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Effects of Growth Temperatures and Elevated CO2 on Respiration Rates in Norway Spruce

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Graduate Program in Biology
A thesis submitted in partial fulfillment of the requirements for the degree in Master of Science
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EFFECTS OF ELEVATED GROWTH TEMPERATURES AND CO_2 ON LIGHT AND DARK RESPIRATION RATES IN NORWAY SPRUCE (*Picea abies*)

Monograph

by

Yulia Kroner

Graduate Program in Biology

A thesis submitted in partial fulfillment

of the requirements for the degree of

Master of Sciences

The School of Graduate and Postdoctoral Studies

The University of Western Ontario

London, Ontario, Canada

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Abstract

Projected increase in growth temperatures and CO_2 may affect carbon balance in Norway spruce (*Picea abies*), a dominant coniferous species of the boreal forest ecosystem. To examine the effects of elevated growth temperatures and CO_2 on photosynthesis and respiration rates in this species, I exposed three-year-old seedlings to six treatments: ambient (400 ppm) and elevated (750 ppm) CO_2 concentrations combined with three growth temperatures: ambient, ambient +4 ^oC, and ambient +8 ^oC. I found that while net growth was generally not affected by growth CO_2 or temperature, leaf nitrogen concentrations were reduced, mortality rates were higher, and needles were shorter and thinner in +8 ^oC treatments, compared to cooler treatments. I found that net CO_2 assimilation rates and dark respiration acclimated to temperature but not CO_2, while patterns of acclimation of light respiration in the light varied between years. The highest net CO_2 assimilation rates were found in trees grown at +4 ^oC combined with elevated CO_2, which could indicate that a slight increase in growth temperature with elevated CO_2 may benefit the carbon balance of Norway spruce. However, further warming had negative effects on carbon uptake, with trees from the +8 ^oC treatments showing the lowest CO_2 assimilation and dark respiration rates. The Q_{10} of light respiration was 35% higher than the Q_{10} of dark respiration, so that the ratio of light respiration to dark respiration increased as leaf temperature increased. I conclude that light respiration is not a constant fraction of dark respiration, although both parameters are tightly correlated, and this relationship can be used to improve models of terrestrial vegetation.

Key words: Climate change, Norway spruce, *Picea abies*, temperature, CO_2, photosynthesis, dark respiration, light respiration, acclimation.

Acknowledgments

First of all, I would like to thank my supervisor Dr. Danielle Way for all her support, encouragement and guidance through this degree. I am very grateful for her continuous help and limitless patience. It was an unforgettable experience to be a part of the Way lab. I appreciate all the help "to infinity and beyond" of current and past Way lab members: Joe Stinziano, Vi Bui, Marin Mema, Nick Harris, and of course, numerous volunteers. I thank my committee advisors, Dr. Norman P.A. Hüner and Dr. Sheila Macfie for their incredibly valuable feedback. I also would like to thank the Biotron facility staff, Steve Bartlett and Carrie Hamilton for all their technical support: some of my experiments could not be completed without their continuous help. And, I am grateful to the Biotron Imaging Section staff, Dr. Richard Gardiner and Dr. Karen Nygard for providing me with the imaging equipment and all their assistance during the long hours of the microscopy analysis. I thank the amazing Robarts Research Institute staff for their sample embedding and slide preparation services. I would also like to thank current and past members of the Hüner, Bernards and Sinclair labs for all their help during this project. Of course, the list of thanks cannot be completed without my deepest appreciations of the Biology Department faculty and staff. Finally, I want to thank my family for all their support during the last two years.

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1 Introduction

1.1 Boreal forest and global warming

In the last two centuries, atmospheric CO_2 concentrations have risen from 280 ppm to 400 ppm due to anthropogenic disturbances such as fossil fuel burning and land use change, with projections of CO_2 concentrations of up to 900 ppm by the year 2100. As a result, the Earth's global mean temperature is expected to rise by 2-4 ^{o}C (IPCC 2014), and even more extreme temperature increases (5.5-10 ^{o}C) are predicted at high latitudes (IPCC 2014). These climatic changes will have strong effects on the world's largest terrestrial ecosystem, the boreal forest, which occupies about 1.3 billion ha (FAO 2000). This ecosystem contains *ca* 800 Pg carbon (C), or one-third of all the terrestrial C stores on the planet (Apps et al. 1993). Recent changes in the global C cycle have been attributed to perturbations in the function of high latitude forests and the arctic region due to climatic change (Graven et al. 2013). Considering the significance of boreal forests on the global carbon cycle, understanding how climate change will affect these high latitude forests is important for understanding and predicting future C fluxes from vegetation.

1.2 Role of respiration in the global carbon cycle

Net ecosystem C uptake is determined by the balance of C gain from CO_2 assimilation (A) and C loss via respiration (R). Both processes are co-dependent: R needs the substrate provided by A, and A is dependent on ATP and C skeletons from R that are required for sugar synthesis. On the global scale, A fixes 120.4 Gt C from the atmosphere every year, whereas

about half of this C (~60 Gt C) is lost through plant and soil R (Schlesinger & Andrews 2000, Amthor 1995), and leaf R contributes about 50% of this C loss (Atkin et al. 2007). Although anthropogenic activities also affect the global C cycle, burning of fossil fuels and land-use change only contribute 6.3 and 1.6 Gt C per year, respectively, to the atmosphere (IPCC 2014). Given the scale of the C fluxes of A and R globally, even a minor change in either process can have an effect on atmospheric CO_2 on the order of anthropogenic contributions (Drake et al. 1999, Lindroth et al. 2008).

1.3 Responses of photosynthesis to elevated CO_2 and temperature

Since global climate change is primarily due to increasing CO_2 concentrations, research on how A and R in terrestrial plants will respond to climate change should include both elevated growth temperatures and CO_2. The response of A to future climate conditions is relatively well studied compared to the responses of R (Xu et al., 2013; Way & Yamori, 2014; Way et al. 2015). Elevated CO_2 typically stimulates net CO_2 assimilation rates (A_{net}) (Sage & Kubien 2007, Crous et al. 2008, Crous et al. 2012, Marshall & Linder 2013) by increasing substrate availability for Rubisco (ribulose-1,5-bisphosphate) in the Calvin-Benson cycle. Elevated growth temperatures change the temperature response of A_{net} by increasing the thermal optimum of A_{net} as plants acclimate to the higher growth temperature (Berry & Bjorkman 1980, Way & Sage 2008, Yamori et al. 2014). However, A_{net} measured at the growth temperature may be lower in plants that developed at high growth temperatures, offsetting any enhancement of A_{net} due to a CO_2 effect (Fig. 1.1) (Tjoelker et al. 1998; Benlloch-Gonzalez et al. 2014; Way & Yamori 2014).

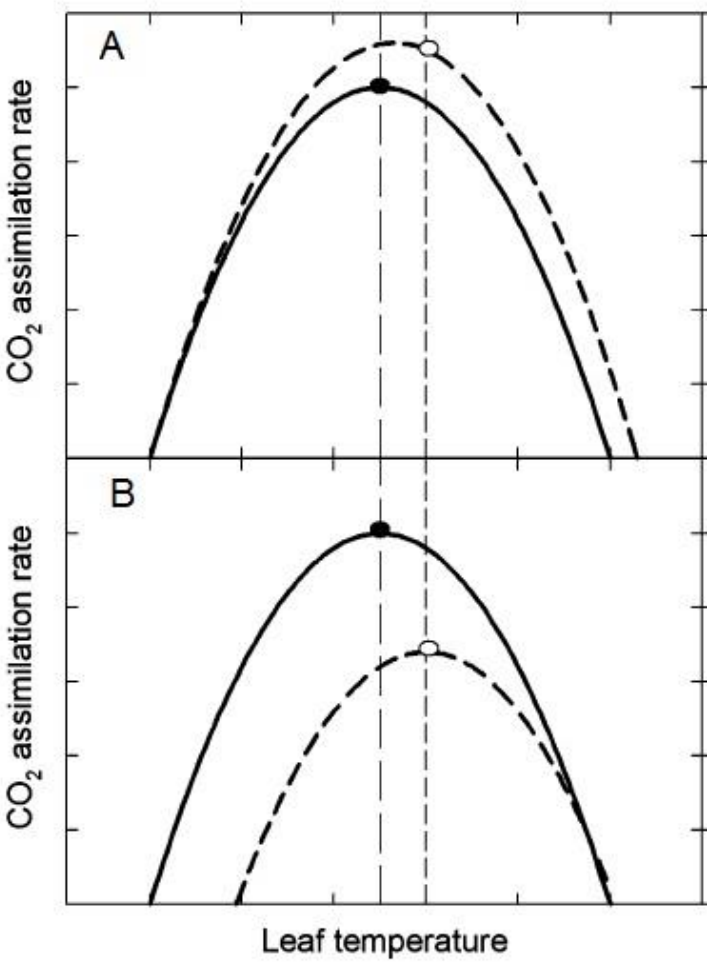

Figure 1.1 Conceptual figure of how long-term warming may affect net CO_2 assimilation rates. In A) net CO_2 assimilation rates are higher in warm-grown plants compared to control plants; in B) net CO_2 assimilation rate are suppressed in warm-grown plants compared to control plants. Solid lines- plants grown at the control temperature, dashed lines- plants grown at a warmer temperature. Vertical lines: long-dashed line represents growth temperature for control plants; short-dashed line represents growth temperature for warm-grown plants. Circles indicate net CO_2 assimilation rates at control (solid) and warmer (open) growth temperatures. Modified from Way and Yamori, 2014.

1.4 Cellular plant respiration in the light

The response of mitochondrial R to future climates is more complicated than that of A_{net}, mainly because leaf respiration rates are different in the light (R_{light}) and in the dark (R_{dark}). The rates of R measured in the dark are usually higher than those in the light, implying that light suppresses non-photorespiratory mitochondrial R (Krömer 1995, Kirschbaum & Farquhar 1987, Villar 1995, Wang et al. 2001). The reduction in R fluxes in the light may be caused by down-regulation of pyruvate dehydrogenase and partial inhibition of the citric acid cycle (Tcherkez et al. 2008). However, the exact mechanism responsible for a decrease in R in the light remains unclear, and the degree of light suppression is not constant. Hurry et al. (2005) highlighted that the degree of suppression may fluctuate between 16 and 77%. Other studies have demonstrated that light suppression of R varies considerably among plant species (Krömer 1995; Atkin et al. 1997; Pärnik and Keerberg 1995), and is also dependent on leaf age, as younger leaves show less inhibition than old ones (Villar et al 1995). Work done by Ayub et al. (2011) suggested that R_{light} can be estimated as ~0.7 R_{dark}, which would be a useful simplification for models that predict C-fluxes of vegetation, because R_{light} is not as easily measured as R_{dark}. However, given the uncertainties in the degree of light-suppression of R, further investigation is needed to improve our understanding of this phenomenon and the accuracy of these models.

1.5 Responses of plant respiration to elevated CO_2 and temperature

The responses of R to changes in CO_2 and growth temperature reported in the literature are variable. Both R_{light} and R_{dark} typically increase exponentially with a linear increase in leaf temperature on short time scales (i.e. minutes) (Villar et al. 1995; Atkin et al. 2005; Way & Sage 2008; Rodriguez-Calcerrada et al. 2010; Silim et al. 2010; Ayub et al. 2011; Crous et al. 2013; Slot et al. 2014). However, when higher irradiances (200-2000 μmol photons m^{-2} s^{-1}) were used, no strong response of R_{light} to T_{leaf} (6-30˚C) was found (Atkin et al. 2000a).

Thermal acclimation of R_{light} and R_{dark} can alter the shape of the temperature response curve of R to a long-term increase in growth temperature by adjusting leaf anatomy and protein composition (Atkin & Tjoelker 2003). Two types of thermal acclimation have been defined, and both are characterized by a decrease in R in warm-grown plants. Type I acclimation is described as a decrease in the Q_{10} of R (the relative change in R for every 10 ^{o}C temperature increase) as a result of changes in enzyme regulation, whereas Type II acclimation is characterized by a decrease in the intercept of the curve in warm-treated plants (Atkin & Tjoelker 2003; Fig. 1.2). In contrast, short –term (i.e. minutes to an hour) changes in CO_2 have no significant effect on R_{light} or R_{dark} (Tissue et al. 2002; Crous et al. 2012), but higher rates of R_{light} and R_{dark} have been observed in plants grown in elevated CO_2 (i.e a long-term CO_2 effect) compared to those than in plants grown in ambient CO_2 (Wang et al. 2001; Shapiro et al. 2004). Although the effect of a combination of higher growth temperatures and elevated CO_2 on R has been studied in a very small number of deciduous species (Tjoelker et al. 1999; Tjoelker et al. 2009; Wang et al. 2001;

Crous et al. 2012), to our knowledge, this has never been studied on both R_{light} and R_{dark}, nor has it been assessed in a coniferous species.

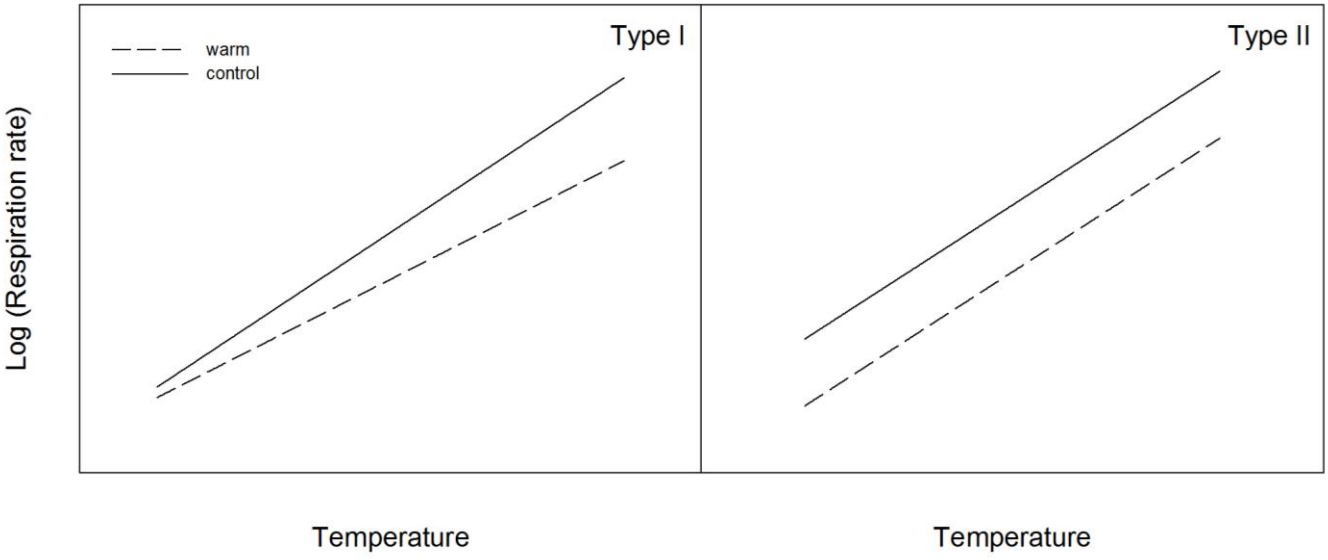

Figure 1.2 Conceptual figure of two forms of thermal acclimation in respiration. In Type I acclimation, the Q_{10} is reduced in trees grown at warmer temperatures; in Type II acclimation, the Q_{10} remains the same for both control and warm-grown plants; but the intercept is lower in warm-treated trees. Adapted from Atkin and Tjoelker, 2003.

1.6 Norway spruce

Norway spruce is an evergreen dominant conifer in Eurasian boreal forests (Eckenwalder 2009), that can reach a maximal height of 62 m and a stem diameter of 2 m (Tjoelker et al. 2008). The optimal climatic conditions for Norway spruce are 6 ^{o}C annual mean temperatures and 490-580 mm of precipitations during the growing season (60-150 days a year depending on geographical location) (Shmidt-Vogt 1977). The species survives very low temperatures (to -60 ^{o}C) (Shmidt-Vogt 1977) and growing season frosts of -7 ^{o}C (Ellenberg 1978). Due to its tolerance of cold climates, it forms a dominant part of the canopy in forests across northern Europe, including large areas of Scandinavia and Siberia, and in higher altitude regions of more southern areas such as Switzerland, Germany, Italy, and the Balkan Peninsula (Tjoelker et al. 2008). Norway spruce has been also successfully introduced and naturalized in North America, from Connecticut to Michigan, including New England. and the southern-eastern regions of Canada (Sullivan 1994). In nature, Norway spruce is rarely exposed to high temperatures, but mature trees can survive up to +50 ^{o}C (Schmidt-Vogt 1977). However, young seedlings cannot tolerate those temperatures for more than several minutes (Kreeb 1979).

1.7 Objectives and Predictions

In this study, R_{light}, R_{dark} and A_{sat} (light-saturated net CO_2 assimilation rates) were measured on Norway spruce seedlings, grown in both ambient and elevated CO_2 and at a range of growth temperatures; these data were then correlated with leaf biochemical and anatomical

traits. My predictions were that: 1) R_{dark} is suppressed by light in all treatments, so that $R_{light} < R_{dark}$, but both R_{light} and R_{dark} increase as short-term leaf temperatures rise; 2) R_{light} and R_{dark} measured at a given leaf temperature are lower in plants grown in elevated temperatures due to thermal acclimation, but growth at elevated CO_2 results in minimal differences across CO_2 treatments; 3) the surface area of mitochondria in leaf cross-sections correlates positively with leaf respiration rates, since a greater volume of mitochondria should increase leaf respiration capacity; 4) not accounting for the difference between R_{light} and R_{dark}, and the effect of growth temperature and CO_2 on R, will lead to substantial errors in modelling of R.

2 Materials and methods

2.1 Experimental design

Three-year-old bare root Norway spruce seedlings were ordered from a nursery (Kendal, ON, Canada, 44˚1´ N, 78˚32´ W) in the springs of 2013 and 2014. One seedling was planted per pot into 11.3 L pots (Myers Industries Lawn & Garden Group, Middlefield, OH, USA). Pots were >10 L, as smaller pot volumes may induce down-regulation of photosynthesis in high CO_2 environments (Wang & Curtis 2002). Pots were filled with Promix BX mycorrhizae soil mix (Premier Tech Horticulture, Rivière-du-Loup, QC, Canada) to prevent nutrient deficiency, and supplemented with a slow-release fertilizer (Slow Release Plant Food, 12-4-8, Miracle Gro®, The Scotts Company, Mississauga, ON, Canada). Seedlings were randomly assigned to six climate-controlled greenhouses, each having a different CO_2 by temperature treatment, at Western University's Biotron facility (43˚0´ N, 81˚16´ W). The six treatments were: ambient temperature

(AT) coupled with 400 μmol mol^{-1} CO_2 (ambient CO_2, AC); AT coupled with 750 μmol mol^{-1} CO_2 (elevated CO_2, EC); AT+4 ^oC coupled with AC; AT+4 ^oC coupled with EC; AT+8 ^oC coupled with AC; and AT+8 ^oC coupled with EC. The AT was calculated from hourly temperature averages for the last five years (2008-2012) from the London, ON airport meteorological station (Environment Canada). Light intensity fluctuated naturally during the day, reaching a maximum of 2056 μmol photons m^{-2} s^{-1} in midsummer. Relative humidity was maintained between 60-80%. Seedlings were watered daily as needed to maintain a moist growth medium.

Stem heights and diameters at the soil surface were measured after planting, and at the end of each of the 2013 and 2014 growing seasons (November 2013 and 2014). Growth was calculated as the change in shoot height and stem diameter over the growth season to focus on growth that occurred during the treatments. Mortality rates and bud production were also assessed at the end of each growing season.

2.2 Physiological measurements

For R_{light}, R_{dark} and A_{sat}, gas exchange measurements were conducted in August and September of each year on new, fully expanded needles that developed under the treatments, using a portable photosynthesis system (Li-Cor 6400XT, opaque and conifer chamber 6400-22; Li-Cor Inc., NE, USA). The photosynthesis system contains an open-path infra-red gas analyzer for measuring CO_2 and water fluxes from leaves as compared to known concentrations of CO_2 and water that enter the cuvette. A branch of each tree with these new needles was placed inside

the chamber and carefully sealed to avoid gas leaks from the cuvette. Four trees per treatment were measured in the first year (2013) and five in the second (2014). Net CO_2 assimilation rates (A_{net}) were measured at different light intensities (1200, 800, 100, 90, 80, 70, 60, 50, 40, 30, 20, 10, 5 and 0 µmol photons m^{-2} s^{-1}) and four leaf temperatures (10 ^{o}C, 20 ^{o}C, 30 ^{o}C, 40 ^{o}C) at a CO_2 concentration of 400 µmol mol^{-1} and relative humidity between 50-60%. Leaf temperatures were achieved by measuring seedlings inside a walk-in growth chamber with regulated ambient temperatures (Environmental Growth Chambers, model M18SI (in 2013) and GR128 (in 2014), Chagrin Falls, OH, USA). R_{light} was assessed using the Kok method (Kok, 1948), based on the assumption that at low light intensities (from ~30 to 80 µmol photons m^{-2} s^{-1}), linear extrapolation of the light response curve allows estimation of R_{light} which cannot be measured directly. R_{dark} was measured at 0 µmol photons m^{-2} s^{-1} after a 25-minute dark period to minimize post-illumination CO_2 bursts, e.g. light–stimulated R_{dark} (Atkin et al. 1998), and A_{sat} was measured at a saturating irradiance of 1,200 µmol photons m^{-2} s^{-1}. All R_{light} values were corrected for changes in intercellular CO_2 (Ci) along the light gradient (Kirschbaum & Farquhar 1987), since a reduction in irradiance reduces A_{net}, and this in turn increases Ci and the substrate for photosynthesis, changes in Ci must be corrected for to assess the direct effects of light on A_{net}. Stomatal conductance (g_s) was also measured at each irradiance to confirm that g_s was not limiting CO_2 fixation. The temperature where net CO_2 assimilation rates were maximal (the thermal optimum, T_{opt}) was calculated for all the treatments by fitting the equation:

$$A_{net} = aT^2 + bT + c \qquad \text{[Equation 1]}$$

to the data and calculating the temperature (T) where A_{net} was maximal, with a, b and c representing constants. The Q_{10} values for R_{light} and R_{dark} (i.e. the relative increase in R for a 10°C temperature increase) were calculated according to Atkin and Tjoelker 2003:

$Q_{10} = 10^{10k}$ [Equation 2]

where k is the slope of log-transformed R plotted against leaf temperature.

To investigate the possibility of elevated CO_2-induced down-regulation of A_{net} in the 11.3 L pots, gas exchange measurements (as A_{net} versus Ci curves) were conducted in 2013 on two trees grown in 11.3 L pots and on two trees grown in a 100 L container. The A_{net} was measured at a range of CO_2 concentrations (50, 100, 200, 400, 800, 1000, 1200, 1500, 1800 μmol mol $^{-1}$), a light intensity of 1200 μmol photons m^{-2} s^{-1}, a relative humidity of 50% and a leaf temperature of 25 ^{o}C. Maximum rates of Rubisco carboxylation (V_{cmax}) and maximum rates of electron transport (J_{max}) were calculated according to Farquhar et al. (1980). Fresh needles from seedlings used for gas exchange were harvested immediately after all the measurements and analyzed for projected leaf area and needle lengths by taking a photo of the needles, and then using ImageJ software (NIH, Bethesda, MD).

2.3 Carbohydrate and nitrogen analysis

Leaf samples from the measured leaves were taken after the leaf area analysis and dried at 70 ^{o}C to constant mass for dry mass and specific leaf area measurements. Samples were then ground with a Wiley mill.

Leaf soluble sugars (glucose, fructose, sucrose) were extracted according to Hendrix (1993), with slight modifications. Briefly, 1 mL of 80% ethanol was added to 10 mg of dried tissue. After 20 minutes of incubation at 80 ^{o}C, a sample was centrifuged at 12,000 RPM for 2

minutes (Centrifuge 5414, S/N 5414 I 12192, Eppendorf, Germany). The process was repeated two more times. One mL of supernatant was transferred to a separate microfuge tube, mixed with activated charcoal to absorb phenolics and tannins that can interfere with enzymatic analyses, and centrifuged at 10,000 RPM for 10 minutes. Sixty µL of the aliquot was pipetted to a 96-well plate and left overnight to evaporate ethanol from the samples, leaving the sugars as residues. The following day, 150 µL of glucose GAHK-20 kit (Sigma-Aldrich Co. LLC.) was added to each well, incubated for 30 minutes on a shaker at room temperature, and NADPH absorbance was read at 340 nm using a spectrophotometer to obtain glucose concentrations (Versa max, Microplate reader, Molecular Devices, S/N BN02815). Twenty µL of phosphoglucose isomerase (1,000 U/mL, Megazyme International Ireland, Bray, Co. Wicklow, Ireland) was added to each well to convert fructose to glucose, and the plate was incubated for 20 minutes on a shaker at 37 ^{o}C before the absorbance was reread at 340 nm. Forty µl of an isomerase solution (150 U/mL in 100 mM acetate buffer (pH 4.5) 2,000 U/mL, Megazyme) was then added to each well to convert sucrose to glucose, the plate was incubated at 37 °C on a shaker for 30 minutes, and the absorbance was reread at 340 nm. Using a standard curve, concentrations of glucose, fructose and sucrose were then determined.

To calculate the percent starch per dry leaf mass, 1 mL of 0.2 M KOH was added to the pellet remaining from the soluble sugar extractions, and incubated for 1 hour at 100 ^{o}C. After the sample was cooled to room temperature, pH was lowered to 6.6-7.5, and 200 µL of α-amylase (~23,400 U/mL, Sigma-Aldrich), diluted to ~2600EU/mL in 1.0 M Tris acetate (pH 7.2) was added to the sample and incubated for 30 minutes at 80 ^{o}C. The sample was cooled to room temperature, and the pH was lowered to 4.8-5.0. Five hundred µL of diluted to ~250EU/mL in 100 mM acetate buffer (pH 4.5) amyloglucosidase (3,260 U/mL, Megazyme) was added and

incubated for 1 hour at 55 ^{o}C. To stop enzymatic digestion, the sample was boiled for 4 minutes, and 500 µL of the solution was mixed with 1 mL of H_2O and centrifuged at 10,000 RPM. Sixty µL of the aliquot was pipetted into a 96-well plate and left overnight. The following day, 150 µL of GAHK-20 was added and incubated for 30 minutes at room temperature, and the absorbance was read at 340 nm.

Leaf %N and %C were analyzed on ground leaves with a CN analyzer (NCS 2500, Carlo Erba, Milan, Italy) which allows quantifying elemental composition of the sample. Between 3-4 mg of dry ground tissue was placed into a tin container, and loaded into the combustion reactor at 1020 ^{o}C. Combusted and evaporated elements such as CO_2 and N_2 were then measured through a chromatographic column, and each of elements read separately.

2.4 Leaf anatomy and mitochondrial surface area

Eight to ten needles per tree were collected on September 20, 2013 from the same four individuals in each treatment used for physiological measurements. The needles were bundled with a cotton thread and cut 2-5 mm from the leaf tip to promote penetration of a mitochondrial staining solution (MitoTracker ® Mitochondrion-Selective Probes, Molecular Probes, Inc, Eugene, OR, USA). The mitochondrial probe solution was applied for 45 minutes and then needles were washed with 0.2 M phosphate buffer with 0.3 M sucrose (pH 6.4). The samples were left overnight in buffered paraformaldehyde at 4 ^{o}C. The next day, samples were washed three times with phosphate-sucrose buffer, moved into 70% (V/V) EtOH and transferred to Robarts Research Institute, Molecular Pathology Core Facility. Automated ethanol dehydration

was processed using a Leica ASP300 (Shandon Histocentre 3, Thermo Electron Corporation), and samples were then embedded in paraffin. The paraffin block was trimmed and the surface soaked for an hour in an ice bath. Two 5 μm-thick cross-sections were taken using a microtome (Leica RM2255). Two additional 5 μm-thick cross-sections were taken at a 200-300 μm distance from the first sample. The samples were placed on slides, dried at 40 ^{o}C overnight, deparaffinised with xylene and prepared for microscopy.

The prepared samples were observed at 1000x magnification using a light microscope (Carl Zeiss Z1 Carl Zeiss Imaging and Microscopy, Germany). Photographs were taken in a two-channel spectrum (DAPI and Alexa 565) with a digital camera (Carl Zeiss AxioCam MR5, Carl Zeiss Imaging and Microscopy, Germany). Cross-sectional leaf area and total mitochondrial cross-sectional surface areas were calculated using ImageJ software (NIH, Bethesda, MD).

2.5 Modelling

To determine whether the differences in R generated by growth in elevated CO_2 and temperature need to be accounted for in current models of ecosystem R, I modeled daily R fluxes for each treatment and compared them to the fluxes from ACAT seedlings and also to R rates derived from 0.7 R_{dark}. To calculate total diurnal R, I used temperature responses of R_{light} and R_{dark} for each of the six treatments, based on the exponential functions fit to the data. These rates were then driven by a 24-hour leaf temperature trace that was collected from a representative seedling from the ACAT treatment. A diurnal leaf temperature trace was measured with a copper-constantan (type T) thermocouple attached to a data logger (Spectrum 1700, Veriteq

Instruments, Richmond, BC, Canada) on a needle in the ATAC treatment on October 19, 2013; data were collected every 30 seconds and averaged into five-minute periods. R_{light} and R_{dark} rates were calculated for each five-minute period based on the temperature-functions for each growth treatment. These treatment-specific R_{light} and R_{dark} were then summed for the 24-hour day for each treatment to compare the effect of acclimation of R on daily needle R fluxes.

2.6 Statistical analysis

Data were analyzed with R (R GUI Version 3.0.2 (R Core Development Team, 2013). For physiological measurements, two-way ANOVAs (T_{growth} and growth CO_2 as factors), three-way ANOVAs (T_{growth}, growth CO_2 and year), and repeated measures ANOVAs (on leaf temperature response curves accounting for individual trees, with T_{growth}, growth CO_2 and year) were applied. Tukey's Post-Hoc test for comparisons of means was used to investigate significant treatment effects.

3 Results

3.1 Growth temperature and CO_2 settings

CO_2 and temperature treatments began May 20, 2013. Ambient, +4 ^oC, and +8 ^oC temperature treatments were successfully maintained in each growing season (Fig. 3.1). CO_2 concentrations generally remained stable during the treatments and the elevated CO_2 was consistently > 300 ppm greater than the ambient CO_2 treatment (Fig. 3.1).

3.2 Mortality rates and growth

Seedlings from 2014 had lower mortality rates than those from 2013 (Table 3.1). There was no significant treatment effect (p=0.1) on mortality in 2013, but in 2014, there were significantly higher mortality rates observed in the +8 ^oC treatment regardless of the CO_2 treatment.

Neither growth temperature (T_{growth}) (p=0.5) nor growth CO_2 (p=0.09) affected shoot height growth in 2013 (Fig. 3.2A), while in 2014, growth CO_2 still had no effect on shoot height, but warming treatments significantly increased shoot height growth in all the EC treatments (Fig. 3.2B).

There was a strong growth CO_2 effect on stem diameter growth in 2013 and 2014, in contrast to a lack of a CO_2 effect on shoot height growth (Figs. 3.2C and 3.2D). In 2013 the stem diameter was greater in ATEC treatment compared to ATAC, while in 2014, +4 ^oC and +8 ^oC

combined with elevated CO_2 treatments increased stem diameter compared to the comparable warmed AC treatments.

Bud production in 2013 showed no significant difference across treatments ($p>0.09$; Fig. 3.2E). In 2014, T_{growth} did not affect the number of buds set in the AC treatment, however bud set was stimulated by warmer temperatures in the EC treatments ($p<0.001$; Fig. 3.2F).

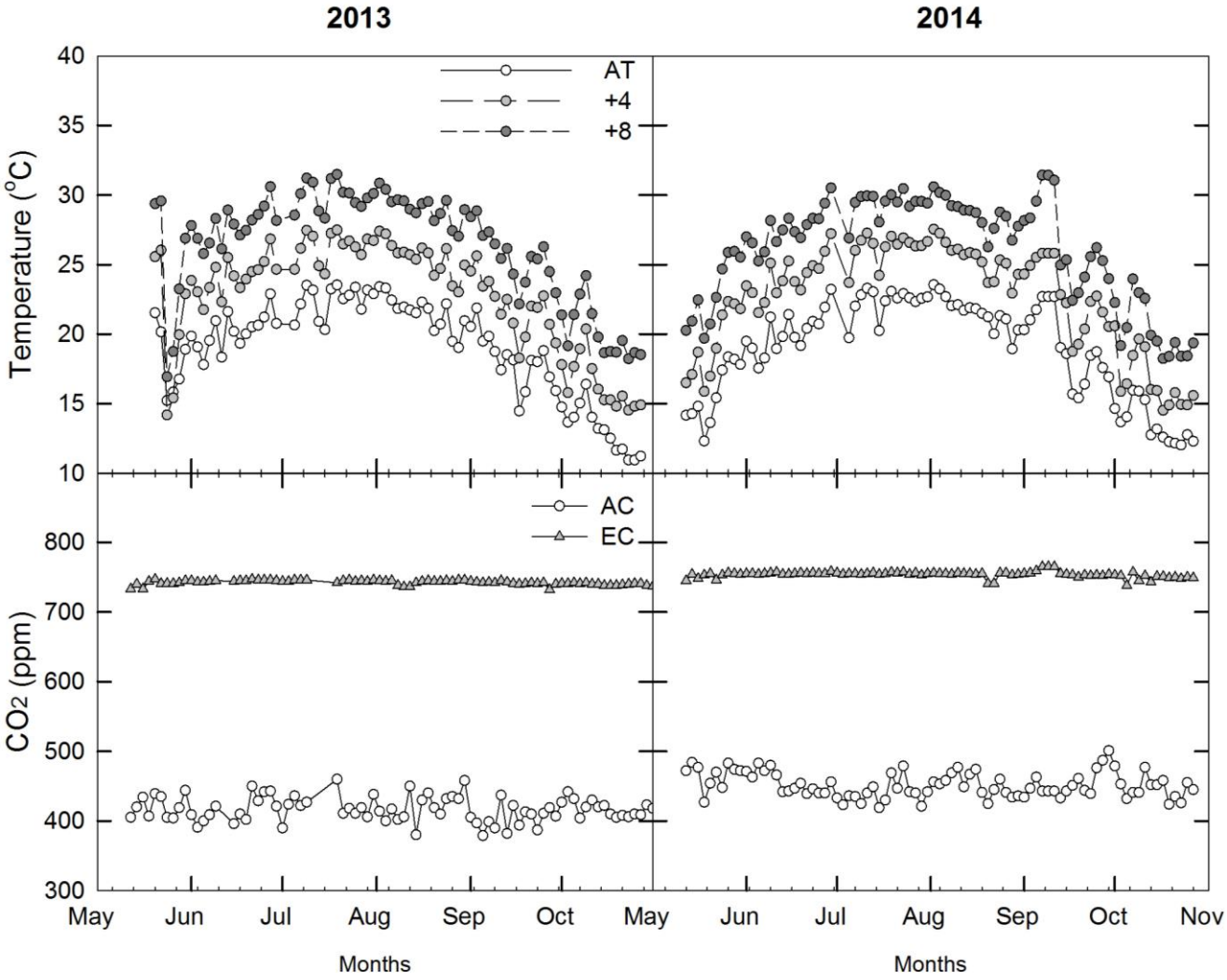

Figure 3.1 2013 and 2014 growth temperature and growth CO_2 conditions for Norway spruce seedlings. AT- ambient temperature, +4- ambient temperature +4 ^{o}C, +8- ambient temperature + 8 ^{o}C. AC- ambient CO_2, EC- elevated CO_2. Data are means of two rooms for each temperature treatment and of three rooms for each CO_2 treatment.

Table 3.1 Mortality rates (% of trees) of Norway spruce seedlings grown at a variety of growth temperatures and CO_2 concentrations in 2013 and 2014. AT-ambient temperature, +4-ambient temperature+4 ^{o}C, +8-ambient temperature + 8 ^{o}C. AC-ambient CO_2, EC-elevated CO_2. Letters indicate significant differences of means within a year.

Treatment	**2013**	**2014**
Chi-square test	**(p=0.1)**	**(p<0.001)**
AT/AC	33% (a)	0.05%(bc)
+4/AC	65% (a)	0% (c)
+8/AC	72% (a)	20% (ab)
AT/EC	40% (a)	0.03% (c)
+4/EC	47% (a)	0.03% (c)
+8/EC	78% (a)	26% (a)

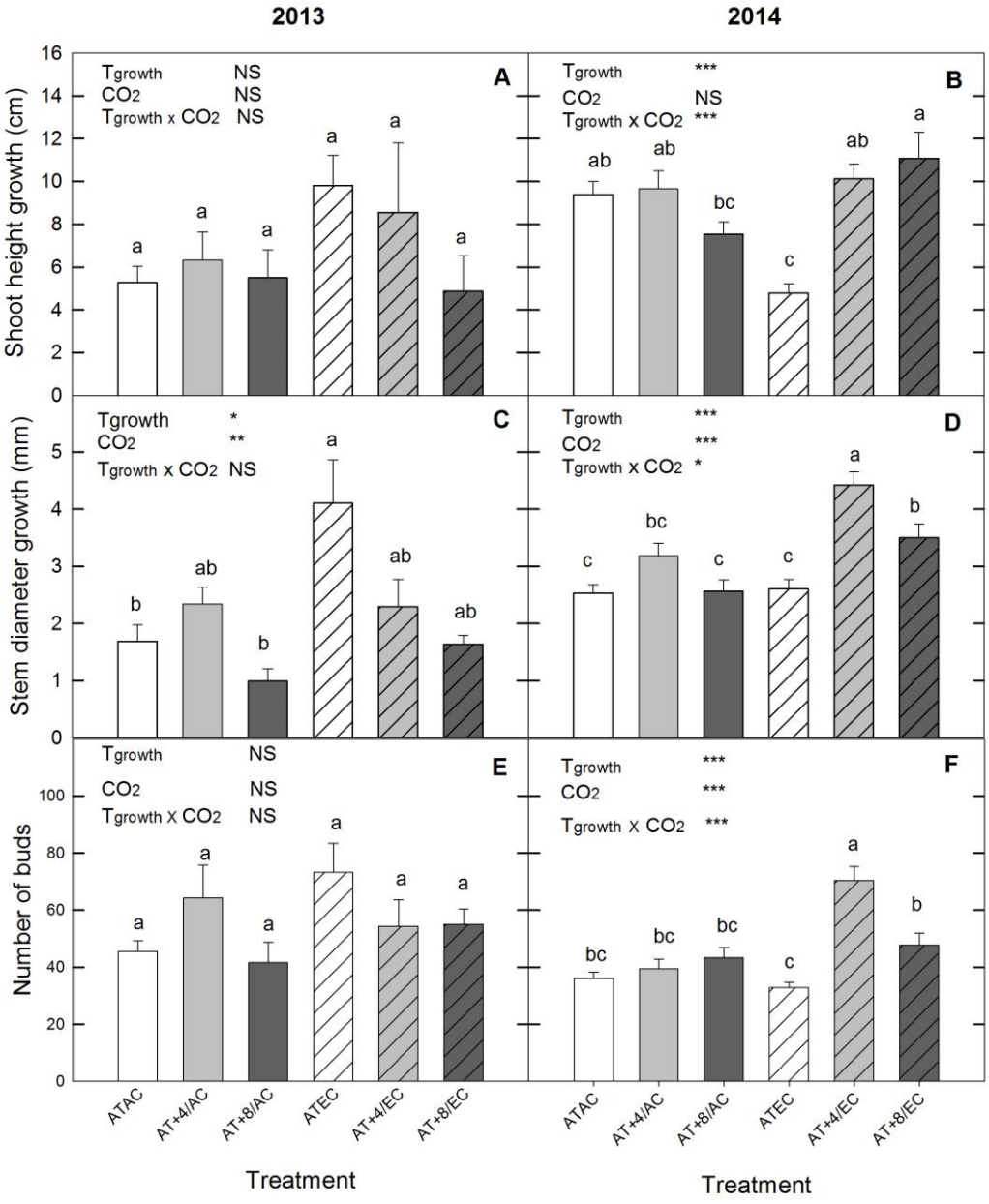

Figure 3.2 Seedling shoot height and stem diameter growth (where growth represents the difference between the initial and final seedling size), and bud set of Norway spruce seedlings grown at a variety of growth temperatures and CO_2 concentrations in 2013 and 2014. Means ± SE, n=4-11 (2013), n=26-35 (2014). Different lower-case letters indicate a significant difference between treatments. AT-ambient temperature, +4-ambient temperature +4 ^{o}C, +8-ambient temperature + 8 ^{o}C. AC-ambient CO_2, EC-elevated CO_2.

3.3 Light-saturated photosynthesis (A_{sat})

As there was no significant interaction of year with T_{growth} or CO_2 (Table S1) for A_{sat}, the data were combined for both seasons (Fig 3.3). A_{sat} was highly dependent on T_{leaf}, rising with increasing T_{leaf} up to a maximum value at the thermal optimum (the leaf temperature where the net CO_2 assimilation rate was highest) in every treatment (Table 3.2; Fig. 3.3). A_{sat} from all the treatments was lowest at a T_{leaf} of 40 ^{o}C (Fig. 3.3). In AC treatments, the highest A_{sat} was observed in the trees from the AT treatment, but in EC treatments, A_{sat} in the AT trees was lower compared to those from the +4 ^{o}C treatment. Regardless of the CO_2 treatment, trees from +8 ^{o}C had the lowest A_{sat} between 10 and 30 ^{o}C (Table 3.2; Fig. 3.3). Above 30 ^{o}C, A_{sat} was similar in all the AC trees; in EC trees, A_{sat} was lowest in +8 ^{o}C trees and highest in +4 ^{o}C trees at these high T_{leaf} values (Fig. 3.3).

The thermal optima for A_{sat} were higher in 2014 than in 2013 (Table 3.3; p=0.005). Thermal optima of A_{sat} shifted to higher values as T_{growth} increased, but the effect of T_{growth} was more pronounced in 2013 ($p<0.001$), than in 2014 (p=0.09). Growth CO_2 had no effect on the thermal optima of A_{sat} in 2013 (p=0.18) or 2014 (p=0.77), and there was no interaction of T_{growth} x CO_2 (2013: p=0.96, 2014: p=0.68).

There was no significant interaction of year with T_{growth} or CO_2 (Table S2) for stomatal conductance (g_s), so the data were combined for both seasons (Fig. 3.4). No significant difference was observed across low, medium and high light intensities ($p>0.05$, Fig. 3.4). At 10 ^{o}C in both years, g_s was 50% higher in AT treatments compared to +8 ^{o}C (T_{growth} effect: p=0.032). Leaf g_s increased with increasing T_{leaf}, peaking around 20 ^{o}C and reaching their lowest values at 40 ^{o}C (Table 3.4; T_{leaf} effect: $p<0.001$). The interaction of T_{leaf} x T_{growth} on g_s was significant ($p<0.001$), but growth CO_2 had no effect on g_s (p=0.87, Table 3.4).

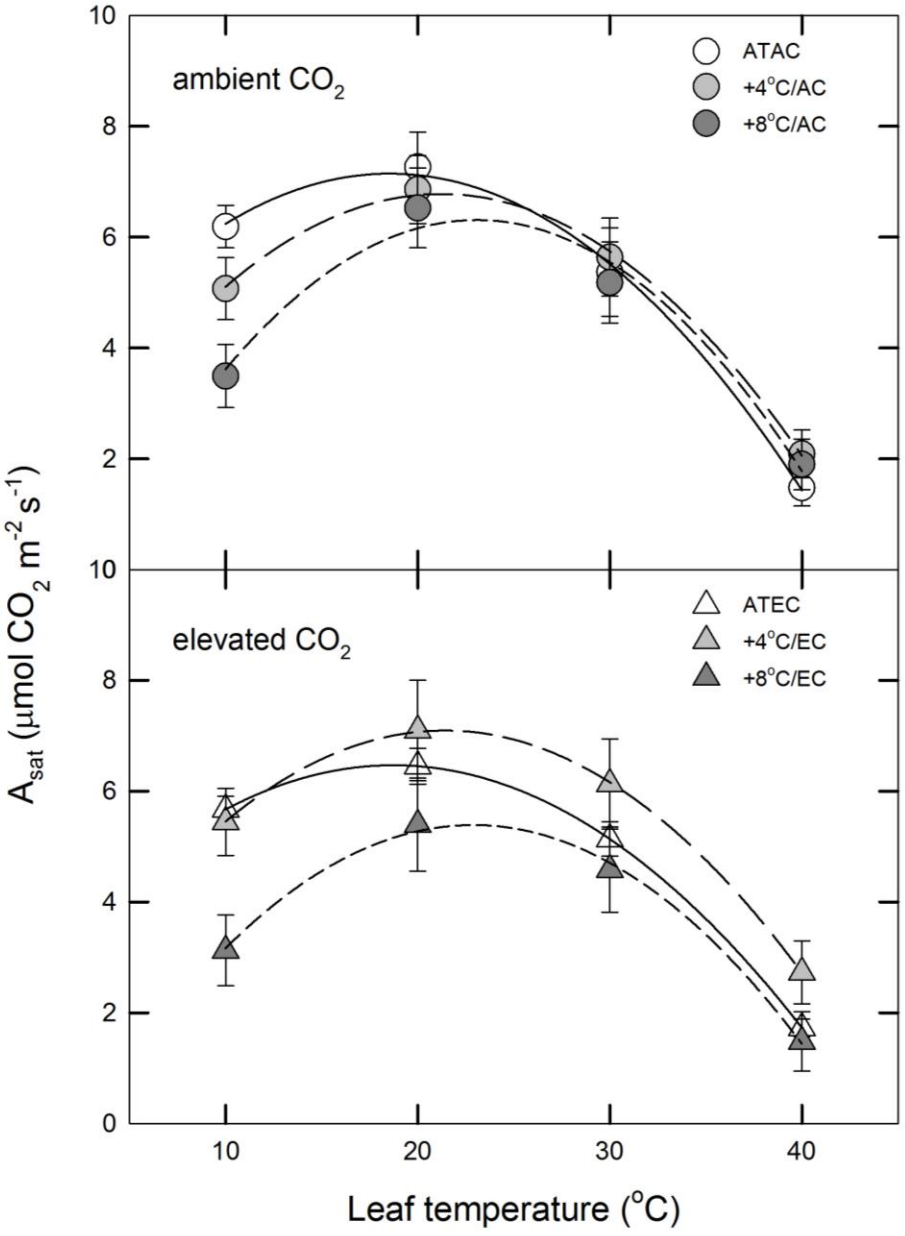

Figure 3.3 Light-saturated net CO_2 assimilation rates (A_{sat}) measured as a function of leaf temperature of Norway spruce seedlings grown at a variety of growth temperatures and CO_2 concentrations in 2013 and 2014. Means ± SE, n=9. AT-ambient temperature, +4-ambient temperature +4 ^{o}C, +8-ambient temperature + 8 ^{o}C. AC-ambient CO_2, EC-elevated CO_2.

Table 3.2 Repeated measures two-way ANOVA for changes in light-saturated photosynthesis (A_{sat}) of Norway spruce seedlings to changes in growth CO_2, growth temperature (T_{growth}), and leaf temperature (T_{leaf}), for the combined 2013 and 2014 growth seasons. Significant results are bolded.

	Df	**Sum of Squares**	**F Ratio**	**p-value**
CO_2	1	1.63	0.46	0.5
T_{growth}	2	55.8	10.68	**0.00015**
T_{leaf}	3	640.71	452.97	**<0.0001**
CO_2 X T_{growth}	2	10.8	0.07	0.94
CO_2 X T_{leaf}	3	3.65	1.2	0.31
T_{growth} X T_{leaf}	6	38.37	7.91	**<0.0001**
CO_2 X T_{growth} X T_{leaf}	6	1.17	0.25	0.96

Table 3.3 Thermal optima of A_{sat} (in degrees ^{o}C), Q_{10} of R_{dark} and Q_{10} of R_{light} of Norway spruce seedlings grown at a variety of growth temperatures and CO_2 concentrations in 2013 and 2014. Means ± SE, n=4 (2013), n=5 (2014). Different letters indicate significant difference within a year (Tukey's Post-Hoc test). AT-ambient temperature, +4-ambient temperature +4 ^{o}C, +8-ambient temperature + 8 ^{o}C. AC-ambient CO_2, EC-elevated CO_2.

Thermal optima	**2013**	**2014**
ATAC	11.5±1.9 (c)	19.8±1.2 (a)
+4/AC	19.4±3.7 (ac)	21±0.9 (a)
+8/AC	21±1 (ab)	23.7±0.6 (a)
ATEC	14.3±2.3 (bc)	20.1±0.7 (a)
+4/EC	21.1±1.5 (ab)	21.3±0.9 (a)
+8/EC	23.7±0.76 (a)	22±2.4 (a)
Q_{10} R_{dark}		
ATAC	1.9±0.07 (a)	1.9±0.09 (a)
+4/AC	1.8±0.07 (a)	2±0.03 (a)
+8/AC	2.1±0.19 (a)	1.9±0.1 (a)
ATEC	2.1±0.14 (a)	2±0.02 (a)
+4/EC	2±0.06 (a)	2±0.07 (a)
+8/EC	2±0.09 (a)	2±0.07 (a)
Q_{10} R_{light}		
ATAC	2.6±0.23 (a)	2.4±0.2 (a)
+4/AC	2.8±0.2 (a)	2.6±0.27 (a)
+8/AC	2.9±0.39 (a)	2.3±0.25 (a)
ATEC	3±0.21 (a)	2.7±0.12 (a)
+4/EC	2.8±0.28 (a)	2.6±0.18 (a)
+8/EC	2.7±0.4 (a)	2.9±0.41 (a)

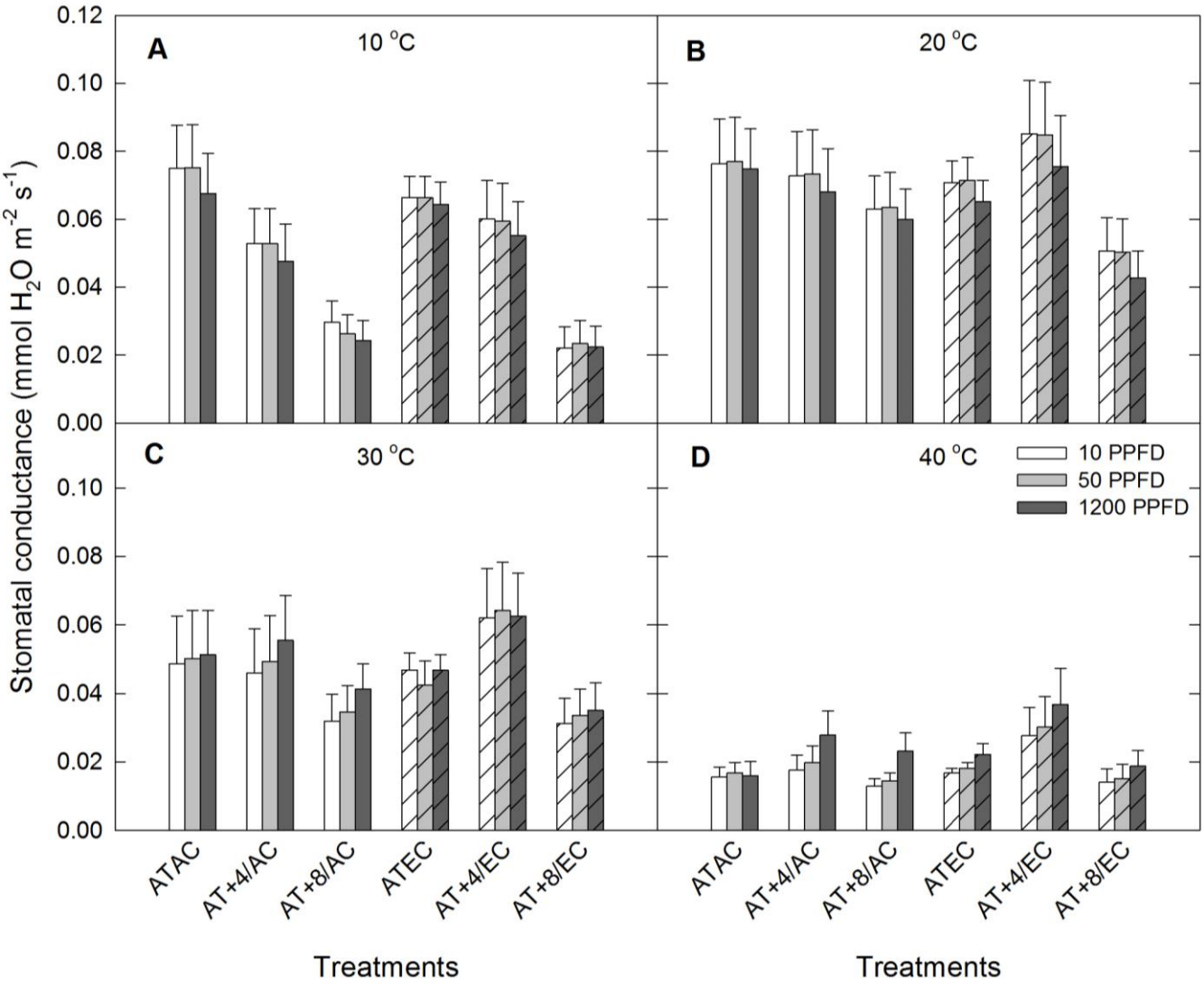

Figure 3.4 Stomatal conductance measured at 10, 50 and 1200 photosynthetic photon flux density (PPFD) of Norway spruce seedlings grown at a variety of growth temperatures and CO_2 concentrations in 2013 and 2014. Means ± SE, n=9. AT-ambient temperature, +4-ambient temperature +4 ^{o}C, +8-ambient temperature + 8 ^{o}C. AC-ambient CO_2, EC-elevated CO_2.

Table 3.4 Repeated measures two-way ANOVA for changes in stomatal conductance (g_s), measured at 1200 photosynthetic photon flux density, of Norway spruce seedlings to changes in growth CO_2, growth temperature (T_{growth}), and leaf temperature (T_{leaf}) in 2013 and 2014 growth seasons. Significant results are bolded.

	Df	Sum of Squares	F Ratio	p-value
CO_2	1	0.00001	0.02	0.87
T_{growth}	2	0.021	3.7	**0.032**
T_{leaf}	3	0.043	62.1	**<0.0001**
CO_2 X T_{growth}	2	0.0023	0.47	0.63
CO_2 X T_{leaf}	3	0.0006	0.86	0.46
T_{growth} X T_{leaf}	6	0.0093	6.71	**<0.0001**
CO_2 X T_{growth} X T_{leaf}	6	0.0004	0.26	0.95

3.4 Light-stimulated dark respiration (R_{dark})

There was no significant difference between the two years in R_{dark}, nor any interactions of year x CO_2 or year x T_{growth} (Table S3), so data were combined for the 2013 and 2014 growth seasons (Fig. 3.5). R_{dark} increased exponentially as T_{leaf} increased in all treatments ($p<0.001$; Fig. 3.5). Trees from +4 ^{o}C and +8 ^{o}C treatments had lower R_{dark} than trees grown in ambient temperatures ($p<0.05$), and growth CO_2 had no effect on R_{dark} ($p=0.2$). Trees grown in +8 ^{o}C had the lowest R_{dark} compared to other T_{growth} treatments.

Table 3.5 Repeated measures two-way ANOVA for combined 2013 and 2014 growth seasons results; response of light-stimulated dark respiration rates (R_{dark}) of Norway spruce seedlings to growth CO_2, growth temperature (T_{growth}), and leaf temperature (T_{leaf}). Significant results are bolded.

	Df	Sum of Squares	F Ratio	p-value
CO_2	1	0.19	0.46	0.5
T_{growth}	2	8.86	10.68	**0.00015**
T_{leaf}	3	112.8	452.97	**<0.0001**
CO_2 X T_{growth}	2	0.05	0.07	0.94
CO_2 X T_{leaf}	3	0.3	1.2	0.31
T_{growth} X T_{leaf}	6	3.94	7.91	**<0.0001**
CO_2 X T_{growth} X T_{leaf}	6	0.12	0.25	0.96

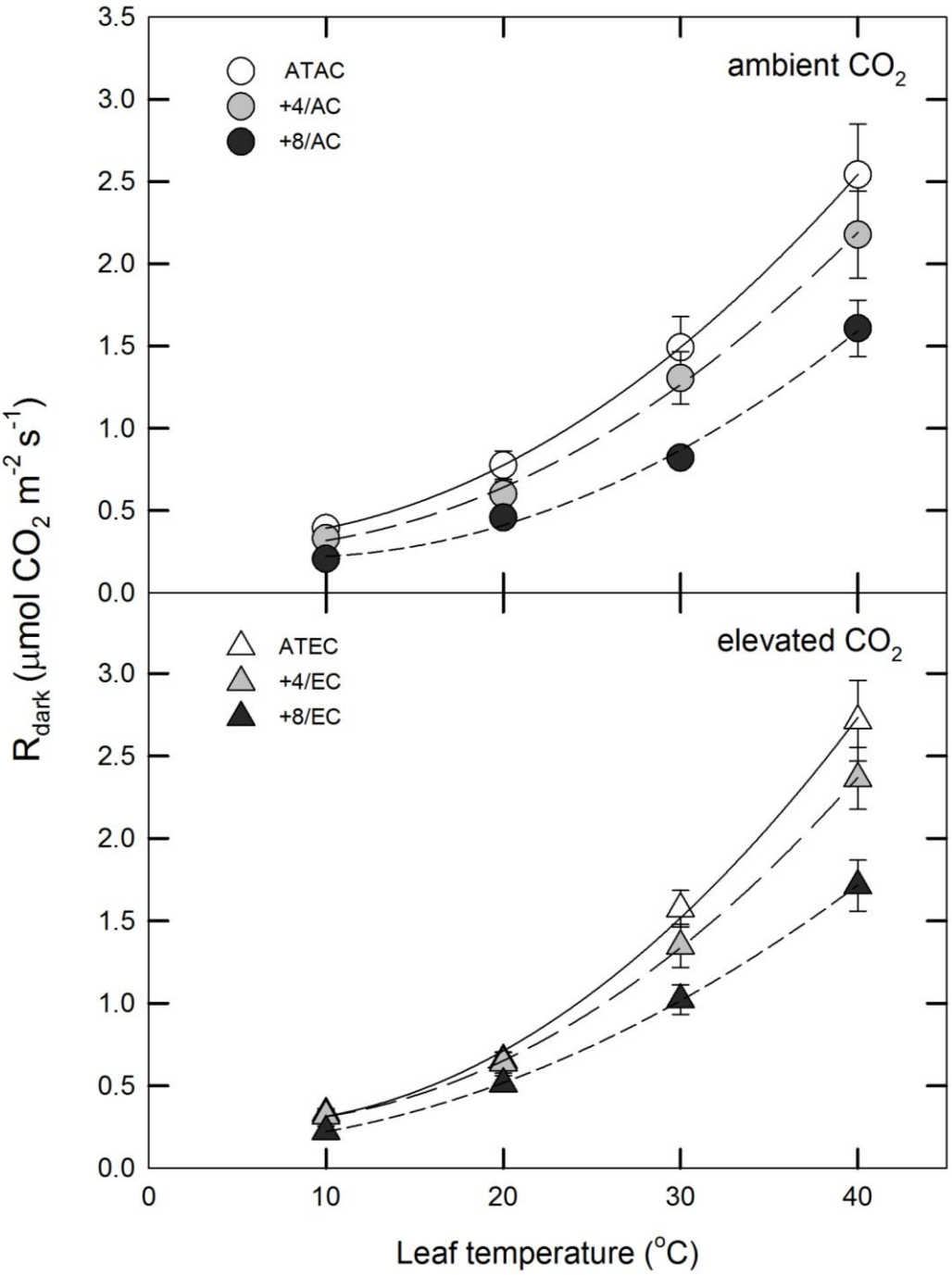

Figure 3.5 Light-stimulated dark respiration rates (R_{dark}) measured as a function of leaf temperature of Norway spruce seedlings grown at a variety of growth temperatures and CO_2 concentrations in 2013 and 2014. Means ± SE, n=9. AT-ambient temperature, +4-ambient temperature +4 ^{o}C, +8-ambient temperature + 8 ^{o}C. AC-ambient CO_2, EC-elevated CO_2.

3.5 Light respiration (R_{light}) measurements

The interaction of year x T_{growth} was significant for R_{light} (Table S4), so R_{light} data are presented separately for both years (Fig. 3.6). Some measurements at a T_{leaf} of 10 ^{o}C were excluded from the analysis due to negative values at low light intensities. The negative values may have been due to low CO_2 flux rates that are within the error of the Li-Cor gas exchange apparatus; they were not due to stomatal closure, as g_s at 10 μmol photons m^{-2} s^{-1} was equal to, or even slightly higher than, those measured at 1200 μmol photons m^{-2} s^{-1} (Fig. 3.4).

Similar to R_{dark}, R_{light} increased as T_{leaf} increased ($p<0.001$; Table 3.6 and 3.7). Trees grown at ambient temperatures had higher R_{light} than trees grown at warmer temperatures. The effect of T_{growth} and the interaction of T_{growth} x T_{leaf} was significant in 2013 ($p<0.001$; Fig. 3.6A and 3.6C) indicating significant acclimation of R_{light} to high growth temperatures. In 2014, although trees grown at +8 ^{o}C appeared to have lower R_{light} compared to other growth temperatures, the effect of T_{growth} was not significant ($p=0.18$). Trees grown in elevated CO_2 had lower R_{light} than trees grown in ambient CO_2 in 2014 (CO_2 effect: $p=0.012$, CO_2 x T_{leaf}:, $p=0.003$; Fig. 3.6B and 3.6D). The response of R_{light} to T_{growth} and CO_2 therefore varied across the two growing seasons.

3.6 R_{light} : R_{dark} ratio as a function of $T_{leaf,}$ and R_{light} to R_{dark} relationship

The Q_{10} values for R were similar in both years (R_{light}: $p=0.25$; R_{dark}: $p=0.47$), and there was no significant treatment effect on the Q_{10} ($p>0.1$, Table 3.3). The Q_{10} of R_{light} was about 35%

higher than the Q_{10} of R_{dark} (Q_{10} of R_{dark} = 2 ± 0.03; Q_{10} of R_{light} = 2.7 ± 0.08, Table 3.3, $p<0.001$).

Because the Q_{10} of R_{light} was larger than that of R_{dark}, the ratio of R_{light}/R_{dark} increased as T_{leaf} increased. The R_{light}:R_{dark} ratio was >1 in AT and +4 ^{o}C treatments at the highest T_{leaf} values in 2013 (Fig. 3.7A and 3.7C), and in +8^{o}C/EC in 2014 (Fig. 3.7D), which means that R_{light} was not suppressed by the light in those cases, but was instead enhanced. When R_{light} was plotted versus R_{dark}, a strong relationship was seen (R^2=0.95; $p<0.001$; Fig. 3.8) regardless of the treatment or year.

3.7 A_{sat} : R_{dark} ratio as a function of T_{leaf}

To determine the CO_2 fraction lost during R_{dark} from A_{sat}, as an index of how much fixed carbon was available for processes other than respiration, I plotted A_{sat} : R_{dark} ratio as a function of T_{leaf}. Ratios of A_{sat} to R_{dark} exhibited a linear decrease as T_{leaf} increased in all the treatments in both years (Fig. 3.9; $p<0.001$). The ratio of A_{sat}:R_{dark} was higher from 10 to 30 ^{o}C in 2014 than in 2013 (T_{leaf} x year effect: p=0.002). There was no effect of either growth CO_2, or T_{growth} treatments on A_{sat}/R_{dark} ($p>0.1$; Fig. 3.9).

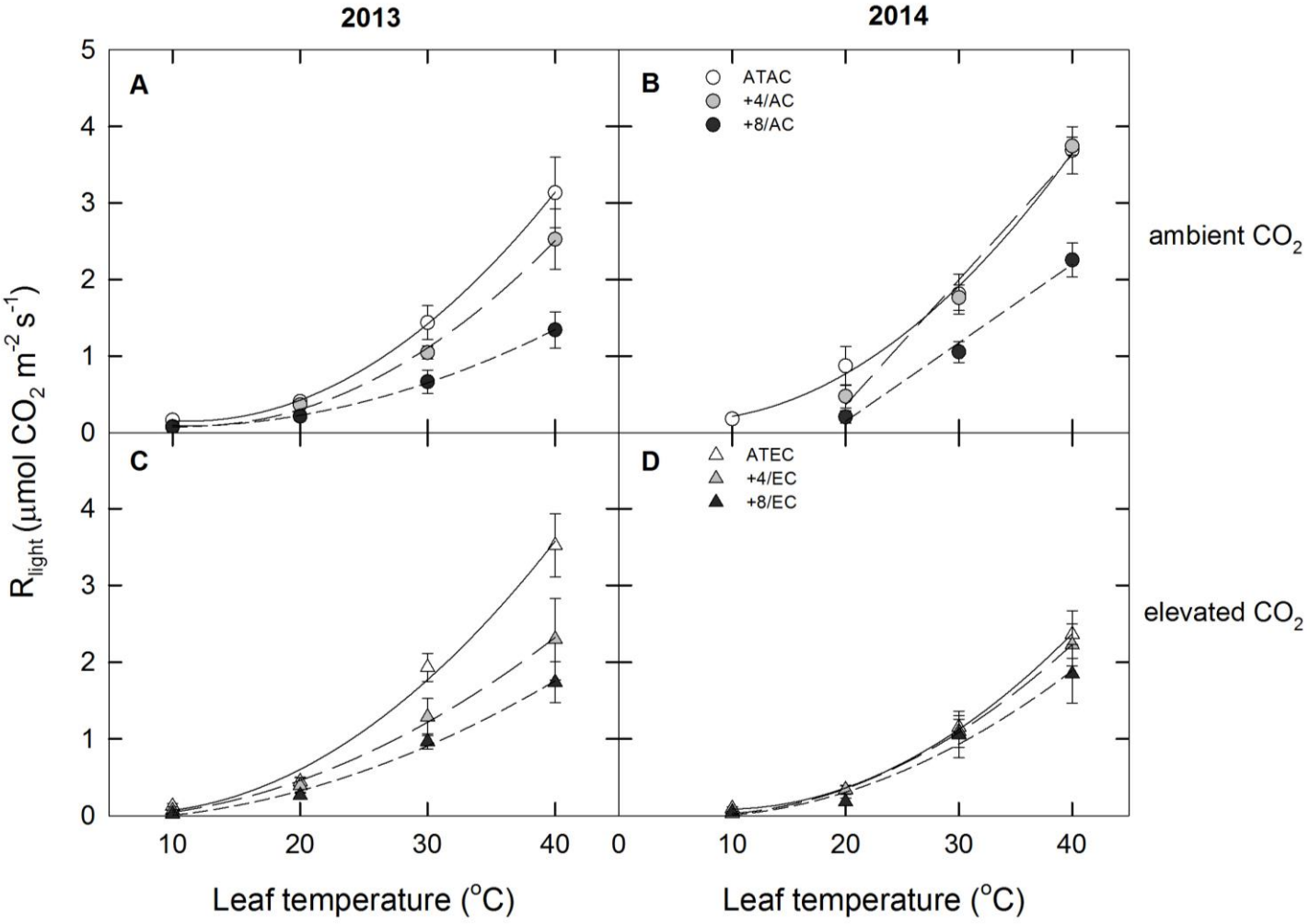

Figure 3.6 Light respiration rates **(**R_{light}) as a function of leaf temperature measured in Norway spruce seedlings grown at a variety of growth temperatures and CO_2 concentrations in 2013 and 2014. Means ± SE, n=4, except for 10 ^{o}C where n=1-4 (2013); n=5 (2014), except for 10 ^{o}C where n=1-4 (no data points for +4/AC and +8/AC), and except for 20 ^{o}C where n=3-5. AT-ambient temperature, +4-ambient temperature +4 ^{o}C, +8-ambient temperature + 8 ^{o}C. AC-ambient CO_2, EC-elevated CO_2.

Table 3.6 Repeated measures two-way ANOVA results for 2013 growth season of Norway spruce seedlings; response in light respiration rates (R_{light}) to changes in growth CO_2, growth temperature (T_{growth}), and leaf temperature (T_{leaf}). Significant results are bolded.

	Df	Sum of Squares	F Ratio	p-value
CO_2	1	0.56	2.11	0.16
T_{growth}	2	8.5	16.01	**0.0001**
T_{leaf}	3	84.52	161.76	**<0.0001**
CO_2 X T_{growth}	2	0.28	0.53	0.6
CO_2 X T_{leaf}	3	0.38	0.72	0.54
T_{growth} X T_{leaf}	6	7.55	7.22	**0.00001**
CO_2 X T_{growth} X T_{leaf}	6	0.34	0.32	0.92

Table 3.7 Repeated measures two-way ANOVA results for 2014 growth season of Norway spruce seedlings; response in light respiration rates (R_{light}) to changes in growth CO_2, growth temperature (T_{growth}), and leaf temperature (T_{leaf}). No measurements for T_{leaf} = 10 ^{o}C included. Significant results are bolded.

	Df	Sum of Squares	F Ratio	p-value
CO_2	1	3.16	6.54	**0.017**
T_{growth}	2	1.41	1.46	0.25
T_{leaf}	2	45.76	219.89	**<0.0001**
CO_2 X T_{growth}	2	0.56	0.57	0.57
CO_2 X T_{leaf}	2	1.13	5.44	**0.007**
T_{growth} X T_{leaf}	4	0.14	0.34	0.85
CO_2 X T_{growth} X T_{leaf}	4	0.45	1.08	0.38

Table 3.8 Repeated measures two-way ANOVA results for the response in R_{light}/R_{dark} to changes in growth CO_2, growth temperature (T_{growth}), and leaf temperature (T_{leaf}) of Norway spruce seedlings in 2013. Significant results are bolded.

	DF	Sum of Squares	F Ratio	p-value
CO_2	1	0.94	6.18	**0.02**
T_{growth}	2	0.022	0.07	0.9
T_{leaf}	2	5.66	87.29	**<0.0001**
CO_2 X T_{growth}	2	0.36	1.2	0.32
CO_2 X T_{leaf}	2	0.013	0.2	0.82
T_{growth} X T_{leaf}	4	0.71	5.46	**0.001**
CO_2 X T_{growth} X T_{leaf}	4	0.27	2.12	0.093

Table 3.9 Repeated measures two-way ANOVA results for the response in R_{light}/R_{dark} to changes in growth CO_2, growth temperature (T_{growth}), and leaf temperature (T_{leaf}) of Norway spruce seedlings in 2014. No measurements for $T_{leaf} = 10\ ^{o}C$ included. Significant results are bolded.

	DF	Sum of Squares	F Ratio	p-value
CO_2	1	1.1	6.85	**0.015**
T_{growth}	2	0.011	0.036	0.96
T_{leaf}	2	6.2	81.25	**<0.0001**
CO_2 X T_{growth}	2	0.42	1.32	0.29
CO_2 X T_{leaf}	2	0.006	0.07	0.93
T_{growth} X T_{leaf}	4	0.83	5.43	**0.0011**
CO_2 X T_{growth} X T_{leaf}	4	0.21	1.4	0.25

Ratios of A_{sat} to R_{dark} decreased as T_{leaf} increased in all the treatments in both years ($p<0.001$). The ratio of A_{sat}:R_{dark} was higher at 10, 20, and 30 ^{o}C in 2014 than in 2013 (T_{leaf} x year effect: $p=0.002$). There was no effect of either growth CO_2, or T_{growth} treatments on A_{sat}/R_{dark} ($p>0.1$; Fig. 3.9) A_{sat}:R_{dark} ratio for all the treatments and both years plotted vs T_{leaf} demonstrated strong relationship (Fig. 3.9; $R^2=0.96$; $p<0.001$).

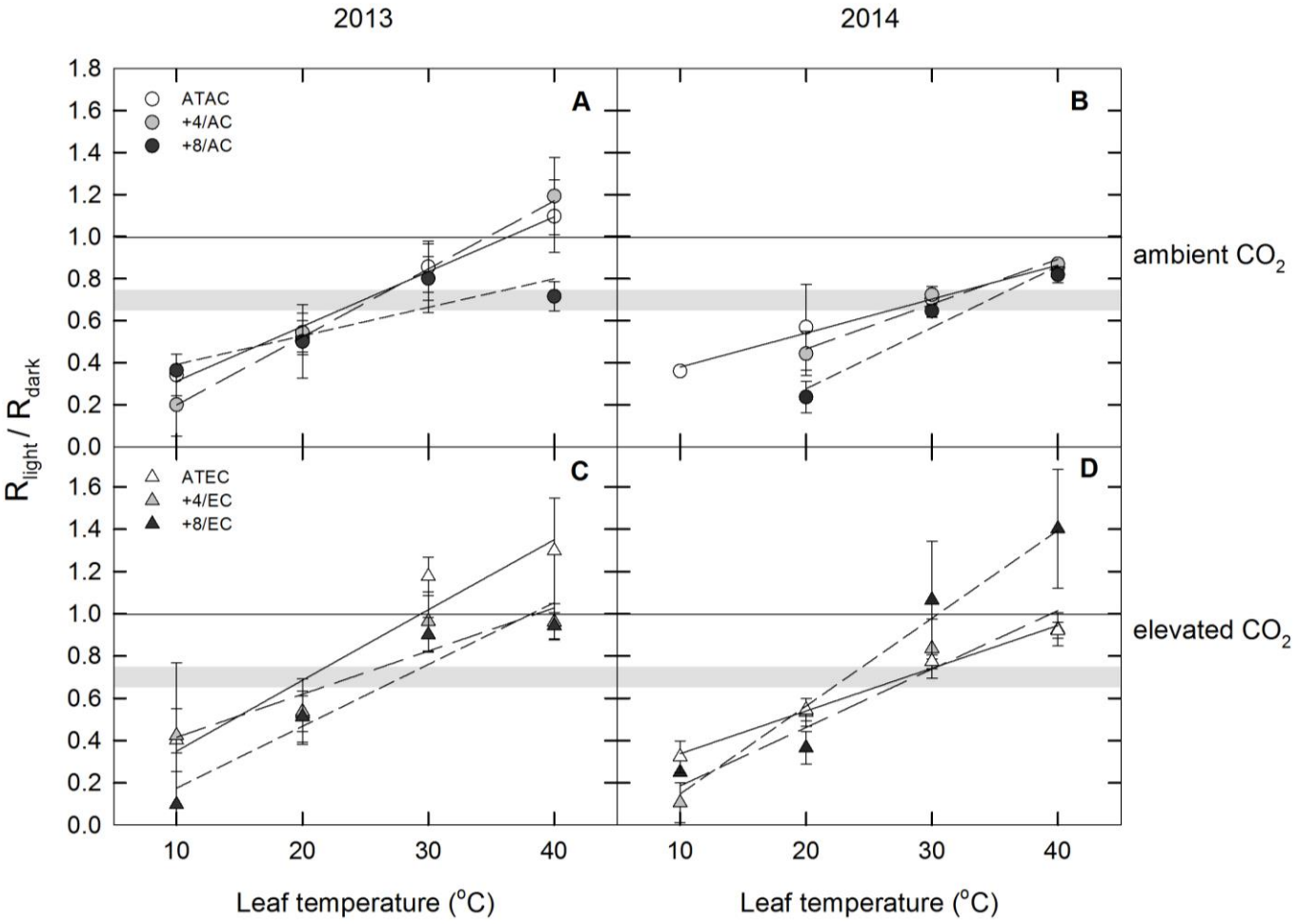

Figure 3.7 The ratio of light respiration to dark respiration (R_{light}/ R_{dark}) as a function of leaf temperature measured in Norway spruce seedlings grown at a variety of growth temperatures and CO_2 concentrations in 2013 and 2014. Means ± SE, n=4 (2013), except for 10 ^{o}C where n=1-4; n=5 (2014), except for 10 ^{o}C where n=1-4 (no data points for +4/AC and +8/AC), and 20 ^{o}C where n=3-5. AT-ambient temperature, +4-ambient temperature +4 ^{o}C, +8-ambient temperature + 8 ^{o}C. AC-ambient CO_2, EC-elevated CO_2. Solid horizontal line indicates when R_{light} equals to R_{dark}; the grey area shows where R_{light}/R_{dark} equals to 0.7.

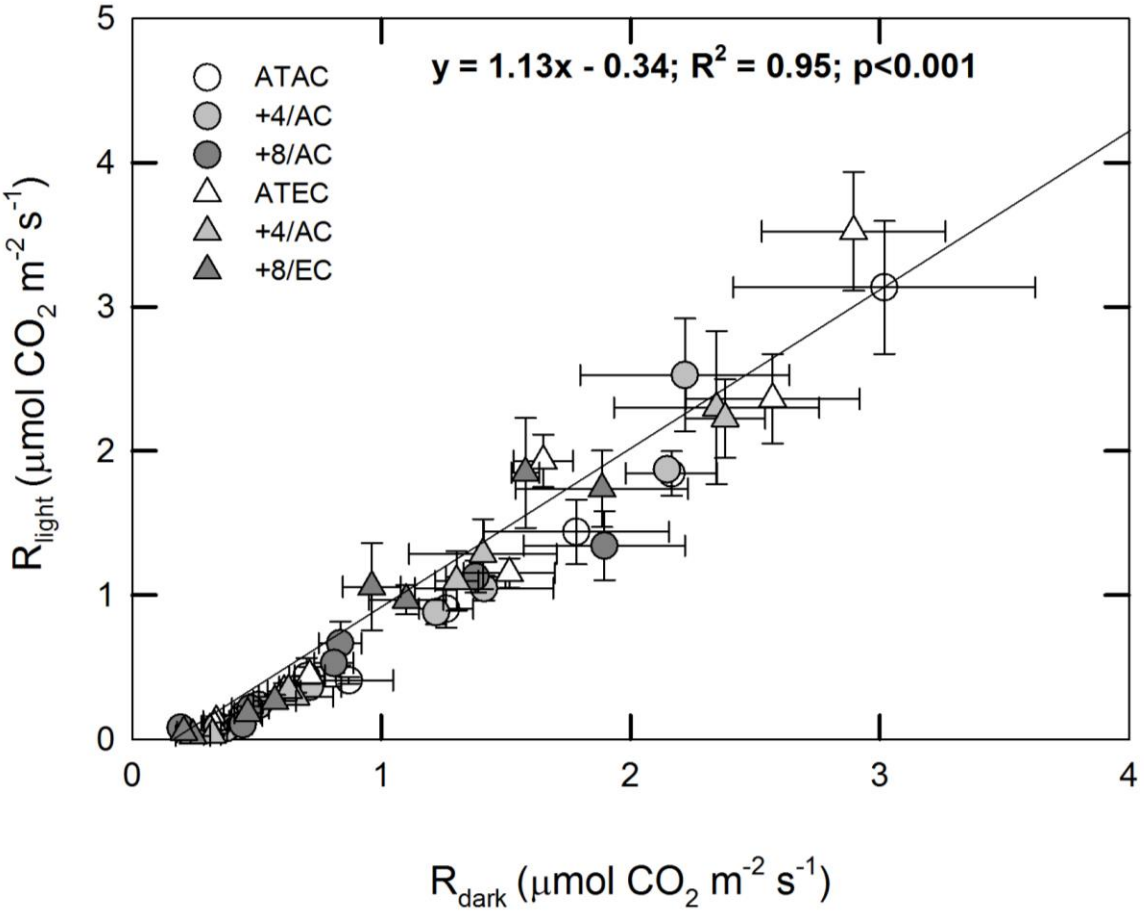

Figure 3.8 Light respiration (R_{light}) versus light-stimulated dark respiration (R_{dark}) in Norway spruce seedlings grown at a variety of growth temperatures and CO_2 concentrations in 2013 and 2014. Means ± SE, n=4 (2013), except for 10 ^{o}C where n=1-4; n=5 (2014), except for 10 ^{o}C where n=1-4 (no data points for +4/AC and +8/AC), and 20 ^{o}C where n=3-5. AT-ambient temperature, +4-ambient temperature +4 ^{o}C, +8-ambient temperature + 8 ^{o}C. AC-ambient CO_2, EC-elevated CO_2.

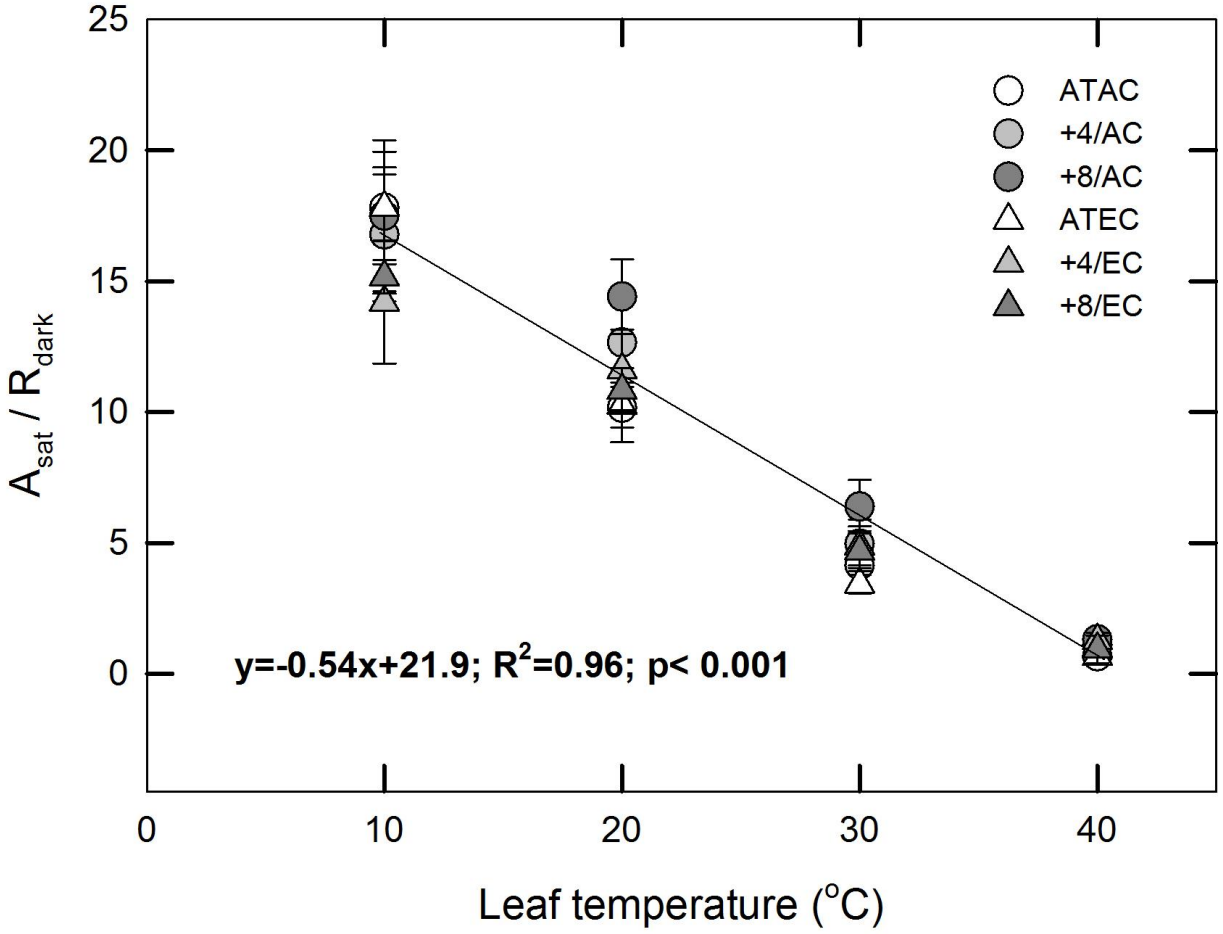

Figure 3.9 The ratio of light-saturated net CO_2 assimilation rate (A_{sat}) to light-stimulated dark respiration (R_{dark}) as a function of leaf temperature measured in Norway spruce seedlings grown at a variety of growth temperatures and CO_2 concentrations in 2013 and 2014. Means ± SE, n=9 (2013 and 2014). AT-ambient temperature, +4-ambient temperature +4 ^oC, +8-ambient temperature + 8 ^oC. AC-ambient CO_2, EC-elevated CO_2.

3.8 CO_2 assimilation in trees grown in 11.3 and 100 liter soil volumes

Trees grown in 11.3 L pots had a higher V_{cmax} ($p=0.037$) than trees grown in 100 L containers, and J_{max} values were not significantly different between pots and containers ($p=0.27$), indicating that there was no evidence for CO_2-induced down-regulation of photosynthesis. Across all the growth temperatures, there was no significant interaction of T_{growth} x soil volume effect on either V_{cmax} or J_{max} ($p> 0.05$; Fig. 3.10).

3.9 Biochemical and anatomical leaf traits

The percentage of total leaf non-structural carbohydrates (NSC = glucose, fructose, sucrose and starch) varied between years, such that trees from 2014 had higher NSC than trees from 2013. However, there was no significant interaction of year x CO_2 or year x T_{growth} on NSC concentrations ($p>0.05$, Table S6), so the data were combined for both years (Table 3.11). The NSC concentration in leaves was not significantly different between treatments, as neither CO_2 nor growth temperature significantly affected NSC concentrations (Tables 3.10 and 3.11).

There was no significant interaction of year x CO_2 or year x T_{growth} on leaf %N ($p=0.53$, $p=0.95$, Table S7), so the data were combined for 2013 and 2014. The %N in dry leaf mass was generally similar across treatments, but %N was lower in +8 ^oC trees, in both CO_2 treatments, and lower in EC than AC trees (T_{growth} effect: $p=0.002$, CO_2 effect: $p=0.007$, Tables 3.11 and 3.12).

Year had no effect on specific leaf area (SLA) ($p=0.14$), and SLA did not vary across treatments ($p>0.1$, Table 3.11).

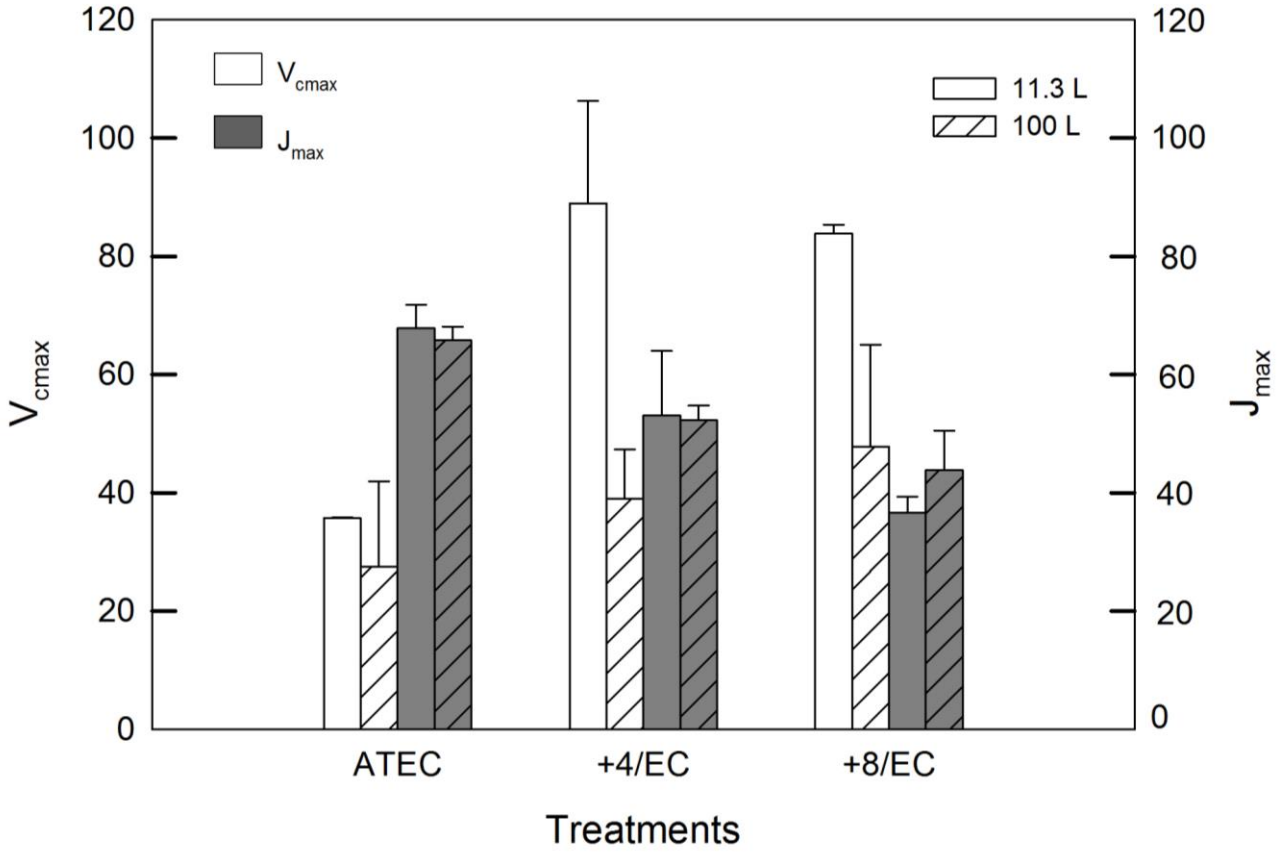

Figure 3.10 V_{cmax} and J_{max} measured at T_{leaf} of 25 ^{o}C in Norway spruce seedlings grown in 11.3 L and 100 L soil volume across three growth temperatures and elevated CO_2 concentrations. Means ± SD, n=2. AT-ambient temperature, +4-ambient temperature +4 ^{o}C, +8-ambient temperature + 8 ^{o}C. EC-elevated CO_2.

Table 3.10 Two-way ANOVA results for changes in non-structural carbohydrates of Norway spruce trees to changes in growth CO_2 and growth temperature (T_{growth}) for 2013 and 2014. Significant results are bolded.

	DF	Sum of Squares	F Ratio	p-value
CO_2	1	0.94	0.601	0.49
T_{growth}	2	2.27	0.724	0.442
T_{growth} X CO_2	2	0.85	0.27	0.764

Table 3.11 Biochemical and anatomical leaf traits. Total non-structural carbohydrates (% per dry leaf mass, %NSC), nitrogen concentration per dry leaf mass (%N), and specific leaf area (SLA) of Norway spruce seedlings grown at a variety of growth temperatures and CO_2 concentrations in 2013 and 2014.Different letters indicate significant differences (Tukey's Post-Hoc test). AT-ambient temperature, +4-ambient temperature +4 ^{o}C, +8-ambient temperature + 8 ^{o}C. AC-ambient CO_2, EC-elevated CO_2.

Treatment	**%NSC**	**%N**	**SLA (cm^2 g^{-1})**
ATAC	2.18±0.52 (a)	2.5±0.12 (a)	107±10.8 (a)
+4/AC	2.03±0.34 (a)	2.56±0.05 (a)	108±13.3 (a)
+8/AC	2.0±0.31 (a)	2.13±0.07 (b)	101±6.8 (a)
ATEC	2.78±0.46 (a)	2.31±0.11 (ab)	89±6.8 (a)
+4/EC	2.23±0.47 (a)	2.2±0.08 (ab)	120±11.1 (a)
+8/EC	1.99±0.36 (a)	2.0±0.11 (b)	93±6.5 (a)

Table 3.12 Two-way ANOVA results for 2013 and 2014 growth seasons of change in leaf nitrogen concentrations of Norway spruce trees to changes in growth CO_2 and growth temperature (T_{growth}). Significant results are bolded.

	DF	Sum of Squares	F Ratio	p-value
CO_2	1	0.675	7.931	**0.00703**
T_{growth}	2	1.239	7.277	**0.00174**
T_{growth} X CO_2	2	0.128	0.75	0.4778

Needle length decreased as T_{growth} increased (Table 3.18; $p<0.001$, but CO_2 had no effect on needle length ($p=0.12$). The cross-sectional area of needles decreased as T_{growth} increased ($p<0.001$) in both ambient and elevated CO_2 treatments, but CO_2 had no effect ($p=0.69$; Fig. 3.11; Table 3.13). In trees grown in ambient CO_2, cross-sectional leaf area was 130 % greater in AT trees than in +4 ^{o}C and +8^{o} C trees; EC trees demonstrated a similar pattern, with a more gradual, but not statistically significant decrease (24-56 %) in cross-sectional area from ambient to warmer T_{growth} treatments (T_{growth} x CO_2 effect: $p<0.001$).

The highest mitochondrial cross-sectional area was also found in trees grown in ambient temperatures ($p<0.001$). Mitochondria occupied between 7-10% of the cross-sectional leaf area in AT and +4 ^{o}C treatments, which is less than the 12% occupied in the +8 ^{o}C treatments (T_{growth} effect: $p<0.001$; Table 3.13). In EC trees, the mitochondrial area in a leaf cross-section was slightly higher in AT treatment compared to +4 ^{o}C and +8 ^{o}C (Table 3.13).

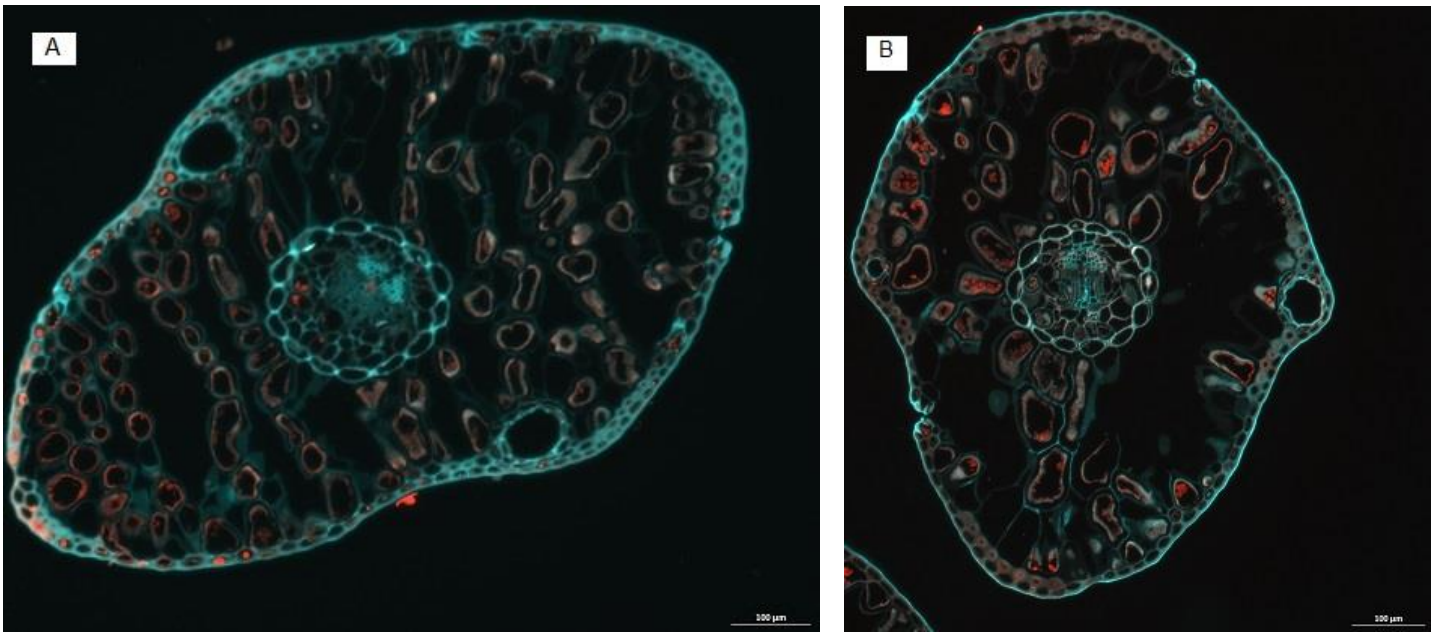

Figure 3.11 Representative microscope images of needle cross-sections of Norway spruce seedlings grown at A) ATAC and B) +8 ^{o}C/AC. Scale bar = 100 μm. Orange staining indicates mitochondria. AT- ambient temperature, +8 ^{o}C-ambient temperature + 8 ^{o}C. AC-ambient CO_2.

Table 3.13 Cross-sectional leaf area, mitochondrial cross-sectional area, and needle length for Norway spruce seedlings grown at a variety of growth temperatures and CO_2 in 2013. Means ± SE, n=23 (ATAC) n=21 (ATEC, +8/EC), n=9 (+4/AC) and n=2 in mitochondria count, n=16 (+4/EC), n=20 (+8/AC); needle length: n=33-47. Letters indicate multiple comparisons of means (Tukey's Post-Hoc test). AT-ambient temperature, +4-ambient temperature +4 ^{o}C, +8-ambient temperature + 8 ^{o}C. AC-ambient CO_2, EC-elevated CO_2.

	Leaf cross-sectional area (μm^2)	**Mitochondrial cross-sectional area (μm^2)**	**%Mitochondria per cross-sectional leaf area**	**Needle length (mm)**
ATAC	431.26±23.5 (a)	31.75±1.74 (a)	7.45±0.26 (c)	13.9±0.5 (a)
+4/AC	177.68±3.62 (c)	12.44±3.35 (c)	6.97±1.74 (bc)	10.47±0.57 (b)
+8/AC	190.49±5.56 (c)	22.0±0.94 (bc)	11.59±0.43 (ab)	8.34±0.36 (c)
ATEC	350.4±26.87 (b)	34.34±3.4 (a)	9.66±0.55 (b)	12.91±0.38 (a)
+4/EC	282.01±15.64 (bc)	27.5±2.75 (b)	9.71±0.63 (b)	10.76±0.38 (b)
+8/EC	224.53±14.75 (b)	26.35±1.31 (b)	12.07±0.57 (a)	7.5±0.26 (c)

3.10 Modelling

To model the total daily CO_2 lost through R, I calculated the rate of R at each temperature point during a 24-hour period, using R_{dark} in the night hours and R_{light} during the light hours. Leaf temperature fluctuated during the day, peaking at 1 pm at 15.4 ^{o}C and decreasing to a minimum of 9 ^{o}C at 12 am the next day (Fig. 3.12).

I used these leaf temperatures to model R_{light} during the day for each treatments, based on results from Figure 3.6 and also for the model where $R_{light} = 0.7\ R_{dark}$, using data from Figure 3.5. I also modeled R_{dark} for the night hours from Figure 3.5 and added these with the modeled R_{light} to generate 24-hour R C-losses.

In 2013 and 2014, AT treatments showed the highest total daily R, while +4 ^{o}C and +8 ^{o}C trees had 14-56% lower R_{light} rates compared to the ATAC treatment (Table 3.14). When R for the light hours were calculated from R_{dark}, using $R_{light} \sim 0.7\ R_{dark}$, diurnal R was overestimated by 11-65% (Table 3.14).

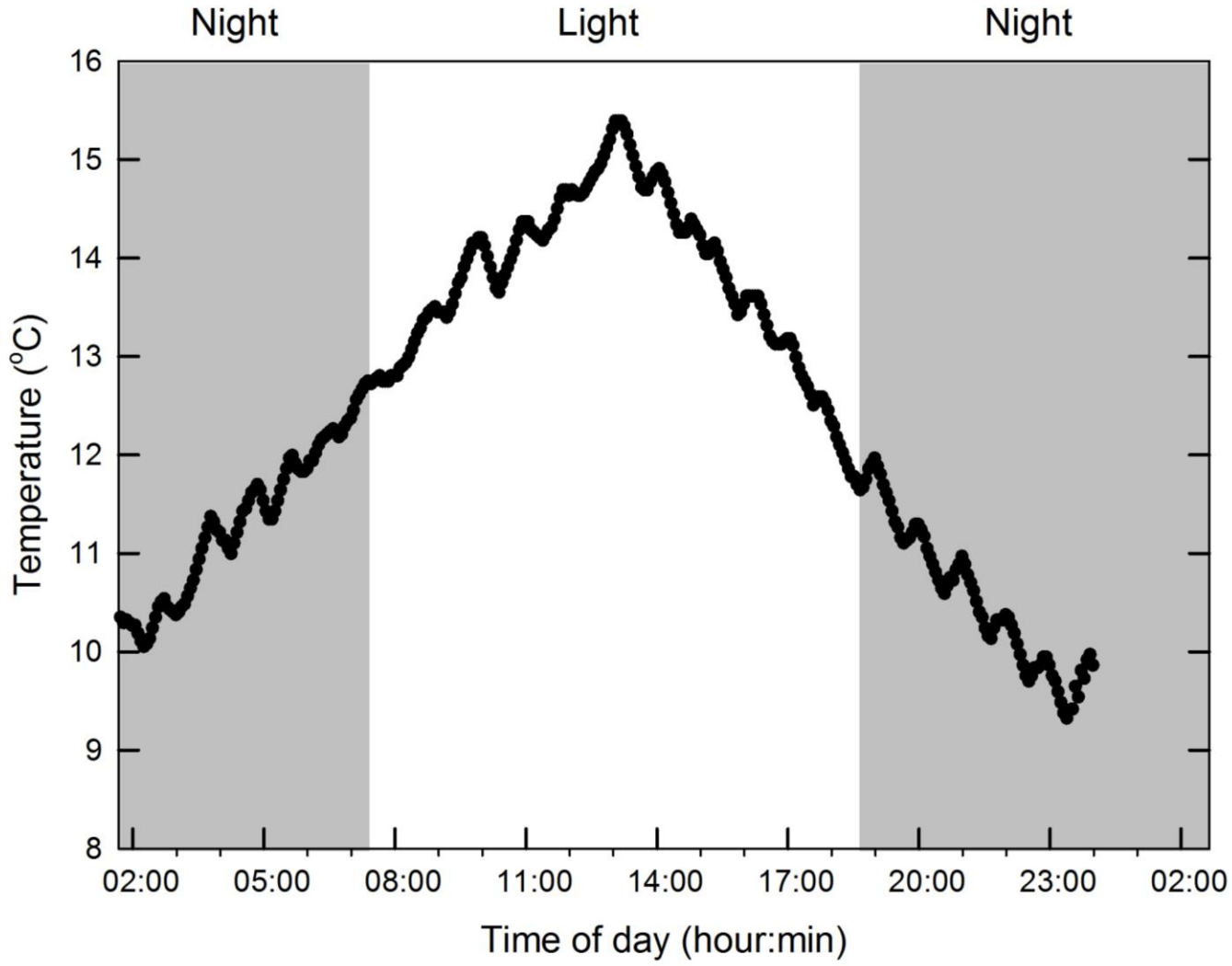

Figure 3.12 Representative 24-hour leaf temperature trace for a Norway spruce grown at ambient temperature and ambient CO_2. Grey areas represent dark periods.

Table 3.14 Total C-loss (μmol CO_2 m^{-2} day^{-1}) of Norway spruce through R, calculated for a 24-hour period for all the treatments for 2013 and 2014. AT-ambient temperature, +4-ambient temperature +4 ^oC, +8- ambient temperature + 8 ^oC. AC-ambient CO_2, EC-elevated CO_2.

2013

	Total 24-hour R (using R_{light} during the light hours)	**Total 24-hour R (using 0.7 R_{dark} during the light hours)**	**Difference in 24-hour R models (%)**	**% of total R compared to ATAC**
ATAC	117.77	154.1	30.8	
+4/AC	77.88	86.85	11.2	66
+8/AC	61.43	69.94	13.9	52
ATEC	101.59	119.1	17.2	86
+4/EC	85.77	111.88	30.4	73
+8/EC	66.13	89.52	35.4	56

2014

	Total 24-hour R (using R_{light} during the light hours)	**Total 24-hour R (using 0.7 R_{dark} during the light hours)**	**Difference in 24-hour R models (%)**	**% of total R compared to ATAC**
ATAC	103.82	125.71	21.1	
+4/AC	48.32	54.6	13	47
+8/AC	56.25	92.68	64.76	44
ATEC	83.87	108.4	29.2	81
+4/EC	80.77	109.65	35.76	78
+8/EC	50.84	71.67	41	49

4 Discussion

4.1 Mortality rates and growth

The 20-39% higher mortality rates in the +8 ^{o}C trees compared to spruce grown in the ambient conditions, were likely due to heat stress. In their native ecological niche, Norway spruce are more frequently exposed to extreme low temperatures rather than to high temperatures. Young seedlings transferred to temperatures close to their survival limits (~35 ^{o}C) are especially sensitive to this temperatures change because of their unlignified shoots (Kreeb 1979). Although +8 ^{o}C treatments enhanced early growth (including bud burst and new branch formation) compared to other temperature regimes, some of the seedlings started to die despite ample of water. I can therefore conclude that much higher than ambient temperatures have a negative effect on survival of Norway spruce. However, the seedlings that survived grew equally well across the treatments, which implies there may be little effect of growth temperature on tree growth across the warming scenarios used here (Lavola et al. 2012; Teskey & Will 1999).

4.2 Thermal and CO_2 acclimation of light –saturated photosynthesis

In this experiment, I excluded the possibility of A_{sat} down-regulation that is often associated with smaller soil volumes, by measuring photosynthetic capacity (V_{cmax} and J_{max}). I found that trees grown in pots had consistently equal or higher V_{cmax} and J_{max} than trees grown in a 10-fold bigger soil volume (Fig. 3.10). Based on the results, I conclude that seedlings grown in the field would have similar CO_2 responses to the experimental trees. While elevated CO_2 can

down-regulate photosynthesis (Leakey et al. 2009, Way et al. 2015), in this experiment I found that across different T_{growth} treatments, there was no significant CO_2 effect on A_{sat} (Table 3.2).

Interestingly, in the 4 ^{o}C treatment combined with elevated growth CO_2, the higher T_{growth} enhanced CO_2 assimilation rates (Figure 3.3), from a T_{leaf} of 10 to 40 ^{o}C, which implies that a slight increase in T_{growth} plus higher growth CO_2 may be beneficial for Norway spruce.

This experiment showed strong thermal acclimation of A_{sat}: the A_{sat} versus T_{leaf} curve in trees grown in higher T_{growth} generally had lower rates of A_{sat} (with the exception of +4 ^{o}C combined with elevated growth CO_2), and simultaneously the thermal optimum (T_{opt}) was found at a higher T_{leaf} (higher T_{growth} = higher T_{opt}). When $T_{leaf} > T_{opt}$, a sharp decline in A_{sat} was observed in all the treatments. It was proposed that at very high temperatures, Rubisco activity is severely reduced (Sage & Kubien 2007; Way & Yamori 2013; Yamori et al. 2014). The lower photosynthetic rates observed in trees grown at higher T_{growth} may be explained by their lower leaf N content, which likely reflects lower Rubisco content and lower photosynthetic enzymes concentrations (Tjoelker et al. 2009; Yamori et al. 2005). Indeed, our results showed that trees grown at +8 ^{o}C had a lower % N in leaves than the trees from other treatment groups.

4.3 Thermal acclimation of R_{dark} and R_{light}

The responses of R_{dark} and R_{light} to a short-term change in T_{leaf} (minutes to hours) are well studied (Atkin et al. 2000b; Teskey &Will 1999; Slot & Kitajima 2015, Way et al. 2015), growing membrane fluidity and increases in specific enzyme functions make R increase exponentially (Amthor 1984, Ryan 1991). My results showed similar trends in both types of R,

as R rates rapidly increased in trees from all the treatments as T_{leaf} rose. The upper thermal threshold of R is usually beyond 40 ^{o}C, where R reaches a high temperature threshold and then decreases as a result of reductions in enzymatic activity (e.g. adenylate kinase) (Atkin et al. 2005, Igamberdiev & Kleczkowski 2006).

Plasticity allows plants to acclimate non-photorespiratory mitochondrial respiration to changes in ambient temperatures (Atkin & Tjoelker, 2003). This ability to adjust the shape of the temperature response curve to a temperature change is related to alterations in leaf anatomy (e.g. changes in leaf density) and protein composition (Atkin et al. 2005). Long- term (weeks to month) exposure to elevated growth temperature had different effects on R_{dark} and R_{light} contingent on the year. In 2013 I found a similar response of both types of R to the warming. However, in this study, only R_{dark} showed a consistent acclimation response to long-term warming during both years (Tables S3 and 3.5). First, I will discuss long-term thermal acclimation of R_{dark}.

When plants were grown at warmer temperatures, R_{dark} was reduced (Fig. 3.5). This acclimation allows a plant to control optimal ATP and C-skeleton balance, and to minimize C lost under warmer conditions. To validate the degree of thermal acclimation, I used the equation from Loveys et al. 2003:

$$Acclim_{setTemp} = (R_{control} \text{ at set T} / R_{warm} \text{ at set T}) \quad \text{[Equation 3]}$$

where $Acclim_{setTemp}$ is acclimation at a set temperature, $R_{control}$ is R of a control plant at this leaf temperature, and R_{warm} is R of a warm-grown plant at the same set leaf temperature.

When $Acclim_{setTemp} > 1$, thermal acclimation has taken place. The values were consistently greater than 1 ($Acclim_{@20\ (AT->+4)} = 1.29$; $Acclim_{@20\ (AT->+8)} = 1.69$). As I discussed previously, there are two types of thermal acclimation for R: Type I and Type II (see Fig. 1.2). In this experiment, the Q_{10} remained stable across the treatments (2±0.03), which confirmed that the spruce trees demonstrated Type II acclimation. To calculate whether the acclimation reached a perfect homeostasis, I used the equation (Loveys et al. 2003):

$$Acclim_{Homeo} = (R_{control} \text{ at } T_{control} / R_{warm} \text{ at } T_{warm}) \quad \text{[Equation 4]}$$

where $Acclim_{Homeo}$ is a degree of homeostasis due to thermal acclimation, $R_{control}$ at $T_{control}$ is R of control plant at its growth temperature, R_{warm} at T_{warm} is R of warm-grown plant at its growth temperature. When $Acclim_{Homeo}$ equals 1, there is a perfect homeostasis. In our study, R_{dark} demonstrated almost perfect homeostasis in +4 ^{o}C and +8 ^{o}C treatments ($A_{Homeo\ (AT->+4)} = 0.93$; $A_{Homeo\ (AT->+8)} = 1.08$). A higher degree of acclimation of R to temperature was found previously in conifers compared to deciduous species (Tjoelker et al. 1999, Teskey & Will, 1999), which supports my findings.

R_{light}, similarly to R_{dark}, thermally acclimated in 2013, but the homeostasis of R_{light} was less perfect than R_{dark} ($Acclim_{Homeo\ (AT->+4)} = 0.85$; $Acclim_{Homeo\ (AT->+8)} = 0.88$). The Q_{10} of R_{light} also remained stable across the treatments (2.7±0.08) which validated Type II thermal acclimation.

4.4 R_{light} acclimation to growth CO_2

In contrast to 2013, in 2014 there was no acclimation of R_{light} to T_{growth}, but instead, there was evidence of acclimation to elevated CO_2 (Fig. 3.6). R_{light} was significantly decreased when growth CO_2 concentration was elevated in AT and +4 ^{o}C trees. These lower rates of R_{light} are associated with higher A_{sat} than was observed in this season compared to 2013.

4.5 R_{light} : R_{dark} ratio and modelling total diurnal R

Across two years and in all treatments, the Q_{10} of R_{light} was consistently 35% higher than the Q_{10} of R_{dark}, therefore R_{light} : R_{dark} increased with an increase in T_{leaf}. This allows me to conclude that the R_{light} : R_{dark} ratio is dictated by the Q_{10}, and is not constant. A change in the R_{light} : R_{dark} ratio as T_{leaf} increases has also observed in other species (Way & Sage 2008; Way & Yamori, 2014). Considering all of the above, the assumed conversion rate of 0.7 R_{dark} to estimate R_{light} (Ayub et al. 2011) cannot be used when modelling C fluxes. Indeed, in this experiment, the R_{light}= 0.7 R_{dark} only matched the data when R was measured at a T_{leaf} of 22-27 ^{o}C (Fig. 3.7, grey area). As such, earlier results indicating that R_{light} is 0.7 R_{dark} may be due to measuring both fluxes at room temperature. However, I propose that in Norway spruce, R_{light} can be calculated at every T_{leaf} based on the equation explaining the relationship of both (Fig. 3.8). The total R for 24-hour period was overestimated up to 65% when the equation R_{light} = 0.7 R_{dark} was used instead of the real R_{light} values measured during the light hours (Table 3.14). This overestimation may significantly skew C fluxes calculations on an ecological scale.

4.6 A_{sat} : R_{dark} ratio

A_{sat} and R_{dark} are co-dependent processes, as A needs the adenylates produced by R_{dark}, and R needs the substrate from A (Krömer 1995). Looking at a short-term temperature response, leaves at very high temperatures utilize much more substrate in R than A_{sat} can provide and the ratio of A_{sat} to R_{dark} decreases (Fig. 3.9), consequently reaching zero at a high T_{leaf} of ~45 ^{o}C. A similar effect was found by Gifford (1995) in wheat (*Triticum aestivum*) grown across 15-30 ^{o}C, by Ziska & Bunce (1998) in soybean (*Glycine max*) grown at 20-35 ^{o}C, (2006), and by Way & Sage (2008) in black spruce (*Picea mariana*) grown at 22-30 ^{o}C. This decline implies the leaf is unable to balance total C on these timescales. However, in contrast to this idea, Atkin et al. (2006) found that while alpine *Plantago* species had the same A/R_{dark} decrease seen there, lowland *Plantago* species maintained a constant A/R_{dark} ratio.

When A_{sat} and R_{dark} acclimate to T_{growth}, it is logical to propose that the A_{sat} : R_{dark} ratio should show similar patterns across the treatments. Indeed, when the A_{sat} : R_{dark} ratio of all the treatments from both years were plotted against T_{leaf}, there was a strong positive correlation (R^2= 0.96; Fig. 3.9).

4.7 Leaf anatomy and biochemical traits of acclimation

When trees acclimate to T_{growth}, resulting in lower A_{sat} and R rates in the +8^{o}C treatment compared to the AT, we expect to see anatomical and biochemical changes. While growth, SLA and NSC were not significantly affected by T_{growth}, I observed thinner and shorter needles in the

+8 ^{o}C treatment (Fig. 3.11, Table 3.13), which supports the theory of anatomical adjustment when thermal acclimation occurs (Atkin et al. 2005). This may explain why trees on one hand assimilated less C, but on the other hand, also lost proportionally less C.

In this experiment I found that the mitochondrial density was highest in +8 ^{o}C treatments regardless of CO_2. It might be suggested that reduced R rates in this treatment group cannot be supported by these findings. However, the effects of environmental stresses on mitochondrial anatomy had been seen in previous studies when plant mitochondrial shape (Kiwimänempää et al. 2001) and functionality (Armstrong et al. 2006) may change during a short period of time (hours to days) according to the cellular needs for energy. Due to the fact that the harvest and microscopy analysis was done only once, it is difficult to conclude whether mitochondrial density reflects my physiological findings.

5 Conclusion

Norway spruce, a dominant European coniferous species, was used as a model in this study, describing how boreal forest species will respond to projected growth temperatures and CO_2. I have found that in the +4 ^oC warmer and elevated CO_2 concentration climate, Norway spruce will likely fix more CO_2, but this will not necessarily lead to better growth. On the other hand, much greater warming (+8 ^oC), although harmful for the young seedlings as shown by higher mortality rates, will trigger acclimation: A_{net} and R_{dark} will simultaneously be reduced in the new climate conditions. However, the response of R_{light} to projected climate conditions remains unclear and requires further investigation, but I propose that neither elevated CO_2 nor higher than current T_{growth} will reduce R_{light} rates.

My work has shown the importance of using measured R_{light} values in models of R. Considering the difficulty of measuring of R_{light}, I propose to use the equation explaining the R_{light} to R_{dark} relationship, and not the conversion rate of 0.7, when modelling C fluxes from vegetation.

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7 Appendices

Table S1. Repeated measures three-way ANOVA results for yearly changes in light-saturated photosynthesis (A_{sat}) of Norway spruce seedlings to changes in CO_2, growth temperature (T_{growth}) and leaf temperature (T_{leaf}) for the combined 2013 and 2014 growth seasons. Significant results are bolded.

	Df	Sum of Squares	F Ratio	p-value
CO_2	1	1.48	0.2	0.66
T_{growth}	2	54.53	3.65	**0.034**
Year	1	127.51	17.07	**0.00017**
T_{leaf}	3	635.74	189.14	**<0.0001**
CO_2 X T_{growth}	2	10.43	0.7	0.5
CO_2 X Year	1	2.77	0.37	0.55
T_{growth} X Year	2	6.54	0.44	0.65
CO_2 X T_{leaf}	3	3.96	1.18	0.32
T_{growth} X T_{leaf}	6	39.29	5.84	**<0.0001**
Year X T_{leaf}	3	13.11	3.9	**0.01**
CO_2 X T_{growth} X Year	2	2.64	0.18	0.84
CO_2 X T_{growth} X T_{leaf}	6	1.08	0.16	0.99
CO_2 X Year X T_{leaf}	3	4.77	1.42	0.24
T_{growth} X Year X T_{leaf}	6	6.64	0.99	0.44
CO_2 X T_{growth} X Year X T_{leaf}	6	4.02	0.6	0.73

Table S2. Repeated measures three-way ANOVA results for yearly changes in stomatal conductance (g_s) of Norway spruce seedlings to changes in CO_2, growth temperature (T_{growth}) and leaf temperatures (T_{leaf}) measured at 1200 photosynthetic photon flux density for the combined 2013 and 2014 growth seasons. Significant results are bolded.

	Df	Sum of Squares	F Ratio	p-value
CO_2	1	0.000067	0.051	0.82
T_{growth}	2	0.018	6.82	**0.003**
Year	1	0.05	38.27	**< 0.0001**
T_{leaf}	3	0.04	67.19	**<0.0001**
CO_2 X T_{growth}	2	0.0023	0.86	0.43
CO_2 X Year	1	0.0014	1.06	0.31
T_{growth} X Year	2	0.007	2.71	0.08
CO_2 X T_{leaf}	3	0.0006	0.93	0.43
T_{growth} X T_{leaf}	6	0.0093	7.25	**<0.0001**
Year X T_{leaf}	3	0.0028	4.3	**0.006**
CO_2 X T_{growth} X Year	2	0.0022	0.85	0.44
CO_2 X T_{growth} X T_{leaf}	6	0.00036	0.28	0.94
CO_2 X Year X T_{leaf}	3	0.001	1.54	0.21
T_{growth} X Year X T_{leaf}	6	0.0016	1.24	0.29
CO_2 X T_{growth} X Year X T_{leaf}	6	0.001	0.79	0.58

Table S3. Repeated measures three-way ANOVA results for yearly changes in light-stimulated dark respiration rates (R_{dark}) of Norway spruce seedlings to changes in CO_2, growth temperature (T_{growth}) and leaf temperature (T_{leaf}) for the combined 2013 and 2014 growth seasons. Significant results are bolded.

	Df	Sum of Squares	F Ratio	p-value
CO_2	1	0.19	0.45	0.5
T_{growth}	2	8.86	10.52	**0.0002**
Year	1	1.48	3.51	0.068
T_{leaf}	3	112.8	453.52	**<0.0001**
CO_2 X T_{growth}	2	0.054	0.064	0.94
CO_2 X Year	1	0.206	0.49	0.49
T_{growth} X Year	2	0.358	0.425	0.66
CO_2 X T_{leaf}	3	0.298	1.2	0.31
T_{growth} X T_{leaf}	6	3.938	7.92	**<0.0001**
Year X T_{leaf}	3	0.694	2.79	**0.043**
CO_2 X T_{growth} X Year	2	0.17	0.2	0.82
CO_2 X T_{growth} X T_{leaf}	6	0.123	0.25	0.96
CO_2 X Year X T_{leaf}	3	0.122	0.49	6.9
T_{growth} X Year X T_{leaf}	6	0.562	1.13	3.5
CO_2 X T_{growth} X Year X T_{leaf}	6	0.129	0.26	0.95

Table S4. Repeated measures three-way ANOVA results for yearly changes in light respiration rates (R_{light}) of Norway spruce seedlings to changes in CO_2, growth temperature (T_{growth}), and leaf temperature (T_{leaf}) for the combined 2013 and 2014 growth seasons. Significant results are bolded.

	Df	Sum of Squares	F Ratio	p-value
CO_2	1	3.037	9.17	**0.004**
T_{growth}	2	7.704	11.63	**<0.001**
Year	1	2.99	9.03	**0.004**
T_{leaf}	3	155.2	383.11	**<0.0001**
CO_2 X T_{growth}	2	0.5	0.75	0.48
CO_2 X Year	1	0.31	0.93	0.34
T_{growth} X Year	2	2.18	3.29	**0.047**
CO_2 X T_{leaf}	3	1.44	3.57	**0.016**
T_{growth} X T_{leaf}	6	4.68	5.77	**<0.0001**
Year X T_{leaf}	3	1.43	3.53	**0.017**
CO_2 X T_{growth} X Year	2	0.24	0.37	0.7
CO_2 X T_{growth} X T_{leaf}	6	0.75	0.92	0.48
CO_2 X Year X T_{leaf}	3	0.5	1.25	0.3
T_{growth} X Year X T_{leaf}	6	3.14	3.87	**0.0014**
CO_2 X T_{growth} X Year X T_{leaf}	6	0.13	0.16	0.99

Table S5. Repeated measures two-way ANOVA results for the response in R_{light}/R_{dark} to changes in growth CO_2, growth temperature (T_{growth}), and leaf temperature (T_{leaf}) of Norway spruce seedlings in 2013 and 2014. Significant results are bolded.

	DF	Sum of Squares	F Ratio	p-value
CO_2	1	3.04	9.17	**0.0042**
T_{growth}	2	7.7	11.63	**0.000096**
Year	1	2.99	9.03	**0.0045**
T_{leaf}	3	155.2	383.11	**<0.00001**
CO_2 X T_{growth}	2	0.5	0.75	0.48
CO_2 X Year	1	0.31	0.93	0.34
T_{growth} X Year	2	2.18	3.29	**0.047**
CO_2 X T_{leaf}	3	1.44	3.57	0.016
T_{growth} X T_{leaf}	6	4.68	5.77	**0.000024**
Year X T_{leaf}	3	1.43	3.53	**0.017**
CO_2 X T_{growth} X Year	2	0.24	0.37	0.7
CO_2 X T_{growth} X T_{leaf}	6	0.75	0.93	0.48
CO_2 X Year X T_{leaf}	3	0.5	1.25	0.3
T_{growth} X Year X T_{leaf}	6	3.14	3.87	**0.0014**
CO_2 X T_{growth} X Year X T_{leaf}	6	0.13	0.16	0.99

Table S6. Three-way ANOVA results for yearly changes in non-structural carbohydrates (NSC) of Norway spruce trees to changes in growth CO_2 and growth temperature (T_{growth}) in 2013 and 2014. Significant results are bolded.

	DF	Sum of Squares	F Ratio	p-value
CO_2	1	0.0001	1.143	0.291
T_{growth}	2	0.0001	1.376	0.294
Year	1	0.004	48.073	**<0.00001**
T_{growth} X CO_2	2	0.00004	0.514	0.602
T_{growth} X Year	2	0.00001	0.127	0.881
CO_2 X Year	1	0.00002	0.183	0.671
T_{growth} X CO_2 X Year	2	0.00003	0.365	0.696

Table S7. Two-way ANOVA results for yearly changes in leaf nitrogen concentration of Norway spruce trees to changes in growth CO_2, growth temperature (T_{growth}), and leaf temperature (T_{leaf}) in 2013 and 2014. Significant results are bolded.

	DF	Sum of Squares	F Ratio	p-value
CO_2	1	0.6751	9.948	**0.003**
T_{growth}	2	1.2389	9.127	**0.0005**
Year	1	0.7755	11.426	**0.0016**
T_{growth} X CO_2	2	0.1277	0.941	0.398
T_{growth} X Year	2	0.0863	0.636	0.534
CO_2 X Year	1	0.0002	0.003	0.954
T_{growth} X CO_2 X Year	2	0.3735	2.751	0.075

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