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
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 \( \mu \)mol m\(^{-2}\) s\(^{-1}\) and 10 points below 20 \( \mu \)mol 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 °C 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 \( \mu \)mol 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):

$$ R = R_0 \exp \left[ \left( \frac{E_0}{R_g} \right) \left( \frac{1}{T_0} - \frac{1}{T_a} \right) \right] $$

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:

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

and,

$$ T_1 - T_2 = 10 \text{ (°C)}.$$  

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 – 20°C 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 (\[\ln R = \ln R_0 + \frac{E_0}{R_g} \times f(T),\]
\[f(T) = \frac{1}{T_0} - \frac{1}{T}\]) 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 \(f(T)\) (\(T=14.9 \, ^\circ C\) for \(E. \, vaginatum\) and \(16.2 \, ^\circ C\) for \(B. \, nana\)); the \(f(T)\times\)treatment and \(f(T)\times\)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 × treatment and temperature × 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 10$^{th}$ to August 20$^{th}$).
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 10\textsuperscript{th} to August 20\textsuperscript{th}) and for three warming scenarios (plus 2.5, 5, and 7 °C 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 \((100 - (R_L/R_D \times 100))\), 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 ($f(T) \times$ Greenhouse interaction, p=0.02) (Table 1). $E_0$ was also different between $R_L$ and $R_D$ (as indicated by $f(T) \times$ 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 $A_{\text{max}}$ at 15 °C in *E. vaginatum* or in *B. nana*. $A_{\text{max}}$ 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 $A_{\text{max}}$ to $R$ (at 15 °C) 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 °C 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 $\delta^{13}$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
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 C3 grass *Triticum aestivum*, however \( R_L \) was less sensitive to temperature than \( R_D \) in the C4 *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*<sub>o</sub> (photorespiration) and inhibition of *R*<sub>L</sub> (reviewed *in*: Griffin & Turnbull, 2013), however its precise mechanism and directionality remain undetermined. Some previous studies have shown high inhibition of *R*<sub>L</sub> under conditions when *V*<sub>o</sub> was also high (Atkin *et al.*, 2000, Hurry *et al.*, 2005, Zaragoza-Castells *et al.*, 2007). In this study, such a relationship between *V*<sub>o</sub> and inhibition of *R*<sub>L</sub> was only observed in *E. vaginatum*, indicating *V*<sub>o</sub> may be partially responsible for suppressing *R*<sub>L</sub> at higher temperatures for this species. Other recent work identified a positive correlation between *V*<sub>o</sub> and *R*<sub>L</sub> in studies that compared *C*<sub>4</sub> versus *C*<sub>3</sub> species and direct *V*<sub>o</sub> manipulations (Griffin and Turnbull, 2013) and in studies that suppressed *V*<sub>o</sub> with high ambient CO<sub>2</sub> (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*<sub>o</sub> and *R*<sub>L</sub> across both species and treatments. This is likely because both *V*<sub>o</sub> (Brooks & Farquhar, 1985, Ku & Edwards, 1977) and *R*<sub>L</sub> (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 $A_{\text{max}}/R_D$ and $A_{\text{max}}/R_L$. For both study species, we observed a significant difference between $A_{\text{max}}/R_D$ and $A_{\text{max}}/R_L$ 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. vaginatum*, 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**
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.

Acknowledgements

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darkness and light in *Eucalyptus saligna* exposed to industrial-age atmospheric CO$_2$ and

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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.

<table>
<thead>
<tr>
<th>Species</th>
<th>Treatment</th>
<th>Respiration</th>
<th>Model parameters and statistics</th>
<th>Equation statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>$R_0$</td>
<td>$E_0$</td>
</tr>
<tr>
<td><em>E. Vaginatum</em></td>
<td>Control</td>
<td>Dark</td>
<td>0.94 (0.15)$^a$</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Light</td>
<td>0.98 (0.10)$^a$</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Greenhouse</td>
<td>Dark</td>
<td>0.90 (0.09)$^a$</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Light</td>
<td>0.81 (0.12)$^a$</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><em>B. Nana</em></td>
<td>Control</td>
<td>Dark</td>
<td>0.44 (0.05)$^{ab}$</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Light</td>
<td>0.26 (0.08)$^c$</td>
<td>0.014</td>
</tr>
<tr>
<td></td>
<td>Greenhouse</td>
<td>Dark</td>
<td>0.44 (0.05)$^a$</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Light</td>
<td>0.31 (0.04)$^b$</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
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).

<table>
<thead>
<tr>
<th>Effect</th>
<th><em>E. vaginatum</em> P value</th>
<th><em>B. nana</em> P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$f(T)$</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Greenhouse</td>
<td>0.04</td>
<td>0.39</td>
</tr>
<tr>
<td>$f(T)$×Greenhouse</td>
<td>0.02</td>
<td>0.35</td>
</tr>
<tr>
<td>Light</td>
<td>0.09</td>
<td>0.002</td>
</tr>
<tr>
<td>$f(T)$×Light</td>
<td>&lt;0.001</td>
<td>0.46</td>
</tr>
<tr>
<td>Greenhouse×Light</td>
<td>0.91</td>
<td>0.34</td>
</tr>
<tr>
<td>$f(T)$×Greenhouse×Light</td>
<td>0.39</td>
<td>0.54</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Effect</th>
<th>$R_L/R_D$ P value</th>
<th>$V_o$ P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species</td>
<td>0.004</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Temperature</td>
<td>0.06</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Species×Temperature</td>
<td>0.002</td>
<td>0.55</td>
</tr>
<tr>
<td>Greenhouse</td>
<td>0.36</td>
<td>0.72</td>
</tr>
<tr>
<td>Species×Greenhouse</td>
<td>0.48</td>
<td>0.15</td>
</tr>
<tr>
<td>Temperature×Greenhouse</td>
<td>0.22</td>
<td>0.43</td>
</tr>
<tr>
<td>Species×Temperature×Greenhouse</td>
<td>0.52</td>
<td>0.01</td>
</tr>
</tbody>
</table>

*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.

<table>
<thead>
<tr>
<th>Species</th>
<th>$E.\ vaginatum$</th>
<th>$B.\ nana$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average temperature (°C)</td>
<td>13.1</td>
<td>9.80</td>
</tr>
<tr>
<td>Leaf GPP (mol C m$^{-2}$ y$^{-1}$ leaf)</td>
<td>27.12</td>
<td>26.46</td>
</tr>
<tr>
<td>Leaf respiration</td>
<td>$R_D$ only</td>
<td>9.23</td>
</tr>
<tr>
<td></td>
<td>$R_D$ and $R_L$</td>
<td>6.98</td>
</tr>
<tr>
<td>Error of not using $R_L$ (%)</td>
<td>+32</td>
<td>+12</td>
</tr>
<tr>
<td>Leaf NPP (mol C m$^{-2}$ y$^{-1}$ leaf)</td>
<td>$R_D$ only</td>
<td>17.91</td>
</tr>
<tr>
<td></td>
<td>$R_D$ and $R_L$</td>
<td>20.14</td>
</tr>
<tr>
<td>Error of not using $R_L$ (%)</td>
<td>-11</td>
<td>-4</td>
</tr>
</tbody>
</table>

(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.

<table>
<thead>
<tr>
<th>Species</th>
<th>$E.\ vaginatum$</th>
<th>$B.\ nana$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Warming scenario (°C over 2006 temperature)</td>
<td>0</td>
<td>2.5</td>
</tr>
<tr>
<td>Leaf GPP (mol CO$_2$ m$^{-2}$ leaf)</td>
<td>Control (non-acclimated)</td>
<td>23.74</td>
</tr>
<tr>
<td></td>
<td>Greenhouse (acclimated)</td>
<td>30.29</td>
</tr>
<tr>
<td></td>
<td>Error (%)</td>
<td>-21</td>
</tr>
<tr>
<td>Leaf Respiration (mol CO$_2$ m$^{-2}$ leaf)</td>
<td>Control (non-acclimated)</td>
<td>5.94</td>
</tr>
<tr>
<td></td>
<td>Greenhouse (acclimated)</td>
<td>4.95</td>
</tr>
<tr>
<td>Leaf NPP (mol CO$_2$ m$^{-2}$ leaf)</td>
<td>Control (non-acclimated)</td>
<td>17.80</td>
</tr>
<tr>
<td></td>
<td>Greenhouse (acclimated)</td>
<td>25.34</td>
</tr>
<tr>
<td></td>
<td>Error (%)</td>
<td>-31</td>
</tr>
<tr>
<td>Contribution to the error of NPP (%)</td>
<td>Photosynthesis (GPP)</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Respiration</td>
<td>-</td>
</tr>
</tbody>
</table>
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*
Figure 2

b  B. nana
Figure 3

**Figure 3**

Graphs showing:
- $Q_{10}$ (10 - 20°C)
- Respiration at 15°C (µmol m$^{-2}$ s$^{-1}$)
- $A_{max}/R$ (15°C)

Comparison between Control and Greenhouse conditions for:
- *Eriophorum vaginatum*
- *Betula nana*
Temperature (°C)

% Inhibition of $R_L$

$V_o$ (µmol m$^{-2}$ s$^{-1}$)

E. vaginatum Control
E. vaginatum Greenhouse
B. nana Control
B. nana Greenhouse

R$^2$=0.30
P=0.0003

R$^2$=0.54
P<0.0001

R$^2$<0.01
P=0.99

R$^2$=0.18
P=0.25

R$^2$=0.37
P=0.0001

R$^2$=0.12
P=0.03

R$^2$=0.89
P=0.001

R$^2$=0.10
P=0.40

Temperature (°C)
SI Figure 2. Leaf respiratory inhibition (a) and $V_0$ 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) $\delta^{13}$C values, (b) $\delta^{15}$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).