Title: **Predicting ecosystem carbon balance in a warming Arctic: the importance of long-term thermal acclimation potential and inhibitory effects of light on respiration**

Running title: temperature response of light respiration

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Abstract

The carbon balance of Arctic ecosystems is particularly sensitive to global environmental change. Leaf respiration (R), a temperature-dependent key process in determining the carbon balance, is not well understood in Arctic plants. The potential for plants to acclimate to warmer conditions could strongly impact future global carbon balance. Two key unanswered questions are (1) whether short-term temperature responses can predict long-term respiratory responses to growth in elevated temperatures and (2) to what extent the constant daylight conditions of the Arctic growing season inhibit leaf respiration. In two dominant Arctic species *Eriophorum vaginatum* (tussock grass) and *Betula nana* (woody shrub), we assessed the extent of respiratory inhibition in the light (R_L/R_D), respiratory response to short-term temperature change, and respiratory acclimation to long-term warming treatments. We found that R of both species is strongly inhibited by light (averaging 35% across all measurement temperatures). In *E. vaginatum* both R_L and R_D acclimated to the long-term warming treatment, reducing the magnitude of respiratory response relative to the short-term response to temperature increase. In *B. nana*, both R_L and R_D responded to short-term temperature increase but showed no acclimation to the long-term warming. The ability to predict plant respiratory response to global warming with short-term temperature responses will depend on species-specific acclimation potential and the differential response of R_L and R_D to temperature. With projected woody shrub encroachment in Arctic tundra and continued warming, changing species dominance between these two functional groups, may impact ecosystem respiratory response and carbon balance.

Keywords: acclimation, Arctic, *Betula nana*, climate change, *Eriophorum vaginatum*, Kok effect, tundra

Introduction

Arctic tundra covers approximately 4.3 million km^2, and is among the most vulnerable ecosystems to climate warming (IPCC, 2007). Given the system's large carbon (C) reservoirs (as much as 1/3 of the global soil C pool (Callaghan *et al.*, 2004)) and low vegetative productivity (Williams & Rastetter, 1999), climate change in the Arctic could dramatically and disproportionately alter the global C cycle. Thus, a better understanding of the physiological processes that control productivity and C exchange is needed (Rastetter *et al.*, 2010).

Plant respiration (R) is a key process determining C balance, yet it is poorly understood in Arctic ecosystems and rarely measured at the leaf-level where a predictive mechanistic understanding is needed. R is particularly difficult to quantify in the constant daylight of the arctic growing season. Although R can be measured in darkened tissues (R_D) (*e.g.* Chapin & Oechel, 1983, Muraoka *et al.*, 2008, Shaver *et al.*, 1998), in the light mitochondrial metabolism is significantly reorganized in comparison to its status in the dark (Hurry *et al.*, 2005). There is likely an inhibitory effect of photosynthetic products on respiratory function (Graham, 1980, Tovar-Mendez *et al.*, 2003, Wang *et al.*, 2001), and an associated downregulation of multiple metabolic processes in the light (Sturm *et al.*, 2005, Tcherkez *et al.*, 2012). Still R continues in the light (R_L) (Kromer, 1995), and is particularly important to Arctic plants because of the extended daylight conditions of their growing season.

R is also subject to long-term thermal acclimation (days to months), and this acclimation itself is species dependent and responsive to environmental conditions (Kruse *et al.*, 2011), including

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ambient temperature and physiological history (Amthor, 1989, Atkin *et al.*, 2000). The precise acclamatory mechanisms are not fully understood and therefore difficult to model (*reviewed in:* Atkin & Tjoelker, 2003, Kruse *et al.*, 2011). Regardless, due to its high thermal sensitivity, R may be influenced by global warming, potentially impacting large-scale ecological processes.

In the short-term (minutes to hours), R rates tend to increase exponentially with higher leaf temperature as a function of enzyme activity, substrate availability or adenylate control (*reviewed in:* Atkin & Tjoelker, 2003). The few studies that exist on the temperature response of R_L, or the degree of light inhibition of R, show varying effects dependent on growth conditions (Ayub *et al.*, 2011, Shapiro *et al.*, 2004). To our knowledge the short-term temperature response of R_L has never been measured in Arctic plants.

An understanding of warming effects on the C balance of Arctic ecosystems requires that respiratory C losses (R_L) be placed in the context of photosynthetic C gains (A). Although A can also thermally acclimate (*reviewed in:* Chen & Zhuang, 2013), species from colder climates have shown a lower capacity to do so (Atkin *et al.*, 2006), suggesting that Arctic plants may be particularly sensitive to warming. If A acclimates less than R, a thermally adjusted R would result in a lower A/R, correlating with slower growth rates (Atkin *et al.*, 1996, Poorter *et al.*, 1990) and a higher proportion of fixed C respired in warmer conditions.

Modeling exercises have demonstrated that assumptions regarding the degree of thermal acclimation of photosynthesis and respiration can impact predictions of ecosystem C balance (King *et al.*, 2006, Wythers *et al.*, 2005). Atkin *et al.* (2005) outlined a means of quantifying

observed respiratory acclimation, but no methods exist for predicting when or where respiratory acclimation will happen, thus complicating models of the effects of warming on ecosystem C balance. Lacking mechanistic information, most models simply predict the long-term temperature response of R from short-term temperature response measurements or, more problematically, ignore the possibility of thermal acclimation. Further, only two studies (Heskel *et al.*, 2013a, Heskel *et al.*, 2012) report R_L in Arctic plants, and a more robust understanding of this process is needed for C balance models of the Arctic that assess gross photosynthesis from net photosynthesis or net ecosystem exchange.

We quantify the short-term temperature response and degree of long-term thermal acclimation of R to better understanding the role of leaf R in the response to warming of *Betula nana* and *Eriophorum vaginatum*. These species represent two dominant functional types with opposite projected distributional responses to warming (Elmendorf *et al.*, 2012). We quantify the degree of light inhibition of R by measuring the Kok effect (Kok, 1948). Specifically we ask whether short-term temperature response curves can be used to predict the effects of long-term warming treatments. Finally we consider the errors associated with various assumptions regarding R_L and respiratory acclimation when modeling leaf respiratory C losses and Net Primary Production (NPP) under various warming scenarios.

Methods

Study Site

The research site is located in tussock tundra vegetation within the Toolik Lake Long Term
Ecological Research site, Alaska (68°38′N, 149°43′W). The site is dominated by graminoids (*E.
vaginatum* and *Carex bigelowii*), and deciduous shrubs (*Betula nana, Salix* spp.) (Shaver &
Chapin, 1991). Greenhouse warming treatments began in June of 1989 with a fully randomized
blocked design with 4 treatment blocks. All treatment plots are 5m×10m, with walkways
between plots for access (Gough & Hobbie, 2003). Greenhouses are rectangular (~2.5 m×5 m)
wooden frames with 65 cm vertical walls and a gabled roof 130 cm high (increased over time
due to shrub growth), covered with polyethylene plastic. The plastic is removed at the end of
August and replaced in May or June. Greenhouse air temperatures are elevated 3-5 degrees
above controls; and effects on soil temperatures, relative humidity and photosynthetically active
radiation (PAR) are outlined in other work (Chapin *et al.*, 1995, Hobbie & Chapin, 1998).

Field measurements

We used the 'Kok effect' (Kok, 1948) to estimate leaf R_L and R_D. This method measures the
response of *A* over incrementally decreasing irradiance at the ambient CO_2 concentration,
effectively eliminating the CO_2 gradient and the potential diffusion of CO_2 into the cuvette. The
'Kok effect' specifically refers to the break in the slope of the measured photosynthetic rate in a
light response (*AQ*) curve when taken with high resolution light increments over low irradiances.
At very low irradiance the slope, or quantum yield, of photosynthesis is relatively steep; at the
vicinity of the light compensation point, a distinct break occurs and the slope decreases.

Extrapolating the linear regression of photosynthesis measured at irradiances below the break to 0 PAR extends to R_D; the regression line for irradiance above the break extrapolates to R_L (SI Figure 1). At high irradiances the photosynthetic rate saturates, so when calculating R_L the only points included are those in the linear section of the *AQ* curve above the light compensation point, but well below the saturated rate (Singsaas *et al.*, 2001). Both of these regressions must be corrected to conditions of constant intercellular CO_2 (Ayub *et al.*, 2011, Kirschbaum & Farquhar, 1987).

Open flow gas exchange measurements were made *in situ* (Li- Cor 6400; Li-Cor Inc., NE, USA) at a series of three different temperatures between 5 and 25 degrees °C, depending on ambient conditions. Three plants were measured in the control and greenhouse treatments in each of the 4 blocks. Light response curves were measured with a minimum of 20 points below 100 μmol m^{-2} s^{-1} and 10 points below 20 μmol m^{-2} s^{-1} PAR. All measurements were made at ambient CO_2. Measurements on *B. Nana* (N=24) were made in July 2005 and measurements on *E. vaginatum* (N=78) were made in July of 2006, with half the measurements taken in the greenhouse treatments and half in the controls. The measurement temperature ranged between 7 to 23°C for *E. vaginatum* and 10 to 20°C for *B. nana* because of fluctuations in the ambient environmental conditions, with mean temperature for *B. nana* slightly higher than the mean for *E. vaginatum* (16.2 versus 14.9 ˚C). A single statistical outlier point was removed from the data set. The photosynthetic rate at saturating irradiance was also measured to estimate the ratio of photosynthesis to respiration. Following (Farquhar & von Caemmerer, 1982), rates of RuBP oxygenation (V_o) by Rubisco in the upper irradiance range used to estimate R_L (100 μmol m^{-2} s^{-1} PPFD, where inhibition of photosynthesis is likely to be maximal) were used to assess

relationships between R_L and associated rates of Rubisco activity (Ayub *et al.,* 2001; Crous *et al.,* 2012).

Statistical analysis

The temperature response curves of R_D and R_L were analyzed by fitting all R (dark or light) measurements of one treatment plot to a modified Arrhenius equation (Lloyd & Taylor, 1994): $R = R_0 \exp [(E_0 / R_g) (1/T_0 - 1/T_a)]$ where R_0 is the respiration rate at a base temperature T_0 (10 °C, 283 K in our study), T_a is the leaf temperature (K) when R is measured, and R_g is the ideal gas constant (8.314 J mol^{-1} K^{-1}). E_0 is equivalent to the overall energy of activation of the processes, similar but not identical to the energy of activation for a single enzyme reaction, so E_0 should simply be considered a temperature response variable. The model was fitted with SigmaPlot (Systat Software Inc., San Jose, CA, USA). The respiration rate at 15 °C was also calculated in order to compare respiration rates at a moderate temperature.

The commonly used Q_{10}, which is a simple parameter to measure respiratory temperature response, can be linked to this model by*:*

$Q_{10} = \exp [(E_0 / R_g) (1/T_2 - 1/T_1)]$

and,

$T_1 - T_2 = 10$ (ºC).

As defined by this model, Q_{10} is temperature dependent (Atkin & Tjoelker, 2003) and is determined by E_0 at a set temperature. In this study, a Q_{10} for the range of 10 – 20ºC was calculated to facilitate comparison with other studies reporting only Q_{10} values. Our approach neglects any response to temperatures approaching or surpassing the temperature optimum for

respiration and thus the interpretation should be constrained to the range of observational

measurement temperatures. The potential for a temperature dependency of the T optimum of

respiration is not addressed.

To examine the effect of the greenhouse treatment and light on the respiratory temperature

responses, the Arrhenius equation was transformed to a linear format ($\ln R = \ln R_0 + E_0/R_g \times f(T)$,

$f(T) = 1/T_0 - 1/T$) and E_0 (the slope) was compared between treatments (greenhouse vs. control)

and light (R_L vs. R_D) with ANCOVA (Warton *et al.*, 2006). Treatment, light, and f(T) were

respectively set as two main factors and the covariate; their main and interactive effects on $\ln R$

were tested. The effect of f(T) indicates the significance level of E_0 for all data; the main effect

of treatment and light indicates whether $\ln R$ was significantly different between treatments or

light levels at the mean of f(T) (T=14.9 °C for *E. vaginatum* and 16.2 °C for *B. nana*); the

f(T)×treatment and f(T)×light interactions respectively indicate different E_0 between treatments

or between R_D and R_L. The analysis was done separately for the two species (Datadesk 6.0, Data

Description, Ithaca NY, USA).

To determine whether R_L/R_D (a measure of light inhibition of R) in each species was different

from zero, we used a one sample, two-tailed t-test. To detect an effect of temperature on R_L/R_D,

linear regressions for each species and each treatment were performed (Systat version 11.0,

Systat Software Inc., San Jose, CA, USA). The magnitude of R_L/R_D and the slope of R_L/R_D

against temperature were compared between species and treatments with ANCOVA. The effect

of species, treatment (main factors) and temperature (covariate) and their interactions on R_L/R_D

and V_o were examined (Datadesk 6.0, Data Description, Ithaca NY, USA). The main effect of

treatments and species indicate whether R_L/R_D was the same (at the mean temperature of all measurements, 16.0 °C) between treatments or species. Significant temperature $\times$ treatment and temperature $\times$ species interactions indicate different temperature response between treatments or species respectively.

Leaf gas exchange modeling

We used a physiological model that incorporates the R_L and R_D responses into a Farquhar based photosynthesis model to examine the net effect of light respiratory inhibition and respiratory thermal acclimation on leaf-level gas exchange (see SI Model Description for details). Gross primary productivity (GPP), respiration, and net primary productivity (NPP) were calculated at the leaf-level. We conducted two modeling exercises to examine the impact of ignoring light respiratory inhibition or respiratory thermal acclimation on estimations of leaf-level carbon exchange.

First, we calculated leaf respiration with and without the Kok effect and estimated the error caused by ignoring light inhibition of R. To incorporate the Kok effect into the model, the leaf respiration rate was calculated with the temperature response of R_L when the light level was above 10 μmol m^{-2} s^{-1} and with the response of R_D for lower light levels. Without the Kok effect, leaf respiration was calculated with parameters of R_D only. The error caused by ignoring light inhibition of R was estimated. We further incorporated photosynthetic parameters to calculate the effect of ignoring light inhibition of R on leaf NPP estimates. This model exercise was conducted for the growing seasons of 2004 to 2006 (June 10th to August 20th).

Second, to estimate the error caused by ignoring leaf thermal acclimation, we used photosynthetic and respiratory parameters of control and greenhouse-acclimated leaves to calculate leaf gas exchange under different warming scenarios. Leaf-level GPP, R and NPP were calculated for the growing season of 2006 (June 10th to August 20th) and for three warming scenarios (plus 2.5, 5, and 7 ºC relative to 2006). Both R_L and R_D were incorporated into the physiological model in this exercise. To estimate leaf gas exchange, we generated parameters for current conditions using leaves from the control treatment for 2006 and by using parameters of greenhouse-acclimated leaves for the various warming scenarios. The error of not incorporating leaf thermal acclimation and the relative contribution of photosynthesis and respiration on the error of leaf-level NPP was estimated.

Results

Kok effect

R of *B. nana* and *E. vaginatum* was inhibited by light in the control and the greenhouse
(p<0.001). The respiration rate in light was reduced to 35% of the rate measured in darkened
leaves (SI Figure 2a) when averaging all measurements conducted under ambient conditions for
both species. The Kok effect $(100-(R_L/R_D\times100))$, or percent inhibition of R in the light, ranged in
E. vaginatum from 2-83% depending on temperature, with a mean of 37%; and in *B. nana* from
3-52% with a mean of 28%. The Kok effect was different between species, with higher percent
inhibition in *E. vaginatum* (SI Figure 2a, p<0.01). *E. vaginatum* showed higher V_o at 100 µmol
PPFD m^{-2} s^{-1} (0.93 µmol O$_2$ m^{-2} s^{-1}) than *B. nana* (0.15 µmol O$_2$ m^{-2} s^{-1}), and there was no
difference between the control and greenhouse (SI Figure 2b).

Short-term respiratory temperature response and thermal acclimation of respiration

Overall, R responded positively to changes in short-term temperature in both species (p<0.0001),
and there were significant effects of treatment and species on both E_0 and on R (Tables 1, 2a). In
E. vaginatum, E_0 (slope) was different between treatments, with the respiration rate less sensitive
to temperature (lower E_0) in the greenhouse than in the control (f(T) × Greenhouse interaction,
(p=0.02) (Table 1). E_0 was also different between R_L and R_D (as indicated by f(T) × Light
interaction, p<0.001) (Table 2a). In *E. vaginatum, R_L* increased with short-term temperature in
the control (p<0.0001), but did not respond significantly to short-term temperature in the
greenhouse (Figure 1); R_D increased with short-term temperature in the control (p<0.0001) and
the greenhouse (p=0.001) (Figure 1). In *E. vaginatum, Q_{10}* values were lower for R_L than for R_D

(Figure 2a), and overall E_0 values were lower for R_L than for R_D (Table 1). R was lower in the greenhouse than in the control when temperature was over 10 ˚C, in both light and dark measurements (Figures 1, 2c).

In *B. nana*, there was no treatment effect on E_0, or Q_{10} (Table 1, 2a, Figure 2b, d). R_L and R_D also had similar E_0 and Q_{10} (Table 1, Figure 2b). R_L increased with short-term temperature in the control (p=0.007) and the greenhouse (p=0.0003) as did R_D (p=0.0003 for control and p<0.0001 for greenhouse).

There were significant species and species by temperature effects on the degree of inhibition of R in the light (R_L/R_D) (Table 2b). R_L/R_D increased with short-term temperature in both control and greenhouse treatments for *E. vaginatum* (p<0.001 in both, Figure 3a). In contrast, we did not detect a significant trend or treatment effect in *B. nana*.

An ANCOVA model including both species suggested significant effects of temperature, species and a 3-way interaction on V_o (Table 2b). On average, V_o of *E. vaginatum* was 0.89 (SE 0.07) μmol O_2 m^{-2} s^{-1} for the control and 0.97 (SE 0.04) μmol O_2 m^{-2} s^{-1} for the greenhouse, while V_o of *B. nana* was 0.18 and 0.11 μmol O_2 m^{-2} s^{-1}, respectively for control and greenhouse. Overall, V_o increased exponentially with temperature. For *E. vaginatum*, the positive V_o-temperature relationship applied to both treatments, but for *B. nana*, this relationship was significant for the control but not greenhouse (Figure 3b).

Photosynthesis to respiration ratio

We did not detect treatment differences in A_{max} at 15 ºC in *E. vaginatum* or in *B. nana*. A_{max} values for *E. vaginatum* were 17.9 (SE 1.3) in the greenhouse and 19.9 in the control (SE 1.5); and for *B. nana* were 13.6 in the greenhouse (SE 0.73) and 12.2 in the control (SE 0.72). In both species, the ratio of A_{max} to R (at 15 ºC) was significantly higher in light than in darkness for the greenhouse, but not for the control (Figure 2e, f).

Leaf gas exchange modeling

Leaf-level GPP was 3 to 4 times leaf R for *E. vaginatum* and 9 to 12 times leaf R for *B. nana* (Table 3a). Ignoring the Kok effect caused substantial error in estimating leaf R, especially for *B. nana* (+33% to +49%), but the impact on estimates of leaf-level NPP was limited (2%-11%).

E. vaginatum displayed strong positive thermal acclimation of photosynthesis, and estimates of leaf GPP increased 38%, 47% and 53% in the plus 2.5, 5 and 7 ºC warming scenarios respectively (bold fonts, Table 3b). Not accounting for thermal acclimation resulted in underestimating leaf GPP up to 21% in the three warming scenarios. R of *E. vaginatum* acclimated to warming, *i.e.* greenhouse grown leaves had lower respiration rates in warming scenarios (5.1 to 5.6 mol CO_2 m^{-2} leaf) than control leaves in 2006 (5.9 mol CO_2 m^{-2} leaf). Ignoring thermal acclimation caused an overestimation of leaf R by as much as 48% in the three warming scenarios. For leaf NPP, ignoring thermal acclimation led to a nearly 30% underestimation of leaf R in *E. vaginatum*.

Estimates of leaf GPP of *B. nana* were almost constant across 2006 and across the three warming scenarios (34 to 39 mol CO_2 m^{-2} leaf for June 10 to August 20). For *B. nana* the error caused by ignoring thermal acclimation in estimating NPP was small (5 to 8% overestimation).

Discussion

Our study species represent important and frequently dominant vegetation in Arctic tundra
(Elmendorf *et al.*, 2012) and thus may be representative of the total leaf respiratory flux from
terrestrial vegetation in this ecosystem. We observed strong respiratory inhibition in the light for
both *E. vaginatum* and *B. nana*, and species-specific patterns in the short-term temperature
response of light inhibition of *R* and in respiratory acclimation to long-term warming.
Furthermore, we found no difference in leaf δ^{13}C values (SI Figure 3a), indicating similar water
use efficiency across treatments, thus a low chance that the higher humidity in the greenhouses
drove our findings. Extrapolating beyond the specific measurement conditions represented in
this study would require a mechanistic interpretation of the underlying processes. However, by
integrating our results into a leaf-level carbon flux model, we demonstrate that the common
practice of substituting R_L with R_D, or ignoring respiratory thermal acclimation causes substantial
error in estimates of leaf respiratory flux.

Kok effect

Our findings of substantial inhibition of *R* in the light, consistent with Heskel *et al.* (2012 &
2013a), emphasize the importance of accounting for light inhibition when calculating the
ecosystem scale leaf respiratory flux in Arctic systems. Currently, all eddy-covariance
measurements face a challenge when attempting to decompose the net CO_2 exchange into the
component mechanistic fluxes and this problem is intensified in the Arctic because of the
extended duration of the light period (Heskel *et al.*, 2013b). Interpreting the net C flux signal
recorded by an eddy-flux tower requires that a modeled respiratory flux (both autotrophic and
heterotrophic) be subtracted to determine the rate of canopy photosynthesis. Our quantification

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of *R* in the light suggests that past efforts to derive these estimates based on *R* measured in the dark overestimate actual *R* and total respiratory C flux. Our results further demonstrate that in *E. vaginatum* the Kok effect increases with short-term temperature change for both treatments, and we saw no change in the magnitude of the effect between the control and greenhouse treatment. This indicates that the importance of the Kok effect may increase with climate warming in this species and thus may have an even larger impact on overall C flux estimates in the future.

The precise causes and mechanisms of the Kok effect are presently unknown, and thus a biochemical or physiological interpretation of our results requires caution. Cellular energy status is often cited as the general driver for the inhibition of respiration in the light (Atkin *et al.,* 2013, Griffin & Turnbull, 2013) with energy demand being met by light driven processes rather than from stored reduced substrates during periods of illumination. Mechanistically this could be realized if excess ATP or redox equivalents generated by the light reactions of photosynthesis were to deactivate the pyruvate dehydrogenase (PDH) complex (Budde & Randall, 1990, Gemel & Randall, 1992). PDH can also be regulated by substrates like pyruvate and CoA, and the product Actyl CoA, linking light inhibition of respiration to photorespiration, amino acid formation and a general reorganization of the TCA pathway (Igamberdiev *et al.*, 2001, Tcherkez *et al.*, 2008, Tcherkez *et al.*, 2005, Tcherkez *et al.*, 2009). Citrate and other stored C compounds may be utilized in the light and would reduce demand for flux through the TCA pathway (Gauthier *et al.*, 2010, Tcherkez *et al.*, 2012). Reorganizing the TCA pathway as suggested by Tcherkez *et al.* (2012), and removing C compounds to support the synthesis of glutamate or other amino acids would increase the transfer of amino groups via photorespiration and reduce overall rates of CO_2 release from the mitochondria. Other suggested mechanisms include NAD(P)H balance between respiration, chloroplastic pentose phosphates and photosynthesis

(Buckley & Adams, 2011, however see Cornic & Jarvis, 1972); a general non-linear response of RuBP oxygenation (V_o) to light, particularly in the low light range (Cornic, 1977); other environmentally driven changes in V_o (Griffin & Turnbull, 2013); and metabolic non-steady-state effects (Tcherkez *et al.*, 2012). Although our understanding of the precise mechanisms of the Kok effect remains incomplete, much progress is being made and it is important that our interpretation of the ecological responses proceeds in a consistent manner.

Thermal acclimation of respiration and light inhibition

Impacts of warming on plants will depend on the acclimation potential of the metabolic apparatus. Respiratory acclimation to temperature occurs in some species (Atkin & Tjoelker, 2003, Teskey & Will, 1999) but not in others (Dillaway & Kruger, 2011, Way & Sage, 2008). The studies above evaluated acclimation using R_D, and the temperature response of R_L and its thermal acclimation is rarely reported (but see Ayub *et al.*, 2011). Here, we report an acclimation response of leaf R to warmer growth temperatures in *E. vaginatum*, but not in *B. nana*.

In contrast to *E. vaginatum*, *B. nana* showed no significant difference in R_0 or E_0 between the greenhouse and control treatments, indicating a lack of acclimation to warmer growth temperatures. Acclimation can be a means to avoid excessive C losses from higher respiration rates, however given *B. nana's* increased individual size and geographic range in tundra during the past several decades (Heskel *et al.*, 2013a, Heskel *et al.*, 2012), this species may instead use excess respiratory products for increased growth in a warmer climate. Net C gain and dynamics of respiratory intermediates need to be examined to test this hypothesis. This capability may contribute to the continued expansion of this shrub.

Few studies have addressed the thermal acclimation of the Kok effect, and results vary by species. Growth temperature had little impact on the degree of light inhibition of leaf R in *Eucalyptus saligna* seedlings (Ayub *et al.*, 2011). By contrast, thermal acclimation of light inhibition was observed in *Quercus ilex*, a Mediterranean tree with long-lived leaves (Zaragoza-Castells *et al.*, 2007). We did not find a significant difference in the temperature response of light inhibition between the control and the greenhouse treatment for the two species in this study, indicating no substantial thermal acclimation of light inhibition. This pattern is similar to *E. saligna* but contrast to *Q. ilex*. The difference in response between our two tundra species and the evergreen *Q. ilex* may be attributable to the short leaf life-span of tundra species versus the long life span of the evergreen oak. The lower investment in leaves, and limited variation of irradiance and temperature during the growing season of the Arctic species, may make thermal acclimation of light inhibition less favored.

Previous studies have addressed the impacts of various other environmental factors besides temperature on R_L, including atmosphere CO_2 concentration (Ayub *et al.*, 2011, Crous *et al.*, 2012, Shapiro *et al.*, 2004, Wang *et al.*, 2001), nutrients (Atkin *et al.*, 2013, Heskel *et al.*, 2013a, Heskel *et al.*, 2012, Shapiro *et al.*, 2004) and water availability (Ayub *et al.*, 2011, Crous *et al.*, 2012). Because of the general correlation between leaf nitrogen and R (Griffin *et al.*, 2002, Reich *et al.*, 2006, Ryan, 1995, Xu *et al.*, 2007), we might expect N availability to impact leaf respiratory response. A strong effect of N on R_L has been reported (Atkin *et al.*, 2013, Shapiro *et al.*, 2004), and recent work suggested that inhibition of R in the light may decrease with increased N fertilization (Heskel *et al.*, 2012, Heskel *et al.*, 2013b). Further research is needed to

elucidate more detailed mechanisms of how foliar N concentrations affect thermal acclimation of R.

Short-term temperature response of R_L and light inhibition

Studies on the temperature response of R_L are limited, and the results are inconsistent. The short-term temperature coefficient of R_L was often lower than R_D for three *Plantago* species (Atkin *et al.*, 2006). By contrast, Shapiro *et al.* (2004) reported a temperature response of R_L in a herb *Xanthium strumarium* in which Q_{10} and E_0 of R_L was similar to or higher than that of R_D. Griffin & Turnbull (2013) reported similar temperature response of R_L and R_D in the C_3 grass *Triticum aestivum,* however R_L was less sensitive to temperature than R_D in the C_4 *Zea mays.* In our study, Q_{10} and E_0 of R_L was far lower than that of R_D in *E. vaginatum* while no significant difference in these parameters between R_L and R_D was observed in *B. nana*. Our results, together with previous works, suggest short-term temperature response of R_L and its association with the temperature response of R_D can vary across species and therefore requires species-specific quantification.

Because of the absence of long-term thermal acclimation of light inhibition of R in our study, the observed short-term temperature responses of light inhibition in each species can provide insights into their relative performance under warmer growth conditions, helping to explain warming's observed positive impacts on *B. nana* and negative impacts on *E. vaginatum*. Recent modeling studies suggest that light inhibition of R is largely governed by photosynthetic adenylate balance (Buckley & Adams, 2011): excess ATP or redox equivalents generated by the light reactions of photosynthesis reduce the demand for respiratory energy in the light compared

to that needed in the dark. The gross photosynthesis rate at low light is not likely to be substantially affected by temperature because of light limitation. In this case, a positive response of light inhibition to temperature in *E. vaginatum* indicates a reduced proportion of ATP and C skeletons supplied by R at higher temperatures. Thus at higher temperatures, in the light, for *E. vaginatum*, the demand for these compounds appears to be eliminated, and growth inhibition may occur. In contrast, constant light inhibition across temperatures in *B. nana* suggests a generally balanced supply and demand of respiratory products, in which case growth is still maintained at high temperatures. This observation is consistent with the fact that *E. vaginatum* is gradually being replaced by *B. nana* as climate warms (Tape *et al.*, 2006).

There is an assumed relationship between V_o (photorespiration) and inhibition of R_L (*reviewed in*: Griffin & Turnbull, 2013), however its precise mechanism and directionality remain undetermined. Some previous studies have shown high inhibition of R_L under conditions when V_o was also high (Atkin *et al*., 2000, Hurry *et al*., 2005, Zaragoza-Castells *et al*., 2007). In this study, such a relationship between V_o and inhibition of R_L was only observed in *E. vaginatum,* indicating V_o may be partially responsible for suppressing R_L at higher temperatures for this species. Other recent work identified a positive correlation between V_o and R_L in studies that compared C_4 versus C_3 species and direct V_o manipulations (Griffin and Turnbull, 2013) and in studies that suppressed V_o with high ambient CO_2 (Ayub *et al.,* 2011; Crous *et al.,* 2012; Shapiro *et al.,* 2004; Tcherkez *et al.,* 2008; Wang *et al*., 2001). This is consistent with our observation that there is a strong positive correlation between V_o and R_L across both species and treatments. This is likely because both V_o (Brooks & Farquhar, 1985, Ku & Edwards, 1977) and R_L (Atkin *et al.* 2006, Shapiro *et al.* 2004, Griffin & Turnbull 2013) are responding independently to

temperature. Overall, our results and other studies indicate that V_o may be suppressing R_L at higher temperatures, or that both processes could be responding independently to temperature.

Impact of light inhibition and thermal acclimation of respiration on leaf –level carbon balance

Understanding the balance between leaf R and photosynthesis (A) is crucial for estimating net plant C gain. In a warmer climate, the ratio of A/R could decrease if R is proportionally more responsive to warming than A. A is often limited by light (Ziska & Bunce, 1998) and insufficient attention has been given to the relationship between A and R_L. In this study, we compared A_{max}/R_D and A_{max}/R_L. For both study species, we observed a significant difference between A_{max}/R_D and A_{max}/R_L in the greenhouse treatment but not in the control. This finding suggests that using R_D rather than R_L to estimate respiratory C loss is likely to lead to more significant error in a warmer climate, with implications for the majority of models that currently assume a fixed A/R ratio based on R_D alone (however see Dufrêne *et al.*, 2005).

In our leaf-level model simulation, ignoring R_L led to overestimation of the cumulative temperature driven respiratory C flux during the growing seasons of 2004 to 2006 (up to 32% for *E. vaginatum* and 49% for *B. nana*), with differences due mainly to interannual variation of temperature. Given the abundance of *E. vaginatum* and *B. nana* in Arctic tundra and the dominance of R_L (relative to R_D) during the growing season, accounting for variations in light inhibition of leaf R for these species will be important for predictions of ecosystem level respiratory C flux. With expected expansion of *B. nana*, the error is likely to increase in models based on R_D only. Though the impact of respiratory light inhibition on estimates of leaf NPP is limited, mainly because of a high *A/R* ratio, such error is still problematic when partitioning CO_2

flux. To integrate R_L into C flux models an empirically determined correction term may be applied to R_D to estimate light inhibition (Shapiro *et al.*, 2004).

Most global models assume that R increases exponentially without acclimation to temperature (Atkin *et al.*, 2008). Despite increasing incorporation of dynamic plant C exchange responses into vegetation models, less than a fifth of models account for autotrophic R acclimation to temperature (Smith & Dukes, 2013). Studies that did incorporate respiratory thermal acclimation found significant differences in the estimates of C dynamics (Atkin *et al.*, 2008, Chen & Zhuang, 2013). However, these studies used algorithms describing respiratory thermal acclimation based on R_D and did not incorporate the effect of light inhibition. We modeled leaf-level CO_2 fluxes under different warming scenarios accounting for light inhibition of R. For *E. vaginatum,* which showed significant thermal acclimation of both R_D and R_L, ignoring thermal acclimation of R caused substantial error (up to 48%) in our estimates of cumulative leaf R. Thus, accounting for thermal acclimation of R in the light is essential to developing accurate C dynamic models of Arctic tundra. In addition, for *E. vaginatm,* the major source of error in leaf NPP estimates was ignoring thermal acclimation of A (3 to 4 times that caused by ignoring acclimation of R, Table 3b). Thus models should include algorithms of thermal acclimation of both A and R. In addition, models for overall C flux should include measurements of soil respiration and its components (Chapin III *et al.*, 2009). Collaborative experiments and modeling studies could address this challenge (Smith & Dukes, 2013).

Conclusions

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We demonstrate for the first time the importance of the combined long-term thermal acclimation and light inhibition in estimating ecosystem level-leaf respiratory flux in Arctic systems. Short-term responses of R to temperature predicted long-term response in *B. nana*, but not in *E. vaginatum* because of acclimation of R in *E. vaginatum* with long term warming. As *E. vaginatum* is a dominant tundra species, models of C cycling in the Arctic must account for thermal acclimation in R, or risk substantially overestimating the leaf respiratory flux with warming. Because of a substantial difference in R_L and R_D and the nearly constant exposure of Arctic plants to light during the growing season, a failure to account for light inhibition of R in this system also will cause significant error in C budget estimates, most pronounced in *B. nana.* Thus, the differential responses to temperature of R_L and R_D and acclimation should be incorporated into C dynamic models to improve estimates of the respiratory C flux.

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Table 1. Respiration-temperature response curve fit for R_L and R_D for *E. vaginatum* and *B. nana* in greenhouse and control treatments. Comparisons between treatments and R_L or R_D were made for $\ln R_0$ and E_0 (kJ/mol) after linear transformation of the respiration model (Warton *et al.*, 2006). Values marked with different letters are significantly different from each other (t-test). Parentheses indicate standard errors.

Species	Treatment	Respiration	Model parameters and statistics				Equation statistics	
			R_0	P	E_0	P	R^2	P
E. Vaginatum	Control	Dark	0.94 (0.15)[a]	<0.001	72.3 (10.9)[a]	<0.001	0.638	<0.001
		Light	0.98 (0.10)[a]	<0.001	25.9 (7.7)[b]	0.001	0.284	0.001
	Greenhouse	Dark	0.90 (0.09)[a]	<0.001	47.0 (8.4)[ab]	<0.001	0.417	<0.001
		Light	0.81 (0.12)[a]	<0.001	3.7 (14.8)[c]	0.803	0.0013	0.826
B. Nana	Control	Dark	0.44 (0.06)[ab]	<0.001	69.1 (10.5)[a]	<0.001	0.873	<0.001
		Light	0.26 (0.08)[c]	0.014	92.8 (24.6)[a]	0.007	0.715	0.004
	Greenhouse	Dark	0.44 (0.05)[a]	<0.001	89.9 (10.5)[a]	<0.001	0.9383	<0.001
		Light	0.31 (0.04)[b]	<0.001	97.3 (10.7)[a]	<0.001	0.954	<0.001

Table 2. ANCOVA models testing the effects of environmental and species factors on R, R_L/R_D and V_o.

(a) Main effects and interactions of short-term temperature (f(T)), long-term warming treatment (Greenhouse), and R_L versus R_D (Light) on R for *E. vaginatum* and *B. nana* at the mean temperature of all measurement points (approximately 15˚C).

Effect	P value	
	E. vaginatum	*B. nana*
f(T)	**<0.001**	**<0.001**
Greenhouse	**0.04**	0.39
f(T)×Greenhouse	**0.02**	0.35
Light	*0.09*	**0.002**
f(T)×Light	**<0.001**	0.46
Greenhouse×Light	0.91	0.34
f(T)×Greenhouse×Light	0.39	0.54

(b) Main effects and interactions of species, temperature, and treatment on the Kok effect (R_L/R_D) and V_o.

Effect	P value	
	R_L/R_D	V_o*
Species	**0.004**	**<0.0001**
Temperature	*0.06*	**<0.0001**
Species×Temperature	**0.002**	0.55
Greenhouse	0.36	0.72
Species×Greenhouse	0.48	0.15
Temperature×Greenhouse	0.22	0.43
Species×Temperature×Greenhouse	0.52	**0.01**

*Analysis was done after log transformation to fulfill the homogeneity of variance

Table 3. Sensitivity analysis showing the impact of not incorporating R_L or thermal acclimation on annual leaf-level gas exchange (calculated for the period of June 10th to August 20th).

(a) Annual leaf gas exchange (GPP, respiration, and NPP) modeled with R_D only or both R_D and R_L for 2004-2006. The error caused by not using R_L is estimated.

Species		*E. vaginatum*			*B. nana*		
Year		2004	2005	2006	2004	2005	2006
Average temperature (˚C)		13.1	9.80	9.35	13.1	9.80	9.35
Leaf GPP (mol C m-2 y-1 leaf)		27.12	26.46	23.74	38.29	38.51	33.81
Leaf respiration (mol mol C m-2 y-1 leaf)	R_D only	9.23	6.83	6.29	4.25	3.18	2.94
	R_D and R_L	6.98	6.09	5.94	3.18	2.17	1.98
	Error of not using R_L (%)	+32	+12	+6	+33	+47	+49
Leaf NPP (mol C m-2 y-1 leaf)	R_D only	17.91	19.62	17.44	34.05	35.33	30.86
	R_D and R_L	20.14	20.37	17.80	35.11	36.34	31.83
	Error of not using R_L (%)	-11	-4	-2	-3	-3	-3

(b) Annual leaf gas exchange (GPP, respiration, and NPP) modeled under different warming scenarios, with and without incorporating thermal acclimation of photosynthesis and respiration. Values in bold fonts show the best estimation of gas exchange in different scenarios. The error caused by not incorporating leaf thermal acclimation, and the relative contribution of GPP and respiration to this error are estimated.

Species		*E. vaginatum*				*B. nana*			
Warming scenario (˚C over 2006 temperature)		0	2.5	5	7	0	2.5	5	7
Leaf GPP (mol CO_2 m^{-2} leaf)	Control (non-acclimated)	**23.74**	25.71	27.46	28.66	**33.81**	36.33	38.55	40.07
	Greenhouse (acclimated)	30.29	**32.69**	**34.84**	**36.29**	32.83	**35.30**	**37.49**	**38.96**
	Error (%)	--	-21	-21	-21	--	+3	+3	+3
Leaf Respiration (mol CO_2 m^{-2} leaf)	Control (non-acclimated)	**5.94**	6.65	7.46	8.20	**1.98**	2.70	3.69	4.75
	Greenhouse (acclimated)	4.95	**5.14**	**5.36**	**5.55**	2.37	**3.32**	**4.66**	**6.11**
	Error (%)	--	+29	+39	+48	--	-19	-21	-22
Leaf NPP (mol CO_2 m^{-2} leaf)	Control (non-acclimated)	**17.80**	19.06	20.00	20.46	**31.83**	33.63	34.86	35.32
	Greenhouse (acclimated)	25.34	**27.55**	**29.48**	**30.74**	30.46	**31.98**	**32.83**	**32.85**
	Error (%)	--	-31	-32	-33	--	+5	+6	+8
Contribution to the error of NPP (%)	Photosynthesis (GPP)	--	82	78	74	--	68	52	45
	Respiration	--	18	22	26	--	32	48	55

Figure Legends

Figure 1. Temperature response of R_D and R_L for *E. vaginatum* and *B. nana*. (a) In *E. vaginatum*, R_L and R_D show responses to changes in both short-term and long-term temperature manipulations. (b) In *B. Nana*, R_L and R_D show responses to changes in short-term temperature, but not to long-term temperature manipulations.   Measurements of each species-treatment combination are fitted to a single curve.

Figure 2. Gas exchange parameters of *E. Vaginatum* and *B. nana* in control and greenhouse growth conditions. The temperature coefficient of respiration (Q_{10}) (a,b), respiration rate (c, d), and A_{max}/R (e, f) at 15˚C are respectively shown for R_D and R_L. Values marked with different letters are significantly different from each other.

Figure 3. Percent inhibition of R in the light (a) and V_o (b) with change in short-term temperature in *E. Vaginatum* and *B. nana* in greenhouse treatments and controls. Temperature responses for the percent inhibition of R in the light were fitted to linear curves and responses for V_o were fitted to exponential growth curves. R^2 and P values of fitted curves are shown.
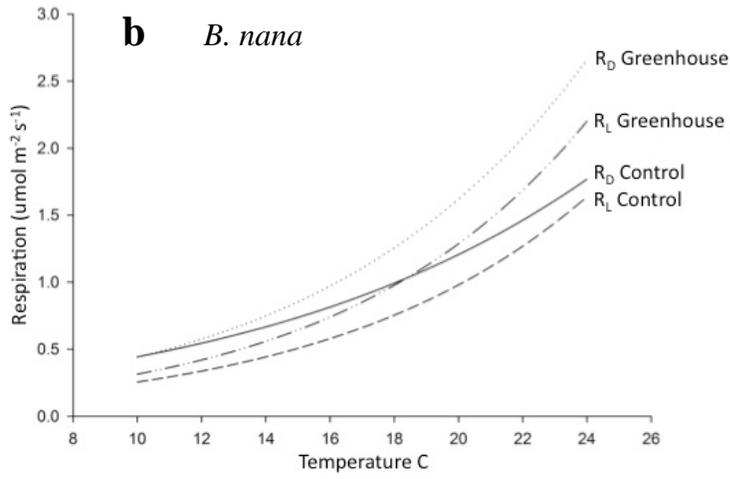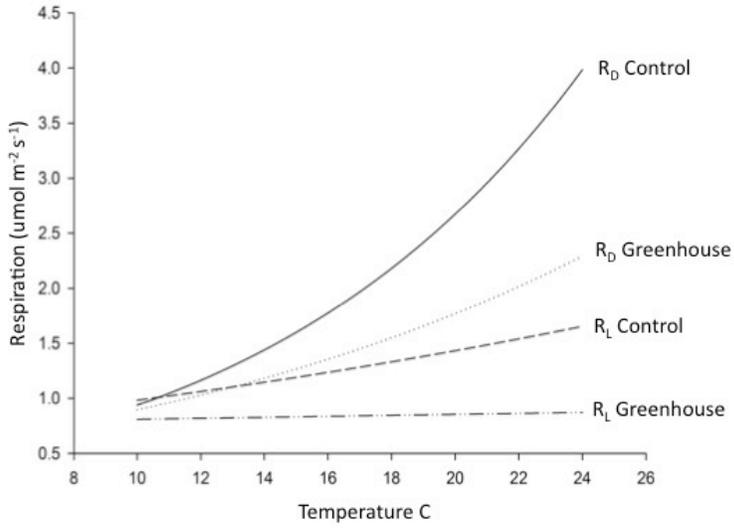
Figure 1

      a   *E. vaginatum*
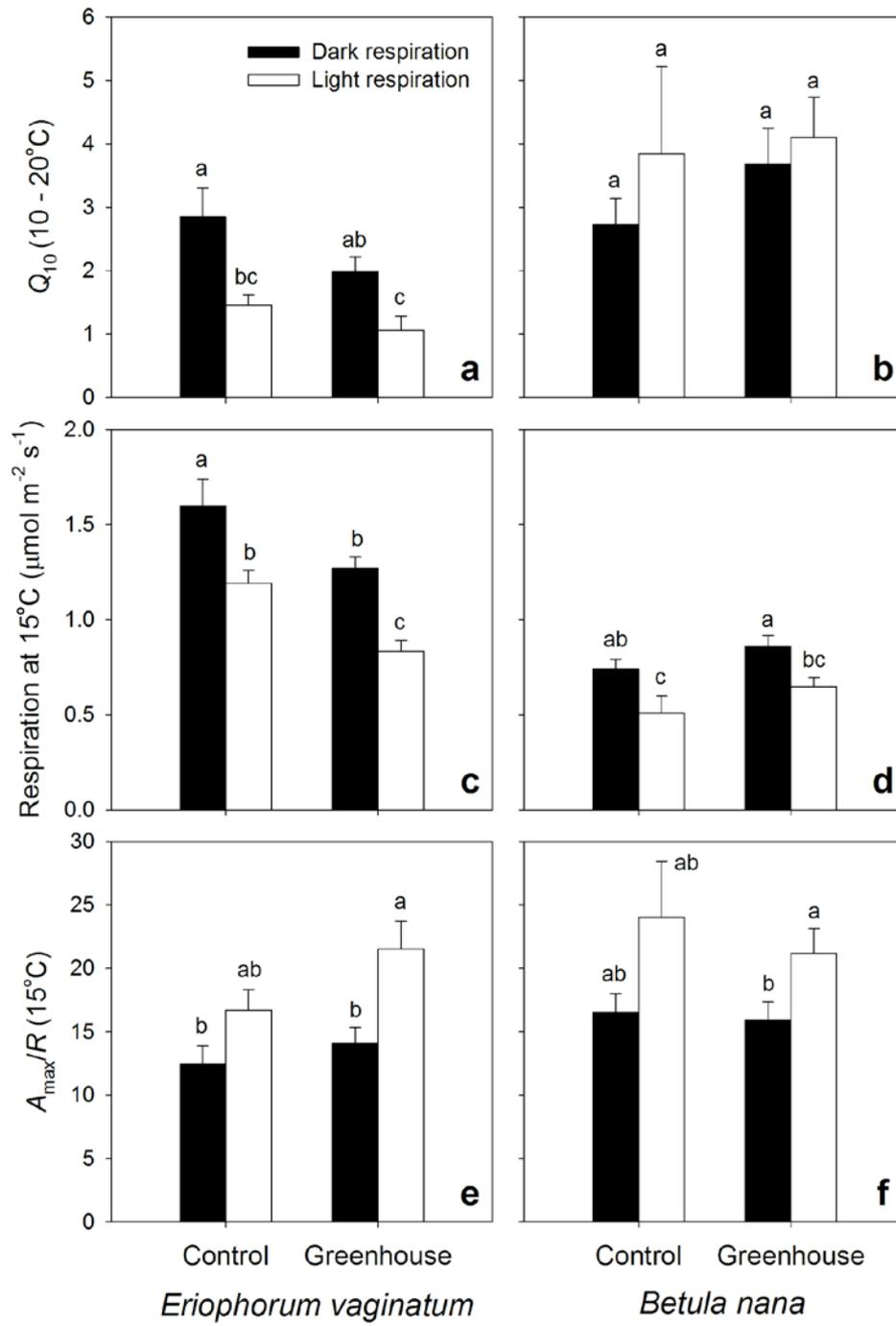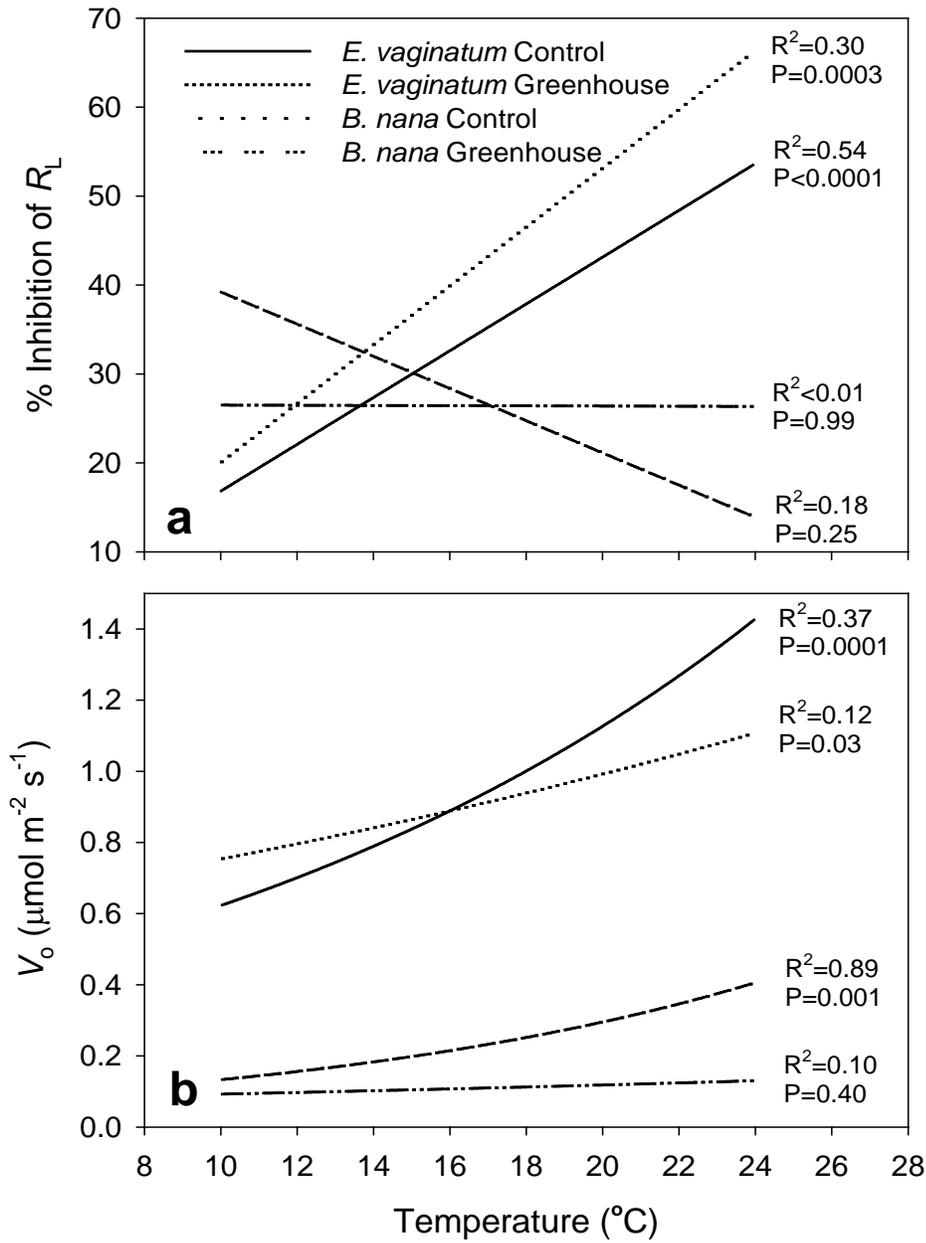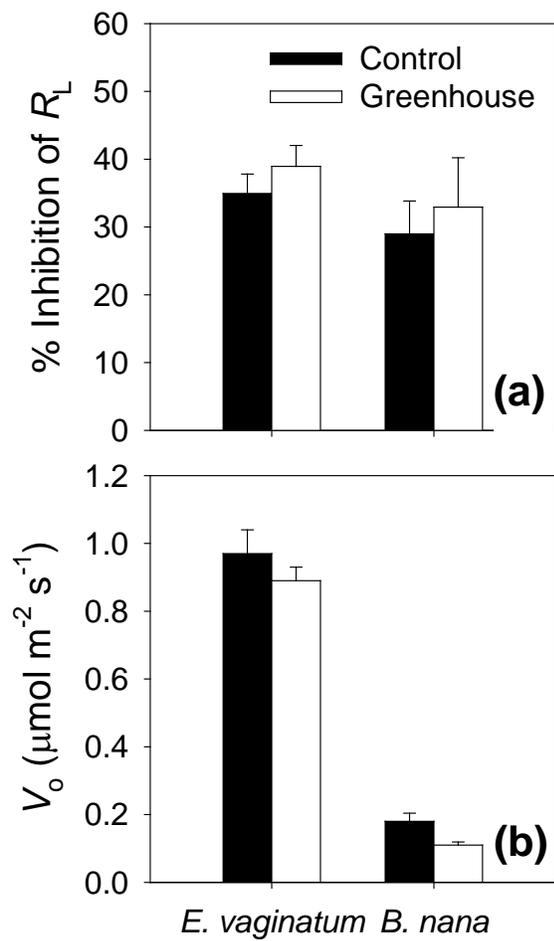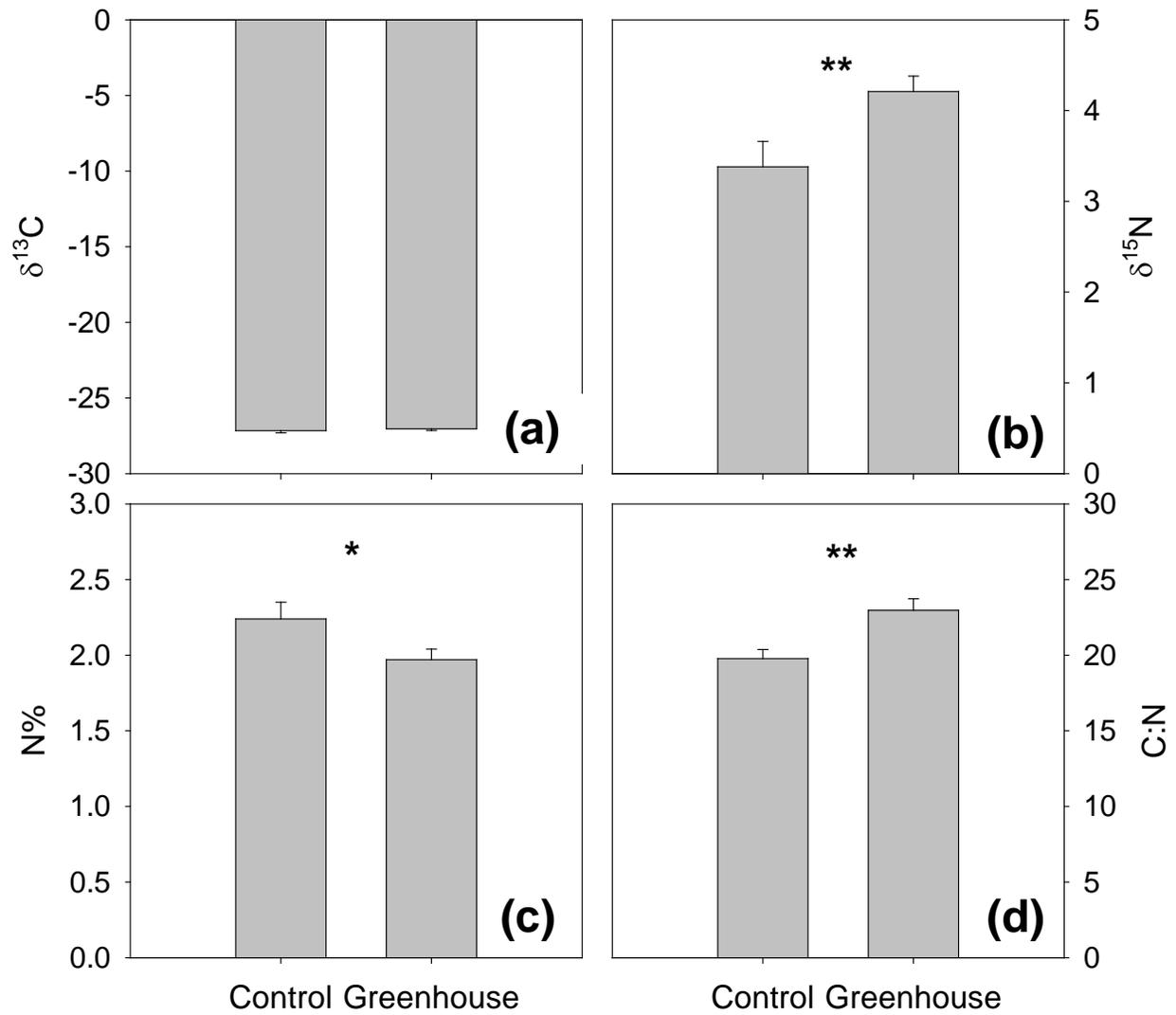
b *B. nana*

Figure 2

Figure 3

SI Figure 2. Leaf respiratory inhibition (a) and V_o at 100 µmol PPFD $m^{-2} s^{-1}$ (b) of *E. vaginatum* and *B. nana* in control and greenhouse treatments.

SI Figure 3. Leaf (a) δ^{13}C values, (b) δ^{15}N values, (c) %N and (d) C/N ratios of *E. vaginatum* in greenhouse and control treatments. Significant differences between the control and greenhouse treatment are marked (* P<0.05, ** P<0.01).