ISSN 1021-4437, Russian Journal of Plant Physiology, 2007, Vol. 54, No. 6, pp. 736–740. © Pleiades Publishing, Ltd., 2007.

RESEARCH PAPERS

Effect of Imitated Global Warming on $\Delta^{13}C$ Values in Seven Plant Species Growing in Tibet Alpine Meadows[1]

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Received June 15, 2006

Abstract—Open-top chambers were used to estimate the possible effects of global warming on $\delta^{13}C$ of seven plant species grown in alpine meadow ecosystem. The $\delta^{13}C$ values of plant species were lower after long-term growth in open-top chambers. In the course of experiment, temperature significantly increased inside the chambers by 4°C. Plant species grown at a lower elevation above sea level had higher $\delta^{13}C$ values as compared to those grown at a higher elevation. This was in accordance with the effect of open-top chamber on $\delta^{13}C$ values in plants. Greater availability of CO_2 and lower water vapor pressure at higher temperature inside the chambers, as indicated by an increase in discrimination against $^{13}CO_2$, probably result in more negative $\delta^{13}C$ values of plants because higher stomatal conductance increases availability of CO_2 and causes greater discrimination against $^{13}CO_2$. The plant species studied could be the indicator species for testing global warming by the change in carbon isotope ratios at the two growth temperatures.

DOI: 10.1134/S1021443707060039

Key words: Kobresia humilis - Saussurea superba - Gentiana straminea - Leontopodium nanum - imitating warming - stable carbon isotope - alpine meadow - Tibet plateau

INTRODUCTION

Stable carbon isotope composition ($\delta^{13}C$) has been used to study physiological conditions of plants since the 1970s [1]. Most investigations were focused on the photosynthetic pathways based on different fixation of CO_2 by C_3-, C_4-, and CAM plants [2–4]. Other researchers concentrated on variations of water use efficiency (WUE) in plants grown under various water availability conditions [5–8]. Global changes refer to global climate change, such as global warming, greenhouse effect, increasing ultraviolet radiation, elevation of sea level, element cycles, and biodiversity decline. Since the 1990s, the governments and scientists all over the world pay more attention to global changes, biodiversity, and sustainable development, especially focusing on the effects of primary production, structure, and functioning of ecosystem. However, there is some uncertainty in the effects of global changes on ecosystems, because some of their components are time- and space-dependent. This uncertainty results in the absence of direct indicator of potential global warming in the future. Some studies considered the effect of global changes on physiological processes in plants [9, 10].

The Qinghai-Tibet plateau, a unique geographic unit, has a great impact on the Eurasia atmospheric circulation and distribution of various ecosystems, especially on their structure, functioning, adaptation, and evolutionary patterns since the plateau is the highest plateau in the world. Due to the high-altitude climate, the ecosystem is very fragile and sensitive to global climate changes [11]. Thus, the plateau is becoming more and more important to study climate changes in terrestrial ecosystems. Prevailing alpine meadow ecosystem has made this region to be a target for investigating the relationship between structure and function of the ecosystem on one hand, and global changes on the other.

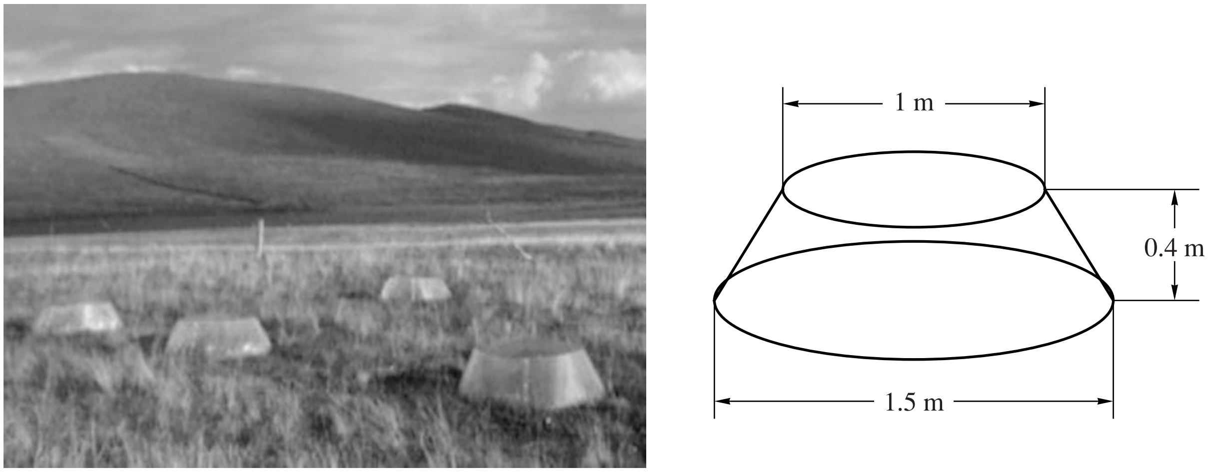
In order to determine the potential effects of global changes on alpine meadow ecosystem, we conducted the experiments with open-top chambers to focus the effect of elevated temperature on the changes in $\delta^{13}C$ values. We performed them in Haibei Alpine Meadow Ecosystem Station of Chinese Academy of Sciences. The main purpose of this study is to test whether $\delta^{13}C$ value of plant can be regarded as an indicator of temperature changes, and to decide what plant species would be sensitive for potential global warming in the near future.

MATERIALS AND METHODS

Study area. The study was conducted near Haibei Alpine Meadow Ecosystem Station established in 1976 in order to investigate the alpine meadow ecosystem, the phenomenon of biodiversity, adaptive and evolu-

Abbreviations and designations: *g*—stomatal conductance; WUE—water use efficiency.

[1] This text was submitted by the authors in English.



Distribution of open-top chambers and a simple description of an open-top chamber used.

tionary strategies of species, and the impact of global changes on grassland ecosystem. The station is located in the region of the Tibetan plateau, in a large valley oriented NW/SE and surrounded by the Qilian Mountains, in latitude 37°29′–37°45′ North and longitude 101°12′–101°23′ East. The average altitude of mountain area is 4000 m above sea level and 3200 m in the case of valley area. The climate is dominated by the southeast monsoon and the high-pressure system of Siberia being continental monsoon type climate, with severe and long winters and short cold summers. The annual average air temperature is –1.7°C with the extremes of 27.6°C and –37.1°C. During the winter months, the average temperature can drop to –20°C in the highland area; during summer, the temperature in the warmest month (July) averages from 14 to 22°C in the valleys and from 4 to 10°C in the mountains. Average annual precipitation ranges from 426 to 860 mm, 80% of which falls during the short summer growing season from May to September. The annual average sunlight is 2462.7 h with 60.1% of total available sunshine. Vegetation is presented mainly by *Kobresia humilis*, *Saussurea superba*, *Gentiana straminea*, and *Leontopodium nanum*, the vegetation height is below 0.3 m, in average.

Establishment of open-top chambers. Open-top chambers introduced during International Tundra Experiment (ITEX) were used to simulate greenhouse effect and investigate the influence of temperature on $\delta^{13}C$ and physiological condition of plants in September, 1997 (figure). Eight conical open-top chambers made of fiberglass (Sun-Lite HP, 0.1 cm thick) were distributed over a fenced off 30×30 m area. Each open-top chamber was 1.5 m in bottom diameter and 0.4 m in height. The fiberglass material provided a high solar transmittance of 86% for the visible wavelengths and a low transmittance (< 5%) for IR radiation. The inclined sides at an angle of 60° to horizontal are designed to maximize both the transmittance of incoming radiation and trapping of the heat. The chambers remained in the meadow throughout the whole year, from 1997 to 2002.

Sample collection and δ^{13}C measurement. Healthy fully expanded leaves were collected for isotope analysis from seven perennial meadow grass plants inside and outside chamber within an area of relatively uniform *Kobresia humilis* meadow. The leaf position on stem was similar in control area and chamber. In the experiment, the leaves were sampled in the middle of the open-top chambers during the exuberant season (July) in 2002. Leaves were dried to constant weight in an oven at 70°C for 48 h, and ground finely prior to analysis. Operation condition for producing CO_2 were the following: oxidizing furnace temperature was 900°C, reducing furnace was 680°C, pillar temperature was 40°C. The resulting CO_2 was purified and injected into a Finnigan MAT DELTA^{PLUS} XL isotope ratio spectrometer (Germany). Interface between element-analysis meter and spectrometer was ConFlow III. Standards consisted of the Peedee Belemnite (PDB) formation from South Carolina, United Stated. The results were expressed in $\delta^{13}C$ relative to the PDB standard in the conventional (per mil notation) as follows:

$$\delta^{13}C = [(^{13}C/^{12}C)_s/(^{13}C/^{12}C)_{sta} - 1] \times 1000,$$

where $^{13}C/^{12}C$ are the isotopic ratios of sample (s) and PDB is the standard (sta). The overall (sample preparation plus analysis) analytical precision is ± 0.2‰.

Data analysis. All data were analyzed by using SPSS (Statistical Package for Social Scientists) for windows 10.5. Stable carbon isotope ratios between the control and the treated materials were tested for significant differences by Independent-Samples *T* Test (Levene's test). All tests were two-tailed.

RESULTS

$\delta^{13}C$ values of seven plant species listed in Table 1 ranged from –24.84‰ to –26.92‰ in control leaves,

Table 1. Effects of exposure to open-top chamber on average $\delta^{13}C$ values in seven plant species grown in Tibet alpine meadows

Species	Control leaves		Leaves after treatment	
	$\delta^{13}C$, ‰	isotopic discrimination factor, ‰	$\delta^{13}C$, ‰	isotopic discrimination factor, ‰
Saussurea superba	–25.13	17.56	–25.66	18.13
Gentiana straminea	–26.92	19.44	–24.96	17.39
Kobresia humilis	–26.83	19.35	–27.58	20.14
Leontopodium nanum	–25.12	17.56	–27.62	20.18
Oxytropis cohrocephala	–25.77	18.24	–28.25	20.84
Amblytropis diversifolia	–24.84	17.27	–31.28	24.03
Lancea tibetica	–26.01	18.49	–26.50	19.00

and from –24.96 ‰ to –31.28 ‰ as measured in the open-top chamber, with the average values of – 25.80‰ and – 27.41‰, respectively. During open-top chamber experiment, the temperature inside the chamber increased by 4°C, however, no significant difference was detected in light intensities, water availability, and CO_2 concentrations. $\delta^{13}C$ values of all plants investigated were lower after exposure to open-top chamber, except *Gentiana straminea* (Table 1). $\delta^{13}C$ value of *Amblytropis diversifolia* dramatically decreased by 6.44‰ ($t = 46.396$, $df = 4$, $P < 0.01$), where t means statistic value under T test, df means degree of freedom, and P is a probability. $\delta^{13}C$ values of *Oxytropis cohrocephala* and *Leontopodium nanum* decreased by 2.5‰, respectively ($t = 16.822$, $df = 4$, $P < 0.01$ and $t = 17.634$, $df = 4$, $P < 0.01$). On the contrary, $\delta^{13}C$ value for *Gentiana straminea* increased by about 2.0‰ ($t = –26.509$, $df = 4$, $P < 0.01$). We also found that three plant species grown on lower elevation (2300 m) had higher $\delta^{13}C$ values as compared with those grown on the experiment areas at elevation of 3200 m (Table 2). At the same time, a simple experiment was carried out with meadow insect (*Orgyia aurollimbta*) inhabiting open-top chambers. The effect of open-top chamber resulted in lower $\delta^{13}C$ values of the insect with 28.50 ± 0.06‰ as compared with –27.91 ± 0.28‰ of the control ($t = –6.666$, $df = 5$, $P = 0.003$). This further proved the decline in $\delta^{13}C$ values under open-top chamber treatment because there was a steady enrichment of $\delta^{13}C$ between *Orgyia aurollimbta* and its food.

Table 2. $\delta^{13}C$ values of three plant species grown at two elevations above sea levels

Species	Elevations, m	
	3200	2300
Plantago asiatica	–26.13 ($n = 8$)	–29.73 ($n = 6$)
Picea crassifolia	–22.52 ($n = 5$)	–25.11 ($n = 6$)
Elymus nutans	–26.32 ($n = 4$)	–29.83 ($n = 8$)

DISCUSSION

Some investigations have demonstrated that open-top chamber increased air temperature in chamber by 4°C compared with that out of the chamber, no significant differences being found in other factors, i.e., water status and CO_2 concentration [12]. The leaf samples were all collected in the middle of the open-top chambers (light irradiation difference can be negligible), so it is reasonable to speculate that lower $\delta^{13}C$ values of plants are mainly due to an increase in temperature within the chambers. Plants grown at various elevations above the sea level differ in $\delta^{13}C$ values from the plants grown at higher elevation by exhibiting more negative $\delta^{13}C$ values (Table 2). Discrimination against $^{13}CO_2$ by plants generally decreases with altitudes [13–17], i.e., $\delta^{13}C$ values of plants usually increase with altitude. Elevation, to certain extent, can lead to lower temperature.

Plants could be useful indicators of climate change if carbon isotope ratios varied in a systematic way with the temperature of plant growing. Thoughton [1] reported that $\delta^{13}C$ values became slightly more negative (by up to 2‰) with increasing temperature in several plant species. Similar results were obtained by other researchers [13]. The temperature influence on variation in isotope composition in plants presumably resulted from changes in the CO_2 availability with temperature because plants would need more CO_2 under higher temperature conditions. However, we failed to gain significant difference in CO_2 concentration within and outside the open-top chamber (about 370 ppm). Temperature effect on plant carbon isotope discrimination is mostly determined by the CO_2 partial pressure in the intercellular airspaces (C_i), which is affected by the ratio of assimilation rate (*A*) to stomatal conductance (*g*) ($C_i = C_a - A/g$), where C_a is atmospheric CO_2 partial pressure). Elevation of temperature in the open-top chambers could increase both *A* and *g*. More efficient discrimination in open-top chambers (Table 1) might be due to more intense stimulation of stomatal conductance than that of the assimilation in the open-top chambers. In this case, greater stomatal conductance increases availability of CO_2 and causes greater discrimination against $^{13}CO_2$ (and thus more negative $\delta^{13}C$ values). Most laboratory studies suggest an increase of $\delta^{13}C$ as temperature decreases [10, 18, 19]. Relationship between $\delta^{13}C$ and temperature was investigated and expressed as 0.2–0.3‰ [11]. Our results indicated that the $\delta^{13}C$ values of plants decreased with the increase in temperature. We calculated the theoretical change in $\delta^{13}C$ expected for the range of temperatures observed along the altitudinal profile. According to this, approximately 50–75% of the variation observed in $\delta^{13}C$ could be explained by temperature variations. Greater availability of CO_2 due to higher conductance of stomata at a higher temperature probably causes more negative $\delta^{13}C$ values because of greater discrimination of plants against $^{13}CO_2$. Zhou et al. [12] indicated that open-top chamber slightly increased CO_2 concentration (or partial pressure) in chamber as compared to the control, but not significantly. Furthermore, $\delta^{13}C$ values of respired CO_2 is more negative than ambient CO_2 because of the preference of plants to ^{12}C release during respiration [8]. In open-top chambers, the occurrence of partial re-fixation of respired CO_2 partly results in more negative $\delta^{13}C$ values of plants, because open-top chamber delays diffusional efflux of respired CO_2 as compared with non-open-top chamber treatment.

Fortunately, we measured $\delta^{13}C$ values of CO_2 both inside (–10.65 ± 0.17‰) and outside (–10.34 ± 0.08‰) the open-top chambers and found no significant differences ($t = -2.431$, $df = 6$, $P = 0.51$) due to low height of the chambers, which indicated that respired CO_2 had a weak effect on more negative $\delta^{13}C$ values in plants. Increase in CO_2 uptake at higher temperature conditions would have contributed too much influence. On the other hand, inside open top chambers the increase in temperature goes along with a change in water vapor pressure, which is lower at higher temperature. This is a combined effect leading to a change in stomatal conductance and hence water use efficiency of the plants inside the chamber. Therefore, stomatal conductance/WUE will play a major role in giving access of CO_2 to the plants and hence have an effect on C isotope discrimination during C fixation. This effect was reproducible, the open-top chamber experiments similarly affected the $\delta^{13}C$ values in seven plant species grown at alpine meadow ecosystem. Some of them, such as *Leontopodium nanum, Oxytropis cohrocephala*, and *Amblytropis diversifolia*, could be used as the indicators of global changes, especially concerning future global warming. Variations in temperature would be an important factor for stable carbon isotope metabolism and fractionation.

ACKNOWLEDGMENTS

The authors thank the Haibei Alpine Research Station for facilitating this research and Foundation of Henan University of Science and Technology (projects nos. 2006ZY005 and 2004018) for partial support of this study.

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