Leaf photosynthesis and simulated carbon budget of *Gentiana straminea* from a decade-long warming experiment

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Abstract

Aims

Alpine ecosystems may experience larger temperature increases due to global warming as compared with lowland ecosystems. Information on physiological adjustment of alpine plants to temperature changes can provide insights into our understanding how these plants are responding to current and future warming. We tested the hypothesis that alpine plants would exhibit acclimation in photosynthesis and respiration under long-term elevated temperature, and the acclimation may relatively increase leaf carbon gain under warming conditions.

Methods

Open-top chambers (OTCs) were set up for a period of 11 years to artificially increase the temperature in an alpine meadow ecosystem. We measured leaf photosynthesis and dark respiration under different light, temperature and ambient CO_2 concentrations for *Gentiana straminea*, a species widely distributed on the Tibetan Plateau. Maximum rates of the photosynthetic electron transport (J_{max}), RuBP carboxylation (V_{cmax}) and temperature sensitivity of respiration Q_{10} were obtained from the measurements. We further estimated the leaf carbon budget of *G. straminea* using the physiological parameters and environmental variables obtained in the study.

Important findings

1) The OTCs consistently elevated the daily mean air temperature by ~1.6°C and soil temperature by ~0.5°C during the growing season.

2) Despite the small difference in the temperature environment, there was strong tendency in the temperature acclimation of photosynthesis. The estimated temperature optimum of light-saturated photosynthetic CO_2 uptake (A_{max}) shifted ~1°C higher from the plants under the ambient regime to those under the OTCs warming regime, and the A_{max} was significantly lower in the warming-acclimated leaves than the leaves outside the OTCs.

3) Temperature acclimation of respiration was large and significant: the dark respiration rates of leaves developed in the warming regime were significantly lower than leaves from the ambient environments.

4) The simulated net leaf carbon gain was significantly lower in the *in situ* leaves under the OTCs warming regime than under the ambient open regime. However, in comparison with the assumed non-acclimation leaves, the *in situ* warming-acclimated leaves exhibited significantly higher net leaf carbon gain.

5) The results suggest that there was a strong and significant temperature acclimation in physiology of *G. straminea* in response to long-term warming, and the physiological acclimation can reduce the decrease of leaf carbon gain, i.e. increase relatively leaf carbon gain under the warming condition in the alpine species.

Keywords: alpine plant • acclimation • experimental warming • open-top chamber • photosynthesis • temperature

INTRODUCTION

Global mean temperatures are increasing and are predicted to increase further into the future (IPCC 2007). Temperature elevation will affect plant carbon budgets through its effects on photosynthesis and respiration (e.g. Luo 2007). The change of terrestrial vegetation carbon budgets can, in turn, directly feedback to affect the CO_2 concentration in the atmosphere (IPCC 2007; Luo 2007). Understanding the changes of plant photosynthesis and respiration in responding to a warming environment is thus essential to further understand and predict the future carbon dynamics in the atmosphere (Atkin and Tjoelker 2003; Dewar et al. 1999; Gunderson et al. 2000).

Photosynthetic and respiration rates generally increase with a temporal increase of environmental temperature below the optimal temperature when other resources are not limiting (e.g. Berry and Björkman 1980; Hikosaka et al. 2006). The physiological processes can also result in acclimation to long-term changes in the temperature environment (e.g. Campbell et al. 2007; Larigauderie and Körner 1995; Pearcy 1977; Xiong et al. 2000; Zhou et al. 2007). The temperature sensitivity and acclimation degree of photosynthesis differ from that of respiration (Bruhn et al. 2007; Morison and Morecroft 2006; Way and Sage 2008). Any changes in the two processes may eventually change the plant carbon budget, which alters the overall ecosystem carbon budget (Atkin et al. 2006; Dewar et al. 1999; Loveys et al. 2002). The degree of acclimation in photosynthesis and respiration is, therefore, an important determinant to predict the future response of ecosystems to elevated temperatures. Moreover, the plant-level response provides a mechanistic understanding that can be masked at the ecosystem level.

Since the magnitude of photosynthetic and respiratory acclimation varies with species and other environmental variables (Berry and Björkman 1980), these processes are still poorly understood, especially under field conditions and/or for long-term acclimation. Laboratory experiments, in which the growth temperature often differs by 5–10°C, can provide useful information for predicting the effects of global warming on plant carbon budgets (see reviews from Hikosaka et al. 2006; Medlyn et al. 2002). The information, however, can also be of limited use partly because the projected magnitude of global warming in the short term is smaller than the experimental conditions simulated in these laboratory studies. Therefore, understanding acclimation under field conditions with more realistic temperature elevation and, if possible, under long-term warming condition is needed to predict plant response to potential climate change in the future.

The impact of global warming on terrestrial ecosystems has been reported to be greater in arctic tundra and high mountain regions than at low latitudes or altitudes (e.g. Beniston 2006; Maxwell 1992; Mitchell et al. 1990). Nevertheless, plant responses—especially ecophysiological responses to temperature elevation in these cold ecosystems—have been scarcely understood, though a few studies suggest that the acclimation response to increasing temperature is not so different among alpine and lowland herbaceous species (Arnone and Körner 1997; Collier 1996; Larigauderie and Körner 1995). Our primary aim was thus to assess physiological acclimation of alpine plants and its consequence to leaf carbon gain under warming conditions. We hypothesized that alpine plants would exhibit photosynthetic and respiration acclimation to temperature elevation, and the acclimation should increase the leaf carbon gain. To test the hypothesis, we used one of the most common species on the Qinghai-Tibetan Plateau, *Gentiana straminea*, growing within the open-top chambers (OTCs) for a period of 11 years.

PLANT MATERIALS AND METHODS

Study site and plant materials

The field site was an alpine *Kobresia humilis* meadow (latitude 37°37′N, longitude 101°12′E, altitude 3250 m), which is located at the northeastern edge of the Qinghai-Tibetan Plateau. The annual mean air temperature of this site is −1.6°C and the annual precipitation is 562 mm (Klein et al. 2004). The OTCs, each being 1.5 m diameter, 40 cm height and 1.0-mm thick fiberglass (Solar Components Corporation, Manchester, NH), were set up in 1997. Similar OTCs have been an important method for simulating warming in the field, especially in tundra or alpine ecosystems (Allen et al. 1992; Arft et al. 1999; Ceulemans and Mousseau 1994; Walker et al. 2006). The OTCs elevated growing season averaged daily air temperature by 0.6–2.0°C, and growing season averaged maximum daily air temperature by 1.9–7.3°C. For additional details on the study site, the experimental design, the micro-climate effects of the treatments and the community and ecosystem responses, see Klein et al. (2004, 2005, 2007, 2008).

Gentiana straminea Maximum. (Gentianaceae) is one of the common forbs in the meadow and is a genus commonly found across the Tibetan Plateau and the Himalayan region. This species is also a medicinal plant that provides an important ecosystem service in the region (Klein et al. 2008). *Gentiana straminea* grows its linear-shaped leaves diagonally from the soil surface to the top of the canopy. Its mature leaves are 20–30 cm long. The inclination angle of fully expanded leaves is 49±9°, the ratio of length to width is approximately 4–5 (Cui et al. 2004).

Assessment of the micro-environmental parameter of inside and outside of OTCs

We installed six quantum sensors (GaAsP Photodiode G1118, Hamamatsu City, Japan) on the horizontal surface at the average height of the *G. straminea* leaves, three temperature sensors (copper–constantan thermocouples) on the leaf surface, three temperature sensors 20 cm above the soil surface and three temperature sensors (copper–constantan thermocouples) at a depth of 5 cm in the soil on the inside and outside of the OTCs, respectively. All quantum sensors

were calibrated under sunlight and artificial shade against a standard quantum sensor (Li-Cor Model 190S, Li-Cor, Lincoln, NE; Tang and Washitani 1995). We recorded photosynthetic photon flux density (PPFD), air and leaf and soil temperatures at 1-min intervals using a datalogger (Thermic 2300A, EtoDenki Ltd., Tokyo, Japan) from 14 July 2008 to 6 August 2008.

Photosynthetic response to PPFD, temperature and intercellular CO_2 concentration

Leaf gas exchange was measured on fully expanded intact leaves in the field with an LI-6400 portable photosynthesis measurement system (Li-Cor, Inc., Lincoln, NE). We replaced the desiccant and soda lime at the beginning of the measurement every day. Leaves from three or four plants, each inside and outside the OTCs, respectively, were measured under PPFD of 0, 25, 50, 100, 250, 500, 1 000, and 1 500 μmol m^{-2} s^{-1} each combined with five temperatures: 10, 15, 20, 25, and 30°C in the sample chamber. We conducted the measurements of photosynthesis and respiration during the periods from 9:00 to 11:00 am and from 2:00 to 5:00 pm. We controlled manually the chamber temperature according to the ambient temperature, i.e. setting a relatively high chamber temperature when the ambient temperature was high in the late morning or the early afternoon. We then were able to avoid a large difference between temperatures within the chamber and in the ambient, as well as to avoid any possible soil water stress in the noon. The temperature difference was often less than ~3°C with occasionally a maximum of ~7°C. Leaf dark respiration was obtained when the PPFD was 0 within the chamber. During these measurements, we maintained the CO_2 concentration at 370 μmol mol^{-1}. The relative humidity was controlled between 60% and 70% by increasing water vapor pressure by bubbling air through a plastic bottle containing water or decreasing water vapor pressure by desiccant.

To obtain the maximum rates of the photosynthetic electron transport (J_{max}) and RuBP carboxylation (V_{cmax}), we measured the CO_2 dependence of photosynthesis under CO_2 concentrations of 0, 50, 100, 150, 370 and 1 000 μL L^{-1} in the ambient air (C_a). Light intensity was set to 1 000 μmol m^{-2} s^{-1} at three temperatures: 15, 20 and 25°C. The J_{max} and V_{cmax} are calculated from the following equations (Farquhar *et al.* 1980):

$$V_{cmax} = (IS) \times \{ \Gamma^* + K_c(1 + O/K_o) \},$$

$$J_{max} = \frac{(A_{1000} + R_d) \times (4C_i + 8\Gamma^*)}{C_i - \Gamma^*}.$$

Where C_i and O are partial pressures of CO_2 and O_2 in the intercellular air space, respectively. A_{1000} is the net photosynthetic rate at the highest C_a of 1000 μL L^{-1} CO_2. IS is the initial slope of the A versus C_i curve obtained with the data measured at C_a of 0, 50, 100 and 150 μL L^{-1}. Γ^* is the CO_2 compensation

point in the absence of day respiration. K_c and K_o are Michaelis constants for carboxylation and oxygenation, respectively.

For K_c and K_o, we used equations of Harley and Tenhunen (1991):

$$K_c = 2 \times \exp(31.95 - \frac{65.0}{R \times T_l}),$$

$$K_o = \exp(19.61 - \frac{36.0}{R \times T_l}),$$

where R is the universal gas constant ($R = 8.314$ J K^{-1} mol^{-1}) and T_l is leaf temperature (K). Γ^* was determined using the following equations (Brooks and Farquhar 1985):

$$\Gamma^* = 44.7 + 1.88(T - 25) + 0.036(T - 25)^2,$$

where T is leaf temperature (°C).

The gasket effect involved in photosynthesis and respiration measurements is an important and complicated issue (Flexas *et al.* 2007; Pons and Welschen 2002; Rodeghiero *et al.* 2007). It includes several factors, such as leaf chamber size, physical properties of leaf, lateral diffusion of CO_2 within leaf, stomata patchiness and the leakage between gaskets. We paid our attention to the leakage problem. Firstly, the leaf of *G. straminea* was very tough and smooth in the surface, which allowed us to be able to close the chamber very firmly without hurting the leaf. Secondly, we used the white gasket (LI-Cor part No. 6564-156) in the upper part of the chamber, which partly reduced the leak.

The response of respiration rate to temperature (Q_{10})

The short-term temperature sensitivity of respiration rates is described with Q_{10} (Bruhn *et al.* 2007):

$$Q_{10} = (R_T/R_{T0})^{[10/(T - T_0)]},$$

where R_{T0} and R_T are values of R measured, respectively, at measuring temperatures T_0 and T.

Leaf morphological and biochemical parameters

In July 2007, we sampled 8–10 leaves from different individual plants inside and outside OTCs, respectively, and then measured the leaf area using a CI-202 portable leaf area meter (CID, Inc., Vancouver, WA). The samples were oven-dried at 80°C for 24 h and weighed. Based on the measured leaf area and dry weight, we calculated the leaf mass per unit area (LMA, gm^{-2}).

To measure the content of Chlorophyll *a* and *b*, the leaves were cut into small pieces after the photosynthetic measurement and then crushed thoroughly with a pestle before extracting in 5 mL of *N,N*-Dimethylformamide (DMF). The resulting solution was stabilized in tightly closed bottles that were kept at ~5°C in the dark for 24 h. Absorbance of the chlorophyll solution was analyzed at wavelengths of 663.8, 646.8 and 750 nm using a spectrophotometer (UV-1601, Shimadzu, Tokyo, Japan). We analyzed DMF as the blank solution and

calculated the Chlorophyll *a* and *b* concentrations as follows (Porra *et al.* 1989):

Chlorophyll

$$a = \frac{(12.00 \times (D_{663.8} - D_{750}) - 3.11 \times (D_{646.8} - D_{750})) \times 5}{1000 \times m} (\text{mg/gfw}),$$

Chlorophyll

$$b = \frac{(20.78 \times (D_{646.8} - D_{750}) - 4.88 \times (D_{663.8} - D_{750})) \times 5}{1000 \times m} (\text{mg/gfw}),$$

where, m is fresh weight; $D_{663.8}$, $D_{646.8}$ and D_{750} are absorbance at 663.8, 646.8 and 750 nm, respectively; and units are mg/gfw = milligrams per gram of fresh weight.

Estimation of the leaf carbon budget

To assess the effects of photosynthetic temperature acclimation on leaf carbon gain, we conducted simulation experiments. We calculated the gross carbon gain and carbon loss using PPFD–photosynthesis curves under different temperature ranges. These curves were obtained from the experiments in this study. The averaged PPFD measured from three sensors above leaf canopy was used for the simulation. Air temperatures measured inside the OTCs were grouped into six classes, i.e. $\leq 5°C$, $5 \leq 10°C$, $10 \leq 15°C$, $15 \leq 20°C$, $25 \leq 30°C$, $30°C>$, respectively. Since all the measurements of temperature and PPFD were conducted at 1-min interval, we first calculated the carbon budgets at 1-min interval. We then obtained the daily carbon budget by summing up the instantaneous values. The leaf carbon loss (R_{leaf}) from leaf dark respiration was obtained by assuming that daytime and nighttime respiration rates showed the same functional relationship to temperature.

We did two simulation experiments as follows:

Simulation experiment 1: The purpose of this simulation was to compare the carbon gain for the *in situ* leaves between the warming regime and the ambient regime. All physiological parameters and temperature and light variables for the simulation were the *in situ* data. The carbon budget for the *in situ* warming regime was estimated from physiological parameters of leaves inside the OTCs in combination with the temperature and PPFD measured within the OTCs; the carbon budget for leaves of the *in situ* ambient regime was from the data obtained all from the ambient regime.

Simulation experiment 2: The aim was to examine the effect of temperature acclimation on leaf carbon budgets. We calculated the leaf carbon budgets from the leaf parameters obtained for the AMBIENT regime combining with the environmental variables WITHIN the OTCs. The leaf carbon budget obtained from the cross-matching was considered as the assumed non-acclimated leaves. The carbon budget for the *in situ* warming-acclimated leaves was the same as that described in the simulation 1.

Data analysis

All measurements were replicated three or four times from different plants. The results are presented as means (M) ± standard deviations (SD). We used Student's *t*-tests and KaleidaGraph software (Synergy Software, Inc., PA) to assess the differences of photosynthetic parameters and simulated carbon budget between plants inside and outside the OTCs. One-way analysis of variance (ANOVA) was used to assess the effect of growth environments on the simulated carbon budget.

RESULTS

Temperature regimes, leaf morphology and biochemistry

The mean daily air temperature (24-h mean) and daytime mean air temperature (from 8:00 am to 8:00 pm) were significantly higher inside than outside the OTCs. The daytime mean temperature was ~3.3°C higher inside than outside the OTCs, while the nighttime mean temperature inside versus outside of the OTCs was not statistically significant somewhat lower inside than outside the OTCs (Table 1). The frequency distribution of daytime temperature showed two peaks both inside and outside the OTCs. One peak appeared within 14–16°C for both the regimes, but the other peak was within 20–22°C outside the OTCs and within 24–26°C inside the OTCs (Fig. 1a). Inside the OTCs, ~30% of daytime experienced temperature >26°C, while ~12% of the daytime experienced temperature >30°C (Fig. 1a). The frequency distribution of nighttime temperature, however, was almost the same inside and outside the OTCs (Fig. 1b). Temperatures between 6°C and 8°C were the most frequent and amounted to 16% of the nighttime temperatures.

Leaf size, leaf mass per unit area and both chlorophyll *a* and *b* tended strongly to be higher inside than outside the OTCs, though no statistical significance was detected in the limited sample size (Table 2).

Table 1: air and soil temperatures measured from 14 July 2008 to 6 August 2008 inside and outside of OTCs

	OTC warming	Ambient
Air temperature (°C)		
Daily mean	13.30 (2.84)[a]	11.70 (2.55)[b]
Daytime mean	21.08 (4.53)[a]	17.76 (3.44)[b]
Nighttime mean	5.53 (3.11)	5.64 (3.05)
Soil temperature (°C)		
Daily mean	14.40 (1.05)[a]	13.92 (0.91)[b]
Daytime mean	14.75 (1.00)	14.13 (0.84)
Nighttime mean	14.05 (1.13)	13.72 (1.01)

a and b on the right of numbers mean statistical difference ($P < 0.01$) between the two regimes in Student's *t*-test.
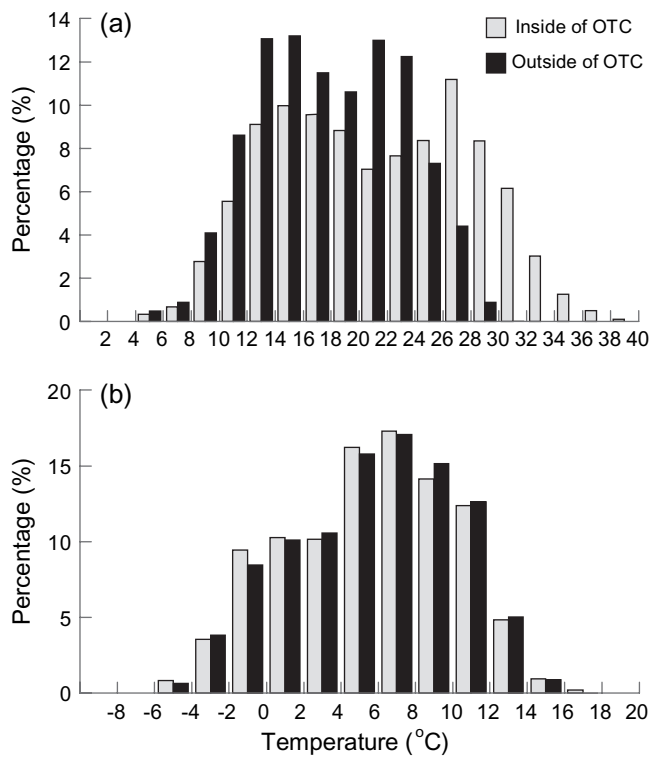
Figure 1: the percent duration of air temperature measured at 1-min intervals from 14 July 2008 to 6 August 2008 (**a**: daytime air temperature; **b**: nighttime air temperature).

Table 2: the leaf morphology and chlorophyll contents of *Gentiana straminea* growing inside versus outside of the OTCs

	OTC warming	Ambient
Leaf size (cm^2)	31.1 (14.5)	21.5 (4.2)
Leaf mass per unit area (LMA, g m^{-2})	109.0 (8.6)	104.6 (7.5)
Chlorophyll *a* content (mg g^{-1})	1.09 (0.19)	0.89 (0.08)
Chlorophyll *b* content (mg g^{-1})	0.46 (0.07)	0.37 (0.04)
Chlorophyll (*a* + *b*) content (mg g^{-1})	1.38 (0.23)	1.12 (0.10)
The ratio of chlorophyll *a* and *b*	2.35 (0.09)	2.38 (0.01)

Student's *t*-test was conducted to compare the values between the two regimes, and none of these parameters showed significant difference.

Response of gas exchange to measurement temperatures

We examined the following parameters to assess the possible acclimation in photosynthetic gas exchange in response to the temperature elevation in the OTCs.

The light-saturated photosynthetic rate (A_{max}).

A_{max} increased with the increase of measurement temperature from 10°C to 15°C for the leaves outside the OTCs but to 20°C for the leaves inside the OTCs. Further increase of the measurement temperature resulted in a rapid decrease of A_{max}

in the leaves for both regimes. The decrease of A_{max} with the increase of temperature was fast in the leaves outside the OTCs. This resulted that the estimated temperature optimum of A_{max} shifted ~1°C higher from the plants under the ambient regime to those within the OTCs. The optimal temperature of A_{max} for leaves within the OTCs is ~19°C.

A_{max} decreased at lower measurement temperatures under the warming condition. A Student's *t*-test showed that the A_{max} was significantly lower in the leaves inside the OTCs at the measurement temperature of 15°C, but no other statistically significant differences in A_{max} between the leaves from the two contrasting temperature regimes at other measurement temperatures, due to the large SDs (Fig. 2a).

The initial slope of PPFD–photosynthesis curve.

The initial slope showed a similar response to measurement temperature like A_{max}, but the maximum of the slope occurred at much higher measurement temperature (Fig. 3a, $P < 0.05$).

Stomatal conductance at A_{max}.

The temperature response of stomatal conductance (g_{sw}) at A_{max} followed almost similar pattern as A_{max} in response to the change of measurement temperature (Fig. 3b, $P < 0.05$). The estimated maximum g_{sw}, however, occurred at much high temperature and showed a larger difference between the plants inside and outside the OTCs: 20°C for the plants inside the OTCs and 10°C for the plants outside the OTCs, respectively.

The light compensation point.

The light compensation point (LCP) showed almost a linear increase with the increase of measurement temperature for the plants both inside and outside the OTCs, and it was significantly higher in the leaves outside than inside the OTCs (Fig. 3c, $P < 0.05$).

The dark respiration rates.

The OTCs warming decreased leaf respiration significantly. The leaf respiration rates were significantly lower in the leaves within the OTCs than in the ambient ($P < 0.01$, in ANOVA for the effect of growth regimes, Fig. 2b). The Q_{10} for the short-term temperature response was 2.10 and 1.73 for the plants from inside and outside the OTCs, respectively.

Temperature response of the RuBP regeneration and RuBP carboxylation

We assessed acclimation in the electron transport and carboxylation by examining the two processes in responding to the changes of measurement temperature. The maximum rate of electron transport (J_{max}) measured under the three different temperatures showed statistically significant difference between the plants from the two contrasting regimes (Fig. 4a, $P < 0.05$ in ANOVA for the effect of growth regimes). However, there was no statistically significant difference in
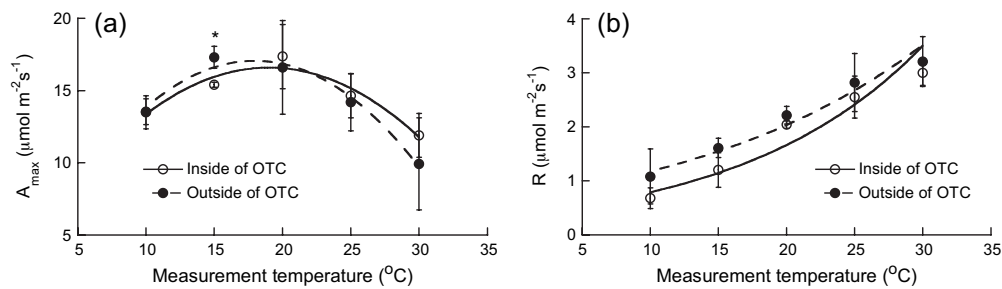
Figure 2: (**a**) Light-saturated photosynthetic rate (A_{max}) and (**b**) dark respiration rate (R) as a function of measurement temperature in *Gentiana straminea* growing inside (open circle, solid line) and outside (filled circle, dotted line) of OTCs. Values represent means ± SDs of three or four samples. A_{max} and R were fitted by two-dimensional polynomial function and exponential function curves of measuring temperature, respectively. [Inside of OTCs: $A_{max} = -0.042T^2 + 1.51T + 2.21$, $r^2 = 0.92$; $R = 0.38\text{EXP}(0.074T)$, $r^2 = 0.92$; Outside of OTCs: $A_{max} = -0.051T^2 + 1.83T + 0.64$, $r^2 = 0.98$; $R = 0.68\text{EXP}(0.055T)$, $r^2 = 0.96$.] Asterisk indicates mean statistical differences for the Student's t-test ($P < 0.01$) between two regimes at the same measurement temperature. Experimental regimes affect significantly ($P < 0.01$) to the temperature response of dark respiration (**b**) examined from one-way factorial ANOVA analysis.

the effect of growth regime on the maximum rate of carboxylation (V_{cmax}) or the ratio of J_{max}/V_{cmax} (Figs 4b and c). The V_{cmax} increased and the J_{max}/V_{cmax} ratio decreased with the increase of the measurement temperature in plants from both the growth regimes (Fig. 4b and c, $P < 0.01$ for linear regression model).

Simulated carbon budget

We first examined the simulated leaf carbon budgets for *in situ* leaves (Fig. 5). The *in situ* leaves showed statistically significantly lower gross carbon gain, as well as lower net carbon gain in the warming-acclimated than the ambient leaves ($P < 0.001$, paired Student's t-test). The estimated optimal temperature for the gross carbon gain was 20.5°C and 17.4°C for the warming-acclimated and ambient leaves, respectively. The estimated optimal temperature for the net carbon gain was 18.9°C and 16.7°C for the warming-acclimated and ambient leaves, espectively.

To assess the effect of temperature acclimation on leaf carbon gain, we examined the carbon gain in the *in situ* warming leaves and the assumed non-acclimated leaves. The estimated average of daily gross CO_2 uptake was significantly higher, while the averages of daily CO_2 loss was significantly lower in the warming-acclimated leaves than the assumed non-acclimated leaves (Table 3, $P < 0.0001$ in paired Student's t-test). Nighttime respiration was also significantly lower in the warming-acclimated leaves than the assumed non-acclimated leaves. These results led to a significant higher daily net carbon gain in the acclimated leaves.

DISCUSSION

Temperature acclimation of photosynthesis and respiration in *Gentiana straminea*

Photosynthetic acclimation.

There was strong tendency that temperature acclimation of photosynthesis occurred under the warming condition. The conclusion is based on the following.

Firstly, the estimated optimal temperature of A_{max} was ~1°C higher in the leaves under the warming regime. In general, plants grown at higher temperatures exhibit a higher optimal temperature of photosynthetic rate. An increase of 1°C in growth temperature can lead to a shift by 0.10–0.59°C in the optimal temperature in different plants (see review by Hikosaka *et al.* 2006). A shift of 1°C in A_{max} suggests a change of temperature environment ranging from 5°C to 10°C. The averaged daytime difference in the daily temperature and daytime temperature was only 1.60 and 3.32°C (Table 1) in our study. This suggests a possible strong acclimation in *G. straminea*, since the temperature differences were much smaller than most laboratory experiments (see reviews from Hikosaka *et al.* 2006; Medlyn *et al.* 2002). In addition, in these laboratory experiments, temperature was set as constant during the daytime. Little information is currently available for characterizing how the optimal temperature shifts in the field conditions. However, the temperature shift mentioned above in our study was derived from a curve fitting, which unfortunately could not be used for a strict statistic test in the current experimental design.

Secondly, the result that A_{max} was statistically significantly lower at the measuring temperature of 15°C under the warming condition may indicate that temperature acclimation occurred, since a shift of A_{max} to higher temperatures under higher measuring temperatures should lead to a decrease of A_{max} under lower measuring temperatures (Fig. 2). This result, on the other hand, provided evidence with statistical significance that the photosynthetic acclimation did occur in the alpine species.

There is current no general pattern of temperature acclimation of photosynthesis in relation to measurement temperature and we have little knowledge about how temperature acclimation of photosynthesis performs under temperatures other than the optimum temperature (Leuning 2002; Medlyn *et al.* 2002). Further studies are needed to clarify the pattern to predict carbon gain of plants under feature climate conditions.
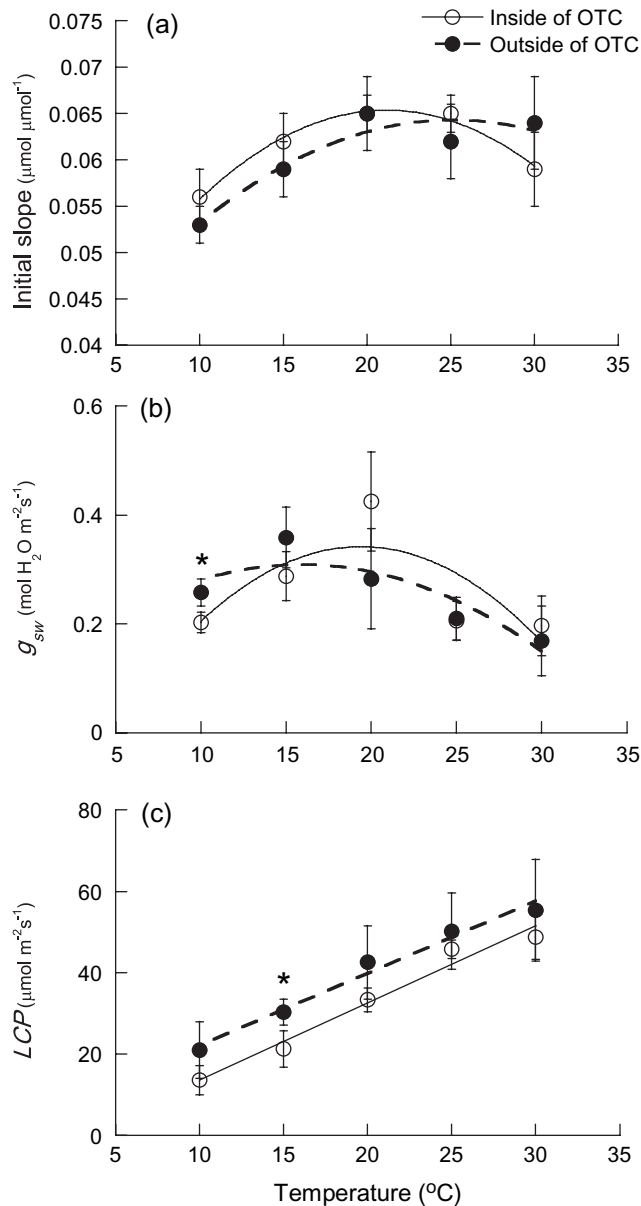
Figure 3: the temperature response of (**a**) the apparent quantum yield of light-photosynthesis curve (α), (**b**) stomatal conductance (g_{sw}), (**c**) and light compensation point (LCP). Mean ± SD for three or four leaves of different ramets from inside (open circle) and outside (filled circle) of OTCs are plotted. Two-dimensional polynomial function curves of measurement temperature for α and g_{sw} and linear function curves for LCP were fitted, respectively ($P < 0.05$). Asterisk indicates mean statistical differences for the Student's *t*-test ($P < 0.05$) between two regimes at the same measurement temperature. Experimental regimes affect significantly ($P < 0.05$) to the temperature response of LCP (c) examined from one-way factorial ANOVA analysis.

Acclimation of leaf respiration.

Leaf respiration can acclimate to changes in temperature regime (Atkin *et al.* 2000; Larigauderie and Körner 1995; Pearcy 1977). Acclimation to higher temperature typically results in decreased respiration rate at the reference temperature, e.g.
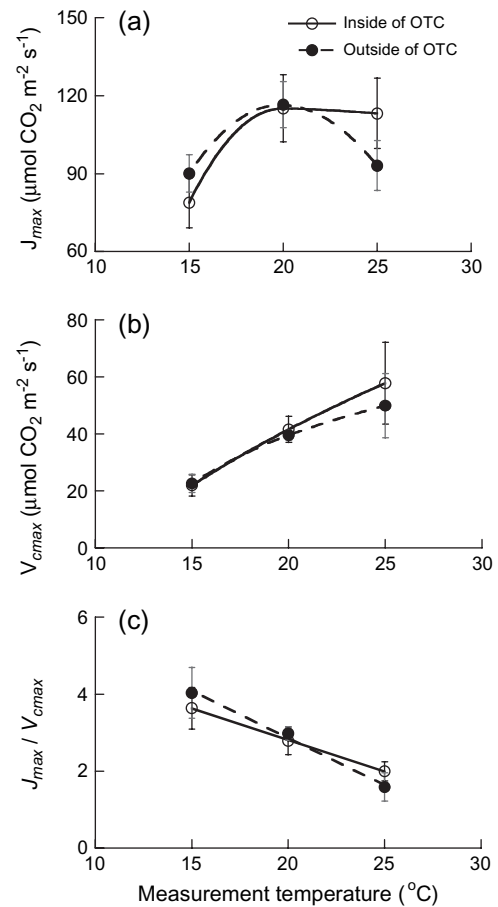

Figure 4: temperature dependencies of (**a**) the maximum rate of electron transport (J_{max}), (**b**) the maximum rate of carboxylation (V_{cmax}), and (c) the ratio of J_{max} to V_{cmax} (J_{max}/V_{cmax}). Abbreviations and symbols are the same as those in Fig. 2. Data represent means ± SD, $n = 3$ or 4. Experimental regimes affect significantly ($P < 0.05$) to the temperature response of J_{max} (a) examined from one-way factorial ANOVA analysis.

25°C (Atkin and Tjoelker 2003). In our experiment, the averaged respiration rate under the measuring temperatures from 10 to 25°C was significantly lower in plants growing under the warming than the ambient condition, which indicates an evident temperature acclimation of respiration.

Atkin and Tjoelker (2003) tried to distinguish two types of respiratory acclimation to temperature, with Type I acclimation changing predominantly in Q_{10} values but no change under low measuring temperature, and Type II acclimation in which respiration rate changes under all measuring temperatures. The results of our study suggest both Type I and Type II acclimation of respiration occurred in *G. straminea*, i.e. both the intercept and the slope of temperature–response function changed under the warming condition. Similar acclimation was reported before (Azcón-Bieto *et al.* 1983; Covey-Crump 2002).

The mechanisms for the two types are not clarified yet, but Type I seems have a larger change in respiratory substance, while Type II in respiratory capacity (i.e. the activity and
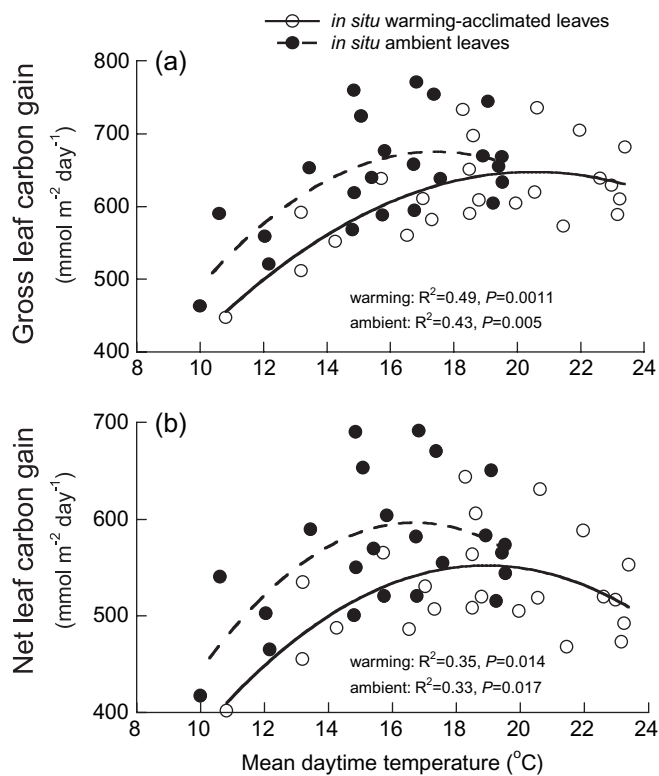
Figure 5: (**a**) mean daytime gross carbon gain and (**b**) net carbon gain in leaves of *Gentiana straminea* in relation to daytime mean air temperature. Open circles and solid line indicate the *in situ* warming-acclimated leaves and closed circles and dotted line indicate *in situ* ambient leaves. Two-dimensional polynomial function curves were fitted to the data.

Table 3: leaf carbon budgets (mean ± SD) estimated for the period from 14 July 2008 to 6 August 2008 ($n = 24$)

Estimated carbon budgets (mmol m^{-2} day^{-1})	Warming-acclimated leaves	Assumed non-acclimated leaves
Daily gross carbon gain	590±64	579±57
Daily carbon loss by respiration	121±25	127±25
Daily net carbon gain	469±54	452±50
Daytime carbon loss by respiration	89±24	92±23
Nighttime carbon loss by respiration	32±6	35±6

The photosynthetic and respiratory parameters for warming-acclimated leaves and assumed non-acclimated leaves were estimated from the measurements of leaves inside and outside the OTCs, respectively. Paired Student's *t*-test showed that all the means were significantly different ($P < 0.001$) in leaves between the regimes.

content of mitochondrion; Atkin and Tjoelker 2003). Large changes in the concentration of soluble sugars have been observed when plants were transferred from one temperature to another (e.g. Covey-Crump 2002; Atkin *et al.* 2000). The change in Q_{10} of *G. straminea* thus perhaps indicates a change

in respiratory substance occurred under the warming condition. However, Q_{10} for leaf respiration varies largely among different species, and no consistent pattern has been observed for Q_{10} of leaves from low- and high-temperature environments (Loveys *et al.* 2003). On the other hand, the larger decrease in respiration under lower temperatures suggests also that plants of *G. straminea* may change their respiratory capacity. Further evidence should be obtained to clarify the underlying mechanism.

Relative temperature acclimation in photosynthesis and respiration.

Many studies have found that thermal acclimation is greater in respiration than in photosynthesis, or even there is no photosynthetic temperature acclimation (e.g. Campbell *et al.* 2007; Ow *et al* 2008a,b; Way and Sage 2008; Yamori *et al.* 2005). These differences in acclimation can have important implications for the plant carbon budget. Campbell *et al.* (2007) reported that the ratio of respiration rate to photosynthetic rate was temperature-dependent with high values in cold-acclimated plants. Consistent with these findings, *G. straminea* showed a larger acclimation response in respiration than in photosynthesis. Although the ecological consequence in the acclimation remains to be further clarified, the decrease of respiration rate under almost all the temperature in the warming condition found in plants of *G. straminea* may increase their net carbon gain if CO_2 uptake would remain no or small change.

Mechanisms underlie the temperature acclimation of photosynthesis

In the biochemical model of photosynthesis in C3 plants, temperature acclimation of photosynthesis can be attributed to the following four variables: intercellular CO_2 concentration, the maximum rate of RuBP carboxylation (V_{cmax}), the maximum capacity of photosynthetic electron transport (J_{max}) and the ratio of J_{max} to V_{cmax}. Hikosaka *et al.* (2006) showed that V_{cmax}, which is likely to be the most important for the change of temperature optimum of photosynthesis, increased in general with increasing growth temperature. In *G. straminea*, V_{cmax} showed a clear tendency of increase in plants from the warming regime at the measuring temperature of 25°C (Fig. 4), but the increase was not statistically significant, which was also due to smaller sample size and large variation of nature environments in the manipulation experiment. Further studies are needed to confirm if the pattern observed in the current study is common in alpine plants, since the changes of V_{cmax} differ largely among species and the information is still limited for figuring out a general pattern for these variable changes in C3 plants (Hikosaka *et al.* 2006; Medlyn *et al.* 2002).

Effects of physiological acclimation on leaf carbon budget

Leaf carbon budget provides a useful conceptual tool as well as a practical approach for studying the consequence of

physiological acclimation of leaves (Pearcy *et al.* 1987). The net leaf carbon gain integrates photosynthesis and respiration and can be used to assess the combined results of temperature acclimation in the two physiological processes. Firstly, the simulation results (Fig. 5) in our study indicated that the *in situ* warming leaves achieved significant lower net leaf carbon gain as compared with the *in situ* ambient leaves. This suggests that the warming condition would decrease leaf net carbon gain in *G. straminea*. However, secondly, the acclimation of photosynthesis and respiration played a very important and significant role to reduce the decrease of leaf net carbon by the warming. The leaf net carbon gain was significantly higher in the warming-acclimated leaves than the assumed non-acclimated leaves (Table 3).

Despite increasing interest in how alpine plants will respond to global warming, detailed information regarding physiological acclimation and its consequences are lacking (Arnone and Körner 1997; Collier 1996; Larigauderie and Körner 1995). Our study provides the first case describing the acclimation of photosynthesis and respiration to long-term temperature elevation in a common alpine plant species. Additional studies are required to examine whether long-term temperature elevation will result in reduced leaf carbon gain for other alpine species and to investigate the underlying mechanisms driving this response.

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