Acclimation of Foliar Respiration and Photosynthesis in Response to Experimental Warming in a Temperate Steppe in Northern China

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Abstract

Background: Thermal acclimation of foliar respiration and photosynthesis is critical for projection of changes in carbon exchange of terrestrial ecosystems under global warming.

Methodology/Principal Findings: A field manipulative experiment was conducted to elevate foliar temperature (T_leaf) by 2.07°C in a temperate steppe in northern China. R_d/T_leaf curves (responses of dark respiration to T_leaf), A_g/T_leaf curves (responses of light-saturated net CO2 assimilation rate to T_leaf), responses of biochemical limitations and diffusion limitations in gross CO2 assimilation rate (A_g) to T_leaf, and foliar nitrogen (N) concentration in Stipa krylovii Roshev. were measured in 2010 (a dry year) and 2011 (a wet year). Significant thermal acclimation of R_d to 6-year experimental warming was found. However, A_g had a limited ability to acclimate to a warmer climate regime. Thermal acclimation of R_d was associated with not only the direct effects of warming, but also the changes in foliar N concentration induced by warming.

Conclusions/Significance: Warming decreased the temperature sensitivity (Q10) of the response of R_d/A_g ratio to T_leaf. Our findings may have important implications for improving ecosystem models in simulating carbon cycles and advancing understanding on the interactions between climate change and ecosystem functions.

Introduction

The balance between respiration and photosynthesis is critical to the exchange of carbon between the atmosphere and the terrestrial biosphere [1–3]. Instantaneous increases in foliar temperature (T_leaf) typically result in an increase in respiration/photosynthesis (R/A) ratio because the response of respiration to T_leaf normally follows an approximate exponential-type curve (at moderate temperatures) while the response of photosynthesis to T_leaf often bears a bell-shaped curve [i.e. the thermal optimum (T_opt) of respiration is higher than that of photosynthesis] [4,5]. In contrast, long-term warming experiments have suggested that R/A ratio is often conservative to changes in growth temperature (T_growth) through acclimation, the metabolic adjustment for compensating changes in T_growth [6–8]. Acclimation could occur via suppression of respiration in response to changes in foliar carbohydrate supplies [4,9]. The thermal acclimation of respiration and photosynthesis is associated with multitudes of signal cascades and networks, which involves the reallocation of resources to achieve and maintain not only optimal R/A ratio but also protective strategies under sustained warming as projected by global climate models [10–12]. However, the mechanisms of thermal acclimation of respiration and photosynthesis to climate warming are far from clear, especially in natural ecosystems.

The acclimation of foliar respiration to warmer T_growth has been found in numerous studies [8,13–17], which may also be associated with plant developmental stage and other abiotic factors, such as drought and nutrient availability [18–21]. Thermal acclimation of respiration might occur via changes in the temperature sensitivity, Q10 or the basal respiration, R_b (respiration at a reference temperature, such as 10°C) [11]. Altered Q10 partially reflects temperature-mediated changes in energy demand and/or available substrates [1,17,20] whereas changes in R_b may be associated with temperature-mediated changes in respiratory capacity, reflecting changes in mitochondrial abundance, structure and/or protein composition [22–24]. As a result, thermal acclimation of respiration may enhance plant net carbon assimilation by reducing carbon loss under warmer T_growth while maintaining basal rates of respiration in colder T_growth for subsequent recovery [12,20,25,26].
The thermal acclimation of the foliar net CO₂ assimilation rate (Aₙ) may involve three primary sets of processes that control the Aₙ/T_leaf curves (response of Aₙ versus T_leaf), namely respiratory, biochemical and stomatal processes [27]. First, Aₙ is the difference between gross CO₂ assimilation rate (A_g) and foliar dark respiration (R_d), Aₙ = A_g – R_d, which requires the decoupling of the two processes because A_g and R_d feature different thermal dynamic properties and thus involve different thermal acclimation processes [28]. This could result in a shift in T_opt and a change in the shape of the Aₙ/T_leaf curve. Therefore, R_d must be evaluated separately and factored out to understand the acclimation mechanisms of Aₙ in response to global warming [3,18,29].

Second, the acclimation of A_g to warmer T_growth deals with the changes in Rubisco activity [29–33] and electronic transport processes [34] where T_growth affects the thermal dependence of various enzymes in the dark and light reactions [35,36]. Therefore, the temperature sensitivity of the maximum rate of Rubisco carboxylation (V_{cmax}) and the maximum rate of photosynthetic electron transport (J_{max}) are associated with the acclimation of A_g [36,37]. In addition, the change in the balance between carboxylation and regeneration of RuBP, indicated by J_{max}/V_{cmax} ratio, may also result in the shift of T_{opt} of A_g due to nitrogen (N) partitioning in the photosynthetic apparatus [3,31,38,39]. Finally, the temperature-dependent diffusion processes of CO₂ to chloroplasts, such as stomatal conductance (g_s) and mesophyll conductance (g_m), can also affect the thermal acclimation of photosynthesis [36,40].

Kirschbaum and Farquhar [41] showed that higher conductance could cause an increase of CO₂ concentrations in the carboxylation site (C_s) and then resulted in a shift in limitation of A_g from Rubisco to electron transport capacity. Since T_{opt} of electron transport-limited A_g is higher than that of Rubisco-limited A_g, T_{opt} of A_g was increased (0.05°C per 1 μmol mol⁻¹ CO₂) [36].

Setaria krylovii Roshev. is a keystone species in the temperate steppe in northern China [42,43]. Climate models predict this region will be 4°C warmer by 2100, which may have severe impacts on Setaria krylovii Roshev. [44]. Examining the respiration and photosynthesis of this species is critical to the steppe productivity and the carbon cycle of the ecosystem. The objectives of the current study are to examine: (1) the acclimation capacity of respiration and photosynthesis to experimental warming under field conditions, and (2) the homeostasis of respiration/photosynthesis ratio in response to experimental warming in the steppe ecosystem.

Materials and Methods

Site Description

The research site (42°02’ N, 116°17’ E, 1324 m a.s.l.) is a typical temperate steppe located in Duolun County, Inner Mongolia Autonomous Region, China. The experiment has received the permits for the field study from the land owner, Institution of Botany, Chinese Academy of Sciences. The mean daily temperature, minimum and mean air temperature (lines) and precipitation (bars) at the study site in 2010 and 2011. The filled rectangles on the top of figure indicate the growing season (May to October) and the open rectangles for the non-growing season (November to April). The arrows mark the timing of field campaigns when the gas exchange measurements were initiated. doi:10.1371/journal.pone.0056482.g002
annual temperature (MAT) is 2.1°C, with monthly mean temperature ranging from −17.5°C in January to 18.9°C in July. The mean annual precipitation (MAP) is approximately 385 mm with approximately 85% falling from May to September. The soils are chestnut (Chinese classification system) or Haplic Calcisols (FAO classification system), with 62.0% sand, 20.3% silt, and 17.0% clay respectively. The soils are characterized as sandy, slightly alkaline and nutrient poor with pH values around 7.7 and bulk density of 1.3 g cm⁻³ and soil total organic C and N concentrations of 16.1 and 1.5 g kg⁻¹ respectively. The plant communities in the temperate steppe are dominated by *Stipa krylovii* Roshev., *Artemisia frigida* Willd., *Potentilla acaulis* L., *Cleistogenes squarrosa* (Trin.) Keng., *Allium bidentatum* Fisch. ex Prokh., and *Agropyron cristatum* (L.) Gaertn.

Warming Experiment

The warming experiment was initiated in April 2006 with infrared heaters (MSR-2420, Kalglo Electronics Inc., USA; radiation output is approximately 1600 W) as the heating source (Fig. 1). Briefly, an infrared heater of 1.65 m in length was suspended at 2.25 m above the ground in each warming plot which features a dimension of 3×4 m. A reflector associated with the heater can be adjusted so as to generate an evenly distributed radiant input to the plant canopy. In the control plots, a ‘dummy’ heater with the same shape and size was suspended at the same height to simulate shading effects of the infrared radiator. The effects of warming on *T*_leaf were measured using a portable infrared thermometer (FLUKE 574, Fluke Inc., USA). The mean daytime *T*_leaf in the warming plots was increased by 2.07°C compared to the control plots. The warming experiment was designed for long-term simulation of global change and it featured a complete random block design with multiple treatments (day warming, night warming, diel warming, and N addition) and six replicates. We took advantage of this multi-factor experiment by selecting the diel warming and control plots with all the other factors kept at control levels. The details of the experiment can be found in Wan et al. [44] and Xia et al. [45].

Gas Exchange Measurements

We measured foliar gas exchange using a portable photosynthesis system (LI-6400, LI-COR Inc., USA) in the middle of the growing seasons (late-July to early-August) in 2010 and 2011 (Fig. 2) to remove the effect of seasonal changes in photosynthetic and respiratory acclimation in *Stipa krylovii* Roshev. [19]. Four individuals (one individual per plot) were measured in each treatment. Eight days were required to complete all field measurements each year. Light, *T*_leaf, humidity, and CO₂...
concentration were independently controlled in a 23 cm cuvette. Given the Trefr control capacity is limited (within ±6°C) with the factory setup of the LI-6400 system, we modified the Trefr control system by adding metal blocks with water channels to heat or cool the peltiers, thermoelectric cooling elements. The water channels were connected to a heating/cooling water bath whose temperature was controlled by adding hot or cold water. This modification allows holding Trefr at any level between 10 and 40°C during the summer growing season in the steppe.

The photosynthetically active photon flux density (PPFD) was provided by the red/blue LED light source built in the foliar cuvette calibrated against an internal photodiode (LI-6400-02B, LI-COR Inc.). The vapor pressure deficit (VPD) in the foliar leaf was controlled by passing the air entering the cuvette through either anhydrous calcium sulfate for the lower Trefr when humidity was high or bubbling air via water at higher Trefr when the air was dry. CO2 concentrations in the cuvette were controlled using an injector system (LI-6400-01, LI-COR Inc.) which allows holding Crefr at 50 and 1200 ppmv CO2.

Table 1. Results (P-values) of one-way ANOVA on the effects of warming on the responses of Amax (the net CO2 assimilation rate), Rg (dark respiration), A0 (the gross CO2 assimilation rate), Vrmax (the maximum rate of Rubisco carboxylation) and Jmax (the maximum rate of photosynthetic electron transport) expressed per unit foliar area and nitrogen to instantaneous change (10–40°C within a 5 h period) in Trefr (foliar temperature) in 2010 and 2011.

| Year | Parameters | c | ΔHg | ΔHd | ΔS | Topt | Q10 | ref10 |
|------|------------|---|-----|-----|----|------|-----|-------|
| 2010 | Amax       | 0.836 | 0.844 | 0.735 | 0.727 | 0.310 | / | 0.816 |
|      | Rg         | 0.027 | 0.046 | /     | /     | 0.049 | 0.090 |
|      | A0         | 0.292 | 0.300 | 0.979 | 0.913 | 0.328 | / | 0.839 |
|      | Vrmax     | 0.055 | 0.064 | /     | /     | 0.062 | 0.784 |
|      | Jmax       | 0.879 | 0.842 | 0.757 | 0.772 | 0.520 | / | 0.181 |
|      | A0/N       | 0.726 | 0.732 | 0.575 | 0.612 | 0.323 | / | 0.955 |
|      | Rg/N       | 0.071 | 0.095 | /     | /     | 0.094 | 0.094 |
|      | A0/N       | 0.977 | 0.976 | 0.138 | 0.178 | 0.302 | / | 0.996 |
|      | Vrmax/N    | 0.150 | 0.158 | /     | /     | 0.142 | 0.546 |
|      | Jmax/N     | 0.474 | 0.468 | 0.678 | 0.646 | 0.874 | / | 0.381 |
| 2011 | Amax       | 0.647 | 0.472 | 0.986 | 0.923 | 0.619 | / | 0.855 |
|      | Rg         | 0.042 | 0.040 | /     | /     | 0.042 | 0.050 |
|      | A0         | 0.403 | 0.400 | 0.600 | 0.529 | 0.637 | / | 0.758 |
|      | Vrmax     | 0.723 | 0.712 | /     | /     | 0.779 | 0.656 |
|      | Jmax       | 0.166 | 0.167 | 0.325 | 0.369 | 0.317 | / | 0.487 |
|      | A0/N       | 0.354 | 0.354 | 0.470 | 0.453 | 0.989 | / | 0.886 |
|      | Rg/N       | 0.010 | 0.010 | /     | /     | 0.011 | 0.026 |
|      | A0/N       | 0.036 | 0.035 | 0.604 | 0.554 | 0.933 | / | 0.698 |
|      | Vrmax/N    | 0.074 | 0.079 | /     | /     | 0.093 | 0.703 |
|      | Jmax/N     | 0.463 | 0.468 | 0.215 | 0.223 | 0.115 | / | 0.657 |

c is a scaling constant, ΔHg is the activation energy, ΔHd is a term for deactivation, ΔS is an entropy term, Topt is the thermal optimum, Q10 is the temperature sensitivity and ref10 is the estimated basal rate at the reference temperature of 10°C. Significant values (P<0.05) are shown bold.
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Table 2. Warming effects on the responses of $A_n$ (the net CO$_2$ assimilation rate), $R_d$ (dark respiration), $A_g$ (the gross CO$_2$ assimilation rate), $V_{cmax}$ (the maximum rate of Rubisco carboxylation) and $J_{max}$ (the maximum rate of photosynthetic electron transport) expressed per unit foliar area and nitrogen to instantaneous change (10–40 °C within a 5 h period) in $T_{leaf}$ (foliar temperature) in the dry growing season (2010).

| Parameters | Treatment | $c$ | $\Delta H_a$ | $\Delta H_d$ | $\Delta S$ | $T_{opt}$ | $Q_10$ | ref_{10} |
|------------|-----------|-----|--------------|--------------|-------------|----------|--------|----------|
| $A_n$ (µmol m$^{-2}$ s$^{-1}$) | Control | 24.24 ± 5.86 | 50.48 ± 14.38 | 166.73 ± 19.47 | 0.56 ± 0.07 | 22.49 ± 1.04 | / | 17.33 ± 3.63 |
| | Warming | 25.73 ± 3.69 | 53.96 ± 8.92 | 158.17 ± 14.21 | 0.53 ± 0.04 | 23.99 ± 0.87 | / | 16.34 ± 1.87 |
| $R_d$ (µmol m$^{-2}$ s$^{-1}$) | Control | 20.03 ± 0.92 | 45.93 ± 2.27 | / | / | 1.83 ± 0.05 | 1.70 ± 0.12 |
| | Warming | 16.59 ± 0.75 | 38.37 ± 1.97 | / | / | 1.66 ± 0.04 | 1.35 ± 0.13 |
| $A_g$ (µmol m$^{-2}$ s$^{-1}$) | Control | 38.57 ± 6.03 | 83.57 ± 14.11 | 134.98 ± 14.15 | 0.46 ± 0.05 | 22.53 ± 1.38 | / | 18.44 ± 3.52 |
| | Warming | 29.77 ± 6.66 | 63.18 ± 11.13 | 135.46 ± 9.63 | 0.45 ± 0.03 | 24.30 ± 0.91 | / | 17.59 ± 1.88 |
| $V_{cmax}$ (µmol m$^{-2}$ s$^{-1}$) | Control | 24.68 ± 1.26 | 49.08 ± 3.08 | / | / | 1.91 ± 0.07 | 46.38 ± 2.43 |
| | Warming | 21.64 ± 0.25 | 41.97 ± 0.62 | / | / | 1.74 ± 0.01 | 45.33 ± 2.73 |
| $J_{max}$ (µmol m$^{-2}$ s$^{-1}$) | Control | 34.22 ± 4.47 | 68.79 ± 10.39 | 126.60 ± 17.24 | 0.43 ± 0.05 | 25.50 ± 0.90 | / | 126.47 ± 15.86 |
| | Warming | 35.24 ± 4.58 | 71.89 ± 10.69 | 132.40 ± 4.80 | 0.44 ± 0.01 | 26.55 ± 1.24 | / | 101.15 ± 5.31 |
| $A_g/N$ (µmol g$^{-1}$ s$^{-1}$) | Control | 22.15 ± 8.29 | 49.63 ± 20.16 | 189.72 ± 13.56 | 0.63 ± 0.05 | 23.42 ± 0.78 | / | 3.18 ± 0.73 |
| | Warming | 27.95 ± 13.45 | 63.21 ± 32.05 | 201.66 ± 14.95 | 0.67 ± 0.06 | 25.29 ± 1.55 | / | 3.13 ± 0.58 |
| $R_d/N$ (µmol g$^{-1}$ s$^{-1}$) | Control | 18.04 ± 0.83 | 45.10 ± 2.03 | / | / | 1.81 ± 0.05 | 0.33 ± 0.02 |
| | Warming | 15.06 ± 1.08 | 38.51 ± 2.64 | / | / | 1.66 ± 0.06 | 0.28 ± 0.02 |
| $A_g/N$ (µmol g$^{-1}$ s$^{-1}$) | Control | 28.54 ± 7.08 | 64.31 ± 17.24 | 131.93 ± 19.92 | 0.44 ± 0.07 | 23.89 ± 0.76 | / | 3.38 ± 0.72 |
| | Warming | 29.01 ± 13.69 | 65.48 ± 32.55 | 176.38 ± 16.66 | 0.58 ± 0.06 | 26.04 ± 1.75 | / | 3.39 ± 0.60 |
| $V_{cmax}/N$ (µmol g$^{-1}$ s$^{-1}$) | Control | 22.47 ± 1.15 | 47.69 ± 2.81 | / | / | 1.87 ± 0.07 | 9.18 ± 0.49 |
| | Warming | 19.79 ± 1.15 | 41.26 ± 2.83 | / | / | 1.72 ± 0.06 | 9.68 ± 0.61 |
| $J_{max}/N$ (µmol g$^{-1}$ s$^{-1}$) | Control | 26.79 ± 5.46 | 55.64 ± 13.06 | 151.52 ± 16.73 | 0.50 ± 0.05 | 27.17 ± 1.47 | / | 23.28 ± 3.43 |
| | Warming | 35.83 ± 10.49 | 77.39 ± 24.84 | 165.48 ± 27.26 | 0.55 ± 0.09 | 27.58 ± 1.98 | / | 19.04 ± 2.88 |

$c$ is a scaling constant, $\Delta H_a$ is the activation energy, $\Delta H_d$ is a term for deactivation, $\Delta S$ is an entropy term, $T_{opt}$ is the thermal optimum, $Q_{10}$ is the temperature sensitivity and $ref_{10}$ is the estimated basal rate at the reference temperature of 10 °C. Values are means (n = 4, ± SE).

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Estimation of $V_{cmax}, J_{max}$ and $g_m$

$A_n/C_a$ curves ($A_n$ versus chloroplastic CO$_2$ concentration) were fitted to estimate $V_{cmax}, J_{max}$, $TPU$ (triose-phosphate utilization) and $g_m$. The spreadsheet-based software of Sharkey et al. [50] was modified (Appendix S1) to fit the $A_n/C_a$ curve by fixing the $R_d$ value which was measured following the $A_n/C_a$ curve. This modification will improve the model performance by reducing the number of estimated parameters and thus decreasing the degree of freedom in fitting the model. As in the original software the optimum of $V_{cmax}, J_{max}$, $TPU$ and $g_m$ was obtained by minimizing the root mean square error (RMSE) of each curve [51,52].

Estimation of Dependence of Reaction Rates on Temperature

The responses of $R_d$ and $V_{cmax}$ to $T_{leaf}$ were fitted to a non-peaked model, following Harley et al. [53], due to the fact that the deactivation of $R_d$ and $V_{cmax}$ was not observed in our study:

$$Parameter(R_d, V_{cmax}) = e^{(c \cdot \Delta H_a / RT_{10})}$$

where $c$ is a scaling constant, $\Delta H_a$ is the activation energy, $R$ is the molar gas constant (0.008314 kJ K$^{-1}$ mol$^{-1}$) and $T_{10}$ is the absolute $T_{leaf}$ (K) [54]. $Q_{10}$ of $R_d$ and $V_{cmax}$ were modeled using the following general function:

$$Parameter(R_d, V_{cmax}) = ref_{10}Q_{10}^{m(T_{leaf} - 10)/10}$$

where $ref_{10}$ is the estimated basal rate at the reference temperature of 10°C, and $T_{leaf}$ is the leaf temperature (°C). The responses of $A_n$, $A_g$ and $J_{max}$ to $T_{leaf}$ were fitted using a peak model in view that the deactivation at high $T_{leaf}$ was substantial:

$$Parameter(A_n, A_g, J_{max}) = \frac{e^{(c \cdot \Delta H_d / RT_{10})}}{1 + e^{(c \cdot \Delta S / RT_{10})}}$$

where $\Delta H_d$ is a term for deactivation and $\Delta S$ is an entropy term [54,55]. The second derivative of Eqn 3 shows that $Topt$ can be calculated [56] as follows if the parameter includes a peak:
**Table 3.** Warming effects on the responses of $A_0$, $R_d$, $A_g$, $V_{\text{max}}$ and $J_{\text{max}}$ expressed per unit foliar area and nitrogen to instantaneous change (10–40°C within a 5 h period) in $T_{\text{leaf}}$ in the wet growing season (2011).

| Parameters | Treatment | $c$ ($\mu$mol m$^{-2}$ s$^{-1}$) | $\Delta H_d$ | $\Delta H_d$ | $\Delta S$ | $T_{\text{opt}}$ | $Q_{10}$ | ref$\alpha$ |
|------------|-----------|----------------------------------|--------------|--------------|------------|----------------|----------|-----------|
| $A_0$      | Control   | $30.91 \pm 9.71$                 | $66.17 \pm 22.71$ | $175.99 \pm 18.90$ | $0.59 \pm 0.06$ | $24.89 \pm 2.47$ | /         | $15.07 \pm 2.49$ |
|            | Warming   | $21.82 \pm 6.90$                 | $44.81 \pm 16.09$ | $175.39 \pm 26.86$ | $0.58 \pm 0.08$ | $26.48 \pm 1.76$ | /         | $15.56 \pm 1.29$ |
| $R_d$      | Control   | $24.93 \pm 1.23$                 | $60.02 \pm 3.13$ | /             | /           | $2.19 \pm 0.09$ | $0.58 \pm 0.09$ |          |
|            | Warming   | $18.95 \pm 1.97$                 | $44.85 \pm 4.88$ | /             | /           | $1.81 \pm 0.11$ | $0.92 \pm 0.11$ |          |
| $A_g$      | Control   | $39.19 \pm 13.54$               | $85.44 \pm 31.59$ | $167.09 \pm 24.98$ | $0.56 \pm 0.08$ | $25.65 \pm 2.77$ | /         | $15.27 \pm 2.45$ |
|            | Warming   | $25.00 \pm 8.06$                 | $52.17 \pm 18.80$ | $148.67 \pm 22.07$ | $0.49 \pm 0.07$ | $27.34 \pm 1.99$ | /         | $16.16 \pm 1.26$ |
| $V_{\text{max}}$ ($\mu$mol m$^{-2}$ s$^{-1}$) | Control | $23.98 \pm 0.80$ | $47.78 \pm 2.07$ | /             | /           | $1.87 \pm 0.05$ | $40.65 \pm 4.82$ |          |
|            | Warming   | $22.25 \pm 1.81$                 | $45.87 \pm 4.47$ | /             | /           | $1.83 \pm 0.11$ | $43.85 \pm 4.84$ |          |
| $J_{\text{max}}$ ($\mu$mol m$^{-2}$ s$^{-1}$) | Control | $27.81 \pm 5.52$ | $54.61 \pm 13.31$ | $167.70 \pm 27.42$ | $0.54 \pm 0.08$ | $31.56 \pm 1.01$ | /         | $102.85 \pm 15.77$ |
|            | Warming   | $41.44 \pm 6.65$                 | $86.59 \pm 15.41$ | $136.76 \pm 8.98$ | $0.46 \pm 0.02$ | $30.03 \pm 0.97$ | /         | $90.79 \pm 4.06$ |
| $A_0/N$ ($\mu$mol g N$^{-1}$ s$^{-1}$) | Control | $28.91 \pm 9.48$ | $64.42 \pm 22.35$ | $277.95 \pm 128.81$ | $0.91 \pm 0.41$ | $26.15 \pm 3.34$ | /         | $4.38 \pm 0.71$ |
|            | Warming   | $18.26 \pm 4.78$                 | $39.33 \pm 11.09$ | $175.08 \pm 35.01$ | $0.57 \pm 0.11$ | $26.09 \pm 1.89$ | /         | $4.50 \pm 0.30$ |
| $R_d/N$ ($\mu$mol g N$^{-1}$ s$^{-1}$) | Control | $24.49 \pm 1.52$ | $62.01 \pm 3.83$ | /             | /           | $2.25 \pm 0.11$ | $0.16 \pm 0.03$ |          |
|            | Warming   | $16.71 \pm 1.44$                 | $42.52 \pm 3.54$ | /             | /           | $1.76 \pm 0.08$ | $0.26 \pm 0.02$ |          |
| $A_g/N$ ($\mu$mol g N$^{-1}$ s$^{-1}$) | Control | $37.55 \pm 11.17$ | $84.51 \pm 26.22$ | $193.11 \pm 55.95$ | $0.64 \pm 0.17$ | $26.25 \pm 3.03$ | /         | $4.37 \pm 0.64$ |
|            | Warming   | $22.93 \pm 6.77$                 | $50.15 \pm 17.56$ | $157.41 \pm 33.58$ | $0.52 \pm 0.10$ | $26.58 \pm 2.18$ | /         | $4.65 \pm 0.29$ |
| $V_{\text{max}}/N$ ($\mu$mol g N$^{-1}$ s$^{-1}$) | Control | $23.24 \pm 0.69$ | $48.97 \pm 1.84$ | /             | /           | $1.90 \pm 0.05$ | $11.67 \pm 1.37$ |          |
|            | Warming   | $21.11 \pm 0.70$                 | $43.81 \pm 1.61$ | /             | /           | $1.78 \pm 0.04$ | $12.26 \pm 0.58$ |          |
| $J_{\text{max}}/N$ ($\mu$mol g N$^{-1}$ s$^{-1}$) | Control | $27.40 \pm 9.59$ | $56.67 \pm 22.90$ | $218.93 \pm 62.97$ | $0.71 \pm 0.20$ | $32.46 \pm 1.55$ | /         | $28.09 \pm 3.70$ |
|            | Warming   | $36.34 \pm 6.18$                 | $77.67 \pm 14.53$ | $131.16 \pm 7.25$ | $0.44 \pm 0.22$ | $29.26 \pm 0.79$ | /         | $26.24 \pm 1.40$ |

Values are means ($n=4$, ± SE). See Table 2 for abbreviations defined.

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\[
T_{\text{opt}} = \frac{\Delta H_d}{\Delta S - R \ln[\Delta H_d/\Delta H_d - \Delta H_d]} 
\]

(4)

**Estimation of Biochemical Limitations to Photosynthesis**

Temperature dependence of $A_g$ limited by RuBP carboxylation ($A_c$), RuBP regeneration ($A_i$) and $TPU$ ($A_p$) were reconstructed as follows:

\[
A_c = \frac{V_{\text{max}}(C_c - \Gamma^*)}{C_c + K_c(1 + O/K_o)} 
\]

(5)

\[
A_i = \frac{J_{\text{max}}(C_c - \Gamma^*)}{4C_c + 8\Gamma^*} 
\]

(6)

\[
A_p = 3TPU 
\]

(7)

where $V_{\text{max}}$, $J_{\text{max}}$ and $TPU$ were derived from fitted kinetic parameters ($c$, $\Delta H_d$, $\Delta H_d$ and $\Delta S$) in our study, $K_c$, $K_o$ and $\gamma$ were derived from a general set of kinetic parameters in Sharkey et al. [50]. $C_c$ was set at 250.8 ppmv in view that the mean $C_c/C_o$ ratio was 0.66 at ambient CO$_2$ concentration (380 ppmv) for all the $A_c/C_o$ curves measured in the current study, $O$ was the partial pressure of oxygen at Rubisco.

**Foliar Characteristics**

Foliar N concentration on an area basis was determined using the foliage covered in the cuvette during the gas exchange measurements. The foliage samples were first used to measure the leaf area with an area meter (Li-3100, Li-Cor Inc.) and then biomass where the samples were dried at 65°C for 48 h. Then the dry samples were ground to powder for measuring the total C and N concentrations with a CN analyzer (NA Series 2, CE Inc., Germany).

**Data Analyses**

The raw data from the gas exchange measurements were cleaned and processed in Excel spreadsheets where the non-linear $A_c/C_o$ curve fitting was performed as in Sharkey et al. [50]. The fitting was improved by fixing $R_d$ with the measured value (Appendix S1). Further statistical analyses were conducted using SPSS (version 17.0, SPSS Inc., USA). One-way ANOVA was used to analyze the effects of warming on (1) the foliar chemical properties (C, N, and C/N ratio) and (2) the thermal dynamic

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properties ($c$, $\Delta H_n$, $\Delta H_d$, $\Delta S$, $Q_{10}$, $T_{opt}$ and $n_{fg}$) of foliar gas exchange ($A$, $R_d$ and $A_g$) and photosynthetic metabolism ($V_{\text{max}}$ and $J_{\text{max}}$).

Differences were considered statistically significant at $P<0.05$. Linear regression was employed to examine relationships between foliar properties and climate (i.e. $T_{growth}$). $T_{growth}$ in the control plots was an average for daytime $T_{air}$ during the 5 d prior to gas exchange measurements in each plot. This choice was based on: (1) our observation that the bulk of individual foliar development by *Stipa krylovii* Roshev. species typically required 4–6 d; and (2) published results indicating that adjustments of foliar metabolism to climate change can occur rapidly (e.g. in a span of 1–5 d following a shift in $T_{growth}$ [13,15,57–61]); (3) Gunderson et al. [60] found that $T_{opt}$ for photosynthesis was

Figure 5. Warming effects on the responses of the maximum rate of Rubisco carboxylation ($V_{\text{max}}$), the maximum rate of photosynthetic electron transport ($J_{\text{max}}$) and the $J_{\text{max}}/V_{\text{max}}$ ratio to foliar temperature ($T_{leaf}$) in 2010 (left panels) and 2011 (right panels). The filled circles indicate the warming plots and the open circles for the control plots. (A) and (B) area-based $V_{\text{max}}$; (C) and (D) area-based $J_{\text{max}}$; (E) and (F) N-based $V_{\text{max}}$; and (G) and (H) N-based $J_{\text{max}}$. Each data point is the average of 4 replicates.

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Figure 6. Warming effects on the responses of stomatal conductance ($g_s$) (A, B), mesophyll conductance ($g_m$) (C, D), carboxylation site CO$_2$ concentrations ($C_c$) (E, F), and $C_c/C_a$ ratio (G, H) to foliar temperature ($T_{leaf}$) in 2010 (left panels) and 2011 (right panels). The filled circles indicate the warming plots and the open circles for the control plots. Each data point is the average of 4 replicates.

Note: $g_m$ is constrained to be 30 (mol m$^{-2}$ s$^{-1}$ Pa$^{-1}$) or less.

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Table 4. Foliar characteristics of *Stipa krylovii* Roshev. grown in the control and warming plots.

| Year | Treatment | N concentration | C concentration | C/N ratio |
|------|-----------|-----------------|-----------------|-----------|
| 2010 | Control   | 5.34±0.07       | 86.66±1.48      | 16.22±0.16|
|      | Warming   | 5.02±0.16       | 92.91±3.42      | 18.48±0.26|
|      | P value   | 0.063           | 0.100           | <0.001    |
| 2011 | Control   | 3.41±0.05       | 78.12±1.35      | 22.92±0.15|
|      | Warming   | 3.68±0.06       | 79.70±1.44      | 21.65±0.11|
|      | P value   | 0.002           | 0.426           | <0.001    |

Warming effects on foliar nitrogen concentrations (g N m$^{-2}$), carbon concentrations (g C m$^{-2}$) and C/N ratio (g g$^{-1}$) were analyzed using one-way ANOVA for each year. Significant values ($P<0.05$) are shown bold (Mean ± SE, N = 28).

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Figure 7. Responses of $Q_{10}$ (the temperature sensitivity) (top panel) and $R_{10}$ (the estimated basal respiration rate at the reference temperature of 10°C) (lower panel) in the control (open) and warming (filled) plots in 2010 (circles) and 2011 (squares) to $T_{\text{growth}}$ (left panel) and foliar nitrogen concentrations (right panel), respectively. Values are means ($n = 4$, ± SE).

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Figure 8. Warming effects on the responses of biochemical limitations in gross CO$_2$ assimilation ($A_g$) to foliar temperature ($T_{\text{leaf}}$) at chloroplast partial pressure of CO$_2$ ($C_c$) of 250.8 ppmv in 2010 (left panels) and 2011 (right panels). The top panels indicate the control plots and the lower panels for the warming plots. $C_c$ was set at 250.8 ppmv considering that the mean $C_c/C_a$ ratio was 0.66 at ambient CO$_2$ concentration (380 ppmv) for all the $A_n/C_i$ curves measured. The response of $A_g$ is delineated by the minimum value of either Rubisco-limited (solid curve), ribulose bisphosphate (RuBP) regeneration-limited (dashed curve) and $P_i$ regeneration-limited (dotted curve). Circle indicates co-limited point, moving from the Rubisco-limited state to RuBP regeneration-limited state.

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strongly correlated with mean daytime $T_{air}$. In addition, $T_{growth}$ in the warming plots were approximatively calculated by adding warming effects (2.07°C) to the mean daytime $T_{air}$ during the 5 d prior to gas exchange measurements in each plot.

**Results**

**Microclimate and Experimental Warming**

The meteorological data collected at the experimental site showed that the growing season of 2010 was dry while the growing season of 2011 was wet (Fig. 2). The daily mean $T_{air}$ between 1 May, the onset of plant growth, and the time of the field measurements (27 July in 2010 and 2011) was 17.2°C in 2010 and 15.6°C in 2011 with the long-term average (1953–2011) of 15.5°C during the same period. Meanwhile, the precipitation during the same period was only 115 mm in 2010 and 183 mm in 2011 with the long-term average of 177 mm. The growing season precipitation in 2010 was only about 63% of that in a normal year, confirming 2010 was a dry year (Fig. 2).

The experimental warming significantly increased daytime $T_{leaf}$ by 2.07°C ($P<0.001$), on average (Fig. 3). Warming increased daytime $T_{growth}$ in the warming plots reaching 28.59 and 23.14°C in 2010 and 2011, respectively. Meanwhile, the daytime $T_{growth}$ in the control plots was only 25.72 and 23.11°C in 2010 and 2011, respectively. The details of the warming effects on microclimate at the study site can be found in Wan et al. [44] and Xia et al. [45].

**Respiration**

Warming significantly decreased respiratory temperature sensitivity, $Q_{10}$, in both years (both $P<0.05$) (Fig. 4, Table 1). $Q_{10}$ of $R_{d}$ on a foliar area basis decreased from 1.83 in the control plots to 1.66 in the warming plots in 2010 ($P=0.049$) (Table 2) and from 2.19 to 1.81 in 2011 ($P=0.042$) (Table 3). Meanwhile, $Q_{10}$ of $R_{d}$ on a foliar N basis marginally decreased from 1.81 to 1.66 in 2010 ($P=0.094$) and significantly decreased from 2.25 to 1.76 in 2011 ($P=0.011$) (Table 2, 3). Warming marginally reduced base respiration rate at 10°C ($R_{10}$) on a foliar area basis from 1.70 to 1.35 μmol m$^{-2}$ s$^{-1}$ in 2010 ($P=0.090$) but increased that from 0.58 to 0.92 μmol m$^{-2}$ s$^{-1}$ in 2011 ($P=0.050$) (Table 2, 3). Warming effects on the $R_{10}$ on a foliar N basis were similar to the area-based $R_{d}$ (Fig. 4).

**Photosynthesis**

The $A_{c}/T_{leaf}$ curves were typically bell-shaped in both warming and control plots (Fig. 4). Warming had little effect on $T_{opt}$ of $A_{c}$ in both years (both $P>0.05$) (Table 1); $T_{opt}$ of $A_{c}$ on a foliar area basis was 22.49 and 23.94°C for the control and the warming plots respectively in 2010, and 24.89 and 26.48°C respectively in 2011 (Fig. 4). $T_{opt}$ of $A_{c}$ on a foliar area basis was 22.55 and 24.50°C for the control and the warming plots respectively in 2010 ($P=0.329$), and 25.65 and 27.34°C respectively in 2011 ($P=0.037$) (Fig. 4). Warming also had little effects on $T_{opt}$ of $A_{c}$ and $A_{c}$ on a foliar N basis in either 2010 or 2011 (all $P>0.05$) (Table 1).

**Biochemical Limitations to Photosynthesis**

The effects of warming on $Q_{10}$ of $V_{c,max}$ were not statistically significant between the warming and the control plots in both years (both $P>0.05$) (Table 1), but we found a general decreasing trend from the control to warming plots (Fig. 5). $Q_{10}$ of $V_{c,max}$ on a foliar area basis was 1.91 and 1.74 for the control and the warming plots respectively in 2010, and 1.87 and 1.73 respectively in 2011 ($P=0.077$) (Fig. 5, Table 2, 3). $Q_{10}$ of $V_{c,max}$ on a foliar N basis was 1.87 and 1.72 for the control and the warming plots respectively in 2010 ($P=0.174$), and 1.90 and 1.78 respectively in 2011 ($P=0.068$) (Fig. 5, Table 2, 3). The warming effects on $Q_{10}$ of $V_{c,max}$ were not be detected in 2010 or 2011 (both $P>0.05$) (Table 1). In addition, the warming effects on the slope and $y$-intercept of the temperature-response curves for $V_{c,max}$ were not statistically significant (all $P>0.05$), though the ratio decreased linearly with the $T_{leaf}$ (Fig. 5).

**Diffusion Limitations to Photosynthesis**

In 2010, a dry year, $g_{s}$ in the warming plots was marginally greater than that in the control plots ($P=0.137$), and $T_{opt}$ for $g_{s}$ was about 17.42°C in the warming plots and less than 10°C in the control plots (Fig. 6). The $g_{s}$ in the warming plots was significantly greater than that in the control plots ($P<0.001$), and $T_{opt}$ for $g_{s}$ appeared at 37.09°C in the warming plots and 27.86°C in the control plots (Fig. 6). $C_{i}$ in the warming plots was approximately 35 ppmv greater than that in the control plots ($P<0.001$), but $C_{i}$ was independent of $T_{leaf}$ in both the warming and the control plots (both $P>0.05$) (Fig. 6). Similarly, $C_{i}/C_{a}$ ratio was constant and
independent of $T_{\text{stem}}$ in the warming and the control plots (both $P>0.05$) (Fig. 6). However, experimental warming significantly increased $C_i/C_o$ ratio in 2010 ($P=0.001$) with an average value of 0.70 in the warming plots and 0.61 in the control plots (Fig. 6).

In 2011, a wet year, Warming had little effect on $g$ and $g_{\text{ms}}$ (both $P>0.05$), which resulted in no difference in $C_i$ between the warming and the control plots ($P>0.860$) (Fig. 6). Experimental warming also had little effect on $C_i/C_o$ ratio in 2011 ($P=0.447$) with an average value of 0.67 in the warming plots and 0.65 in the control plots (Fig. 6).

**Foliar Characteristics**

Warming marginally decreased foliar N concentration in 2010 ($P=0.063$), but significantly increased that in 2011 ($P=0.002$) (Table 4). Warming had little effect on foliar carbon concentration in both years (both $P>0.05$), Foliar C/N ratio was significantly higher in the warming plots than in the control plots in 2010 ($P<0.001$) and the opposite was true in 2011 (Table 4).

**Discussion**

**Acclimation of Respiration**

$R_i$ was sensitive to $T_{\text{stem}}$ with the $R_i/T_{\text{stem}}$ relationship following a typical exponential curve, but warming reduced the magnitude (Fig. 4, Table S1). Our results are consistent with previous studies [19,20,62] that the temperature sensitivity of $R_i$ is negatively related to the $T_{\text{growth}}$ (Fig. 7). According to the respiratory acclimation mechanisms proposed by Atkin and Tjoelker [11], the temperature-mediated change in $Q_{10}$ is determined by the maximum enzyme activity and/or substrate availability [1,17,20]. Earlier results from the same warming experiment confirmed that day warming significantly reduced foliar starch concentrations (-6.1%, $P=0.009$), suggesting the reduction in $Q_{10}$ in the current study might be attributed to the lower substrate concentrations.

Foliar N concentrations induced by experimental warming in our study may also affect the temperature sensitivity of $R_i$, $Q_{10}$ (Fig. 7). To date, few studies have examined the role of N in the change in $Q_{10}$. Turnbull et al. [63] found that $Q_{10}$ of $R_i$ for the trees in a temperate rainforest increased with increasing N availability along a soil chronosequence in New Zealand. However, Ose et al. [64] have reported that N had little or no impact on $Q_{10}$ of $R_i$ when saplings grown at high and low N availabilities were transferred to a different $T_{\text{growth}}$ regime. Here, we found a negative correlation between $Q_{10}$ of $R_i$ and foliar N concentrations (Fig. 7). The detailed mechanisms are not clear, but the confounding effect of foliar N concentrations with other factors, such as temperature and precipitation, may have played an important role in the “apparent” $Q_{10}$ [11,65,66].

In the current study we found that experimental warming marginally reduced base respiration rate at 10°C ($R_{i0}$) in 2010 but increased that in 2011 (Table 2, 3). This could have been attributed to the differential responses of foliar N concentration to warming in the two hydrologically contrasting growing seasons. Warming marginally decreased foliar N concentration in the dry growing season (2010), but increased that in the wet growing season (2011) (Table 4). A growing number of studies [6,14,17], including our current study, have found that foliar N concentration was strongly related to $R_{i0}$ (Fig. 7). Therefore, we believed that foliar N concentration played an important role in the diverging responses of $R_{i0}$ to warming in both years.

**Acclimation of Photosynthesis**

Photosynthesis has long been known to acclimate to prevailing $T_{\text{growth}}$ by shifting the $T_{\text{opt}}$ [67]. For example, Gunderson et al. [60] have reported that a 3-year warming of 2–4°C has resulted in a higher $T_{\text{opt}}$ of $A_g$ for five species of deciduous trees. In the current study we found that a 6-year warming of 2.07°C did not result in changes in $T_{\text{opt}}$ of $A_g$ (Fig. 4, Table S1). We also found that there were not statistically significant differences between the shift in $T_{\text{opt}}$ of $A_g$ and $A_{\text{ms}}$ in 2010 ($P=0.896$) or 2011 ($P=0.984$). This suggests that the instantaneous response of photosynthesis was independent of changes in $R_i$.

It has been proposed that the increase in the temperature sensitivity of $V_{\text{max}}$, indicated by $\Delta H_{\text{i}}$ of $V_{\text{max}}$, contributed to the thermal acclimation of photosynthesis to experimental warming [36,61,68]. However, in the current study we found that warming slightly decreased $\Delta H_{\text{i}}$ of $V_{\text{max}}$ (Fig. 5, Table 1). Biochemically, the change in $\Delta H_{\text{i}}$ of $V_{\text{max}}$ is closely related to the temperature dependence of Rubisco activity [69], Rubisco activation status [70,71], dimorphism of Rubisco [31], and the amount of Rubisco [72]. The lower $\Delta H_{\text{i}}$ of $V_{\text{max}}$ obtained from the warming plots indicated that warming slightly decreased the temperature sensitivity of those processes.

Previous studies found that RuBP regeneration processes may play an important role in the thermal acclimation of photosynthesis [34,39,73]. The increase in the thermal stability of photosystem II, indicated by $\Delta H_{\text{i}}$ of $V_{\text{max}}$, has been shown to be related to the thermal acclimation of $A_g$ to warming [34–36,74]. However, in the current study we found only minor response of $\Delta H_{\text{i}}$ of $V_{\text{max}}$ to warming (Fig. 5, Table 1). This is also confirmed by our results that the RuBP regeneration seldom limited $A_g$ (Fig. 8).

A number of studies have reported that the balance between the carboxylation and the regeneration of RuBP, indicated by $J_{\text{max}}/V_{\text{max}}$, can also affect the thermal acclimation of photosynthesis [39,75]. In our study, the experimental warming had little effect on the linear trend of $J_{\text{max}}/V_{\text{max}}$ ratio to $T_{\text{stem}}$ (Fig. 5). Nevertheless, in this study we found that $J_{\text{max}}/V_{\text{max}}$ ratio declined sharply and linearly with the instantaneous increase in $T_{\text{stem}}$ (Fig. 5). Many ecosystem models, such as Biome-BGC [76], have set $J_{\text{max}}/V_{\text{max}}$ ratio as a constant (2:1) which is independent of $T_{\text{stem}}$.

Wullschlager [77] analyzed 164 $A_g/C_o$ curves for 109 C3 plant species which were measured under $T_{\text{stem}}$ ranging from 13 to 35°C and found the average $J_{\text{max}}/V_{\text{max}}$ ratio was 2.1. Others found that $J_{\text{max}}/V_{\text{max}}$ ratio was not a constant instead varying with $T_{\text{stem}}$ through a linear [51,78–80] or nonlinear relationship [81]. Our current results show that the relationship (between $J_{\text{max}}$ and $V_{\text{max}}$) itself is highly temperature dependent, suggesting that photosynthesis models have to consider the temperature dependence of $J_{\text{max}}/V_{\text{max}}$ ratio.

In addition to biochemical limitations, the thermal acclimation of photosynthesis may also relate to CO2 diffusion processes in leaves and chloroplasts, such as $g_s$ and $g_{\text{ms}}$ because changes in $T_{\text{growth}}$ may affect CO2 diffusivity, solubility, membrane permeability and stomatal movement [82–85]. Previous studies have found that increasing $g_s$ and/or $g_{\text{ms}}$ can cause the increase of $T_{\text{opt}}$ of $A_g$ [36,40,41,67,86]. In the current study we found that warming increased $g_{\text{ms}}$ (Fig. 6) in 2010 which might contribute to the modest variation in $T_{\text{opt}}$ of $A_g$ in 2010. However, we found smaller increases in $g_s$ and $g_{\text{ms}}$ (Fig. 6) in 2011, which may explain the weaker acclimation in 2011 (Fig. 4). The differential responses of CO2 diffusion process to warming in the two hydrologically contrasting growing seasons could have been attributed to changes in soil moisture and N availability induced by warming [57]. It is noted that, so far, no consistent conclusions have been achieved on the warming effect on $g_s$ and $g_{\text{ms}}$. Some researchers found that
warming increased $g_d$ [39,88–90] and $g_m$ [91], and others found warming decreased $g_d$ [92] and $g_m$ [61], or no effect on $g_d$ [93] and $g_m$ [40]. Those various studies suggest that other factors, such as warming-induced water depletion and change in $N$ availability, may have interacting effects on responses of $CO_2$ diffusion process to warming. These results call for multi-factor experiments, such as the combination of warming with water manipulation and fertilization [21], for understanding the mechanisms of thermal acclimation of photosynthesis under future global change.

Balance between Respiration and Photosynthesis

The acclimation of foliar respiration and photosynthesis is also reflected in $R/A$ ratio which indicates the balance between carbon gain, loss and accumulation [1,2]. Our results show that the instantaneous (<5 h) warming at foliage level has non-linearly increased $R_d/A_g$ ratio, indicating proportionally more carbon loss through $R_d$ as $T_{leaf}$ goes up (Fig. 9). However, the 6-year experimental warming has resulted in thermal acclimation of the grasses as evidenced by the decrease of the curvature of the response curve of $R_d/A_g$ ratio to $T_{leaf}$ (Fig. 9). It is important to note that though the balance between $R_d$ and $A_g$ was re-established through the thermal acclimation [6,8,9,18], $R_d/A_g$ ratio was still increasing with $T_{growth}$ in a wet year (Fig. 9). This means that, at foliage level, acclimation can only partially compensate the negative impact from the global warming.

Supporting Information

Table S1 Results (P-values) of two-way ANOVA on the effects of warming, year, and both interactions on the responses of $A_g$ (the net $CO_2$ assimilation rate), $R_d$ (dark respiration), $A_u$ (the gross $CO_2$ assimilation rate), $V_{cmax}$ (the maximum rate of Rubisco carboxylation) and $J_{max}$ (the maximum rate of photosynthetic electron transport) expressed per unit foliar area and nitrogen to instantaneous change (10–40°C within a 5 h period) in $T_{leaf}$ (foliar temperature). $e$ is a scaling constant, $\Delta H^*_d$ is the activation energy, $\Delta S^*$ is an entropy sensitivity, and $r_{f10}$ is the estimated basal rate at the reference temperature of 10°C. Significant values ($P<0.05$) are shown bold.

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Appendix S1 User’s guide for the $A/C_v$ curve fitting model with measured respiration, modified based on Sharkey et al.’s [50] Microsoft Excel spreadsheet-based software to reduce the number of fitting parameters ($R_d$ is fixed in the model), version 1.2 (Last updated 25 July, 2012).

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Author Contributions

Conceived and designed the experiments: YC MX SW. Performed the experiments: YC RS QY. Analyzed the data: YC MX QY. Contributed reagents/materials/analysis tools: BH SW. Wrote the paper: YC MX RS QY BH SW.
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