Environmental Controls of Foliar Respiration in Arctic Tundra Plants

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ABSTRACT

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The Arctic is warming at rapid, unprecedented rates, causing cascading ecological and environmental changes that threaten to destabilize the vast amounts of carbon stored in the vegetation and soils of the tundra. Foliar gas exchange, which is responsible for the initial fixation of carbon, is likely to respond to warming and associated environmental change in tundra plants, though the direction and degree of these responses are not well studied. This dissertation aims to quantify multiple cellular and leaf-level processes underlying carbon cycling in tundra plants, and to address the responses of these processes to abiotic and biotic effects of warming in the Arctic.

To assess the impact of environmental change on foliar gas exchange physiology of tundra plants, a series of empirical studies were conducted on common and abundant plant species located near Toolik Lake, on the North Slope of Alaska. Long-term manipulated treatment plots that simulate the effects of climate change in this region, including elevated growth temperature and increased soil nutrient availability, served as the research setting for multiple experiments that addressed the response of variables such as foliar photosynthesis, respiration, photorespiration, mitochondria and chloroplast size and density, and physical leaf traits. Due to the extreme photoperiod experienced by arctic vegetation, respiration in the light (estimated using the Kok method) was quantified in addition to dark respiration for a more accurate depiction of plant carbon fluxes.
Individual studies, presented as dissertation chapters, examine the responses of the aforementioned variables to a gradient of soil nitrogen and phosphorus availability; decades-long warming and fertilization; seasonal timing and short-term intra-season temperature fluctuations; and canopy position within a shrub community. Collectively, the results of these studies find respiration to be more sensitive to long- and short-term environmental variation than photosynthesis, indicating a decoupling of the processes controlling foliar carbon cycling. Across all species and environmental conditions, respiration is inhibited by light, emphasizing the need for the estimation of this physiological phenomena and its inclusion in regional terrestrial ecosystem carbon models. Also, foliar carbon fluxes in woody shrub species are significantly higher than non-shrub species across experiments, a finding that demands attention given the general trend of increasing shrub cover associated with warming in the Arctic tundra. The results presented in this study on the environmental controls on leaf-level gas exchange allow for a more thorough understanding of the current carbon balance of this region and provides new data the can inform predictions and models of its future status.
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DEDICATION

I dedicate this work to my grandmother, Helene Rotman Heskel, and the memories of my deceased grandparents, Martha Henrietta Miller Aiken, Jesse Edward Aiken, and Milton Morris Heskel. For me, they represent the values they promoted in their own lives - intellectual curiosity and skepticism, steadfast sensibility, hard work, the embracement of change, and a deep love of nature and beauty.
CHAPTER ONE

Introduction

To begin: respiration enables life. The process of converting nutrients to energy and releasing waste products exists in all living organisms. In photosynthetic organisms, which harvest light energy to create chemical energy, respiration provides metabolic support in all cells of all tissues at all times, facilitating the growth and maintenance of all chlorophyll-containing species from green algae to canopy trees. The enormous annual exchange of carbon between terrestrial ecosystems and the atmosphere is driven primarily by photosynthesis and respiration, and results in approximately $0.9 \pm 0.6 \text{ Gt yr}^{-1}$ of stored carbon in the terrestrial environment in recent years (IPCC 2007). Plant respiration releases approximately half of all CO$_2$ assimilated via photosynthesis, with leaves contributing approximately half of whole plant CO$_2$ release (Poorter, Remkes & Lambers 1990; Atkin, Scheurwater & Pons 2007). As a result, subtle changes in photosynthesis and respiration can result in significant variation in global fluxes, thus demanding observation and experimental measurement for informed modeling of the terrestrial carbon cycle (Trumbore 2006).

The cellular pathways comprising respiration in plants, glycolysis, the tri-carboxylic acid cycle, and the electron transport chain/oxidative phosphorylation, collectively consume oxygen and glucose, and produce reductant, energy in the form of ATP, carbon skeletons, and CO$_2$. The resulting foliar release of CO$_2$ can vary depending on tissue type, plant functional type, and environmental conditions. An important item to note when considering respiration in plants is that it occurs simultaneously with photosynthesis. Photosynthesis and respiration, though both energy-producing, are far from redundant; together, these processes allow

Decades of research illuminate the environmental sensitivity of foliar respiration. Some of the reported environmental controls of respiration (those relevant to arctic climate change, and therefore this thesis) - temperature, light, nitrogen and phosphorus – are described in the following literature review. Other important environmental influences on respiration, such as atmospheric CO$_2$ concentration and water availability, though not covered in the following chapters, also figure prominently in ecophysiological studies. Because respiration can respond differently to these environmental factors than photosynthesis, it is likely climate change will affect the balance between these processes, and in turn, impact net ecosystem carbon flux and allocation (Ryan 1991). Collectively, the effects of these environmental controls on respiration relate back to foliar energy requirements and metabolic efficiency. Generally, factors promoting enhanced rates of photosynthesis (i.e. nitrogen, CO$_2$, light, temperature) also produce higher rates of respiration in the short term, though issues such as longer-term acclimation and light inhibition, complicate this relationship.

A major source of concern is how plant respiration will respond to warming and other consequences related to climate change, especially in geographic regions experiencing rapid environmental and ecological change (Ryan 1991; King et al. 2006; Atkin, Millar & Turnbull 2010). The Arctic tundra is a region of prioritized research interest as its vegetation and soils store a large proportion of the global organic carbon pool, and rapid warming threatens to change this millennia-old carbon sink to a source (Gorham 1991; Oechel et al. 1993; Oechel et al. 2000; Mack et al. 2004; Ping, Michaelson & Jorgenson 2008; Tarnocai et al. 2009).
Arctic warming, which is occurring at an unprecedented rate (Serreze et al. 2000), is associated with a host of cascading ecological effects, many of which indirectly promote further warming and environmental change through a network of positive feedbacks (Sturm et al. 2005a; Wookey et al. 2009; Myers-Smith et al. 2011a; Loranty & Goetz 2012).

The ensuing chapters portray change through the lens of foliar respiration. Each chapter tackles an ecologically relevant issue in the Arctic tundra, examines its implications, and quantifies its effect on foliar gas exchange physiology. Following this introduction is a thorough review of the scientific literature on the changing arctic landscape and the physiology and environmental sensitivity of respiration. This review provides the necessary background on foliar respiratory processes, suggests future research endeavors, and provides the context for hypotheses and experimentation in the following data based chapters. The subsequent four chapters are based on data collected from tundra plants located near Toolik Lake, on the North Slope of Alaska, between 2009-2011. Three of the four studies presented here utilized the nearly 30-year-old global change experiments that have been continuously maintained at the Arctic Long Term Research field site. All studies sampled from ecologically important, abundant (and often dominant) species that represented different plant functional types in order to best characterize the main effects of environmental change on foliar physiology. These species include *Eriophorum vaginatum* (“cotton grass”), a tussock-forming sedge that is distributed widely over Alaska’s North Slope, northern Canada, and northern Eurasia, *Betula nana nana* L. (“dwarf birch”) and *Salix pulchra* (“tealeaf willow”), both deciduous woody shrubs associated with woody shrub expansion in the Arctic, and *Rubus chamaemorus* “cloudberry”), a widespread herbaceous perennial forb.
Chapter Three (published as Heskel et al., 2012 in the American Journal of Botany) examines the impact of increasing nitrogen and phosphorus availability on foliar respiration in the light and dark, photosynthesis, mitochondria and chloroplast size and densities, as well as other leaf traits (specific leaf area, leaf nitrogen and phosphorus concentrations) in *B. nana* and *E. vaginatum*. This chapter compares species that represent the current and historic (*E. vaginatum*) and future predicted (*B. nana*) tundra, using a recently established fertilization gradient simulating predicted environmental change. Chapter Four (currently in press at Ecology and Evolution as Heskel et al., 2013) expands upon the ideas presented in Chapter Three. This study quantifies rates of gas exchange (respiration in the light and dark, photosynthesis, photorespiration) to estimate foliar carbon use efficiency and relate it to the community composition of *B. nana*, *E. vaginatum*, and *R. chamaemorus* under long term warming and fertilization. Similar to Chapter Three, this study aims to evaluate physiological advantages that may facilitate woody shrub expansion under predicted environmental change.

Chapter Five (under review at Functional Ecology) considered the seasonality of gas exchange in leaves of *B. nana* and *E. vaginatum* grown under ambient conditions and long-term warming. This study quantifies photosynthetic variables (maximum photosynthesis, maximum carboxylation rate of RuBP, and electron transport rate), respiration in the light and dark, and leaf traits on short (4-5 day) intervals to capture a high temporal resolution view of foliar carbon cycling. Models evaluate the relative influence of ambient temperature, which range widely without pattern in this region, on foliar fluxes. The final data based chapter (6) focuses on the allocation of resources within shrub canopies to see if vertical patterns emerge, as documented in other systems. Variables measured in *B. nana* and *S. pulchra* (same as in Chapter 5) relate to leaf area index to a varying degree, without clear indication of
optimization. These woody shrub species are likely to be increasingly representative of tundra vegetation under change, and characterizing their carbon cycling and how they modify intra-canopy microenvironment will be informative for carbon balance assessments.

Dear Reader, the following chapters encapsulate nearly five years of literature review, fieldwork, data analysis and interpretation, and written composition. With this thesis, I enthusiastically contribute new information about the environmental controls of foliar respiration in the ecologically important and rapidly changing Arctic tundra with the hope that it will be meaningful for scientific community.
CHAPTER TWO

Review of the Literature on the Environmental Controls of Foliar Respiration in Arctic Tundra Plants

1. Introduction

The Arctic tundra faces drastic ecological consequences from climate change, and a particular source of concern is disruption of the terrestrial carbon cycle, the flow of carbon between the land and atmosphere (Shaver et al. 1992; Post et al. 2009). Plant gas exchange – photosynthesis and respiration - and soil respiration, comprise the basis of the terrestrial carbon cycle and determine a region’s “carbon budget”, the amount of carbon stored in vegetation and soils and subsequently released into the atmosphere. Northern latitude vegetation and soils store approximately one third of the world’s carbon, and rapid warming has altered the balance of this system, potentially changing this millennia-old carbon “sink” to a “source”, with a positive net release of carbon dioxide (CO₂) to the atmosphere (Gorham 1991; Oechel et al. 1993; Chapin et al. 2005; Ping, Michaelson & Jorgenson 2008; Tarnocai et al. 2009). It is likely the physiology governing gas exchange in plants will respond to the dynamic changes in the Arctic, where mean annual temperatures are increasing faster than average global rates (Chapman & Walsh 1993; Serreze et al. 2000). Plant respiration, which releases nearly half of all carbon assimilated through photosynthesis, could determine the future carbon balance of this system, but the specific responses of this process to the changing environment remain unknown (Schimel 1995; Schlesinger 1997). The size of the flux and the environmental sensitivity of the underlying processes, make understanding plant respiration a priority in the carbon-rich, rapidly warming Arctic tundra.
Foliar respiration, and how it will respond to rapid environmental change, represents a large unknown when considering arctic tundra vegetation. This review summarizes current and predicted change in the Arctic tundra, addresses the difficulty of obtaining accurate estimations of mitochondrial respiration, describes the multiple abiotic and biotic factors that control respiration in the Arctic tundra, and identifies future research needs.

2. A Changing Arctic

Arctic warming has resulted in multiple cascading ecological and environmental effects that individually and collectively threaten to profoundly impact the carbon cycle. The direct and indirect effects include the thawing of permafrost, deepening of the soil active layer, lengthening of the growing season, increased soil microbial activity and nutrient availability, and the alteration of the plant community composition due to the northward encroachment of woody shrub species (Chapin et al. 1995; Sturm, Racine & Tape 2001; Chapin et al. 2005; Tape, Sturm & Racine 2006). In the Arctic, the encroachment and expansion of woody shrub species into tundra is altering the ecology of the historically sedge-dominated landscape (Sturm, Racine & Tape 2001; Tape, Sturm & Racine 2006; Walker et al. 2006; Hudson & Henry 2010; Myers-Smith et al. 2011a). Shrub expansion in the tundra increases surface roughness, affecting the regional energy balance (Chapin et al. 2005), and is associated with earlier snowmelt, which can decrease albedo and promotes a positive feedback for further warming (Liston et al. 2002; Bonfils et al. 2012; Loranty & Goetz 2012). Shrub-dominated communities allow for greater snow depth, and the relatively taller canopy height of shrubs compared to tussock-dominated communities, create an insulating layer that warms soil during winter months, thus stimulating winter soil microbial activity (Liston et al. 2002;
Schimel, Bilbrough & Welker 2004; Sturm et al. 2005b). Also, the addition of shrubs’ recalcitrant leaves and woody stems can affect the litter decomposition rate, as well as its nutrient status, though this may be complicated by uncoupled fine-root decomposition rates (Hobbie 1996; Cornelissen et al. 2007; Hobbie et al. 2010). Shifting litter composition and increased soil microbial activity, allow for faster rates of nutrient turnover, which can add nitrogen to a system historically limited by cold temperatures (Hobbie, Nadelhoffer & Höögberg 2002; Jonasson, Castro & Michelsen 2004; Björk et al. 2007).

In addition to the indirect effects via the woody shrub invasion, warming can directly impact soil microbial activity, nutrient release and CO$_2$ efflux. Arctic soil carbon reserves are sensitive to warmer temperatures and stimulated decomposition increases CO$_2$ release from soils (Grogan & Chapin 2000) and plants (Johnson et al. 2000; Mack et al. 2004). However, this increased decomposition also can increase nitrogen mineralization, leading to greater primary production and the potential to counteract the stimulated release of CO$_2$ through respiration, though reports of the degree of this compensation vary (Tinker & Ineson 1990; Hobbie & Chapin 1998; Jonasson, Michelsen & Schmidt 1999; Schmidt, Jonasson & Michelsen 1999). Experimental evidence shows differential effects of warming on species, with shrub species often benefitting to a stronger degree than non-shrub species in terms of biomass and community composition (Chapin & Shaver 1996; Walker et al. 2006; Elmendorf et al. 2012).

Warming can also influence seasonal timing in the Arctic. Timing of snowmelt in Northern Alaska has advanced in the past half-century by approximately eight days (Stone et al. 2002), consequently affecting the energy balance of the system. As snow-covered tundra has a much higher albedo than snow-free tundra, earlier snowmelt contributes to a positive
temperature-albedo feedback (Chapin et al. 2005; Sturm et al. 2005a; Pomeroy et al. 2006; Bonfils et al. 2012; Loranty & Goetz 2012). Earlier snowmelt can also alter plant phenology and lengthen the growing season, and increase time for primary production (van Wijk & Williams 2003), though the details of the coupled biological and environmental alterations require more research attention. For example, while earlier snowmelt caused by warming extends the growing season, the “new” light conditions in the early season create a novel environment for the vegetation. However, as previously noted, carbon release from soil microbial activity and plant respiration may counter the carbon uptake via photosynthesis.

A network of dynamic factors of multiple spatial and temporal scales control the carbon balance in this system. Foliar respiration, which can release approximately half of assimilated carbon in tundra plants (Hicks-Pries, Schuur & Crummer 2013), must be considered to accurately estimate the current and future fate of carbon in the Arctic. For this reason, and to advance the current state of knowledge on respiration across all biomes, a deeper understanding of the environmental and biotic controls of leaf respiration through experimentation, observation, and modeling is needed.

3. Physiology and measurement of respiration in the light

Arctic tundra species experience near complete to complete daylight during the growing season, and for this reason, measures of foliar gas exchange may be complicated due to the light inhibition of respiration. While previously published studies have not incorporated this phenomenon into their measurements, it will likely yield a more accurate estimation of foliar carbon cycling. Here, the underlying physiological mechanisms controlling respiration during photosynthesis are presented.
Foliar respiration is comprised of multiple co-occurring cellular pathways that results in the production of adenosine tri-phosphate (ATP) and carbon skeletons and releases CO₂. Unlike photosynthesis, respiration occurs in all cells of all plants at all times, though characterizing foliar respiration can be difficult due to its environmental sensitivity (described in depth below), and as a result, is often over-simplified in carbon models. A further complication is the light inhibition of respiration, which must be considered when evaluating plant carbon cycling during the night-less growing season of the Arctic. Accurately capturing carbon fluxes in leaves in the light is difficult for two reasons: (1) respiration and photosynthesis, which release and take up CO₂, respectively, occur concurrently; and (2) light inhibits plant respiration. The physiological mechanisms associated with the light inhibition of respiration and the measurement of this flux are presented in the sections below.

3.1. Respiration during photosynthesis

Photosynthesis and respiration are the basis of autotrophic metabolism, responsible for the conversion of light energy into chemical energy and providing plant cells with necessary carbon skeletons and ATP for growth and maintenance. These processes interact through multiple pathways and the reciprocal use of byproducts to maintain metabolic efficiency and prevent over-oxidation. Photosynthesis provides carbon compounds needed for respiratory metabolism, and in turn mitochondrial respiration supports light associated processes of photosynthesis, photorespiration, nitrogen assimilation, and the oxidation of excess reductant (Raghavendra, Padmasree & Saradadevi 1994; Kromer 1995; Raghavendra & Padmasree 2003; Noguchi & Yoshida 2008)
The light inhibition of respiration in photosynthetic cells has long been documented in the literature (Kok 1948; Kok 1956; Ishii & Murata 1978; Ishii, Shibayama & Murata 1979; Sharp, Matthews & Boyer 1984), and recent advances are revealing the underlying biochemical and cellular controls of this phenomenon, and highlights from these endeavors are presented here. In the light, pyruvate dehydrogenase and malic enzyme, precursors to the tri-carboxylic acid cycle are lessened in the light (Budde & Randall 1990; Hill & Bryce 1992; Tovar-Méndez, Miernyk & Randall 2003). Light is also linked to glycolysis and promotes a reorganization of the TCA cycle that makes it decidedly un-cyclical, using stored citrate in place of acetyl-coenzyme A and results in the production of glutamate/glutamine, thought to support photorespiration (Tcherkez et al. 2005; Tcherkez et al. 2008; Tcherkez et al. 2009; Tcherkez et al. 2012). As both photosynthesis and respiration produce ATP, this redundancy may also limit respiratory rates in the light. While the ratio of cytosolic ATP:ADP is related to the inhibition of respiration in the light, this is found to be true only at high values (Dry & Wiskich 1982; Peltier & Thibault 1985).

Photorespiration, which is associated with the regulation of precursors to the TCA cycle (Budde & Randall 1990; Gemel & Randall 1992; Tovar-Méndez, Miernyk & Randall 2003; Tcherkez et al. 2005), is often correlated to the degree of inhibition of respiration in the light (Griffin & Turnbull 2013). Because this process occurs simultaneously with mitochondrial respiration in the light, it confounds measurements as they both consume O_2 and release CO_2. Previous studies show that these processes can be compensatory, and the ratio of their rates can be sensitive to environmental factors such as ambient CO_2 and O_2 concentration, irradiance, and temperature (Leegood et al. 1995; Pärnik & Keerberg 1995; Hurry et al. 1996; Hurry et al. 2005; Tcherkez et al. 2008; Griffin & Turnbull 2013).
Refixation, which occurs when the carbon released via mitochondrial respiration is reintegrated into photosynthetic processes, and thus not released into the atmosphere, can create a reduction in carbon efflux via respiration. Pinelli and Loreto (2003) found that respiration in the light was inversely related to photosynthetic rate, suggesting the refixation of emitted carbon. At elevated CO\textsubscript{2}, the respiratory CO\textsubscript{2} release in the light was lower than in plants exposed to ambient and low CO\textsubscript{2} levels, suggesting that optimal photosynthetic conditions of high CO\textsubscript{2} led to increased rates of intercellular CO\textsubscript{2} refixation and thus less efflux from the leaf to the atmosphere (Loreto, Velikova & Marco 2001; Pinelli & Loreto 2003; Busch et al. 2012). However, \textsuperscript{14}C labeling experiments have shown that even when taking into account refixation, there is still true inhibition of the TCA cycle (Pärtink, Ivanova & Keerberg 2007b)

3.2 Quantifying respiration in the light

Given the multiple co-occurring fluxes of O\textsubscript{2} and CO\textsubscript{2} in the light, respiration in the light (R\textsubscript{L}) cannot be measured directly. However, indirect estimates of R\textsubscript{L} can be obtained through a variety of methodologies, ranging from the use of stable isotopes (Weger et al. 1988; Turpin et al. 1990; Pinelli & Loreto 2003), to \textsuperscript{14}C (McCashin, Cossins & Canvin 1988; Pärnik & Keerberg 1995; Hurry et al. 1996) and gas exchange (Kok 1948; Laisk 1977; Sharp, Matthews & Boyer 1984; Brooks & Farquhar 1985; Villar, Held & Merino 1995; Peisker & Apel 2001). Stable isotopes and radiocarbon techniques can be useful for the measurement of R\textsubscript{L}, as they control for the multiple CO\textsubscript{2} and O\textsubscript{2} fluxes. Parnik and Keerberg (1995) used \textsuperscript{14}CO\textsubscript{2} and known concentrations of O\textsubscript{2} to determine the distinct rates of decarboxylation by mitochondrial respiration and photorespiration (Pärnik & Keerberg 1995; Hurry et al. 2005).
Similarly, application of $^{13}$CO$_2$ can distinguish photorespiratory and mitochondrial respiratory rates (Delfine, Marco & Loreto 1999; Loreto, Velikova & Marco 2001).

There are three primary methods for the detection of $R_L$ at the leaf level using infrared gas exchange techniques: the Laisk method (Brooks & Farquhar 1985; Villar, Held & Merino 1995; Laisk & Loreto 1996), the Peisker method (Peisker & Apel 2001), and the Kok method (Kok 1948; Sharp, Matthews & Boyer 1984). Both the Laisk and Peisker methods utilize intercellular CO$_2$ concentration response ($A$-$c_i$) curves to estimate respiration in the light. The Laisk method estimates the rate of respiration in the light from the intersection of three $A$-$c_i$ curves measured at different light levels. The intercellular CO$_2$ concentration at this point ($c^*$) indicates where CO$_2$ assimilation is equal to the negative value of respiration in the light ($A = - R_L$). In contrast, the Peisker method estimates $R_L$ and $c^*$ through a linear regression of the CO$_2$ compensation concentration ($\Gamma$) and the product of the respiration rate in the dark ($R_D$) and the intercellular resistance for CO$_2$ fixation. Where the Laisk method assumes the degree of inhibition is independent of irradiance, the Peisker method assumes the degree of inhibition to be independent of $R_D$ and photosynthetic performance (Peisker & Apel 2001).

The Kok method estimates $R_L$ from CO$_2$ concentration values collected via infrared gas analysis within a cuvette when leaf material is exposed to decreasing light levels in a constant CO$_2$ environment. This method is based on the observation that the quantum yield of photosynthesis usually decreases abruptly above a certain level of light intensity—often near the light compensation point, where carbon flux is zero (Kok 1948). This leads to a noticeable non-linearity, or “bend”, in the otherwise linear lower range of the light-response curve, which is interpreted as the saturation point of light inhibition of respiration. Since respiration is assumed to be constant above this point, an extrapolation to 0 $\mu$mol m$^{-2}$ s$^{-1}$ PAR of the
linear portion of the curve above this point is assumed to give the rate of respiration in the light. The Kok method assumes the CO$_2$ assimilation rate responds only to light, and thus corrections must be made to account for changes in internal CO$_2$ ($c_i$) at different light levels. As rates of photosynthesis slow under decreasing light intensity, CO$_2$ tends to accumulate within the leaf, increasing $c_i$, which in turn affect the shape of the light curve by decreasing rates of photorespiration at lower light levels. However, correcting to a constant $c_i$ can minimalize this effect (Kirschbaum & Farquhar 1987; Ayub et al. 2011).

4. Environmental controls on foliar respiration in the Arctic

Few direct measures of foliar respiration in arctic plants across environmental treatments or gradients exist in the literature. This represents a large gap in knowledge, and one that must be filled in to more accurately predict the future carbon balance in the rapidly changing Arctic tundra. Foliar respiration can be sensitive to many environmental factors, and measurements of this metabolic flexibility can provide information for modeling carbon release, as well as lead to a better understanding of the physiological underpinnings of these fluxes. Here, major environmental controls of respiration are described, with special attention to respiration in the light and experimental evidence from the Arctic.

4.1. Irradiance and photoperiod

Light influences respiration at different temporal scales due to changes in substrate supply and physiological regulation. Respiration in the light is generally inhibited due to cellular pathways described earlier, so rates are less than respiration measured in darkness. After short-term illumination, plants exhibit two pulses of CO$_2$ efflux in the dark, the first due to
photorespiration and the second due to light enhanced dark respiration (Atkin, Evans & Siebke 1998). The amount of these respiratory bursts after illumination is dependent on the level of irradiance and correlated with the degree of inhibition of respiration by the light (Atkin, Evans & Siebke 1998). This light-enhanced dark respiratory burst is likely due to an increase in substrate made available through photosynthesis under the short-term high irradiance. Plants grown under high light conditions also exhibit higher rates of dark respiration when compared to low-light grown plants, which corresponds to greater rates of photosynthesis in the light periods preceding measurement (Pärnik, Ivanova & Keerberg 2007b). Similarly, both leaf level and extracted mitochondria of shade and sun plants show adaptations in both photosynthesis and dark respiration that help maintain carbon efficiency and avoid over-reduction and its resulting cellular damage (Gauhl 1976; Noguchi et al. 2005; Yoshida & Noguchi 2009). Photoperiod can also influence foliar respiration: extreme photoperiods, like those experienced by arctic tundra plants, are likely to play a role in the control of carbon gain and flowering time (Randerson et al. 1999; Heide 2005). Reducing photoperiod length in plants adapted to full-day light also reduces the rate of dark respiration (McNulty & Cummins 1989), likely due to a diminished energy demand.

4.2. Temperature

Temperature is integral to respiration and can have a range of effects that vary over time. For the sake of brevity and relevance to the following dissertation chapters, this section of the review will focus on the impact of growth temperature on the light inhibition of respiration and respiratory response to elevated growth temperature in arctic plants.
A limited number of studies have examined temperature effects on $R_L$ and the degree of inhibition by light, and none specifically addressing tundra plants prior to the data published in following chapters. Early work on the influence of measurement temperature and the Kok effect found values for higher light compensation point and respiration in the dark with higher measurement temperature, which in these cases, corresponded to more significant ‘bends’ in the low light response curves (Ishii & Murata 1978; Sharp, Matthews & Boyer 1984). A later study found decreasing inhibition of respiration by light with increasing measurement temperature in plants grown under low light, but the opposite effect in plants grown at higher irradiances (Atkin et al. 2000). In addition, $R_L$ increases with measurement temperature to a greater degree in plants grown under elevated CO$_2$, and inhibition was most relaxed under elevated CO$_2$ and high nitrogen conditions (Shapiro et al. 2004). In a similar study on the interactive effects of temperature, CO$_2$, and water availability, values of $R_L$ were higher in plants grown at the relatively lower temperature across different ambient CO$_2$ concentrations (Ayub et al. 2011). The degree of inhibition of respiration by light in this study was more sensitive in plants grown at an elevated temperature, with the most inhibition occurring at the highest CO$_2$ level (Ayub et al. 2011). While not considering temperature as an independent factor, but rather an aspect of seasonality, values of $R_L$ were greatest during the peak of the growing season, during the warmest months, and this corresponded with the least inhibition of respiration by light (Crous et al. 2012). In sum, elevated temperatures are associated with higher rates of $R_L$ and lower degrees of light inhibition across multiple species and growth conditions. As temperatures increase in many ecologically important regions of the world, more field measurements of $R_L$ under a range of temperatures are needed to better estimate how foliar carbon release will respond in the future.
Direct measurements of foliar respiration in arctic plants, though not as common as those of photosynthesis, exist for multiple species (McNulty, Pellizzari & Cummins 1988; Atkin & Cummins 1994; Shaver et al. 1998; Muraoka et al. 2008), though few were made in leaves grown under different temperatures. Of these, *Eriophorum angustofolium*, a congeneric of the common *Eriophorum vaginatum*, which features prominently in the following chapters, respired at a lower rate under long term warming compared to ambient growth temperature (Shaver et al. 1998), and *Ranunculus glacialis* respired less at common measurement temperatures when grown in 18°C conditions than 8°C conditions (Arnone & Korner 1997). Both studies suggest potential thermal acclimation of respiration in leaves grown under warm temperatures. However, this effect does not necessary translate to the ecosystem scale, where soil respiration overwhelms the plant signature and warming increases ecosystem respiration across multiple tundra types (Shaver et al. 1998; Grogan & Chapin 2000; Welker, Fahnestock & Jones 2000; Biasi et al. 2008; Huemmrich et al. 2010; Natali et al. 2011).

4.3. *Nutrient availability*

Plant respiration correlates to nitrogen (N) across tissue types, functional group and biomes (Reich et al. 1998; Reich et al. 2006; Reich et al. 2008). This relationship, like that of photosynthesis and N, is though to relate the N-rich enzymes of metabolism with the rates of metabolic processes. Though many studies have related soil nutrient status to direct measures of respiration, the work presented in the following chapters will nearly double the number of published studies that examine the impact of nutrient availability on $R_L$. Shapiro et al. (2004) found higher rates of respiration in the light under elevated soil N, and this effect was
amplified under elevated CO$_2$; the inhibition of $R_L$ was also lowest under high-N treatments, suggesting the crucial role of nutrient status on the control of $R_L$. A recent study looked at this response in field grown species through a natural gradient of nutrient availability of a soil chronosequence, and found the least inhibition of respiration by light in leaves with the highest N and P, which corresponded with the youngest soil sites (Atkin et al. 2013), again showing the importance of soil fertility in controlling the light inhibition of respiration.

Phosphorus (P) is also correlated with whole plant and leaf level photosynthesis (Reich & Schoettle 1988). This influence corresponds to the leaf level N: P relationship, with differing effects in different biomes (Reich, Oleksyn & Wright 2009). P-deficient plants can exhibit lower rates of photosynthesis, respiration and photorespiration (Terry & Ulrich 1973). In the Arctic, N is often a limiting factor for vegetation; the cold temperature inhibits rates of soil nutrient turnover of N and P, thus slowing their availability for uptake by plant roots. Experiments that added N in the form of nitrate and ammonia to tundra soils have noted increases in both photosynthesis and respiration (Atkin & Cummins 1994; Arens, Sullivan & Welker 2008). N and P availability can also result in saturation, with a non-linear tapering at high input levels (Baddeley, Woodin & Alexander 1994; Arens, Sullivan & Welker 2008). Also, after constant fertilization, plant carbon gain from increased photosynthetic rates may be eclipsed by soil carbon loss under fertilization (Mack et al. 2004).

4.4. Canopy Position

Irradiance decreases through a canopy due to the layers of overlapping leaves that create self-shading. This attenuation is generally characterized by the Lamber-Beer law in models to calculate how light is absorbed by individual leaves at different heights of a canopy (Hirose &
Werger 1987). Generally, wind speed also declines exponentially within the canopy (Landsberg & James 1971; Oliver 1971), thus the boundary layer conductance is lower within the canopy. Boundary layer conductance affects stomatal conductance by creating a more humid environment around the leaf, which then decreases rates of transpiration (Jarvis & McNaughton 1986). The environmental factors of light, temperature and moisture control rates of CO₂ exchange through photosynthesis and respiration in a canopy. Leaf N concentration can be distributed according to the light availability within the canopy (Field 1983; Hirose & Werger 1987). Nitrogen is generally strongly correlated with both photosynthesis and respiration (Evans 1989; Reich et al. 2006), and plant canopies of single and multi-species, may allocate resources in order to maximize canopy photosynthesis (Evans 1993b; Hirose & Werger 1994b; Anten, Schieving & Werger 1995; Anten 1997) Multi-layer models of canopies take these distributions into account when estimating canopy CO₂ exchange, avoiding some of the oversimplification of single-leaf measurements (de Pury & Farquhar 1997).

While photosynthetic parameters and related leaf traits are well outlined in a canopy (Ellsworth & Reich 1993), controls of respiration remain elusive and difficult to study. Respiration responds to many internal and external factors, including substrate supply, metabolic demand, growth, development, light inhibition, and thermal acclimation as discussed previously. While rates of respiration are linked strongly to photosynthesis and leaf N content (Azcon-Bieto & Osmond 1983; Reich et al. 2006), respiration is inhibited in the light, causing complications when modeling gas exchange in a canopy. Thus, while high respiratory rates could be assumed in leaves in the upper canopy due to the distribution of N and carbohydrate substrate available from photosynthesis, the exposure to high light levels
may inhibit respiration during the day. Respiration physiology is not static within a canopy, and can vary in rate and temperature response depending on canopy position (Griffin, Turnbull & Murthy 2002). Canopy position can also influence the foliar respiratory response to elevated CO$_2$ (Griffin et al. 2001b; Tissue et al. 2002) and may depend on leaf age (Brooks et al. 1991).

The tundra ecosystem, composed of heath, tussock and wet-sedge vegetation types, is characterized by its low-lying canopy and leaf area index around ~1 (van Wijk, Williams & Shaver 2005). However, warming is quickly changing this scenario, as the advancement of shrub species will increase surface roughness and canopy height (Thompson et al. 2004; Myers-Smith et al. 2011a). This change in community composition will increase canopy complexity, requiring more parameters to be incorporated into ecosystem-level gas exchange estimates. In lower latitude systems, respiration can be modeled based on the previous days’ photosynthesis (Whitehead et al. 2004), but respiration of arctic tundra vegetation occurs in the light throughout the whole growing season. For this reason, measures of $R_L$ and the degree of inhibition of $R_L$ are necessary to fully characterize the carbon fluxes in the tundra.

**4.5. Seasonality**

Seasonal variation in carbon fluxes increase with higher latitude (Falge et al. 2002). In northern latitude ecosystems, winter and early spring temperatures are warming (Chapman & Walsh 1993), which is correlated with an increase in seasonal CO$_2$ amplification (Chapin et al. 1996). This amplification on an annual basis can be explained by both an increase in soil and plant respiration during the snow-covered winter months as well as an increase in
photosynthesis due to earlier leaf out in the spring, extending the growing season (Chapin et al. 1996; Randerson et al. 1999; Starr, Oberbauer & Ahlquist 2008).

Within the growing season, variation in rates of photosynthesis and respiration exist, and neglecting this inter-season variation can lead to model over-estimation of ecosystem and canopy level carbon uptake (Wilson, Baldocchi & Hanson 2001). Early season photosynthesis usually increases with leaf area index and leaf photosynthetic capacity (Baldocchi, Vogel & Hall 1997). Abiotic factors such as increased photoperiod and air temperature also contribute to increased carbon uptake during this time period. However, the effect of phenology on gas exchange physiology is difficult to quantify because of the correlation between temperature and available light with phenological development. Many of the studies that examine seasonal carbon cycling focus on the ecosystem scale and use eddy-covariance for measurement (Greco & Baldocchi 1996; Baldocchi, Vogel & Hall 1997; Falge et al. 2002). While this gives accurate estimates of total net ecosystem exchange for an area, partitioning the individual rates of photosynthesis, heterotrophic and autotrophic respiration is difficult. Leaf level respiration, as well as that of stems, branches and roots, can vary within the growing season, and relates to N content (Vose & Ryan 2002; Dungan, Whitehead & Duncan 2003). In addition, the temperature response of respiration is found to vary seasonally, and with respect to canopy position (O'Grady et al. 2010). However, further investigation is needed to address the relationship between phenology, seasonality and the light inhibition of respiration.

5. Future research needs

This analysis and review of the literature indicates many advances in understanding drivers of change in the Arctic as well as in understanding environmental controls of foliar respiration.
Though, large gaps of data and understanding exist in the integration of environmental change in arctic tundra plants with foliar physiological mechanisms. Future research endeavors should work to minimize these gaps through experimentation, observation, and modeling; suggestions on areas of priority are described below.

5.1. Ecosystem respiration partitioning and scaling

Partitioning carbon fluxes at the ecosystem scale can be challenging, and this is further complicated by the known light inhibition of foliar respiration when considering the light environment of the tundra. Failing to include the inhibited respiratory rates can lead to inaccurate estimates of gross primary productivity and net primary productivity. While there is no easy answer, a few studies have attempted to incorporate this phenomenon when approaching carbon flux portioning, either through the inclusion of reduced rates at low light levels in a model (Wohlfahrt et al. 2005b), regressing eddy covariance data at low light similar to the Kok method (Bruhn et al. 2011), or just straightforwardly reducing respiration rates based on published values (Janssens et al. 2001; Chambers et al. 2004). Current application of similar methods to eddy covariance, chamber, and foliar data in arctic Alaska may more clearly interpret these data and partition carbon fluxes while incorporating measured, species-specific values of the light inhibition of respiration (Griffin et al. unpublished).

5.3. Enhancing global change experiments

Global change experiments in Arctic tundra have been incredibly fruitful for research on belowground and aboveground ecological interactions, and allowed many highly informative
pan-Arctic, cross-site comparisons (Walker et al. 2006; Cahoon et al. 2012; Elmendorf et al. 2012). However, a need exists for more multi-factor experiments, and to a greater extent, multi-level experiments that employ gradients of global change factors, in lieu of large pulsed additions of fertilizer or a single heating treatment. A more subtle treatment regime of tundra vegetation should reveal thresholds among many variables of interest that will help to inform terrestrial carbon models. In addition, due to the limited funding periods of many grants, short-term treatment effects may be more prominent in the literature than long-term effects. The impact of treatment duration poses an interesting issue, and one that can be assessed through data synthesis and modeling. For critics of global change experiments, arguing unrealistic treatment protocols on tundra vegetation, there is likely to be a healthy research future in space-for-time observational measurements that sample from regions of the Arctic that are experiencing different rates of change, whether it be shrub expansion, permafrost thaw, or warming.

5.4. **Realistic representation of respiration in models**

Finally, respiration is often oversimplified in terrestrial carbon models, merely a constant function of temperature. Increasing amounts of studies show the temperature response of respiration is nonlinear and environmentally sensitive, proving this representation to be highly limited (Atkin & Tjoelker 2003; Atkin, Bruhn & Tjoelker 2005; Atkin et al. 2008). Again, the light inhibition of respiration must also be considered, especially in carbon-rich, night-less high latitude regions. While these phenomena may be difficult to incorporate in global vegetation models, the current parameterization of respiration is likely to be weak in its contribution to accurate carbon forecasts.
6. Conclusions

As autotrophic respiration represents half of the carbon balance equation of plants, its response to climate change must be investigated for accurate assessments of ecosystem carbon exchange. Warming is rapidly altering many aspects of the Arctic tundra that impact how carbon is stored and cycled. The response of foliar gas exchange physiology of tundra vegetation to environmental change associated with warming is not well documented, though may be crucial in accurately estimating carbon exchange in this ecologically important region. This review provides an overview of major environmental controls of leaf level respiration and suggests how these factors may influence tundra plants. As these plants face a near 24-hour photoperiod, the light inhibition of respiration, an aspect of gas exchange often overlooked in ecosystem-level study, is crucial to producing an accurate carbon budgets of individual plants as well as for vegetation as a whole within a region. Similarly, more research on the downstream effects of warming and woody shrub expansion on plant CO₂ efflux will shed light on future dynamics. Empirical study and modeling of photosynthesis and respiration will provide insight into the plants’ adaptations for this extreme environment and allow for more accurate predictions about the future of arctic carbon cycling.
CHAPTER THREE

Leaf- and cell-level carbon cycling responses to a nitrogen and phosphorus gradient in two Arctic tundra species

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ABSTRACT

Consequences of global climate change are detectable in the historically nitrogen- and phosphorus-limited Arctic tundra landscape, and have implications for the terrestrial carbon cycle. Warmer temperatures and elevated soil nutrient availability associated with increased microbial activity may influence rates of photosynthesis and respiration. This study examined leaf-level gas exchange, cellular ultrastructure, and related leaf traits in two dominant tundra species, *Betula nana*, a woody shrub, and *Eriophorum vaginatum*, a tussock sedge, under a 3-year-old treatment gradient of nitrogen (N) and phosphorus (P) fertilization in the North Slope of Alaska. Respiration increased with N and P addition - the highest rates corresponding to the highest concentrations of leaf N in both species. The inhibition of respiration by light (‘Kok effect’) significantly reduced respiration rates in both species ($p < 0.001$), ranged from 12-63% (mean 34%), and generally decreased with fertilization for both species. However, in both species, observed rates of photosynthesis did not increase and photosynthetic nitrogen use efficiency generally decreased under increasing fertilization. Chloroplast and mitochondrial size and density were highly sensitive to N and P fertilization ($p < 0.001$), though species interactions indicated divergent cellular organizational strategies. Results from this study demonstrate a species-specific decoupling of respiration and photosynthesis under N and P fertilization, implying an alteration of the carbon balance of the tundra ecosystem under future conditions.

**Keywords**: Arctic tundra; carbon cycling; chloroplasts; light inhibition; mitochondria; nitrogen; phosphorus; photosynthesis; respiration
INTRODUCTION

The effects of increased nitrogen (N) and phosphorus (P) availability on carbon cycling in Arctic tundra vegetation are important for the current understanding and future predictions of net carbon (C) balance. Arctic tundra is characterized by its vast carbon reservoirs (Gorham 1991; Ping, Michaelson & Jorgenson 2008) and N and P limitation (Shaver & Chapin 1986; Shaver et al. 1992; Hobbie, Nadelhoffer & Högb erg 2002), with C accumulation being due to low soil temperatures sustaining a deep layer of organic matter in permafrost (Dowding et al. 1981; Robinson & Wookey 1997). It has been suggested that global change may alter this state (Shaver et al. 1992; Chapin et al. 1995). Consequences of climate change on Arctic tundra ecosystems are evidenced through multiple cascading environmental and ecological effects, with implications for soil nutrient availability, changes in soil microbial mineralization, and carbon cycling. Increases in winter and summer temperatures (Serreze et al. 2000; Chapin et al. 2005; Anderson 2010), lengthening of snow-free seasons (Stone et al. 2002), and decreased albedo due to warming and land-surface changes (Chapin et al. 2005; Lundberg & Beringer 2005) all affect biological processes underlying N and P resources for vegetation. Enabled by warmer conditions, the northward expansion of woody shrubs into tussock-dominated tundra continues to change plant community composition (Sturm, Racine & Tape 2001; Tape, Sturm & Racine 2006). During winter months, the taller canopy height of shrubs allows for greater snowpack, creating an insulating layer that warms soil, and in turn can increase soil nutrient availability (Sturm et al. 2004). This, as well as observed differences in litter quality, decomposition rates, and soil microbial community structure, exhibit how ecosystem C, N and P cycling can be altered under a shift to a more shrub-dominated landscape (Hobbie 1996; Weintraub & Schimel 2005; Wallenstein, McMahon & Schimel...
Further, warmer soil temperatures may increase rates of N and P mineralization (Nadelhoffer et al. 1991; Jonasson, Castro & Michelsen 2004), adding to the available N pool. Also, despite the remoteness of the Arctic, N deposition from fossil fuel combustion is detectable (Woodin 1997; Hodson et al. 2005) and is likely to increase in the future (Galloway et al. 2004). Though the direct anthropogenic source of N is relatively minimal, the combined impact of warming-mediated biological and environmental processes with deposition, will likely lead to a greater overall availability of limiting nutrients.

Long-term manipulation experiments of soil nutrient fertilization on Arctic tundra vegetation demonstrate the controlling influence of N and P on above ground primary productivity (Shaver & Chapin 1986; Johnson et al. 2000; Bret-Harte et al. 2004; Mack et al. 2004). Community responses to nutrient fertilization are well documented in tundra ecosystems (Shaver & Chapin 1986; Shaver et al. 1998; Johnson et al. 2000; Hobbie, Nadelhoffer & Högberg 2002; Bret-Harte et al. 2004; Mack et al. 2004). In tussock tundra, N+P fertilization can result in increased aboveground biomass, attributed to a compositional shift to a greater abundance of woody shrub species like Betula nana (Shaver & Chapin 1986; Chapin et al. 1995; Bret-Harte, Shaver & Chapin 2002; Mack et al. 2004). This suggests species and functional group differences that may be reflected in plant gas exchange. In wet sedge tundra, the community-level CO₂ fluxes of net ecosystem exchange (NEE), ecosystem respiration (ER), and gross ecosystem productivity (GEP) all increase under N+P fertilization, with the response to P being proportionally greater than that of N (Shaver et al. 1998). Similarly, when Johnson et al. (2000) considered the seasonal and diel impacts of CO₂ exchange in the same experimental plots, both ER and GEP were elevated significantly under
N+P addition and exhibited the greatest divergence from the control plots during the peak season.

Species differences in carbon cycling can be especially important when considering the shifting ecological dynamics of the Arctic tundra. As woody shrubs like *B. nana* expand both in individual size and geographic range, comparisons of leaf-level photosynthesis and respiration with the historically dominant tundra tussock *Eriophorum vaginatum* may strengthen predictions about the future tundra ecosystem function. Despite the large literature on tundra responses to nutrient addition, there are relatively few studies that examine leaf-level rates of gas exchange (Baddeley, Woodin & Alexander 1994; Chapin & Shaver 1996; Shaver *et al.* 1998; Bret-Harte *et al.* 2001). Of these, reports of N and P effects on photosynthetic and respiratory rates range from stimulation (Baddeley, Woodin & Alexander 1994; Chapin & Shaver 1996), to no change or inhibition (Shaver *et al.* 1998; Bret-Harte *et al.* 2001). Also, many studies on tundra vegetation utilize single-dose nutrient addition experiments, limiting the mechanistic understanding of these processes and their response to the environment. Single-dose fertilization experiments, when not compared with multi-level, multi-nutrient treatments, while informative, cannot capture possible threshold limits of physiological processes by N or P availability that may be observed at the community- (Arens, Sullivan & Welker 2008) or leaf-scale (Baddeley, Woodin & Alexander 1994). Previous work by Arens *et al.* (2008) in dwarf-shrub tundra reported a decline in GEP under higher N+P fertilization. Leaf measurements under graded nutrient availability may also reveal species interactions with N and P, and more detailed seasonal effects (Baddeley, Woodin & Alexander 1994). However, for both leaf- and community-level measurements of
carbon exchange under fertilization, the duration of nutrient addition must be considered, and comparisons across different treatment time frames may be limiting in their interpretation.

To acquire a more accurate description of carbon cycling, measurements of respiration in the light ($R_L$) are needed in addition to photosynthesis and dark respiration, as plants experience a near 24-hour photoperiod during the growing season in the carbon-rich Arctic tundra, and neglecting this measurement can lead to inaccurate estimations of leaf carbon gain. Autotrophic respiration rates are inhibited by the light - a phenomena known as the Kok effect - through multiple enzymatic pathways preceding or associated with the tri-carboxylic acid cycle (Budde & Randall 1990; Hill & Bryce 1992; Hoefnagel, Atkin & Wiskich 1998; Tovar-Méndez, Miernyk & Randall 2003; Tcherkez et al. 2005; Rasmusson & Escobar 2007). Previous studies show the degree of light inhibition can be sensitive to environmental factors such as ambient CO$_2$ concentration, growth irradiance, temperature, and water availability (Hurry et al. 1996; Wang et al. 2001; Shapiro et al. 2004; Hurry et al. 2005; Pärnik, Ivanova & Keerberg 2007b; Tcherkez et al. 2008). However, given the multiple O$_2$ and CO$_2$ fluxes that occur concurrently in the light, respiration in the light cannot be measured directly. Methods have been developed to estimate $R_L$ indirectly using stable isotopes (Weger et al. 1988; Turpin et al. 1990; Pinelli & Loreto 2003), $^{14}$C (McCashin, Cossins & Canvin 1988; Pärnik & Keerberg 1995; Hurry et al. 1996) and gas exchange (Kok 1948; Laisk 1977; Sharp, Matthews & Boyer 1984; Brooks & Farquhar 1985; Villar, Held & Merino 1995; Peisker & Apel 2001). Here, the Kok method (Kok 1948), which calculates $R_L$ based on the extrapolation of a low-light CO$_2$ assimilation curve is employed for this study based on its relative ease in measurement for a high volume of replicates in the field.
In addition to gas exchange measurements, the characterization of pertinent leaf cellular organelles can be meaningful when addressing physiological responses to the environment. Mitochondria and chloroplasts mediate autotrophic carbon fluxes, and changes in their respective densities (based on direct counts using transmission electron microscopy) can indicate underlying adaptations, as evidenced in responses to elevated CO$_2$ (Robertson & Leech 1995; Robertson et al. 1995; Griffin et al. 2001a; Tissue et al. 2002; Wang, Anderson & Griffin 2004), canopy position (Tissue et al. 2002), and temperature (Armstrong, Logan & Atkin 2006; Armstrong et al. 2006). Direct count measurements of mitochondria are rare and rarer still in field grown species. Moreover, to our knowledge no study has yet assessed how organelle abundance is affected by nutrient availability, either in arctic or non-arctic species. Such data could provide important insights into the underlying factors responsible for nutrient-dependent changes in metabolic rates in Arctic plants.

Given the carbon-rich, N- and P-limited setting of the Arctic tundra, as well as the potential sensitivity of tundra vegetation to warming-mediated environmental changes, a detailed characterization of leaf- and cell-level carbon-cycling processes in tundra vegetation will allow for greater understanding of the current and future carbon balance in this region. To examine the physiological, sub-cellular, and physical responses of Arctic plant leaves to elevated soil nutrient availability, we measured variables related to foliar carbon cycling in two dominant Arctic species of contrasting growth forms, *Betula nana* and *Eriophorum vaginatum*, under increasing levels of combined nitrogen and phosphorus addition. Using multi-level fertilized plots established in 2007 at the Arctic LTER in Toolik Lake, Alaska, measurements of respiration in the light and the dark, photosynthesis, mitochondrial and chloroplast densities, and related leaf traits were conducted using samples collected in July.
2009. These data were analyzed to test hypotheses about leaf- and cell-level carbon cycling: (1) that rates of photosynthesis and respiration would increase with combined nitrogen and phosphorus availability in a coupled manner; (2) the inhibition of respiration by light would decrease with increased fertilization, as suggested in Shapiro et al. (2004); and (3) the underlying cellular ultrastructure would correspond to leaf level fluxes. To our knowledge, this study is the first to characterize rates of leaf respiration taking place in the light in Arctic plants, as well as the first to report on nutrient-mediated changes in organelle abundance.

**MATERIALS AND METHODS**

*Site Description and Species* - The study took place in July 2009 at the Arctic Long Term Ecological Research field station at Toolik Lake, located in the foothills region of the Brooks Range, North Slope, Alaska (68° 38'N, 149° 43'W, elevation 760 m). Soils in this area consist of a 30-50 cm of peaty organic layer and a silty mineral layer, with both atop permafrost. The average growing season of this region lasts approximately 10-12 weeks, beginning in early to mid-June. Ambient light and temperature levels during the sampling period in late July fluctuated diurnally (Environmental Data Center Team 2009, Fig. 1), and no large precipitation event occurred during this time.

Leaves were sampled from plots in a randomized block design (four replicate blocks) of seven different fertilization treatments, created in 2007 by Gaius Shaver and colleagues on a large area of moist acidic tundra (MAT). Each year after snowmelt and prior to leaf-out, graded amounts of N and P, in the form of granular ammonium nitrite and triple superphosphate, are evenly distributed on the 5 x 20 m plots according to fertilization treatment. The grades of treatment increased proportionally: “CT” or “0N” (control, no
fertilization), “0.5N” (0.5 g m⁻² NH₄NO₃-N + 0.25 g m⁻² P); “1N” (1 g m⁻² NH₄NO₃-N + 0.5 g m⁻² P); “2N” (2 g m⁻² NH₄NO₃-N + 1 g m⁻² P); “5N” (5 g m⁻² NH₄NO₃-N + 2.5 g m⁻² P); and “10N” (10 g m⁻² NH₄NO₃-N + 5 g m⁻² P). The experimental plots also included “nitrate-only” (5 g m⁻² NaNO₃-N + 2.5 g m⁻² P) and “ammonium-only” (5 g m⁻² NH₄Cl-N + 2.5 g m⁻² P) plots. For this study, leaves were sampled from the control (0N), 5N, and 10N plots only.

It should be noted that these treatment levels, while denoted as 0N, 5N, and 10N, represent a combined dosage of nitrogen and phosphorus.

The species selected for this study represent the dominant tundra vegetation of the North Slope. *Eriophorum vaginatum* (“cottongrass”) is an evergreen tussock sedge, whose range spans the Arctic tundra and sub-boreal latitudes. *Betula nana* (“dwarf birch”) is a deciduous shrub, whose distribution covers Arctic, sub-Arctic, and alpine regions.

Physiological measurements were made on fully illuminated leaves sampled from the top of the tundra canopy in July 2009. Care was taken to ensure a sampling of fully expanded leaves of a similar size and age for both species.

*Foliar gas exchange* - CO₂ assimilation rate as a response of light was measured under ambient (400 ppm) CO₂ concentration using an infrared gas analyzer (Li-Cor 6400xt, Li-Cor, Lincoln, NE). Four replicate measurements were made for each species under the three fertilization growth conditions corresponding to the four replicate blocks of the experimental plots. Cuvette block temperature was set to 20 °C for all measurements to control for leaf temperature effects, and is representative of temperatures experienced by leaves during the measurement period (Fig. 1). Relative humidity inside the leaf chamber was maintained between 30-60% during measurements, and leaf vapor pressure deficit average was 0.709 ±
0.075 (SE) across all treatments. Leaves were enclosed in the cuvette at high light conditions to acclimate prior to measurement. Measurements of CO₂ assimilation were recorded at photosynthetic photon flux density (PPFD) of 1500, 1200, 800, 400, 200, 100, and then by every 5 µmol m⁻² s⁻¹ decreasing to 0 µmol m⁻² s⁻¹. This range of light levels fully encompasses the light environment experienced by both species in their natural environment (Fig. 1). To estimate theoretically maximal light-saturated photosynthetic rate ($A_{\text{max}}$) curve-fitting software was employed, using a rectangular hyperbolic function. Dark respiration ($R_D$) was measured as the CO₂ efflux at zero irradiance. Photosynthetic rates at PPFD of 900 µmol m⁻² s⁻¹ ($A_{900}$) were also measured, as this light level is representative of the average light environment during sampling and measurement times (Fig. 1). For gas exchange, all leaves sampled were cut in the field, re-cut while submerged under water, and measured inside in the laboratory. Previous experiments on these species showed no difference in rates of gas exchange and stomatal conductance between field-measured and lab-measured, cut leaves (Griffin, unpublished). Multiple ($n \cong 10$) E. vaginatum leaves were laid across the base of the cuvette to cover the 6 cm² area. Betula leaves, which were smaller than the 6 cm² cuvette area, were measured for leaf area after IRGA measurement and assimilation values were calibrated to that area for calculations. Values of photosynthesis are expressed on an area and mass basis. Photosynthetic N use efficiency (PNUE) is expressed as $A_{\text{max}}$ per milligram N.

Quantifying respiration in the light using the Kok effect - Respiration in the light ($R_L$) was indirectly measured using the Kok method (Kok 1948), which plots CO₂ assimilation as a response of light at low PPFD (<100 µmol m⁻² s⁻¹). Using the light curves described above, the low light points were analyzed using solver software (Excel Solver, Microsoft, Redmond,
WA) to distinguish and fit two lines, one above and one below the “breakpoint” that occurs under low-light measurements (Shapiro et al. 2004). Where the line extrapolated from points above the breakpoint intersects the y-axis is considered $R_L$. The line fitted from points below the breakpoint intersects the y-axis at the $R_D$ value, where PPFD = 0 µmol m$^{-2}$ s$^{-1}$. Respiration in the light was estimated after correcting for changes in internal CO$_2$ concentration according to Kirschbaum and Farquhar (1987), as described in Ayub et al. (2011). This correction accounted for changes in CO$_2$ concentration at the site of carboxylation that occur under decreasing light. The degree of inhibition of respiration by light is expressed as $I_{RL} = 1 - (R_L/R_D)$. These measurements were taken at a relative humidity of approximately 40-60%, and CO$_2$ concentration of 400 ppm. Potential diffusion in and out of the cuvette was accounted for, as was diffusion through the gasket, according to corrections presented in the Li-Cor 6400 Instructional Manual.

**Measurement of O$_2$ consumption of leaves** - Oxygen consumption by leaf tissue was measured using a Clark-type liquid phase oxygen electrode (Rank Brothers, Cambridge, U.K. and Hansatech Instruments, Norfolk, UK). Prior to measurement in the electrode, leaf tissue was sliced into small segments with a razor in a 20 mM MES buffer (pH = 6.0) and incubated in the dark for approximately 30 minutes. For uncoupled rates of oxygen consumption, 20 µM carbonylcyanide m-chlorophenylhydrazone was added to the incubation and measurement buffers to achieve uncoupled (near maximal) rates of mitochondrial respiration. The electrode cuvette contained 2 ml of the same MES buffer for measurements, which were conducted at 20°C. The depletion of oxygen was recorded over ~5 minutes and rates were calculated based
on a dry mass basis. Two replicate measurements were made for each species under each growth condition per replicate block for a total $n = 8$.

*Organelle ultrastructure* - Leaves were collected in late July and then fixed at 5°C in buffered glutaraldehyde (2% wt/vol in 0.05 M potassium phosphate buffer), placed in sealed glass 20 ml vials, and transported in insulated containers from Alaska to Lamont-Doherty Earth Observatory in Palisades, NY. Samples were post-fixed in 2% (wt/vol) phosphate buffered osmium tetroxide, dehydrated in graded acetone series, embedded in catalyzed epon (TAAB resin, Energy Beam Sciences, Agawan, MA), and polymerized at 65°C. Ultrathin sections were obtained using a Porter-Blum MT-2 ultramicrotome, collected on uncoated copper grids, stained with Reynold’s lead citrate, and examined with a Philips 201 transmission electron microscope (Einthoven, The Netherlands) operated at 60 kV accelerating voltage. The total number of mitochondria and chloroplasts were counted per cell section ($n = 45$ per species and treatment combination), and measured for size ($n = 30$, from a subset of cells measured for density), as described in Griffin et al. (2001). Each cell was measured for area, and mitochondrial and chloroplast density (number per unit cell area, not including cell walls) was calculated.

*Physical leaf traits and foliar nutrients* - All leaf samples used for gas exchange measurements were also measured for leaf area using a flatbed scanner and computer with the WinRhizo program (Regent Instruments, Quebec, CA). After the area was measured, leaf samples were dried in an oven at 60°C for a minimum of two days before mass was determined. After transport to New York, these samples were ground, weighed, and packaged
for elemental analysis to determine [CHN] (2400 Series II, Perkin-Elmer, Boston, MA).

Remaining ground leaf samples were bulked by replicate block (n = 4) and sent to the North Carolina State University Environmental and Agricultural Testing Service (Raleigh, NC, USA) for analysis by wet digestion to determine total phosphorus concentration.

Statistical Design and Analysis - Rates of photosynthesis and respiration, TEM organelle data, and leaf characteristics were analyzed using a two-way analysis of variance (ANOVA) with species and fertilization treatment as factors. Differences between fertilization levels were determined with post hoc Tukey’s test. All data obtained from the experiment are expressed as means ± S.E. All analyses used the statistical programming software R version 2.11.1.

RESULTS

Leaf nutrients and physical leaf traits – Leaf nitrogen concentration exhibited differences between species (p < 0.001, $F_{1,22} = 112.70$), treatments (p < 0.001, $F_{2,9} = 33.28$), and interaction effects were also found to be significant (p < 0.005, $F_{2,18} = 10.113$). In leaves of *E. vaginatum*, N concentration showed a general increasing trend from 0N and 5N to 10N, and in leaves of *B. nana*, this trend was significant (p < 0.001, Fig. 2). Highest N concentration for both species was measured in leaves grown under the 10N treatment (Fig. 2). Leaf carbon (C) concentration differed between species (p < 0.05, $F_{1,22} = 6.411$), with *B. nana* higher than *E. vaginatum* at all treatment levels, though there was no significant difference across treatments. Leaf C concentration was greatest at the 5N treatment level for both species. The ratio of C:N differed between species (p < 0.001, $F_{1,22} = 88.80$), across treatments (p < 0.001, $F_{2,9} = 26.23$), and species-treatment interactions (p < 0.05, $F_{2,18} =$...
5.30). *E. vaginatum* had higher C:N values at all treatment levels, with the greatest value observed at the 5N treatment level (30.33 ± 3.77). Values for C:N decreased in *B. nana* with increasing fertilization (*p < 0.01*). Leaf phosphorus values (Fig. 2) were limited statistically due to the small number of replicates, though the highest values for both species were recorded in leaves grown under the 10N treatment.

Specific leaf area (SLA), a measurement of leaf area per unit mass, differed at the species level (*p < 0.001, F_{1, 22} = 29.74*), and from the 0N to 10N treatment in *E. vaginatum* (*p < 0.05, Fig. 3*). For both species, the highest mean SLA leaves were observed at the 10N treatment level, and values for *B. nana* were higher than *E. vaginatum* at each treatment level, though structural differences due to leaf type likely drive these differences. Leaf dry matter content (DMC) also differed between species (*p < 0.01, F_{1, 22} = 8.67*) and treatment (*p < 0.05, F_{2, 9} = 4.54*). Greater DMC was found in *B. nana*, and in both species, DMC decreased with increasing fertilization. Values of fresh mass per area (FMA) differed highly between species (*p < 0.0001, F_{1, 22} = 749.74*), and non-significant fertilization and interaction effects were observed; *B. nana* increased slightly under nutrient addition, and FMA of *E. vaginatum* decreased by nearly 20% between the 0N to 10N levels.

*Foliar gas exchange across a fertilization gradient* - Area-based rates of maximal photosynthesis (*A_{max}*), displayed no significant differences between the species or across the N and P treatment gradient, though mass-based rates of maximal photosynthesis were greater in *B. nana* (*p < 0.05*), and interaction effects were observed (*p < 0.01; Table 1*). The highest rates of carbon assimilation were observed under the 5N treatment for *B. nana* and under the 10N treatment for *E. vaginatum*; whereas the lowest rates were observed at 0N in *B. nana* and
5N for *E. vaginatum* (Table 2). Area- and mass-based rates of $A_{900}$ differed in species-treatment interaction (Table 1), following the same trends as observed in $A_{\text{max}}$ (Table 2). $A_{\text{max}}$ displayed no clear, cross-taxa trend when expressed as a response to leaf N concentration (Fig. 4). PNUE exhibited treatment effects ($p < 0.01$) and was higher in *E. vaginatum* ($p < 0.001$). Greatest PNUE was observed under the 5N treatment in *B. nana*, and PNUE decreased under higher levels of fertilization in *E. vaginatum* (Fig. 5).

Area- and mass-based mitochondrial dark respiration rates were greater in *B. nana* then *E. vaginatum* ($p < 0.001$ and 0.01, respectively), but displayed no treatment or interaction effects (Table 1). Highest area-based rates of $R_D$ were observed under the 10N treatment level for both species (*B. nana*: $4.5 \pm 1.02 \ \mu\text{mol CO}_2 \ \text{m}^{-2} \ \text{s}^{-1}$; *E. vaginatum*: $1.8 \pm 0.25 \ \mu\text{mol CO}_2 \ \text{m}^{-2} \ \text{s}^{-1}$), and *B. nana* displayed a general increasing trend in rates of $R_D$ across the fertilization gradient on an area and mass basis (Fig. 6a, 6c). When expressed on a mass basis, respiration decreased under increasing fertilization in *E. vaginatum* (Fig. 6d). A general cross-taxa increase in $R_D$ is observed when expressed as a response to average leaf N concentration (Fig. 4). Respiration in the light also differed by species ($p < 0.001$) with *B. nana* exhibiting higher rates at all fertilization levels (Fig. 6). Area- and mass-based rates of $R_L$ did not differ significantly, but displayed an increasing trend in *B. nana* from the 0N to 10N level (Fig. 6a, 6c). Overall, average $R_L$ increases with average leaf N concentration, though this relationship is stronger in *B. nana* (Fig. 4).

The net foliar carbon exchange, a ratio of photosynthetic to respiratory rates, was greater in *E. vaginatum* for both measurements of respiration ($p < 0.001$, Tables 1-2). *E. vaginatum* had a higher net exchange than *B. nana* at the 0N and 10N levels for $A:\!R_D$, but not $A:\!R_L$. No difference was observed across fertilization treatments for either species for $A:\!R_D$.
and $A:R_L$, though an interaction effect between species and treatment level was observed in $A:R_D$ ($p < 0.01$, Table 1). The highest mean net exchange occurred in leaves grown in the 5N treatment plots for $B. nana$ and in the 10N treatment plots for $E. vaginatum$ (Table 2). Conversely, the lowest mean net exchange (considering both $R_D$ and $R_L$) occurred in leaves from the highest fertilization treatment plots for $B. nana$ and the 5N treatment plots for $E. vaginatum$ (Table 2).

Measurements of coupled and uncoupled rates of dark respiration differed between species ($p < 0.01$, $F_{1, 48} = 10.02$), but not in treatment level or interaction effects. The highest coupled rates of respiration were found in leaves grown in the 5N treatment plots for $B. nana$ and the 10N treatment plot for $E. vaginatum$ (Table 3); whereas the highest uncoupled rates were found in leaves grown under the 10N treatment in $B. nana$ and the 0N treatment in $E. vaginatum$. The effect of the uncoupling agent CCCP varied in elevation in oxygen consumption rates (Table 3), with a mean stimulating effect of $13 \pm 4\%$ across both species and all treatments.

**Light inhibition of respiration** - Respiration was inhibited by light among all replicates ($p < 0.001$, $F_{1, 21} = 359.15$), though species and treatment differences in the inhibition of respiration by light ($I_{RL}$) were not detected (Table 1). However, in both species, $I_{RL}$ showed a decreasing trend with higher levels of N and P fertilization. The degree of the Kok effect ranged from 12-62% with a mean of $33.5 \pm 2.7\%$ across both species and all treatments. Though not significant, $B. nana$ exhibited less light inhibition than $E. vaginatum$ at all three treatment levels. Across both species, when expressed as a response to leaf N concentration, $I_{RL}$ decreases with higher leaf N concentration (Fig. 4).
Organelle ultrastructural characteristics - Chloroplast and mitochondrial densities and sizes were influenced by fertilization treatments for both species (Fig. 7, Table 4). Chloroplast density exhibited treatment ($p < 0.001$), but not species or interaction effects (Table 4). Within species, *B. nana* chloroplast density increased from 0N and 5N to the 10N treatment level (both $p < 0.05$), and in *E. vaginatum* it increased from 0N to 10N ($p < 0.01$).

Chloroplast size differed among effects based on species, treatment, and species-treatment interaction (all $p < 0.001$, Table 4). The largest mean chloroplast sizes were 3.3 µm$^2$ in *B. nana* under no fertilization treatment and 3.1 µm$^2$ in *E. vaginatum* under 10N treatment. *E. vaginatum* exhibited a general, though not significant, decreasing trend in chloroplast size from 0N to 10N, while *B. nana* increased from 0N to 5N and 5N to 10N. The mean total chloroplast area per cell increased 35% and 28% from 0N and 5N to 10N respectively, in *E. vaginatum*; whereas, the same measure in *B. nana* increased over 500% from 0N to 10N (Fig. 8a, 8d).

Mitochondrial density differed by species ($p < 0.001$), fertilization treatment ($p < 0.001$), and the interaction effect ($p < 0.05$, Table 4). Mitochondrial density was greatest for both species at the 5N treatment level (*B. nana*: 0.18 mito µm$^2$ cell area; *E. vaginatum*: 0.11 mito µm$^2$ cell area), and higher densities were observed in *B. nana* at all treatment levels. Mitochondrial size was influenced by species, fertilization level, and the interaction ($p < 0.01; 0.001; \text{ and } 0.001$, respectively, Table 4). Although no significant increase was observed between the 0N and 5N treatment in, mean mitochondrial size increased under 10N in *E. vaginatum* ($p < 0.001$). *B. nana* did not show a similar increasing trend in mitochondrial size under fertilization. The largest mitochondria were observed in leaf sections sampled from the
10N treatment plot for *E. vaginatum* (0.24 µm²), and in leaf sections from the control plots for *B. nana* (0.21 µm²). The mean total mitochondrial area per cell increased 267% in *E. vaginatum*, and 27% in *B. nana* as fertilization increased from 0N to 10N (Fig. 8b, 8e). The ratio of total average chloroplast area to total average mitochondria area per cell was greatest under the 10N treatment in *B. nana* (Fig. 8c) and under the control treatment in *E. vaginatum* (Fig. 8f).

**DISCUSSION**

This study presents data on the responses of photosynthesis and respiration under increasing fertilization levels in two dominant Arctic tundra species. We also report, for the first time, measurements of the light inhibition of respiration in these species, and the effects of increased N and P availability on this effect, as well as on mitochondria and chloroplast density and abundance. We evaluated these leaf-level and cellular fine structure responses to increased N and P fertilization in arctic tundra plants with the hypothesis that gas exchange rates, related chloroplast and mitochondria size and density per unit cell area, and leaf traits will respond in a coupled manner.

*Decoupled rates of gas exchange under nutrient addition*

The photosynthetic rates (*A*$_{\text{max}}$ and *A*$_{900}$) of both *B. nana* and *E. vaginatum* diverged from a direct trend in response to N and P fertilization, and did not correlate with leaf N concentration. Previous work by Chapin *et al.* (1996), using the same fertilization treatment as the 10N level for this study for approximately the same duration (~3 years), found a significant increase in leaf-level photosynthesis under nutrient addition for *B. nana* and *E.*
*vaginatum* that was also observed in two other tundra species. In contrast, Bret-Harte *et al.* (2001) observed a decrease in leaf-level photosynthetic rate in *B. nana* grown for eight years under the same fertilization application as Chapin and Shaver (1996), suggesting either potential long-term acclimation or diminishing impact of increased soil nutrient availability. These examples suggest an influence of treatment duration on gas exchange measurements in these species. A similar response was documented in another tussock sedge, *Eriophorum angustifolium*, under the same treatment application for approximately five years – photosynthesis decreased, while rates of respiration were unaffected by fertilization (Shaver *et al.* 1998). Another mechanistic explanation for this response may be N saturation, where increased leaf-N is not accompanied by a coupled increased in photosynthetic capacity (Aber *et al.* 1989), and this has been documented in tundra vegetation at the community scale (Arens, Sullivan & Welker 2008). Further, both species in this study exhibit the lowest PNUE at the highest fertilization treatment (Fig. 5) and highest leaf-N, suggesting the allocation of N to non-photosynthetic proteins, such as nitrate storage or cell wall material (Takashima et al. 2004). This is underscored by the tandem decrease in SLA under the 10N treatment in the evergreen *E. vaginatum* (Fig. 3). Seasonal and developmental timing, which can produce interactive effects on gas exchange in tundra vegetation under nutrient addition, may also explain differences in measured responses across studies (Atkin & Cummins 1994; Baddeley, Woodin & Alexander 1994; Illeris & Jonasson 1999). Environmental heterogeneity across measurement years (Boelman *et al.* 2005; Arens, Sullivan & Welker 2008) may further complicate cross-study comparisons of nutrient influences on carbon cycling. The decoupling of carbon assimilation with leaf nitrogen and fertilization treatment and the pronounced
species differences emphasize the need to evaluate the broader dynamics of leaf carbon balance when considering the response of Arctic ecosystems to environmental change.

Respiration generally responded more directly to fertilization in *B. nana* than *E. vaginatum* for both *R*<sub>L</sub> and *R*<sub>D</sub>, underscored by differences in leaf concentrations of N and P (Figs. 2, 4). These differences may be explained by functional differences between the species, such as (1) their source of N acquisition - *B. nana* forms ectomycorrhizal associations and *E. vaginatum* is non-mycorrhizal (Chapin, Moilanen & Kielland 1993; Hobbie & Hobbie 2006) and (2) their growth forms – *B. nana* is a deciduous shrub requiring rapid and efficient growth during the short growing season (Bret-Harte *et al.* 2001) and *E. vaginatum* is an evergreen that retains its thick tillers through multiple winters (Fetcher & Shaver 1983). However, considering these species together, respiration rates corresponded to leaf N and P in a linear manner (Fig. 4), as described previously in plants from different biomes (Reich *et al.* 1998). Comparison of coupled and uncoupled (maximal) dark respiration rates (Table 3), further emphasize species differences and suggest differing mechanisms under high nutrient fertilization: the small difference between coupled and uncoupled rates in *E. vaginatum* implies little unused respiratory potential, while *B. nana* exhibits the largest difference between coupled and uncoupled rates, perhaps indicating an increased capacity for energy production and carbon release. The ratios of photosynthesis to respiration also showed significant species effects (Table 1), with *E. vaginatum* demonstrating no variation in net foliar carbon exchange across fertilization levels, while *B. nana* increased from control to the 5N treatment, but decreased from 5N to 10N (Table 2). The decoupled effect of increased respiration and decreased photosynthesis resulted in the lowest net carbon exchange under the highest fertilization, which mirrors measures of net ecosystem exchange from chamber
studies (Illeris & Jonasson 1999; Arens, Sullivan & Welker 2008). Therefore, while rates of respiration seem more closely linked to fertilization treatments and foliar N, the lack of a tandem increase in photosynthesis suggests the potential for greater carbon loss from Arctic plants under predicted future environmental conditions.

This study is the first to address the inhibition of respiration by light across a soil nutrient gradient and demonstrates the consistent depression of respiration rates in the light in these species. The general decrease in inhibition with higher soil nutrient availability and leaf N concentration (Fig. 4) supports the idea that inhibition may be relaxed under environmental conditions associated with enhanced photosynthesis to meet the increased cellular energy demand, as observed in Xanthium strumarium grown under elevated CO$_2$ (Wang et al. 2001) and elevated CO$_2$ and high N availability (Shapiro et al. 2004). Similarly, the lower inhibition and higher respiration rates of B. nana may be attributable to its being a deciduous shrub; shrubs allocate a substantial amount of energy expenditure to foliar growth and woody biomass accumulation, which both are enhanced under fertilization during the short Arctic growing season (Bret-Harte et al. 2001; Shaver et al. 2001; Bret-Harte, Shaver & Chapin 2002). Considering these explanations, it is possible the potential overestimation of leaf and ecosystem respiration when the Kok effect is not measured or incorporated into models may be less pronounced in a future shrubbier, more N-rich tundra system.

**Sensitivity of cellular organization to fertilization**

In addition to describing the rates of gas exchange, this study also aimed to characterize the underlying cellular organelles that serve as the setting for photosynthesis and respiration, and, to our knowledge, represents the only report of these variables in Arctic
tundra species and plants under nitrogen and phosphorus treatments. Mitochondria and chloroplasts of mesophyll cells responded to fertilization treatment in terms of density, size, and total cell volume occupied (Table 4), though differed in response patterns between species (Fig. 8). The fine-structure organization of these cells appears to be dynamic and adaptable in both species. Instead of simply increasing the number of mitochondria and chloroplasts as reported in other species grown under elevated CO$_2$ (Robertson et al. 1995; Griffin et al. 2001a; Wang, Anderson & Griffin 2004), both the size and number of these organelles appear to shift to adapt to the new energy demand. In *B. nana*, this resulted in decoupled increases in organelle total volume that favored chloroplast growth over mitochondrial growth under the highest N and P availability (500% and 27%, respectively); whereas the opposite was the case in *E. vaginatum* (35% and 267%). These contrasting responses of organelle response to fertilization, and therefore assumed energy demand, underscores the differences in short-term adaptations across taxa.

Scaling from the organelles to leaf-level rates of photosynthesis and respiration did not yield obvious functional relationships, though the related increases in total organelle volume per cell corresponded slightly with respiration in both species, and with photosynthesis in *E. vaginatum*. However, in *B. nana*, an increase in chloroplast total volume under the highest fertilization did not correlate with the depressed rates of photosynthesis. Comparison of $A:R_L$ and $A:R_D$ with chloroplast:mitochondria ratios also shows species differences in organelle-to-process relationships: the maintenance of $A:R$ despite a large drop in the relative total volume of chloroplast to mitochondria in *E. vaginatum* suggests an increased efficiency in chloroplasts under higher N and P availability. This may be due to a restructuring of grana and stroma thylakoids in response to potential higher photosynthetic activity, as evidenced in
leaves under elevated CO$_2$ (Griffin et al. 2001a; Teng et al. 2006). However, in B. nana the opposite is evident – an increase in chloroplast to mitochondria ratio was not reflected in the lower $A:R$ ratio under higher fertilization. These discrepancies may be explained by differences in regulation - organelle biogenesis, development, and degradation occur on longer time scales than photosynthesis and respiration, which can shift according to short-term environmental cues. Also, it is possible that more or larger organelles do not necessarily mean they are all of equal functional efficiency (Jeong et al. 2002). Further, measurement techniques may complicate direct correlations of counts from visualizations of cell fine structure and foliar gas exchange. Organelle measurements made using transmission electron microscopy, which images ultrathin sections of leaf tissue for analysis, may limit the potential for direct scaling compared to confocal microscopy that can localize functionally active mitochondria through staining (Logan & Leaver 2000; Gomez-Casanovas et al. 2007). This study reports significant relationships in organelle organization under increasing fertilization in two arctic species that suggest adaptive cellular responses, and highlights the potential for future research to further elucidate and quantify the connections between these scales.

Our study incorporates measurements of photosynthesis, respiration, organelle sizes and densities, and leaf nutrients to more accurately evaluate leaf-level carbon cycling in Arctic tundra species under increased N and P availability, as is predicted under warmer temperatures. Results presented highlight the need for leaf-level measurements to describe and predict carbon cycling in a changing environment. This is especially true when considering foliar respiration, which is inhibited by light and can be sensitive to environmental drivers such as N and P fertilization. For accurate estimations of foliar net carbon exchange, the inhibition of respiration of light must be considered, especially in arctic
ecosystems where dark respiration measurements do not reflect reality during the night-less growing season. Neglect of this measurement can lead to inaccurate estimations of carbon exchange at the leaf, organism, and ecosystem levels. Our study also provides the first known characterizations of mitochondria and chloroplasts in Arctic species, providing further insight on the cellular adaptations to increased nutrient availability. While the decoupled and species-specific variation in foliar carbon cycling rates under increasing fertilization treatments may complicate predictions, these findings represent another degree of complexity that must be considered in the Arctic tundra under climate change.
**TABLES AND FIGURES**

**Table 1.** Two-way ANOVA results for photosynthetic and respiratory variables comparing *B. nana* and *E. vaginatum* (Species), nitrogen and phosphorus fertilization treatment levels (NP), and interaction effects (Species x NP). Degrees of freedom for all variables were:

<table>
<thead>
<tr>
<th>Species (df = 1)</th>
<th>NP (df = 2)</th>
<th>Species x NP (df = 2)</th>
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<tr>
<td></td>
<td>$F$- ratio</td>
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<tr>
<td>$A_{\text{max}}$ (area)</td>
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<td>0.083</td>
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<td>***</td>
</tr>
<tr>
<td>$R_D$ (mass)</td>
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<td>**</td>
</tr>
<tr>
<td>$I_{RL}$</td>
<td>18.170</td>
<td>***</td>
</tr>
<tr>
<td>$A:R_D$</td>
<td>11.304</td>
<td>**</td>
</tr>
<tr>
<td>$A:R_L$</td>
<td>2.160</td>
<td>0.160</td>
</tr>
<tr>
<td>$A:R_D$</td>
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<td>***</td>
</tr>
<tr>
<td>$A:R_L$</td>
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Variables analyzed include area- and mass-based photosynthesis rates ($A_{\text{max}}, A_{900}$), quantum yield of photosynthesis ($QY$), dark respiration ($R_D$), respiration in the light ($R_L$), degree of respiratory inhibition by light ($I_{RL}$), ratios of photosynthesis to respiration rates ($A:R_D$ and $A:R_L$), coupled respiration via oxygen consumption ($R_C$), and uncoupled respiration via oxygen consumption ($R_U$). For $A_{\text{Max}}, QY, R_D, R_L, I_{RL}, A:R_D,$ and $A:R_L$, $n = 3-4$ for *B. nana* and $n = 4$ for *E. vaginatum*. Stars represent significance as follows: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. 


Table 2. Photosynthetic rates of *B. nana* and *E. vaginatum* under fertilization treatments, including maximal photosynthesis ($A_{\text{max}}$) and photosynthesis at 900 µmol m$^{-2}$ s$^{-1}$ PPFD ($A_{900}$), on an area- and mass-basis ($n = 4$). Values for the ratio of photosynthesis to respiration in the dark ($A:R_D$) and light ($A:R_L$) are also shown. Values presented are means ± SE.

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<th>$A_{\text{max}}$ (mass)</th>
<th>$A_{900}$ (mass)</th>
<th>$A:R_D$</th>
<th>$A:R_L$</th>
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<td></td>
<td>(µmol CO$_2$ m$^{-2}$ s$^{-1}$)</td>
<td>(nmol CO$_2$ g$^{-1}$ s$^{-1}$)</td>
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</tr>
<tr>
<td>0N</td>
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<td>9.4 ± 0.80</td>
<td>111.3 ± 8.77</td>
<td>73.0 ± 6.53</td>
<td>5.2 ± 0.63</td>
<td>8.15 ± 1.12</td>
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<td>17.6 ± 2.30</td>
<td>189.9 ± 28.40</td>
<td>136.9 ± 20.12</td>
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<td>107.2 ± 9.38</td>
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Table 3. Control and uncoupled (maximal) rates of oxygen consumption (nmol O$_2$ g DM$^{-1}$ s$^{-1}$) in leaf slices of *B. nana* and *E. vaginatum*, and the percent change under uncoupling treatment ($n = 8$). Values presented are means ± SE.

<table>
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<tr>
<th></th>
<th>Control</th>
<th>Uncoupled</th>
<th>% Change</th>
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<tr>
<td><strong>B. nana</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0N</td>
<td>5.5 ± 0.23</td>
<td>6.2 ± 0.47</td>
<td>+10.83</td>
</tr>
<tr>
<td>5N</td>
<td>6.5 ± 0.63</td>
<td>6.8 ± 0.41</td>
<td>+4.93</td>
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<tr>
<td>10N</td>
<td>5.4 ± 0.52</td>
<td>6.9 ± 0.74</td>
<td>+27.52</td>
</tr>
<tr>
<td><strong>E. vaginatum</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>0N</td>
<td>6.4 ± 0.24</td>
<td>7.6 ± 0.51</td>
<td>+19.56</td>
</tr>
<tr>
<td>5N</td>
<td>6.5 ± 0.51</td>
<td>7.4 ± 0.41</td>
<td>+14.18</td>
</tr>
<tr>
<td>10N</td>
<td>6.8 ± 0.30</td>
<td>6.8 ± 0.51</td>
<td>+0.49</td>
</tr>
</tbody>
</table>
Table 4. Results of a two-way ANOVA comparing *B. nana* and *E. vaginatum* and fertilization treatment effects on chloroplast (Chloro) and mitochondria (Mito) size ($n = 30$) and density ($n = 45$). Stars represent significance as follows: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

<table>
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<tr>
<th></th>
<th>Species (df = 1)</th>
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<tr>
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<td>$P$</td>
<td>$F$- ratio</td>
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<td>Chloro density</td>
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<td>0.345</td>
<td>11.160 ***</td>
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<tr>
<td>Chloro size</td>
<td>7.759 ***</td>
<td>8.744 ***</td>
<td>23.313 ***</td>
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<td>Mito density</td>
<td>12.239 ***</td>
<td>11.363 ***</td>
<td>4.553 *</td>
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<tr>
<td>Mito size</td>
<td>5.039 **</td>
<td>15.851 ***</td>
<td>9.707 ***</td>
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</table>
Figure 1. Light (PAR; dashed line) and temperature (solid line) during the sampling period in July 2009, measured nearby within 100 m of the treatment plots at Toolik Lake, Alaska.
Figure 2. Leaf nitrogen (unshaded, left axis) and phosphorus (shaded, right axis) concentration of *B. nana* and *E. vaginatum* grown under the three fertilization treatments (*n* = 4). Values shown are mean ± SEM, with alphabetic notation signifying significance at p < 0.05 between the treatment levels. Limited sample size prohibited error to be calculated for phosphorus values.
**Figure 3.** Specific leaf area (SLA) of *B. nana* and *E. vaginatum* under the three treatment levels (*n* = 4). Values are mean ± SEM and alphabetic notation signifying significance at *p* < 0.05 between the treatment levels.
Figure 4. Rates of foliar CO$_2$ exchange, including maximum photosynthesis ($A_{max}$), dark respiration ($R_{Dark}$), respiration in the light ($R_{Light}$), and degree of inhibition of respiration by light (% Inhibition) in B. nana (filled) and E. vaginatum (unfilled) as a response of leaf N and P concentrations ($n = 4$). Values shown are mean ± SEM and adjusted $R^2$. 
Figure 5. Photosynthetic nitrogen use efficiency (PNUE) of *B. nana* and *E. vaginatum* across the three fertilization treatments (*n* = 4). Values shown are mean ± SEM.
Figure 6. Area- and mass-based rates of dark respiration (shaded) and respiration in the light (unshaded) from leaves of *B. nana* (A and C) and *E. vaginatum* (B and D) grown under fertilization treatments of 0 N (control), 5 N, and 10 N (*n* = 4). Values shown are mean ± SEM.
**Figure 7.** Chloroplasts and mitochondria in leaf mesophyll cells of *E. vaginatum* grown under control (A) and 10 N fertilization (B); and *B. nana* under control (C) and 10 N fertilization (D). Starch granules were present in chloroplasts of *B. nana* under fertilization (D). Scale bars are equal to 2 µm.
Figure 8. Average total area of gas exchange organelles per 100 µm² mesophyll cell area in B. nana (A, B) and E. vaginatum (D, E). Values shown are the product of average chloroplast and mitochondria density and average mitochondria and chloroplast size. The ratio of the average total chloroplast area to average total mitochondria area is shown in panels C and F. Error bars are absent for these metrics, as they are calculated based on averages.
CHAPTER FOUR

Differential physiological responses to environmental change promote woody shrub expansion

MARY HESKEL, HEATHER GREAVES, ARI KORNFELD, LAURA GOUGH, OWEN K. ATKIN, MATTHEW H. TURNBULL, GAUIS SHAVER, and KEVIN L. GRIFFIN

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ABSTRACT

Direct and indirect effects of warming are increasingly modifying the carbon-rich vegetation and soils of the Arctic tundra, with important implications for the terrestrial carbon cycle. Understanding the biological and environmental influences on the processes that regulate foliar carbon cycling in tundra species is essential for predicting the future terrestrial carbon balance in this region. To determine the effect of climate change impacts on gas exchange in tundra, we quantified foliar photosynthesis ($A_{\text{net}}$), respiration in the dark and light ($R_D$ and $R_L$, determined using the Kok method), photorespiration, carbon gain efficiency (CGE, the ratio of photosynthetic CO$_2$ uptake to total CO$_2$ exchange of photosynthesis, photorespiration and respiration), and leaf traits of three dominant species - *Betula nana*, a woody shrub, *Eriophorum vaginatum*, a graminoid, and *Rubus chamaemorus*, a forb, grown under long term warming and fertilization treatments since 1989 at Toolik Lake, Alaska. Under warming, *B. nana* exhibited the highest rates of $A_{\text{net}}$ and strongest light inhibition of respiration, increasing CGE nearly 50% compared to leaves grown in ambient conditions, which corresponded to a 52% increase in relative abundance. Gas exchange did not shift under fertilization in *B. nana* despite increases in leaf N and P and near-complete dominance at the community scale, suggesting a morphological rather than a gas-exchange physiological response. *R. chamaemorus*, exhibited minimal shifts in foliar gas exchange, and responded similarly to *B. nana* under treatment conditions. By contrast, *E. vaginatum*, did not significantly alter its gas exchange physiology under treatments and exhibited dramatic decreases in relative cover (warming: -19.7%; fertilization: -79.7% warming with fertilization: -91.1%). Our findings suggest a foliar physiological advantage in the woody shrub *B. nana* that is further mediated
by warming and increased soil nutrient availability, which may facilitate shrub expansion and in turn alter the terrestrial carbon cycle in future tundra environments.

**Keywords:** respiration, photosynthesis, carbon gain efficiency, Kok effect, tundra shrub encroachment, *Betula nana nana, Eriophorum vaginatum, Rubus chamaemorus*
INTRODUCTION

The vegetation and soils of northern latitudes account for approximately 33-50% of the global terrestrial carbon pool, and impacts associated with climate change are altering the carbon balance of this system (Gorham 1991; Oechel et al. 1993; Ping, Michaelson & Jorgenson 2008; Tarnocai et al. 2009). Arctic warming has resulted in multiple cascading ecological and environmental effects that individually and collectively threaten profound impacts on the terrestrial carbon cycle (Shaver et al. 1992; Post et al. 2009). Of these, one of the drivers of change with the greatest potential impact is the rapid conversion of low, mostly non-woody tundra vegetation to taller vegetation dominated by deciduous shrubs (Chapin et al. 2005), and several recent reports suggest that this conversion is happening in many tundra regions (Sturm, Racine & Tape 2001; Tape, Sturm & Racine 2006). As both warming and shrub encroachment increasingly modify tundra, it is essential to understand how abundant species assimilate, store, and respire carbon, necessitating accurate measures of independent foliar carbon cycle components in this quickly changing landscape.

Shrubs can alter tussock-tundra physically and biologically on multiple scales, with many impacts attributable to their higher canopy stature. The taller canopies of shrubs increase snow depth, which leads to greater insulation during winter months, increasing soil microbial activity and in turn soil nutrient availability during spring snowmelt (Schimel, Bilbrough & Welker 2004; Sturm et al. 2005b). Taller, denser canopies also intercept more solar radiation in the summertime, leading to decreases in soil thaw and cooler soil temperatures in the summer (Chapin et al. 1995, Blok et al. 2011, Shaver et al. in press). Further, taller canopies increase surface roughness of the tundra landscape, altering its energy balance (Liston et al. 2002; Chapin et al. 2005), and may alter timing of snowmelt (Pomeroy...
et al. 2006), which has implications for landscape albedo (Sturm et al. 2005a) and species’ phenology (van Wijk & Williams 2003). Like shrubs, graminoid species also show measurable responses to climate change impacts on the tundra in terms of relative community cover and leaf chemistry and morphology (Chapin et al. 1995). Evidence suggests that these responses may be mediated by, and arguably are a direct result of, the growth and proliferation of shrubs, such as Betula nana, measured in our study, at the individual, community, and ecosystem level (Shaver et al. 2001; Wookey et al. 2009). Eriophorum vaginatum, a common tundra graminoid, is particularly well-studied in previous studies of leaf, whole plant, and community levels due to its high abundance. Its ecology, under current and potential future conditions, is well documented (Wein & Bliss 1974; Shaver & Chapin 1986; Chapin & Shaver 1996). A non-dominant but abundant species, Rubus chamaemorus, also considered in this study, fills a different ecological niche as a low-lying, shade-tolerant deciduous forb that may also play a more important role in future tundra composition and production.

Long-term field experiments underscore the effects of environmental change on community and ecosystem properties in Arctic tundra (Chapin et al. 1995; Hobbie & Chapin 1998; Elmendorf et al. 2012), and a detailed look at foliar fluxes in common tundra species may clarify these relationships. Understanding the biological and environmental influences on the processes that regulate the foliar processes in tundra species is essential for predicting the future terrestrial carbon balance in this region, because only at the leaf-level can the relevant photosynthetic and respiratory parameters may be measured. Given the inherent differences among the common tundra species measured in this study – a woody, deciduous, clonal, ectomycorrhizal shrub; a tillering, non-mycorrhizal tussock sedge; and a deciduous, shade-
loving, arbuscular mycorrhizal forb – it is likely that leaf-level gas exchange will differ amongst species according to metabolic demand, and potentially shift differentially under warming and nutrient availability. Knowledge of these intra- and interspecies differences in photosynthesis and respiration should yield more accurate estimations of larger-scale carbon fluxes under current and future warming.

The balance of photosynthetic and respiratory rates controls foliar carbon fluxes, and here we define a novel metric of carbon gain efficiency (CGE) as a ratio of the rate of carbon assimilated through photosynthesis to the total carbon assimilated through photosynthesis and released via photorespiration and respiration. A greater CGE may indicate lower respiratory costs for tissue maintenance, ion transport, and other energy demanding processes, or enhanced photosynthetic capacity (Lambers, Chapin & Pons 2008). Conversely, a decreased CGE may result in decreased overall carbon storage. The enzyme-mediated pathways of photosynthesis and respiration respond to changes in ambient air and soil temperature and soil nutrient availability associated with climate change in the Arctic tundra (Shaver et al. 1992; Wookey et al. 2009), and this adaptive shift may promote the migration and establishment of shrubs through increases in CGE of shrubs relative to non-shrub species.

Plants in the Arctic tundra experience near-complete or complete 24-hour photoperiods during the growing season, and this extreme light environment should be considered when evaluating foliar gas exchange, as multiple leaf-level carbon fluxes are sensitive to light. Thus, accurate evaluation of foliar carbon exchange and CGE in tundra plants requires estimates of both respiration in the light ($R_L$) and photorespiration ($PR$) in addition to photosynthesis ($A_{net}$) and dark respiration ($R_D$). Though important to quantify as a baseline maximum rate of potential mitochondrial respiration, incorporation of only $R_D$ would
not reflect *in vivo* rates of mitochondrial carbon efflux in arctic plants, as foliar respiration is reduced in light compared to respiration in the dark due to alterations in multiple enzymatic pathways associated with the tri-carboxylic acid cycle (Kok 1948; Sharp, Matthews & Boyer 1984; Budde & Randall 1990; Atkin *et al.* 1997; Hoefnagel, Atkin & Wiskich 1998; Tcherkez *et al.* 2005). $R_L$ can be sensitive to multiple environmental factors including atmospheric CO$_2$ concentration, irradiance, temperature, and water availability (Leegood *et al.* 1995; Pärnik & Keerberg 1995; Hurry *et al.* 1996; Wang *et al.* 2001; Shapiro *et al.* 2004; Hurry *et al.* 2005; Pärnik, Ivanova & Keerberg 2007; Tcherkez *et al.* 2008). Currently, little is known about the degree of biochemical inhibition of respiration in the light in arctic plants, or if they become adapted to limit or eliminate this inhibition in an environment with constant daylight during the growing season. Here, we also consider photorespiration, another environmentally sensitive foliar CO$_2$ efflux (Collatz 1977; Brooks & Farquhar 1985; Leegood & Edwards 2004), because the neglect of these fluxes could lead to a potentially significant miscalculation of respiration, gross photosynthesis, and the resulting ecosystem carbon balance. A predictive and mechanistic understanding of ecosystem carbon flux in Arctic tundra requires knowledge of how both processes respond to current and predicted environmental change.

We argue that differences observed at the community and ecosystem scale associated with woody shrub expansion may be consistent with, and in part attributable to, tundra plant species’ foliar carbon cycling and metabolic efficiency. We hypothesized that shrubs of the Alaskan Arctic tundra would have a higher carbon gain efficiency at the leaf level than other abundant tundra species, and that this difference could represent a physiological advantage that is consistent with increasing shrub density under arctic climate change. To test this under
current and predicted environmental scenarios, we quantified photosynthesis, respiration in the dark and light, photorespiration, and related leaf traits in three dominant tundra species representing three different functional groups under ambient and experimental conditions that simulated impacts associated with climate change, specifically warming and increased soil nutrient availability. A related objective was to quantify the light inhibition of respiration in these species and to examine the importance of this phenomenon in foliar carbon fluxes and metabolism. Our study represents one of the first reports of respiration in the light and photorespiration, two environmentally sensitive, potentially substantial, yet often neglected C fluxes, in these dominant arctic tundra species under long-term warming and fertilization. The new detailed data on these mechanistic processes will allow for a better understanding on the carbon efficiency in these species, and their potential relationships with known community level shifts in arctic vegetation under simulated global change.

MATERIALS AND METHODS

Field Site and Species - The study took place during the peak growing season from mid-June through mid-July 2009 at the Arctic Long Term Ecological Research (LTER) field site near Toolik Lake (68°38’N, 149°36’W) on Alaska’s North Slope, located 254 km north of the Arctic Circle. All leaves were fully expanded and from the upper canopy, and were sampled from experimental plots in moist acidic tundra (MAT) established and maintained by the LTER since 1989 (similar to an older experiment described by Chapin and Shaver (1985)). The MAT site consists of four randomized blocks of 5 x 20 m treatment plots separated by a 1 m buffer arrayed two-by-two on a slightly-sloped, poorly-drained hillside. Treatments manipulate the ambient environment to reflect predicted impacts of climate change on tussock
tundra, including increased soil and air temperature using greenhouses (GH), increased
nitrogen and phosphorus availability using fertilizers (NP), and a combined treatment of
warming and fertilization (GHNP). Wood-framed greenhouses, covered with transparent 0.15
mm plastic sheeting, passively increase air temperature by approximately 5°C during the
growing season, while insignificantly affecting light intensity, humidity, and thaw depth
(Gough and Hobbie 2003). In the NP plots, 10 g m\(^{-2}\) of granular NH\(_4\)NO\(_3\)-N and 5 g m\(^{-2}\) of
granular P\(_2\)O\(_5\)-P is applied each year in early June after snowmelt. In the GHNP treatment
plots, tundra vegetation is enclosed by the same greenhouses as in the GH plots and treated
with the same fertilizers, in the same amounts, as in the NP plots.

The focal species for our study are common and abundant at the MAT site:

*Eriophorum vaginatum* L. (“cottongrass”), the tussock-forming sedge that gives “tussock
tundra” its name and is widely distributed over much of Alaska’s North Slope, northern
Canada, and northern Eurasia; *Betula nana nana* L. (“dwarf birch”), a deciduous woody shrub
that is also abundant and often dominant over much of the entire Arctic region; and *Rubus
chamaemorus* (“cloudberry”), a widespread and often abundant herbaceous perennial forb. In
this work on leaf properties of these species, care was taken to ensure a sampling of only fully
expanded leaves of a similar size and age.

*Foliar gas exchange* - CO\(_2\) fluxes of photosynthesis and respiration were measured using an
infrared gas analyzer (IRGA; LI-6400XT Portable Photosynthesis System, LI-COR, Lincoln, NE). All measurements were taken on clipped leaves collected from the long-term MAT
treatment plots, re-cut under water in the field, and transported in water to the laboratory.
Preliminary tests on these species showed no difference in rates of gas exchange and stomatal
conductance between field-measured and lab-measured leaves (Griffin, unpublished), as has been shown previously in other species (Mitchell, Bolstad & Vose 1999; Turnbull et al. 2003). This sampling technique was required to provide a greater degree of temperature control, maximize the number of replicates, and minimize time between replicates over the short growing season and during potentially rapidly changing environmental conditions in the field. For each E. vaginatum sample, we collected approximately ten leaves from a single tussock in order to provide sufficient leaf area in the leaf chamber (~6 cm²). B. nana leaves, which were smaller than the 6 cm² cuvette area, were measured for leaf area after IRGA measurements and gas exchange values were corrected relative to that area for analysis. Individual leaves of R. chamaemorus covered the entire leaf cuvette for gas exchange measurements. Two replicate measurements per species were made on leaves collected from each treatment block.

Prior to measurement, leaves were enclosed in the cuvette at high light conditions to acclimate. CO₂ assimilation was measured under ambient (400 ppm) CO₂ concentration under 26 levels of photosynthetically active radiation (PAR): 1500, 1200, 800, 400, 200, 100, and every 5 PAR between 100 and 0 μmol m⁻² s⁻¹. This range of light fully encompasses the light environment experienced by the three species in their growth environment (Heskel et al. 2012). After 10 minutes in darkness, it was assumed that no photosynthesis or photorespiration was taking place, and all CO₂ flux could be attributed to mitochondrial respiration in the dark (RD). All measurements were taken at a relative humidity of approximately 40-60%, and potential diffusion in and out of the cuvette was accounted for, as was diffusion through the gasket, according to corrections presented in the Li-Cor 6400 Instructional Manual. Average leaf vapor pressure deficit was 0.717 ± 0.081 (SE) across all
treatments. To account for the influences of leaf temperature on gas exchange variables, cuvette block temperature was set to 20° C for all measurements, which is representative of temperatures experienced during the growing season (Heskel et al., 2012). Maximal light-saturated net photosynthetic rate ($A_{\text{net}}$) was estimated by fitting the data to a rectangular hyperbolic function (Excel Solver, Microsoft, Redmond, WA). Photorespiration ($PR$), which represents the CO$_2$ flux release associated with the oxygenation of Rubisco at saturating light, was calculated according to equations presented by von Caemmerer and Farquhar (1981), as described in Ayub et al. (2011). Using values of photosynthesis, photorespiration, and respiration in the light ($R_L$, see Kok effect below), carbon gain efficiency (CGE) was calculated ($CGE = A_{\text{net}} / (A_{\text{net}} + R_L + PR)$ to estimate the proportional carbon gain per carbon exchanged. Values of photosynthesis and respiration are expressed on an area, mass, and nitrogen basis.

*Quantifying respiration in the light using the Kok effect* - To estimate respiration in the light, we used the Kok method, which is convenient for field measurements and can be used under ambient atmospheric conditions. This method is based on the observation that the quantum yield of photosynthesis usually decreases abruptly above a certain level of light intensity—often near the light compensation point, where carbon flux is zero (Kok 1948). This leads to a noticeable non-linearity, or “bend”, in the otherwise linear lower range of the light-response curve, which is interpreted as the saturation point of light inhibition of respiration ($I_{RL}$, explained in detail in Shapiro et al. 2004). Since respiration is assumed to be constant above this point, an extrapolation to 0 μmol m$^{-2}$ s$^{-1}$ PAR of the linear portion of the curve above this point is assumed to give the rate of respiration in the light (Fig. S1). Here, the irradiance range
we used to calculate $R_L$ spanned from 25-90 \( \mu \text{mol m}^{-2} \text{ s}^{-1} \). The percent inhibition of light respiration is calculated from the ratio of the difference between dark and light respiration rates to the rate of dark respiration \( \% I_{RL} = ([R_D - R_L] / R_D) \times 100 \).

The Kok method assumes the \( \text{CO}_2 \) assimilation rate responds only to light, and thus corrections must be made to account for changes in internal \( \text{CO}_2 (c_i) \). As rates of photosynthesis slow under decreasing light intensity, \( \text{CO}_2 \) tends to accumulate within the leaf, increasing \( c_i \), which in turn affect the shape of the light curve by decreasing rates of \( PR \) at lower light levels. We corrected all measurements to a constant \( c_i \) to account for these effects, according to Kirshbaum and Farquhar (1987), as described in Ayub et al. (2011), to achieve a more accurate extrapolation of $R_L$.

**Physical leaf traits and foliar nutrients** - All leaf samples used for gas exchange measurements were measured for leaf area using a rotating-belt leaf area meter (LI-3100C Area Meter, LI-COR, Lincoln, NE). Samples were then dried in an oven at 60°C for a minimum of two days before mass was determined. After transport to Columbia University in New York, all samples were ground, weighed, and packaged for elemental analysis of [CHN] (2400 Series II, Perkin-Elmer, Boston, MA). Remaining ground leaf samples were bulked by replicate block \( (n = 4) \) and sent to the North Carolina State University Environmental and Agricultural Testing Service (Raleigh, NC, USA) for analysis by wet digestion to determine total phosphorus concentration.

**Relative species abundance** - The cover of mosses, lichens, and all vascular plant species was measured in eight 1 x 1 m² plots in each block in mid-July 2007 by visual estimation as
described by Gough and Hobbie (2003). The relative cover was calculated by dividing the individual specie’s cover by the total plant cover for each measured quadrat (Gough & Hobbie 2003). Major functional group and species level community shifts correlated with warming, fertilization, and warming with fertilization treatments that occurred prior to 2007, so these data should represent the vegetation that occurred in these plots in 2009 with reasonable confidence (Gough, unpublished).

Statistical Analyses - The effects of environmental treatment on gas exchange variables and leaf characteristics were analyzed using a three-way analysis of variance (ANOVA) in R (v2.7.0, The R Foundation for Statistical Computing), assigning species, fertilization, and warming as explanatory variables. Block effects were not found to be significant for the variables measured. Tukey’s Honestly Significant Difference test was used for multiple comparisons of means. Community data were analyzed using a two-way MANOVA with B. nana, E. vaginatum, R. chamaemorus, and “other” (an aggregate of all other species found in the plots but not considered in this study) as dependent variables, and warming and fertilization as factors. A Wilks-Lambda test statistic was used to determine significance. Differences were considered significant if \( p < 0.05 \).

RESULTS

Species-mediated variation in leaf chemistry and traits

Leaf N was greatest in the deciduous species, B. nana and R. chamaemorus, while E. vaginatum exhibited the lowest concentrations (main effect of species: \( p < 0.001, F_{2, 94} = 14.414 \)). A significant interaction between warming and fertilization was present among all
species \((p < 0.01, F_{1, 95} = 11.072)\), where warming dampened the magnitude of increase under fertilization. Within individual species, leaf N of \(E. vaginatum\) and \(B. nana\) increased in NP plots (Table 1). In leaf P, a significant interaction effect between warming and fertilization was observed \((p < 0.01, F_{1, 47} = 7.549)\), driven by differences in \(B. nana\) and \(R. chamaemorus\), where the combined treatment values were lower than NP plots alone. Similar to leaf N, the lowest concentrations were observed in \(E. vaginatum\) (main effect of species: \(p < 0.001, F_{2, 46} = 12.679\)). No significant warming effect independent of fertilization was detected upon analysis. In terms of foliar C:N, the interaction between warming and fertilization \((p < 0.001, F_{1, 95} = 14.159)\) resulted in greater values between species. Foliar C:N varied among species (main effect of species: \(p < 0.001, F_{2, 94} = 22.234\)), with greatest values observed in leaves of \(E. vaginatum\). Among species, fertilization altered foliar C:N, mainly through higher measures of N in leaves grown under fertilization (Table 1, \(p < 0.001, F_{1, 95} = 33.403\)). Foliar N:P did not vary significantly among species, though was predictably influenced by fertilization treatment \((p < 0.001, F_{1, 47} = 25.099)\). This trend was also apparent when considering individual species, where N:P was considerably lower under fertilized growth conditions compared to the control leaves (Table 1).

For all species, SLA was significantly affected by warming \((p < 0.05, F_{1, 95} = 5.457)\), mainly driven by \(R. chamaemorus\), of which SLA increased with elevated growth temperature (species-treatment interaction effect: \(p < 0.01, \text{Fig. S2}\)). Similarly, fertilization did not increase SLA, except in the case of \(R. chamaemorus\) (Fig. S2).

*Species and warming drive differences in carbon exchange*
Mean values of net photosynthesis ($A_{\text{net}}$) were greatest in $B. \ nana$ and lowest in $E. \ vaginatum$ (Table 2), and species’ effects were highly significant on an area, mass, or N basis (all $p < 0.001$, Table S1). Considering all replicates, neither fertilization nor warming treatments significantly altered photosynthesis. However, leaves of $E. \ vaginatum$ exhibited a general decline in rates under warming in both the non-fertilized and fertilized plots. Similarly, across all replicates, photorespiration ($PR$) in $E. \ vaginatum$ was significantly lower than in $B. \ nana$ and $R. \ chamaemorus$ (Table 2). Between species, $PR$ calculated in leaves grown under the combined GHNP treatment was elevated compared to the individual treatments, NP and GH alone (Table 2). Under warming, $PR$ of $E. \ vaginatum$ was lower than in leaves of both $B. \ nana$ ($p < 0.05$) and $R. \ chamaemorus$ ($p = 0.197$), though when combined with fertilization this effect was not found.

Dark respiration ($R_D$) did not vary significantly among species when expressed by area, though highest rates were reported in $B. \ nana$ when expressed on a mass- and N-basis. Among species, warming decreased area-based $R_D$ (-21.7% ± 6.8, Table S1), and mass-based $R_D$ (-21.4 ± 11.1, Table S1). Within species, no significant treatment effects were observed in $R_D$ (Fig. 1). Light significantly inhibited respiration ($p < 0.0001$) across all replicates, and the percentage inhibition of respiration ($% \ I_{RL}$) ranged widely (Fig. 1), with a mean of 27% ± 2 across species and treatments. Rates of respiration in the light ($R_L$), like $R_D$, were lowest in $E. \ vaginatum$ (Fig. 1) and varied among species on an area- ($p < 0.05$), mass- ($p < 0.001$), and N-basis ($p < 0.001$, Table S1). Warming further reduced rates of respiration among species when expressed by area: -36.9% ± 8.3 ($p < 0.001$), mass: -22.3% ± 9.1 ($p < 0.001$), and N: -20.1% ± 8.1, ($p < 0.05$). Fertilization treatment had a similar, though less significant effect, with mean
values of $R_L$ lower than control values across species, though only by area ($p < 0.05$) and N ($p < 0.01$). No significant interactions between warming and fertilization were found.

Carbon gain efficiency (CGE) values were greatest in $B. nana$, and significantly lower in both $R. chamaemorus$ ($p < 0.05$) and $E. vaginatum$ ($p < 0.001$, Fig. 2). Across species, both warmed and fertilized growth conditions minimally influenced CGE, with effects of $3.4\% \pm 1.7$ and $3.5\% \pm 1.8$, respectively. Within an individual species, only rates of CGE in $B. nana$ exhibited significant elevation: warming more than doubled CGE compared to control values ($p < 0.05$, Fig. 2). Further, under warming, CGE of $B. nana$ was significantly greater than the other two species (both $p < 0.01$). There were no clear significant correlations of mass-based fluxes with N, P, or N:P (Fig. S3), though $B. nana$ and $R. chamaemorus$ showed higher rates of gas exchange and CGE than $E. vaginatum$ per unit N or P.

**Shrub dominance and tussock vulnerability under manipulated environmental conditions**

At the community scale, fertilized and warming treatments exhibited a significant interaction effect on relative species abundance considering all measured species, and among individual species, only $B. nana$ had a significant interaction effect (Table 3). Relative species abundance, both overall and considering individual species, was significantly influenced by fertilization (Table 3). Warming affected the relative species abundance overall, but only affected $E. vaginatum$ individually, significantly decreasing its abundance ($p < 0.005$, Fig. 3). Mean relative abundance values of $B. nana$ increased under warming, further under fertilization and the combined treatment (all $p < 0.05$), compared to control values, whereas $R. chamaemorus$ increased in cover under fertilization and $E. vaginatum$ significantly decreased under both warming and fertilization treatments (Fig. 3). Overall, $B. nana$ responded most in
terms of expanding cover under long-term treatments, becoming the dominant species in the NP and GHNP plots, while in those same plots, *E. vaginatum* was most negatively affected.

**DISCUSSION**

This study presents new insights into leaf-level carbon exchange in the light in three common and abundant arctic species, under current ambient and predicted future environmental conditions, with special attention to mechanistic responses in light, specifically mitochondrial respiration in the light and photorespiration, given the nightless environment of the tundra growing season. Incorporating these novel measurements to quantify carbon gain efficiency provides relevant measures of carbon loss in Arctic plants, and considered together, our findings suggest a foliar physiological advantage in the leaves of woody shrub *B. nana* over the graminoid *E. vaginatum* and forb *R. chamaemorus* that may enable community-scale dominance and be further mediated by warming and increased soil N and P availability.

*Species-level physiological differences under current conditions*

Our results indicate strong species-driven differences in leaf-level gas exchange and underlying leaf nutrient composition under ambient environmental conditions. We found higher rates of CO₂ assimilation in leaves of *B. nana* than the other species, mirroring differences in net photosynthesis measured in nearby plots nearly three decades ago (Chapin & Shaver 1996). Rates of respiration in the light and dark were lowest in *E. vaginatum* and highest in *B. nana* and *R. chamaemorus* (Fig. 1) potentially reflecting potential differences in energy demand required for new leaf growth and development. Similarly, when compared to *E. vaginatum*, both *B. nana* and *R. chamaemorus* exhibited greater leaf N and P, which
corresponded to greater net photosynthetic and respiratory rates (Fig. S3). \( R_t \) and \( PR \) are important variables to quantify as autotrophic respiration may account for nearly 50% of ecosystem respiration in tundra system (Hicks-Pries \textit{et al.} 2013). The inclusion of these two parameters allowed the first known calculation of foliar carbon gain efficiency in these species. \textit{B. nana} exhibited the highest rate of CGE in ambient growth conditions due to the higher proportion of \( \text{CO}_2 \) assimilation to \( \text{CO}_2 \) loss through \( PR \) and \( R_t \), and it is possible much of that carbon that would be allocated towards woody stem growth and expansion (Bret-Harte, Shaver \& Chapin 2002). At the community scale, our results also show similar species composition in control plots to previous measurements (Shaver \& Chapin 1986; Shaver \textit{et al.} 2001), indicating long-term consistency in composition under ambient environmental conditions. This consistency exhibited in the control plots provide a stable background against which experimental manipulations of key environmental changes can be measured.

\textit{Physiological responses to warming and fertilization promote shrub expansion}

Environmental treatments simulating future impacts of climate change on the Alaskan Arctic tundra facilitated a restructuring of the community. The woody shrub, \textit{B. nana}, increased in abundance disproportionally under elevated air temperature, and to a stronger degree under increased soil N and P availability and in a combination of the two treatments, ultimately replacing the graminoid \textit{E. vaginatum} and leading to a stark alteration of relative species composition (Fig. 3). Despite similar trends at the community-scale, the foliar physiology within- and across-species responded differently to these environmental treatments both on a mass and area basis, suggesting important distinctions in the physiological and morphological mechanisms that may facilitate shrub expansion in a future climate.
B. nana exhibited the greatest rates of CGE under warming (Fig. 2), due to the compounded effect of a high rate of $A_{net}$ and lower rates of $PR$ and $R_L$ compared to control plants. E. vaginatum and R. chamaemorus exhibited general increases in CGE, mainly due to decreases in $PR$ and $R_L$ in E. vaginatum, and decreases in $R_L$ in R. chamaemorus. $A_{net}$ showed no increase under warming, as reported previously in arctic tundra vegetation at the leaf- (Chapin & Shaver 1996) and ecosystem-scale (Welker et al. 2004; Biasi et al. 2008; Huemmrich et al. 2010), potentially indicating a long-term loss of stimulatory effect. However, for E. vaginatum, $A_{net}$ in leaves grown under warming was lower than in leaves grown in ambient conditions, suggesting this species is operating beyond its temperature optimum. It is also possible that in vivo leaf temperature may be altered by increased cover density in a shrubbier environment, potentially lowering rates through an acclimation to the cooling effect of shading in a taller canopy. The influence of warming on photosynthesis is likely to depend on the duration and degree of the warming treatment, and is shown to vary across region and microclimate (Oberbauer et al. 2007; Elmendorf et al. 2012). All species in this study exhibited a general trend of greater inhibition of respiration in the light under warming, which contrasts with results from a previous study (Atkin et al. 2006). The lower mean $R_D$ and $R_L$ values under warming across the three species also suggest long-term thermal acclimation of respiration (Atkin & Tjoelker 2003). Taken as a whole, foliar gas exchange is modified under warmer temperatures, and allows for significantly greater C accumulation in B. nana, creating a competitive physiological advantage compared to the non-woody species. When considering the community, this may lead to more relative cover of woody shrubs under warming, mirroring the trend already established in Arctic tundra (Chapin et al. 1995; Tape, Sturm & Racine 2006; Walker et al. 2006). In addition to altering leaf physiology in B.
nana, warming can stimulate belowground growth and N uptake (Hobbie & Chapin 1998; Gough & Hobbie 2003). Further, warming may alter components of the associated ectomycorrhizal community that promote nutrient acquisition (Deslippe et al. 2011) and belowground carbon transfer in B. nana (Deslippe and Simard 2011). Conversely, the decrease in relative cover in the historically abundant graminoid E. vaginatum may be in part related to leaf-level physiology due to its tendency to decrease $A_{\text{net}}$ under warming (Table 2), its less photosynthetic N-use efficiency compared to B. nana, as well as herbivory (Gough et al. 2012) and interspecies competition for resources such as light and soil nutrients in a shrubbier canopy (Fetcher 1985).

In contrast to warming, long-term fertilization did not impact carbon gain efficiency in any of the study species. B. nana and R. chamaemorus, both mycorrhizal-forming, had higher leaf N and P concentrations than E. vaginatum, though this did not translate to enhanced rates of carbon exchange (Fig. S3). An earlier study including these three species found a similar lack of stimulatory effect of fertilization on maximal photosynthesis despite increases in shoot growth (Bigger & Oechel 1982). Previous experiments reported elevated photosynthesis under the same fertilization treatment after ~3 yr (Chapin & Shaver 1996), though decreases were reported by Bret-Harte et al. (2001) after ~8 yr, which may suggest a temporal duration of fertilization effect, and potentially foliar N-acclimation after a relatively short time period. Values of N:P found in this study do not indicate photosynthetic limitation by either N or P (Tessier & Raynal 2003). Moreover, a lack of a clear photosynthesis-N relationship (Fig. S3) in these species, also observed along water tracks (Griffin & Turnbull 2013) suggests the allocation of N towards other cellular processes, such as nitrate assimilation and storage or
cell wall material (Onoda, Hikosaka & Hirose 2004; Takashima, Hikosaka & Hirose 2004), or wood production (Bret-Harte et al. 2001; Bret-Harte, Shaver & Chapin 2002).

While foliar carbon fluxes exhibited little positive response to greater N and P availability, significant changes were observed at the community scale (Fig. 3a). Fertilization, under both ambient and warmed temperatures enabled the dominance of *B. nana* and the near disappearance of *E. vaginatum* from the treatment plots. *R. chamaemorus*, though not as responsive as *B. nana*, also expanded its presence under elevated N and P (Fig. 3b). This expansion of the woody shrub *B. nana* is noted in previous studies in the tundra of Arctic Alaska (Chapin et al. 1995; Shaver et al. 2001; Bret-Harte, Shaver & Chapin 2002; Mack et al. 2004; Walker et al. 2006) and appears to be a widespread phenomenon. In contrast to the foliar physiological response observed under warming, this may indicate a morphological adaptive strategy under fertilization, where shoot initiation and expansion, and thus total leaf area, increases; but the physiology of those leaves is relatively unaltered. This implies a strong relationship between total foliar N, leaf area index, and gross primary productivity (Williams & Rastetter 1999; van Wijk, Williams & Shaver 2005), although this may be limited by self-shading or decreased photosynthetic N-use efficiency (Street et al. 2007). In addition, considering the similar rates of CGE in the species under fertilization, the concurrent dominance of *B. nana* at the community level suggests belowground interactions may be responsible for driving plant composition changes. This is supported by earlier studies in which a marked decline in the fine root production of *E. vaginatum* under long term N and P fertilization (Sullivan et al. 2007), and increases in ectomycorrhizal fungi associated with *B. nana* (Clemmensen et al. 2006) were associated with greater aboveground dominance of *B. nana*. Further, though long-term elevated soil N and P may enhance total net primary
production (NPP) and aboveground C and N pools, over time belowground C losses from soil may outpace gains from gross primary productivity (Chapin et al. 1995; Mack et al. 2004).

The combined growth treatment of warming with fertilization revealed decoupled impacts on leaf physiology. For all study species, leaf N and P declined under the combined treatment compared to fertilization alone (Table 1), suggesting, in the case of B. nana, a dilution of tissue N and P due to greater shoot initiation and expansion under the combined treatment. Foliar respiration in the coupled treatment reflected similar trends as seen under warming alone, suggesting thermal acclimation for all three species, and an elevation of the inhibition of respiration in the non-shrub species. The effect of the coupled treatment did not lead to either enhanced photosynthesis or CGE, in concordance with previous measurements at the ecosystem scale (Chapin & Shaver 1996; Boelman et al. 2005). However, the combined treatments still strongly altered community composition with comparable decreases in E. vaginatum and increases in R. chamaemorus and B. nana as observed under fertilization alone. Together, these data confirm N and P to be a greater limitation to shrub growth and expansion, and in turn a greater threat to tundra biodiversity, than temperature, and underscore the importance of multi-factor global change experiments to understand terrestrial carbon cycling (Templer & Reinmann 2011).

*Implications and conclusions*

When considering the carbon reservoir of the Arctic tundra and its future fate, it is important to include estimates of leaf-level physiological responses of tundra species and how they may change under future predicted conditions. To that end, we present the first ecologically meaningful estimates of respiration to consider the known inhibition of respiration caused by
light from the well-studied, long-term global change experiment at Toolik Lake, Alaska. By combining estimates of $R_L$ with carefully measured photosynthetic rates and calculated rates of photorespiration, we present for the first time estimates of carbon gain efficiency in arctic tundra species. While the different environmental treatments result in a similar phenomenological response, this study demonstrates that a number of different physiological mechanisms are responsible. Our results indicate a shift in foliar gas exchange physiology that may provide an advantage to the dominant woody shrub species $B. nana$, facilitating its expansion into the historically tussock-dominated tundra. Further, we describe the possibility of a more morphological/developmental strategy on the part of $B. nana$ that may mediate aboveground expansion when soil N and P are not limiting. Prior to our experiments, it was unknown to what degree these two mechanisms contributed to the observed increase in shrub growth, and only through the quantification of the relevant physiological measurements could these relationships be determined. Therefore, despite similar plot-level responses, the underlying mechanisms vary, providing critical information for modelling, and requiring further study to fully understand the long-term ecosystem consequences. Also, the synergistic impacts of both warming and increased N and P show interactions that do not reflect an additive effect, which suggest the need for more research on multiple factors on these processes.

The use of physiological measurements suitable to quantifying the mechanistic responses in the unique Arctic environment of continuous daylight provides new insights into the regulation of carbon in this important ecosystem. The incorporation of these findings into vegetation models will more accurately reflect the mechanistic underpinnings of the
ecosystem response to global change and improve our predictive understanding. Future modelling studies can benefit from integrating these foliar physiological processes and their temperature and seasonal responses at the canopy and ecosystem level, as they may reveal new insights into carbon allocation. Ultimately, knowing more about species’ individual and community growth responses under environmental change and the corresponding relative carbon fluxes will enable more accurate estimates of ecosystem carbon storage. Our results suggest that different functional strategies under the individual treatments may allow for the continued encroachment and expansion of woody shrubs into the Arctic tundra as temperatures warm in this region, and this has significant implications for both biodiversity and carbon storage.
Table 1. Foliar element concentrations and ratios of the three study species grown under control and treatment conditions. N \((n = 7-9)\) and P \((n = 4)\) values represent mmol g\(^{-1}\) of leaf area, and N:P \((n = 4)\) is a ratio of those values, however, C:N \((n = 7-9)\) represents a ratio of the percent of dry leaf matter for both elements. Means ± SE are presented. Alphabetical notation is used to indicate significance within a species \((p < 0.05)\).

<table>
<thead>
<tr>
<th>Species</th>
<th>Treatment</th>
<th>N (mmol g(^{-1})) ± SE</th>
<th>P (µmol g(^{-1})) ± SE</th>
<th>C:N ± SE</th>
<th>N:P ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. nana</td>
<td>CT</td>
<td>1.92 ± 0.03(^{a})</td>
<td>98.5 ± 5.5</td>
<td>17.78 ± 0.33(^{a})</td>
<td>19.58 ± 0.79(^{a})</td>
</tr>
<tr>
<td></td>
<td>GH</td>
<td>1.94 ± 0.09(^{a})</td>
<td>106.5 ± 3.2</td>
<td>17.35 ± 0.57(^{a})</td>
<td>18.30 ± 1.20(^{a})</td>
</tr>
<tr>
<td></td>
<td>NP</td>
<td>2.51 ± 0.16(^{b})</td>
<td>200.2 ± 7.4(^{bc})</td>
<td>13.23 ± 0.63(^{b})</td>
<td>12.58 ± 0.65(^{b})</td>
</tr>
<tr>
<td></td>
<td>GHNP</td>
<td>2.03 ± 0.10(^{a})</td>
<td>158.2 ± 2.9(^{b})</td>
<td>17.14 ± 0.85(^{a})</td>
<td>12.82 ± 0.74(^{b})</td>
</tr>
<tr>
<td>E. vaginatum</td>
<td>CT</td>
<td>1.69 ± 0.09(^{a})</td>
<td>85.3 ± 2.3(^{a})</td>
<td>20.08 ± 1.11(^{a})</td>
<td>19.85 ± 1.06(^{a})</td>
</tr>
<tr>
<td></td>
<td>GH</td>
<td>1.62 ± 0.05(^{a})</td>
<td>92.3 ± 1.5(^{a})</td>
<td>20.35 ± 0.54(^{a})</td>
<td>17.64 ± 0.38(^{ab})</td>
</tr>
<tr>
<td></td>
<td>NP</td>
<td>2.10 ± 0.05(^{b})</td>
<td>126.8 ± 3.7(^{b})</td>
<td>15.73 ± 0.33(^{b})</td>
<td>16.78 ± 0.79(^{ab})</td>
</tr>
<tr>
<td></td>
<td>GHNP</td>
<td>1.85 ± 0.05(^{a})</td>
<td>143.7 ± 6.5(^{bc})</td>
<td>17.75 ± 0.54(^{a})</td>
<td>12.97 ± 0.69(^{b})</td>
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<tr>
<td>R. chamaemorus</td>
<td>CT</td>
<td>2.11 ± 0.11(^{a})</td>
<td>138.8 ± 11.0(^{a})</td>
<td>15.51 ± 0.84(^{a})</td>
<td>15.79 ± 1.23(^{a})</td>
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<tr>
<td></td>
<td>GH</td>
<td>2.06 ± 0.08(^{a})</td>
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<tr>
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<td>12.24 ± 0.66(^{a})</td>
</tr>
<tr>
<td></td>
<td>GHNP</td>
<td>2.05 ± 0.11(^{a})</td>
<td>126.4 ± 22.2(^{a})</td>
<td>15.79 ± 0.86(^{a})</td>
<td>17.92 ± 1.98(^{a})</td>
</tr>
</tbody>
</table>
Table 2. Foliar photosynthesis, photorespiration for the three species under control and treatments \((n = 7-10)\). Values are expressed on an area basis and represent means ± SE; significance was tested for species, warming, and fertilization effect by three-way ANOVA. Species that do not share a superscript capital letter, growth conditions that do not share a superscript number, and individual means that do not share a lower-case superscript letter are significantly different \((p < 0.05)\).

<table>
<thead>
<tr>
<th>Species</th>
<th>A\textsubscript{net} (µmol m\textsuperscript{-2} s\textsuperscript{-1})</th>
<th>PR (µmol m\textsuperscript{-2} s\textsuperscript{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CT\textsuperscript{1}</td>
<td>GH\textsuperscript{1}</td>
</tr>
<tr>
<td>B. nana\textsuperscript{A}</td>
<td>16.99 ± 1.50</td>
<td>16.95 ± 1.63</td>
</tr>
<tr>
<td>E. vaginatum\textsuperscript{B}</td>
<td>10.02 ± 1.38</td>
<td>11.75 ± 1.64</td>
</tr>
<tr>
<td>R. chamaemorus\textsuperscript{C}</td>
<td>12.24 ± 0.80</td>
<td>11.65 ± 1.22</td>
</tr>
</tbody>
</table>
**Table 3.** Results from a two-way MANOVA on the relative abundance of the three focal species, with fertilization (NP) and warming (GH) as factors. Stars represent significance as follows: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; F-values were not reported for non-significant results for clarity.

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>$F$</th>
<th>$P$</th>
<th>df$^*$</th>
<th>$F$</th>
<th>$P$</th>
<th>$F$</th>
<th>$P$</th>
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<td>Overall</td>
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<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>NP</td>
<td>3, 91</td>
<td>204.76***</td>
<td>1.93</td>
<td>299.95***</td>
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<td>ns</td>
<td>ns</td>
<td>10.19***</td>
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<tr>
<td>NP*GH</td>
<td>3, 91</td>
<td>9.45***</td>
<td>1.93</td>
<td>11.55***</td>
<td>ns</td>
<td>ns</td>
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</table>

$^*$Degrees of freedom for *B. nana* F-tests were the same for the other two species.
Figure 1. Respiration in the dark (shaded) and light (unshaded) and the corresponding inhibition of respiration by light (diagonally striped) in *B. nana*, *E. vaginatum*, and *R. chamaemorus*. Values represent means ± SE, *n* = 8 for all variables. Alphabetical notation was not used for clarity.
Figure 2. Carbon gain efficiency of *B. nana* (diagonal stripe), *E. vaginatum* (unshaded), *R. chamaemorus* (lightly shaded) under control conditions and the three treatments (*n* = 8). Values represent means ± SE; alphabetical notation indicates significance between treatments within a species at *p* < 0.05.
Figure 3. Relative species cover across treatments (A) and percent change in species cover relative to control plot measurements (B); *B. nana* (diagonal stripe), *E. vaginatum* (unshaded), *R. chamaemorus* (lightly shaded), and all other measured species were combined (darkly shaded).
Table S1. ANOVA results for gas exchange variables, comparing the three study species under all growth conditions. Variables analyzed include area-, mass-, and nitrogen-based photosynthesis ($A_{\text{net}}$), dark respiration ($R_D$), respiration in the light ($R_L$), degree of respiratory inhibition by light ($\% I_{RL}$), photorespiration (PR), and carbon use efficiency (CGE). For all variables measured or calculated, $n = 7-10$ for all species-treatment combinations. Stars represent significance as follows: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

<table>
<thead>
<tr>
<th>Species</th>
<th>NP</th>
<th>Warming</th>
<th>NP x Warming</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>d.f.</td>
<td>F</td>
<td>P</td>
</tr>
<tr>
<td>$A_{\text{net-area}}$</td>
<td>2, 94</td>
<td>18.08</td>
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</tr>
<tr>
<td>$R_D$-area</td>
<td>2, 94</td>
<td>6.80</td>
<td>ns</td>
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<tr>
<td>$R_L$-area</td>
<td>2, 94</td>
<td>3.18</td>
<td>*</td>
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<tr>
<td>$A_{\text{net-mass}}$</td>
<td>2, 94</td>
<td>86.51</td>
<td>***</td>
</tr>
<tr>
<td>$R_D$-mass</td>
<td>2, 94</td>
<td>63.31</td>
<td>**</td>
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<td>41.88</td>
<td>***</td>
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<td>$A_{\text{net-N}}$</td>
<td>2, 94</td>
<td>82.03</td>
<td>***</td>
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<td>$R_D$-N</td>
<td>2, 94</td>
<td>53.95</td>
<td>***</td>
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<td>2, 94</td>
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<td>***</td>
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<tr>
<td>$% I_{RL}$</td>
<td>2, 94</td>
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<td>PR</td>
<td>2, 94</td>
<td>5.40</td>
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<tr>
<td>CGE</td>
<td>2, 94</td>
<td>11.80</td>
<td>***</td>
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</tbody>
</table>
Figure S1. Example light-response curve at low PAR displaying the Kok effect. Unshaded points above the bend in the slope extrapolate to $R_L$ on the y-axis. Shaded points below the breakpoint decrease at a faster rate and terminate at $R_D$ where PAR = 0 µmol m$^{-2}$ s$^{-1}$. 

![Diagram of light-response curve with annotations $R_L$, $R_D$, CO$_2$ flux (µmol m$^{-2}$ s$^{-1}$), and PAR (µmol m$^{-2}$ s$^{-1}$).]
Figure S2. Specific leaf area of the three study species grown under treatment conditions \((n = 8)\). Values presented are means ± SE; alphabetic notation denotes significance between treatments within a species at \(p < 0.05\).
Figure S3. Mass-based rates of photosynthesis, respiration in the light, and carbon gain efficiency of *B. nana* (circle), *E. vaginatum* (triangle), *R. chamaemorus* (square) plotted against mass-based N, P, and N:P. Values represent means under treatments ± SE.
CHAPTER FIVE

Seasonality of foliar respiration in two dominant plant species from the Arctic tundra: response to long-term warming and short-term temperature variability

MARY A. HESKEL, OWEN K. ATKIN, MATTHEW H. TURNBULL, and KEVIN L. GRIFFIN

ABSTRACT

Warming in the Arctic tundra is correlated with earlier snowmelt and lengthening of the growing season, which has implications for the terrestrial carbon cycle. It is necessary to understand leaf-level carbon cycling, photosynthesis and respiration, as well as the important, yet often unmeasured light inhibition of respiration, in dominant tundra species to accurately estimate ecosystem carbon exchange. Here, we examined seasonal variation in foliar gas exchange using infrared gas analysis and related leaf traits in two common tundra species under ambient and warmed growth conditions, and the response of these fluxes to intra-season temperature variability, during the 2010 growing season at the Arctic Long Term Ecological Research site at Toolik Lake, Alaska. Species differences and seasonal timing drove most of the variation in photosynthesis, respiration in the dark and in the light (estimated using the Kok method), and leaf nitrogen and SLA. The impact of long-term warming was not consistent in its effect on physiological fluxes, though thermal acclimation led to significantly lower rates of respiration in both species. However, the influence of shorter-term ambient temperature variability, though significant, did not exhibit clear, predictive trends for either photosynthesis or respiration. The inhibition of respiration by light declined through the growing season in both species ($p < 0.001$). This study extends our mechanistic understanding of photosynthesis and respiration during the night-less, highly environmentally variable arctic tundra growing season. New data on seasonal variation and temperature responses of photosynthetic parameters and evidence of a cross-taxa within-season alteration of the light inhibition of respiration will result in more accurate estimation of ecosystem carbon exchange in this changing landscape.

**Keywords:** respiration; photosynthesis, Kok effect, long-term warming, seasonality
INTRODUCTION

Recent climate change in the Arctic tundra has resulted in a cascade of warming-mediated ecological changes (Post et al. 2009; Wookey et al. 2009), many of which directly and indirectly impact terrestrial carbon (C) cycling in this carbon-rich region (Shaver et al. 1992; Chapin et al. 1995). Among these changes, warming is associated with the lengthening of the Arctic tundra growing season through the promotion of earlier spring snowmelt (Stone et al. 2002), often linked to increased woody shrub cover (Chapin et al. 2005; Pomeroy et al. 2006), and delayed snow cover in the fall. The potentially two-tailed extension of the snow-free season and warmer ambient growth temperatures may alter the carbon balance of this system by increasing the duration of carbon assimilation via photosynthesis and carbon release through plant and soil respiration. However, seasonal dynamics of many aspects of the terrestrial carbon cycle, especially at the leaf-level, remain unclear. As the tundra becomes increasingly modified via warming, it is important to understand the relationship of foliar gas exchange, photosynthesis and respiration, to seasonal timing and temperature variability, to strengthen predictive ability for understanding C dynamics in this landscape.

Previous studies have examined growing season C exchange in Arctic tundra, primarily at the ecosystem scale using data from eddy covariance (Vourlitis & Oechel 1999; Vourlitis et al. 2000; Loranty et al. 2010; Rocha & Shaver 2010) and large chamber methods (Oerbauer, Starr & Pop 1998; Oechel et al. 2000; Welker et al. 2004; Sullivan et al. 2008; Natali et al. 2011). Similar to lower-latitude ecosystems, these measurements generally exhibit increases in gross ecosystem photosynthesis (GEP) with an increasing leaf area index (LAI) as the growing season progresses, and concurrent, though not necessarily proportionally coupled, increases in ecosystem respiration (ER). Experimental warming treatments, applied
to predict future C exchange under elevated temperatures, vary in their effect on growing
season net ecosystem exchange (NEE). For example, experimental warming can enhance both
ER and GEP, creating little difference seasonal NEE and maintaining a role as a C sink,
though tundra type and variation in inter-annual environmental conditions, such as water
availability and the ambient light, can influence this effect (Welker, Fahnestock & Jones
2000; Welker et al. 2004; Oberbauer et al. 2007; Sullivan et al. 2008). Alternatively, other
cases exhibit changes of NEE through an increase in ER, leading to a C source in both
experimentally warmed sites (Natali et al. 2011) and non-manipulated sites with generally
warmer soil and canopy temperatures (Cahoon et al. 2012). Given the difficulty of
partitioning soil and autotrophic CO$_2$ fluxes in tundra field studies, few studies examine the
effect of experimental warming on C exchange in vegetation alone, though some information
is known about gas exchange in individual species (Chapin & Shaver 1996; Shaver et al.
1998; Starr, Oberbauer & Ahlquist 2008).

Short-term, intra-season temperature responses of photosynthesis and respiration
should be considered in concert with long-term warming responses to mechanistically
understand and robustly predict rates of foliar and ecosystem carbon cycling in the Arctic
tundra, given the highly fluctuating temperature environment during growing season. Short-
term ambient temperature conditions may drive significant change in both ecosystem and leaf
carbon balance, in addition to the current and dramatic warming trend in this region (Serreze
et al. 2000). For example, foliar respiration can be sensitive to small rapid shifts in
temperature, and can thermally acclimate over time (Atkin & Tjoelker 2003; Atkin, Bruhn &
Tjoelker 2005). In both warm-grown and warm-treated leaves, this flexibility can lead to
decreased respiration compared to cold-grown and cold-treated, when measured at a constant
temperature (Atkin & Tjoelker 2003), which may be attributed to reorganization of the mitochondrial alternative and cytochrome pathways (Armstrong et al. 2006; Armstrong et al. 2008; Searle et al. 2011). Kornfeld et al. (2013) recently reported a significant decrease in respiration in the two focal species of our current study, *Betula nana* and *Eriophorum vaginatum*, grown under the same long-term warming treatment conditions, suggesting potential thermal acclimation at a longer timescale. While the Kornfeld et al. (2013) study demonstrated the potential role of underlying mitochondrial mechanisms in response to long-term warming, it is still unclear how this long-term warming will impact temperature sensitivity at shorter time scales through the growing season.

Many aspects of the seasonality of foliar C cycling, and the influence of long-term warming and shorter-term intra-seasonal temperature variation on these processes, remain unknown in the C-rich tundra landscape. In our current study, we examine foliar photosynthesis, respiration, and associated leaf traits in two abundant species, *Betula nana*, a woody shrub, and *Eriophorum vaginatum*, a tussock-forming graminoid, to acquire accurate estimates of the species’ contributions to the ecosystem carbon balance during the growing season. This study also investigates the relationship of these mechanistic responses to seasonal timing, long-term warming treatment, and short-term ambient temperature variability. Additionally, a related objective of this study was to quantify the light inhibition of respiration through the growing season; as arctic tundra plants experience near-complete or complete 24-hour photoperiods during the growing season, estimates of respiration in the light ($R_L$), which account for the known inhibition of respiration in light (the “Kok effect”), were quantified in addition to dark respiration ($R_D$). We hypothesize that seasonal dynamics of gas exchange will correspond to energy demand of the growing vegetation: (1) rates of
photosynthesis should increase as leaves develop, though this trend may be stronger in the deciduous shrub *Betula* than the evergreen graminoid *Eriophorum*; (2) respiration rates will be greatest in the early growing season when energy demand for growth is highest; and (3) the elevated energy demand for growth will be associated with relaxed levels of light inhibition of respiration, as shown in elevated CO$_2$ studies (Shapiro et al. 2004; Crous et al. 2012). Further, we hypothesize that growth under long-term warming will suppress the respiratory response to temperature due to thermal acclimation (Atkin & Tjoelker 2003). To test these hypotheses in tundra vegetation, we utilized global change experimental plots that have been continuously maintained for nearly 30 years by the Arctic Long Term Ecological Research site (ARC LTER) at Toolik Lake, Alaska, and measured foliar gas exchange and related leaf traits at condensed intervals over eight weeks during the 2010 growing season. Our study represents a high temporal resolution examination of the mechanisms controlling plant carbon balance through the tundra growing season, and the first to quantify the seasonal response of the light inhibition of foliar respiration, maximum rates of RuBP carboxylation and RuBP regeneration in these common tundra species.

**MATERIALS AND METHODS**

*Field Site and Species* - The study took place over eight weeks during the 2010 growing season (June 6$^{th}$ - July 28$^{th}$) at the ARC LTER field site near Toolik Lake (68°38’N, 149°36’W) on Alaska’s North Slope, located 254 km north of the Arctic Circle. Air temperature and precipitation were all measured and recorded at this site by the Toolik Field Station Environmental Data Center (EDC Team, 2011). All leaves were sampled from experimental plots in moist acidic tundra (MAT) established and maintained by the LTER
since 1989 (similar to an older experiment described by Chapin et al. (2005)). The MAT site consists of four randomized blocks of 5 x 20 m treatment plots separated by a 1 m buffer arrayed two-by-two on a slightly-sloped, poorly-drained hillside. Leaves were sampled from control (CT) plots and plots that have been warmed via greenhouses (GH) since 1989. These wood-framed greenhouses, covered with transparent 0.15 mm plastic sheeting, passively increase air temperature by approximately 5°C during the growing season, while insignificantly affecting light intensity, humidity, and thaw depth (Gough and Hobbie 2003). The focal species for our study are common and abundant at the MAT site: *Eriophorum vaginatum* L. (“cottongrass”; henceforth referred to as “*Eriophorum*”), the tussock-forming sedge that is widely distributed over much of Alaska’s North Slope, northern Canada, and northern Eurasia and *Betula nana nana* L. (“dwarf birch”; henceforth referred to as “*Betula*”), a deciduous woody shrub that is also abundant and often dominant over much of the entire Arctic region.

*Foliar carbon exchange -* CO₂ fluxes of photosynthesis and respiration were measured using an infrared gas analyzer (IRGA; LI-6400XT Portable Photosynthesis System, LI-COR, Lincoln, NE). All measurements were made on clipped leaves of *Eriophorum* and small branches for *Betula* collected from the long-term MAT greenhouse and control plots, re-cut under water in the field, and transported in water to the laboratory as in Heskel et al. (2012). Preliminary tests on these species showed no difference in rates of gas exchange and stomatal conductance between field-measured and lab-measured leaves (Griffin, unpublished), as has been shown previously in other species (Mitchell, Bolstad & Vose 1999; Turnbull et al. 2003). This sampling technique was required to provide a greater degree of temperature
control, maximize the number of replicates, and minimize time between replicates over the short growing season and during potentially rapidly changing environmental conditions in the field. For each *Eriophorum* sample, we collected approximately ten leaves from a single tussock in order to provide sufficient leaf area in the leaf chamber (~6 cm²). For *Betula*, the 1-2 measured leaves from the sampled branch, which were smaller than the 6 cm² cuvette area, were measured for leaf area after IRGA measurements and gas exchange values were corrected to that area for analysis. To obtain high resolution seasonal monitoring of gas exchange, leaves of both species were sampled from each of the four CT and GH plots at the MAT site (*n* = 4) approximately every 4-5 days, comprising a measurement round. For twelve days in mid-July, there was a gap in measurement, as equipment was being used for another research undertaking.

Prior to measurement in the laboratory, leaves were enclosed in the cuvette at high light conditions to activate photosynthesis, though this exposure was kept to a minimum time to reduce the potential for photoinhibition. CO₂ assimilation was measured under ambient (400 ppm) CO₂ concentration under 26 levels of PAR: 1500, 1200, 800, 400, 200, 100, and every 5 PAR between 100 and 0 μmol m⁻² s⁻¹. This range of light fully encompasses the light environment experienced by the species in their growth environment. Following the light-response curve, leaf samples were treated to 900 μmol m⁻² s⁻¹ PAR and 400 ppm CO₂ for 5-10 minutes before CO₂ assimilation was measured in response to 11 levels of increasing [CO₂]: ~0, 50, 100, 150, 200, 300, 400, 600, 800, 1200, and 1500 ppm. Following the CO₂-response curve measurement, and after 10 minutes in darkness at 400 ppm CO₂, it was assumed that no photosynthesis or photorespiration was taking place, and all CO₂ flux could be attributed to mitochondrial respiration in the dark (*R*₉₉).
All measurements were taken at a relative humidity of approximately 40-60%, and potential diffusion in and out of the cuvette was accounted for, as was diffusion through the gasket, according to corrections presented in the Li-Cor 6400 Instructional Manual. Average leaf vapor pressure deficit was 0.942 ± 0.32 kPa (SE) for the light-response curves, and 1.142 ± 0.29 across both species and treatments. Cuvette block temperature was set to 20° C for all measurements to control for leaf temperature, and is representative of temperatures experienced during the growing season (Fig. 1), and leaf temperatures in situ ranged from 19-23° C across both species. Maximal light-saturated net photosynthetic rate ($A_{\text{max}}$) was estimated by fitting data from the light-response curve to a rectangular hyperbolic function (Excel Solver, Microsoft, Redmond, WA). CO$_2$-response curves were analyzed for the maximum carboxylation velocity of Rubisco ($V_{\text{cmax}}$) and maximum rate of electron transfer ($J$) using the $A/C_{\text{i}}$ curve fitting utility (version 2007.1) provided and detailed in Sharkey et al. (2007). Examples of light- and CO$_2$-response curves can be seen in Fig. S1. Values of photosynthesis and respiration are expressed on an area, mass, and nitrogen basis.

Quantifying respiration in the light using the Kok method - To estimate respiration in the light ($R_{\text{Light}}$), we used the Kok method, which is convenient for field measurements and can be used under ambient atmospheric conditions. This method is based on the observation that the quantum yield of photosynthesis usually decreases abruptly above a certain level of light intensity—often near the light compensation point, where carbon flux is zero (Kok 1948). This leads to a noticeable non-linearity, or “bend”, in the otherwise linear lower range of the light-response curve, which is interpreted as the saturation point of light inhibition of respiration (explained in detail in Shapiro et al. 2004). Since respiration is assumed to be
constant above this point, an extrapolation to 0 \( \mu \text{mol m}^{-2} \text{s}^{-1} \) PAR of the linear portion of the curve above this point is assumed to give the rate of respiration in the light. Here, the irradiance range we used to calculate \( R_{\text{Light}} \) spanned from 25-90 \( \mu \text{mol m}^{-2} \text{s}^{-1} \) (Fig. S1b).

The Kok method assumes the CO\(_2\) assimilation rate responds only to light, and thus corrections must be made to account for changes in internal CO\(_2\) (\( c_i \)). As rates of photosynthesis slow under decreasing light intensity, CO\(_2\) tends to accumulate within the leaf, increasing \( c_i \), which in turn affect the shape of the light curve by decreasing rates of photorespiration at lower light levels. We corrected all measurements to a constant \( c_i \) to account for these effects, according to Kirshbaum and Farquhar (1987), as described in Ayub et al. (2011), to achieve a more accurate extrapolation of \( R_{\text{Light}} \).

**Physical leaf traits and foliar nutrients** - All leaf samples used for gas exchange measurements were measured for leaf area using a rotating-belt leaf area meter (LI-3100C Area Meter, LI-COR, Lincoln, NE). Samples were then dried in an oven at 60°C for a minimum of two days before mass was determined. After transport to Columbia University in New York, all samples were ground, weighed, and packaged for elemental analysis of [CHN] (2400 Series II, Perkin-Elmer, Boston, MA).

**Statistical Analyses** - The effect of seasonal timing on foliar gas exchange and related leaf characteristics was analyzed using a linear mixed-effects model framework to perform a three-way repeated measures analysis of variance (RM-ANOVA) in R (v2.7.0, The R Foundation for Statistical Computing), assigning measurement round (the 4-5 day period when replicates from each species-treatment combination were measured), species (*Betula*
and *Eriophorum*), and warming (CT or GH) as explanatory variables, and treatment block was treated as a random effect. Prior to analysis, data were tested for normality (Shapiro-Wilk) and heteroscedasticity (Breusch-Pagan), and log_{10}-transformed when necessary. Post-hoc multiple comparisons were made using Tukey’s test. To address the relationships between foliar physiological rates and ambient temperature or leaf traits, correlation analyses were employed. In addition, multiple models that integrated the effects of species, measurement round, warming treatment, and ambient temperature to assess select gas exchange variables were evaluated using corrected Akaike Information Criteria (AIC<sub>c</sub>; in supplemental materials). For all analyses, *P*-values < 0.05 were considered significant.

**RESULTS**

*Seasonal temperature variability* - Full snowmelt at the long-term experimental warming and control plots occurred by ~June 9, 2010 (Toolik EDC). During the measurement period of Julian Day (JD) 162-209 (June 11-July 28), the maximum and minimum temperature and precipitation were highly variable (Fig. 1). During this period, the highest recorded temperature (23.9°C) occurred on July 9<sup>th</sup>, and the lowest minimum temperature post-snowmelt occurred on July 6<sup>th</sup>, merely three days prior. Average temperature during the measurement period was 10.7°C, with an average minimum temperature of 4.8°C and an average maximum temperature of 14.3°C. In passively warmed plots, this translates to an average maximum temperature of 19.3°C, nearly equal to the leaf sample measurement temperature. Three large precipitation events occurred during the measurement period, corresponding with lower ambient air temperatures (Fig. 1).
Specific leaf traits and chemistry - Due to the contrasting leaf forms of Betula, which produces small, flat, circle-shaped leaves each season, and Eriophorum, which grows elongated, three-sided tillers that are retained through the snow-covered winter, it is not surprising the corresponding specific leaf area (SLA) measurements differ greatly between the species taken as a whole (Table 1; Fig. S2), and within both control and warming treatments ($p < 0.0001$). Though individual measurement rounds show some variation in SLA, across the growing season, elevated growth temperature did not affect either species compared to leaves in the control (Fig. S2). Leaf C concentration was greater in Betula than Eriophorum ($p < 0.0001$), and warming treatment, and seasonal timing (Round) did not affect this in either species (Fig. S3a,d, Table 1). Eriophorum decreased in proportional N content as the season progressed, while Betula also decreased through the season, though it was less pronounced (Fig. S3); warming did not elevate N content in either species. The species-specific trends in N caused the related increases in C:N over the seasonal course (Fig. S3).

Intra-seasonal dynamics of photosynthesis and respiration - The impacts of species, warming treatment, and measurement round through the season on area-, mass-, and N-based gas exchange rates (measured at 20°C), as expressed by the results of a three-way ANOVA, are shown in Table 2. Variation in area-based maximal photosynthetic rates (Fig. 2) were primarily explained through species differences, with Betula consistently exhibiting greater rates of carbon assimilation across all measurements, as well as when considering measurements in the warmed plots ($p < 0.01$). Area-based $A_{\text{max}}$ showed no significant response to the measurement round (Fig. 2), though mass-based rates yielded an interactive effect of species and round (Fig. S3a-d; Table 2), suggesting the effect of leaf structural
differences between the species when assessing photosynthetic rates. Only when expressed on a per-N rate did $A_{\text{max}}$ vary with measurement round, driven by the increases in per-N photosynthesis in the later season related to lower foliar N-concentration (Fig. S3e-h). Similarly, there were no observed changes in rates under long-term warming treatment in either species, and *Betula* consistently exhibited higher rates in both warmed and control plots compared to *Eriophorum*. When considering the processes underlying photosynthetic efficiency, $V_{\text{cmax}}$ was significantly affected by the interaction of species and measurement round. Mean rates of $V_{\text{cmax}}$ in *Betula*, particularly under warming, increased slightly through the season (Fig. 3). Rates of $V_{\text{cmax}}$ and $J$ were highly correlated ($R^2 = 0.856$). Growth under long-term warming did not significantly influence either variable (Fig. 3; Table 2).

Across all measures of mitochondrial dark respiration, *Betula* exhibited higher rates than *Eriophorum* (Table 2). A significant interaction term between species and measurement round highlighted differences in mitochondrial dark respiration (area- and N- based), between *Eriophorum* and *Betula* over the course of the growing season; *Eriophorum* displayed a gradual decrease in rates around day 180 (June 29) that was not observed in *Betula* (Fig. 2c-d). Further, considering all measurements, long-term growth warming was associated with lower rates of $R_{\text{Dark-area}}$ in both species, though this effect is only observed in *Eriophorum* considering $R_{\text{Dark-N}}$. On a mass basis, dark respiration decreased through the growing season in both species (Table 2), with highest mean rates for both species occurring in the first two weeks of the growing season.

One of the main objectives of this study was to obtain a better understanding of the light inhibition of respiration in these species, and how this phenomenon may relate to other leaf traits. Across species and growth conditions, light significantly suppressed respiration
rates ($p < 0.0001$), with individual leaf values ranging widely. $R_{\text{Light}} : R_{\text{Dark}}$ was less in *Eriophorum* than *Betula* under both growth conditions ($p < 0.001$), and decreased further under warming in *Eriophorum* ($p < 0.01$). For both species and growth conditions, $R_{\text{Light}} : R_{\text{Dark}}$ decreased over the growing season (Fig. 4). The resulting $R_{\text{Light}}$ values (Fig. 2e-f), though reduced due to inhibition, followed similar effects as $R_{\text{Dark}}$: warming decreased rates in only *Eriophorum* for area- and N-based rates ($p < 0.05$ for both), but not mass-based rates, which found warming to significantly lower rates when considering all measurements ($p < 0.01$). Further, the effect a species-measurement round interaction ($p < 0.05$) was evidenced in the different trends of respiratory release over the course of the season (Fig. 2e-f; Fig. S4).

The ratio of carbon losses through respiration in the light to gross photosynthesis ($R_{\text{Light}} : A_{\text{Gross}}$) decreased through the season in both species (Fig. 5), though at different rates. These proportional respiratory losses were significantly greater in *Betula* than *Eriophorum* and warming significantly decreased these values, considering all measurements across the season (Table 2). No clear relationships were found between the degree of inhibition and foliar fluxes or traits in these species; regression analysis between the degree of light inhibition and % N, $A_{\text{max}}$, and $V_{\text{cmax}}$ produced $R^2 < 0.10$ (data not shown).

*Intra-seasonal ambient temperature influences on gas exchange* - The temperature variability of the arctic tundra may likely control the *in situ* rates of photosynthesis and respiration in dominant plants there, as both processes can be sensitive to short-term changes in thermal environment. Despite statistical significance of measurement timing found for foliar gas exchange variables (Table 2), to further explain the variation in these rates we evaluated and compared models that incorporated ambient temperature values from the day of, and mean
values from the week prior to, gas exchange measurement. Table SM 1 shows the results of models incorporating the effect of species, warming treatment, day-of and week-before minimum, maximum, and average temperatures on area-based \(A_{\text{max}}\), \(R_{\text{Dark}}\), \(R_{\text{Light}}\) and \(R_{\text{Light}:}\) \(R_{\text{Dark}}\), evaluating by comparing relative AICc weights. The best model for photosynthesis (measured at 20\(^\circ\)C) incorporates only species, with the next best only incorporating species and warming treatment, suggesting no significant effect of short-term ambient air temperature on rates of \(A_{\text{max}}\). In contrast, both \(R_{\text{Dark}}\) and \(R_{\text{Light}}\) (also measured at 20\(^\circ\)C) were more sensitive to recent ambient air temperatures, with the best models incorporating the effect of species, growth conditions, and either the prior week’s average temperature (\(R_{\text{Dark}}\)) or the measurement day’s minimum temperature (\(R_{\text{Light}}\); SM Fig. 5), while the top three models for \(R_{\text{Light}}: R_{\text{Dark}}\) incorporated minimum temperature values. For \(R_{\text{Light}}\), when considered alone, a clear predictive response is not obvious (Fig. S5), though significant relationships were observed between \(R_{\text{Light}}\) and day-of minimum temperature (\(p < 0.01\)); day-of maximum temperature (\(p < 0.01\)); day-of average temperature (\(p < 0.01\)), week-prior minimum temperature (\(p < 0.01\)), though not with the prior week’s average or maximum temperature, suggesting greater sensitivity to short-term and/or colder conditions. Another analysis that correlated the \(R^2\) value from the relationship of \(R_{\text{Light}}\) to a previous time window’s average temperature (in increments of five day intervals) found no clear acclimation temperature for a given period, except in the case of Betula grown under warming, which was best correlated with ambient temperature averaged over 25 days prior to measurement (data not presented).

The model comparison (SM Table 1), in addition to the three-way ANOVA results (Table 2), highly support the importance of species differences in rates of gas exchange; models without species as a factor were ranked lowest among the tested models.
DISCUSSION

The primary objective of this study was to evaluate the mechanistic physiological responses of foliar photosynthesis and respiration to long-term warming conditions and natural variations in ambient temperature through the growing season in two dominant arctic plants. We hypothesized that the within-growing season rates of photosynthesis, respiration, and the inhibition of respiration will relate to growth-induced energy demand, and these rates will acclimate to long-term warming and be responsive to short-term temperature fluctuations. Our results show stronger seasonal and thermal responses in respiration than in photosynthesis (when both are measured at 20°C), which was further influenced by species differences and growth under long-term warming. This study also provided the opportunity to expand upon existing knowledge about biotic and environmental controls on foliar $R_{\text{Light}}$ and how seasonal timing and temperature variability may mediate the ratio of $R_{\text{Light}}$ to $R_{\text{Dark}}$. Together these findings provide a more detailed mechanistic understanding of the control of carbon exchange in tundra ecosystems.

Species-mediated influences of seasonal and environmental effects on carbon exchange - In our study, rates of photosynthesis and respiration of Betula, when measured at a common temperature, tended to be higher than those of Eriophorum during the arctic tundra growing season (Fig. 2; Table 2), while grown under both control and long-term passive warming. The higher rates of carbon exchange in Betula were consistent with previous studies that sampled plants at mid-season when grown under warming (Chapin & Shaver 1996) and soil nitrogen and phosphorus addition (Heskel et al. 2012). The difference in $A_{\text{max}}$ between Betula and Eriophorum was greatest directly after leaf out (Fig. 2), which emphasizes the impact of
growth form on foliar physiological processes. *Betula*, a deciduous woody shrub is likely to have a relatively high energy demand immediately post snowmelt to accommodate efficient aboveground and belowground growth during the short growing season, and this may be further enhanced under warming (Chapin *et al.* 1995; Sullivan *et al.* 2007). By contrast, *Eriophorum*, a tiller-producing graminoid, can retain leaves for multiple years (Fetcher & Shaver 1983) and for this reason may act less opportunistically upon snow-melt in the spring in terms of nutrient acquisition via fine root growth (Sullivan *et al.* 2007) and quick leaf production. These trends are supported by previous work on deciduous and evergreen species, where seasonal photosynthesis and respiration were consistently significantly lower in the evergreen (Ow *et al.* 2010). The species contrast is underscored by the respective leaf architectures and composition: inter-species comparisons show a less dense (greater SLA), higher N-content leaf in *Betula* than *Eriophorum* (Table 1, Figs. S2 and S3).

We found a general, though not significant, decline in $A_{\text{max}}$ in leaves sampled from long-term warming plots, suggesting a potential, duration-dependent effect at the leaf level that may be contributing to a similar phenomenon previously reported in ecosystem processes (Shaver *et al.* 2000; Elmendorf *et al.* 2012). Also, these tundra species may be pushed beyond their photosynthetic thermal optimum under elevated temperatures (Sage & Kubien 2007), which could be a likely scenario in arctic populations that may be locally adapted for colder growth temperatures. Because we controlled for leaf temperature across measurements, there is no apparent seasonal arch of $A_{\text{max}}$, $V_{\text{cmax}}$, and $J$ rates, which generally characterizes growing season carbon assimilation of leaves measured at ambient leaf temperatures, as was previously reported (for $A_{\text{max}}$) in these species (Starr, Oberbauer & Ahlquist 2008). At the leaf-level, highest rates of $A_{\text{max}}$, $V_{\text{cmax}}$, and $J$ can occur in the early growing season (Dungan,
This discrepancy may be explained by the highly variable ambient temperature and precipitation conditions (Fig. 1), though model evaluation found no strong relationship between photosynthetic variables (measured at 20°C) and short-term temperature values (Table S1). Also, because we measured all leaves at the same temperature (20 °C), we controlled for the ambient temperature influence on leaf temperature that can affect photosynthetic rates, and allow for comparison across changes in ambient temperature to assess thermal acclimation.

Respiration rates of both species were generally highest in the first few weeks post-snowmelt (Fig. 2e-f), which is more apparent in $R_{\text{Light}}$ than $R_{\text{Dark}}$. In both Betula and Eriophorum, higher energy demand, and therefore respiratory rates, in the early season relative to the mid- and late- season, may be attributable to new leaf growth and development (Vose & Ryan 2002; Xu, Schuster & Griffin 2007; Ow et al. 2010), and possibly a higher density of mitochondria in younger leaves (Armstrong, Logan & Atkin 2006). This respiratory release can be further enhanced by the colder ambient temperatures experienced by plants in the early growing season due to a short-term cold acclimation (Atkin & Tjoelker 2003; Armstrong, Logan & Atkin 2006). It should be noted that similar measured rates of respiration rates across species may not necessarily equate to similar energy efficiency; Betula is reported to exhibit greater respiratory efficiency than Eriophorum through the differential use of the alternative and cytochrome pathways, potentially lending a competitive advantage in growth and development (Kornfeld et al. 2012). In addition, the ratio of respiratory loss in the light to gross photosynthesis, $R_L: A_{\text{Gross}}$, decreased over the growing season (Fig. 5), driven by lower values of $R_L$ as the season progressed, rather than any significant change in photosynthesis, which is discordant with the idea that the processes would increase in a
coupled manner based on carbohydrate substrate supply and demand. We were unable to quantify foliar carbohydrate values in these species through the season, though future studies could include this informative measurement to further relate the mechanistic links between these processes.

Both species exhibited lowest values of $R_{\text{Light}} : R_{\text{Dark}}$ towards the end of the season in late July (Fig. 4), suggesting a potential developmental and/or energy demand related control on the inhibition of respiration by light that allows for greater respiratory energy production in the early season during leaf expansion. The only other known study that explored the seasonal response of $R_{\text{Light}} : R_{\text{Dark}}$, observed the least inhibition earlier in the growing season, prior to the warmest month (Crous et al. 2012). This response, similar to our study, despite a starkly different ecological system, supports the idea of shared seasonal timing-mediated biochemical controls on the light inhibition of respiration across species. The degree of inhibition is known to show a relaxed response under environmental conditions that can stimulate growth due to increased demand for energy and C-skeletons, as exhibited under elevated CO$_2$ (Wang et al. 2001; Shapiro et al. 2004) and increased soil nutrient availability (Heskel et al. 2012), though this is not always the case (Tissue et al. 2002; Ayub et al. 2011). Long-term warming also mediated $R_{\text{Light}} : R_{\text{Dark}}$, with lower values observed in warm-grown leaves, with differences between treatments larger in the mid-to-late season, though measurement temperature can also impact these relationships (Atkin, Scheurwater & Pons 2006).

Mechanistic explanations for the relationships between growth demand and cellular energy status and the degree of light inhibition of respiration have been previously reported. The short growing season and approximate 24-hour diel light exposure in the Arctic tundra may exert increased growth demand on arctic species, especially woody shrubs like Betula,
known to exhibit secondary growth rates under current conditions, which may influence $R_{\text{Light}} < R_{\text{Dark}}$ (Bret-Harte et al. 2001; Bret-Harte, Shaver & Chapin 2002). In the light, adenylate supply from photosynthesis may decrease energy demand from mitochondrial respiration. Additionally, photorespiration is associated with the inactivation of pyruvate dehydrogenase, a pre-cursor to the tri-carboxylic acid (TCA) cycle (Budde & Randall 1990), though previous estimations of photorespiration in these species did not find a strong correlation with the inhibition of respiration in the light (Heskel et al. 2013). The TCA cycle can also be significantly altered in the light to support N-assimilation, which effectively reduces CO$_2$ release (Tcherkez et al. 2005; Tcherkez et al. 2008; Tcherkez et al. 2009). This effect may be enhanced in tundra plants when soil N is less limiting; under these conditions, growth rates increase (Bret-Harte, Shaver & Chapin 2002), and both the growth rates and the inhibition of respiration in the light are observed to be slightly increased in fertilized soils (Heskel et al. 2013). Additionally, the oxidative pentose phosphate pathway (OPPP), which contributes necessary reductant for the synthesis of multiple metabolites in darkness, may be relaxed in the light when reductants are provided by photosynthesis, diminishing CO$_2$ release by the OPPP. Though the OPPP comprises a proportionally smaller CO$_2$ flux compared to that from the TCA cycle, in the light this decrease may contribute to lower overall CO$_2$ release, causing $R_{\text{Light}} < R_{\text{Dark}}$ (Buckley and Adams 2011). In our current study, the apparent relaxation of the light inhibition of respiration in leaves of both study species (Fig. 4), resulting in the greatest proportional CO$_2$ losses (Fig. 5), suggests that high growth demand for ATP and carbon skeletons outweigh potential carbon losses in the early season.
In addition to quantifying the effects of long-term warming on the growing season foliar gas exchange, this study evaluated the influence of intra-seasonal short-term temperature variability on these physiological processes. Arctic tundra growing seasons can exhibit dramatic shifts in temperature and precipitation within days, as was observed in this study during summer 2010 in Toolik Lake, Alaska (Fig. 1), and this variability is likely to influence the temperature-sensitive processes controlling leaf carbon cycling. When modeled against the minimum, average, and maximum ambient temperature of the measurement day, as well as the prior weeks’ averages for these variables, and accounting for the effects of species and growth environment, some patterns emerge that help to characterize the nature of the temperature sensitivity of these processes processes (measured at 20°C) to day-to-day shifts in ambient air temperature (Table S1). However, photosynthesis, which is known to respond to short-term changes in temperature in field grown plants (Berry & Björkman 1980; Poyatos et al. 2012), did not show strong relationships to daily or weekly variation in temperature, and instead species’ effects explained the most variation (Table S1). The underlying photosynthetic machinery maintains consistent rates of carbon assimilation (measured at a constant temperature) throughout the growing season, though daily fluctuations in temperature and precipitation episodes in the Arctic may influence in vivo rates. Plant species from alpine and arctic ecosystems can exhibit local adaptation to the extreme variability experienced in those locations (Korner & Larcher 1988), which may translate to a limited acclimation (Atkin, Scheurwater & Pons 2006). Further, the ambient temperature conditions of this region fluctuate so unpredictably in short time periods through the season, any analysis using temperature averages of previous time windows might not be as useful for estimating
thermal acclimation as in regions with more predictable seasonal temperature patterns (Ow et al. 2010; Searle et al. 2011).

In contrast, respiratory rates in the light and dark, when measured at a constant temperature, appeared to be more correlated to short-term ambient temperature variation. The AICc analysis shows the strongest model for $R_{\text{Dark}}$ included species and warming effects and the prior week’s average temperature, whereas the strongest model for $R_{\text{Light}}$ included the former parameters and the minimum temperature from the measurement day (Table S1). The model relationships may suggest a short-term thermal sensitivity of respiration that may be more responsive to temperature minimums, though this is not clearly reflected in regressions of $R_{\text{Light}}$ with the ambient temperature values (Fig. S5). This sensitivity of respiration to temperature minimums has been observed previously in roots as well, and suggests that thermal acclimation in cold growth temperatures may regulate metabolic activity to meet demands of growth and maintenance at the expense of greater carbon loss (Covey-Crump, Attwood & Atkin 2002). The lowest minimum, average, or maximum ambient temperatures were not associated with the highest rates of respiration when measured at a common temperature, as would be expected under respiratory cold acclimation (Atkin & Tjoelker 2003). However, it is possible that developmental timing may also play into the leaves’ respiratory rates and ability to acclimate especially in Betula in the early season when its leaves are still expanding (Armstrong, Logan & Atkin 2006). Through a relatively simple modeling exercise, we found some evidence of differential temperature controls on photosynthesis and respiration in Betula and Eriophorum; we suggest that future work should address more detailed environmental and biotic factors, including soil temperature, daily
photosynthetic radiation, and leaf carbohydrate data, to more accurately inform the seasonal
temperature sensitivity of foliar carbon exchange.

Conclusions and implications -

The physiological measurements collected in this study allowed for the quantification of
mechanistic responses in the night-less and highly variable growing season of the Arctic
tundra, and provide new insights into foliar carbon regulation. We present new information on
the seasonal trends of foliar carbon cycling in two dominant tundra species, and relate these
fluxes to short-term temperature variability and long-term warming. Our study presents the
first published values of photosynthetic parameters $V_{cmax}$ and $J$ at multiple points during the
season in these species, which can inform and refine parameterization of vegetation models at
many scales. Also, we present the first report of the seasonal flexibility of the degree of
inhibition of respiration in the light under long-term warmed growth conditions, in these or
any species, thus enabling more accurate calculations of ecosystem respiration. Based
on these measurements, a seasonal estimate (not accounting for potential temperature
sensitivity discussed above; see SM eq.1) shows the neglect of the inhibition of foliar
respiration in the light in arctic tundra vegetation could lead to overestimations of foliar
carbon loss of $\sim 4$ mol m$^{-2}$ leaf ($\sim 48$ g) over the growing season, even more ($\sim 4.2$ mol m$^{-2}$)
when considering plants grown under warmed conditions. Further, our study shows few clear,
predictive relationships between foliar carbon exchange fluxes and the short-term ambient
temperature environment, which is important for ecosystem carbon exchange models, as
calculations only parameterizing respiration from ambient temperature may not provide
accurate estimates. Our study contributes important data that allows for increased
understanding of dominant tundra species’ responses to environmental change, both long- and short-term, and will allow for more predictive power in models estimating ecosystem carbon storage.
**TABLES AND FIGURES**

**Table 1.** Results from three-way repeated measures ANOVA analyzing carbon (% C) and nitrogen (% N) concentrations and their ratio (C:N) of dry leaf material and specific leaf area (SLA) in *Betula* and *Eriophorum*. Asterisks denote significance at the $p < 0.05$ (*), $< 0.01$ (**), and $< 0.001$ (***)..

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<td>F-stat</td>
<td>p</td>
<td>F-stat</td>
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Table 2. Results from three-way repeated measures ANOVA analyzing foliar gas exchange variables, including maximal photosynthesis ($A_{\text{max}}$), dark respiration ($R_{\text{dark}}$), and respiration in the light ($R_{\text{light}}$) expressed on an area, mass, and nitrogen (N) basis, the ratio of $R_{\text{light}}$ to $R_{\text{dark}}$ ($R_{\text{light}}$: $R_{\text{dark}}$), the ratio of photosynthetic carbon assimilation to respiratory carbon release ($A$:R), and the maximum carboxylation rate of Rubisco ($V_{\text{cmax}}$) and the electron transport rate ($J$, both measured at ambient leaf temperature, and corrected for a constant temperature of 25°C) and in both species. Asterisks denote significance at the $p < 0.05$ (*), $< 0.01$ (**), and $< 0.001$ (***)..

<table>
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**Figure 1.** Growing season maximum and minimum air temperature (a) and precipitation (b). Gas exchange measurements on leaf samples occurred between the dashed lines (Julian days 162-209, or June 11 – July 28, 2010).
Figure 2. Maximum photosynthesis ($A_{\text{max}}$), dark respiration ($R_{\text{dark}}$), and respiration in the light ($R_{\text{light}}$) through the growing season for *Betula* (circles) and *Eriophorum* (triangles) grown in ambient (unfilled symbols) and passively warmed (filled symbols) conditions ($n = 4$).
Figure 3. Photosynthetic parameters (maximal carboxylation rate, $V_{\text{cmax}}$, and electron transport rate, $J$, both corrected to 25 °C) of both species over the growing season under ambient (open) and warmed (filled) growth conditions. Values shown are means with standard errors.
**Figure 4.** Means and standard errors of the ratio of respiration in the light to dark respiration through the growing season in *Betula* (circles) and *Eriophorum* (triangles) under ambient, control (CT, open symbols) and passively warmed (WG, filled symbols) growth conditions (*n* = 4).
**Figure 5.** The proportional carbon loss through respiration in the light to gross photosynthesis in both species over the growing season. Values represent means and SE; error bars are not visible for many values due to their small value.
Supplemental Materials

Figure S1. Examples of light (A), Kok effect (C; PAR < 100 μmol m⁻² s⁻¹), and CO₂ (C) response curves taken for this study. Regression fitted to the 25-90 PAR range and extrapolated to the y-axis results in \( R_{\text{Light}} \); whereas \( R_{\text{Dark}} \) is equal to the carbon released when PAR = 0 μmol m⁻² s⁻¹. Values shown are means with standard errors of carbon assimilation of *Eriophorum* grown under passive warming measured on July 2, 2010.
**Figure S2.** Specific leaf area of both species across the growing season; open symbols represent control conditions, and filled symbols represent warmed growth conditions.
Figure S3. Growing season trends in leaf carbon (C), nitrogen (N), and C:N values in *Betula* (A-C) and *Eriophorum* (D-F). Open symbols represent values from leaves grown under ambient control conditions, and filled symbols represent values from leaves grown under a passive warming treatment.
Figure S4. Rates of photosynthesis and respiration in the light of *Betula* and *Eriophorum* over the growing season presented on mass (A-D) and nitrogen (E-H) bases. Values presented are means and standard error.
**Figure S5.** Respiration in the light ($R_{\text{Light - Area}}$) as a response to minimum, maximum, and average temperatures from the day of measurement (A-C), and from the averages from the week prior to measurement (D-F). Filled symbols represent leaves grown under warming (WG), and open symbols represent leaves grown under ambient conditions (CT).
Table S1. AIC<sub>c</sub> comparison of models used to evaluate the effect of species and environment on gas exchange variables. Effects evaluated include species (S), elevated growth temperature (W), minimum (dMinT), maximum (dMaxT), and average (dAvgT) ambient temperature from the day of measurement, and minimum (wMinT), maximum (wMaxT), and average (wAvgT) temperature from the week prior to measurement.

<table>
<thead>
<tr>
<th>model</th>
<th>k</th>
<th>Δ&lt;sub&gt;i&lt;/sub&gt;</th>
<th>w&lt;sub&gt;i&lt;/sub&gt;</th>
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<td></td>
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</tr>
<tr>
<td>S</td>
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<td></td>
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</tr>
<tr>
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<td>-</td>
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<tr>
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<tr>
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Species (S) are Betula nana and Eriophorum vaginatum, passive warming treatment (W) was either present or absent. Higher w<sub>i</sub> scores are better; only models with w<sub>i</sub> > 0.05 are shown, which is why there are few models for photosynthesis; the best model is in bold text. Columns are: model – the candidate linear mixed effect models, where “+” indicates an additive effect; k – the number of parameter estimates in the model, plus one; Δ<sub>i</sub> – the difference in AIC<sub>c</sub> value for that model and the best model; w<sub>i</sub> – the Akaike weight, the relative support of the model compared to all models; rank – a display of relative model strength: each asterisk represents ~20% of the total weight.
Equation S1. Seasonal estimation of the difference between per-leaf-area respiratory release in the light and in the dark.

\[
\text{Seasonal } (R_{Dark} - R_{Light}) = \sum_{i=\text{Round 1}}^{\text{Round 10}} [R_{Dark(day)Round_i} \times \text{length of Round}_i] - \sum_{i=\text{Round 1}}^{\text{Round 10}} [R_{Light(day)Round_i} \times \text{length of Round}_i]
\]
CHAPTER SIX

Examining intra-canopy carbon cycling patterns in an Arctic tundra shrub community

MARY A. HESKEL, MATTHEW H. TURNBULL, and KEVIN L. GRIFFIN

Submitted to *Physiologia Plantarum* on April 16, 2013.
ABSTRACT

Arctic warming is facilitating ecological change, including the encroachment and expansion of woody shrubs in the tundra, impacting the terrestrial carbon cycle in this region. The ‘greening’ of Arctic tundra can lead to denser and taller shrub communities, altering the microenvironment of canopy leaves, and in turn influencing the foliar physiology controlling carbon exchange. To better understand intra-canopy patterns of leaf traits and gas exchange, we measured a variety of associated foliar and environmental variables in multiple shrub communities near Toolik Lake, on the North Slope of Alaska during the 2010 and 2011 growing season. In both years, light and temperature varied with canopy depth, creating vertical differentiation in abiotic factors known to influence foliar carbon cycling. Specific leaf area also varied with canopy depth, and related strongly to leaf nitrogen, with greatest values at the top of the canopy. However, these physical traits were not reflected in any significant, predictable changes in photosynthesis or respiration, suggesting a disconnect between environmental drivers and physiological rates of gas exchange. This discrepancy may be explained by mechanisms such as photoinhibition, leaf age, and stomatal conductance, as well as the unique arctic light environment during the short growing season. Though significant vertical patterns of gas exchange are not seen in these shrub canopies, data presented here represent a highly-detailed characterization of biotic and abiotic factors that control foliar carbon cycling in this rapidly changing landscape and can inform ecosystem carbon models enabling more robust predictions of C exchange in a shrubbier, taller tundra community.

**Keywords:** canopy optimization, nitrogen, photosynthesis, respiration, resource allocation, woody shrub expansion
INTRODUCTION

Rapid warming in the Arctic is facilitating ecological change (Serreze et al. 2000; Hinzman et al. 2005; Post et al. 2009), including the well documented ‘greening’ of the tundra (Jia, Epstein & Walker 2003; Myers-Smith et al. 2011a). The Arctic tundra is becoming ‘shrubber’, a trend observed through comparative photography (Sturm, Racine & Tape 2001; Tape, Sturm & Racine 2006) and satellite remote sensing (Stow et al. 2004), reported in first-hand accounts (Thorpe et al. 2002), and supported by long-term experimental warming (Walker et al. 2006; Elmendorf et al. 2012). Woody shrub expansion in the Arctic results in increased leaf area and canopy height, and is associated with a cascade of ecological effects that can influence regional climate, energy balance, and carbon cycling (Chapin et al. 2005; Sturm et al. 2005a; Bonfils et al. 2012; Cahoon et al. 2012). In light of the growing shrub dominance in Arctic tundra, and the observed and potential future increases in shrub community stature, it is important to understand the canopy dynamics of foliar physiological mechanisms and nitrogen allocation, and the resulting influences on carbon exchange.

The arctic tundra ecosystem is characterized by environmental extremes, which, in tandem with current and predicted climate change, shape the regional carbon balance. Permafrost beneath the soil active layer limits plant rooting depth, and colder soil temperatures, while preserving large stores of organic matter, can constrain soil microbial activity, and in turn, soil nutrient availability (Schimel, Kielland & Chapin III 1996; Schuur et al. 2009; Tarnocai et al. 2009). Concealed by snow for a majority of the year, tundra vegetation experiences near-to-complete 24-hour photoperiods and highly variable ambient temperature and precipitation during the short growing season (~June-August). Moreover, the warming-mediated woody shrub expansion is actively changing the ecology and
biogeochemistry of the system in ways that may impact foliar physiology. These changes include the modification of soil nutrient availability via increased microbial activity under elevated snow cover in winter months (Sturm et al. 2005b), and altered litter input (Hobbie 1996; Buckeridge et al. 2010); the potential cooling of soil through increased ground shading by larger canopies (Blok et al. 2011); and of particular interest to our study, greater canopy complexity due to increased canopy height and leaf area index (LAI) (Hollister, Webber & Tweedie 2005; Walker et al. 2006; Myers-Smith et al. 2011b).

Previous studies in tundra communities across the Arctic have found strong correlations relating LAI and total leaf nitrogen (N) when LAI is relatively low (between 0 – 1 m² m⁻²), implying nutrient availability limitation on leaf production (Williams & Rastetter 1999; van Wijk, Williams & Shaver 2005). As leaf area and N are key controls on photosynthetic carbon (C) assimilation, these relationships may be used to infer gross primary productivity (GPP) of tundra canopies regardless of community composition (Williams & Rastetter 1999; Street et al. 2007). However, these studies also show that at high leaf area (LAI ≥ 2 m² m⁻²) the tight, predictive correlations of LAI and N and GPP weaken (van Wijk, Williams & Shaver 2005; Street et al. 2007), suggesting an alteration in canopy foliar organization that affects C cycling. Further, despite this knowledge on general ecosystem controls on GPP, it remains unknown if in taller shrub communities (LAI ≥ 2 m² m⁻²), leaves which may experience varying thermal and light micro-environments exhibit vertical patterns of foliar N allocation and C cycling within individuals.

Changes in canopy structure can affect the amount of light available to individual leaves of woody species, and can influence patterns of foliar photosynthetic capacity and nitrogen partitioning within a canopy (Field 1983; Hirose & Werger 1994; Hollinger 1996).
dense stands, the distribution of N can exhibit trends nearing optimization, facilitating the
maximization of whole canopy photosynthesis (Werger & Hirose 1991), though this is not
always observed (Evans 1993; Hollinger 1996). The relationship between leaf N and
photosynthetic capacity has long been established (Field & Mooney 1986; Evans 1989), and
similarly, general, scalable relationships between N and foliar respiration have also been
documented in many plant species (Ryan 1995; Reich et al. 1998; Reich et al. 2008; Atkin et
al. 2013). The consistency of these intra-canopy trends in light, N, and foliar carbon exchange
has not yet been addressed in arctic shrubs, nor has the potential influence of environmental
drivers on these relationships. The accurate estimation of within-canopy patterns of carbon
cycling in tundra shrubs requires the incorporation of multiple biotic and abiotic variables. As
with lower-latitude studies, multiple measures of the light and thermal environment, and
corresponding values of foliar nitrogen and photosynthetic and respiratory capacity are
necessary to examine potential allocation and physiological patterns within a tundra shrub
canopy. Additionally, due to the night-less environment of the Arctic tundra, estimates of
respiration in the light ($R_{\text{Light}}$) and the degree of inhibition of mitochondrial respiration ($R_{\text{Dark}}$)
by light, which are known to vary in arctic species under environmental change (Heskel et al.
2012; Heskel et al. 2013) will provide more meaningful representation of canopy carbon
exchange.

Here, we closely examine shrub canopies dominated by Betula nana and Salix
pulchra, two common deciduous species, in the North Slope of Alaska to address questions
about the nature of the relationships between LAI, N, and C exchange. We hypothesize that
foliar nitrogen and gas exchange rates of woody shrubs will vary with LAI and corresponding
light availability. This study presents the first published values of photosynthetic, respiratory,
and leaf nutrient variables, in response to LAI and corresponding thermal and light microenvironment within a shrub canopy. Understanding within-shrub distribution of physiological activity is important for scaling up to shrub community canopy and tundra landscape carbon gain. Results from this study will inform ecosystem carbon models and enable more robust predictions of C exchange in a shrubbier, taller tundra.

**MATERIALS AND METHODS**

*Field site, species, and environmental monitoring* - The study took place during July 12-19, 2010 and July 6-23, 2011 at three distinct shrub-dominated sampling sites on the border of Toolik Lake, less than 2km from Toolik Field Station on the North Slope of Alaska (68°38’N, 149°36’W), located 254 km north of the Arctic Circle. Sites were selected for their height (> 0.5 m) while still being representative of the surrounding vegetation, covered an approximately 1.5m x 1.5m area, and were dominated by *Betula nana* and *Salix pulchra*, though contained ‘understory’ forbs, mosses, and lichens. The area of sampling within a given site did not exceed a 1 m radius from the center to ensure similar soil conditions. Both *Betula nana* L. (“dwarf birch”) and *Salix pulchra* (“tealeaf willow”) are deciduous woody shrubs that are abundant and often dominant over much of the Arctic region.

In 2010, the selected shrub site was partitioned into three categorical levels for sampling that represented thirds of the height of the canopy: “Low”, “Mid”, and “Top”. Above the canopy, and at approximately 1/3 and 2/3 from the top of the canopy, small quantum sensors (SQ-110 Sun Calibration Quantum Sensor, Apogee Instruments, Logan, UT) and 24 gauge copper constantan wire thermocouples (Omega Engineering, Stamford, CT)
were attached to rods in the ground continuously measured photosynthetically active radiation (PAR) and ambient air temperature. Thermocouples were cross-calibrated and properly shaded to reflect the ambient air temperature environment at those heights. A data logger (CR10X, Campbell Scientific, Logan, UT) collected these data continuously during the sampling period, though a battery malfunction at the end of the sampling period led to a regrettable loss of the environmental data for the 2010 sampling period. Leaf area index (LAI) was measured using a plant canopy analyzer (LAI-2200, LI-COR, Lincoln, NE). Five leaf samples were selected from *B. nana* and *S. pulchra* for gas exchange and leaf chemistry analysis as described below.

In 2011, shrub canopy sampling was altered slightly to better capture the ambient growth environment and increase the sampling power. We established two shrub canopy sites, within 100 m of each other. Instead of categorically partitioning the canopy into three levels, we set up ten microenvironment monitoring sites at various heights within the larger canopy representing different leaf environments to get a more detailed characterization of the ambient light and temperature environment experienced by leaves of *B. nana* and *S. pulchra*. PAR and temperature data was collected continuously during the sampling period and logged in a data logger, as described above. At each micro-environmental monitoring site within the shrub sites, LAI was measured in the same manner as in the 2010 shrub site in order to relate LAI to intercepted PAR in the same shrub region.

*Foliar carbon exchange* - CO₂ fluxes of photosynthesis and respiration were measured using an infrared gas analyzer (IRGA; LI-6400XT Portable Photosynthesis System, LI-COR, Lincoln, NE). All measurements were made on clipped leaves collected from the shrub sites,
recut under water in the field, and transported in water to the laboratory as described in Heskel et al. (2012). Preliminary measurements showed no difference in rates of gas exchange and stomatal conductance between field-measured and lab-measured leaves (Griffin, unpublished), as previously observed in other woody species (Mitchell, Bolstad & Vose 1999; Turnbull et al. 2003). This sampling technique provided a greater degree of temperature control, and allowed for us to maximize the number of replicates, and minimize time between replicates.

*S. pulchra* leaves were generally large enough to occupy the entire leaf cuvette (~6 cm²), and were trimmed to only the area that occupied the cuvette after IRGA analysis for leaf area and mass measurement (see below). *B. nana* leaves, which were smaller than the 6 cm² cuvette area, were measured for leaf area after IRGA measurements and gas exchange values were corrected to that area for analysis. In 2010, five replicates were sampled from each categorical canopy level, for a total of 15 unique replicates per species. In 2011, two replicates of both species (*n = 2*) were sampled from each environmentally monitored canopy location, totaling 20 unique measurements per species throughout the shrub canopy at each site, and 80 unique replicates for the study. However, to avoid pseudoreplication, replicates of from the same LAI level were bulked by species.

Prior to IRGA measurement in the laboratory, leaves were enclosed in the cuvette under high light conditions (~1200 µmol m⁻² s⁻¹ PAR) to acclimate to the measurement conditions. CO₂ assimilation was measured under ambient (400 ppm) CO₂ concentration under 26 levels of decreasing PAR: 1500, 1200, 800, 400, 200, 100, and every 5 µmol m⁻² s⁻¹ between 100 and 0 µmol m⁻² s⁻¹. This range fully encompasses the light environment experienced by tundra species in their growth environment. Following the light-response
curve, leaf samples were treated to 900 μmol m$^{-2}$ s$^{-1}$ PAR and 400 ppm CO$_2$ for 5-10 minutes before CO$_2$ assimilation was measured in response to 11 levels of increasing [CO$_2$]: ~0, 50, 100, 150, 200, 300, 400, 600, 800, 1200, and 1500 ppm. Immediately after CO$_2$-response curve measurement, leaves were kept in the cuvette in darkness for 10 minutes at 400 ppm CO$_2$. During this time, it was assumed that no photosynthesis or photorespiration was taking place, and all CO$_2$ flux could be attributed to mitochondrial respiration in the dark ($R_D$).

All IRGA measurements were taken at a relative humidity of approximately 40-60%, and potential diffusion in and out of the cuvette was accounted for, as was diffusion through the gasket, according to corrections presented in the Li-Cor 6400 Instructional Manual. Cuvette block temperature was set to 20°C for all measurements to control for leaf temperature, and is generally representative of temperatures experienced by these species during the growing season. Maximal light-saturated photosynthetic rate ($A_{max}$) was estimated by fitting data from the light-response curve to a rectangular hyperbolic function (Excel Solver, Microsoft, Redmond, WA). We analyzed CO$_2$-response curves for the maximum carboxylation velocity of Rubisco ($V_{cmax}$) and maximum rate of electron transfer ($J$) using the $A$-$C_i$ curve fitting utility (version 2007.1) provided and detailed in Sharkey et al. (2007). Respiration and photosynthesis values are expressed on an area, mass, and nitrogen basis. For the 2011 measurements, using a poromoter (SC-1 Leaf Porometer, Decagon Devices, Pullman, WA), we measured stomatal conductance in leaves from the ‘upper’ (samples no more than 20 cm from maximum canopy height) and ‘lower’ (samples from the bottom third of total canopy height) regions of the shrub canopy. The porometer also recorded leaf temperature, and these values were considered statistically to control for differences at different canopy levels.
Quantifying respiration in the light using the Kok method - To estimate respiration in the light ($R_L$), we used the Kok method, convenient for field measurements and employable under ambient atmospheric conditions, as demonstrated previously in arctic species in similar conditions (Heskel et al. 2012; Heskel et al. 2013). This method is based on the observation that the quantum yield of photosynthesis usually decreases abruptly above a certain level of light intensity—often near the light compensation point, where carbon flux is zero (Kok 1948). A noticeable non-linearity, or “bend”, in the otherwise linear lower range of the light-response curve is observable, interpreted as the saturation point of light inhibition of respiration (explained in detail in Shapiro et al., 2004). Respiration is assumed to be constant above this point, and an extrapolation to 0 µmol m$^{-2}$ s$^{-1}$ PAR of the linear portion of the curve above this point is assumed to give the rate of respiration in the light. Here, the irradiance range we used to calculate $R_L$ spanned from 25-90 µmol m$^{-2}$ s$^{-1}$, as in Heskel et al. (2012) and Heskel et al. (2013).

The Kok method assumes the CO$_2$ assimilation rate responds only to light, and thus corrections must be made to account for changes in internal CO$_2$ ($c_i$). As photosynthestic rates slow under decreasing light intensity, CO$_2$ tends to accumulate within the leaf, increasing $c_i$, affect the shape of the light curve by decreasing rates of photorespiration at lower light levels. Here, we corrected measurements to a constant $c_i$ to account for these effects to achieve a more accurate extrapolation of $R_L$, according to Kirshbaum and Farquhar (1987), as described in Ayub et al. (2011).
*Foliar chemistry and leaf traits* - In both 2010 and 2011, after infrared gas analysis, sampled leaves were measured for leaf area using a rotating-belt leaf area meter (LI-3100C Area Meter, LI-COR, Lincoln, NE). Samples were then dried in an oven at 60°C for no less than 48 hours before mass was determined. Specific leaf area (SLA, cm² g⁻¹), a metric approximating leaf density, could then be calculated from these variables. After transport to Columbia University in New York, all samples were ground, weighed, and packaged for elemental analysis of [CHN] (2400 Series II, Perkin-Elmer, Boston, MA). In 2010 only, additional ground leaf sample was sent to the Laboratory for Biotechnology and Bioanalysis in the School of Biological Sciences at Washington State University (Pullman, WA) to determine δ¹³C and δ¹⁵N.

*Statistical analyses* - The main effects of species and LAI on foliar physiological processes and physical traits were determined with a two-way ANOVA in R (v2.7.0, The R Foundation for Statistical Computing). To account for interannual differences, data collected in 2010 was analyzed separately from data collected in 2011. For the 2011 data, “site” was considered a random factor to control for intra-site variation in environmental variables, and also to control for temporal differences in ambient temperature between the two sampling periods. Prior to analysis, data were tested for normality (Shapiro-Wilk) and heteroscedasticity (Breusch-Pagan), and log₁₀-transformed when necessary. Post-hoc assessment of species and canopy level differences were made using Tukey’s HSD test. To address the relationships between foliar physiological rates and ambient temperature or leaf traits, correlation analyses were employed.
RESULTS

Micro-environmental variation within a tundra shrub canopy

Leaf area index, measured at the ten different micro-environmental sensors located within the selected tundra shrub sites, allowed for a detailed depiction of the intra-shrub light environment. Maximum LAI at the lowest sensor measured was 2.35 m$^2$ m$^{-2}$ in 2010, and 3.03 m$^2$ m$^{-2}$ in 2011. The PAR values corresponding to micro-environmental sensors in 2011 show a clear difference in light environment between the top (LAI < 0.5 m$^2$ m$^{-2}$) and the rest of the canopy (Fig. 1). The average, minimum, and maximum PAR recorded by the top two sensors were greater than those recorded at lower canopy heights. In terms of temperature, average values did not show any trends with LAI, though minimum and maximum temperature values reflected a pattern similar to that observed in PAR measurements, with highest values measured at the canopy crown (Fig. 1). Both PAR and temperature measurements varied with leaf area within a relatively low canopy, especially considering the range between leaves at the crown and leaves closer to the ground.

Leaf physical and chemical traits

The quantification of foliar chemistry and physical traits through the tundra shrub canopy allowed for closer examination of potential variation in leaf traits in the two dominant shrub species. In both 2010 and 2011, values of SLA, a measure that approximates leaf density, were lowest at the top of the canopy, and increased with canopy depth (Fig. 2). The trend of decreasing SLA with increasing LAI was nearly parallel in B. nana and S. pulchra in 2010, and no species effects were detected, though in 2011, when a greater range of LAI was
sampled, SLA in *B. nana* was significantly greater than *S. pulchra* (Table 1). No interactive effects between species and LAI were found for either measurement year.

LAI did not influence foliar C concentration in either study year, though a significant species effect was found: leaves of *B. nana* had greater concentrations of C than *S. pulchra* in both 2010 and 2011 (Fig. S1). Differences in foliar N, when expressed as a concentration (%), were found between species in 2010, but not 2011, and not influenced by LAI (Table 1). In contrast, when foliar N was expressed per unit leaf area (N_{area}, g m^{-2}), *B. nana* had significantly larger values than *S. pulchra* in 2010 (*p* < 0.05) and lower values than *S. pulchra* in 2011 (*p* < 0.001). Further, N_{area} increased in a similar manner in both species as LAI values decreased towards the crown of the shrub canopy (Fig. 3). In 2010, for both species, the top canopy level had significantly higher values of N_{area} when compared to the lower two levels (both *p* < 0.0001). In 2011, a similar relationship was observed between N_{area} and LAI (*p* < 0.001, $R^2 = 0.46$ for both species), with greatest values at the top of the canopy. Though, the strong N_{area}-LAI relationship (Fig. 3), is likely a by-product of the strong SLA-LAI gradient in these species: as leaves at the top of the canopy have less area per unit mass (and inversely, more mass per unit area), more N will be found in samples with greater mass per unit area. The correlation between N_{area} and leaf mass per unit area (data not shown), yields strong predictive relationships in both measurement years ($R^2 = 0.80$ in 2010, and $R^2 = 0.74$ in 2011).

The ratio of concentrations of C and N (C:N) varied significantly between species in 2010, though not in 2011, with values in *B. nana* lower than *S. pulchra*, likely due variation in C more than variation in N (Table 1, Fig. S1). Significant effects of LAI on C:N were not observed in either species (Table 1).
In 2010, stable C and N isotopes were measured in leaf samples of both species at the three canopy levels (low, middle, and top). *B. nana* had lower values of δ\(^{13}\)C than *S. pulchra* at each canopy level, with the lowest values for both species at the bottom of the canopy, and the least negative δ\(^{13}\)C values in leaves sampled from the top of the canopy (Fig. 4). A similar trend occurs in δ\(^{15}\)N, where the most negative values occur in *B. nana* at the lowest canopy level, though *S. pulchra* does not exhibit any significant variation across canopy levels (Table 1, Fig. 4).

**Photosynthesis and respiration through a tundra shrub canopy**

One of the main objectives of this study was to quantify variation in photosynthetic parameters within the tundra shrub canopy, and examine if these measures related to other leaf traits, such as $N_{\text{area}}$ and SLA. Maximum photosynthesis rates were significantly higher in *B. nana* than *S. pulchra* in both measurement years (Table 2, Fig. 5). Despite variation in $N_{\text{area}}$ through the canopy, there was no significant relationship between LAI and $A_{\text{max}}$ (Table 2). This lack of trend can be observed when $A_{\text{max}}$ is expressed on an area-, mass-, and N-basis (Table 2; only area is graphically depicted in Fig. 5). There were no significant species or LAI effects on either $V_{\text{cmax}}$ or $J$ for both measurement years (Table 2; Fig. 5). In 2010 there was a slight, non-significant trend with higher mean values of both parameters at the top of the canopy (Fig. 6), though this trend was not apparent in data from 2011. Considering both species and measurement years, $V_{\text{cmax}}$ and $J$ are significantly correlated with $N_{\text{area}}$ ($p < 0.005$ and $p < 0.05$, respectively), though not in a clearly predictive way ($R^2 < 0.10$ for both variables; Fig. S2). In 2010, $A_{\text{max}}$ was significantly correlated to $N_{\text{area}}$ across species ($p < 0.01$; $R^2 = 0.30$), though *S. pulchra* exhibited a stronger correlation than *B. nana* (Fig. 8). In 2011,
the cross-taxon $A_{\text{max}} - N_{\text{area}}$ relationship was not significant or strongly correlated ($R^2 = 0.02$), though considering only $B. nana$, this relationship was significant ($p < 0.05$; Fig. 8).

In 2010, mitochondrial dark respiration exhibited significant species differences, with values from leaves of $B. nana$ greater than $S. pulchra$ (Fig. 5); though in 2011, the species effect was not significant (Table 2). In 2011, a significant influence of LAI on $R_{\text{Dark}}$ was found when expressed on an area-basis, though this trend did not extend when $R_{\text{Dark}}$ was expressed on mass- and N-bases; in 2010 there were significant interaction effects of species and LAI, mainly due to the high mean values of $B. nana$ at the middle canopy level. With the exception of $B. nana$ in 2010, generally the highest values of $R_{\text{Dark}}$ were at the top of the canopy. Inter-annual differences were also observed in the relationship between $R_{\text{Dark}}$ and $N_{\text{area}}$: in 2010 in $S. pulchra$ (though not $B. nana$) it was significantly correlated ($p < 0.05$; Fig. 8), though this was not observed in 2011 in either species.

Measures of respiration in the light in 2010 were highest in leaves of $B. nana$ from the middle portion of the canopy, paralleling trends in $R_{\text{Dark}}$ (Fig. 5). In $S. pulchra$, which had lower rates of $R_{\text{Light}}$ at each level, the highest mean rates were reported in leaves from the top of the canopy, and more closely linked to trends in photosynthesis (Fig. 5). Species effects on $R_{\text{Light}}$ were significant when expressed on an area-, mass- and N-basis, though a significant species-LAI interaction term highlights the large difference in mean rates of $R_{\text{Light}}$ at the middle of the shrub canopy. In 2011, no significant species effects were observed in $R_{\text{Light}}$, and values generally fell within the range measured in 2010 (Table 2, Fig. 5). Also, no significant effect of LAI was found in 2011, though generally the highest values were measured in leaves at the top of the canopy (Fig. 5). Similar to trends in $R_{\text{Dark}}$, $R_{\text{Light}}$ was only significantly correlated with $N_{\text{area}}$ in 2010 in $S. pulchra$. Considering individual species, $R_{\text{Light}}$: 
$R_{\text{Dark}}$ did not vary with canopy position for either measurement year (Table 2). However, considering both species, there was a general, though not statistically significant, increasing trend of $R_{\text{Light}}: R_{\text{Dark}}$ with LAI ($p = 0.08$; Fig. 7). The ratio of respiration in the light to gross photosynthesis, an estimate of proportional carbon loss to total carbon exchanged in tundra species, differed between species in both years (Table 2), though in contrasting ways: in 2010, mean $R_{\text{Light}}: A_{\text{gross}}$ values were lower in *S. pulchra*, but in 2011, *B. nana* had lower mean values (Fig. 7). In 2011, LAI significantly influenced $R_{\text{Light}}: A_{\text{gross}}$ values (Table 2), with a general trend towards higher proportional carbon release at the top of the shrub canopy in both species (Fig. 7).

Stomatal conductance was measured in 2011 only, on both species in leaves at the top and bottom of the shrub canopy. Across all measurements, *S. pulchra* had higher conductances than *B. nana*, and there were no significant differences between time of day and canopy position within species (Table 3). Within *S. pulchra*, leaves sampled from the top of the canopy had significantly greater stomatal conductance than leaves sampled from the bottom of the canopy ($p < 0.01$, Table 3).

**DISCUSSION**

We present for the first time a highly detailed description of foliar traits and physiological processes at different canopy positions to characterize how light, N, and carbon cycling mechanisms are distributed within the canopy of a tundra shrub community. Micro-environmental monitoring throughout the shrub canopy allowed for the confirmation that, despite the relatively short stature of tundra vegetation, there is indeed stratification of
temperature and light that has the potential to promote foliar physiological differentiation. While leaf physical traits of both species responded to canopy position, this did not translate into a predictable pattern of photosynthesis or respiration through the canopy. However, findings from this study, including the first published values for $V_{\text{cmax}}$, $J$, and $R_{\text{Light}}$ in *S. pulchra* and, to our knowledge, the first published physiological data of tundra species at multiple canopy heights, allow for new insight into how shrubs regulate carbon exchange in a “greening” tundra landscape.

**Variation in the tundra shrub micro-environment**

Prior to this study, the variation in temperature and degree of light extinction in the field in relatively taller tundra shrub canopies was unknown; here, we quantified differences that allow for a more realistic description of the leaf micro-environment. As expected, generally the highest temperatures and PAR values were recorded at the sensors located at the top of the shrub canopy (Fig. 1), with lower values at higher values of LAI. Though average temperatures did not vary greatly between canopy positions, there was a stark contrast between the top of the canopy and all other positions when considering the minimum temperature, which parallels trends in light. The concurrent increases in environmental complexity with shrub stature poses new challenges for considering carbon cycling, given the light and temperature sensitivity of photosynthesis and respiration.

**Canopy carbon exchange in woody shrubs**

The foliar carbon cycling variables measured in this study did not exhibit clear vertical patterns that would imply potential canopy optimization in the tundra shrub canopy.
Differences in light and temperature micro-environment associated with changes in LAI (Table 1) influenced leaf physical structure, creating denser leaves at the top of the canopy and thinner leaves at the bottom of the canopy (Fig. 1). This trend, observed in both species in both measurement years, reflects a cross-taxa intra-canopy morphological organization that is found in other systems (Tissue et al. 2002; Lloyd et al. 2010) and may be associated with an increase in cell height in sun-exposed leaves to accommodate more Rubisco per unit area and an increase of mesophyll surface area to promote higher rates of photosynthesis (Terashima, Miyazawa & Hanba 2001; Terashima et al. 2011). Foliar nitrogen (N_{area}) generally increased with LAI in both measurement years (Fig. 3), though the relationship was not as clear for SLA, suggesting a potential delay between leaf structural changes and corresponding physiological processes that is supported by the absence of greater rates of photosynthesis and respiration at the top of the canopy (Fig. 5). The isotopic nitrogen signature in these species supported previously revealed species differences in their ectomycorrhizal associations (Hobbie & Hobbie 2006), with little intra-canopy variation.

The consistency in physiological and physical leaf traits, including foliar C concentration and C:N ratio (Fig. S1), rates of respiration in the light and dark (Fig. 5), photosynthetic parameters (Fig. 5-6), the degree of inhibition of respiration, and ratio of carbon release to assimilation in the light (Fig. 7), at different canopy heights, despite variation in micro-environment suggest a potential lack of short-term plasticity within a canopy in this shrub community. Previous work on these species at Toolik Lake under long-term environmental manipulation shows a large range of physiological and morphological responses (Chapin & Shaver 1985; Chapin et al. 1995; Chapin & Shaver 1996; Heskel et al. 2013), though duration of treatment probably influences the degree of these effects for most
treatments. When considering a shrub canopy, leaves towards the ground may only be effectively shaded later in the growing season as the canopy fills in. With only approximately one month before senescence, there may be little time for foliar functional reorganization that would be metabolically efficient; thus, leaves from the lower canopy may exhibit rates more similar to those of leaves at the top of the canopy, compared to leaves grown under an experimental shading treatment.

In this study, we also examined less frequently measured gas exchange variables, specifically $R_{\text{Light}}$ and the ratio of $R_{\text{Light}}$ to $R_{\text{Dark}}$ and $A_{\text{gross}}$, to evaluate the effect of canopy position (and the corresponding light and temperature micro-environment) on respiratory performance in shrubs located in the night-less Arctic tundra. Rates of foliar photosynthesis and respiration in the light and dark fit within the range of previously published measurements in $B. nana$ and $S. pulchra$ grown in moist acidic tundra (Bret-Harte et al. 2001; Heskel et al. 2012; Heskel et al. 2013; Patankar et al. 2013), and little variation was observed at different canopy heights (Fig. 5). The ratio of respiratory carbon loss in the light to that in the dark did not increase significantly with canopy height as leaves are more exposed to light, which suggests that respiratory metabolism does not respond to short-term variation in light and temperature – environmental controls known to affect $R_{\text{Light}}$: $R_{\text{Dark}}$ over longer time scales (Atkin et al. 2000; Heskel et al. 2013). While little variation was detected in $R_{\text{Light}}$: $R_{\text{Dark}}$ through the canopy, the environmental data obtained in our study underscores the importance of including $R_{\text{Light}}$ when estimating arctic carbon exchange: average PAR values indicate most of the leaves in these shrub canopies would experience light inhibition of respiration (Fig. 1). Foliar carbon efficiency, here quantified as the proportional amount of carbon released in the light to the total carbon exchanged $R_{\text{Light}}$: $A_{\text{gross}}$, increased minimally with LAI in 2011(though
not significantly in 2010). This subtlety parallels responses seen in canopy trees where leaves at the top of the canopy were most carbon efficient (Griffin et al. 2001), though even in this forest system, major differences were only observed in the upper 40% of the canopy. Though the arctic shrub communities examined in our study are merely ~1m, it is possible that continued warming in this area will lead to increases in stature that surpass a height threshold and greater differences in physiological traits through the canopy will be observed.

The absence of any clear, significant indication of an ‘optimized’ canopy in this shrub community may be explained by multiple biotic and environmental aspects of the Arctic tundra. First, stomatal conductance was only significantly higher between the lower and upper canopy leaves in *S. pulchra*, though not in *B. nana*, suggesting a species-specific potential limitation to photosynthetic efficiency in leaves at the crown (Table 3). This corresponds to the less negative δ^{13}C values at the top of the canopy (Fig. 4) that indicate less discrimination by Rubisco, despite greater stomatal conductance (Table 3) and similar rates of photosynthesis (Figs. 5-6), suggesting intra-canopy variation in water use efficiency and a less than optimal use of foliar N for carbon assimilation. These variables are influenced by canopy position, with self-shading due to the closing shrub canopies likely at the root of some of these differences (Street et al. 2007). Second, photoinhibition of photosynthesis may limit rates of carbon assimilation in leaves at the crown, especially in tundra species that are subjected to near-constant light through the growing season. Though examination of light response curves of leaves in our study does not immediately provide evidence of photoinhibition, fluorescence data (F_v/F_m) in leaves of *B. nana* and *S. pulchra* collected in 2012 at shrub study sites near Toolik Lake indicate potential light stress in leaves at the top of the canopy (Formica and Griffin, unpublished). Despite higher values of N_area in leaves at the
crown (Fig. 3), rates of photosynthesis were not greater towards the top of the canopy (Figs. 5-6), and perhaps this less efficient carbon assimilation prevents the accumulation of reactive oxygen species or other potentially harmful by-products of excessive, prolonged light exposure, though more research is needed to measure how phenolic compounds and the xanthophyll cycle are involved in these species.

It is also possible that the short length of the growing season, and therefore the young age of the deciduous shrub leaves, may modify how resources are allocated within arctic shrub canopies. Leaf age is generally equal across the canopy in deciduous woody shrubs in the tundra, and the costs of any redistribution of foliar N within a canopy to optimize carbon assimilation is likely to outweigh the benefits within a short time scale (Field 1983). Also, the history of populations of B. nana and S. pulchra within the Arctic tundra context may influence how these species distribute nitrogen and cycle carbon within their canopies. Prior to current warming (and the associated woody shrub expansion), the North Slope of Alaska had not experienced a high density of these woody shrub species since the Holocene Thermal Maximum circa 11-9,000 ka (Eisner & Colinvaux 1992; Kaufman et al. 2004), and for millennia did not experience competition for light that may create a need for more optimal carbon assimilation via the reallocation of resources within a canopy.

Finally, in this study, canopy position and light availability were approximated through measures of LAI and flat-surfaced quantum sensors positioned at different heights within the shrub community, which while detailed in their description of the leaf micro-environment, may not adequately record the relevant light environment in this system. Generally in this region, and in our study, arctic shrubs in expanding communities are clumped through the landscape, often in locations with higher water and nutrient availability.
(Tape et al. 2012), and surrounded by lower-statured vegetation. This distribution pattern results in a large edge effect that likely modifies the assumed relationship between the position within the canopy and foliar light availability, as leaves lower in the canopy, but at the edge, may receive equivalent amounts of light as leaves towards the crown. Additionally, during the growing season at Arctic latitudes, the edge effect may be further amplified by the path of the sun and large solar zenith angle (Stow et al. 2004), effectively decoupling LAI and light availability for edge leaves towards the bottom of shrub canopies.

**Implications and conclusions**

The absence of clear, predictive trends in leaf N and gas exchange characteristics through arctic shrub canopies is important and informative for models of tundra carbon exchange. Significant species differences within the same plant functional type are apparent in nearly every variable measured and these differences should be integrated where appropriate to further refine models. This may be helpful by simplifying spatially explicit models that predict differential species dominance in shrub expansion hotspots (i.e. alongside streams and water tracks, in rims of tundra polygons, and on sites of permafrost failure and thermal erosion). While not considered in this study, the measured increase in LAI is likely to affect the functioning and composition of “understory” vegetation, as the increasing height of many shrubs may spur belowground competition for soil nutrients and aboveground competition for light. While our null hypotheses - that leaf physiological traits would not vary with canopy position and LAI - were accepted, this work can serve as experimental support for theoretical and modeling studies that assess the effect of LAI and canopy height on foliar mechanisms in the Arctic (Kiepe et al. 2013), and provide important species-specific information on how
photosynthetic and respiratory variables scale with nitrogen (Figs. 8 & S2). Few measurements of LAI in our study were comparable to values found in other optimization studies (Hirose & Werger 1987; Schieving et al. 1992; Griffin et al. 2001) and it is possible if current trends in shrub expansion associated with warming continue, a threshold in density will be crossed, ultimately leading to more ‘optimized’ canopies. We suggest that future research continues to examine how canopy height and density and environmental thresholds influence how leaf N is distributed and carbon cycling occurs within shrub canopies.

Our study presents a multi-year characterization of foliar carbon cycling and two dominant woody shrub species that are known to be expanding in the Alaskan Arctic (Tape, Sturm & Racine 2006; Myers-Smith et al. 2011a; Tape et al. 2012). Our data thus provides a detailed description of intra-canopy physiological processes and leaf traits and serves as a background for future study on the effect of canopy complexity on carbon exchange. As the Arctic tundra continues to ‘green’, with shrub communities expanding and becoming denser and taller, it is important for future research to address how these community structural changes impact intra-canopy micro-environmental variation and foliar and ecosystem functioning.
**TABLES AND FIGURES**

**Table 1.** Two-way ANOVA results of leaf carbon and nitrogen concentration, d13C and d15N, and specific leaf area in *B. nana* and *S. pulchra* from 2010 and 2011. In 2010, only three LAI levels were sampled from for the gas exchange measurements. In 2011, 10 levels were sampled from. Asterisks denote significance at the $p < 0.05$ (*), $< 0.01$ (**), and $< 0.001$ (***).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Species</th>
<th>LAI</th>
<th>Species x LAI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F-stat</td>
<td>p</td>
<td>F-stat</td>
</tr>
<tr>
<td>% C</td>
<td>10.871</td>
<td>**</td>
<td>0.219</td>
</tr>
<tr>
<td>% N</td>
<td>41.49</td>
<td>***</td>
<td>0.765</td>
</tr>
<tr>
<td>C: N</td>
<td>14.373</td>
<td>***</td>
<td>0.397</td>
</tr>
<tr>
<td>$\delta^{13}$C</td>
<td>24.436</td>
<td>***</td>
<td>39.723</td>
</tr>
<tr>
<td>$\delta^{15}$N</td>
<td>21.174</td>
<td>***</td>
<td>3.837</td>
</tr>
<tr>
<td>SLA</td>
<td>0.225</td>
<td>0.638</td>
<td>35.102</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>F-stat</th>
<th>p</th>
<th>F-stat</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>% C</td>
<td>14.751</td>
<td>***</td>
<td>0.010</td>
<td>0.920</td>
</tr>
<tr>
<td>% N</td>
<td>0.157</td>
<td>0.694</td>
<td>2.300</td>
<td>0.133</td>
</tr>
<tr>
<td>C: N</td>
<td>0.064</td>
<td>0.801</td>
<td>0.031</td>
<td>0.861</td>
</tr>
<tr>
<td>SLA</td>
<td>35.415</td>
<td>***</td>
<td>18.264</td>
<td>***</td>
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Asterisks denote significance at the $p < 0.05$ (*), $< 0.01$ (**), and $< 0.001$ (***)
Table 2. Statistical results from a two-way ANOVA analyzing foliar gas exchange in *S. pulchra* and *B. nana* at difference canopy levels represented by leaf area index (LAI). Asterisks denote significance at the $p < 0.05$ (*), $< 0.01$ (**), and $< 0.001$ (***)..

<table>
<thead>
<tr>
<th>Variable</th>
<th>2010 Species</th>
<th>LAI</th>
<th>Species x LAI</th>
<th>2011 Species</th>
<th>LAI</th>
<th>Species x LAI</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>$F$-stat</td>
<td>$p$</td>
<td>$F$-stat</td>
<td>$p$</td>
<td>$F$-stat</td>
<td>$p$</td>
</tr>
<tr>
<td>$A_{\text{max}}$-Area</td>
<td>10.216</td>
<td>**</td>
<td>1.889</td>
<td>0.173</td>
<td>0.528</td>
<td>0.596</td>
</tr>
<tr>
<td>$R_{\text{dark}}$-Area</td>
<td>67.773</td>
<td>***</td>
<td>3.036</td>
<td>0.067</td>
<td>6.181</td>
<td>**</td>
</tr>
<tr>
<td>$R_{\text{light}}$-Area</td>
<td>43.999</td>
<td>***</td>
<td>3.584</td>
<td>*</td>
<td>5.552</td>
<td>*</td>
</tr>
<tr>
<td>$A_{\text{max}}$-Mass</td>
<td>11.291</td>
<td>**</td>
<td>0.311</td>
<td>0.734</td>
<td>0.326</td>
<td>0.724</td>
</tr>
<tr>
<td>$R_{\text{dark}}$-Mass</td>
<td>55.490</td>
<td>***</td>
<td>1.929</td>
<td>0.168</td>
<td>4.294</td>
<td>*</td>
</tr>
<tr>
<td>$R_{\text{light}}$-Mass</td>
<td>40.905</td>
<td>***</td>
<td>2.769</td>
<td>0.082</td>
<td>4.317</td>
<td>*</td>
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<tr>
<td>$A_{\text{max}}$-N</td>
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<td>0.206</td>
<td>0.815</td>
<td>0.519</td>
<td>0.601</td>
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<tr>
<td>$R_{\text{dark}}$-N</td>
<td>43.681</td>
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<td>1.696</td>
<td>0.205</td>
<td>5.174</td>
<td>*</td>
</tr>
<tr>
<td>$R_{\text{light}}$-N</td>
<td>31.623</td>
<td>***</td>
<td>2.436</td>
<td>0.108</td>
<td>4.745</td>
<td>*</td>
</tr>
<tr>
<td>$R_{\text{light}}:R_{\text{dark}}$</td>
<td>2.971</td>
<td>0.097</td>
<td>1.221</td>
<td>0.312</td>
<td>1.517</td>
<td>0.241</td>
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<tr>
<td>$R_{\text{light}}:A_{\text{gross}}$</td>
<td>8.036</td>
<td>**</td>
<td>1.843</td>
<td>0.180</td>
<td>2.950</td>
<td>0.071</td>
</tr>
<tr>
<td>$V_{\text{cmax}}$</td>
<td>1.762</td>
<td>0.196</td>
<td>3.379</td>
<td>0.050</td>
<td>1.271</td>
<td>0.298</td>
</tr>
<tr>
<td>$J$</td>
<td>1.238</td>
<td>0.276</td>
<td>2.028</td>
<td>0.152</td>
<td>0.761</td>
<td>0.478</td>
</tr>
</tbody>
</table>
Table 3. Stomatal conductance (mm m$^{-2}$ s$^{-1}$) in leaves of *B. nana* and *S. pulchra* sampled from the crown (high) and the base (low) of shrub canopy. Different alphabetical notation denotes significant differences.

<table>
<thead>
<tr>
<th></th>
<th><em>B. nana</em></th>
<th><em>S. pulchra</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>High$^e$</td>
<td>Low$^f$</td>
</tr>
<tr>
<td>Morning$^e$</td>
<td>277.04 ± 42.41$^l$</td>
<td>318.90 ± 41.75$^l$</td>
</tr>
<tr>
<td>Afternoon$^e$</td>
<td>348.03 ± 31.02$^f$</td>
<td>184.14 ± 36.2$^f$</td>
</tr>
</tbody>
</table>
**Figure 1.** Photosynthetically active radiation (PAR) and temperature values corresponding to leaf area index measured in the first shrub site of 2011 recorded during July 5-12. Maximum temperature values were scaled to maximum temperature values measured during this period collected by Toolik Field Station Environmental Data Center due to a measurement inaccuracy in quantum sensors in the shrub canopy site.
Figure 2. Specific leaf area of *B. nana* and *S. pulchra* at different canopy heights corresponding to leaf area index in 2010 and 2011. In 2010, replicates were taken from the same height levels that represented the crown, middle, and lower thirds of the canopy and corresponded to measured LAI values, while in 2011, for graphical clarity, variables were binned (*n* =4) by similar LAI values.
Figure 3. Foliar nitrogen (g m\(^{-2}\) leaf area) in *B. nana* (closed circles) and *S. pulchra* (open circles) at different mean values of LAI measured in 2010 and 2011 (binned for clarity).
Figure 4. Values of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in *B. nana* and *S. pulchra* at three canopy heights measured in 2010. Alphabetical notation signifies significant differences between species and levels.
Figure 5. Foliar carbon fluxes plotted against measured LAI values in *B. nana* and *S. pulchra* in 2010 and 2011. Bars represent standard error.
Figure 6. Rates of $V_{c\text{max}}$ and $J$ (both corrected for 25°C) in *B. nana* (closed circles) and *S. pulchra* (open circles) at different values of LAI measured in 2010 and 2011. Error bars represent standard error.
Figure 7. Ratio of respiratory rates in the light and dark and the ratio of respiration in the light to gross photosynthesis in 2010 and 2011 for *B. nana* (closed symbols) and *S. pulchra* (open symbols).
**Figure 8.** Gas exchange fluxes in 2010 and 2011 in *B. nana* and *S. pulchra* plotted against foliar N_{area}. Linear trendlines were fitted to data (within a species) when $R^2 > 0.30$. 

![Graphs showing gas exchange fluxes in 2010 and 2011 for *B. nana* and *S. pulchra* with trendlines and R^2 values.](figure8.png)
**Figure S1.** Foliar C and C: N values measured in leaves of *B. nana* and *S. pulchra* in 2010 and 2011 at different LAI levels in a tundra shrub canopy. Bars represent standard error.
**Figure S2.** Photosynthetic parameters $V_{c_{\text{max}}}$ and $J$, temperature, corrected to 25°C, for *B. nana* (closed symbols) and *S. pulchra* (open symbols) from 2010 (circles) and 2011 (squares). Though significantly correlated, $R^2$ values for both relationships were under 0.10, and for this reason linear trend lines are not depicted.
CHAPTER SEVEN

Bringing the Kok effect to light: Integrating daytime respiration and net ecosystem exchange

MARY A. HESKEL, OWEN K. ATKIN, MATTHEW H. TURNBULL, and KEVIN L. GRIFFIN

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ABSTRACT

Net ecosystem exchange (NEE) represents the difference between carbon assimilated through photosynthesis, or gross primary productivity (GPP), and carbon released via ecosystem respiration (ER). NEE, measured via eddy covariance and chamber techniques, must be partitioned into these fluxes to accurately describe the carbon dynamics of an ecosystem. GPP and daytime ER may be significantly overestimated if the light inhibition of foliar mitochondrial respiration, or “Kok effect” is not accurately estimated and further integrated into ecosystem measurements. The light inhibition of respiration, a composite effect of multiple cellular pathways, is reported to cause between 25-100% inhibition of foliar mitochondrial respiration, and for this reason needs to be considered when estimating larger carbon fluxes. Partitioning of respiration between autotrophic and heterotrophic respiration, and applying these scaled respiratory fluxes to the ecosystem-level proves to be difficult, and integrating the light inhibition of respiration will require new interpretations and analysis of carbon exchange in terrestrial ecosystems.

**Keywords:** net ecosystem exchange, eddy covariance, Kok effect, photosynthesis, respiration
**Introduction**

The terrestrial carbon cycle, the flow of carbon between the land and atmosphere, accounts for immense fluxes of carbon dioxide globally. These fluxes vary interannually and are influenced by human activity and climate patterns, resulting in the storage of ~1-5 Pg C yr$^{-1}$ in terrestrial systems according to recent model estimations (IPCC 2007, Le Quere *et al.* 2009). The tremendous exchange of carbon between the atmosphere and terrestrial ecosystems is driven primarily by photosynthesis and respiration, fixing atmospheric carbon dioxide (CO$_2$) into C compounds for structural use and energy metabolism, and converting C compounds into chemical energy for cell maintenance and growth and releasing CO$_2$ back to the atmosphere. However, there is a great discrepancy on how photosynthesis and respiration are treated in models of carbon exchange: photosynthesis can be accurately predicted from a mechanistic model (Farquhar *et al.* 1980), while respiration is often modeled as a function of temperature or foliar nitrogen (de Pury & Farquhar 1997, Ryan 1991), a set fraction of photosynthetic carbon gain (DeLucia *et al.* 2007), or based on estimates of multiple separate processes (Cannell & Thornley 2000).

Ecosystem respiration (ER), the process which returns fixed carbon to the atmosphere, accounts for a large portion of the terrestrial carbon cycle and can originate from heterotrophic and autotrophic sources (Trumbore 2006). Autotrophic respiration, specifically that of plants, represents approximately half of overall ER, with leaves contributing approximately half of whole plant CO$_2$ release (Amthor 2000). Net ecosystem exchange (NEE), the difference between the carbon acquired through photosynthetic fixation (gross primary productivity, GPP) and the carbon released through ER, can be measured through eddy covariance techniques (Baldocchi *et al.* 1988). While these methods allow for estimation
of ecosystem scale CO$_2$ flux, partitioning and interpretation of this value is difficult and requires the consideration of multiple scales and environmentally sensitive processes (Chambers et al. 2004, Gilmanov et al. 2007, Griffis et al. 2004, Lasslop et al. 2010, Zobitz et al. 2008). Here, we present an important and often overlooked phenomena that impacts plant carbon cycling – the light inhibition of foliar respiration - and urge for its incorporation in calculations of ecosystem carbon exchange.

light ($R_L$) could result in overestimations in both ER and GPP. Our paper addresses the measurement and partitioning of NEE into different fluxes of carbon dioxide, and suggests how $R_L$ should be evaluated and incorporated into models of ecosystem carbon cycling.

**Net ecosystem exchange and its measurement**

The difference between the fluxes of photosynthesis and respiration at the ecosystem scale is represented as NEE. NEE is measured directly and serves as the basis for the calculation of GPP, where $GPP = NEE - ER$. ER refers to ecosystem respiration, the combined fluxes of autotrophic respiration ($R_A$, the respiration of leaves, stems and roots) and heterotrophic respiration ($R_H$) from soil microorganisms, fungi, and the miniscule signal from any animals in the footprint. The resulting value for NEE can be either positive or negative, denoting the measured ecosystem as a carbon source or a carbon sink, respectively. NEE can be measured by eddy covariance techniques or by smaller chamber measurements. Eddy covariance (EC) provides direct, continuous measurements of CO$_2$ fluxes between the terrestrial ecosystem and the atmosphere by measuring the covariance between changes in wind velocity and CO$_2$ mixing ratio (Baldocchi 2003, Baldocchi 2008), and allows for seasonal, annual and multi-year exchange estimates of NEE (Baldocchi 2003). However, EC requires specific physical and environmental conditions for accurate measurement, including flat terrain and relatively large and uniform vegetation distribution within the tower footprint (Baldocchi 2003, Finnigan *et al.* 2003). When these conditions are not met, bias can accrue in the data, causing inaccuracies that must be considered and corrected (Baldocchi 2003). Over long periods of time, intermittent technical issues may create gaps in the data, but these can be filled using statistical and empirical models (Falge *et al.* 2001, Moffat *et al.* 2007, Ruppert *et al.* 2006).
Due to the large scale, continuous, non-destructive, and accurate measurements, EC towers number in the hundreds across the globe and are often integrated into cross-site networks at the regional, continental and global scale (Baldocchi 2008). The chamber method determines NEE and ER of the enclosed area by employing an infrared gas analyzer to measure the CO$_2$ concentration within the chamber. Clear plastic chambers are used for NEE measurements, and then darkened to measure ER. Subtracting ER from NEE can then estimate photosynthesis within the chamber footprint (Griffis et al. 2000). From these estimates, values for NEE can be scaled upward when leaf area index (LAI) is known. The chamber method can be labor intensive, but allows for true replicates unlike EC, where there is often only a single tower at each measurement site. For both methods, difficulty lies in the interpretation of the CO$_2$ flux values. NEE measurements must be partitioned into the two major fluxes. As GPP is calculated in both cases using ER estimates, an accurate measure of respiration is crucial to understanding the whole system.

**Respiration in NEE models**

Respiration in plants, unlike photosynthesis occurs in all living cells of all organisms at all times. Due to the complications of measuring foliar respiration in daylight, ER is usually only measured at night or in darkened chambers. ER measurement at night by EC methods can be error prone and lead to inaccurate estimates due to the suppression of turbulence at night when friction velocity is too low (Goulden et al. 1996). Daytime measurements of ER using darkened chambers can often lead to higher than expected estimates when scaled to the ecosystem level (Bolstad et al. 2004, Lavigne et al. 1997, Law et al. 1999, Wohlfahrt et al. 2005a), potentially due to transient increases in foliar respiration that often occur when
illuminated plants are exposed to darkness (Atkin et al. 2000, Atkin et al. 1998, Barbour et al. 2011, Gilmanov et al. 2007, Xue et al. 1996). Given these issues, extensive study has gone into modeling ER to obtain indirect estimates of CO$_2$ efflux based on environmental parameters. To overcome the bias introduced by the low turbulence, some models estimate ER by making ecosystem-scale light response curves (Gilmanov et al. 2007, Lasslop et al. 2010, Wohlfahrt et al. 2005a). Using the corresponding irradiance and NEE measures, a hyperbolic curve is fitted to describe the relationship between CO$_2$ flux and light. From these data, the fitted curve can be extrapolated back to the $y$-intercept to estimate the CO$_2$ efflux in the absence of light (Falge et al. 2001, Gilmanov et al. 2007, Griffis et al. 2003, Wohlfahrt et al. 2005a). To create this curve, many values of NEE from a wide span of irradiance are required. However, as these light levels correspond to different times of day or through a season, they must be standardized to control for the temperature response of ER. Reichstein et al. (2005) provides a thorough comparison of both aforementioned methods highlighting their advantages and drawbacks.

NEE models are simplified in terms of the assumptions about the temperature dependence of autotrophic respiration. Respiratory Q$_{10}$ values are likely nonlinear, and using fixed values could lead to over- or under-estimation of ER over longer time scales (Davidson et al. 2006, Tjoelker et al. 2001, Xu et al. 2007). Similarly, temperatures experienced during the day are warmer than experienced at night and represent a portion of the temperature response curve that do not correspond to night ER fluxes (Tjoelker et al. 2001). In addition, thermal acclimation of foliar respiration observed in plants complicates modeled temperature responses of vegetation (Atkin et al. 2008, Atkin et al. 2005, Atkin & Tjoelker 2003).

Another area of oversight in NEE models is the failure to incorporate the light inhibition of
The Kok effect

In the mid-20th century, Bessel Kok, using algal suspensions and aquatic plants, measured carbon assimilation as a response of light intensity and found a “sharp bend” at low light intensity, creating two distinct linear parts of the curve (Kok 1948, Kok 1949, Kok 1956). The two linear sections yield different intercepts on the y-axis (Fig. 1), which Kok interpreted to reveal the inhibitory effect of light and photochemical processes on respiration. The degree of this effect has been measured in many species through various techniques, and is reported to vary widely in degree of inhibition of mitochondrial respiration in the light (Atkin et al. 2000, Atkin et al. 1998, Atkin et al. 1997, Ayub et al. 2011, Brooks & Farquhar 1985, Crous et al. 2012, Heskel et al. 2012, Hurry et al. 2005, Ishii et al. 1979, Kirschbaum & Farquhar 1987, Kromer 1995, McCashin et al. 1988, Shapiro et al. 2004, Villar et al. 1994, Villar et al. 1995, Wang et al. 2001).

Research over the past half-century supports this inhibitory effect of light (Ishii & Murata 1978, Ishii et al. 1979, Sharp et al. 1984) and has identified a number of processes responsible for this phenomenon (Table 1). Multiple cellular pathways link photosynthesis and respiration directly and indirectly (Cournac et al. 2002, Hoefnagel et al. 1998, Kok 1949, Kromer 1995, Raghavendra et al. 1994, Riazunnisa et al. 2008), and many of these pathways serve as feedbacks to maintain efficient energy metabolism and avoid over-reduction or the
accumulation of reactive oxygen species that can damage the cell (Forti 2008, Noguchi & Yoshida 2008, Raghavendra et al. 1994, Saradadevi & Raghavendra 1992). However, as a result of these overlapping processes, respiration rates are controlled and inhibited by light through gene regulation and associated enzyme and substrate concentrations (Hoefnagel et al. 1998, Rasmusson & Escobar 2007). Pyruvate dehydrogenase and malic enzyme, precursors to the tri-carboxylic acid (TCA) cycle, are both light inhibited (Budde & Randall 1990, Hill & Bryce 1992, Tovar-Méndez et al. 2003). Light is also linked to the reduction of glycolysis and reorganization of the TCA cycle (Tcherkez et al. 2008, Tcherkez et al. 2005, Tcherkez et al. 2012). As both photosynthesis and respiration produce energy in the form of adenosine triphosphate, this redundancy is thought to control $R_L$. While the ratio of cytosolic adenosine triphosphate to adenosine diphosphate (ATP:ADP) is related to the degree of inhibition of respiration, this is found to be true only at high values (Dry & Wiskich 1982, Peltier & Thibault 1985).

Photorespiration, the oxygenation of rubilose-1,5-bisphosphate in the light, can be associated with the down-regulation of precursors to the TCA cycle and correlated with the degree of light inhibition of respiration (Budde & Randall 1990, Gemel & Randall 1992, Tcherkez et al. 2005, Tovar-Méndez et al. 2003). However, rates of respiration in the light may increase under increasing photorespiration, reflecting the demand for TCA cycle carbon skeletons associated with amino transfer reactions in the peroxisome (Griffin & Turnbull 2013, Tcherkez et al. 2008). Previous studies show that these processes may be compensatory, and the ratio of their rates can be sensitive to environmental factors such as ambient CO$_2$ concentration, irradiance, and temperature (Hurry et al. 2005, Hurry et al. 1996, Leegood et al. 1995, Pärnik et al. 2007, Pärnik & Keerberg 1995, Tcherkez et al. 2008).
Photorespiration occurs concurrently with $R_l$ and it can confound measurement using gas exchange techniques as both processes consume oxygen ($O_2$) and release $CO_2$.

Refixation, which occurs when the carbon released via mitochondrial respiration is reintegrated into photosynthetic processes, and thus not released into the atmosphere, can create a reduction in carbon efflux via respiration. Pinelli and Loreto (2003) found that respiration in the light was inversely related to photosynthetic rate, suggesting the refixation of emitted carbon (Loreto et al. 2001, Pinelli & Loreto 2003). At elevated $CO_2$, the respiratory $CO_2$ release in the light was lower than in plants exposed to ambient and low $CO_2$ levels, suggesting that optimal photosynthetic conditions of high $CO_2$ led to increased rates of intercellular $CO_2$ refixation and thus less efflux from the leaf to the atmosphere (Busch et al. 2012, Loreto et al. 2001, Pinelli & Loreto 2003). However, $^{14}C$ labeling experiments have shown that even when taking into account refixation, there is still true inhibition of the TCA cycle (Pärnik et al. 2007).

In addition to the cellular controls of the light inhibition of respiration, multiple studies identified environmental influences on the degree of this effect. Elevated atmospheric $CO_2$ growth conditions can increase respiratory carbon loss in the light, and this may be further enhanced under higher measurement temperatures (Shapiro et al. 2004, Wang et al. 2001), and modified by seasonal timing and exposure to drought (Ayub et al. 2011, Crous et al. 2012). Growth under warmed conditions can affect the degree of inhibition of respiration across species (Heskel et al. 2013), and the warming effect can be further mediated by light conditions (Zaragoza-Castells et al. 2007) and the measurement temperature (Atkin et al. 2006, Ayub et al. 2011). Further, increased soil nutrient availability can relax the degree of inhibition of respiration in the light in multiple field-grown arctic (Heskel et al. 2012, Heskel
et al. 2013) and rainforest species (Atkin et al. 2013), and in lab-grown Xanthium strumarium (Shapiro et al. 2004). Knowing these trends, the environmental sensitivity of the light inhibition of respiration needs to be further investigated to evaluate potential cross-taxa patterns that may inform larger-scale predictive carbon models.

Analytical models may also help elucidate the behavior of the biochemical mechanisms underlying the inhibition of respiration in the light. Buckley and Adams (2011), using a model based around flux-balance equations for cellular adenylate and reductant, found the suppression of respiration in light to be highly variable and controlled predominantly by photosynthetic ATP:ADP. This model supported findings from empirical study, mainly the inverse relationship of respiratory inhibition in the light with energy demand (Buckley & Adams 2011). This and hopefully additional future analytical models will expand the study of light inhibition of respiration through their theoretical insights. While plant biochemists and physiologists continue to reveal the direct and indirect causes of this inhibition at the cell level, ecophysiologists, ecosystem ecologists, and modelers can move forward in measuring the degree of this inhibition at different scales across ecosystems.

**Measuring respiration in the light**

Multiple $O_2$ and $CO_2$ fluxes occur concurrently in the light (Table 2), complicating direct measurement of respiration. However, methods have been developed in order to obtain estimates of $R_L$ indirectly. These approaches vary in their methodologies, from the use of stable isotopes (Pinelli & Loreto 2003, Turpin et al. 1990, Weger et al. 1988), to radiocarbon (Hurry et al. 1996, McCashin et al. 1988, Pärnik & Keerberg 1995) and gas exchange (Brooks & Farquhar 1985, Kok 1948, Peisker & Apel 2001, Villar et al. 1994). Stable
isotopes and radiocarbon techniques can be useful for the measurement of $R_L$, as they can determine pathway-specific rates of fluxes. While isotopic methods can reveal intricacies of metabolic pathways, they are not practical for larger scale observations that would be necessary for scaling up to the ecosystem level. For this reason, we will not consider these techniques here.

There are three primary methods for the detection of the Kok effect at the leaf level using gas exchange techniques: the Laisk method (Brooks & Farquhar 1985, Laisk 1977, Villar et al. 1995), the Peisker method (Peisker & Apel 2001), and the Kok method (Kok 1948, Sharp et al. 1984), though new methods are being developed, if not widely applied (Yin et al. 2011). Both the Laisk and Peisker methods utilize intercellular CO$_2$ concentration response ($A$-$c_i$) curves to estimate $R_L$. The Laisk method estimates the rate of $R_L$ from the intersection of three $A$-$c_i$ curves measured at different light levels. The intercellular CO$_2$ concentration at this point ($c^*$) indicates where CO$_2$ assimilation is equal to the negative value of $R_L$ ($A = -R_L$). The Peisker method estimates $R_L$ and $c^*$ through linear regression of the CO$_2$ compensation concentration ($\Gamma$) and the product of the respiration rate in the dark ($R_D$) and the intercellular resistance for CO$_2$ fixation. Where the Laisk method assumes the degree of inhibition is independent of irradiance, the Peisker method assumes the degree of inhibition to be independent of $R_D$ and photosynthetic performance (Peisker & Apel 2001).

The Kok method estimates $R_L$ from CO$_2$ exchange values collected via infrared gas analysis within a cuvette when leaf material is exposed to decreasing light levels. Unlike the Peisker and Laisk method, the Kok method measures CO$_2$ exchange as a response of light, not intercellular CO$_2$ concentration. In the field, this aspect is highly important, as large differentials in CO$_2$ concentration between the gas exchange chamber and ambient air can
At low light, or when values of photosynthetically active radiation (PAR) are less than 100 µmol m$^{-2}$ s$^{-2}$, CO$_2$ uptake slows and eventually reaches the light compensation point (LCP). The LCP represents the PAR value where CO$_2$ efflux from respiration is equal to CO$_2$ consumption from photosynthesis. Around the LCP a breakpoint in the linear trend of CO$_2$ concentration occurs (Fig. 1). Extrapolating a line to the y-axis from the points above this breakpoint will yield a value assumed to be the amount of CO$_2$ respired in the light, whereas the y-intercept derived from the line created from the points below this breakpoint result in the dark respiration value (Fig. 1). Using these two values, the degree of inhibition of respiration by light is calculable, where inhibition = 1 - $R_L / R_D$.

Of the gas exchange methods, the Kok method is the most practical protocol for multiple field-based measurements and allows for relatively simple analysis – both of these are required for broad-scale ecological surveys. Also, for scaling reasons, the Kok method is the only of the gas exchange methods that could easily correspond with eddy covariance methods, as light and CO$_2$ flux are the only required parameters.

**Integrating $R_L$ into NEE estimates**

FLUXNET, a network of eddy covariance towers, approximated the overestimation of GPP by the neglect of the inhibition of respiration to be no more than 15%. These coarse estimations of $R_L$ attempt to incorporate a physiological phenomenon whose underlying mechanisms are intricate and not well understood.

More involved integrations of the $R_L$ into NEE estimation, like those by Wohlfahrt et al. (2005) and Bruhn (2011) may yield more accurate descriptions of ecosystem carbon cycling. Wohlfahrt et al. (2005b) applied the light inhibition of $R$ to a model based on EC flux measurements based in the Austrian Alps to estimate ecosystem GPP. Two estimates of the degree of inhibition were put into the model – 50% and 85% - corresponding to low and high light levels, respectively. The results yielded an 11-17% reduction in estimated GPP compared to models that did not incorporate the light inhibition of respiration (Wohlfahrt et al. 2005b). This study acknowledges that the degree of overestimation of GPP by neglecting this inhibitory effect is highly dependent on ecosystem attributes including the ratio of $R_H$ to $R_A$ and total leaf area in the measurement location (Wohlfahrt et al. 2005b).

Bruhn et al. (2011) scaled $R_L$ to the ecosystem level by applying the leaf-level Kok method to NEE estimates measured through EC. CO$_2$ flux values were drawn from afternoon measurements where PAR was greater than the ecosystem LCP, but less than 550 µmol m$^{-2}$ s$^{-1}$ (Bruhn et al. 2011). Similar to foliar measurements, a line is fitted to these values and extrapolated to the y-axis to determine the estimate of ecosystem respiration in the light (ER$_L$). Ecosystem respiration in the dark (ER$_D$) was estimated using nighttime measurements of ER that were corrected to a constant temperature so they may be compared to ER$_L$ values (Bruhn et al. 2011). While noting both methods of estimating nighttime respiration (Falge et al. 2002, Lasslop et al. 2010), Bruhn et al. (2011) use nighttime ER estimates without
mention of the potential error caused by low turbulence (Goulden et al. 1996). Effects of other sources of respiration (e.g. stem, root, soil) and light attenuation through the canopy were accounted for, resulting in a 52% and 82% inhibition of respiration when scaled to the canopy-level (Bruhn et al. 2011). Similarly, the authors note the environmental variability that occurs due to phenology and changes in soil moisture and canopy composition over longer time periods, and for this reason, warn against using blanket inhibition estimates (Bruhn et al. 2011). As these studies employ no new collection methodologies and only require new data analysis, further application should be pursued to evaluate potential specific environmental controls on light inhibition.

In addition to EC, a combined approach using stable isotope applications may reveal new information on ecosystem carbon release from leaves in the light. Foliar respiratory CO$_2$ release in the light carries a different isotopic signature than that released in the dark; day respiration produces $^{13}$C-depleted CO$_2$ likely due to the fractionation against $^{13}$C by both pyruvate dehydrogenase and the TCA cycle, which may be further enhanced under light inhibition of those processes (Hurry et al. 2005, Tcherkez et al. 2008, Tcherkez et al. 2005). Tcherkez et al. (2010) found a slight $^{13}$C depletion in CO$_2$ respired from leaves in the light compared to the $^{13}$C enriched respiratory release in the dark that could be identified at the mesocosm scale where it corresponded with isotopic fractionation measured in fluxes from a canopy of sunflower leaves in a growth chamber (Tcherkez et al. 2010). A logical continuation in terms of experimental application would be to test if the depleted $^{13}$C signal of foliar respiration in the light could be detected in a less controlled environment, similar to the scaling of the enriched $^{13}$C signal produced by light enhanced dark respiration from leaf to ecosystem at the leaf-level (Barbour et al. 2011). Though more experimentally intensive than
EC measurement, stable isotope applications may allow for in situ direct quantification of foliar $R_L$ in multiple species compared to the estimation of $ER_L$ via EC, which cannot easily partition plant and soil sources of CO$_2$.

The studies above provide insight into the potential for the light inhibition of foliar respiration to be scaled and applied to the ecosystem level. However, it is necessary to realize that what holds true at the leaf level may not at the ecosystem level; and an aggregate estimate of the degree of light inhibition of respiration of an ecosystem may not be easily scaled. Future research should consider the scaling properties of respiration and how environmental and species influences on the variation in light inhibition can be reconciled within and across different systems.

**Implications and Conclusions**

Neglecting to include the light inhibition of respiration can lead to overestimations of both GPP and ER (Amthor & Baldocchi 2001, Bruhn et al. 2011, Janssens et al. 2001, Morgenstern et al. 2004, Wohlfahrt et al. 2005b); as GPP is derived from ER, inaccurate assessments of ER will confound GPP. Including $R_L$ into estimations for daytime ER will lead to more realistic approximations of carbon fluxes. Environmental variation will likely influence the degree to which the Kok effect impacts GPP estimates (Wohlfahrt et al. 2005b). For instance, in ecosystems that are mainly evergreen and can assimilate carbon year round, the $R_L$ will have a larger influence on GPP estimates than ecosystems that only bear leaves for a portion of the year (Wohlfahrt et al. 2005b). This effect would also hold true in ecosystems that have a high $R_A : R_H$ ratio, where ecosystem CO$_2$ efflux is more controlled by leaf respiration (Lohila et al. 2003, Wohlfahrt et al. 2005b). Inversely, for ecosystems with low
leaf area index and where ER is dominated by soil respiration, the influence of the light inhibition of respiration on GPP may be minimal (Bolstad et al. 2004, Janssens et al. 2001, Lavigne et al. 1997, Law et al. 1999, Wohlfahrt et al. 2005b). In stands with high leaf area, self shading may limit this inhibitory effect for much of the canopy, dampening its effect on GPP (Wohlfahrt et al. 2005b).

For accurate estimates of both GPP and ER, the light inhibition of respiration must be integrated into evaluations of NEE. The Kok effect, detectable at the leaf level and responsible for varying degrees of respiratory inhibition, could have large implications for ecosystem scale carbon fluxes. Its measurement and application to the ecosystem scale will require new analysis and interpretation of eddy covariance measurements, along with the physiological ‘ground-truthing’ of foliar and chamber measurements at the individual and community levels across different environmental conditions.
### Table 1. Identified causes of light inhibition of mitochondrial respiration

Tabled mechanisms associated with respiration that are down regulated in the light, causing an inhibitory effect. Locations of these processes and enzymes include the mitochondria (M), cytosol (C), chloroplast (CP), and peroxiosome (P).

<table>
<thead>
<tr>
<th>Mechanism</th>
<th>Process</th>
<th>Location</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malic enzyme</td>
<td>Oxidation of malate in the TCA cycle</td>
<td>M</td>
<td>Hill and Bryce, 1992.</td>
</tr>
</tbody>
</table>

* Refixation does not directly inhibit respiratory processes, but can produce an observable “inhibitory” effect due to the reduced CO₂ efflux from the leaf.
Table 2. Simultaneous CO₂ and O₂ fluxes that occur in the light that can complicate direct measurement of $R_L$.

<table>
<thead>
<tr>
<th>Process</th>
<th>Location</th>
<th>Consumes O₂</th>
<th>Releases CO₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mitochondrial respiration</td>
<td>Mitochondria</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Photorespiration</td>
<td>Chloroplast stroma, peroxiosome, mitochondria</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Chlororespiration</td>
<td>Thylakoid membrane</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Mehler Reaction</td>
<td>Thylakoid membrane</td>
<td>X</td>
<td></td>
</tr>
</tbody>
</table>
**Figure 1.** A visualization of a low-light CO$_2$ assimilation curve depicting the Kok effect. At low PAR levels (below 100 µmol m$^{-2}$ s$^{-1}$), a break in the linear light response curve occurs around the light compensation point. Points above the breakpoint (unshaded) are used to extrapolate a line to the y-axis will yield the $R_L$ estimate, whereas the measured data point when PAR = 0 will yield the $R_D$ estimate.
CHAPTER EIGHT

Synthesis

The preceding chapters present new information on the environmental controls of foliar respiration in the rapidly changing Arctic tundra. Prior to the studies included in this dissertation, little was known about how leaf-level respiration varies across tundra species and responds to current and predicted change associated with warming. These chapters reveal trends in carbon cycling in ecologically important species that will help in the future refinement of terrestrial carbon models in this warming-modified, carbon-rich setting. This final chapter serves to highlight the main findings of the presented studies, evaluate their influence on future research, and propose future directions.

The empirical research conducted in this dissertation represents an exhaustive examination of foliar CO$_2$ exchange in common and abundant tundra species under multiple environmental conditions. Chapter Three (Heskel et al. 2012), looks closely at two dominant tundra species, B. nana and E. vaginatum, grown under increasing levels of soil nitrogen and phosphorus to determine if photosynthesis, respiration, and related organelles and leaf traits respond linearly to the historically limited nutrients. The decoupled responses suggest a greater sensitivity to nitrogen and phosphorus in respiration than photosynthesis that may result in greater carbon loss in a warmer climate. This study is the first to characterize the response of mitochondria and chloroplast size and density in these or any arctic species, showing the highly plastic response of sub-cellular organization across species that underlies carbon cycling under different growth conditions. Finally, the inhibition of respiration by light
was lowest in leaves under high fertilization, indicating a relaxed control under increased energy demand.

Chapter Four (Heskel et al. 2013) continued to examine the impact of manipulated growth conditions, though on a much longer timescale. This study relates community composition to foliar physiology through a close examination of carbon gain efficiency in multiple species under fertilization and warming. The foliar carbon cycling responses in *B. nana* differ despite community composition dominance; warming is associated with higher rates of carbon efficiency, whereas little physiological change is seen under fertilization. Compared to the previous chapter, the lack of large affect of additional nitrogen and phosphorus suggests a treatment duration effect that must be considered when comparing across years, sites, and studies. While helpful in setting ranges of responses, the treatments used in this study (especially in the fertilization plots) may exaggerate the future predicated response. For this reason, it is important to couple these measurements with those made in the preceding chapter that provided a more subtle, graded approach to altered soil environment.

The first two data-based studies do not control for the timing of measurement with respect to the short growing season of the Arctic tundra. Chapter Five focuses on the intra-seasonal variation in foliar gas exchange under warming and ambient growth conditions to determine developmental, seasonal, and long- and short-term temperature influences in *B. nana* and *E. vaginatum*. Species effects drove most of the differences found in this study, however, warming acclimation seemed to influence respiration across both species, lowering rates across the season. Warming treatment had been applied for nearly 30 years, so long-term thermal acclimation found in these leaves is a likely response for tundra under current and future warming. The highly variable temperature and precipitation of the tundra during the
growing season may limit short-term thermal acclimation, as conditions vary widely within days. Perhaps the most interesting aspect of this study found a significant trend in the behavior of light inhibition of respiration, decreasing over the season, which is likely related to energy demand related to leaf development.

The increasing community dominance of woody shrub species inspired the final empirical study (Ch. 6), which evaluates patterns of gas exchange and leaf traits through a shrub canopy. The relatively high leaf area index of the measured shrub canopies creates micro-environmental variation for leaves of *B. nana* and *S. pulchra*. Despite some significant differences in physical leaf traits, physiological measurements do not vary greatly with leaf area index. However, this study did not examine extreme canopy heights, and thresholds of canopy cover and intra-canopy variation may likely lead to a significant variation in canopy distribution of nitrogen and other resources. Finally, a focused review (Chapter 7) on the light inhibition of respiration and its incorporation into net ecosystem exchange argues for a more accurate estimation of ecosystem carbon fluxes.

The encompassing patterns and trends found in these studies portray respiration as an environmentally sensitive process that is likely to increase CO$_2$ efflux in a warmer, shrubbier, more nitrogen-rich tundra. This dissertation mainly focuses on the CO$_2$ of photosynthesis, respiration, and photorespiration, with special attention to the inhibition of respiration in the light given the extreme photoperiod of the Arctic growing season. Respiration, considering all species and environmental conditions, shows varying and often significant inhibition by light (Fig. 1), on average decreased by 37.4 ± 1.0 %. The inhibition of respiration by light in these samples does not relate in any linear predictable manner with foliar nitrogen or specific leaf area, nor do fluxes of maximal photosynthesis or respiration. This is highly important to note,
as leaf nitrogen and specific leaf area are easy to measure traits that are often used in predictive models as a substitute for direct measures of physiological processes; though they may not accurately portray the wide range of values. Neglecting the broad array of leaf physiological responses to the environment over simplifies complex mechanisms, especially when responses are nonlinear, (Ch. 4) or exhibit acclimation over time (Ch. 5-6).

Recent studies detail the many issues concerning the terrestrial carbon balance in the tundra – the landscape continues to become “shrubbier”, which has a host of implications (Myers-Smith et al. 2011a), tundra fires are likely to increase in frequency with warmer temperatures (Rocha et al. 2012), and permafrost continues to thaw, releasing unprecedented amounts of CO₂ (Jorgenson, Shur & Pullman 2006; Cory et al. 2013). Considering these changes, close examination of carbon release from plants and soils associated with these environmental alterations is needed.

While the data included in these chapters can help inform predictive models, future research should expand knowledge of carbon cycling at all scales. Promising new studies on

![Figure 1. Respiration in the light regressed against respiration in the dark; best linear fit (red), significantly lower than 1:1 line (black). Data points represent all species under all treatment and growth conditions.](image)
plant respiration in field settings is revealing new insights at the cellular and ecosystems levels. Studies from the Turnbull Lab at University of Canterbury integrate mass-spectrometry and protein analysis to differentiate between use of the alternative and cytochrome oxidase pathways under different environmental stresses and growth conditions in the field (in New Zealand in Searle et al. 2011; in Alaska in Kornfeld et al. 2012). The use of stable and radioactive isotopes also further refines how respiration is measured in the field, and can account for numerous fluxes, as demonstrated in tundra (Natali et al. 2011; Hicks-Pries, Schuur & Crummer 2013) and grasslands (Barbour et al. 2011), and individual species across plant functional groups (Priault, Wegener & Werner 2009). Though these methods are not used in the studies presented in this dissertation, their application in tundra will likely be meaningful in the quantification and modeling to individual component CO₂ fluxes.

In conclusion, the data in this dissertation fills a large gap in knowledge about foliar carbon fluxes in arctic Alaskan tundra. Prior to these studies, few (~10) published studies reported leaf-level fluxes of photosynthesis and respiration in these species, and fewer under multiple environmental treatments. The two literature reviews and four empirical studies presented here highlight the sensitivity of foliar carbon fluxes, and emphasize the likelihood of an altered carbon balance under change. Hopefully, these can inform models of carbon cycling and emphasize the need for direct foliar measurements of respiration in the light.
REFERENCES


exchange into gross primary productivity and ecosystem respiration using light response function analysis. *Agriculture, Ecosystems & Environment*, 121, 93-120.


