PHOTOSYNTHETICA 48 (3): 437-445, 2010

Different growth and physiological responses to experimental warming of two dominant plant species *Elymus nutans* and *Potentilla anserina* in an alpine meadow of the eastern Tibetan Plateau

F.S. SHI, Y. WU, N. WU+, and P. LUO

ECORES Lab, Chengdu Institute of Biology, Chinese Academy of Sciences, P.O. Box 416, Chengdu 610041, People's Republic of China

Abstract

The effects of experimental warming on the growth and physiology of grass *Elymus nutans* and forb *Potentilla anserina* were studied by using open-top chambers (OTCs) in an alpine meadow of the eastern Tibetan Plateau. The warming treatment increased mean air and soil surface temperatures by 1.53°C and 0.50°C, respectively, but it reduced soil relative water content in the surface layer. Experimental warming enhanced the growth and gas exchange of *E. nutans*, while it reduced those of *P. anserina*. Experimental warming resulted in an increased efficiency of photosystem II (PSII) in *E. nutans*, while decreasing it in *P. anserina*; significantly stimulated non-photochemical quenching, antioxidative enzymes and non-enzymes in both species; and significantly reduced malondialdehyde content in *E. nutans*, while promoting it in *P. anserina*. The results of this study indicated that the two species showed different growth responses to experimental warming and their different physiological performances further indicated that experimental warming alleviated the negative effect of low temperature on the growth and development of *E. nutans*, but limited the competitive ability of *P. anserina* in the study region.

Additional key words: *Elymus nutans*; experimental warming; growth; physiology; *Potentilla anserina*.

Introduction

Evidence indicated that the global mean surface temperature of the Earth has increased over last century by approximately 0.6°C, and the annual mean temperature will continue to rise at a more rapid rate as a result of increasing gas concentrations in the coming decades (IPCC 2007). The stresses of this warming could have been marked by ecological effects on terrestrial ecosystems, as well as on individual species, *e.g.*, from the viewpoints of plant physiology and production (Peters and Lovejoy 1992).

Plant responses to experimental warming can be due to direct or indirect temperature effects (Jonasson *et al.* 1999, Rustad *et al.* 2001). Some plants will grow better than others under a changing climate, and the changes in the exchanges of mass, energy and momentum resulting from atmospheric warming will influence the establishment, survival and reproduction of plants (Woodward 1992, Loik *et al.* 2004). Indeed, our initial results from a warming experiment in the eastern Tibetan Plateau indicated an increase in soil temperature and a decrease in soil moisture content for plots in OTC, and experimental warming increased the cover degree and biomass accumulation in the grasses, but reduced them in the forbs (Shi *et al.* 2008). Similar responses of different plant groups to a warming experiment have also been demonstrated in the Tibetan Plateau (Zhang and Welker

Received 8 January 2010, *accepted* 21 May 2010.

+Corresponding author; fax: +86-28-85222753, e-mail: wuning@cib.ac.cn

Abbreviations: AOS – activated oxygen species; APX – ascorbate peroxidase; AQY – apparent quantum yield; CAT – catalase; C_i – intercellular CO_2 concentration; E – transpiration rate; F_v/F_m – maximal PSII efficiency; g_s – stomatal conductance; LCP – photosynthetic light compensation point; MDA – malondialdehyde; NPQ – non-photochemical quenching; OTC(s) – open-top chamber(s); PAR – photosynthetically active radiation; PFD – photon flux density; P_{max} – maximum net photosynthetic rate; P_N – net photosynthesis rate; POD – peroxidase; q_p – photochemical quenching; R_D – dark respiration rate; SOD – superoxide dismutase; yield – actual photochemical efficiency of PSII in the light.

Acknowledgements: The authors are very grateful to Prof. Xu Xiao for his help in laboratory work and the Key Lab of Ecological Restoration and Biodiversity Conservation of Sichuan (ECORES).The work was financially supported by the National Natural Science Foundation of China (40971178, 30870396), the National "11th Five-year Plan" Science & Technology Program of China.

1996, Zhou *et al*. 2000) and other cold regions (Harte and Shaw 1995, Arft *et al*. 1999, Henry and Molau 1997, Loik *et al*. 2000).

Plant physiological processes are highly sensitive to temperature and plant can acclimate to prevailing temperature at both short- and long-term temporal scales. A change in temperature will result in an immediate alteration of each physiological process (Atkin *et al*. 2006). Thus, prediction of ecosystem responses to climatic warming in a future world strongly relies on our understanding of plant physiological acclimation (Zhou *et al*. 2007). Acclimation of physiological processes has been reported in the literature when plants were exposed to changed temperatures (Edward and Smith 1988, Battaglia *et al*. 1996, Loik *et al*. 2004, Xiong *et al*. 2000, Bolstad *et al*. 2003, Lee *et al*. 2005, Yamori *et al*. 2005). Among physiological parameters, light-saturated photosynthetic rate, apparent quantum yield of the photochemical efficiency of photosystem II and activities of antioxidant enzymes are temperature-dependent and the most sensitive parameters in response to global warming (Atkin *et al*. 2005, Xiong *et al*. 2000, Jarvis *et al*. 2004, Xin and Browse 2000). Such acclimation may vary for functional groups with different evolutionary backgrounds and survival strategies (Loik *et al*. 2000). In this regard, the changes in aboveground biomass accumulation exhibited by grasses and forbs exposed to a warming manipulation in the eastern Tibetan Plateau (Shi *et al*. 2008) lacks a mechanistic explanation.

The present study is an in-depth research based on our previous work. In the present study, we examined the different growth responses for the grass *E. nutans* and forb *P. anserina* exposed to experimental warming. The objective of this study was to identify how the two species were adapted to the elevated temperature by different physiological adjustments. The reasons for choosing the two species included: (*1*) they belong to two different functional groups, *E. nutans* is a graminoid whereas *P. anserina* is a forb; (*2*) they naturally coexist within our study site; (*3*) they are two dominant plant species at the study site (Shi *et al*. 2007, 2008).

Materials and methods

Study site: The study site (30 × 30 m) was selected in a livestock-excluded pasture (since 2002) where vegetation types and micro-topography were fairly consistent, located at Kakagou, Songpan County, Sichuan Province (3,400 m a.s.l., 32°51′N, 103°33′E). The annual mean August temperature there was 15.38°C. There was no absolute frost-free period. The annual mean precipitation was 717.7 mm, of which 80% occurred from May to October. The soil at the site was typical mountain brown one. The major plant species in the study site included some perennial grasses such as *E. nutans*, *Deschampsia cespitosa* and *Festuca ovina*, and an annual forb *Arenaria serpyllifolia*, as well as some perennial forbs such as *P. anserina*, *Geranium pylzowianum*, *Thlaspi arvense* and *Rumex acetosa*.

Experimental design: This experiment followed the methods of the International Tundra Experiment using OTCs as a passive warming device to generate an artificially warmed environment. On March 28, 2006, five OTCs were installed at the study site, and a control plot was also established close to each OTC. The OTCs, which were 1.5 m high (just higher than the highest plants at the study site) with vertical sides, were constructed with 3 mm thick translucent Plexiglas. This material had high solar transmittance in visible wavelengths (about 90%), and low transmittance in the infrared (heat) range (<5%). Both the warmed and the control plots were 1.5 × 1.5 m in size. The distance between the OTC plots and control plots varied from 3 to 4 m, and the distance between two OTC plots ranged from 10 to 15 m.

OTC performance was assessed *in situ* by simultaneous measuring of the air temperature at a height of 15 cm above ground, soil surface temperature at 5-cm belowground, soil relative water content at 0–5 cm and 10–15 cm belowground both inside and outside of randomly selected OTC′s. Measurements were taken continuously at 30-min intervals throughout the experimental period between May and October by alternating among sensors connected to a data logger (*Campbell AR5*, Avalon, USA).

Growth parameters: It is difficult to count the tiller number of *E. nutans*, so the biomass of *E. nutans* was measured by harvesting five 0.25 m^2 square subplots in each of the warmed and the control plots, and the biomass of *P. anserina* was assessed by harvesting 50 individuals in both the warmed and the control plots (n = 10 per replicate). The measurements were carried out on August 31, 2008. All the plant samples were oven-dried for 72 h at 80°C and then weighed.

Physiological measurements: Because of the short growing season in the study site, which lasts just from the end of May to the end of September, the biomass accumulation of most alpine plants reaches and keeps peak values in late August, and all the physiological and chemical variables of the plants are relatively stable during this period. So we measured the physiological and chemical characters of the two plant species at their developmental stages once a week starting from August 16 to August 30, 2008 (3 times in total).

Gas exchange: To characterize warming-induced shifts in carbon acquisition and instantaneous gas exchanges of the two species, the fully expanded and exposed leaves were measured between 08:00 and 11:30 h (local time) on clear days under controlled optimal conditions using an open-mode portable photosynthesis system (model *LI-6400, Li-Cor, Inc*., Lincoln, NE, USA). Three individuals per replicate were randomly selected for sampling at each time. A series of five measurements taken on the same leaf were averaged for each individual, and the mean value of the three individuals was used as one replicate for statistical analysis. The photon flux density (PFD) was maintained at *ca*. 1,000 ± 50 μmol m^{-2} s^{-1} using the *LI-6400* artificial light source, and temperature was maintained at 15 ± 1.5°C, with a relative humidity of 36–55% inside the leaf chamber during the measurement. The CO_2 concentration within the leaf measurement chamber was maintained at 300 ± 10 μmol mol^{-1}. The net photosynthetic rate (P_N), transpiration rate (E), stomatal conductance (g_s) and intercellular CO_2 concentration (C_i) were measured. The resultant net photosynthetic rates were expressed on a projected leaf area basis (unit leaf area). After the measurements, the measured leaves were collected and their projected areas were determined with a scanner and a *UTHSCSA ImageTool* analysis system (*University of Texas Health Science Center*, San Antonio, TX, USA).

***P_N*-PAR response curve**: All measurements were made by the same sampling method as described above. The block temperature was held at 15 ± 1.5°C and the relative humidity was at 36–55%. The response to PAR was measured at 0, 20, 50, 80, 100, 200, 300, 400, 600, 800, 1,000; 1,200; 1,600; 1,800, and 2,000 μmol m^{-2} s^{-1}, respectively. The light-response curve of photosynthesis was fitted to a nonrectangular hyperbola (Hirose and Werger 1987):

$$P_N = [\varphi I + P_{max} - ((\varphi I + P_{max})^2 - 4\varphi I\theta P_{max})^{0.5}]/2\theta - R_D$$

where φ is the initial slope of the curve, I is the photosynthetically active radiation (PAR), P_{max} is the maximum net photosynthetic rate, θ is the convexity, and R_D is the dark respiration rate. First, from the linear regression of the photosynthetic rate on PAR at 0–200 μmol m^{-2} s^{-1}, φ, R_D, and photosynthetic light compensation point (LCP) were obtained as the slope, y intercept and x intercept of these regressions, respectively. Then, a nonrectangular hyperbola was fitted to the whole curve using the φ and R_D values to obtain P_{max} and θ (Hikosaka *et al*. 2004).

Measurements of chlorophyll (Chl) fluorescence: To evaluate the photoinhibition of the two plant species in both the OTC plots and the control plots, Chl fluorescence was measured on the same leaves as those used for gas-exchange measurements, using a modulated fluorometer (*PAM-2100, Walz*, Effeltrich, Germany), as described by Bilger *et al*. (1995). The leaves were kept in clip cuvettes for dark adaptation for 30 min before the measurements. The intensity of the saturation pulse to determine the maximal fluorescence emission in the presence (F_m') and in the absence (F_m) of quenching on the upper surface of the leaf was 8,000 μmol m^{-2} s^{-1}, 0.8 s duration, whereas the 'actinic light' was 600 μmol m^{-2} s^{-1}. The minimal fluorescence yield of a dark-adapted sample (F_0), minimal fluorescence yield of a pre-illuminated sample (F_0') and actual fluorescence intensity at any time (F) were measured according to Van Kooten and Snel (1990). Then the ratio F_v/F_m (where $F_v = F_m - F_0$) were determined according to Rosenqvist and van Kooten (2003), $q_p = (F_m' - F)/(F_m' - F_0')$, $NPQ = F_m/F_m' - 1$ and yield = $(F_m' - F)/F_m'$ were determined according to Bilger and Björkman (1990).

Assessments of malondialdehyde (MDA): The concentration of MDA was measured as described by Heath and Packer (1968). Fresh leaves (0.3 g), five replicates for each species, were homogenized in 5 ml of 5% trichloroacetic acid (TCA) solution each time. The homogenate was centrifuged at 4,000 × *g* for 10 min. Then, 1 ml of supernatant was mixed with 2 ml 0.6% thiobarbituric acid (TBA), which was boiled for 10 min and then cooled at room temperature. The mixture was centrifuged at 3,000 × *g* for 15 min and the supernatant was assayed for the MDA concentration. The absorbance of MDA was measured at 532, 600, and 450 nm. The MDA concentration can be estimated by using the formula C [μmol l^{-1}] = 6.45 ($A_{532} - A_{600}$) – 0.56 A_{450}. The MDA concentration was expressed as μmol g^{-1}(DM).

Assays of antioxidant enzymes: Fresh leaf samples (0.4 g), five replicates for each species, were ground in liquid nitrogen using a mortar and pestle. The ground samples were homogenized on an ice bath in 4 ml of single extraction solution containing 50 mM Tris-HCl (pH 7.0), 1 mM EDTA, 20% glycerin, 1 mM ascorbic acid (AsA), 1 mM dithiothreitol (DTT), 1 mM glutathione (GSH), and 5 mM $MgCl_2$, and extracted at 4°C each time. The homogenate was centrifuged at 8,000 × *g* at 4°C for 15 min. This method was modified from that described by Knorzer *et al*. (1996) and Lei *et al*. (2006). The supernatant was stored in a volume of 0.4 ml at –70°C for the analysis of antioxidant enzymes. All experiments were performed at 15°C and completed within 2 days each time.

Superoxide dismutase (SOD, EC 1.15.1.1): The SOD activity was measured spectrophotometrically based on the inhibition in the photochemical reduction of nitroblue tetrazolium (NBT) (Giannopolitis and Ries 1997), modified as follows: The reaction mixture contained 50 mM Tris-HCl buffer (pH 7.8), 0.1 mM EDTA, and 13.37 mM methionine. 5.7 ml of the reaction mixture was mixed with 200 μl 0.1 mM riboflavin (containing 50 mM

Tris-HCl, 0.1 mM EDTA, pH 7.8) and 0.1 ml of the enzyme source. Finally, riboflavin was added and the reaction was initiated by placing the glass test tubes under fluorescent lamps. The reaction was terminated after 30 min by removing the test tubes from the light source. Nonilluminated identical tubes were used as blanks. An illuminated blank without protein gave the maximum reduction of NBT, thus, the maximum absorbance at 560 nm. In this assay, 1 unit of SOD was defined as the amount of enzyme inhibiting the photoreduction of NBT by 50%.

Catalase (CAT, EC 1.11.1.6): A modification of the procedure of Aebi (1984) was used to analyze the CAT activity. The CAT activity was determined by directly measuring the decomposition of H_2O_2 at 240 nm. The reaction mixture contained 50 mM Tris-HCl buffer (pH 7.0) and 0.1 mM EDTA. The amount of 2.9 ml of the reaction mixture was mixed with 50 µl enzyme solution, then, 50 µl 750 mM H_2O_2 was added to stimulate the reaction. The absorbance at 240 nm was read every 30 s. The CAT activity was followed by decreasing absorbance between 0.5 and 3 min.

Peroxidase (POD, EC 1.11.1.7): The guaiacol peroxidase (G-POD) activity was measured using a modification of the method of Chance and Maehly (1995). The assay mixture contained 50 mM Tris-HCl (pH 7.0), 0.1 mM EDTA, 10 mM guaiacol and 5 mM H_2O_2. Then, 50 µl of the enzyme solution was added to the reaction mixture, and the reaction was stimulated in a total volume of 3.0 ml. The changes in the absorbance of the brown guaiacol at 470 nm between 0.5 and 3.5 min were recorded for calculating the POD activity.

Ascorbate peroxidase (APX, EC 1.11.1.11): The APX activity was measured using a modification of the procedure made by Nakano and Asada (1981). The reaction mixture contained 50 mM Tris-HCl buffer (pH 7.0), 0.1 mM EDTA and 0.1 mM H_2O_2. Then, 2.93 ml of the reaction mixture was homogenized with 50 µl of the enzyme solution, and the reaction was stimulated by 20 µl of 30 mM AsA (a total volume of 3 ml). The H_2O_2-dependent oxidation of ascorbate was followed by a decrease in the absorbance at 290 nm within 1 min.

Statistical analysis: Data were processed separately for the two species (Xiao *et al*. 2003). Standard error (SE) of each treatment was calculated. To test the temperature effects, *t*-test was performed for each species. The *SPSS 16.0 for Windows* was used for all statistical analyses.

Results

Warming effects of OTCs: On average from 2006 to 2008 between May and October, air and soil surface temperatures were 1.53°C and 0.50°C higher in the OTC manipulation than in the controls, based on data logger measurements (Table 1). Experimental warming caused the decline of soil relative water content at 0–5 cm depth, and the average values in the warmed plots and the control plots were 38.92% and 52.44%, respectively. However, warming manipulation had little effect on soil relative water content at 10–15 cm depth in the warmed plots compared to the control plots (Table 1). The physiological measurements were carried out from August 16 to August 30, 2008, during which period air temperature and soil surface temperature both remained at a steady level at the study site (Fig. 1).

Growth properties: The two species showed different responses to experimental warming in biomass accumulation compared to the control. Experimental warming significantly promoted the biomass accumulation of *E. nutans* (Fig. 2*A*), but significantly reduced it in *P. anserina* (Fig. 2*B*).

Table 1. Mean soil temperature at 5 cm below ground and mean air temperature at 15 cm above ground, mean soil relative water content at 0–5 cm and 10–15 cm below ground between May and October in the warmed plot and the control plot in 2006 to 2008.

Year		Temperature [°C]		Soil relative water content [%]	
		Soil	Air	0–5 [cm]	10–15 [cm]
2006	OTC	12.01	8.87	36.17	45.76
	Control	11.48	7.26	50.34	48.29
	Difference	0.53	1.61	–14.17	–2.53
2007	OTC	12.03	8.73	42.03	53.58
	Control	11.56	7.33	55.52	54.18
	Difference	0.46	1.40	–13.49	–0.60
2008	OTC	12.27	9.07	38.58	46.22
	Control	11.75	7.48	51.46	50.34
	Difference	0.52	1.59	–12.88	–4.12

Photosynthetic parameters: P_N, E, g_s, P_{max}, R_D, and AQY of *E. nutans*, and LCP of *P. anserina* significantly increased in the warmed plots compared to those in the control plots (Table 2). However, the P_N, E, g_s, P_{max}, R_D and AQY of *P. anserina*, and the LCP of *E. nutans* significantly decreased in the warmed plots compared to those in the control plots (Table 2).

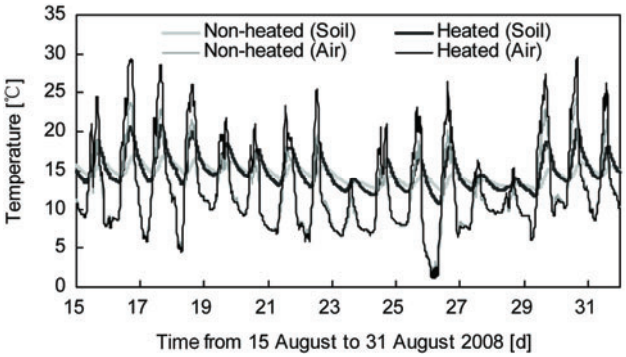

Fig. 1. Mean soil temperature at 5 cm below ground and mean air temperature at 15 cm above ground between 15 August and 31 August 2008 in the warmed plot and the control plot.

Chl fluorescence parameters: The two species showed different responses of F_v/F_m, Yield, q_p and NPQ in the warmed plots and the control plots. Experimental warming significantly increased F_v/F_m, yield and q_p of *E. nutans* (Fig. 3*A,B,C*), and NPQ of *P. anserina* (Fig. 3*D*), but significantly reduced F_v/F_m, yield and q_p of *P. anserina* (Fig. 3*A,B,C*).

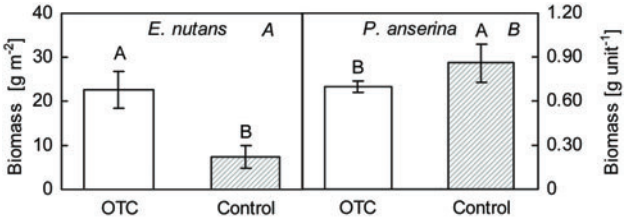

Fig. 2. Effects of experimental warming on biomass accumulation of *E. nutans* (*A*) and *P. anserina* (*B*). The values are means ± SE (n =5 per replicates), biomass of *P. anserina* was measured by harvesting 10 individuals per replicate. The treatments (OTC and control) for each species with different letters are significantly different at $P<0.05$ according to *t*-test.

MDA and activities of antioxidant enzymes: Experimental warming significantly suppressed MDA of *E. nutans*, but significantly promoted MDA of *P. anserina* in the warmed plots compared to that in the control plots (Table 3). The contents of all antioxidant enzymes of the two species were increased in the warmed plots compared to the control plots. Significant increases in SOD and APX were detected in *E. nutans*, and significant increases in SOD, CAT, POD, and APX were in *P. anserina* (Table 3).

Discussion

In any given community, some species will grow better than others under a changing climate; as a result, competitive balances will shift, due to changes in species growth (Alward *et al*. 1999, Sternberg *et al*. 1999). In the present study, the biomass accumulation of *E. nutans* exposed to experimental warming significantly increased. However, the biomass accumulation of *P. anserina* significantly decreased in the warming treatment. The results from our study were corroborated with our previous works (Shi *et al*. 2008), and also coincided with some warming experiments carried out in cold-climate ecosystems; that is, while grasses were particularly stimulated by experimental warming, the effects on forbs were less pronounced or even negative (Campbell *et al*. 1995,

Table 2. Effects of experimental warming on photosynthetic parameters of *E. nutans* and *P. anserina*. All the measurements were done once a week starting from August 16 to August 30, 2008. The values are mean ± SE (n = 15 per replicate). P_N – net photosynthetic rate; E – transpiration rate; g_s – stomatal conductance; C_i – intercellular CO_2 concentration; P_{max} – maximum net photosynthetic rate; R_D – dark respiration rate; AQY – apparent quantum yield; LCP – photosynthetic light compensation point. The treatments (OTC and control) for each species with different letters are significantly different at $P<0.05$ according to *t*-test.

Photosynthetic parameters	*E. nutans*		*P. anserina*	
	OTC	Control	OTC	Control
P_N [μmol m^{-2} s^{-1}]	8.86 ± 1.44^a	7.22 ± 1.95^b	7.46 ± 1.57^b	9.08 ± 1.99^a
E [mmol m^{-2} s^{-1}]	5.16 ± 0.68^a	3.51 ± 0.57^b	5.93 ± 0.74^b	6.95 ± 0.82^a
g_s [mol m^{-2} s^{-1}]	0.50 ± 0.12^a	0.38 ± 0.07^b	0.56 ± 0.18^b	0.71 ± 0.10^a
C_i [μmol mol^{-1}]	136.48 ± 6.11^a	135.72 ± 3.03^a	134.88 ± 4.49^a	138.48 ± 7.73^a
P_{max} [μmol m^{-2} s^{-1}]	10.80 ± 1.04^a	8.57 ± 0.85^b	8.28 ± 0.93^b	10.61 ± 0.72^a
R_D [μmol m^{-2} s^{-1}]	0.76 ± 0.25^a	0.58 ± 0.12^b	0.87 ± 0.18^b	1.05 ± 0.33^a
AQY [mol(CO_2) $mmol^{-1}$]	0.043 ± 0.003^a	0.027 ± 0.003^b	0.031 ± 0.003^b	0.048 ± 0.005^a
LCP [μmol m^{-2} s^{-1}]	17.40 ± 2.26^b	21.71 ± 3.14^a	28.06 ± 2.59^a	21.78 ± 1.62^b

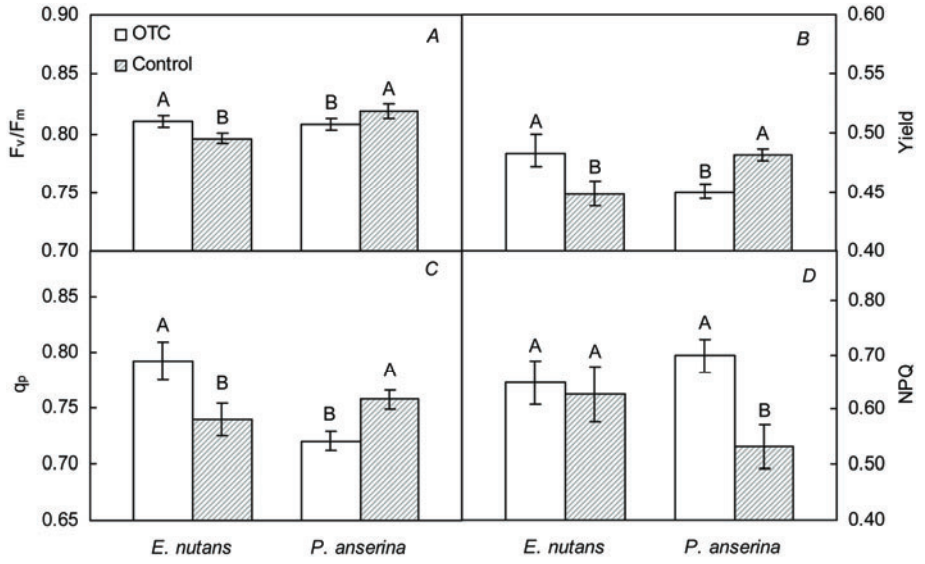

Fig. 3. Effects of experimental warming on chlorophyll fluorescence of *E. nutans* and *P. anserina*. F_v/F_m – maximal PSII efficiency (*A*); yield – actual photochemical efficiency of PSII in the light (*B*); q_p, – photochemical quenching (*C*); NPQ – non-photochemical quenching (*D*). All the measurements were done between 09:30 to 12:00 h (local time) after the same leaves had been used for gas-exchange measurements once a week from August 16 to August 30, 2008. The values are means ± SE (n = 15 per replicate). The treatments (OTC and control) for each species with different letters are significantly different at $P<0.05$ according to *t*-test.

Table 3. Effects of experimental warming on MDA and activities of antioxidant enzymes of *E. nutans* and *P. anserina*. All the measurements were done once a week starting from August 16 to August 30, 2008. The values are mean ± SE (n = 15 per replicate). MDA – malondialdehyde; SOD – superoxide dismutase; CAT – catalase; POD – peroxidase; APX – ascorbate peroxidase. The treatments (OTC and control) for each species with different letters are significantly different at $P<0.05$ according to *t*-test.

Parameters	*E. nutans* OTC	Control	*P. anserina* OTC	Control
MDA [μmol g^{-1}(DM)]	13.1 ± 1.41[b]	15.5 ± 1.32[a]	29.7 ± 1.75[a]	26.7 ± 2. 04[b]
SOD [unit mg^{-1}(protein)]	29.41 ± 5.38[a]	19.29 ± 3.13[b]	36.10 ± 4.29[a]	23.75 ± 4.72[b]
CAT [nmol(H_2O_2) mg^{-1}(protein) min^{-1}]	47.53 ± 2.25[a]	41.87 ± 4.23[a]	398.37 ± 42.49[a]	306.14 ± 23.70[b]
POD [μmol(guaiacol) mg^{-1}(protein) min^{-1}]	375.15 ± 25.43[a]	366.87 ± 24.05[a]	60.43 ± 8.38[a]	40.79 ± 6.42[b]
APX [μmol(AsA) mg^{-1}(protein) min^{-1}]	11.21 ± 1.58[a]	5.62 ± 0.87[b]	10.55 ± 1.72[a]	8.23 ± 1.35[b]

Dormann and Woodin 2002, Harte and Shaw 1995, Zhang and Welker 1996, Parsons *et al*. 1995, Walker *et al*. 1994, Gunn and Farrar 1999).

The different growth forms of *E. nutans* and *P. anserina* could be due to their different responses of P_N to experimental warming. In this study, significantly positive warming effect on leaf P_N was observed in *E. nutans*. P_N responses of the two species to experimental warming explained quite well the warming effects on their growth. The different growth forms of *E. nutans* and *P. anserina* also could be due to their different morphologies. While the photosynthetically active biomass of *E. nutans* was distributed more or less uniformly over the canopy height, the leaves of *P. anserina* were restricted to the lower parts of the canopy close to soil surface (Shi *et al*. 2008). Additionally, experimental warming dried the soil surface layer in our study, and this may also have contributed to species-specific changes in growth forms. Plants responded to drought in a number of ways, which had been reviewed by Chaves *et al*. (2002). Generally, drought had a negative influence on plant growth (Haupt-Herting and Fock 2002, Shah and Paulsen 2003). *P. anserina* could not tolerate a hot and dry environment, which might be one of the reasons why the growth of *P. anserina* was hampered in the present study.

The responses of gas exchange to experimental warming have been shown to vary largely for different species (Loik *et al*. 2000, He and Dong 2003, Xiao *et al*. 2003). In this study, the E, g_s, P_{max}, R_D, and AQY of *E. nutans* significantly increased and the LCP of this species significantly decreased in the warming treatment. The results indicated that experimental warming enhanced the gas exchange of *E. nutans*. The positive effects of warming on gas exchange of some plant species had been reported by other studies (Chapin and Shaver 1996, Bergh and Linder 1999, Loik *et al*. 2004). However, the gas exchange of *P. anserina* significantly decreased in the warming treatment and this could be

attributed to the effect of warming on soil drying, which had been reported by Loik *et al*. (2000). Giorio *et al*. (1999) demonstrated that the decrease of gas exchange under the condition of water stress owed to the limitation of stomata or photosynthetic organelles. Based on this conclusion, we found that the stomatal conductance of *P. anserina* significantly decreased in the warming treatment of this experiment, but its C_i was kept almost at the same level between the two treatments. The findings suggested that the limitation of photosynthetic organelles was the key factor which reduced the gas exchange of *P. anserina* in the present study.

The *in vivo* Chl fluorescence provided basic information on the functioning of the photosynthetic apparatus, as well as on the capacity and performance of photosynthesis. Experimental warming tended to enhance the PSII efficiency of *E. nutans* in terms of increases in F_v/F_m, yield and q_p, which were related to higher excitation energy transferring through PSII, chloroplast electron transport rate and activity. Our results suggested that *E. nutans* showed a stronger adaptability to the warming treatment. In contrast, experimental warming impaired the PSII efficiency of *P. anserina*. Reductions in PSII efficiency are species-specific and depend upon temperature and water stress (Valentini *et al*. 1995). Our results were in agreement with that of Loik and Harte (1996), who reported that the effects of warming on soil drying could result in a loss of excitation energy transfer, and cause an impairment of the PSII efficiency of plant species.

In general, various types of environmental stresses mediated their impacts through oxidative stress caused by the generation of activated oxygen species, AOS (Smirnoff 1998), which were highly reactive and toxic molecules that could cause oxidative damage to membranes, DNA, proteins, and lipids (Apel and Hirt 2004). To avoid the cellular damage due to AOS generation, plants produced a number of antioxidant enzymes that induced and provided secondary protection against oxidative stress (Mittler 2002, Apel and Hirt 2004, Mittler *et al*. 2004). In the present study, experimental warming increased the activities of antioxidative enzymes and stimulated the role of non-enzymes in *E. nutans*, which helped to create a balance in maintaining AOS metabolites for this plant species. As a result, lipid peroxidation in terms of MDA content significantly decreased in the warming treatment, which alleviated to some extent the negative effects of low temperature on the growth and development of *E. nutans*. Though increased NPQ of *P. anserina* could impair oxidative stress to some degree, the AOS accumulation might still exceed the defense ability of antioxidative systems and non-enzymes functions. As a result, the MDA content of *P. anserina* still significantly increased in the warming treatment compared to that in the control. It could be, thus, estimated that the competitive ability of *P. anserina* would possibly further decrease in the alpine meadow under the global warming in the future.

In conclusion, the results of this study has shown that the grass *E. nutans* and the forb *P. anserina* have different growth responses to experimental warming, *i. e.*, experimental warming stimulated the growth of *E. nutans*, but had a negative effect on the growth of *P. anserina*. Furthermore, the different physiological performances of the two species indicated that experimental warming alleviated the negative effect of low temperature on the growth and development of *E. nutans*, but reduced the competitive ability of *P. anserina* in the study region.

References

Aebi, H.: Catalase *in vitro*. – J. Meth. Enzymol. **105**: 121-126, 1984.

Alward, R.D., Detling, J.K., Milchunas, D.G.: Grassland vegetation changes and nocturnal global warming. – Science **283**: 229-231, 1999.

Apel, K., Hirt, H.: Reactive oxygen species: metabolism, oxidative stress, and signal transduction. – Annu. Rev. Plant Biol. **55**: 373-399, 2004.

Arft, A.M., Walker, M.D., Gurevitch, J., Alatalo, J.M., Bret-Harte, M.S., Dale, M., *et al.*: Responses of tundra plants to experimental warming: Meta-analysis of the international tundra experiment. – Ecol. Monogr. **69**: 491-511, 1999.

Atkin, O.K., Bruhn, D., Hurry, V.M., Tjoelker, M.G.: The hot and the cold: unraveling the variable response of plant respiration to temperature. – Funct. Plant Biol. **32**: 87-105, 2005.

Atkin, O.K., Scheurwater, I., Pons, T.L.: High thermal acclimation potential of both photosynthesis and respiration in two lowland *Plantago* species in contrast to an alpine congeneric. – Global Change Biol. **12**: 500-515, 2006.

Battaglia, M., Beadle, C., Loughhead, S.: Photosynthetic temperature responses of *Eucalyptus globules* and *Eucalyptus nitens*. – Tree Physiol. **16**: 81-89, 1996.

Bergh, J., Linder, S.: Effects of soil warming during spring on photosynthetic recovery in boreal Norway spruce stands. – Global Change Biol. **5**: 245-253, 1999.

Bilger, W., Björkman, O.: Role of the xanthophyll cycle in photoprotection elucidated by measurements of light-induced absorbance changes, fluorescence and photosynthesis in leaves of *Hedera canariensis*. – Photosynth. Res. **25**: 173-185, 1990.

Bilger, W., Fisahn, J., Brummet, W., Kossmann, J., Willmitzer, L.: Violaxanthin cycle pigment contents in potato and tobacco plants with genetically reduced photosynthetic capacity. – Plant Physiol. **108**: 1479-1486, 1995.

Bolstad, P.V., Reich, P., Lee, T.: Rapid temperature acclimation of leaf respiration rates in *Quercus alba* and *Quercus rubra*. – Tree Physiol. **23**: 969-976, 2003.

Campbell, B.D., Laing, W.A., Greer, D.H., Crush, J.R., Clark, H., Williamson, D.Y., Given, M.D.J.: Variation in grassland populations and species and the implications for community responses to elevated CO_2. – J. Biogeogr. **22**: 315-322, 1995.

Chance, B., Maehly, A.C.: Assay of catalase and peroxidase. – J. Method Enzymol. **2**: 764-775, 1995.

Chapin, F.S., III., Shaver, G.R.: Physiological and growth responses of arctic plants to a field experiment simulating climatic change. – Ecology **77**: 822-840, 1996.

Chaves, M.M., Pereira, J.S., Maroco, J., Rodrigues, M.L., Ricardo, C.P.P., Osorio, M.L., Carvalho, I., Faria, T., Pinheiro, C.: How plants cope with water stress in the field. Photosynthesis and growth. – Ann. Bot. **89**: 907-916, 2002.

Dormann, C.F., Woodin, S.J.: Climate change in the Arctic: using plant functional types in a meta-analysis of field experiments. – Funct. Ecol. **16**: 4-17, 2002.

Edwards, J.A., Smith, R.L.: Photosynthesis and respiration of *Colobanthus quitensis* and *Deschampsia antarctia* from the maritime Antarctica. – Brit. Antarct. Surv. Bull. **81**: 43-63, 1988.

Giannopolitis, C.N., Ries, S.K.: Superoxide dismutases. 1. Occurrence in higher plants. – Plant Physiol. **59**: 309-314, 1997.

Gunn, S., Farrar, J.F.: Effects of a 4 degrees C increase in temperature on partitioning of leaf area and dry mass, root respiration and carbohydrates. – Funct. Ecol. **13**: 12-20, 1999.

Harte, J., Shaw, R.: Shifting dominance within a montane vegetation community: results of a climate-warming experiment. – Science. **267**: 876-880, 1995.

Haupt-Herting, S., Fock, H.P.: Oxygen exchange in relation to carbon assimilation in water-stressed leaves during photosynthesis. – Ann. Bot. **89**: 851-859, 2002.

He, W.-M., Dong, M.: Plasticity in physiology and growth of *Salix matsudana* in response to simulated atmospheric temperature rise in the Mu Us Sandland. – Photosynthetica **41**: 297-300, 2003.

Heath, R.L., Packer, L.: Photoperoxidation in isolated chloroplast I. Kinetics and stoichiometry of fatty acid peroxidation. – Arch. Biochem. Biophys. **25**: 189-198, 1968.

Henry, G.H.R., Molau, U.: Tundra plants and climate change: the International Tundra Experiment (ITEX). – Global Change Biol. **3**: 1-9, 1997.

Hikosaka, K., Kato, M.C., Hirose, T.: Photosynthetic rates and partitioning of absorbed light energy in photoinhibited leaves. – Physiol. Plantrum **121**: 699-708, 2004.

Hirose, T., Werger, M.J.A.: Nitrogen use efficiency in instantaneous and daily photosynthesis of leaves in the canopy of a *Solidago altissima* stand. – Physiol. Plant. **70**: 215-222, 1987.

IPCC.: Climate Change 2007: The Physical Science Basis. – Summary for Policymakers. WMO and UNEF, Geneva. 2007.

Jarvis, A.J., Stauch, V.J., Schulz, K., Young, P.C.: The seasonal temperature dependency of photosynthesis and respiration in two deciduous forests. – Global Change Biol. **10**: 939-950, 2004.

Jonasson, S., Michelsen, A., Schmidt, I.K., Nielsen, E.V.: Responses in microbes and plants to changed temperature, nutrient, and light regimes in the arctic. – Ecology **80**: 1828-1843, 1999.

Knorzer, O.C., Durner, J., Boger, P.: Alterations in the antioxidative system of suspension - cultured soybean cells (*Glycine max*) induced by oxidative stress. – Physiol. Plant **97**: 388-396, 1996.

Lee, T.D., Reich, P.B., Bolstad, P.V.: Acclimation of leaf respiration to temperature is rapid and related to specific leaf area, soluble sugars and leaf nitrogen across three temperate deciduous tree species. – Funct. Ecol. **19**: 640-647, 2005.

Lei, Y., Yin, C., Li, C.: Differences in some morphological, physiological, and biochemical responses to drought stress in two contrasting populations of *Populus przewalskii*. – Physiol. Plant. **127**: 182-191, 2006.

Loik, M.E., Redar, S.P., Harte, J.: Photosynthetic responses to a climate-warming manipulation for contrasting meadow species in the Rocky Mountains, Colorado, USA. – Funct. Ecol. **14**: 166-175, 2000.

Loik, M.E., Still, C.J., Huxman, T.E., Harte, J.: *In situ* photosynthetic freezing tolerance for plants exposed to a global warming manipulation in the Rocky Mountains, Colorado, USA. – New Phytol. **162**: 331-341, 2004.

Mittler, R.: Oxidative stress, antioxidants and stress tolerance. – Trends Plant Sci. **7**: 405-410, 2002.

Mittler, R., Vanderauwera, S., Gollery, M., Van Breusegem, F.: Reactive oxygen gene network of plants. – Trends Plant Sci. **9**: 490-498, 2004.

Nakano, Y., Asada, K.: Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. – Plant Cell Physiol. **22**: 867-880, 1981.

Parsons, A.N., Press, M.C., Wookey, I.A., Welker, J.M., Robinson, C.H., Callaghan, T.V., Lee, J.A.: Growth-response and of *Calamagrostis lapponica* to simulated environmental-change in the sub-arctic. – Oikos. **72**: 61-66, 1995.

Peters, R.L., Lovejoy, T.L.: Global Warming and Biological Diversity. – Yale University Press, New Haven, Connecticut 1992.

Rosenqvist, E., van Kooten, O.: Chlorophyll fluorescence: a general description and nomenclature. – In: DeEll, J.R., Toivonen, P.M.A. (ed.): Practical Applications of Chlorophyll Fluorescence in Plant Biology. Pp. 31-78. Kluwer Academic Publishers, Dordrecht 2003.

Rustad, L.E., Campbell, J.L., Marion, G.M., Norby, R.J., Mitchell, M.J., Hartley, A.E., Cornelissen, J.H.C., Gurevitch, J.: A metaanalysis of the response of soil respiration, net nitrogen mineralization, and aboveground plant growth to experimental ecosystem warming. – Oecologia. **126**: 543-562, 2001.

Shah, N.H., Paulsen, G.M.: Interaction of drought and high temperature on photosynthesis and grain-filling of wheat. – Plant Soil. **257**: 219-226, 2003.

Shi, F., Wu, N., Luo, P.: Effect of temperature enhancement on community structure and biomass of subalpine meadow in Northwestern Sichuan. – Acta Ecologica Sinica **28**: 5286-5293, 2008.

Shi, F., Wu, N., Luo, P., Yi, S.L., Wu, Y., Wang, Q., Li, Y.L., Chen, H., Gao, Y.H.: Effect of enclosing on community structure of subalpine meadow in Northwestern Sichuan, China. – Chin. J. Appl. Environ. Biol. **13**: 767-770, 2007.

Smirnoff, N.: Plant resistance to environmental stress. – Curr. Opin. Biotechnol. **9**: 214-219, 1998.

Sternberg, M., Brown, V.K., Masters, G.J., Clarke, I.P.: Plant community dynamics in a calcareous grassland under climate change manipulation. – Plant Ecol. **143**: 29-37, 1999.

Valentini, R., Epron, D., De Angelis, P., Matteucci, G., Dreyer, E.: *In situ* estimation of net CO_2 assimilation, photosynthetic electron flow and photorespiration in Turkey oak (*Q. cerris* L.) leaves: diurnal cycles under different levels of water supply. – Plant Cell Environ. **18**: 631-640, 1995.

Van Kooten, O., Snel, J.F.H.: The use of chorophyII fluorescence momenclature in plant stress physiology. – Photosynth. Res. **25**: 147-150, 1990.

Walker, M.D., Webber, P.J., Arnold, E.H., Ebert-May, D.:

Effects of interannual climate variation on aboveground phytomass in alpine vegetation. – Ecology **75**: 393-408, 1994.

Woodward, F.I.: A review of the effects of climate on vegetation: ranges, competition, and composition. – In: Peters, R.L., Lovejoy, T.E. (ed.): Global Warming and Biological Diversity. Pp. 105-123. Yale University Press, New Haven, Connecticut 1992.

Xiao, C.W., Zhou, G.S., Ceulemans, R.: Effects of elevated temperature on growth and gas exchange in dominant plant species from Maowusu sandland, China. – Photosynthetica **41**: 565-569, 2003.

Xin, Z., Browse, J.: Cold comfort farm: the acclimation of plants to freezing temperatures. – Plant Cell Environ. **23**: 893-902, 2000.

Xiong, F.S., Mueller, E.C., Day, T.A.: Photosynthetic and respiratory acclimation and growth response of Antarctic vascular plants to contrasting temperature regimes. – Amer. J. Bot. **87**: 700-710, 2000.

Yamori, W., Noguchi, K., Terashima, I.: Temperature acclimation of photosynthesis in spinach leaves: analyses of photosynthetic components and temperature dependencies of photosynthetic partial reactions. – Plant Cell Environ. **28**: 536-547, 2005.

Zhang, Y.Q., Welker, J.M.: Tibetan alpine tundra responses to simulated changes in climate: Aboveground biomass and community responses. – Arctic Alpine Res. **28**: 203-209, 1996.

Zhou, H.K., Zhou, X.M., Zhao, X.Q.: A preliminary study of the influence of simulated greenhouse effect on a *Kobresia humilis* meadow. – Acta Phytoecol. Sinica **24**: 547-553, 2000.

Zhou, X.H., Liu, X.Z., Wallace, L.L., Luo, Y.Q.: Photosynthetic and respiratory acclimation to experimental warming for four species in a tallgrass prairie ecosystem. – J. Integr. Plant Biol. **49**: 270-281, 2007.