatic conditions cannot be foreseen[3] (Green, 1995; Webb, 1997; Shindell et al., 1998).

Since the first detection of the ozone hole above the Antarctica in the 1980s, it has aroused scientific concerns and attention among international governments, and a large number of studies have been made regarding the impacts of UV-B on the yield of plants and especially crops. Caldwell et al. (1998) found, in their review on the papers published in the past decades concerning UV-B's effects on plants, that a majority of studies among around 600 publications were conducted with herbs or crops as experimental objectives in the greenhouse or laboratory conditions, and less than 5% of researches were finished outdoors. Presently, international scientists shed much light on naturally growing plants, but attention to vegetation in mature forests remains little yet. Field investigation is rather important in the assessment of UV-B's impacts on plants because the impacts are likely to vary with environmental factors such as light, temperature, water and nutrients (Cardwell et al., 1998). In addition, field experiments could provide us an actual balance of UV-B, UV-A and photosynthetically active radiation (PAR) (Kim et al., 1996). Although China lags behind other nations in the aspect of UV-B's effects on plants, many scholars have carried out in-depth researches in recent years on crops, psychrophytes and subtropical forest vegetation (Hou et al., 1998; Li and Wang, 1998; Shi et al., 2001; Sun et al., 2001).

Plant's water use efficiency (WUE) is an objective index for assessing the water use state of plants. Farquhar and Richards (1984) found a close and positive correlation between stable carbon isotopic composition and WUE and it from then became a reliable indicator of water use efficiency. However, a more actual reflection of WUE requires taking into account the leaf-to-air vapor pressure deficit (VPD) (Farquhar et al., 1989). The uptake and balance of important nutrient elements are necessary for the growth and development of plants, and a study by Murali and Teramura (1985) suggested that UV-B radiation would influence the uptake of nutrient elements. Li and Wang (1998) measured the contents of nutrient elements in different parts of wheat under various UV-B radiation strengths and at different developmental stages. The effects of UV-B radiation on the content of nutrient elements were also observed in other plants (Dohler et al., 1987; Hatcher and Paul, 1994). However, in general, present reports which are concerning the effects of UV-B on plant nutrients remain few (Caldwell et al., 1995).

Water shortage has long been the major inhibitor for the growth and distribution of plants in most areas of northern China (Yan et al., 2001). It is estimated that evapotranspiration and water deficit in these areas will intensify due to the further global changes and especially climbing temperature caused by greenhouse effects. Moreover, with the decline of ozone content in the stratosphere, excessive ultraviolet radiation will penetrate the atmosphere and reach the surface of the earth, as a result, plants in these areas will be subjected to the harm of both UV-B radiation and water deficit. In ecologically weak warm-temperate zone, researches on woody species aiming at biodiversity conservation will provide us with empirical data which can be used to estimate the developing trends of these degraded, weak ecosystems in the global climatic changes. On the basis of the achievements of previous studies, this study aims at answering the following questions: 1) what effects enhanced UV-B radiation will pose on the WUE of woody plants in warm-temperate deciduous forests; 2) whether gas exchange technique and stable carbon isotopic technique are complementary to each other in the measurement of the indices; and 3) what effects enhanced UV-B radiation will produce on the nutrient conditions and morphological characteristics of woody plants in warm-temperate deciduous forests.

In order to answer the questions mentioned above, Spiraea pubescens were selected as material in a warm-temperate deciduous forest. An enhanced UV-B radiation and the control were designed for a comparative study. A successive three-year observation and measurements were made to investigate the effects of enhanced UV-B radiation treatment on water physiology, nitrogen nutrient and leaf tissue morphology.

2 Materials and methods

2.1 Theoretical background

The $\delta^{13}C$ analysis is an effective method to evaluate intercellular CO_2 concentration in C_3 plant leaves (Chen et al., 2002). According to Farquhar et al. (1982), $\delta^{13}C$ in plants could be expressed by Eq. (1)

$$\delta^{13}C_p = \delta^{13}C_a - a - (b - a) \times C_i/C_a \quad (1)$$

where $\delta^{13}C_p$ and $\delta^{13}C_a$ are respectively carbon isotopic ratios of plant and air, a is the carbon isotopic difference (4.4‰) in the diffusion of CO_2 from the air into leaves through stomas, b is the $^{13}CO_2$ discriminant value in the CO_2 assimilation by rubisco, and C_i and C_a are the intercellular and air CO_2 concentration, respectively.

According to the definition of water use efficiency, plant WUE could be calculated by Eq. (2)

$$WUE = P_n/T_r = C_a(1 - C_i/C_a)/1.6\ \Delta W \quad (2)$$

where P_n and T_r are the photosynthetic rate and transpiration rate, respectively, and ΔW is the vapor pressure difference between inside and outside leaves.

$\delta^{13}C$ can hence reveal indirectly the long-term water use efficiency of plants

$$WUE = C_a[(\delta^{13}C_p - \delta^{13}C_a + b)/(b - a)]/1.6\ \Delta W \quad (3)$$

[3] World Meteorological Organization (1995). Scientific Assessment of Ozone Depletion: 1994. Report No. 37. WMO

2.2 Experimental site and materials

The experimental site is set in the forest ecosystem station of Beijing, the Chinese Academy of Sciences, geographically located at the Dongling Mountain, Xiaolongmen Village, Mentougou District of west Beijing (39°58′N, 115°26′E). Warm-temperate deciduous broad-leaved forest is the typical vegetation type in this region with the widest distribution. Dominant species can singly develop into dominant community such as *Quercus liaotungensis*, *Betula dahurica*, *Populus davidiana*, and *Juglans mandshurica*, or form into mixed forest (Chen and Huang, 1997). Table 1 presents environment factors in recent years.

Spiraea pubescens, a shrub species up to 1–2 m in height, was selected as the experimental material. The species can be commonly found in shrub layer of this region (Chen and Huang, 1997) and grows in mixed forests on high-elevation dry rocky slopes (He, 1986).

2.3 Experimental design and artificial ultraviolet treatment

Around experimental site, five well-grown bushes of *S. pubescens* were selected and five metal frames (1.5 m in length, 1.2 m in width and 2.5 m in height) were fixed above them respectively. Two of the fames were set as the control, and six UV-B fluorescence lamps (UVB-318, manufactured in Beijing) were fixed on each of the other three frames. The fluorescence tubes were vertically placed above the plant and wrapped by 0.125-mm thick cellulose acetate membrane to filter a small amount of fatal ultraviolet C (UV-C, $<$280 nm) radiation. The cellulose acetate membrane was replaced every half month to ensure the quality of fluorescence radiation after filtration. The amount of enhanced UV-B radiation was controlled by the switch number of opened tubes: two third of them were opened at 11:30–14:30 and the rest at 10:00–11:30 and 14:30–16:00. The UV-B radiation strength was determined by an ultraviolet meter (manufactured by Beijing Normal University), and the reading value was changed into radiation dose by the formula proposed by Caldwell (1971) and by an empirical formula by Wang Xunling from Lanzhou University (personal communication). The calculation of biological effective radiation

$$\text{UV-B} = (140.2 \times (\text{reading value}) \times n \times 3{,}600/1{,}000{,}000) - 0.482{,}3$$

where n represents the UV-B radiation time (h). The enhanced UV-B radiation dose is 9.4 kJ/(m$^2 \cdot$day), a level that simulated a 17% depletion in stratospheric ozone (the radiation factor value is 2.3 (Cardwell, 1971)).

2.4 Measurement methods

The experiments in this study were carried out from June to September of 2004. The following data were measured and samples collected.

2.4.1 Environmental factors at the experimental site

1) Meteorological data

The meteorological data were provided by the forest ecosystem station of Beijing, the Chinese Academy of Sciences, including air temperature, relative air humidity, precipitation and air pressure.

2) Leaf-to-air vapor pressure deficit

The leaf-to-air VPD (Pa) was determined according to Murray (1967)

$$VPD = e_s(T_a) - e_m \tag{4}$$

where $e_s(T_a)$ is the saturated vapor pressure when the mean daily temperature is T_a (°C), e_m is the ambient actual vapor pressure (Pa). The vapor pressure could be obtained by the following formula

$$e_s(T_a) = 610.78\exp\left[\frac{17.269T_a}{237.3 + T_a}\right] \tag{5}$$

$$e_m = 610.78\exp\left[\frac{17.269T_m}{237.3 + T_m}\right] \times \frac{RH_{mean}}{100} \tag{6}$$

Usually, it is assumed that vapor pressure inside the leaves is saturated and then substitute T_a with leaf temperature T_l, replace T_m with ambient air temperature T_a. RH_{mean} in Eq. (6) is the mean relative humidity at the site. Leaf temperature T_l was measured using a ΔT AP4 dynamic porometer (manufactured by ICT Co., Australia).

Table 1 Environmental factors of the experimental site in recent three years

Time		2002				2003				2004			
		June	July	August	September	June	July	August	September	June	July	August	September
Monthly precipitation (MP) /mm		118.1	125.0	60.4	90.5	78.6	87.2	61.3	82.7	78.1	227.2	95.8	57.4
Relative humidity (RH) /%		79	84	89	85	78	86	86	86	77	87	90	82
Monthly mean temperature (MT) /°C		16.1	19.0	17.6	11.8	15.5	17.6	16.5	13.0	16.4	17.6	15.9	12.1
Leaf-to-air vapor pressure	UV-B	–	–	–	–	–	–	–	–	8.65	7.24	6.06	6.06
deficit (VPD) /hPa	Control	–	–	–	–	–	–	–	–	8.67	7.12	6.07	6.26
Air CO_2 concentration (C_a) /(μmol·mol^{-1})		–				–				370.4			
Carbon isotope ratio (δ^{13}C) /‰		–				–				–10.34			

3) Air CO_2 concentration

The air CO_2 concentration was determined using LI-6400 (Li-Cor, USA).

4) Carbon isotopic ratio of air ($\delta^{13}C_a$)

The air around the *S. pubescens* canopy was sampled using a vacuum steel tube at 10:00–11:00. The sampling was done on June 17, July 14 and September 16. The $\delta^{13}C_a$ was measured using a DELTA^{plus} mass spectrograph and a PRECON gas analyzer (Thermo Finnigan Co., Germany) in the Laboratory of Stable Isotopes, Institute of Botany, the Chinese Academy of Sciences.

5) Soil water content

Considering the stability of both biological characteristics of *S. pubescens* and the water content in deep soil, three points at the experimental site wer chosen from June to mid-September, 2004 and soils at 30–40 cm deep were sampled using a soil drill. The soil water content was measured adopting the oven drying method.

2.4.2 Measurements of leaf indices *in vivo*

At 70–80 cm under the fluorescent tubes, three to four well-grown, undamaged leaves from the control and UV-B treated plants were collected (the second or third leaf under the branch top), their stomatal conductance was measured using a ΔT AP4 dynamic porometer, and then they were sealed in envelopes for the later measurement of $\delta^{13}C$. Ten leaves from each plant were sampled *in vivo* for the determination of leaf nitrogen content. After taken back into laboratory, the sampled leaves were weighted using a digital balance, and their leaf areas were determined using a Li-Cor Model 3000 leaf area meter (manufactured by Li-Cor Co., USA). Thereafter, they were air-dried at 60°C for 24 h and their dry weight determined. The leaf water content was measured by oven drying method according to the following formula

$$LWC\ (\%) = (\text{Fresh leaf weight} - \text{dry leaf weight}) / \text{dry leaf weight} \times 100.$$

The leaf samples were ground and passed an 80 mesh sifter for use. The $\delta^{13}C$ was measured using a DELTA^{plus} mass spectrograph and a FLASH EA solid analyzer (Thermo Finnigan Co., Germany) in the Laboratory of Stable Isotope of Institute of Botany, the Chinese Academy of Sciences; leaf total nitrogen content was determined by the Kjeldahl method.

2.4.3 Statistical analysis

The statistics were analyzed by the software SPSS 11.5 (SPSS, 2002).

3 Results

3.1 Effects of UV-B radiation on leaf water content and stomatal conductance

Enhanced UV-B radiation has little impact on the leaf water content and stomatal conductance ($p > 0.05$) (Table 2), and it does not change with seasons. Nevertheless, the seasonal factors have marked effects on these two indices ($p < 0.05$): the leaf water content of UV-B treated plants declines over time (Fig. 1); enhanced UV-B improves leaf free water content by 1.3%–5.3%, although statistically insignificant (Table 2), and the increment fluctuates with season.

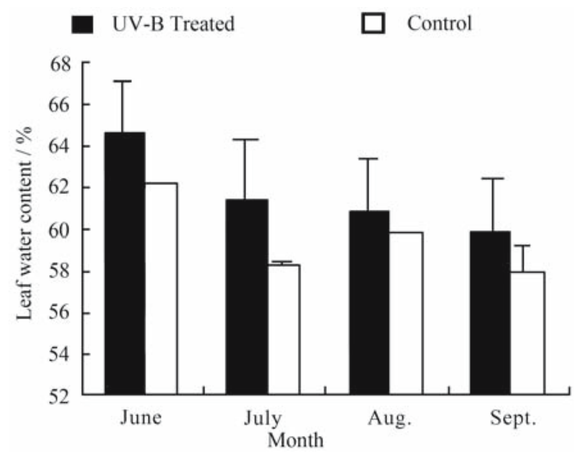

Fig. 1 Effects of UV-B and season on the leaf water content

Table 2 Effects of UV-B, time and their interactions on stomatal conductance, $\delta^{13}C$, leaf water content, water use efficiency (WUE), intercellular CO_2 concentration/air CO_2 concentration (C_i/C_a), leaf area, specific leaf weight (SEW)and total N content

Variation		*df*	*F*							
			Stomatal conductance	Carbon isotope ratio	*WUE*	Leaf water content	C_i/C_a	Leaf area	*SLW*	Total N content
Main effects	UV-B	1	2.105[a)]	0.699[a)]	1.165[a)]	4.034[a)]	0.936[a)]	23.601***	3.283[a)]	15.766**
	Time	3	5.810*	4.073*	24.457***	3.580*	4.014*	0.291[a)]	2.726[a)]	1.257[a)]
Two-factor interaction effects	UV-B × time	3	0.658[a)]	1.179[a)]	1.164[a)]	0.228[a)]	0.927[a)]	0.096[a)]	0.296[a)]	1.764[a)]

Note: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; [a)] no significance.

The stomatal conductance of control and UV-B treated *S. pubescens* leaves rises at first and then declines over time, and enhanced UV-B radiation reduces the stomatal conductance (Fig. 2). A comparison between the control and treatment statistics reveals that the difference reaches its maximum (24.7%) in July when rainfall is rather rich; in June and September when it precipitates much less the stomatal conductance of treated leaves is even higher than that of the control. It was found through correlation analysis that the correlation coefficients between stomatal conductance and water content of deep soil are, respectively, 0.275 and 0.711 for the treated leaves and the control, which is suggested that UV-B reduces the sensitivity of leaf stomatal conductance to soil water content (Table 3).

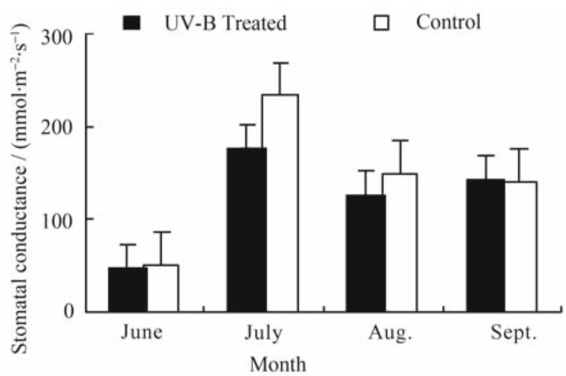

Fig. 2 Effects of UV-B and season on stomatal conductance

3.2 Effects of UV-B radiation on $\delta^{13}C$, WUE and C_i/C_a

Table 2 shows that UV-B enhancement has no significant effects on $\delta^{13}C$, but seasonal factor does ($p < 0.05$). In the whole growing season, the leaf $\delta^{13}C$ has a decreasing tendency generally both in the control and in the treated leaves except that the leaf $\delta^{13}C$ of treated plants rises slightly, which indicates that enhanced UV-B improves the leaf $\delta^{13}C$ to some extent (Fig. 3), but not notably (Table 2). Comparatively, $\delta^{13}C$ is more sensitive than stomatal conductance to UV-B and water content: $\delta^{13}C$ of the treated plants is 7‰ lower than the control in June and September when water content in deep soil is rather low; in July and August when there is plenty water in deep soil, $\delta^{13}C$ of the treated plants is, on the contrary, 10‰–31‰ higher than the control (Fig. 3). Correlation analysis shows that correlation coefficients between $\delta^{13}C$ and water content of deep soil are, respectively, −0.078 and −0.608 for the treated leaves and the control, which suggests that UV-B reduces the sensitivity of $\delta^{13}C$ to soil water content (Table 3).

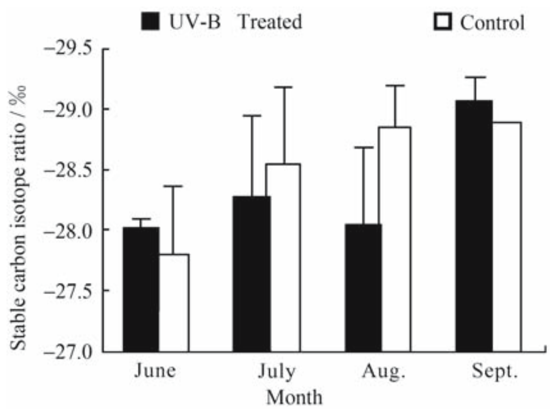

Fig. 3 Effects of UV-B and season on $\delta^{13}C$

The water use efficiency of leaves was calculated using Eq. (3), and the results show little correlation between UV-B and season. The effect of season on WUE, however, is significant ($p < 0.001$), and WUEs of treated plants and the control increase gradually over time, reaching the maximum in August and declining thereafter (Fig. 4). In general, UV-B improves WUE of *S. pubescens* leaves at different levels in different months, although statistically not significant. The UV-B enhances leaf WUE by 9.6% in August but slightly in other months; in June, the WUE of treated leaves is even a little bit lower than the control. The correlation analysis shows a significant relationship between the WUE of treated plants and soil water content (Table 3), indicating that UV-B radiation strengthens the sensitivity of WUE to the ambient soil water content. The ratios of intercellular to air CO_2 concentration (C_i/C_a) of different plants calculated from Eq. (1) show that C_i/C_a difference tends to increase with season: in June, that of treated plants is 1.7% higher than the control, while in July and August, it is 1.6% and 6.3% lower than the control, respectively, and it is roughly the same in September (Fig. 5). The intercellular CO_2 concentration of UV-B treated plants has a similar declining tendency as stomatal conductance. The results of the correlation analysis indicate that the correlation coefficient between deep soil water content and C_i/C_a of treated plants and the control is 0.073 and 0.540

Table 3 Correlation coefficients between soil water content and water use efficiency (WUE), leaf water content, $\delta^{13}C$, intercellular CO_2 concentration/air CO_2 concentration (C_i/C_a), stomatal conductance

		WUE	Leaf water content	Carbon isotope ratio	C_i/C_a	Stomatal conductance
UV-B	Pearson correlation	0.623*	−0.467	−0.078	0.073	0.275
	Sig. (2-tailed)	0.030	0.126	0.810	0.822	0.475
	n	12	12	12	12	9
Control	Pearson correlation	0.604	−0.731*	−0.608	0.540	0.711
	Sig. (2-tailed)	0.113	0.040	0.110	0.168	0.114
	n	8	8	8	8	6

Note: * $p < 0.05$.

respectively (Table 3), suggesting that UV-B reduces the susceptibility of C_i/C_a to soil water.

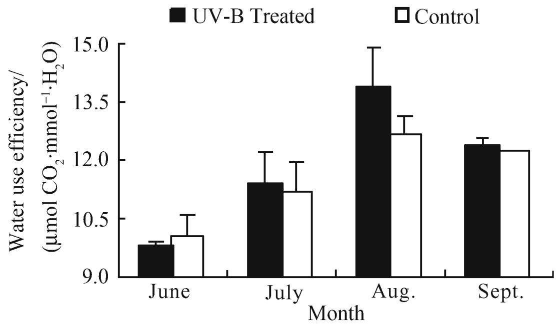

Fig. 4 Effects of UV-B and season on WUE

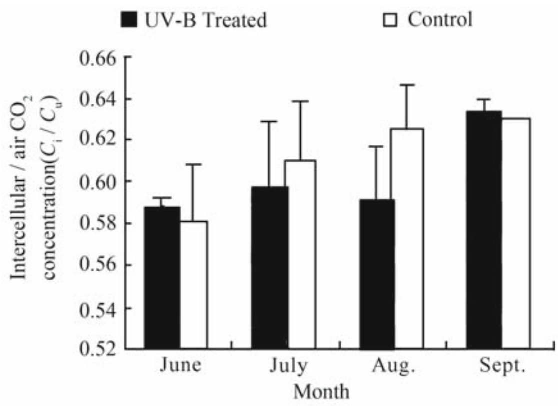

Fig. 5 Effects of UV-B and season on C_i/C_a

3.3 Effects of UV-B radiation on leaf total nitrogen content

The UV-B enhancement has the same impact on leaf total nitrogen content in different months: it improves notably the leaf total nitrogen content of *S. pubescens* leaves ($p<0.001$) without any correlation with seasonal changes. The leaf total nitrogen content of treated plant leaves tends to decline with seasonal transition, but that of the control is in contrast steady, with their difference dropping from 224% in the beginning of growing season to 48% in September (Fig. 6). Taking Fig. 4 into account, it can be found that UV-B radiation improves both WUE and leaf total nitrogen content at the same time.

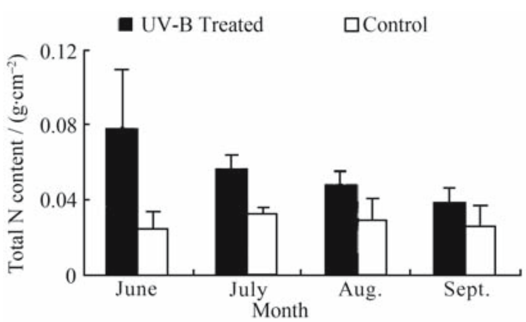

Fig. 6 Effects of UV-B and season on total N content

3.4 Effects of UV-B radiation on morphological characteristics

Enhanced UV-B has an extremely significant effect on leaf area ($p<0.001$) but not on SLW ($p>0.05$) (Table 2). The leaf area of treated plants and the control has little variation with the seasonal change, but leaf area of treated plants has an obvious decrease of 40.0%–55.2% compared with the control (Fig. 7). In the whole growing season, the SLW of the treated plants and the control varies insignificantly, and has even little increase when subjected to UV-B radiation (only by 2.4%–8.4%) (Fig. 8). Statistical analysis shows that the level is insignificant (Table 2), indicating that the leaf thickness is little influenced by enhanced UV-B radiation.

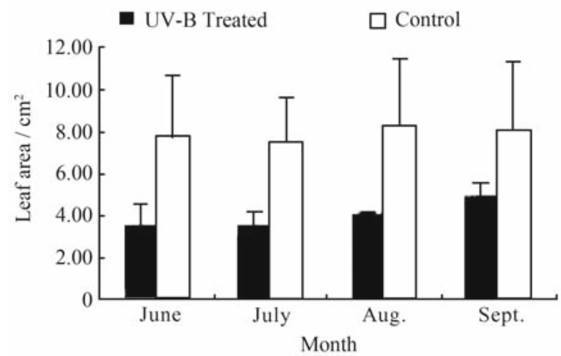

Fig. 7 Effects of UV-B and season on the leaf area

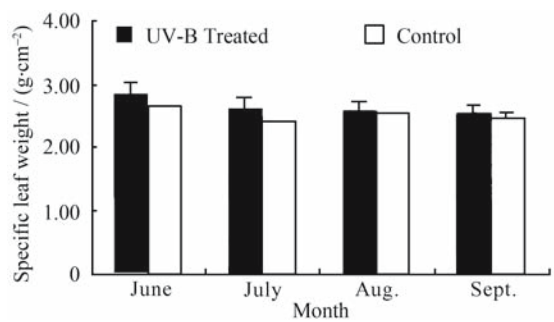

Fig. 8 Effects of UV-B and season on SLW

Morphologically, the leaves of treated plants curl, diminish, speckle and lose green color. Enhanced UV-B delays the blossoming period slightly but accelerates the aging of leaves markedly.

3.5 Seasonal change of soil water content at the sampling site

As shown in Fig. 9, the soil water content tends to rise at first and reaches the peak, thereafter drops slowly. Comparing Table 1 and Fig. 9, it can be found that the soil water content in June is rather low due to the inadequate rainfall, but it increases following the increasing amount of precipitation. In July, the soil water content culminates for the plenty rainfall, and it will remain at a high level in the following months although the precipitation is reduced. Regardless of the inadequate rainfall in September, the soil water content is still higher than in June.

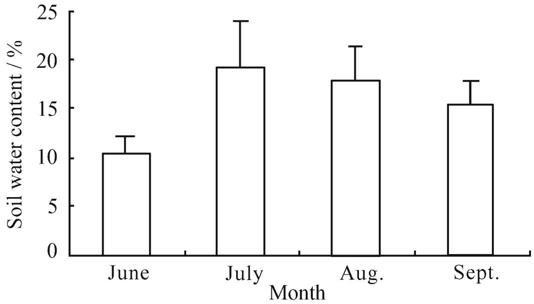

Fig. 9 Average value of season of soil water content (30–40 cm)

4 Discussion

Enhanced UV-B radiation will affect a wide range of species to different degrees. It was generally recognized that wildly grown plants have stronger adaptation to UV-B than those cultivated in growth chamber or greenhouse (Teramura and Murali, 1986; Caldwell et al., 1998). This difference in many cases is caused by visible light (Shi et al., 2001). Photolyase is a specific DNA recovery enzyme in plant cells, which is activated in the presence of both blue light and UV-A to recover the UV-B induced injures (Rupert and Tu, 1976). It has been proven that this photoreactivation will be invalid in the condition of low visible light (Britt et al., 1993). Hence the imbalance of UV-B and other lights in growth chamber or greenhouse might strengthen plant's susceptibility to UV-B.

In this study the artificial enhancement of UV-B radiation markedly reduces the leaf area and improves leaf total nitrogen content of *S. pubescens*, which agrees well with previous reports (Hatcher and Paul, 1994; Li and Wang, 1998; Gitz et al., 1999; Musil et al., 2003; Sullivan et al., 2003). However, UV-B radiation has no statistically significant difference in its effects on stomatal conductance, δ^{13}C, WUE, C_i/C_a, leaf water content and SLW, which indicates that UV-B did not harm *S. pubescens* physiologically.

In semi-arid and arid areas, physiological activities and growth are substantially affected by water condition. An efficient usage of the limited water resources in the environment is crucial for plants to well complete their life history, and WUE is just such an effective indicator for this capacity of different plants (Polley et al., 1993). The results in the present study suggest that enhance UV-B radiation tends to improve WUE of plant leaves. The WUE calculated from δ^{13}C often correlates closely with the ratio of C_3 plant biomass to the evapotranspiration, but it is influenced by the leaf-to-air VPD (Farquhar and Richards, 1984). The UV-B radiation in this study triggers the stomatal closing of *S. pubescens* and therefore reduces evapotranspiration, resulting in an improvement of WUE. The declining intercellular CO_2 concentration obtained from δ^{13}C also demonstrates that the comparatively higher WUE of UV-B treated plants is a result of weakened evapotranspiration (Polley et al., 1993). Greitner and Winner (1988) also once reported that the impact of UV-B on carbon fixation is smaller than on stomatal conductance and therefore improves WUE of plants.

Water stress is a common phenomenon in nature. It was reported that some plant species was more sensitive to water stress than to UV-B radiation under the joint effects of both factors (Teramura et al., 1984). However, this was not observed in field conditions (Teramura and Murali, 1986). It was pointed out by Teramurea (1980) that in the normal water condition, the transpiration intensity of soybean reduced considerably during the developmental period of reproductive organs; on the other hand, the transpiration rate, stomatal conductance and water potential remained unchanged in the water stress, regardless of the impact of UV-B radiation. Relevant studies on soybean suggest that the negative impacts of UV-B on photosynthesis and growth only take effects when soil water is sufficient, and such effects are not notable during the water stress (Sullivan and Teramura, 1990). Furthermore, the content of flavonoids in water stressed plant leaves keeps high, which reduces the UV-B's harm to plants. The results of the present study agree with the findings mentioned above. According to Manetas et al. (1997), UV-B delayed and reduced the injury of water stress on *Pinus pinea*. It was found from this study that the correlation among the free water content of *S. pubescens* leaves, δ^{13}C, C_i/C_a, stomatal conductance and deep soil water content has a decreasing tendency due to UV-B radiation; on the other hand, WUE is more susceptible to the change of soil water content due to the enhanced UV-B radiation.

The enhanced UV-B radiation had a remarkable improvement on leaf nitrogen content of *S. pubescens*, which might stem more directly from that UV-B reduced the biomass and changed its distribution in plants than from the impacts of UV-B on nutrient uptake. Many studies also indicated that the content of nutrients usually increased in the expense of the reduction of biomass. Although the changes of biomass of *S. pubescens* were not measured, the significant decrease of leaf area of UV-B treated plants partly suggested that the plant growth was inhibited apparently; in addition, because nitrogen transfers mainly to actively growing organs or tissues, which leads to a less nitrogen requirement in leaves and twigs, the nitrogen retention in leaves therefore increases. The increasing nitrogen content in plant tissues under UV-B radiation was in some studies attributed to the decline of herbivorous insects' visit (Caldwell et al., 1998) or the effect of other nutrients. Yue et al. (1998) considered the enhanced nitrogen content as the result of changed metabolism of nutritional elements caused by UV-B radiation. However, Björn et al. (1997) found that UV-B radiation had no effects on the content of N, P and K in plant leaves.

The rise of leaf water content in UV-B treated plants is a direct evidence of the inhibited growth of *S. pubescens*. The declining growth of leaves and evapotranspiration lead to the increase in leaf water content. Another possible factor might correlate to the substance assimilating ability of UV-B. Lautenschlager-Fleury (1995) once extracted water-soluble substances from undamaged *Vicia faba* leaf epidermis belt and found that highly UV-absorbable epidermis compounds are all water soluble while remaining components in leaf epidermis have much less absorbability to UV. Caldwell (1968) also found that most UV-absorbable substances in leaf epidermis belt are soluble in CH_4-H_2O-HCl mixture extract. In this study, leaf thickness was little affected by UV-B while leaf water content increased slightly, which needs further studies to determine whether it was because the plant improves physiological activities of UV-absorbing substances by means of increasing leaf water content.

5 Conclusions

The results of this study indicate that the leaf nitrogen content and leaf area of the commonly planted shrub in northern China, *S. pubescens*, are susceptible to enhanced UV-B radiation, and consequently might change the plant morphology and nitrogen cycling. According to the findings, due to the impacts of UV-B radiation, the shape of leaves and branches of UV-B treated plants will shrink, the surplus nitrogen accumulate in leaves will transfer to roots to promote the development of root system, thus enlarging the root/canopy ratio of plant. In this study, the water use efficiency of *S. pubescens* was little affected by UV-B, therefore, the simulation of exposure to enhanced UV-B radiation will not pose fatal harm to *S. pubescens*. According to monitoring information made by NASA (2005) to ozone layer, up till 2004, the area of ozone holes above the Antarctic keeps enlarging since the first detection in 1982. However, it is worthy to be highlighted that the enlarging rate declines considerably since 1990 (the area of ozone holes increased strikingly 20 times from 1982 to 1990, but only 25% from 1990 to 2004), which could be attributed in a large part to the public efforts to reduce the use of chlorocarbon and fluorocarbon in the world communities. However, the current situation is not so encouraging. The latest investigation shows that the area of ozone holes rose to an even higher level in 2004 regardless of a considerable decline in 2003 (NASA, 2005). If the global changes, featured by the increasing CO_2 concentration, temperature and UV-B radiation, intensify in the future, the physiological metabolism and growth of *S. pubescens* and other woody plants in warm temperate zones will be challenged by enhanced UV-B radiation.

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