Early season respiration in *Betula nana nana* and *Eriophorium vaginatum*, two important tundra plant species

Danielle Bitterman
Department of Ecology, Evolution, and Environmental Biology
Columbia University

Mentor:
Kevin Griffin, Ph.D.
Department of Earth and Environmental Sciences
Columbia University

Adviser:
Matthew Palmer, Ph.D.
Department of Ecology, Evolution, and Environmental Biology
Columbia University

December 14, 2010

Abstract

The Arctic tundra has warmed faster than any other part of the earth in the past 50 years. The respiration rate of plants is directly related to temperature, and so warming may create a positive feedback loop where increased plant respiration releases more CO_2, trapping more heat. We studied how the respiration rates of two tundra species, a dwarf shrub, *Betula nana nana,* and a sedge, *Eriophorum vaginatum*, grown under control and greenhouse conditions, change during the first four weeks of the growing season. The basal respiration rate (R_{10}) and the temperature sensitivity of respiration (Q_{10}) were significantly different between species ($p<0.0001$ for R_{10} and Q_{10}), but not treatment ($p=0.77$ for R_{10} and $p=0.11$ for Q_{10}), and changed significantly throughout the experiment ($p<0.0001$ for R_{10} and Q_{10}). The foliar carbon:nitrogen ratio of *Betula* and *Eriophorum* also were also significantly different between species ($p<0.001$) and changed significantly throughout the experiment ($p<0.001$) but were not significantly affected by growth temperature ($p=0.22$). Overall, the respiration rate at 10°C over the first half of the growing season was significantly lower for *Betula* (14.7±0.7 μmol CO_2 g^{-1} s^{-1}) than for *Eriophorum* (24.±1.1 μmol CO_2 g^{-1} s^{-1}), regardless of growth temperature ($p<0.0001$ for species and $p=1.0$ for growth temperature). The lack of acclimation to greenhouse conditions and the variability of R_{10}, Q_{10}, and nutrient composition suggest that the respiration rates of these two species were driven by phenology, not temperature. Understanding the seasonal dynamics of respiration will allow for more accurate predictions of the fate of these species and carbon balance in the Arctic in a warmer climate.

Table of Contents

List of Figures

List of Tables

Introduction

The global average temperature is expected to rise 1.1-6.4 °C by the end of the 21st century due to increasing concentrations of greenhouse gases in the atmosphere (IPCC 2007). Climate change is of particular concern in the Arctic, which is warming faster than most other parts of the globe (Comiso, 2003; Kaufman et al., 2009). Historically, the Arctic tundra has been a net sink of carbon dioxide: it is estimated that photosynthesis in the Arctic has accumulated as much as one-third of the earth's global soil carbon, now stored in permafrost in Arctic tundra ecosystems (Gorham, 1991; Ping et al., 2008). However, warming may cause the ecosystem to become a net source of CO_2 via an increase in plant respiration, which would release the carbon stored deep in the permafrost (Oechel et al., 1993).

The rate of carbon storage and therefore the fate of Arctic ecosystems as a source or sink of carbon depends at least partially on the amount of carbon fixed by plant tissue through photosynthesis, and released through respiration. Prior to being stored in soil, carbon must be converted from the gaseous form in the atmosphere into solid organic matter through the process of photosynthesis. Conversely, plant respiration, which releases energy and gaseous carbon from organic matter, is a major source of CO_2 to the atmosphere; for example, 30-80% of daily photosynthetic gain is re-released into the atmosphere as CO_2 by respiration (Loveys et al., 2002). About 120 PgC yr^{-1} is sequestered from the atmosphere by photosynthesis, while about 60 PgC yr^{-1} is released into the atmosphere as CO_2 by plant respiration (Houghton, 2007). By comparison, only about 8.4±0.5 PgC is released by fossil fuels each year (Friedlingstein et al., 2010).

The rates of respiration and photosynthesis in plants are positively related to temperature, since biochemical processes proceed more rapidly at higher temperatures. As the Arctic continues to warm, the rates of the two processes will change, and the balance of uptake and release of CO_2 in plants may shift. If the rate of respiration increases relative to the rate of photosynthesis, the ecosystem may shift from a net sink to a net source of carbon.

The warming climate has already altered Arctic ecosystems. In Alaska's tundra, deciduous shrubs have been encroaching into higher latitudes, out-competing the tundra tussock-forming sedges traditionally found in the biome (Chapin et al., 1995). In order to accurately model climate change in the future, we must have a better understanding of how climate will alter the rates of photosynthesis and respiration in the dominant Arctic plant species (Sage et al, 2005; Atkin & Tjoelker, 2003).

Respiration is the process by which plants reduce the compounds synthesized during photosynthesis to yield energy for biosynthesis, cellular maintenance, and active transport in plants (Raven et al., 2007). Cellular respiration can be divided into two functional components: growth and maintenance (McCree, 1969; Amthor, 1984; Wullschlege et al., 1992). Growth respiration yields energy and carbon skeletons used to generate new biomass. Maintenance respiration takes place when energy is used for transport (phloem loading), to re-synthesize components of metabolic pathways, to maintain ion gradients, and to alter biosynthetic pathways to adapt to environmental stress (Amthor, 1984).

As most of the nitrogen in leaves is incorporated into enzymes that drive maintenance activity, nitrogen content can serve as a proxy for maintenance respiration in

plants (Lexander, 1970; Ryan, 1995; Reich et al.,1996). More active plants are expected to have higher nitrogen content and to respire more (Amthor, 1984). Foliar carbon content is also positively associated with rates of respiration. Non-structural carbohydrates (sugars and starches) are composed primarily of carbon, and are the substrates for glycolysis and mitochondrial respiration (Azcón-Bieto et al., 1983; Azcón-Bieto & Osmond, 1983; Tjoelker, 1999). Since respiration requires both enzymes and substrates, both of these can act as limiting factors in the biosynthetic pathway and thereby alter the respiration rate. Substrate availability determines respiration rate when carbohydrate content is limiting, and enzymatic capacity determines respiration rate when nitrogen in limiting (Noguchi & Terashima,1997, Vanderwef et al., 1993). Since foliar carbon and nitrogen content depends on species, phenological stage, and the nutrient content of the soil, these factors must be considered when predicting the respiratory capacity of a plant (Hooper & Vitousek, 1998, Lal et al., 2001).

Temperature is also an important determinant of respiration rate (Atkin et al., 2005). In the short-term, a rise in temperature increases the rate of respiration (R), releasing more CO_2 into the atmosphere (Bunce, 2007). However, several studies provide evidence of plant respiration rates acclimating to temperature (Larigauderie & Korner, 1995; Will, 2000; Atkin et al., 2005). During acclimation, the rate of increase of R with temperature is reduced in warm-acclimated plants and the rate of increase of R with temperature is increased in cold-acclimated plants in an attempt to achieve homeostasis, a state where similar respiration rates are exhibited across all temperatures (Figure 1, Mooney, 1963; Atkin & Tjoelker, 2003). Although complete homeostasis is not always achieved, even partial acclimation helps plants maintain stable carbon levels across a

range of temperatures. Without acclimation, plants will respire at a faster rate at ambient temperatures and could lose biomass if global warming continues. Acclimation must be taken into account to understand how changes in temperature will affect CO_2 flux into the atmosphere from plants, because ignoring this process may lead to an overestimation of the amount of CO_2 respired at elevated temperatures.

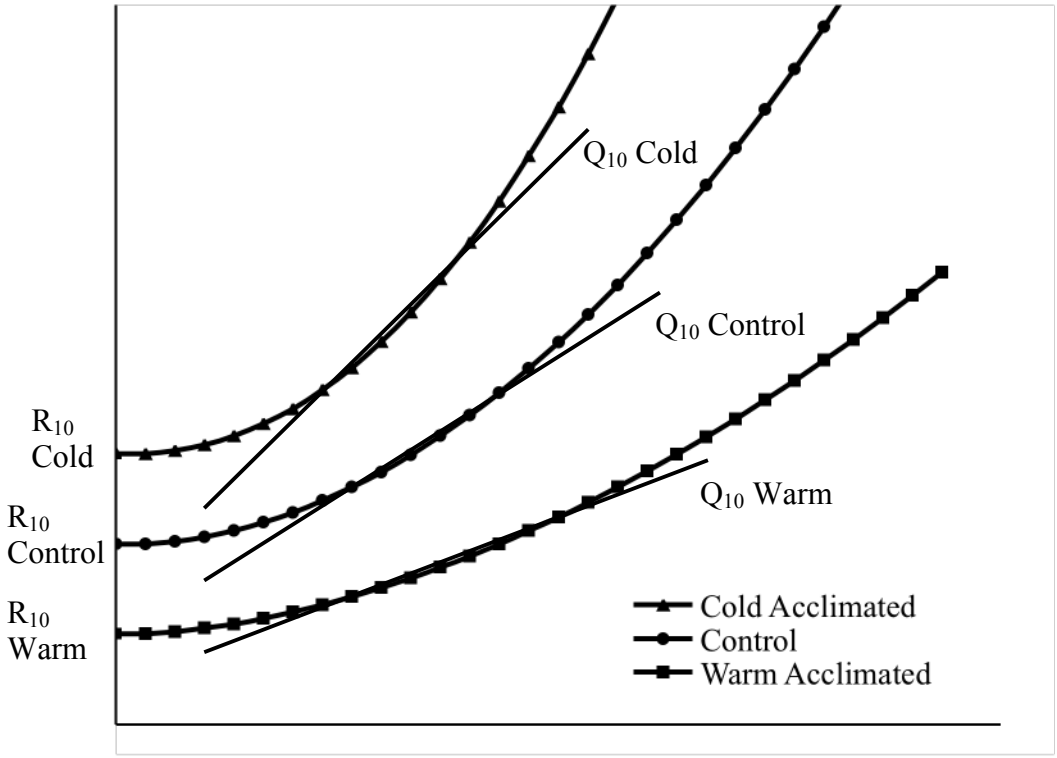

Figure 1. Conceptual figure of the respiration response to temperature. R_{10} is the respiration rate when temperature is 0°C. R_{10} is higher in cold acclimated plants and lower in warm acclimated plants. Q_{10} is the temperature sensitivity of the plant, and is the instantaneous slope of the curve of respiration vs. temperature. Cold acclimated plants have higher slopes than control plants at a given temperature, and so are more sensitive to increases in temperature. Warm acclimated plants have lower slopes than control plants at a given temperature, and so are less sensitive to increases in temperature.

The mechanisms underlying respiratory acclimation to temperature are not entirely understood. The nutrient content of leaves at different temperatures may account for changes in respiration rates. In moderately cold-acclimated plants, a build-up of substrate from photosynthesis due to decreased R may later lead to increased R when the

plant experiences short-term warming (Atkin et al., 2000; Covey-Crump et al., 2002). In warm-acclimated plants, conversely, increases in respiration may be minimized at moderately high temperatures as substrate becomes limiting due to the high R in these plants, since respiration reactions proceed more rapidly at higher temperatures. Additionally, it has been suggested that respiration becomes enzyme-limited at extremely high and low temperatures, conditions in which proteins become damaged and denatured (Covey-Crump et al., 2000). The extent of acclimation varies widely between species, and the physiological mechanisms controlling respiration acclimation to temperature are still poorly understood (Atkin & Tjoelker, 2003; Laurigauderie & Korner, 1995; Loveys et al., 2003).

Foliar respiration, dependent on nutrient content and temperature, may change by alterations in the basal respiration rate (R_{10}) or the sensitivity of plant respiration to changes in temperature (Q_{10}) (Figure 1). Strictly defined, Q_{10} is the proportional change in R when temperature is raised. Q_{10} is usually assumed to be equal to 2, but this value varies proportionally with measurement temperature (Loveys et al., 2003). The instantaneous Q_{10} is equal to the slope of the line tangent to the curve at a given temperature (Figure 1). It has been proposed that mature leaves acclimate their respiration through changes in Q_{10} only, while leaves alter their R_{10} based on the temperature at the time of initiation. (Atkin & Tjoelker, 2003).

A comprehensive understanding of plant respiration in the tundra ecosystem is necessary to predict the role this critical environment will play in the global carbon cycle as climate change continues. The short growing season in the Arctic tundra provides an

excellent opportunity to understand how the respiration rates of the tundra plants change throughout the entire growth period.

Previous studies at Toolik Lake, Alaska, an Arctic Long Term Ecological Research site, have found that that *Betula nana nana,* a common shrub species, respires less at warm temperatures than *Eriophorum vaginatum,* a common tussock-forming sedge (Greaves, 2009). Warm-grown *Betula* and *Eriophorum* also exhibited significant respiration acclimation to temperature (Griffin et al., 2009). Since the growing season in the Arctic is only about eight weeks, it is important for plant species to be able to efficiently use their carbon resources. I studied the extent of early-season respiratory acclimation to temperature of *Betula* and *Eriophorum* in both control and elevated-temperature conditions at Toolik Lake in order to develop a more nuanced understanding of the carbon use of these plants at ambient and elevated temperatures during the first half of the growing season. I hypothesized that a shift in species composition could be attributed in part to the ability of *Betula* to acclimate its respiration rates more rapidly to elevated temperatures at the beginning of the growing season than *Eriophorum*. A better understanding of the temporal patterns of respiration and the ability to acclimate to climate change in these dominant Arctic species is essential to predict the effects of future warming.

Methods

Sampling Site and Species Description

Samples were taken from tussock tundra and shrub vegetation in moist acidic tundra from the Toolik Lake LTER site in the foothills of the Brooks Range, Alaska (68°38'N, 149°43'W, elevation 720 m). The soil consists of a peaty organic layer, which is approximately 30-50 cm thick, over a silty mineral soil; permafrost underlies the soil. Experimental greenhouse and control treatments were established in June, 1989 at the site and are applied to vegetation plots annually in late May or early June. Temperature data were collected hourly by thermocouples mounted 3 meters above the soil in a control plot and 1 meter above the soil in a greenhouse plot.

Leaf Sampling

Leaves of *Eriophorum* and *Betula* were sampled on June 7-June 11, June 11-June 15, June 16-June 19, June 21-June 23, and June 24-June 29, the first half of the growing season. These sampling periods will be referred to using the date in the middle of each sampling period (e.g. June 9 for the first sampling period). Samples were taken from four plots of land, each containing a control and greenhouse treatment. Each sampling period, approximately seven *Eriophorum* tillers and two 10 cm long *Betula* shoots were sampled from both treatments in all plots. Collected shoots were immediately cut under water and transported back to the laboratory, where they were dark-acclimated for 20 minutes prior to gas analysis.

Gas Analysis

An instrument was built to measure the response of respiration to changing temperature. Leaves were placed in a darkened, temperature-controlled cuvette attached to a

circulating water bath. Dry air was passed through the cuvette at a rate of 0.45 L/hr and into an LI-800 infrared gas analyzer (LI-COR, Lincoln, Nebraska) which measured the flux of CO_2 out of the leaf as it was heated from 5°C to 35°C. The Q_{10} for each leaf from 5°C-35°C, the temperature range the plants normally experience in the wild, was modeled using the equation:

$$Q_{10} = (R_2 / R_1)^{[10(T_2 - T_1)]} \quad \text{(Equation 1)}$$

Where R_1 and R_2 are the rates of respiration at temperatures T_1 and T_2, respectively. The temperature response of respiration was modeled using a modified Arhennius equation that assumes that reaction rate increases exponentially with temperature: (Ryan, 1991; Tjoelker, 1999):

$$R = R_{10} \cdot e^{[(Q_{10}/g)(1/T_o - 1/T_a)]} \quad \text{(Equation 2)}$$

Where Q_{10} is the temperature sensitivity at a reference temperature, g is the ideal gas constant, 8.314 J mol^{-1} K^{-1}, T_o is the reference temperature, and T_a is the average ambient temperature on the day each leaf collected was collected. 10°C was used as the reference temperature in this model, as these plants often experience this temperature during the growing season. The average Q_{10} and R_{10} corresponding to each leaf's species, treatment, and the T_a for each leaf was used to the average R of each species and treatment for the entire first half of the growing season, as well as for each individual sampling period.

Carbon and Nitrogen Content

Samples were dried for 48 hours at 40°C after gas analysis and ground to measure carbon and nitrogen content. A 2400 CHN/O Analyzer (PerkinElmer, Waltham, Massachusetts) was used to measure carbon and nitrogen percent dry mass of the leaves for the June 9,

June 13, June 18, and June 26 sampling periods. Leaf material from June 22 was lost during processing.

Data Analysis

The R_{10} and Q_{10} of each leaf were calculated from the respiration vs. temperature curve obtained through gas analysis by fitting the data to Equation 2 using Sigmaplot (Version 8.0, SPSS Inc.). The respiration data were log transformed for a three-way ANOVA comparing R_{10} or Q_{10} across species, treatment, and period. Foliar carbon content, foliar nitrogen content, and foliar carbon:nitrogen ratio (C:N) were also compared across species, treatment, and period with a three-way ANOVA. A Tukey Multiple Comparisons of Means Analysis (95% family-wise confidence level) was performed on the R_{10} and Q_{10} at 10°C to compare the means across sampling periods. The average R at 10°C from for the entire experiment was compared across species and treatment with a two-way ANOVA. Linear regression analyses were used to correlate temperature with R_{10}, Q_{10}, and nutrients, and nutrients with R_{10} and Q_{10}. A three-way repeated measures ANOVA was used to compare R at 10°C at each sampling period across species, treatment, and sampling period; species and treatment were considered as between subject factors, and sampling period was treated as a within subject factor. Linear regressions and two-way ANOVA analyses were carried out on Microsoft Excel (2004). ANOVA and Tukey Multiple Comparisons of Means analyses for R_{10}, Q_{10}, and nutrients were carried out on R (Version 2.12, R Development Core Team 2010). Three-way repeated measures analyses were carried out on PASW Statistics 18 (Version 18.0.3, SPSS Inc.).

Results

There were no clear trends in the temperature at the Toolik LTER from June 7th to June 29th. The average ambient temperature was 13.2±0.6°C in the control treatments and 17.9±1.0°C in the greenhouse treatments, representing a 5±3°C difference in average ambient temperature (Figure 2).

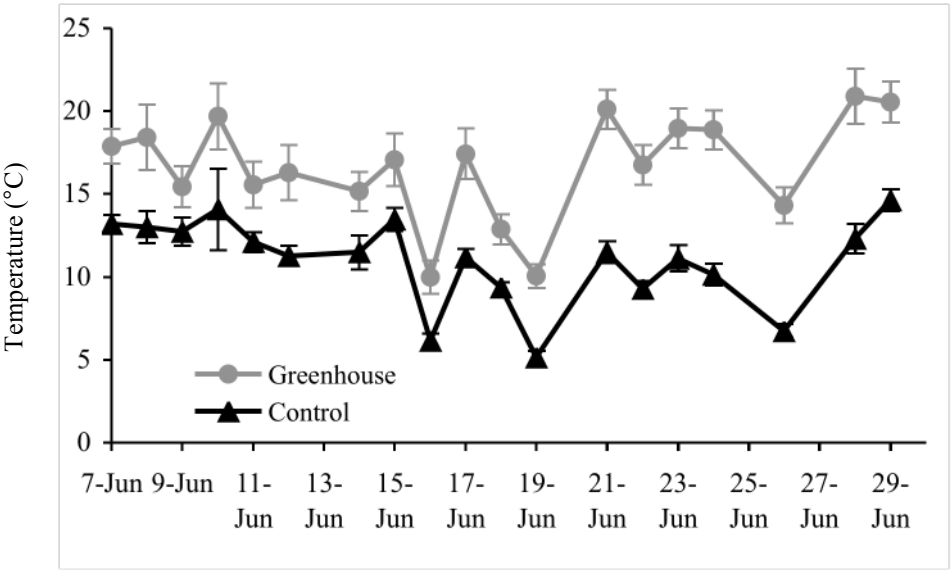

Figure 2. Average temperature on sampling days in the control (black triangles) and greenhouse (grey circles) treatments (mean±1 s.e.).

Although the temperature did not show a temporal trend, in the three-way ANOVA the respiratory responses of *Betula* and *Eriophorum* did change as the season progressed (Figures 3 and 5). R_{10} did not vary significantly with treatment, but did vary significantly with species and sampling period (Table 1). Most notably, the *Eriophorum* R_{10} dropped dramatically during the last sampling period (Figure 3). For both *Betula* treatments, the June 18 and June 26 sampling period were significantly different from one another, but not from the other sampling periods (Figure 4). Only the June 26 R_{10} of the *Eriophorum* treatments was significantly lower than the prior sampling periods (Figure

4). No correlation was found between R_{10} and average temperature on the day each leaf was sampled.

	R_{10}	Q_{10}	Foliar Carbon Content	Foliar Nitrogen Content	C:N	R at 10°C
Species	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.001**	**<0.001**
Sampling Period	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.01**
Treatment	0.77	0.11	0.61	0.19	0.22	0.08

Table 1. Results of three-way ANOVA for R_{10}, Q_{10}, foliar carbon content, foliar nitrogen content, and C:N ratio. Results of three-way repeated measures ANOVA for R at 10°C during each sampling period; sampling was considered as a within subject factor, and species and treatment were considered as between subject factors. Values shown are *p*. Bold indicates statistically significant results.

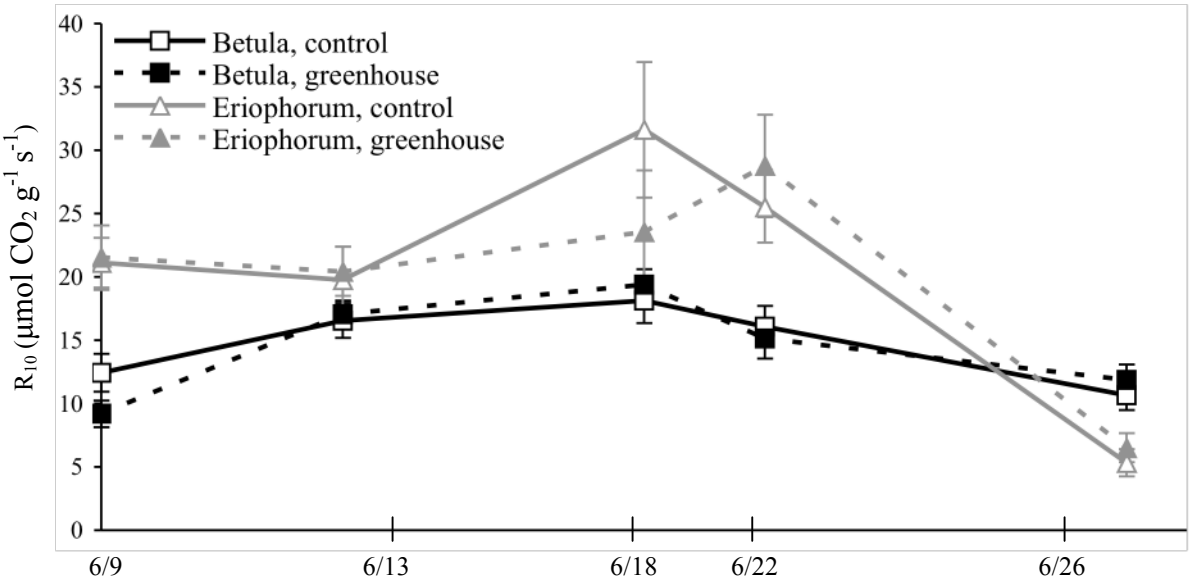

Figure 3. Average R_{10} of each species and treatment (mean±1 s.e., n=4) at each sampling period (*Betula*, control, black open square; *Betula*, greenhouse, black solid square; *Eriophorum*, control, grey open triangle; *Eriophorum*, greenhouse, grey solid triangle). Species (Three-way ANOVA, $p<0.0001$) and sampling period (Three-way ANOVA, $p<0.0001$), but not treatment (Three-way ANOVA, $p=0.77$) had a significant effect of R_{10}.

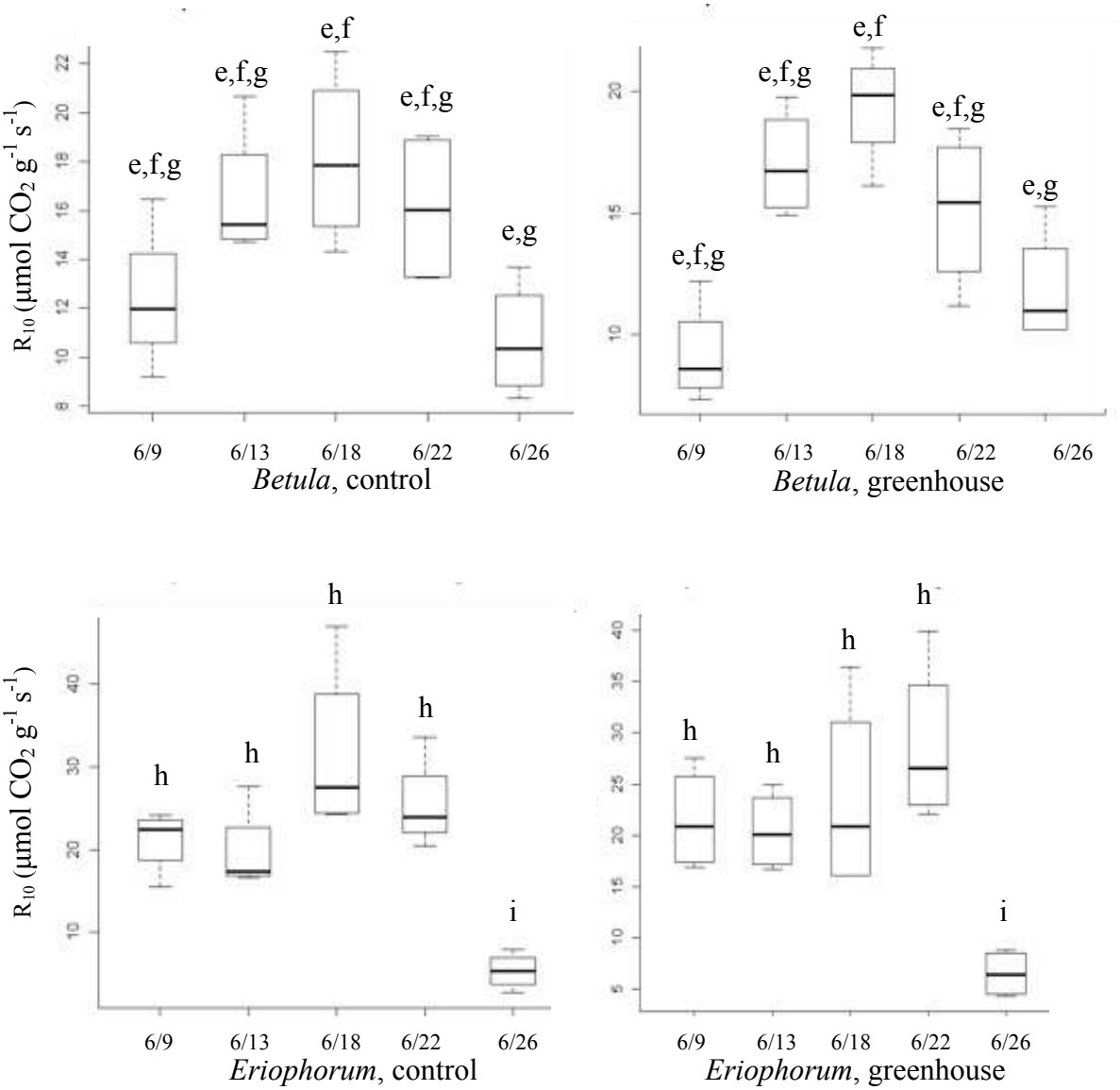

Figure 4. R_{10} boxplots across sampling periods (Tukey Multiple Comparisions of Means, 95% family-wise confidence level, n=4). There was no significant difference found between periods labeled with the same letter, but a significant difference was found between periods labeled with different letters. For both *Betula* treatments, the June 18 and June 26 sampling period were significantly different from one another, but not significantly different from the other sampling periods. The June 26 sampling period was significantly different from prior sampling periods in both *Eriophorum* treatments.

Similarly, the Q_{10} at 10°C of both species did not vary significantly with treatment, but did vary significantly with species and sampling period and (Table 1). *Betula* exhibited no significant difference in the mean Q_{10} at 10°C across all sampling periods, but the *Eriophorum* Q_{10} at 10°C was significantly higher during the June 26 sampling period than the prior sampling periods (Figures 5 and 6). Interestingly, the Q_{10} at 10°C for both *Betula* and *Eriophorum* exhibited a response opposite to R_{10}, decreasing with increasing R_{10} and increasing with decreasing R_{10}. No correlation was found between Q_{10} at 10°C and average temperature on the day each leaf was sampled.

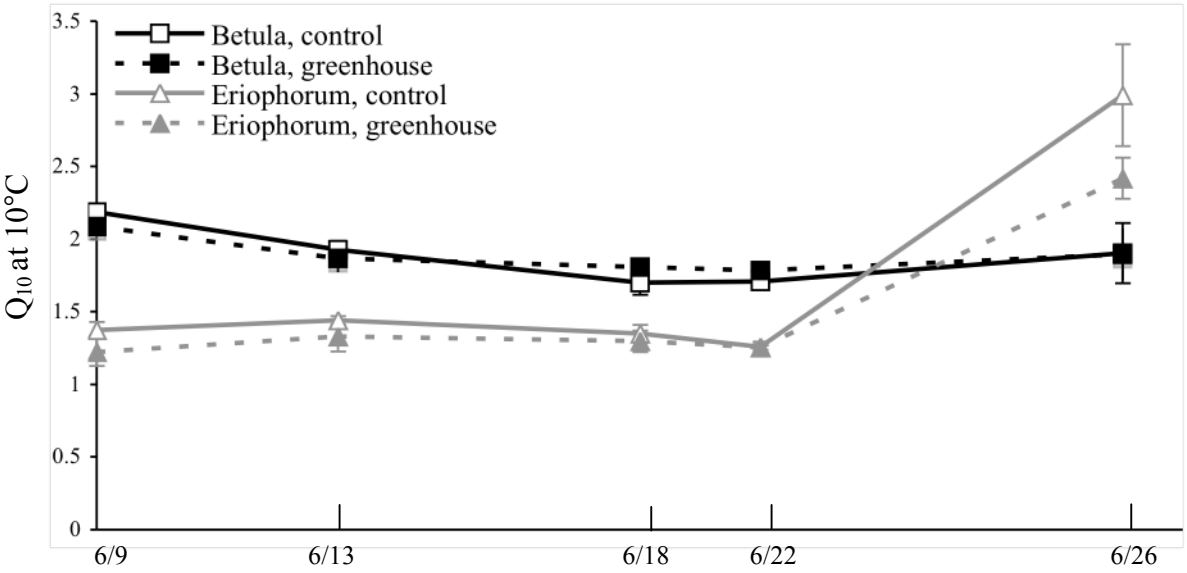

Figure 5. Average Q_{10} at 10°C of each species and treatment (mean±1 s.e., n=4) at each sampling period (*Betula*, control, black open square; *Betula*, greenhouse, black solid square; *Eriophorum*, control, grey open triangle; *Eriophorum*, greenhouse, grey solid triangle). Species (Three-way ANOVA, $p<0.0001$) and sampling period (Three-way ANOVA, $p<0.0001$), but not treatment (Three-way ANOVA, $p=0.11$) had a significant effect on Q_{10}. Note that some of the error bars are too small to see.

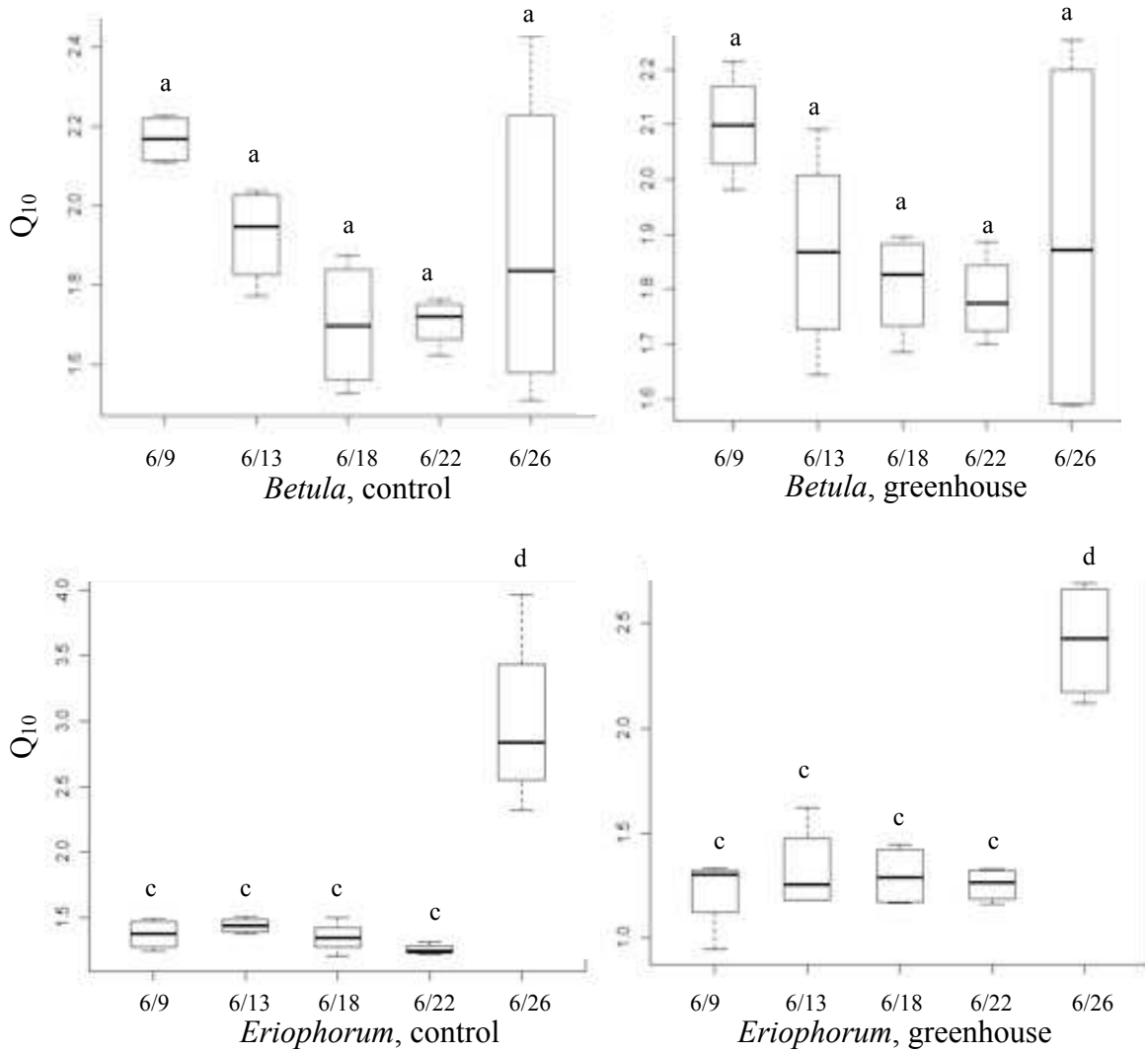

Figure 6. Q_{10} boxplots across period (Tukey Multiple Comparisions of Means, 95% family-wise confidence level). There was no significant difference found between periods labeled with the same letter, but a significant difference was found between periods labeled with different letters. No significant change was seen between sampling period in either *Betula* treatment. The June 26 sampling period was significantly different from prior sampling periods in both *Eriophorum* treatments.

Although R_{10} and Q_{10} varied for both species across the sampling periods, these differences were not great enough to drastically change the respiration response to temperature over the first half of the season. Except for the June 13 sampling period, the shapes of the respiration response to temperature curves are very similar, and are almost identical for the last three sampling periods (Figure 7). Interestingly, R of *Betula* is lower

than that of *Eriophorum* at lower temperatures, and the R of *Eriophorum* is lower at higher temperatures. In addition, except for the June 13 sampling period, the R of *Betula* appears to increase exponentially with temperature, while the R of *Eriophorum* increases linearly with temperature.

The average respiration rate at 10°C for each species and treatment was modeled for the first half of the growing season as well as for each individual sampling period, using the average Q_{10} and R_{10} from the sampling period in which each leaf was sampled and the average ambient growth temperature on the day of sampling (Figure 8). The seasonal pattern of R is similar to that of R_{10}. Sampling period and species, but not treatment, had a significant effect on R at 10°C (Table 1). The average respiration rate at 10°C over the first half of the growing season was significantly lower for *Betula* (14.7±0.7 μmol CO_2 g^{-1} s^{-1}) than for *Eriophorum* (24.±1.1 μmol CO_2 g^{-1} s^{-1}), regardless of growth temperature (Two-way ANOVA, $p<0.0001$ for species and $p=1.0$ for growth temperature). The R of *Betula* was lower on every individual sampling period except during the June 26 sampling period.

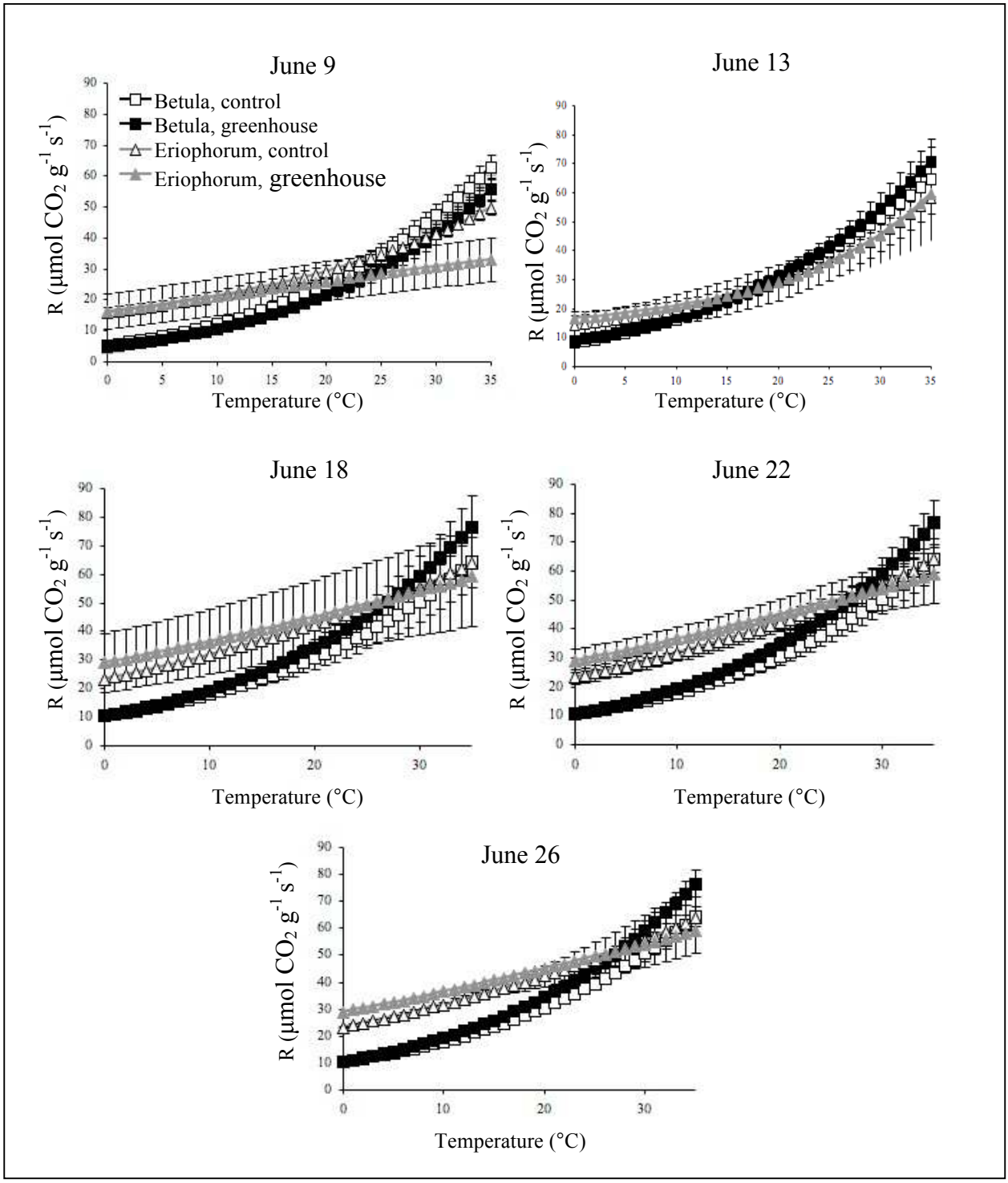

Figure 7. Respiration response to temperature curves for each sampling period (mean±1 s.e., n=4) (*Betula*, control, black open square; *Betula*, greenhouse, black solid square; *Eriophorum*, control, grey open triangle; *Eriophorum*, greenhouse, grey solid triangle).

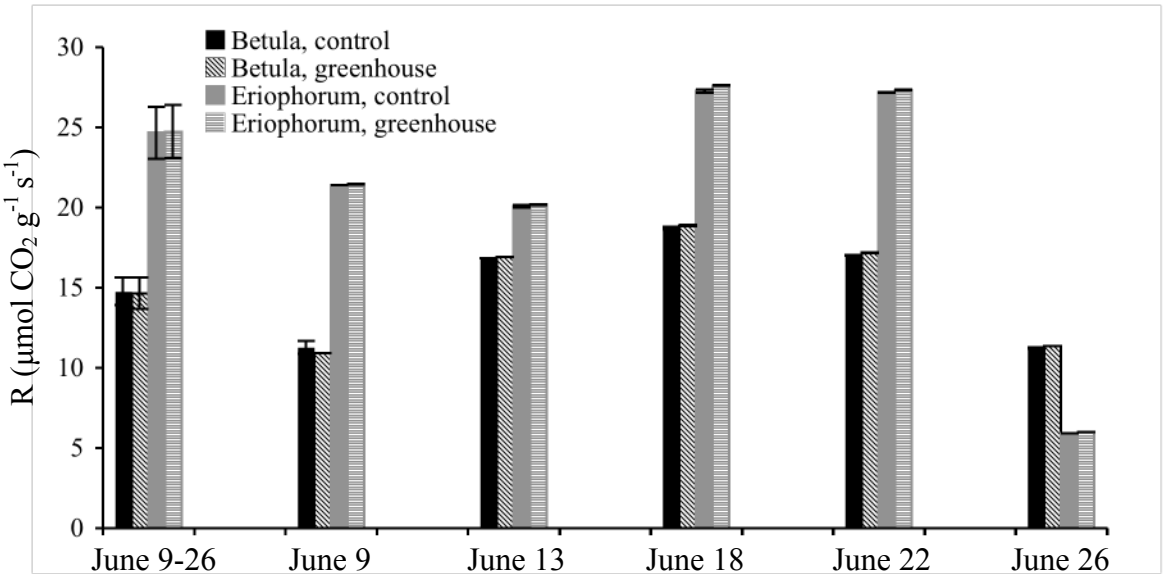

Figure 8. Average R at 10°C from June 9-June 26, and for each sampling period (mean±1 s.e., n=20 for the entire season and n=4 for each sampling period) for each species and treatment (*Betula.* control, black; *Betula*, greenhouse, black dashed; *Eriophorum*, control, grey; *Eriophorum*, greenhouse, grey striped). The R value from June 9-26 represents the average respiration rate at 10°C for each species and treatment over the first half the growing season. The R for each sampling period is the average respiration rate at 10°C for each species and treatment during each sampling period. The ambient temperature of each leaf was used to measure respiration. Note that some of the error bars are too small to see.

The foliar carbon content, nitrogen content, and C:N ratio all exhibited a significant species and sampling period effect, but no treatment effect (Table 1). The carbon content was higher for *Betula* than for *Eriophorum* during all sampling periods (Figure 9). The *Betula* carbon content started at 52.3±0.5% dry mass on June 9, and decreased to 48.2±0.5% dry mass on June 26. The *Eriophorum* carbon content was fairly constant, with 46.5±0.3% dry mass at the beginning of the season and 46.0±0.2% dry mass on June 26. In addition, the nitrogen content of *Betula* was higher than that of *Eriophorum* until June 26, when *Eriophorum* exhibited higher nitrogen content (Figure 10). The *Betula* nitrogen content decreased throughout the growing season, from 4.7±0.3% dry mass on June 9 to 2.4±0.1% dry mass on June 26, while *Eriophorum*'s

nitrogen content increased slightly from 2.5±0.1% dry mass on June 9 to 2.8±0.2% dry mass on June 26. The C:N ratio was higher for *Eriophorum* until June 26, when the C:N ratio of *Betula* was higher (Figure 11). The C:N ratio of *Eriophorum* decreased from 18.8±1.0 on June 9 to 14.0±1.7 on June 13, and then increased to 16.2±1.1 by June 26, while the C:N ratio of *Betula* increased steadily throughout the first half of the season, from 11.3±0.7 on June 9 to 19.9±1.1 on June 26. No significant relationship was found between nutrients and temperature, between nutrients and R_{10}, or between nutrients and Q_{10}.

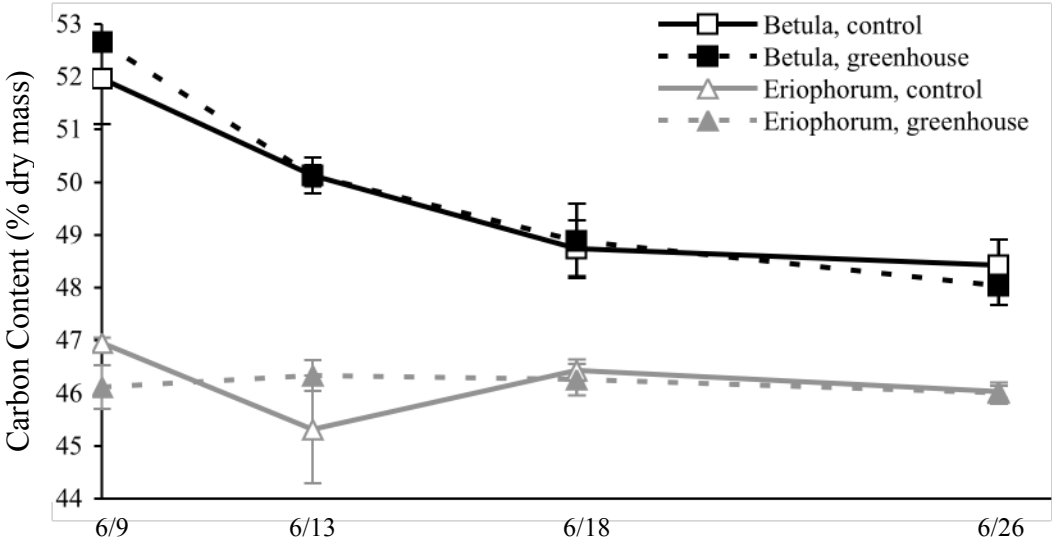

Figure 9. Foliar carbon content (mean±1 s.e., n=4) of each species and treatment on June 9, June 13, June 18, and June 26 (*Betula*, control, open square; *Betula*, greenhouse, solid square; *Eriophorum*, control, open triangle; *Eriophorum*, greenhouse, solid triangle). Species (Three-way ANOVA, $p<0.0001$) and sampling period (Three-way ANOVA, $p<0.0001$) had a significant effect on foliar carbon content, but not treatment (Three-way ANOVA, $p=0.61$).

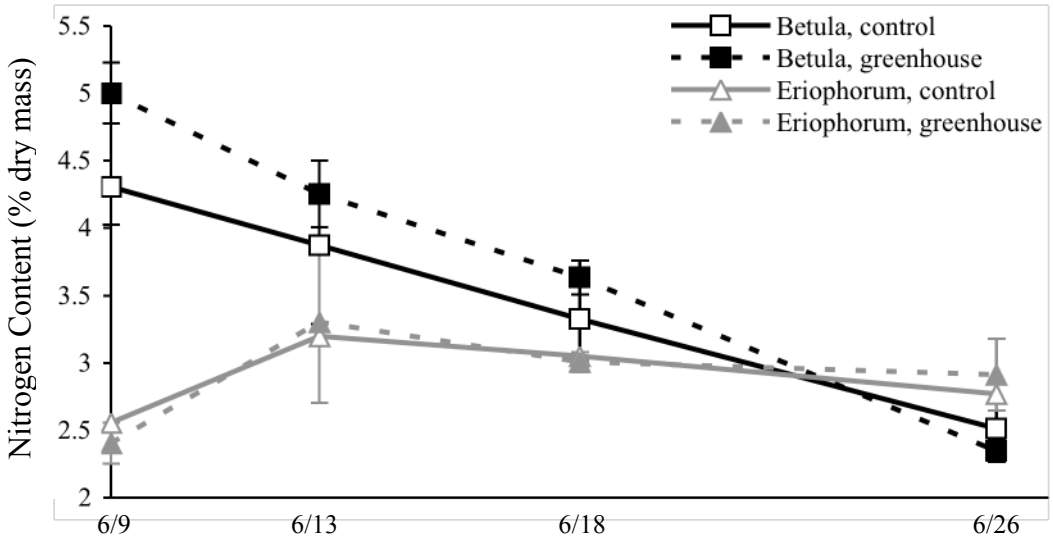

Figure 10. Foliar nitrogen content (mean±1 s.e., n=4) of each species and treatment on June 9, June 13, June 18, and June 26 (*Betula*, control, open square; *Betula*, greenhouse, solid square; *Eriophorum*, control, open triangle; *Eriophorum*, greenhouse, solid triangle). Species (Three-way ANOVA, $p<0.0001$) and sampling period (Three-way ANOVA, $p<0.0001$) had a significant effect on foliar nitrogen content, but not treatment (Three-way ANOVA, $p=0.19$).

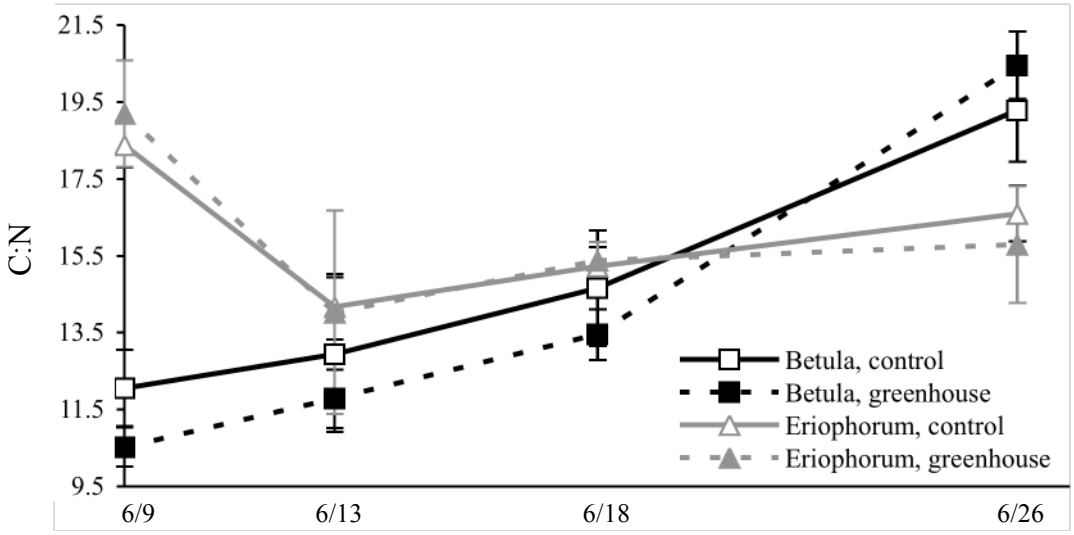

Figure 11. Foliar carbon to nitrogen ratio (mean±1 s.e., n=4) of the leave of each species and treatment on June 9, June 13, June 18, and June 26 (*Betula*, control, open square; *Betula*, greenhouse, solid square; *Eriophorum*, control, open triangle; *Eriophorum*, greenhouse, solid triangle). Species (Three-way ANOVA, $p<0.001$) and sampling period (Three-way ANOVA, $p<0.0001$), had a significant effect on C:N, but not treatment (Three-way ANOVA, $p=0.22$).

Discussion

Although there was little difference between the greenhouse and control treatments, the interspecific variation in respiration and nutrient content of *Betula* and *Eriophorum* may account for the observed replacement of the tussocks by the shrubs. This finding suggests that phenology, not temperature, primarily determined the respiration rates of these species over the first half of the growing season. Since the temperature at which each leaf was grown did not affect R_{10}, Q_{10}, and R, neither *Betula* nor *Eriophorum* exhibited significant respiratory acclimation to temperature, suggesting that both species may be unable to maintain respiratory homeostasis. In a warmer climate, these species may not down-regulate respiration, but may instead exponentially increase respiration with temperature until either substrate or enzymatic capacity becomes limiting. This may produce a positive-feedback loop and contribute the shift of the Arctic ecosystem from a carbon sink to a carbon source.

In addition, no direct correlation was found between temperature and leaf nutrients, R_{10}, or Q_{10}, or between nutrients and R_{10} and Q_{10}. . This finding agrees with studies that found no correlation between foliar nitrogen content and acclimation of R_{10} and Q_{10} to temperature in *Pinus radiata* (Ow et al., 2008). However, a positive relationship between leaf dark respiration and foliar nitrogen content and non-structural carbohydrates has been found in other species *(*Azcón-Bieto et al., 1983, Reich et al., 1998, Tjoelker et al., 2009). These contradictory findings may suggest that respiration may not have been limited by substrate availability or by enzymatic capacity in *Eriophorum* or *Betula*, and demonstrates the variability of respiration between species.

The R_{10}, Q_{10}, R, and nutrient data changed significantly throughout the season, regardless of growth temperature, suggesting that the phenologies of these two species may explain the observed differences seen in their respiration rates. *Betula*, a deciduous shrub, loses its leaves over the winter, growing new leaves each year (Pop et al., 2000). *Eriophorum*, however, overwinters its tillers, and grows new tillers on top of old tillers each growing season to form a tussock (Shaver & Laundre, 1997). These two phenological patterns led to very different uses of sequestered carbon, especially in the early growing season. The rapid spike in the Q_{10} at 10°C values and drop in R_{10} values observed in *Eriophorum* at the end of the experiment may have occurred because this was when the majority of the new tillers appeared (Figures 4 and 6). If this is the case, only the June 26 data reflect the respiration values of the new tillers. These tillers were more sensitive to temperature change but had a lower basal respiration rate than the last year's tillers, which agrees with other findings that new leaves are more temperature-sensitive than mature leaves (Armstrong et al., 2006; Covey-Crump et al., 2002, Loveys et al., 2002).

Interestingly, the R_{10} of *Betula* increases from June 9-June 18, and decreases from June 18-June 26. Most studies observe a very high R_{10} at the beginning of the growing season, which steadily decreases as tissues expand (Armstrong et al., 2006; Evans et al., 2000). The ability of *Betula* to keep its R_{10} low early in the season when its leaves are growing may be an advantage in maintaining its carbon stores. As we were only able to measure one R_{10} value for the new *Eriophorum* tillers, we cannot speculate as to the R_{10} dynamics of new leaves in this species.

The variations in the nutrient concentrations of the two species also reflect the differences in their phenologies. At the end of the growing season, plants reabsorb nitrogen from their leaves before dropping them to conserve nitrogen, as Arctic plants are limited by low levels of soil nitrogen (Atkin, 1996). *Betula* puts the absorbed nitrogen into new leaves at the beginning of each season. This is evident in the nitrogen-rich *Betula* leaves, whose nitrogen stores decreased linearly throughout the first half of the growing season, perhaps as biosynthetic processes used the nitrogen. *Eriophorum* also reabsorbs foliar nitrogen at the end of the growing season, but the nitrogen-deficient tillers are still present the following season. Nitrogen levels were low in the tillers sampled early in the season, but rose throughout the experiment, as the percentage of new tillers that were sampled increased (Figure 10). This pattern is even more evident in the C:N ratio. At the beginning of the season, *Eriophorum* has a higher C:N content than *Betula* (Figure 11). During the June 13 and June 18 sampling periods, the two species had similar foliar C:N content, as nitrogen-enriched *Eriophorum* tillers began to appear, and as the *Betula* leaves lost foliar nitrogen content. By the June 26 sampling period, *Eriophorum* had a lower C:N content than *Betula*, most likely because the majority of nitrogen-rich new tillers had come out and most of the sampled tillers were new, which likely corresponded to the decrease in R_{10} increase in Q_{10}.

It is also possible that the increasing C:N ratio, particularly in *Betula*, is due to the allocation of nitrogen to the leaves at the beginning of the season, which is not lost throughout the season, but diluted by carbohydrates as leaves expand. A dilution of nitrogen by carbohydrates, however, does not explain the initial drop in C:N in *Eriophorum* from June 9 to June 13 (Figure 13). The leaf mass per area ratio of both

species must be measured across the season to clarify what caused the observed seasonal nutrient patterns.

The finding that R_{10} and Q_{10} vary inversely for both species has also been reported in other studies (Xu & Griffin, 2006, Searle et al., unpublished data). If this pattern is found to be widespread, it may be a useful tool in modeling respiration, as R_{10} could be used to predict Q_{10}, and vice versa. Further, R seems to follow the seasonal pattern of R_{10}, not Q_{10}, suggesting that R_{10} is a better predictor of R than Q_{10} for these species.

The respiration responses to temperature demonstrate how *Betula* and *Eriophorum* responded to short-term increases in temperature differently (Figure 10). For all of the sampling periods, *Betula* exhibited the expected exponential response to temperature, but *Eriophorum* exhibited a linear response to temperature, except during the June 13 sampling period. A linear response may indicate that *Eriophorum* was insensitive to short-term increases in temperature. In addition, the R of *Betula* was lower than that of *Eriophorum* at lower temperatures, while the R of *Eriophorum* was lower than that of *Betula* at higher temperatures. Since the average ambient temperature during the first half of the season was 13.2±0.6°C, and never rose above 19.7°C, *Betula* respired at a lower rate in the conditions these plants usually experience.

A comparison of R for the entire season clearly shows that *Betula* respired at a much lower rate than *Eriophorum* during the first half of the growing season, regardless of growth temperature. This indicates that *Eriophorum* may lose more carbon to the atmosphere, potentially giving *Betula* a competitive advantage, at least during the first half of the growing season. The interspecific and intraspecific changes in R for each

sampling period make the seasonal component of respiration extremely evident. It is insufficient to use a single measurement of R_{10} and Q_{10} to accurately model the flux of carbon through these two common Arctic species. The seasonal variability of R of the two species must be taken into account to accurately model the atmospheric CO_2 output of the ecosystem.

Conclusions

Although *Betula* did not acclimate its respiration more efficiently than *Eriophorum* when grown at elevated temperatures as we hypothesized, this experiment demonstrates the very different early-season respiration dynamics of the two species. It is evident that the phenology of the different species affected the total carbon they released through foliar respiration. Overall, the lower R of *Betula* allowed it to retain more carbon than *Eriophorum*, which may give it a competitive advantage. The observed encroachment of the shrubs on the tussock-forming sedge may also be due to the ability of *Betula* to keep its R_{10} low as it initiated growth, though more research must be done on the growth of new *Eriophorum* tillers to verify this. As the climate warms, growing seasons may begin earlier and last longer, and so understanding how these plants respire as they begin growing will become increasingly important. Assuming the respiration rates of these species is constant throughout the growing season is a gross oversimplification, and a nuanced knowledge of how respiration changes throughout the season is necessary to model climate change in the future. The findings of this study suggest that respiration rate is primarily determined by the phenology of the plant, not by its growth temperature. In addition, the inability of either species to acclimate to warmer growth temperatures

suggests that the respiration of these species will form a positive feedback loop with global warming, as more and more carbon is released from the permafrost into the atmosphere as CO_2. Understanding the respiration response to temperature of Arctic species will help predict whether the Arctic will be a net sink or source of carbon in the future.

Recommendations

More research needs to be done to understand the seasonal effect of respiration over the remainder of the growing season to have a complete understanding of the respiration response to temperature of *Betula* and *Eriophorum.* Specifically, since the new *Eriophorum* tillers were sampled only once, more work must be done to understand how the new tillers respire in response to temperature change. The leaf mass per area ratio of the leaves of both species across the growing season must be found to understand what mediates the observed nutrient patterns. Additionally, repeating this experiment on plants that have been grown in nitrogen and phosphorous-fertilized plots, mimicking the increased nutrient availability predicted in the future environment, will help us understand more accurately how the respiration rates of *Betula* and *Eriophorum* will change in the future. Finally, measuring the photosynthetic temperature response at ambient and elevated growth temperature throughout the season is necessary to understand the net carbon released from the permafrost each season.

Acknowledgments

I would like to thank Mary Heskel, Jen Levy, Kristen Gore, and Julia Sable for their help with this project. I also thank James Laundre for providing the temperature data for this experiment. I am very grateful to Stephanie Searle for her advice and support. Thank you to the Earth Institute at Columbia University and to the Department of Ecology, Evolution, and Environmental at Columbia University for funding.

Works Cited

Amthor, J.S. (1984) The role of maintenance respiration in plant growth. *Plant, Cell, Environ.*, **7**, 561-569.

Armstrong, A.F., Badger, M.R., Day, D.A., Barthet, M.M., Smith, P.M.C., Millar, A.H., Whelan, J., Atkin, O.K. (2008) Dynamic changes in the mitochondrial electron transport chain underpinning cold acclimation of leaf respiration. *Plant Cell Environ.,* **31**, 1156-1169.

Armstrong, A.F., Logan, D.C., Atkin, O.K. (2006) On the developmental dependence of leaf respiration: responses to short- and long-term changes in growth temperature. *Am. J. Bot.*, **93**, 1633-1639.

Atkin, O.K. (1996) Reassessing the nitrogen relations of Arctic plants: A mini-review. *Plant Cell Environ.*, **19**, 695-704.

Atkin, O.K., Bruhn, D., Hurry, V.M., Tjoelker, MG. (2005) The hot and the cold: Unravelling the variable response of plant respiration to temperature. *Funct. Plant Biol.,* **32**, 87-105.

Atkin, O.K., Holly, C., Ball, M.C. (2000) Acclimation of snow gum (Eucalyptus pauciflora) leaf respiration to seasonal and diurnal variations in temperature: the importance of changes in the capacity and temperature sensitivity of respiration. *Plant Cell Environ.* **23**, 15-26.

Atkin, O.K. and Tjoelker, M.G. (2003) Thermal acclimation and the dynamic response of plant respiration to temperature. *Trends Plant Sci.*, **8**, 343-351.

Azcón-Bieto, J., Lambers, H., Day, D.A. (1983) Effect of photosynthesis and carbohydrate statues on respiratory rates and the involvement of the alternative pathway in leaf respiration. *Plant Physiol.* **72**, 598-603.

Azcón-Bieto, J. and Osmond, B.C. (1983) The effect of carbohydrate status on the rate of CO_2 production by respiration in darkened and illuminated wheat leaves. *Plant Physiol.* **71**, 574-581.

Berry, J., and Bjorkman, O. (1980) Photosynthetic response and adaptation to temperature in higher-plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol.*, **31**, 491-543.

Bunce, J.A. (2007) Direct and acclamatory responses of dark respiration and translocation to temperature. *Ann. Bot.,* **100**, 67-73.

Campbell, C., Atkinson, L., Zaragoza-Castells, J., Lundmark, M., Atkin, O., Hurry, V. (2007) Acclimation of photosynthesis and respiration is asynchronous in response to temperature regardless of plant functional group. *New Phytol.,* **176**, 375-389.

Chapin, F.S. III, Shaver, G.R., Giblin, A.E., Nadelhoffer, K.J., Laundre, J.A. (1995) Responses of Arctic Tundra to Experimental and Observed Changes in Climate. *Ecology*, **76**, 694-711.

Comiso, J.C. (2003) Warming trends in the Arctic from clear sky satellite observations. *J. Clim.,* **16**, 3498-3510.

Covey-Crump, E.M., Attwood, R.G., Atkin, O.K. (2002) Regulation of root respiration in two species of Plantago that differ in relative growth rate: the effect of short- and longterm changes in temperature. *Plant Cell Environ.*, **25**, 1501-1513.

Evans, J.R., Schortemeyer, N., Macfarlane, N., Atkin, O.K. (2000) Photosynthetic characteristics of 10 *Acacia* species grown under ambient and elevated atmospheric CO_2. *Aust. J. Plant Physiol.* **27**, 13-25.

Friedlingstein, P., Houghton, R.A., Marland, G., Hackler, J., Boden, T.A., Conway, T.J., Canadell, J.G., Raupach, M.R., Ciais, P., Le Quéré, C. (2010) Update on CO_2 Emissions. *Nature Geo*., **3**, 811-812.

Greaves, H. (2009) The Role of Leaf Carbon Exchange in Arctic Shrub Expansion. Department of Ecology, Evolution, and Environmental Biology, Columbia University.

Griffin, K.G., Turnbull, M.H., Atkin, O.K. O. Roger, A. (2009) IPY: Autotrophic respiration in a changing Arctic Climate: Mechanistic responses and ecosystem consequences, Annual Report 2009. Columbia University.

Gorham, E. (1991). Northern peatlands – role in the carbon cycle and probable responses to climatic warming. *Ecol. App.,* **1**, 182-195.

Havaux, M., and Tardy, R. (1996) Temperature-dependent adjustment of the thermal stability of photosystem II in vivo: Possible involvement of the xanthophyll-cycle pigments. *Planta*, **198**, 324-333.

Hooper, D.U., Vitousek, P.M. (1998) Effects of plant composition and diversity on nutrient cycling. *Ecol. Monogr.*, **68**, 121-149.

Houghton, R.A. (2007) Balancing the global carbon budget. *Annu. Rev. Earth Planet. Sci.,,* **35**, 313-347.

IPCC Fourth Assesment Report-Climate Change, 2007. The Scientific Basis. Cambridge University Press, Cambridge.

Kaufman, D.S., Schneider, D.P., McKay, N.P., Ammann, C.M., Bradley, R.S., Briffa, K.R., Miller, G.H., Otto-Bliesner, B.L., Overpeck, J.T., Vinther, B.M. (2003) Recent Warming Reverses Long-Term Arctic Cooling. *Science,* **325**, 1236-1239.

Lal, C.B., Annapurna, C., Raghubanshi, A.S., Singh, J.S. (2001) Foliar demand and resource economy of nutrients in dry tropical species. *J. Veg. Sci.*, **12**, 5-14.

Larigauderie, A., and Korner, C. (1995) Acclimation of leaf dark respiration to temperature in alpine and lowland plant-species. *Ann. Bot.,* **76**, 245-252.

Lexander, K., Carlsson, R., Schalén, V., Simonsson, A., Lundberg, T. (1970) Quantities and qualities of leaf protein concentrates from wild species and crop species grown under controlled conditions. *Ann. Appl. Biol.*, **66**, 193-216.

Lipova, L., Krchnak, P., Komenda, J., Ilik, P. Heat-induced disassembly and degradation of chlorophyll-containing protein complexes in vivo. *Biochim. Biophys. Acta,*, **1797**, 63-70.

Loveys, B.R., Atkinson, L.J., Sherlock, D.J., Roberts, R.L., Fitter, A.H., Atkin, O.K. (2003) Thermal acclimation of leaf and root respiration: an investigation comparing inherently fast- and slow-growing plant species. *Global Change Biol*., **9**, 895-910.

Loveys, B.R. Scheurwater, I., Pons, T.L., Fitter, A.H., Atkin, O.K. (2002) Growth temperature influences the underlying components of relative growth rate: an investigation using inherently fast- and slow-growing plant species. *Plant Cell Environ.,* **25**, 975-987.

Macfarlane, C., Hansen, L.D., Florez-Sarasa, I., Ribas-Carbo, M. (2009) Plant mitochondria electron partitioning is independent of short-term temperature changes. *Plant Cell Environ.,* **32**, 585-591.

Maxwell, K., and Johnson, G.N. (2000) Chlorophyll fluorescence – a practical guide. *J. Exp. Bot.*, **51**, 659-668.

McCree, K.J. (1970) *Prediction and measurement of photosynthetic productivity. Proceedings of the IBP/PP Technical Meeting, Trebon, 14 - 21 September 1969*. Wangeningen: Centre for Agricultural Publishing and Documentation.

Millenaar, F.F., and Lambers H. (2003) The alternative oxidase: *in vivo* regulation and function. *Plant Biol.,* **5**, 2-15.

Mooney, H.A. (1963) Physiological ecology of coastal, subalpine, and alpine populations of *Polygonum bistortoides*. *Ecology,* **44**, 812-816.

Moore, A.L., and Siedow, J.N. (1991) The regulation and nature of the cyanide resistant alternative oxidase of plant mitochondria. *Biochim. Biophys Acta.,* **1059**, 121-140.

Nishiyama, Y., Allakhverdiev, S.I., and Murata, N. (2006) A new paradigm for the action of reactive oxygen species in the photoinhibition of photosystem II. *Biochim. Biophys. Acta.,* **1757**, 742-749.

Noguchi, K. and Terashima, I. (1997) Different regulation of leaf respiration between Spinacia oleracea, a sun species, and Alosacasia odora, a shade species. *Physiol. Plant.*, **101**, 1-7.

Oechel, W.C., Hastings, S.J., Vourlitis, G., Jenkins, M., Riechers, G., Grulke, N. (1993) Recent change of arctic tundra ecosystems from a net carbon-dioxide sink to a source. *Nature,* **361**, 520-523.

Ow L.F., Whitehead, D., Walcroft, A.S., Turnbull, M.H. (2008) Thermal acclimation of respiration but not photosynthesis in Pinus radiata. *Funct. Plant Biol.*, **35**, 448-461.

Ping, C.L., Michaelson, G.J., Jorgenson, M.T., Kimble, J.M., Epstein, H., Romanovsky, V.E., Walker, D.A. (2008) High stocks of soil organic carbon in the North American Arctic Region. *Nature Geosci.,* **1**, 615-619.

Pop, E.W., Oberbauer, S.F., Starr, G. (2000) Predicting vegetative bud break in two arctic deciduous shrub species, *Salix pulchra* and *Betula nana*. *Oecologia*, **124**, 176-184.

Raven, P.H., Johnson, G.B., Losos, J., Singer, S. (2007) *Biology, 8th edition*. New Jersey: McGraw-Hill Higher Education.

Reich, P.B., Oleksyn, J., Tjoelker, M.G. (1996) Needle respiration and nitrogen concentration in Scots Pine populations from a broad latitudinal range: A common garden test with field-grown trees. *Funct. Ecol.*, **10**, 768-776.

Reich, P.B., Walters, M.B., Ellsworth, D.S., Vose, J.M., Volin, J.C., Gresham, C., Bowman, W.D. (1998) Relationships of leaf dark respiration to leaf nitrogen, specific leaf area and leaf life-span: a test across biomes and functional groups. *Oecologia*, **114**, 471-482.

Ryan, M.G. (1991) Effects of climate change on plant respiration. *Ecol. App.*, **1**, 157-167.

Ryan, M.G. (1995) Foliar maintenance respiration of subalpine and boreal trees and shrubs in relation to nitrogen content. *Plant Cell Environ.,* **18**, 765-772.

Sage, R.F., Santrucek, J. and Grise, D.J. (1995) Temperature effects on the photosynthetic response of C-3 plants to long-term CO2 enrichment. *Vegetatio*, **121**, 67-77.

Schreiber, U., and Berry, J.A. (1977) Heat-induced changes of chlorophyll fluorescence in intact leaves correlated with damage of photosynthetic apparatus. *Planta*, **136**, 233-238.

Shaver, G.R., and Laundre, J. (1997) Exsertion, elongation, and senescence of leaves of *Eriophorum vaginatum* and *Carex bigelowii* in Northern Alaska. *Global Change Biol.*, **3**, 146-157.

Tjoelker, M.G., Oleksyn, J., Lorenc-Plucinska, G., Reich, P.B. (2009) Acclimation of respiratory temperature responses in northern and southern populations of Pinus banksiana. *New Phyt.*, **181**, 218-229.

Tjoelker, M.G., Reich, P.B., Oleksyn, J. (1999) Changes in leaf nitrogen and carbohydrates underlie temperature and CO_2 acclimation of dark respiration in five boreal tree species. *Plant, Cell Environ.,* **22**, 767-778.

Vanderwerf, A., Visser, A.J., Schieving, F., Lambers, H. (1993) Evidence for optimal partitioning of biomass and nitrogen at a range of nitrogen availabilities for a fast-growing and slow-growing species. *Funct. Ecol.,* **7**, 63-74.

Will, R. (2000) Effect of different daytime and night-time temperature regimes on the foliar respiration of *Pinus taeda*: prediction the effect of variable temperature on acclimation. *J. Exp. Bot.,* **51**, 1733-1739.

Way, D.A., and Sage, R.F. (2006) Thermal acclimation of photosynthesis in black spruce [Picea mariana (Mill.) BSP]. *Plant Cell Environ.* **31,** 1250-1262.

Wullschleger, S.D., Norby, R.J., Gunderson, C.A. (1992) Growth and maintenance respiration in leaves of Liriodendron tulipifera L. exposed to long-term carbon dioxide enrichment in the field. *New Phyt.,* **121**, 515-523.

Xu, CY and Griffin, K.L. (2006) Seasonal variation in the temperature response of leaf respiration in *Quercus rubra*: foliage respiration and leaf properties. *Funct. Ecol.,* **20**, 778-789.

Works Referenced

Amthor, J.S. (1991) Respiration in a future, higher-CO_2 world. Plant, Cell and Environ., **14**, 13-20.

Armstrong, A.F. *et al.* (2008) Temperature and the mitochondrial electron transport chain. *Plant, Cell and Environ.,* **31**, 1156-1169.

Booth, M.S., Stark, J.M., and Rastetter, E. (2005) Controls on nitrogen cycling in terrestrial ecosystems: A synthetic analysis of literature data. *Ecol. Monogr.*, **75**, 139-157.

Edwards, G.E., Baler, N.R. (1993) Can CO_2 assimilation in maize leaves be predicted accurately from chlorophyll fluorescence analysis? *Photosynthesis Research,* **37**, 89-102.

Fiorani, F., Umbach, A.L., and Siedow, J.N. (2005) The alternative oxidase in plant mitochondria is involved in the acclimation of shoot growth at low temperature. A study of Arabidopsis *AOX1a* transgenic plants. *Plant Physiol.*, **139**, 1795-1805.

Ghouil, H., Montpied, P., Epron, D., Ksontini, M., Hanchi, B., Dreyer, E. Thermal optima of photosynthetic functions and thermostability of photochemistry in cork oak seedlings. *Tree Physiol.*, **23**, 1031-1039.

Griffin, K.G., Turnbull, M.H., Atkin, O.K. O. Roger, A. (2008) IPY: Autotrophic respiration in a changing Arctic Climate: Mechanistic responses and ecosystem consequences. Columbia University.

Poorter, H. (1993) Interspecific variation in the growth response of plants to an elevated ambient CO_2 concentration. *Vegetatio*, **104/105**, 77-97.

Rastetter, E., Perakis, S.S., Shaver, G.R., Agren, G.I. (2005) Terrestrial C sequestration at elevated CO_s and temperature: The role of dissolved organic N loss. *Ecol. App.*, **15**, 71-86.

Schimel, D.S. (1995) Terrestrial ecosystems and the carbon-cycle. *Global Change Biol.*, **1**, 77-91.

Shaver, G.R., Giblin, A.E., Nadelhoffer, K.J., Thieler, K.K., Downs, M.R., Laundre, J.A., Rastetter, E.B. (2006) Carbon turnover in Alaskan tundra soils: effects of organic matter quality, temperature, moisture and fertilizer. *J. Ecol.*, **94**, 740-753.

Tcherkez, G., Bligny, R., Gout, E. *et al.* (2007) Respiratory metabolism of illuminated leaves depends on CO_2 and O_2 conditions. *Plant Biol.*, **105**, 797-802.

Tcherkez, G., Cornic, G., Bligny, R., Gout, E., Ghashghale, J. (2005) In vivo respiratory metabolism of illuminated leaves. *Plant Physiol.*, **138**, 1596-1606.

Yoshida, K., Terashima, I., Noguchi, K. (2007) Up-regulation of mitochondrial alternative oxidase concomitant with chloroplast over-reduction by excess light. *Plant Cell Physiol.* **48**, 606-614.