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Influence of simulated warming using OTC on physiological–biochemical characteristics of *Elymus nutans* in alpine meadow on Qinghai-Tibetan plateau

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ABSTRACT

Elymus nutans Griseb. is a typical important plant species in the alpine meadow of Qinghai-Tibetan plateau. To examine the effects of temperature elevation on its physiological and chemical characteristics, a simulation study was conducted in situ with open-top chambers (OTC) followed the method of International Tundra Experiment (ITEX) from November 2002 to September 2007, and these OTCs were designed five kinds of size with bottom diameters of 0.85, 1.15, 1.45, 1.75, 2.05 m so as to rise different air temperatures. The air temperature inside OTCs increased by 2.68, 1.57, 1.20, 1.07 and 0.69 °C with increase of OTC diameter compared with ambient air. We found that with increase of air temperature, the soluble sugar content and SOD (superoxide dismutase) activity in leaves of *E. nutans* increased first, and then decreased, whereas, the soluble protein content and GSH (Glutathione) content decreased first and increased then, the chlorophyll a and total chlorophyll contents were decreased, but the contents of chlorophyll b were higher than that of control. Increased temperature enhanced the above-ground biomass and blade height of *E. nutans*. These results indicated that elevated temperature had significant and complicated effects on physiological–biochemical characteristics of *E. nutans* on Qinghai-Tibet plateau, when the temperature increased within the range of 0.69–1.57 °C, it may have positive effects on plant growth and development, and *E. nutans* could adapt even develop defensive strategy to the changes of a certain ecological environment changes.

1. Introduction

Evidences indicate that the earth is experiencing climate warming [1,2]. According to the fourth appraisal report content of Intergovernmental Panel on Climate Change (IPCC), 2007, global mean temperature will increase by 1.8–4.0 °C till the end of this century (2100) [3]. In the past 100 years, mean temperature of China has been increased by 0.4–0.6 °C, and it may increase 1.7 °C till 2030, and 2.2 °C till 2050 [4]. Responses of ecosystem to climate warming may be more sensitive and rapid in high-latitude regions and high altitude regions because of (1) the strong role of climate in structuring and regulating alpine ecosystems; (2) inhibited migration as a result of topography and lack of soil formation; and (3) low temperatures and short growing season [5,6]. The Qinghai-Tibetan plateau, with average latitude above 4000 m, is an extensive alpine zone and a sensitive area of climate change and ecological fragile zone, and its temperature has increased at the rate of 0.32 °C per 10 years in the past 30 years on the global climate warming and human activities background [7,8]. Moreover, this region is predicted to experience "much greater than average" increase in surface temperatures in the future [9]. Thus, it is an ideal region to study the response mechanism of terrestrial ecosystems to climate change.

Study from International Tundra Experiment (ITEX) using OTCs warming manipulation have illustrated significant changes in plant phonology, biomass, growth and reproduction, litter decomposition, physiological performance and species composition [10–17] in response to experimental warming, as well as decreasing species diversity and shifts in species dominance were also documented. However, these study were often focus on effects of climate warming on community structures and functions. In high altitude areas [18–21], the physiological, biochemical and eco-physiology response of typical species to climate warming are poorly studied. Low temperatures and short growing seasons are considered to be among the most important limiting factors for the performance of alpine plants. Alpine vegetation is thus thought to be especially vulnerable to global warming.

Elymus nutans Griseb. is a typical important plant species in the alpine meadow of Qinghai-Tibetan plateau. In present paper, we used the OTCs manipulation simulated the climate warming for three consecutive years, examined how experimental warming affected the physiological–biochemical characteristics of typical

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species. According to these studies, we hope to explain the internal mechanisms which species' response or adaptations to globe climate change, to provide the theoretical basis and experimental data supported for climate change and decision-making in the future [10].

2. Materials and methods

2.1. Study region and site description

We conducted our research at the Geduo committee of Animal husbandry in Da-wu township, Ma-qin country, Guo-luo Tibetan autonomous prefecture, Qinghai province, which is situated at 34°17′–34°25′N, 100°26′–100°43′E. Mean altitude is 4120 m above the sea, mean annual temperature is −2.6 °C, and mean annual precipitation is 513.02 mm, 85.20% of which falls during the summer monsoon season (437.10 mm, from May to September). A detailed site description can be found in document [22]. Climatic type is alpine continental climate, which has a long cold season and short warm season, smaller year temperature difference and obvious daily temperature difference and strong solar radiation. Soil types are alpine meadow soil and alpine bush-meadow soil; there are plentiful organic matters in upper soil layer and sub-top soil [22]. *Kobresia humilis* meadow is the main two main summer-grazed and winter-grazed meadows, in which *K. humilis* and *E. nutans* are the dominant species in the community.

By taking *E. nutans* as the research object plant, we established our experiment in typical *K. humilis* meadow in September 2002, placed the conical OTCs on the plots, and fenced without grazing. The OTCs, were designed five kinds of size with bottom diameters of 0.85, 1.15, 1.45, 1.75, 2.05 m, top diameters of 0.40, 0.70, 1.00, 1.30, 1.60 m, and 40 cm in height (i.e., labeled A, B, C, D, E for different sizes) (Fig. 1) [23]. To examine how well the OTCs simulated the effect of climate warming, we make the meadow near the OTCs as the control plots, which was also fenced in September 2002.

2.2. Plant materials

Samples for analyses, the fresh leaves of *E. nutans*, were randomly selected and taken in experiment plots and control plots during August 25 and September 5, 2006. We carried the samples with ice storage tank to the laboratory of Xining, after which chlorophyll contents, soluble sugars content, soluble protein content, glutathione enzyme activity in leaves was determined. Tissue samples were either assayed immediately or frozen until assayed.

2.3. Analytical methods

2.3.1. Physiological and biochemical parameters

Total chlorophyll contents were measured in acetone extract by using the methods of Lichtenthaler, expressed as mg g^{-1} fresh weight (FW). [24]. Soluble sugars content was determined with anthrone method by using the methodology of Zhang et al. [25]. Soluble protein and glutathione (GSH) contents were determined by the method described in document [26], respectively. The measurements of superoxide dismutase activity (SOD activity) were analysed using the NBT with KO_2–DMSO solution as the source of superoxide ions [27]. The blue color developed due to the formation of formazon dye was measured immediately at 560 nm against an appropriate blank. The unit SOD activity was expressed as the concentration of the complex causing 50% inhibition of KO_2–DMSO mediated formazon dye formation. The content of superoxide anion radical in leaves was estimated by the methods of Wang and Luo [28].

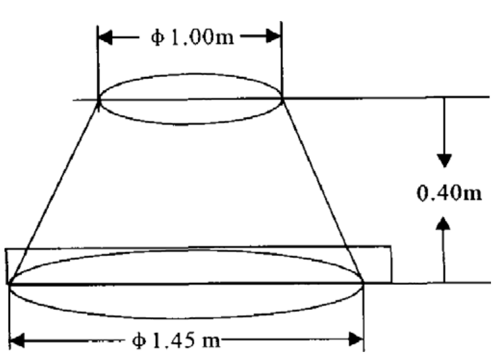

Fig. 1. The sketch of the OTCs in treatment C.

2.3.2. Yield parameters

In this paper, blade height and above-ground biomass of *E. nutans* were estimated as the yield parameters. During the early of start of growing season (mid-May), we labeled 20 strains of *E. nutans* in both experimental and control plots, respectively, determine the blade height in early September, and clipped above-ground parts in late September (last growth stage of plants). Above-ground parts were separated and oven dried at 80 °C till constant weight for biomass determination.

2.4. Statistical analysis

Growth and biomass data were subjected to one-way ANOVA test for assessing the significance of quantitative changes in different parameters due to different warming treatments. Data of physiological characteristics were analyzed for significance of changes due to treatments using one way ANOVA test. Duncan's comparison were performed as post hoc on parameters subjected to ANOVA tests. All the statistical tests were performed using SPSS software (SPSS Inc., version 16.0) and all pictures were drawled by using sigma plot software (version10.0). The significant levels in this paper is 0.05 level.

3. Results and analysis

3.1. Warming effects of OTCs

The OTCs, has advantages of low-cost, simple operation, easy to repeat, are commonly employed to study the effects of climate warming on ecosystems particularly in long-term fields observation [15,16,18]. OTCs can achieve a warming effect because of its conical structure (the side of the OTCs is incline with respect to horizontal), which can reduce the wind speed and trap heat [29]. Some researchers have suggested that the OTCs both increased the soil temperature and air temperature obviously, the warming effect of OTCs in early growth period was stronger than that in late growth period [16]. Moreover, there was a season difference in warming effect of OTCs that amplitudes of warming were always higher in summer and autumn than that in winter and spring. In our study, the OTCs elevated growing season average daily air temperature by 2.68, 1.57, 1.20, 1.07, 0.69 °C and increased growing season averaged soil temperature by 1.74, 1.06, 0.80, 0.60 and 0.30 °C, respectively. All of these warming amplitudes we observed in our study were within the appraisal report content of IPCC2007 and similar with published reports of OTC effects on magnitude of air warming.

3.2. Effect of simulated warming on blade height and above-ground biomass of E. nutans

Blade height and above-ground biomass of *E. nutans* in OTCs exhibited an increase, confirmed with assumption of other

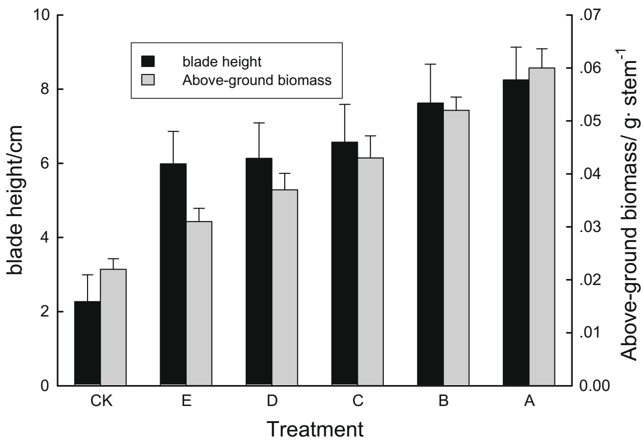

Fig. 2. Effect of simulated warming on blade height and above-ground biomass of *E. nutans.* Black bars represent the blade height of *E. nutans* plus with standard error. Gray bars represent the above-ground biomass of *E. nutans* plus with standard error.

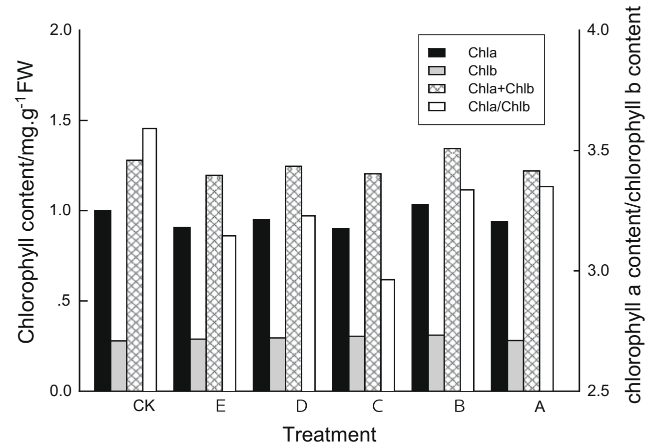

Fig. 3. Effect of simulated warming on chlorophyll content in the leaves of *E. nutans*. Bars represent the content of chlorophyll. Black bars indicate content of chlorophyll a. Gray bars represent the content of chlorophyll b. Cyclone bars represent total chlorophyll content. White bars represent the ratio of chlorophyll a content and chlorophyll b content.

scientist that experimental warming can increase leaf dry weight and leaf blade length [23], and this trend became more obvious from treatment E to treatment A. Treatment A exhibited the highest above-ground biomass, reach a average of 0.06 g per stem, and the highest blade height, an average of 7.9 cm. In contrast, the blade height and above-ground biomass of *E. nutans* in control plots (outside the OTCs) was, respectively, 3.5 cm 0.02 g per stem, both smaller than that of all treatment plots (see Fig. 2).

3.3. Effect of simulated warming on physiological and biochemical parameters

We found that experimental warming reduced both total chlorophyll content and content of chlorophyll a except the treatment B, but no significant differences were observed. And content of chlorophyll b increased in OTCs as compared to the control though there were also no significant differences existed. The decrease of total chlorophyll content was depends mainly on the decrease of content of chlorophyll a. However, with respect to the ratio of chlorophyll a content and chlorophyll b content (chla/chlb), the control plots was the highest with (3.59), as compared with treatment plots with values between 2.50 and 3.50. This result was opposite dramatically to that of other researches, which may practically contribute to the type of object plants such as herbaceous plant and woody plants. Different from the other treatment plots, both the total chlorophyll content and content of chlorophyll a and content of chlorophyll b were higher than those of the control plots [29] (see Fig. 3).

Soluble sugar is an important osmotic adjustment substance in plant tissue [30]. Soluble sugar content in the leaves of *E. nutans* increased significantly by 14.65%, 13.71% and 13.20%, compare with that of control, respectively (Fig. 4). Inversely, soluble sugar content decreased slightly in treatments D and E as compare to the control plots, but no significant difference was observed ($p > 0.05$).

Relationship between plant stress resistance and soluble protein in leaves of plant had been widely studied [30]. Our results showed that the soluble protein content in treatments E and D was higher than the control. But in treatments A, B and C, it decreased significantly by 28.58%, 21.57% and 16.91%, respectively ($p < 0.05$), compare with the control. However, the soluble protein content in leaves of *E. nutans* maintained higher level in all treatment plots and control plots despite of decrease and increase due to treatment effects. The conditions of water-shortage and

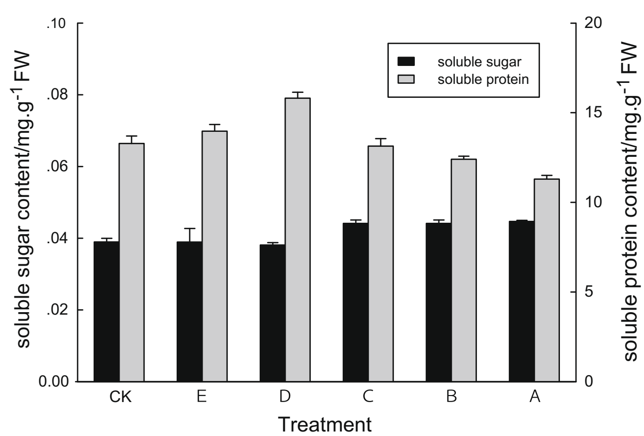

Fig. 4. Effect of simulated warming on soluble sugar content and soluble protein content in the leaves of *E. nutans*. Black bars indicate soluble sugar content. Gray bars represent the soluble protein content.

low temperature in later stage of growth could be responsible for the responses of higher soluble protein level to warming.

Glutathione (GSH), the primary antioxidant in plant, is involved in several redox reactions, including directly reacting with reactive oxygen species and joining in AsA-GSH circle to clean H_2O_2 [31]. The values of GSH content were shown in Fig. 5. GSH content showed increment in treatments D and C, but significant difference was only observed in treatment E (i.e., higher by 28.39%) compared to the control. In treatments B and A, GSH content significantly reduced by 38.35% and 14.16%, respectively, as compared to the control.

Superoxide dismutase (SOD), which catalyzes the dismutation of the superoxide anion (O_2^-) into hydrogen peroxide and molecular oxygen, is one of the most important enzymes in the front line of defense against oxidative stress [32]. Under the warming effects of OTCs, SOD activity showed a trend of fall first, and then rise from treatment E to treatment A, but both of them were lower than SOD activity in control plots, as followed in Fig. 5. And SOD activity of treatment B was lower than that of treatment C; there were significant differences between treatments. Similar trend was found in the content of superoxide anion (O_2^-) in leaves of *E. nutans*.

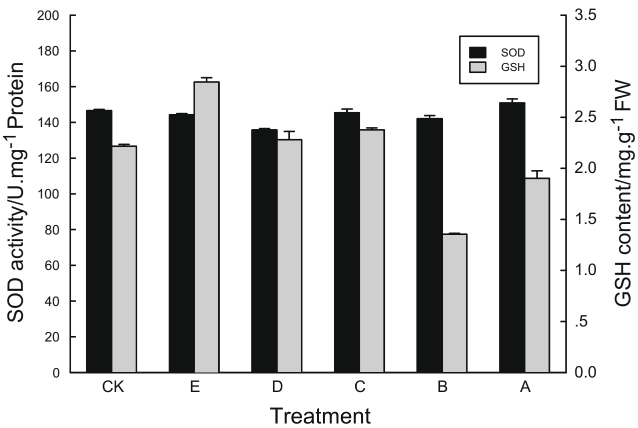

Fig. 5. Effect of simulated warming on GSH content and SOD activity in the leaves of *E. nutans*.

4. Discussion

4.1. Experimental warming and growth characteristics of E. nutans

In this study, we found that the warming increased blade height and above-ground biomass of *E. nutans*. It is possible that the change of community environment and water and heat regime caused by warming effect were the driving, which mainly contribute, but were not solely responsible for the increment of blade height and above-ground biomass. Temperature is considered to be one of the most important limiting factors for the performance of alpine plants, the OTCs changed the microclimate of community, the evapotranspiration near the soil surface (included the evaporation of soil and transpiration of vegetation) in OTCs were always higher than that of control, which conducted a temporary, relatively dry and low-moisture habitat beneficial to the growth of grass group such as the *E. nutans*. As a result, the final tiller number and biomass of *E. nutans* increased. Moreover, the OTCs were fenced for 3 years, which leaded to an accumulation of litter biomass. Increased litter cover reduced air permeability in OTCs, trapped heats and increased the humic substances in bottom of canopy, make other plants such as prostrate plants suffered higher temperature and susceptible to occasional overheating. In contrast, *E. nutans* was not likely to susceptible to those warming-caused stresses and got more competitive advantage of community because of its beneficial location in top of canopy.

Inconsistent results were also found in study of other researchers. Li et al. [33] suggested that the community biomass decrease with the experimental warming. Probably because experimental warming changed the structure of plant community and the competitiveness of plants. Accumulation of litter, change in nutrient availability and soil carbon storage and other changes both would result in a change in the recruitment of the plant community, even a succession occurred. The increment in competitively species did not compensate the decrement of other species, the community biomass decreased at unitary level of community.

4.2. Physiological and biochemical parameters of E. nutans and experimental warming

Some researchers have suggested that the background value of chlorophyll content in leaves of plants was lower in alpine regions because of its climate characteristics of high altitude, low average temperature, low day–night temperature and strong solar radiation, which inhibited the synthesis of chlorophyll [9]. Moreover, the chlorophyll a was prior to chlorophyll b in process of chlorophyll synthesis, chlorophyll b was transformed from chlorophyll a, catalyzed by chlorophyll a oxygen-nasal (CAO) [34]. The chlorophyll content and the ratio of chlorophyll a and chlorophyll b (chla/chlb) varied correspondingly with the temperature, light intensity and other environmental factors of habitat, chlorophyll b degrade faster than chlorophyll b at low temperature [35]. When the warming effects was mild (treatment B to treatment E, with warming amplitudes from 0.69 to 1.57 °C), content of chlorophyll b increased. Increment of chlorophyll b can increase the content and stability of light harvesting complex II (LHCII), which reduced the sensitivity of plants to photo inhibition, promoted the absorption of light and improved efficiency of photosynthesis. Increased chlorophyll b also contributed to an increment of total chlorophyll content, as the results founded in treatment B.

However, in treatment A, the total chlorophyll and chlorophyll b content were decreased as compare to the treatment B. It is possible that *E. nutans* was suffered a double stress of drought and overheat occasionally due to warming amplitudes of 2.68 °C. As a result, total chlorophyll was decreased because of its more decomposed and little synthesized effects. The highest value of chla/chlb in control plots as compare to the treatments, suggesting that the chla/chlb was also the main driver and mechanism that plant maintained a higher ratio of chlorophyll a and chlorophyll b to dampened effects of photo inhibition as an adaption to alpine environment though under the condition of experimental warming.

Generally, the photosynthetic rate of plant was lower when the air temperature lowers than optimum temperature range of photosynthesis [9]. If the temperature increases at a moderate degree, photosynthetic rate of plant would increase obviously. Current researches indicated that climate warming (include experimental warming) were increased nighttime temperature instead of daytime temperature. And on Qinghai-Tibetan plateau, plants were suffered a nighttime frost and freezing damage and a daytime photo inhibition caused by strong solar radiation. Thus, if the air temperature increased mildly, plants could be decoupled from nighttime low-temperature stress and promote the photosynthetic rate at daytime. However, the responses of photosynthesis to climate warming were complicated and mixed. If the temperature increases at large amplitude, the photosynthetic rate would still be restrained due to the discomposing effect of chlorophyll when plant suffered overheating. A problem worthy to be pointed out is that different results may be obtained if the stimulated experiment conducted at the different environment and other backgrounds. Therefore, influence of simulated warming on alpine plants and ecosystem may be complex.

As osmotic adjustment substance, soluble sugar plays an important role in growth and anti-adversity of plants [36]. As follows from Fig. 4, the content of soluble sugar decreased slightly in treatments D and E, and then increased significantly in treatments A, B and C, compared with the control. There are several explanations for these patterns. When warming amplitudes were relatively small, plant suffered a habitat shift from stress of low temperature to suitable environment, the content of soluble sugar decreased as a result of migration of stress. However, complied with increased warming amplitudes, the low atmospheric pressure in alpine meadow, the occasionally higher air temperature in daytime in OTCs, make plant leaves suffered high leaf transpiration rate. Therefore, the increment in amount of soluble sugar could decrease leaf water potential, prevent plant from drought stress and acquisition of desiccation tolerance [37]. Furthermore, the soluble sugar accumulated as dry matter was also the reason of its increment in content. The correlation between soluble sugar and dry matter were always significantly positive or very significantly positive.

Similar with soluble sugar, the amount of soluble protein has been implicated in the anti-adversity of plant. It has been reported that there was a positive correlation between the soluble protein and anti-adversity. Accumulation of soluble protein in leaves could prevent plant cells from dehydration and protect biological macromolecules in vivo without deactivation [38]. In our study, however, the amount of soluble protein decreased dramatically with the rising amplitudes of warming (in treatments A, B and C). It is possible that the soluble protein had been changed into other osmotic adjustment substance such as oxyneurine, or took participated in the structuring and rebuilding of plant itself. The mechanism explains for this is not explicitly, but both of them could result in decrease of soluble protein. Moreover, amount of soluble protein maintained higher lever in all treatment plots and control plots, suggest that the soluble protein was still the most important osmotic adjustment substance in leaves of *E.* nutans.

Some researchers assert alpine plants had high background value of SOD activity, which prevent the plant cell from damage of membrane lipid peroxidation caused by superoxide anion free radical (O_2^-) due to atrocious environment [38]. In this study, the SOD activity maintained higher lever over the entire growing season, well coincided with the theory documented above. The SOD activity exhibited a decreased trend in treatments D, C and E, mainly because of alleviation of temperature stress, the production of reactive oxygen, species of reactive oxygen have been changed was also a plausible reason.

GSH is the most prevalent non-protein thiol in cells. Its de novo and salvage synthesis serves to maintain a reduced cellular environment. GSH is the most powerful intracellular antioxidant and plays a role in the detoxification of a variety of electrophilic compounds and peroxides via catalysis by glutathione-S-transferases (GST) and glutathione peroxidases (GPx). A deficiency in GSH puts the cell at risk for oxidative damage. GSH was often determined as a physiological index of cold resistance, the effect of warming on its relative content has not been reported. There were several plausible reasons explained for the increase of GSH content in treatments D, C and E as compare to the control. These include (1) GSH content kept in a steady state through its own antioxidant system in control, when temperature increased, GSH content increased as response of heat shock, even indirect water stress to changed environment. (2) Photosynthesis of plant increased with slight warming, and more superoxide anion free radical (O_2^-) and H_2O_2 were generated. As a result, GSH in chloroplasts increased to inhibit superoxide generation by AsA-GSH circle, and GSH may be the mainly superoxide scavenger and mechanism to protect photosynthesis of plant in this stage. (3) Confirmed with the explanation of SOD activity decrease in treatments D, C and E, production of reactive oxygen, or species of reactive oxygen have been changed, GSH increased as a responder of antioxidant system of plant.

However, in treatment A, with warming amplitudes of 2.68 °C, *E. nutans* suffered a double stress of higher temperature and water deficiency, O_2^- were generated though photosynthesis has been inhibited. Excessive production of O_2^- oxidized GSH, its content decreased as a result. A reason explained for lowest GSH content in treatment B was that *E. nutans* adapted the warming environment as a new appreciate habitat.

4.3. OTCs and its effects on microclimate

There were many methods or experimental designs used to simulate climate warming and its profound effects on terrestrial ecosystem, which can broadly be defined as passive or active. Active warming methods include buried hearting cables and overhead infrared (IR) heaters, passive warming methods include closed greenhouse and open-top chambers (OTCs) [39]. The OTCs were used in studies of International Tundra Experiment (ITEX) and becoming more prevalent in recent years. Unlike other power needed and costly warming methods, OTCs can used in remote locations where electrical applications are problematic such as filed stations. Moreover, its feasibility and practicability was widely accepted by scientists of the research field. The top of the chamber was open to allow for gas exchange with the atmosphere, and to allow precipitation, minimize the influence of other environmental disturbance.

Some researchers reported that increased temperature could decrease relative humidity (RH) in OTCs, but according to published studies of Havstorm et al. [14], it was clearly indicated that the influence of RH difference existed inside and outside of OTCs can be neglected when experiments were conducted in high-latitude where temperature was the most important limiting factors of growth of plants. Moreover, in order to reduce the enclosure effects of OTCs on plant microclimate, which is a much discussed question in global warming experiments methods, the vertical height of OTCs in our study was just 40 cm above on ground surface. And according to studies of Zhao [10] in Haibei Alpine Meadow Ecosystem Research Station (HBAMERS), China, there were no significant differences in CO_2 concentration and RH between OTCs and the ambient air.

5. Conclusion

In this study, experimental warming influenced the physiological–biochemical characteristics of *E. nutans* at different extent, results obtained indicated that *E. nutans* could adapt or develop tolerance to the temperature changes. If the temperature increased within the range of 0.69–1.57 °C, it may have positive effects on growth and development of *E. nutans.* Physiological–biochemical characteristics of *E. nutans* in treatment B. However, when air temperature in OTCs increased at a amplitude of 2.68 °C, the negative influences of global warming occurred significantly. The hypothesis of the study is that there is a more optimized temperature (in treatment B) in process of global warming which offer more suitable habitat for plants. However, elevated temperature would have a longer, profound and more permanent impact on growth and development of plant because temperature, in combination with indirect warming effects (soil moisture, soil water content, quality of organic matter rate of decomposition and nutrient mineralization and availability, etc.), may influence virtually the growth, reproduction and phenology of alpine plants, resulting in long-term changes in competition, species composition and richness, and the synthesis efforts of these factors will in turn influence ecosystems. Therefore, our study results have certain limitations because only influences of temperature as substitute proxies of climatic changes on some physiological–biochemical characteristics of *E. nutans* were determined and assayed. In addition, *E. nutans* is a perennial plant species, further experimental proofs were need to confirm the warming effects on its growth and development is long-term (three consecutive years) effects or a short-term (the third year) effects.

The rising trend of greenhouse gas concentration in atmosphere and the subsequent global warming is an accepted phenomenon. For the last 20 years, A large number of studies have been conducted on responses of various ecosystems to global warming caused by elevated CO_2, and elevated temperature effects across ecosystems is a more important factor to be understood in global change research. Our findings suggest that the plants in alpine meadows are already responding to recent temperature changes, and alpine plants would more susceptible to climate warming. These findings underscore the importance and need to investigate the performance of other alpine plants, and only by understanding how climate change can influence growth and development of alpine plants, will changes in ecosystems structure and function be well explained and policy makers and land managers be better able to cope with a changing environment.

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References

[1] N. Oreskes, The scientific consensus on climate change, Science 306 (2004) 1986.
[2] Z.F. Xu, T.X. Hu, Y.B. Zhang, J.R. Xian, K.Y. Wang, Responses of phenology and growth of *Betula utilis* and *Abies faxoniana* in a sub-alpine timberline ecotone to simulated global warming, Western Sichuan, China, Journal of Plant Ecology 32 (5) (2008) 1061–1071.
[3] IPCC, Climate Change 2007, The Scientific Basis, Cambridge Press, 2007.
[4] D.H. Qin, Facts, impacts, adaptation, and mitigation strategy of climate change, Chinese Science Bulletin Fund 1 (2003) 1–3.
[5] F.S. Chapin III, R.L. Jefferies, J.F. Reynolds, Arctic plant physiological ecology in an ecosystem context, in: F.S. Chapin III, R.L. Jefferies, J.F. Reynolds (Eds.), Arctic Ecosystems in a Changing Climate: An Eco-Physiological Perspective, Academic Press, San Diego, California, 1992, pp. 441–452.
[6] G. Grabher, M. Gottfried, H. Pauli, Climate effects of mountain plants, Nature 369 (1994) 448–450.
[7] H.L. Sun, D. Zheng, The Formation, Evolution and Development of Qing-Tibetan Plateau, Guangdong Science and Technology Press, Guangzhou 1998, pp. 1–45.
[8] X.D. Liu, B.D. Chen, Climatic warming in the Tibetan Plateau during recent decades, International Journal of Climatology 20 (2000) 1729–1742.
[9] Julia A. Klein, John Harte, Xin-Quan Zhao, Experimental warming causes large and rapid species loss, dampened by simulated grazing, on the Tibetan Plateau, Ecology Letters 7 (2004) 1170–1179.
[10] X.Q. Zhao, G.M. Cao, Y.N. Li, Alpine Meadow Ecosystem and Global Change, 521, Science Press, 2009, pp. 1–60.
[11] F.S. Chapin III, G.R. Shaver, Individualistic growth response of tundra plant species to environmental manipulations in the field, Ecology 66 (1985) 564–576.
[12] A.M. Arft, M.D. Walker, J. Gurevitch, J.M. Alatalo, et al., Responses of tundra plants to experimental warming: meta-analysis of the International Tundra Experiment, Ecological Monographs 69 (1999) 491–511.
[13] G.H.R. Henry, U. Molau, Tundra plants and climate change: the International Tundra Experiment (ITEX), Global Change Biology 3 (1997) 1–9.
[14] M. Havstorm, T.V. Callaghan, S. Jonasson, Differential growth responses of Cassiope tetragona, an arctic dwarf-shrub, to environmental perturbations among three contrasting high and sub-arctic sites, Oikos 66 (1993) 389–402.
[15] M.D. Walker, C.H. Wahren, R.D. Hollister, et al., Plant community responses to experimental warming across the tundra biome, Proceedings of the National Academy of Sciences USA 103 (5) (2006) 1342–1346.
[16] H.K. Zhou, X.M. Zhou, X.Q. Zhao, A preliminary study of the influence of simulated greenhouse effect on a *Kobresia humilis* meadow, Journal of Plant Ecology 24 (5) (2000) 547–553.
[17] Julia A. Klein, John Harte, Xin-Quan Zhao, Experimental warming, not grazing, decreases rangeland quality on the Tibetan plateau, Ecological Applications 17 (2) (2007) 541–557.
[18] Julia A. Klein, John Harte, Xin-Quan Zhao, Decline in medicinal and forage species with warming is mediated by plant traits on the Tibetan plateau, Ecosystems 11 (2008) 775–789.
[19] F.L. Marchand, S. Mertens, F. Kockelbergh, Performance of High Arctic tundra plants improved during but deteriorated after exposure to a simulated extreme temperature event, Global Change Biology 11 (2005) 2078–2089.
[20] J.H.C. Cornelissen, P.M. van Bodegom, R. Aerts, et al., Global negative vegetation feedback to climate warming responses of leaf litter decomposition rates in cold biomes, Ecology Letters 13 (2007) 619–627.
[21] M.D. Walker, C.H. Wahrenb, R.D. Hollisterc, et al., Plant community responses to experimental warming across the tundra biome, Proceedings of the National Academy of Sciences 103 (2006) 1342–1346.
[22] W. Liu, H.K. Zhou, L. Zhou, Biomass distribution pattern of degraded grassland in alpine meadow, Grassland of China 27 (2) (2005) 9–15.
[23] J.Z. Zhao, W. Liu, H.K. Zhou, Effects of simulated greenhouse effect on growth characteristics of *Kobresia humilis*, Acta Botnica Boreali-Occidentalia Sinica 26 (12) (2006) 2533–2539.
[24] H.K. Lichtenthaler, Chlorophylls and earotenoids pigments of photosynthetic bio-membranes, Methods in Enzymology 148 (1987) 351–383.
[25] J.D. Zhang, G.P. Liu, Y.N. Shi, Experimental Methods of Plant Physiology, Jiangxi People's Press, Nanchang, 1982. pp. 172–182.
[26] Institute of Shanghai Plant Physiology of Chinese Academy of Science. Experimental Guide of Modern Plant Physiology. Science Press, Beijing, 2004.
[27] C.N. Giannopiolitis, S.K. Ries, Superoxide dismutase. II. Purification and quantitative relationship with water soluble protein in seedlings, Plant Physiology 59 (1997) 315–318.
[28] A.G. Wang, G.H. Luo, Quantitative relation between the reaction of hydroxylamine and superoxide amion radicals plants, Plant Physiology Communication 26 (6) (1990) 55–57.
[29] H.J. Yin, T. Lai, X.Y. Cheng, X.M. Jiang, Q. Liu, Warming effects on growth and physiology of seedlings of *Betula albo-sinensis* and *Abies faxoniana* under two contrasting light conditions in subalpine coniferous forest of western Sichuan, China, Journal of Plant Ecology 32 (5) (2008) 1072–1083.
[30] X.Y. Tian, Y.J. Liu, Y. Guo, Effect of salt stress on Na^+, K^+, proline, soluble sugar and protein of NHC, Pratacultural Science 25 (10) (2008) 34–38.
[31] B. Zeng, F.J. Wang, C. Zhu, Z.X. Sun, Effect of ascorbate–glutathione (AsA–GSH) cycle on $Hg2^+$ tolerance in rice mutant, Acta Agronomica Sinica 34 (5) (2008) 823–830.
[32] Y.Z. Jiang, Patrice Prognon, Raw material enzymatic activity determination: a specific case for validation and comparison of analytical methods – the example of superoxide dismutase (SOD), Journal of Pharmaceutical and Biomedical Analysis 40 (5) (2006) 1143–1148.
[33] Y.N. Li, L. Zhao, X.Q. Zhao, H.K. Zhou, Effects of a 5-years mimic temperature increase to the structure and productivity of *Kobresia humilis* meadow, Acta Agrestia Sinica 12 (3) (2004) 236–239.
[34] C.A. Guo, F. Liu, X.M. Xu, Chlorophyll-b deficient and photosynthesis in plants, Plant Physiology Communications 42 (5) (2006) 967–973.
[35] L.L. Eggink, H. Park, J.K. Hoober, The role of chlorophyll b in photosynthesis: hypothesis, BMC Plant Biology 1 (2001) 2.
[36] F. Han, X.G. Yue, S.B. Shi, Physiological characteristics in cold resistance of several alpine plants in Qinghai-Tibet Plateau, Acta Botnica. Boreali-Occidentalia Sinica 25 (12) (2005) 2502–2509.
[37] G.X. Xue, H.Y. Gao, P.M. Li, Q. Zou, Effect s of chitosan treatment on physiological and biochemical characteristics in cucumber seedlings under low temperature. Journal of Plant Physiology and Molecular Biology 30(4) (2004) 441–448.
[38] F. Han, D.W. Zhou, Z.H. Teng, W.Y. Zhu, S.B. Shi, Comparison of anti-oxidative system in *Kobresia humlis* grown at different altitudes on Qinghai-Tibet Plateau, Acta Botnica. Boreali-Occidentalia Sinica 23 (9) (2003) 1491–1496.
[39] S.L. Niu, X.G. Han, K.P. Ma, S.Q. Wan, Field facilities in global warming and terrestrial ecosystem research, Journal of Plant Ecology 31 (2) (2007) 262–271.