

# Thermal acclimation of leaf respiration varies between legume and non-legume herbaceous

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## Abstract

### Aims

Ubiquitous thermal acclimation of leaf respiration could mitigate the respiration increase. However, whether species of different plant functional groups showing distinct or similar acclimation justifies the simple prediction of respiratory carbon (C) loss to a warming climate.

### Methods

In this study, leaf dark respiration ( $R_d$ ) of illinois bundleflower (IB, legume), stiff goldenrod (GR, C<sub>3</sub> forbs), indian grass, little bluestem and king ranch bluestem (IG, LB and KB, C<sub>4</sub> grass) were measured with detached leaves sampled in a 17-year warming experiment.

### Important Findings

The results showed that  $R_d$  at 20°C and 22°C ( $R_{20}$  and  $R_{22}$ ) were significantly lower in the warming treatment for all the five species. Lower  $R_{22}$  in warmed than  $R_{20}$  in control in GR, KB, LB and IG imply

acclimation homeostasis, but not in IB. The significant decline in temperature sensitivity of respiration ( $Q_{10}$ ) of GR resulted in the marginal reduction of  $Q_{10}$  across species. No significant changes in  $Q_{10}$  of C<sub>4</sub> grasses suggest different acclimation types for C<sub>3</sub> forbs and C<sub>4</sub> grass. The magnitude of acclimation positively correlated with leaf C/N. Our results suggest that non-legume species had a relative high acclimation, although the acclimation type was different between C<sub>3</sub> forbs and C<sub>4</sub> grasses, and the legume species displayed no acclimation in  $R_d$ . Thus, the plant functional types should be taken into account in the grassland ecosystem C models.

**Keywords:** acclimation, dark respiration, warming, legume, plant functional types

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## INTRODUCTION

Plant respiration, half of which comes from leaves (Atkin *et al.* 2007), releases ~60 Pg C per year (Canadell *et al.* 2007; King *et al.* 2006). Leaf dark respiration ( $R_d$ ) in plants is highly responsive to immediate changes in ambient temperatures. However, the relationship between  $R_d$  and temperatures may vary with growth environment. Thus, warming-induced changes in  $R_d$  are expected to have a large impact on simulated terrestrial ecosystem net carbon (C) exchange and C storage (Piao *et al.* 2010), and on the feedback to the future climatic warming (Huntingford *et al.* 2013). Nevertheless, acclimation of  $R_d$  in plants, an integration of biochemical and physiological adjustments of an individual plant to environmental change,

calls the long-term predictions of respiratory loss of C based on short-term temperature response functions into question (King *et al.* 2006). The inclusion of  $R_d$  acclimation in terrestrial ecosystem C models can increase the net primary production (Slot *et al.* 2014) thus dampen the positive feedback between warming and rising atmospheric CO<sub>2</sub> (Lombardozzi *et al.* 2015; Smith and Dukes 2013).

Acclimation of  $R_d$  to warming could decrease  $R_d$  at a set temperature (Ow *et al.* 2008, 2010; Reich *et al.* 2016; Slot and Kitajima 2015; Tjoelker *et al.* 1999, 2009), which may occur within a few days or longer exposure to a temperature change. Two types of  $R_d$  acclimation have been recognized to reflect different physiological mechanisms that underlie the acclimation process. In the case of type I acclimation,

$R_d$  is downregulated at a higher temperature but remains unchanged at low temperature ( $Q_{10}$ , temperature sensitivity coefficient: the proportional change in respiration with a 10°C temperature change, decreases with high temperature). Under type II acclimation,  $R_d$  decreases at all temperature (no change in  $Q_{10}$ ). Models using the temperature-driven basal respiration algorithm (similar to type II) reduce the estimated  $R_d$  at high temperature but elevate it at low temperature. And models using a temperature-variable  $Q_{10}$  (similar to type I) can considerably reduce  $R_d$  compared with those that used the static  $Q_{10}$  (Wythers *et al.* 2005). Thus, it will be valuable to identify acclimation types to aid in predictions of changes in respiratory fluxes (Slot and Kitajima 2015).

To get a general pattern and to understand the regulating factors of  $R_d$  acclimation for a universal use in dynamic vegetation C models, a lot of field and laboratory studies have been conducted on different species, original place of plants, plant functional types and biomes (Atkin and Tjoelker 2003; Atkin *et al.* 2005; Campbell *et al.* 2007; Dillaway and Kruger 2011; Larigauderie and Körner 1995; Reich *et al.* 2016; Slot and Kitajima 2015; Tjoelker *et al.* 1999). Unfortunately, diverse or even contradictory results of acclimation within the same or different plant functional types are reported. For instance, Tjoelker *et al.* (1999) and Zhou *et al.* (2007) showed conifer species and  $C_4$  grass have a higher degree of  $R_d$  acclimation than broad-leaved trees and  $C_3$  forbs while Campbell *et al.* (2007) and Slot and Kitajima (2015) found forbs, grasses, and evergreen trees share similarities in the extent of  $R_d$  acclimation. Species difference in  $R_d$  acclimation (Tjoelker *et al.* 1999) or a similar  $R_d$  acclimation regardless of the biome of origin and growth forms (Slot and Kitajima 2015) preclude/permit a simple prediction of the response of respiratory C loss to an elevated temperature at the ecosystem scale. Thus, an understanding of the species-specific differences of acclimation in this regard is critical to predict plant response to potential climate change.

Leaf nitrogen ( $N_{\text{leaf}}$ ) and carbon concentration ( $C_{\text{leaf}}$ ) are most frequently examined factors in explaining variations in  $R_d$  acclimation (Aspinwall *et al.* 2016; Atkin and Tjoelker 2003; Chi *et al.* 2013; Reich *et al.* 1998, 2008; Tjoelker *et al.* 1999) because  $N_{\text{leaf}}$  could represent a number of respiratory enzymes as well as protein contents and maintenance costs in terms of the abundance of mitochondrial proteins, i.e. alternative oxidase (AOX) (Atkin *et al.* 2015; Campbell *et al.* 2007; Nijs *et al.* 1995; Reich *et al.* 1998, 2008). However, whether the magnitude of  $R_d$  acclimation to an elevated temperature is affected by the warming-induced change in  $N_{\text{leaf}}$  is still under hot debate (Aspinwall *et al.* 2016; Chi *et al.* 2013; Lee *et al.* 2005; Ow *et al.* 2010; Tjoelker *et al.* 1999).  $N$  availability positively/negatively relates to  $Q_{10}$  of  $R_d$  in a temperate forest/grassland ecosystem (Chi *et al.* 2013; Turnbull *et al.* 2005), whereas it has little or no impact on  $Q_{10}$  and  $R_d$  when saplings are transferred to a different growth temperature regime (Ow *et al.* 2010). Investigation of  $R_d$  acclimation of nitrogen-fixing plants (NFP) might resolve this controversy since the

inclusion of them could provide an opportunity to investigate the relationship between  $N_{\text{leaf}}$  and  $R_d$  acclimation in a wide range of  $N_{\text{leaf}}$ . However, even in a comprehensive study of the global variability of  $R_d$  and its relation to climate and plant functional types (Reich *et al.* 2008), NFP species are not adequately presented. No constraint of  $N_{\text{leaf}}$  on  $R_d$  of NFP (Adams *et al.* 2016), and higher  $R_d$  in  $C_3$  forbs/grasses than in shrub and trees at any given  $N_{\text{leaf}}$  (Atkin *et al.* 2015) imply a high sensitivity of  $R_d$  in  $C_3$  forbs/grasses and a resistance of  $R_d$  in NFP to warming-induced change in  $N_{\text{leaf}}$ . NFP is not limited to legumes, but legumes are the primary NFP plants. Thus, we hypothesized a difference in acclimation pattern of  $C_3$  forbs,  $C_4$  grasses and legumes, and leaf traits like  $N_{\text{leaf}}$  and  $C_{\text{leaf}}$  might play a role for the difference.

## MATERIALS AND METHODS

### Site description

The warming experiment was conducted in McClain County, Oklahoma (34°59' N, 97°31' W). The field site is an old-field tallgrass prairie abandoned from agriculture 40 years ago without grazing for 35 years. The warming treatment launched from mid-August 1999 and continued. Mean annual temperature is 16.3°C, with monthly air temperature ranging from 3.3°C in January to 28.1°C in July. Mean annual precipitation is 915 mm, with monthly rainfall ranging from 30 mm in January to 135 mm in May (average values from 1948 to 1998; Oklahoma Climatological Survey). A silt loam soil in the grassland includes 35.3% sand, 55.0% silt, and 9.7% clay (Zhou *et al.* 2007). Soil carbon content is 1.42% with a mass base (Luo *et al.* 2001). The grassland is used to being dominated by  $C_4$  perennial grasses of *Schizachyrium scoparium*, *Sorghastrum nutans*, *Eragrostis curvula* and two  $C_3$  forbs, *Ambrosia psilostachya* and *Xanthocephalum texanum* (Zhou *et al.* 2007). The five species are visible in all pairs of plots at the beginning of the study. However, after decadal years of warming manipulation, species composition has changed. The grassland is currently dominated by the  $C_4$  perennial grasses, such as *S. scoparium* and *S. nutans*, and the  $C_3$  perennial forbs such as *A. psilostachya*, *Solidago rigida* and a few subordinate legume species, such as *Dalea candida*, *Desmanthus illinoensis* (Shi *et al.* 2015).

### Experimental design

The experiment used a paired factorial split-plot design. Warming was the primary factor and clipping was nested with warming (Luo *et al.* 2001). Within each of six pairs of 2 m × 2 m plots, one plot was heated continuously by using an infrared heater (100 W m<sup>-2</sup>; Kalglo Electronics, Inc., Bethlehem, PA) hung 1.5 m above the ground since 1999, while the other plot, equipped with a “dummy” heater, served as a control. The distance between each pair of plots was ~5 m. Each plot was divided into four 1 m × 1 m subplots. Plants in the diagonal subplots were clipped at the height of 10 cm above the ground annually. In this study, to rule out the interactive

effect of warming and clipping, leaves were only sampled in unclipped plots.

### Measurement of leaf respiration response curve to temperature

Thermal response of  $R_d$  was measured using a Li-Cor 6400 portable photosynthesis system (Lincoln, NE). A total of 133 temperature response curves were measured from May to August 2016. A severe drought in late July resulted in senescence of some leaves. Therefore, we cannot sample the similarly developed leaves for the control and warmed treatments. Since newly developed leaves and pre-existing leaves tend to show the different magnitude of acclimation (Slot and Kitajima 2015), we excluded the data in August in the following analysis.

Temperature response curves were measured on detached leaves from fully expanded, ruddy leaves both in the control and warming treatments. Leaves of stiff goldenrod (GR, *S. rigida*, C<sub>3</sub> forbs), illinois bundleflower (IB, *D. illinoensis*, legume), king ranch bluestem (KB, *Bothriochloa ischaemum*, C<sub>4</sub> grass), little bluestem (LB, *S. scoparium*, C<sub>4</sub> grass) and indian grass (IG, *S. nutans*, C<sub>4</sub> grass) were sampled pre-sunrise once a month and kept hydrated using plastic bags with watered paper towels in a growth chamber (VWR 2020 BOD low temperature incubator, MA) that can efficiently achieve a broad range of measurement temperatures (12, 15, 20, 28 and 35°C) for multiple leaves simultaneously. Sampling across experimental treatments was very carefully balanced for each species across different pairs of plots. We chose to measure detached leaves for two reasons: to increase the number of leaves sampled due to rapid heating and cooling of leaf temperatures in the measurement chamber and to increase the range of measurement temperatures in each response curve. Previous studies showed that  $R_d$  of detached tree leaves is stable under these conditions for several hours and longer (Reich et al. 2016; Slot et al. 2014). Also,  $R_d$  rates for detached leaves are comparable with a former *in situ* measurement in the study site (Zhou et al. 2007). Although the use of detached leaves entails several compromises, there is no apparent reason that detachment would change the thermal response of  $R_d$ , and more importantly, even if detachment influenced the thermal responses, it should affect leaves from ambient and warmed treatment similarly (Sendall et al. 2015).

Each morning, sampled leaves were placed in the growth chamber, which was set to 12°C. The leaf chamber of an infrared gas analyses system (LI-6400 portable photosynthesis systems, Li-Cor, NE) was also placed in the growth chamber, and leaf temperature in the cuvette was set to correspond with the growth chamber temperature. Once both chambers reached the target temperature, leaves were clamped in the leaf chamber for a minimum of 10 min, and 2–3 measurements were logged at a 30-s interval based on the stability status. Airflow was set at ~500  $\mu\text{mol s}^{-1}$ . Humidity within the leaf chamber was controlled to the best of ability. We route the incoming air through desiccant at low temperatures to keep relative

humidity ~85% and to avoid condensation.  $R_d$  was measured for each leaf at 12°C, after which both the growth chamber and leaf temperature were set to 15°C, and the process was repeated for all measurement temperatures in increasing order.

### Leaf carbon and nitrogen measurement

In each month of the  $R_d$  measurement, leaves were air dried and stored. At the end of July, leaves of each species of either the control or the warmed were mixed and ground in liquid *N* in a mortar. The ground samples were separated into three sub-samples. All the samples were sent to the Oklahoma State University soil testing lab (Still water, Oklahoma).

### Data analysis

Calculating the  $R_d$  temperature response curves rather than measuring  $R_d$  at a single temperature for all samples enables us to differentiate the different types of acclimation. Exponential equation with a fixed  $Q_{10}$  coefficient and a log-polynomial equation with temperature-sensitive  $Q_{10}$  were tested to establish the best equation for quantifying  $R_d$  as a function of short-term leaf temperature variation. The exponential model is:

$$R_d = a \times e^{bT}$$

where  $R_d$  is the measured respiration at target temperatures of a sample, the estimated coefficient,  $a$ , describes the basal respiration rate when the temperature is zero and  $b$  represents the  $Q_{10}$  of respiration.  $Q_{10}$  was calculated as:  $Q_{10} = e^{10b}$

In the log-polynomial model,  $R_d$  was fitted as following

$$R_d = e^{a+bT+cT^2}$$

$$\ln Q_{10} = 10b + 20cT + 100c$$

Comparison between the two models showed that they provided similar results for fitting short-term  $R_d$ —temperature (133 curves were successfully fitted with median  $R^2$  of 0.94 and 0.98, respectively). We, therefore, reported only the  $Q_{10}$  and  $R_d$  at standard measurement temperatures of 20°C ( $R_{20}$ ) and 22°C ( $R_{22}$ ) of each leaf calculated from the exponential model as Reich et al. (2016).

We calculated  $R_d$  at temperatures of 20°C and 22°C for both control and warmed treatments as they represent a temperature (20°C) that has been commonly used in other studies (Gunderson et al. 2000; Reich et al. 2016), and a temperature (22°C) due to the warming treatment (Wan et al. 2002). The  $R_d$  at 20°C and 22°C were used in following statistical analyses, and to calculate the acclimation. Acclimation ratios according to the set temperature method (Acclim<sub>SetTem</sub>) and the homeostasis method (Acclim<sub>Homeo</sub>) were calculated as Loveys et al. (2003).

$$\text{Acclim}_{\text{SetTem}} = \frac{R_{20} \text{ at control}}{R_{20} \text{ at warmed}}$$

If acclimation has taken place  $\text{Acclim}_{\text{SetTem}} > 1.0$ . To determine to what extent acclimation approached complete homeostasis of respiration across temperatures we used the homeostasis method (Loveys *et al.* 2003).

$$\text{Acclim}_{\text{Homeo}} = \frac{R_{20} \text{ at control}}{R_{22} \text{ at warmed}}$$

When acclimation is completely hemostatic,  $\text{Acclim}_{\text{Homeo}}$  is 1.0; the value of  $\text{Acclim}_{\text{Homeo}}$  less than 1.0 indicates that acclimation of the warmed leaf is not completely homeostatic.

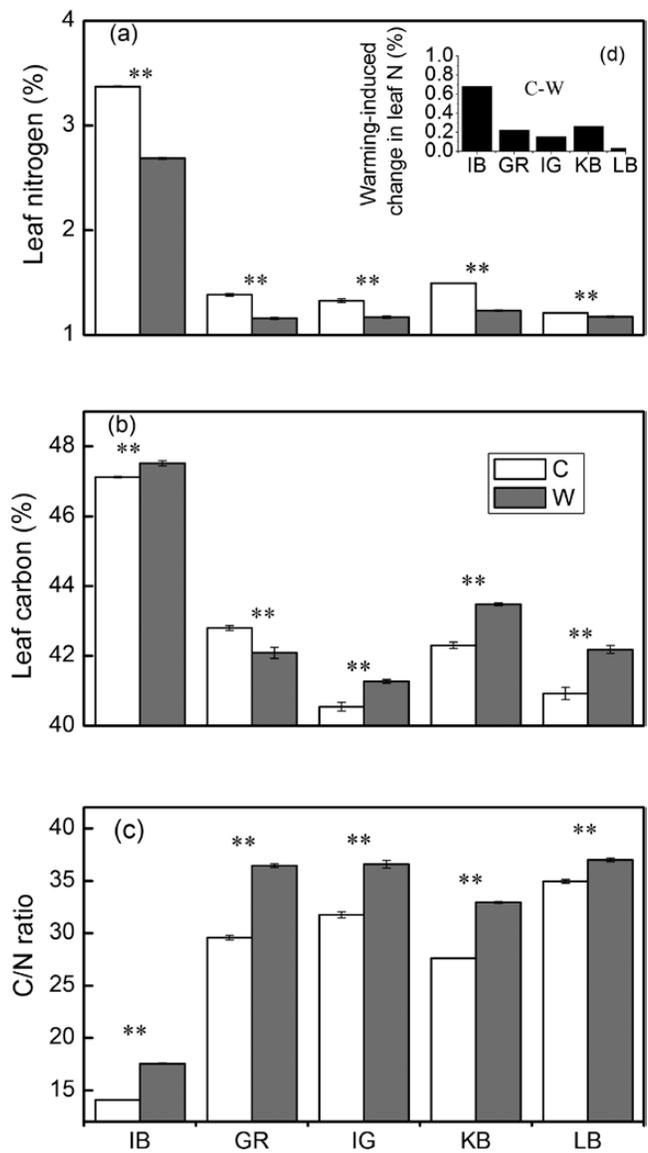
To accurately assess which type of acclimation has occurred, it is more common to compare the slopes and intercepts of log-transformed temperature response curves (Slot and Kitajima 2015). The  $R_d$  at each measuring temperature were averaged across all individuals of each species, and the averaged  $R_d$  was then fitted against temperature by using the log-polynomial function. The  $T$ -test was used to test the difference between fitting slopes and intercepts. The  $R_{20}$  at control and  $R_{22}$  at warmed plots were fitted by a general linear model against the  $N_{\text{leaf}}$  and  $C_{\text{leaf}}$  of each species at control and warmed plots, respectively to investigate the relationship between acclimation and leaf traits. In addition,  $\text{Acclim}_{\text{SetTem}}$  and  $\text{Acclim}_{\text{Homeo}}$  were fitted against C/N to study the coupling effect of  $N_{\text{leaf}}$  and  $C_{\text{leaf}}$  on acclimation magnitude.

The two-way ANOVA analysis tested warming effects and species difference in  $N_{\text{leaf}}$ ,  $C_{\text{leaf}}$ , C/N,  $R_{20}$ ,  $R_{22}$ , and  $Q_{10}$ .

## RESULTS

### Leaf carbon, nitrogen and C/N ratio

$N_{\text{leaf}}$ ,  $C_{\text{leaf}}$  and C/N varied significantly among species (Table 1) with the highest  $N_{\text{leaf}}$  in IB (Fig. 1a). Warming significantly increased the  $C_{\text{leaf}}$  by 1% whereas decreased the  $N_{\text{leaf}}$  by 15.3% across species, thus resulted in an increase in C/N (Table 1, Fig. 1). The warming-induced change in  $N_{\text{leaf}}$  and  $C_{\text{leaf}}$  also varied among species with IB has the highest absolute and relative reduction (20.4%) of  $N_{\text{leaf}}$  (Fig. 1a and d). The response of  $C_{\text{leaf}}$  to warming in GR was different from other species (Fig. 1b). Even reduced, the  $N_{\text{leaf}}$  of IB in warmed treatment was still higher than that in control of other species (Fig. 1a). C/N increased by 16.3% in the warmed treatment (average across all species). C/N of IB was lower than 20 even warming increased it whereas that of other species were all higher than 30 (Fig. 1c).



**Figure 1:** leaf nitrogen (a), carbon (b), C/N ratio (c), and warming-induced change in leaf nitrogen (d) of Illinois bundleflower (IB), Stiff golden Rod (GR), Indian Grass (IG), King Bluestem (KB) and Little Bluestem (LB) in the control (C) and warming (W) treatments. IB is a legume species; GR is  $C_3$  annuals, IG, KB and LB are  $C_4$  perennials. \*\*Statistically significant at  $P < 0.01$ .

**Table 1:** results ( $F$  values) of two-way ANOVA analysis of stimulated warming and species on the leaf dark respiration at 20°C and 22°C, temperature sensitivity of leaf respiration, leaf nitrogen concentration ( $N_{\text{leaf}}$ ), leaf carbon concentration ( $C_{\text{leaf}}$ ) and C/N ratio

Variance source	$N_{\text{leaf}}$	$C_{\text{leaf}}$	C/N	$R_{20}$	$R_{22}$	$Q_{10}$
Warming	3280.02**	56.75**	2023.62**	5.51**	6.15**	2.37 <sup>^</sup>
Species	21965.81**	1755.28**	5228.62**	7.97**	9.05**	0.34
Warming × Species	531.15**	57.58**	67.24**	0.49	0.54	0.52

\*\* $P < 0.01$ , \* $P < 0.05$ , <sup>^</sup> $P < 0.1$ .

## Leaf respiration

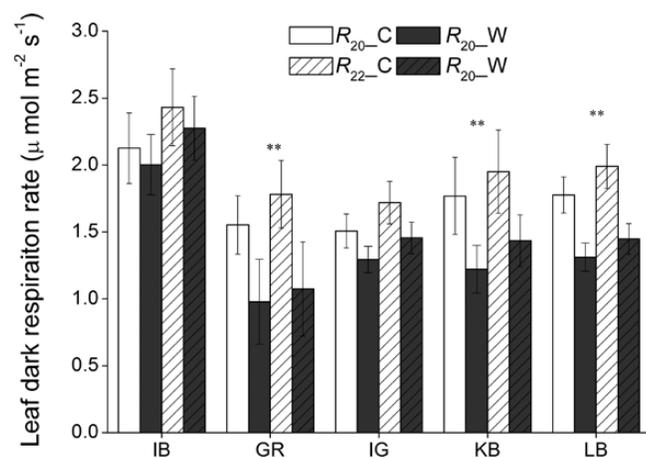
Across the species, plants in the +2°C warmed treatment had significantly lower  $R_{20}$  ( $1.74 \pm 0.09$  and  $1.39 \pm 0.093$  in C and W,  $P = 0.006$ ) and  $R_{22}$  ( $1.97 \pm 0.10$  and  $1.54 \pm 0.10$  in C and W,  $P = 0.003$ ) than in the control treatment (Fig. 2). The +2°C treatment marginally decreased the  $Q_{10}$  of  $R_d$  ( $1.88 \pm 0.07$  and  $1.78 \pm 0.07$  in C and W,  $P = 0.059$ ) indicating acclimation to increased growth temperature. The  $R_{20}$  in the control treatment was higher than  $R_{22}$  in the warmed treatment, especially for the non-legume species (Fig. 2), indicating acclimation homeostasis. The  $\text{Acclim}_{\text{Homeo}}$  was  $1.44 \pm 0.12$ ,  $1.03 \pm 0.04$ ,  $1.23 \pm 0.09$ ,  $1.22 \pm 0.04$  in GR, IG, KB and LB, respectively, implying an acclimation overcompensation (leaves in warmed treatment respire less at a high temperature than ambient-grown leaves at a low temperature), and that of IB was less than 1 indicating a partial acclimation.

The significant difference of fitting slopes was only for the GR (Fig. 3,  $T = 3.86$ ,  $n = 5$ ,  $P < 0.01$ ) and marginally significant intercept difference was observed in KB ( $T = 1.67$ ,  $n = 5$ ,  $P < 0.1$ ).

$R_{20}$  and  $R_{22}$  in either the control or warmed positively correlated with the  $N_{\text{leaf}}$  and  $C_{\text{leaf}}$  (Fig. 4). The fitting slope between  $N_{\text{leaf}}$  and  $R_d$  was significantly higher in the warmed than in control ( $T = 2.80$ ,  $n = 10$ ,  $P < 0.05$ , Fig. 4a); whereas that between  $C_{\text{leaf}}$  and  $R_d$  had no significant difference between the two treatments ( $T = 1.46$ ,  $n = 10$ ,  $P > 0.05$ , Fig. 4b).

## Across-species correlates of acclimation

We explored whether the difference in the degree of acclimation of the five species correlated with leaf traits that are associated with plant metabolism, using trait means of control and warmed leaves to characterize the species.  $\text{Acclim}_{\text{SetTem}}$  marginally correlated with average C/N of each species ( $R = 0.81$ ,



**Figure 2:** leaf dark respiration rate at 20°C and 22°C ( $R_{20}$  and  $R_{22}$ ) in the control (C) and warming treatments (W).  $R_{20}$  and  $R_{22}$  were estimated from the individual fitting of respiration against temperature. The \*\* above the columns mean the statistical difference of  $R_{20}$  in control treatment and  $R_{22}$  in warming treatment. Sample size by warming treatment is ambient = 68, warmed = 64.

$P = 0.093$ ,  $n = 5$ ).  $\text{Acclim}_{\text{SetTem}}$  and  $\text{Acclim}_{\text{Homeo}}$  exponentially related to leaf C/N (Fig. 5). With the infinite lower value of C/N, the  $\text{Acclim}_{\text{SetTem}}$  and  $\text{Acclim}_{\text{Homeo}}$  were 1.005 and 0.87, respectively, which means even  $N$  availability does not constraint plant growth  $R_d$  still can acclimate to warm temperature ( $\text{Acclim}_{\text{SetTem}} > 1$ ) but is not completely homeostatic ( $\text{Acclim}_{\text{Homeo}} < 1$ ).

## DISCUSSION

### $N_{\text{leaf}}$ and $C_{\text{leaf}}$ response to warming

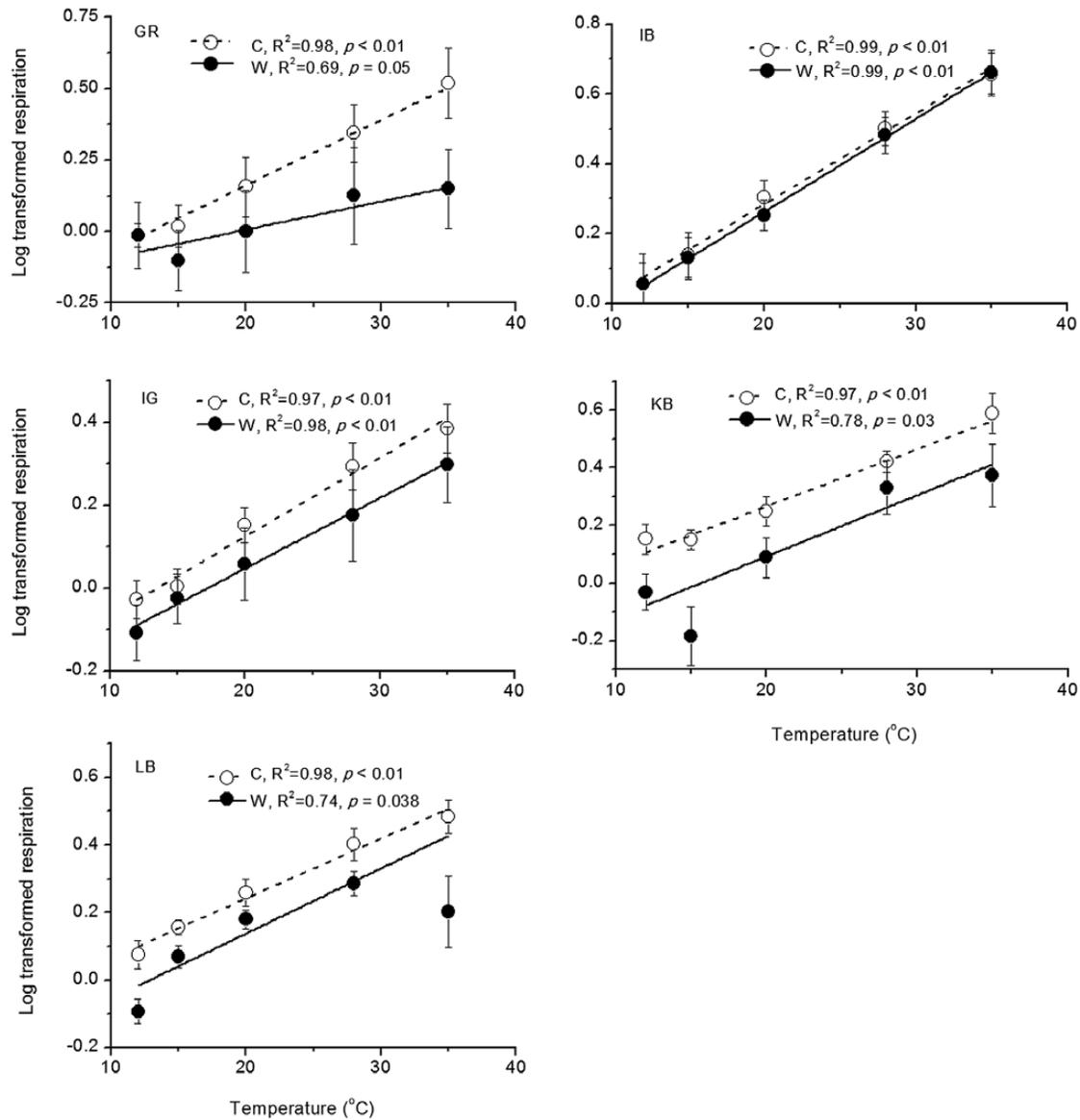
$N_{\text{leaf}}$  and  $C_{\text{leaf}}$  are widely used to explain the  $R_d$  acclimation in a warmer climate (Aspinwall et al. 2016; van De Weg et al. 2013; Tjoelker et al. 1999) as they generally correlate with  $R_d$  (Lee et al. 2005; Reich et al. 1998, 2008; Rowland et al. 2017). The reduction in  $N_{\text{leaf}}$  of all the species due to warming is in agreement with a study in a temperate grassland (Chi et al. 2013).  $N_{\text{leaf}}$  of tree species is more responsive than  $C_{\text{leaf}}$  to changes in growth temperature (Tjoelker et al. 1999). Our result of the higher change in  $N_{\text{leaf}}$  (15.3%) relative to  $C_{\text{leaf}}$  (1%) indicates the response of herbaceous  $N_{\text{leaf}}$  to warming is similar to tree species. The small positive effect of warming on  $C_{\text{leaf}}$  but the large adverse effect on  $N_{\text{leaf}}$  thus increased leaf C/N (Fig. 1).

The depressed net soil  $N$  mineralization in the study site (An et al. 2005) might contribute to the  $N_{\text{leaf}}$  reduction regardless of species. Unlike other species with mineral  $N$  as the sole source of nutrients, legumes can rely on  $N_2$ -fixation. The increased soil temperature and the associated reduction in soil moisture (Wan et al. 2002) might decrease the rhizobia living in the rhizosphere of legumes (Zahran 1999) thus force the legumes to rely more on mineral soil  $N$  in a warmer climate. The lack of significant response in  $R_d$  with additional  $N$  but without additional phosphorus ( $P$ ) suggests the co-limitation of  $N$  and  $P$  in controlling  $R_d$  (van De Weg et al. 2013; Heskell et al. 2014). The warming-induced decline in available  $P$  in grassland ecosystem (Geng et al. 2017) thus, might explain the higher relative  $N$  reduction in IB than other species since legumes are sensitive to  $P$  deficiency (Bargaz et al. 2015).

The fitted slopes between  $N_{\text{leaf}}$  and  $R_{20}$  (or  $R_{22}$ ) of the grasses species (Fig. 4) are comparable with foliage of boreal trees and shrubs (Reich et al. 1998, Tjoelker et al. 1999).  $N_{\text{leaf}}$  of IB in our study is much higher than *Populus deltoides* even with  $N$  addition (Ow et al. 2008). The inclusion of legume species enlarges the  $N_{\text{leaf}}$  range and might provide different insights into  $R_d$  acclimation for the grasses. The higher fitting slope between  $N_{\text{leaf}}$  and  $R_d$  in warmed treatment (Fig. 4) suggests that  $R_d$  is constrained by  $N$  availability in tallgrass prairie.

### Acclimation

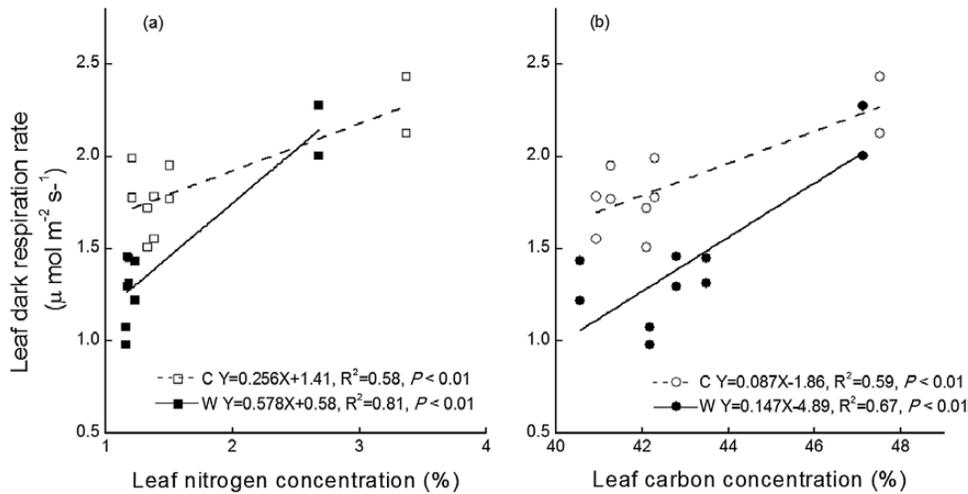
Acclimation could potentially halve the increase in  $R_d$  in a warmer climate (Vanderwel et al. 2015). The observed acclimation of IG in our study using detached leaves and measured acclimation of this species in the field (Zhou et al. 2007) support the reliability of using detached leaves to explore the  $R_d$  response of grass species to increase in growth temperature.



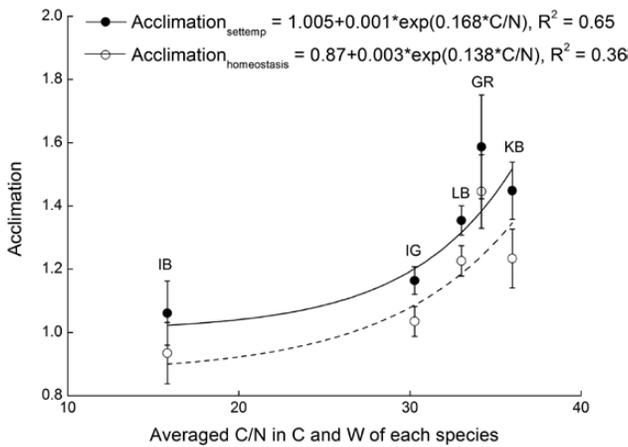
**Figure 3:** log-transformed fittings between measured leaf respiration against in the control (dashed lines) and warming (solid lines) treatment, respectively for five species. See Fig. 1 for the abbreviations of the five species. Respiration rate at a particular temperature was the average of measurements in May, June, and July (mean  $\pm$  SE).

Non-legume species showed a high  $R_d$  acclimation (Fig. 2,  $\text{Acclim}_{\text{Homeo}} = 1.23$  mean of four species, measurement temperature range: 12–35°C) relative to a review of prior studies (Slot and Kitajima 2015,  $\text{Acclim}_{\text{Homeo}} = 0.73 \pm 0.35$ , measurement temperature range: 5–21°C). Similar high acclimation has been reported recently in multi-year warming experiments with tree species (Drake *et al.* 2016; Reich *et al.* 2016). Substantial seasonal and day-to-day variation in growth temperature tends to result in higher acclimation potential in temperate than in tropical ecosystems (Cunningham and Read 2003). Thus, the relative high acclimation of tallgrass species (Fig. 2) could be a result of considerable variation in growth temperature.

Downward acclimation of  $R_d$  is associated with changes in leaf morphology and chemistry at higher temperatures (Aspinwall *et al.* 2016; Atkin and Tjoelker 2003; Atkin *et al.* 2005; Bostad *et al.* 2003; Harrison *et al.* 2009; Hartman and Nippert 2013; Lee *et al.* 2005; Tjoelker *et al.* 1999). Unlike the tree species (Aspinwall *et al.* 2016; Bostad *et al.* 2003), sharper fitting slopes of  $R_d$  with  $N_{\text{leaf}}$  than with  $C_{\text{leaf}}$  (Fig. 4), and the change in fitting slopes between  $R_d$  and  $N_{\text{leaf}}$  in warmed (Fig. 4a) imply the  $R_d$  acclimation is more likely to be controlled by  $N$  availability rather by leaf C in tallgrass prairie. Given that most dynamic global vegetation models use plant functional types to characterize vegetation, it would be valuable to identify systematic differences in acclimation potential



**Figure 4:** relationship of respiration with leaf nitrogen (a) and leaf carbon concentration (b). Blank symbols are the C/N ratio, and  $R_{20}$  and  $R_{22}$  of five species in the control treatment, filled ones are the C/N ratio, and  $R_{20}$  and  $R_{22}$  of them in warming treatment.



**Figure 5:** relationship of leaf dark respiration with leaf carbon to leaf nitrogen concentration ratio. Blank symbols are the averaged estimated respiration at 20°C and 22°C in control treatment for five species, and filled ones are the estimated respiration in warming treatment.

among plant functional types if such differences existed (Reich et al. 2016). Experimental and review studies show plants with different growth forms do not differ in their acclimation capacity (Campbell et al. 2007; Slot and Kitajima 2015). The difference in  $N_{leaf}$  and  $C_{leaf}$  could not account for the interspecific differences in the degree of  $R_d$  acclimation of hydroponically grown herbaceous, grass and tree seedlings (Loveys et al. 2003). However, different acclimation magnitudes and types among plant functional types (Figs 2 and 3) and C/N regulation on acclimation magnitude (Fig. 5) were observed in our study. The positive correlation between  $C_{leaf}$  and  $R_d$  (Fig. 4), and the reduction in  $C_{leaf}$  of GR (Fig. 1b), therefore, could contribute to the lower  $Q_{10}$  in GR in warmed treatment (Fig. 3) and the type I acclimation in GR. Type II acclimation is always accompanied by a decrease in  $N_{leaf}$  (Atkin et al.

2005; Lee et al. 2005; Tjoelker et al. 1999). Although warming declined the  $N_{leaf}$ , and IB has the highest reduction in  $N_{leaf}$ , the  $N_{leaf}$  of IB in the warmed was still much higher than other species in control (Fig. 1a), which might lead to the no acclimation in the IB. In addition to the particular importance of C and N, the C/N is also essential for the regulation of plant growth and development (Martin et al. 2002). However, several studies showed that N or C/N had little or no impact on the extent of thermal acclimation (Aspinwall et al. 2016; Bostad et al. 2003; Ow et al. 2008, 2010), which differs with the positive correlation between C/N and acclimation extent (Fig. 5). Across species and treatments,  $R_d$  acclimation of tree leaves to temperature is widely associated with changes in  $N_{leaf}$  and carbohydrates concentration (Tjoelker et al. 1999), which lends credence to the positive correlation between C/N and acclimation magnitude (Fig. 5). Different with a study in which C/N determines the acclimation type (Cheesman and Winter 2013), C/N only affects the acclimation magnitude in our study (Fig. 5) although the mechanism remains unclear. The difference probably stems from two aspects. One is the plants used (pot-grown seedlings vs. field grown individuals), and the other is the inclusion of legume species in our study which widens the range of  $N_{leaf}$  in examining the relationship between acclimation magnitude and leaf chemistry.

### Implication for the C cycles in tallgrass prairie

Plants adjust  $R_d$  to maintain a positive C balance to tolerate predicted temperature increase. Increased starch concentration under a warming treatment (Di Iorio et al. 2016) leads to C accumulation in acclimated ecosystems. For instance, using a variable ratio of  $R_d$  to photosynthetic capacity with temperature (accounting for  $R_d$  acclimation) rather than a fixed ratio of 10% in an ecosystem model (no consideration of  $R_d$  acclimation) results in an increase in predicted ANPP ~18% of the tallgrass prairie (Wythers et al. 2005). Such C-savings strategy

conforms to the findings of high net ecosystem exchange (NEE) in a warmer climate. Greater acclimation in  $C_3$  forbs than in  $C_4$  grasses (Fig. 2) could in part explain the slight increase in NEE in  $C_4$ -dominated grassland, but significant increase NEE in a  $C_3$ -dominated grassland (Niu *et al.* 2013). Results of a meta-analysis (Slot and Kitajima 2015) suggest that the acclimation can be addressed in most models without the need to define plant functional type-specific acclimation potential other than distinguishing evergreen from deciduous vegetation. While different types of acclimation for the  $C_3$  forbs and  $C_4$  grasses, and legume species implies that plant functional type, at least the legume and non-legumes should be considered in dynamic vegetation models.

## CONCLUSION

In this field study, warming declined the  $N_{\text{leaf}}$  with the highest reduction in legume species.  $C_{\text{leaf}}$  had a lower decrease, consequently resulted in an increase in C/N regardless of species and plant functional types. Legume species showed no acclimation. Non-legume species had a relatively higher acclimation magnitude than tree seedlings.  $C_3$  forbs showed the type I (decline in  $Q_{10}$ ) and the  $C_4$  grasses demonstrated type II acclimation (no change in  $Q_{10}$ ). The  $N_{\text{leaf}}$  positively correlated with the  $R_d$ , suggesting  $R_d$  acclimation is more likely to be controlled by N availability in tallgrass prairie. The higher  $N_{\text{leaf}}$  of legume species even in the warmed may ensure no acclimation of its  $R_d$ . Higher leaf C/N ratio corresponds with greater acclimation magnitude in other non-legume species.

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