



Contrasting acclimation responses to elevated CO₂ and warming between an evergreen and a deciduous boreal conifer

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Abstract

Rising atmospheric carbon dioxide (CO₂) concentrations may warm northern latitudes up to 8°C by the end of the century. Boreal forests play a large role in the global carbon cycle, and the responses of northern trees to climate change will thus impact the trajectory of future CO₂ increases. We grew two North American boreal tree species at a range of future climate conditions to assess how growth and carbon fluxes were altered by high CO₂ and warming. Black spruce (*Picea mariana*, an evergreen conifer) and tamarack (*Larix laricina*, a deciduous conifer) were grown under ambient (407 ppm) or elevated CO₂ (750 ppm) and either ambient temperatures, a 4°C warming, or an 8°C warming. In both species, the thermal optimum of net photosynthesis (T_{optA}) increased and maximum photosynthetic rates declined in warm-grown seedlings, but the strength of these changes varied between species. Photosynthetic capacity (maximum rates of Rubisco carboxylation, V_{cmax} , and of electron transport, J_{max}) was reduced in warm-grown seedlings, correlating with reductions in leaf N and chlorophyll concentrations. Warming increased the activation energy for V_{cmax} and J_{max} (E_{aV} and E_{aJ} , respectively) and the thermal optimum for J_{max} . In both species, the T_{optA} was positively correlated with both E_{aV} and E_{aJ} , but negatively correlated with the ratio of J_{max}/V_{cmax} . Respiration acclimated to elevated temperatures, but there were no treatment effects on the Q_{10} of respiration (the increase in respiration for a 10°C increase in leaf temperature). A warming of 4°C increased biomass in tamarack, while warming reduced biomass in spruce. We show that climate change is likely to negatively affect photosynthesis and growth in black spruce more than in tamarack, and that parameters used to model photosynthesis in dynamic global vegetation models (E_{aV} and E_{aJ}) show no response to elevated CO₂.

KEYWORDSacclimation, boreal conifers, climate change, evergreen and deciduous, *Larix laricina*, *Picea mariana*, V_{cmax} and J_{max}

1 | INTRODUCTION

Anthropogenic greenhouse gas emissions, such as carbon dioxide (CO₂), have already increased global mean surface air

temperatures, which are predicted to increase another 1–4°C by the year 2100 (Stocker et al., 2013). High latitudes will experience more extreme warming of ~8°C by the end of the century (Collins et al., 2013; Stocker et al., 2013), and the boreal forest has

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already experienced warming of 2°C in some areas (Hasper et al., 2016; Price et al., 2013). Growth of high latitude plants, such as those in boreal ecosystems, is often assumed to be temperature- and nutrient-limited, and climate warming is therefore expected to enhance boreal productivity (Högberg, Näsholm, Franklin, & Högberg, 2017; Huang et al., 2019; Jarvis & Linder, 2000; Nicault et al., 2015; Stinziano & Way, 2014; Tucker et al., 2001). In addition, rising CO₂ concentrations should benefit forests via CO₂ fertilization (Norby et al., 2005) and an increase in water use efficiency (Battipaglia et al., 2013; Giguère-Croteau et al., 2019; Keenan et al., 2013), which may benefit boreal tree species currently affected by drought stress (Girardin, Bouriaud, et al., 2016) in a warmer, drier future climate. Boreal forests account for 32% of the total carbon sequestered by the world's forests (Pan et al., 2011) and play a critical role in climate regulation and global biogeochemical cycles (Bonan, 2008; Brandt, 2009). Therefore, climate change-induced shifts in the functioning of this biome will significantly impact regional ecosystem services (Gauthier, Bernier, Kuuluvainen, Shvidenko, & Schepaschenko, 2015), global carbon cycling, and the rate of increase in atmospheric CO₂ concentrations (Bonan, 2008; Graven et al., 2013; Kasischke, 2000).

Despite expectations, recent responses of boreal forests to climate change have not been consistent with predictions of enhanced tree growth. Instead, studies show either no change (Giguère-Croteau et al., 2019; Girardin, Bouriaud, et al., 2016; but see Lloyd, Bunn, & Berner, 2011) or a decline in tree growth and survival (Chen & Luo, 2015; D'Arrigo, Wilson, Liepert, & Cherubini, 2008; Girardin, Hogg, et al., 2016; Lloyd & Bunn, 2007; Ma et al., 2012; Marchand, Girardin, Hartmann, Gauthier, & Bergeron, 2019; Peng et al., 2011; Walker & Johnstone, 2014). The few studies that report positive responses to climate change show that tree growth is enhanced by warming only in wet regions (Hember, Kurz, & Coops, 2017). These results, and recent experimental work (Reich et al., 2018), imply that boreal trees might not benefit from warming under water limitations. However, even studies in well-watered conditions can show a negative effect of higher temperatures on boreal tree species (Tjoelker, Oleksyn, & Reich, 1998; Way & Sage, 2008a, 2008b), suggesting that their growth might be directly reduced by rising temperature. Given the low tree biodiversity of the boreal (Girardin, Bouriaud, et al., 2016), responses to climate change factors of these few species (Girardin, Bouriaud, et al., 2016; Kurepin et al., 2018; Marchand et al., 2019; Tjoelker et al., 1998) might explain observed large-scale productivity trends in the boreal region (Girardin, Bouriaud, et al., 2016; Lloyd et al., 2011), and will ultimately dictate the trajectory of carbon cycling in these forests. However, we still lack an understanding of how the processes that govern leaf carbon fluxes (photosynthesis and respiration), and thus carbon availability for growth, acclimate to climate change in dominant boreal tree species.

Photosynthesis and respiration regulate plant carbon balance, and are key players in the carbon exchange between vegetation and the atmosphere. Both processes are temperature dependent. Increases in leaf temperature stimulate photosynthesis (net CO₂ assimilation rates, A_{net}) up to the thermal optimum (T_{opt}), while A_{net}

declines above the T_{opt} (Berry & Bjorkman, 1980; Way & Yamori, 2014; Yamori, Hikosaka, & Way, 2014). Below the T_{opt} and at current CO₂ levels, A_{net} is often limited by the capacity of Rubisco (ribulose-1,5-bisphosphate carboxylase/oxygenase) to fix CO₂ (Hikosaka, Ishikawa, Borjigidai, Muller, & Onoda, 2006). Above the T_{opt} , the decline in A_{net} is associated with increased photorespiration (Sage & Kubien, 2007), reduced stomatal conductance (Lin, Medlyn, & Ellsworth, 2012), and reduced performance of Rubisco activase, a chaperone protein that maintains the activity of Rubisco (Sage, Way, & Kubien, 2008; Yamori, Suzuki, Noguchi, Nakai, & Terashima, 2006; Yamori & von Caemmerer, 2009). At supraoptimal temperatures, A_{net} can also be limited by electron transport rates, which depend on thylakoid membrane stability (Murakami, Tsuyama, Kobayashi, Kodama, & Iba, 2000; Sage & Kubien, 2007). Respiration also continues in the light (Atkin, Evans, Ball, Lambers, & Pons, 2000), and since mitochondrial respiration rates increase exponentially with increasing leaf temperatures, this also contributes to decreases of A_{net} at high leaf temperatures (Way, 2019).

When plants are grown in elevated temperatures, they acclimate by increasing the T_{opt} of A_{net} ($T_{\text{opt}A}$), as well as the T_{opt} of maximum Rubisco carboxylation rates (V_{cmax}) and maximum electron transport rates (J_{max} ; Kumarathunge et al., 2019; Sendall et al., 2015; Smith & Dukes, 2017; Way & Yamori, 2014). However, shifting these T_{opt} s to higher temperatures does not necessarily improve photosynthetic rates in warmer environments (Way & Sage, 2008a; Way & Yamori, 2014; Zhang et al., 2015). In contrast, dark respiration usually acclimates to warming such that the basal rate of respiration and/or the respiratory temperature sensitivity is reduced in warm-grown plants, decreasing leaf carbon losses (Atkin, Bruhn, Hurry, & Tjoelker, 2005; Atkin & Tjoelker, 2003; Slot & Kitajima, 2015; Smith & Dukes, 2017). Boreal trees effectively acclimate respiration to warming (Benomar et al., 2017; Kroner & Way, 2016; Kurepin et al., 2018; Reich et al., 2016; Wei et al., 2017; Zhang et al., 2015), but A_{net} at growth temperature tends to be reduced in evergreen woody species grown in warmer conditions (Dusenke, Duarte, & Way, 2019; Way & Yamori, 2014). This suggests that changes in carbon fluxes in boreal tree species under future climates could be largely dictated by the extent of photosynthetic acclimation. There is also growing evidence that conifers in the genus *Picea* are particularly sensitive to warming: both A_{net} and biomass are reduced in warm-grown *Picea* compared to control plants in a range of species and studies (Kroner & Way, 2016; Kurepin et al., 2018; Tjoelker et al., 1998; Way & Sage, 2008a, 2008b; Zhang et al., 2015). Although there are few studies on *Larix*, A_{net} was unchanged across a 12°C warming in *Larix laricina* (Tjoelker et al., 1998), suggesting that this deciduous conifer might show strong photosynthetic acclimation under warming conditions.

Both A_{net} and tree growth usually increase under elevated CO₂ (Norby et al., 2005). Net photosynthesis is stimulated by elevated CO₂ as a result of increased substrate availability to Rubisco (Ainsworth & Rogers, 2007; Medlyn et al., 1999). However, A_{net} acclimates to elevated CO₂ such that the initial stimulation of A_{net} is reduced (e.g. Warren, Jensen, Medlyn, Norby, & Tissue, 2014). This photosynthetic downregulation is a consequence of reduced photosynthetic

capacity, which is strongly linked to sink limitations (Ainsworth & Long, 2005; Leakey et al., 2009; Medlyn et al., 1999), and is more common under nutrient limitation (e.g., Ellsworth et al., 2017; Norby, Warren, Iversen, Medlyn, & McMurtrie, 2010; Warren et al., 2014). The latter point is particularly relevant in boreal forests, which are low-nutrient habitats (Gower & Richards, 1990), for nitrogen in particular. Elevated CO₂ thus has little impact on boreal tree productivity in field experiments without nutrient addition (Hättenschwiler & Körner, 1997; Sigurdsson, Medhurst, Wallin, Eggertsson, & Linder, 2013; Sigurdsson, Thorgeirsson, & Linder, 2001), while in well-fertilized boreal conifer seedlings, elevated CO₂ stimulated A_{net} equally in *Picea mariana*, *Pinus banksiana*, and *L. laricina* (Tjoelker et al., 1998). Decreased photosynthesis at high CO₂ has also been correlated with reduced stomatal conductance associated with elevated CO₂-induced abscisic acid signaling and OST1 and SnRK2 kinase activity (Ainsworth & Rogers, 2007; Hsu et al., 2018). Therefore, contrary to warming, which negatively affects *Picea*, the impact of growth CO₂ may not vary considerably between evergreen and deciduous conifers growing in similar soils. The effects of elevated CO₂ on respiration are more variable than they are on A_{net} (Way, Oren, & Kroner, 2015). In the short-term, there is no effect of CO₂ on dark respiration (Amthor, 2000; Amthor, Koch, Willms, & Layzell, 2001), but on longer timescales, respiration can be altered by elevated CO₂, with increased dark respiration rates linked to increased leaf carbohydrate and decreased respiration rates associated with reduced nitrogen concentrations (reviewed in Way et al., 2015).

Both air temperature and atmospheric CO₂ increase concomitantly, and efforts to improve our understanding on the impacts of these combined factors on photosynthesis and respiration are growing (e.g., Broughton et al., 2017; Crous et al., 2013; Kroner & Way, 2016; Kurepin et al., 2018; Lamba et al., 2018; Lewis et al., 2015; Tjoelker et al., 1998; Tjoelker, Reich, & Oleksyn, 1999; Uddling & Wallin, 2012; Wallin et al., 2013; Wang, Kellomaki, & Laitinen, 1995; Zha, Kellomaki, & Wang, 2003). The combined effect of CO₂ and temperature can either enhance or offset their independent effects on leaf physiology (Long, 1991). High CO₂ suppresses photorespiration (which increases with temperature), thus stimulating A_{net} (Wujeska-Klaue, Crous, Ghannoum, & Ellsworth, 2019) and increasing the T_{optA} (Sage & Kubien, 2007); elevated CO₂ thus often enhances plant carbon uptake at high growth temperatures (Uddling & Wallin, 2012; Wallin et al., 2013; Wang et al., 1995). Elevated CO₂ and temperature can also have additive effects on carbon uptake (Dusenke et al., 2019). For instance, elevated CO₂ reduces stomatal conductance (Ainsworth & Rogers, 2007), as does the higher vapor pressure deficit (VPD) that often accompanies increased air temperatures (Oren et al., 1999), which reduces A_{net} and alters the T_{optA} (Mercado et al., 2018). But strong effects of CO₂ on photosynthesis and respiration in warm-grown plants are rare in studies on boreal trees (Kroner & Way, 2016; Tjoelker et al., 1998; Wallin et al., 2013). Indeed, there are generally only weak (Tjoelker et al., 1999; Wallin et al., 2013; Wang et al., 1995; Zha et al., 2003) or no apparent interactions (Kroner & Way, 2016; Kurepin et al., 2018; Lamba et al., 2018; Tjoelker et al., 1998) between elevated temperatures and CO₂

on photosynthetic and respiratory performance in boreal conifers in the studies available to date.

Terrestrial biosphere models (TBMs) use the Farquhar, Caemmerer, and Berry (1980) photosynthesis model to simulate leaf-level photosynthesis and scale it up to estimate biosphere-atmosphere interactions (Rogers, Medlyn, et al., 2017). This model requires data on the photosynthetic capacity variables V_{cmax} and J_{max}, which are key parameters for estimating CO₂ fixation rates. Both V_{cmax} and J_{max} are affected by environmental factors such as growth CO₂ (Ainsworth & Long, 2005) and temperature (Kattge & Knorr, 2007; Smith & Dukes, 2017), and values of V_{cmax} and J_{max} vary across plant functional types (Rogers, 2014). In TBMs, V_{cmax}, J_{max}, and the ratio of J_{max}/V_{cmax} are important input parameters (Rogers, Medlyn, et al., 2017; Rogers, Serbin, Ely, Sloan, & Wullschlegel, 2017b), as are the thermal sensitivity of V_{cmax} and J_{max} (Mercado et al., 2018). The instantaneous temperature responses of V_{cmax} and J_{max} are often modelled by a peaked Arrhenius function (Medlyn et al., 2002; Kumarathunge et al., 2019; but see Smith & Dukes, 2017):

$$f(T_k) = k_{opt} \frac{H_d \exp\left(\frac{E_a(T_k - T_{opt})}{T_k R T_{opt}}\right)}{H_d - E_a \left(1 - \exp\left(\frac{H_d(T_k - T_{opt})}{T_k R T_{opt}}\right)\right)}, \quad (1)$$

where k_{opt} is the process rate (i.e., V_{cmax} or J_{max}; μmol m⁻² s⁻¹) at the optimum temperature (V_{cmaxopt} or J_{maxopt}, respectively), H_d (kJ/mol) is the deactivation energy term that describes the decline in enzyme activity above the thermal optimum, E_a (kJ/mol) is the activation energy term that describes the increase in enzyme activity below the thermal optimum, R is the universal gas constant (8.314 J mol⁻¹ K⁻¹), and T_{opt} and T_k are the optimum and given temperatures of the process rate. Both V_{cmax} and J_{max}, as well as their temperature sensitivity parameters (i.e., E_a, T_{opt}, H_d, V_{cmaxopt} and J_{maxopt}), change with growth temperature (Crous et al., 2018; Hikosaka et al., 2006; Kattge & Knorr, 2007; Kumarathunge et al., 2019; Smith & Dukes, 2017; Yamaguchi et al., 2019; Yamaguchi, Nakaji, Hiura, & Hikosaka, 2016; but see Stefanski, Bermudez, Sendall, Montgomery, & Reich, 2019; Yin, Schapendonk, & Struik, 2019). However, few TBMs include thermal acclimation of photosynthesis, largely due to data scarcity, particularly for tropical and boreal biomes (Kumarathunge et al., 2019; Lombardozzi, Bonan, Smith, Dukes, & Fisher, 2015; Mercado et al., 2018). In addition, we lack data on the impacts of combined elevated temperature and CO₂ on the temperature sensitivity of photosynthetic capacity, particularly in boreal species (Mercado et al., 2018; Smith & Dukes, 2013). The only two studies on boreal conifers to explore the effects of both warming and elevated CO₂ on V_{cmax} found different responses: in mature trees of *Picea abies*, V_{cmax} acclimated to CO₂ with no effect of warming (Lamba et al., 2018), while in seedlings of *Pinus sylvestris*, both warming and high CO₂ increased V_{cmax} (Kellomaki & Wang, 1996). Therefore, more data are needed to improve our understanding of the interactive effects of CO₂ and temperature on photosynthetic and respiratory physiology in these important northern trees.

In boreal forests, *Larix* and *Picea* spp. are common, and their distribution extends through North America and across Eurasia

(Gower & Richards, 1990; Schmidt, 2003). In North American boreal forests, *P. mariana* (black spruce) accounts for 44% of the Canadian forested area, while *L. laricina* (tamarack) accounts for ~3.4% (Girardin, Bouriaud, et al., 2016), but is also widespread and frequently co-occurs with black spruce (Gower & Richards, 1990). Black spruce is an evergreen, late-successional conifer (Taylor, Hart, & Chen, 2013), whereas tamarack is a deciduous, early-successional conifer (Gower & Richards, 1990). Based on the different plant functional types of these species, we may expect contrasting responses to climate change. Evergreens have a weaker ability to acclimate growth and photosynthesis to warming compared to deciduous tree species (Dusenge et al., 2019; Way & Oren, 2010; Way & Yamori, 2014). Moreover, a recent study showed that a late-successional species, *P. abies*, had a limited ability to acclimate to warming compared to *P. sylvestris*, an early-successional species (Kurepin et al., 2018). Based on potential differences in how these species may respond to climate change, we investigated the responses of black spruce and tamarack to moderate (+4°C) and extreme (+8°C) warming combined with ambient (407 ppm) or elevated growth CO₂ concentrations (750 ppm). We tested the following hypotheses:

1. A_{net} will acclimate to warming in tamarack (a deciduous conifer), but in black spruce (an evergreen conifer), A_{net} will respond negatively to warming. The A_{net} of both species will increase with elevated CO₂ across the warming treatments.
2. Photosynthetic capacity (i.e., V_{cmax} and J_{max}) will be reduced by elevated CO₂, but will not be altered by growth temperature in either species.
3. Dark respiration will acclimate to temperature (i.e., be reduced in warm-grown plants), but not to CO₂, in both species.
4. Moderate warming will stimulate growth in tamarack, but reduce growth in black spruce, while extreme warming will reduce growth in both species. Elevated CO₂ will stimulate growth equally in both species.

2 | MATERIALS AND METHODS

2.1 | Plant material and experimental growth conditions

Picea mariana (Mill.) B.S.P. (black spruce) and *L. laricina* (Du Roi) K. Koch (tamarack) were grown from seed. Seeds were provided by the National Tree Seed Centre (Canadian Forest Service; *P. mariana*: seedlot 20,083,181.0 [46°2'N, 82°5'W] *L. laricina*: seedlot 7,930,280.1 [45°2'N, 75°0'W]). Seed lots were selected to match ambient temperature and photoperiod conditions of London, ON, Canada, where the experiment was carried out. This region represents the southern range of the natural distribution of these two species. Prior to sowing seeds on 1 May 2016, 98 pots (49 per species) filled with growth medium were randomly assigned to each of six climate-controlled glasshouses at Western University's Biotron facility

(43°0'N, 81°16'W). Each glasshouse had a different combination of CO₂ and temperature: either ambient CO₂ (407 μmol/mol CO₂; AC) or elevated CO₂ (750 μmol/mol CO₂; EC) crossed with either ambient (0T), ambient +4°C (4T) or ambient +8°C (8T; Figure 1). The ambient temperature regime was derived from hourly temperature averages for 2008–2012 from the London, ON airport meteorological station (Environment Canada). Relative humidity was maintained around 60% in each treatment. CO₂ concentrations were controlled by blowing pure CO₂ into the elevated CO₂ glasshouses, using Argus Control Software (Surrey, BC, Canada) TITAN version 1 (Build 00226B.00, Site ID: 1960); CO₂ concentrations were recorded every 3 hr with an infrared gas analyzer.

Five to 10 seeds of a single species were sown in 11.6 L pots filled with Pro-Mix BX Mycorrhizae growth medium (Premier Tech Horticulture) and fertilized with all-purpose slow release fertilizer (Slow Release Plant Food, 12-4-8, Miracle Gro®; The Scotts Company). Watering was provided as needed to maintain a moist growth medium. Seedlings were thinned to avoid competition

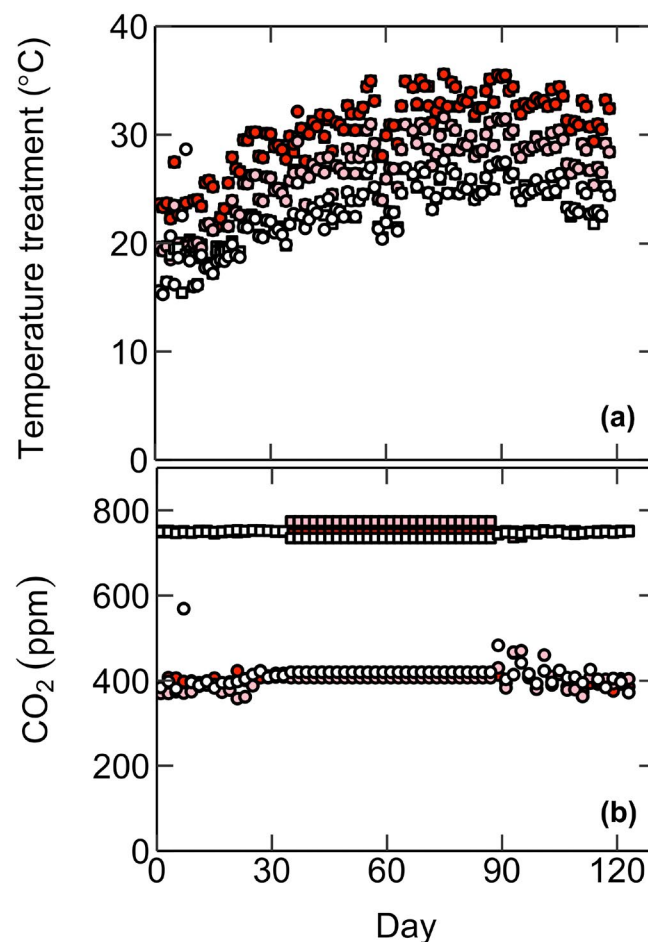


FIGURE 1 Environmental data for the six climate-controlled glasshouses. (a) Air temperature and (b) CO₂ concentrations from the beginning of the experiment till the start of the gas exchange data collection. Symbols represent growth CO₂ concentration (ambient, AC = circle; elevated, EC = square); colors represent growth temperature (ambient, 0T = white; ambient +4°C, 4T = pink; ambient +8°C, 8T = red). AC, ambient CO₂; EC, elevated CO₂

once they established, such that only one seedling per pot remained. After 60 days postplanting, some tamarack in EC8T began to show chlorosis and needle browning. To ensure that this was related to temperature stress and not nutrient limitations, we applied half-strength Hoagland's fertilizer to all seedlings during all treatments once a week for 4 weeks, but with no effect on these symptoms.

By the end of the experiment (31 October 2016), 15 tamarack seedlings (all from AC8T) died, as did one black spruce seedling (also from the AC8T treatment). At the end of the experiment, all remaining seedlings were measured and dried for biomass.

2.2 | Gas exchange measurements

Leaf gas exchange was measured when seedlings were 5 months old. Five healthy (i.e., with no brown needles) seedlings per species were randomly selected from each treatment and measured using one of two portable photosynthesis systems (Li-Cor 6400XT and opaque conifer chamber 6400-22 with 6400-18 RGB light source; Li-Cor Inc.). To achieve the targeted leaf temperatures, a well-watered, potted seedling and the Li-Cor cuvette were placed inside a walk-in growth chamber to ensure that the seedling was exposed to the same temperature as the leaf in the cuvette.

To assess the temperature response of photosynthetic capacity, light-saturated net CO₂ assimilation rates (A_{net}) were measured at varying intercellular CO₂ concentrations (C_i) to build A-C_i curves, using a photosynthetic photon flux density of 1,400 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ and four leaf temperatures (10, 20, 30, and 40°C). The A-C_i curve was started once gas exchange was stable at a reference CO₂ of 400 $\mu\text{mol/m}^2$; ambient CO₂ concentrations (C_a) were then changed sequentially to 400, 200, 150, 100, 50, 400, 750, 1,200, 1,600 and 2,000 and 400 $\mu\text{mol/m}^2$.

To assess the temperature response of A_{net} and dark respiration, separate measurements of A_{net} were conducted on another five seedlings at 10, 15, 20, 25, 30, 35, and 40°C. At each temperature, light-saturated A_{net} was first assessed at a cuvette CO₂ concentration of 400 ppm, then at 750 ppm. The cuvette CO₂ was then returned to 400 ppm, the cuvette irradiance was set to 0 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, and dark respiration (R_d) was measured after a 20 min dark-acclimation period. Only one measurement CO₂ (400 ppm) was used in the R_d measurements, as there is no direct effect of measurement CO₂ on R_d (Amthor et al., 2001). Since the VPD increases with air temperature, which reduces stomatal conductance (Lin et al., 2012), we moistened the soda lime column at 40°C to minimize stomatal closure associated with high VPDs. The VPD was maintained between approximately 0.5 and 4.5 kPa across the full 30°C range of measurement temperatures for all gas exchange measurements. This measurement procedure was repeated at each of the seven measurement temperatures, resulting in two A_{net} temperature response curves (one at 400 ppm CO₂, the other at 750 ppm) and one R_d temperature

response curve for each tree. After R_d measurements, the lights were switched back on, the next temperature was set, and leaves were acclimated for ~40 min before starting A_{net} measurements at the next temperature.

We measured in five consecutive cycles, with each cycle comprising measurements going from the 8T treatment down to the 0T treatment to distribute any effects of growth temperature or CO₂ on developmental stage of the seedlings over the course of the measurements.

2.3 | Parameterization of photosynthesis models

The C₃ photosynthesis model by Farquhar et al. (1980) was used to estimate V_{cmax} and J_{max} from the A-C_i curves. Mesophyll conductance was not measured, therefore only apparent V_{cmax} and J_{max} based on C_i , rather than the CO₂ concentration at the site of carboxylation (C_c), are reported. V_{cmax} was calculated from:

$$A_c = \frac{V_{\text{cmax}}(C_i - \Gamma^*)}{\left[C_i + K_c \left(1 + \frac{O}{K_o} \right) \right]} - R_{\text{day}}, \quad (2)$$

where V_{cmax} is the maximum rate of Rubisco carboxylation, C_i and O are the intercellular concentrations of CO₂ and O₂, respectively, K_c and K_o are the Michaelis-Menten coefficients of Rubisco activity for CO₂ and O₂, respectively, Γ^* is the CO₂ compensation point in the absence of mitochondrial respiration, and R_{day} is mitochondrial respiration in the light. R_{day} was not measured and the value used was the one generated after fitting the equation.

J_{max} was calculated from electron transport rates (J) using:

$$A_j = \left(\frac{J}{4} \right) \times \frac{(C_i - \Gamma^*)}{(C_i + 2\Gamma^*)} - R_{\text{day}}, \quad (3)$$

where J is the rate of electron transport. J_{max} was then calculated by:

$$\theta J^2 - (\alpha Q + J_{\text{max}})J + \alpha Q J_{\text{max}} = 0, \quad (4)$$

where J_{max} is the potential rate of electron transport, θ is the curvature of the light response curve, and α is the quantum yield of electron transport. The values of θ and α were fixed at 0.9 and 0.3 mol electrons/mol photon (Medlyn et al., 2002). Since the two species are cold-adapted, we used values of K_c , K_o , and Γ^* from spinach, a cool-adapted C₃ plant (Galmés, Hermida-Carrera, Laanisto, & Niinemets, 2016). The thermal sensitivity of K_c and K_o was modeled from Medlyn et al. (2002):

$$K_c = 274.6 \exp \left[\frac{80,500(T_k - 298)}{(298RT_k)} \right], \quad (5)$$

$$K_o = 419.8 \exp \left[\frac{14,500(T_k - 298)}{(298RT_k)} \right], \quad (6)$$

where T_k denotes the leaf temperature and R is the universal gas constant ($8.314 \text{ J mol}^{-1} \text{ K}^{-1}$).

Γ^* was calculated based on von Caemmerer (2000):

$$\Gamma^* = \frac{0.5O}{S_{c/o}}, \quad (7)$$

where O is the air oxygen concentration (210 mmol/mol). The temperature sensitivity of $S_{c/o}$ was modeled from Medlyn et al. (2002):

$$S_{c/o} = 2.321 \exp \left[-\frac{29,000(T_k - 298)}{(298RT_k)} \right], \quad (8)$$

which generated a Γ^* at 25°C of $45.2 \text{ } (\mu\text{mol/mol})$, close to the $42.75 \text{ } (\mu\text{mol/mol})$ value measured for tobacco at 25°C (Medlyn et al., 2002).

During parameterization of V_{cmax} and J_{max} , we applied a restriction to the model fitting such that data points with a C_i below 100 or above 1,000 $\mu\text{mol/mol}$ were forced to be V_{cmax} - and J_{max} -limited, respectively (as in Vårhammar et al., 2015). At high C_i (above 1,000 $\mu\text{mol/mol}$) and low leaf temperatures (10 and 20°C), a few trees were limited by triose phosphate use (data not shown). V_{cmax} and J_{max} were parameterized when the model fitting efficiency to the raw data was at least 96%.

2.4 | Modelling temperature responses of A_{sat} , V_{cmax} , J_{max} , and R_d

The temperature response of A_{net} at growth CO_2 was fitted using a quadratic regression (Kroner & Way, 2016):

$$A_{sat} = aT_{leaf}^2 + bT_{leaf} + c, \quad (9)$$

where A_{sat} represents net photosynthesis measured at saturating light and growth CO_2 , T_{leaf} is the measuring leaf temperature, and a , b and c are fitted constants. Maximum rates of net photosynthesis (A_{opt}) and the T_{opt} of A_{sat} were derived from Equation (9). The temperature responses of V_{cmax} and J_{max} were fitted using Equation (1) to derive values for E_{av} , E_{aj} , T_{optV} , T_{optJ} , $V_{cmaxopt}$, and J_{maxopt} . The value of H_d value was fixed at 200 kJ/mol to avoid overparameterization (Kattge & Knorr, 2007; Medlyn et al., 2002).

The temperature response of R_d was fitted with a quadratic regression on natural log-transformed R_d values (Heskel et al., 2016). The Q_{10} (the quotient of increase in respiration for a 10°C rise in leaf temperature) in the range of 10 – 40°C was calculated as in Atkin & Tjoelker (2003):

$$Q_{10} = \left(\frac{R_2}{R_1} \right)^{\frac{10}{(T_2 - T_1)}}, \quad (10)$$

where R_1 and R_2 are the respiration rates at the lower (T_1) and higher temperature (T_2).

2.5 | Leaf structural and chemical analyses

After gas exchange measurements, the needles from each of the 10 measured trees per treatment were collected. Part of each sample was oven-dried at 60°C to a constant mass, ground into fine powder with a Wiley mill (Thomas Scientific), and analyzed for carbon and nitrogen content using an elemental analyzer (Carlo-Erba NA-1500). Another part of each sample was directly frozen in liquid N_2 and stored at -80°C for quantification of chlorophyll.

2.6 | Total leaf chlorophyll measurements

For the five seedlings on which the A - C_i curves were conducted, a subsample of the measured needles was collected for chlorophyll concentration quantification. These needles were ground in liquid N_2 , pigments were extracted in 100% methanol, and the absorption values of the supernatant were measured at 470, and 653 and 666 nm for total carotenoids (xanthophylls and carotenes), chlorophyll a (Chl_a), and chlorophyll b (Chl_b) concentrations, respectively, as outlined in Wellburn (1994):

$$[\text{Chl}_a] = 15.65 \times A_{666} - 7.34 \times A_{653}, \quad (11)$$

$$[\text{Chl}_b] = 27.05 \times A_{653} - 11.21 \times A_{666}, \quad (12)$$

$$[\text{Carotenoids}] = \frac{1,000 \times A_{470} - 2.86 \times [\text{Chl}_a] - 129.2 \times [\text{Chl}_b]}{221}, \quad (13)$$

where A_{470} , A_{653} , and A_{666} represent absorption at the wavelength 470, 653, and 666 nm, respectively.

2.7 | Growth and biomass

At the end of the experiment, all remaining trees were harvested and dried at 60°C to a constant mass ($n = 12$ – 16 for black spruce and $n = 12$ – 15 for tamarack except in AC8T, where tamarack $n = 4$). Roots, stem, and needles were weighed separately to obtain the dry mass for each component.

2.8 | Statistical analyses

All data are reported as means \pm SE. Two-way ANOVAs were used to analyze the main effects of growth temperature and CO_2 , and their interactions, on temperature response parameters of gas exchange (Figures 2 and 3), biomass traits (Figure 5), and leaf biochemical traits (Figure 6). Two-way repeated measures ANOVAs were used to analyze the main effects of growth temperature, and CO_2 , and their interactions, on the responses of A_{net} , g_s , C_i/C_a , V_{cmax} , J_{max} , R_{dark} , and J_{max}/V_{cmax} ratio to changes in leaf temperature. Post hoc Tukey's honest significance tests were used to evaluate responses

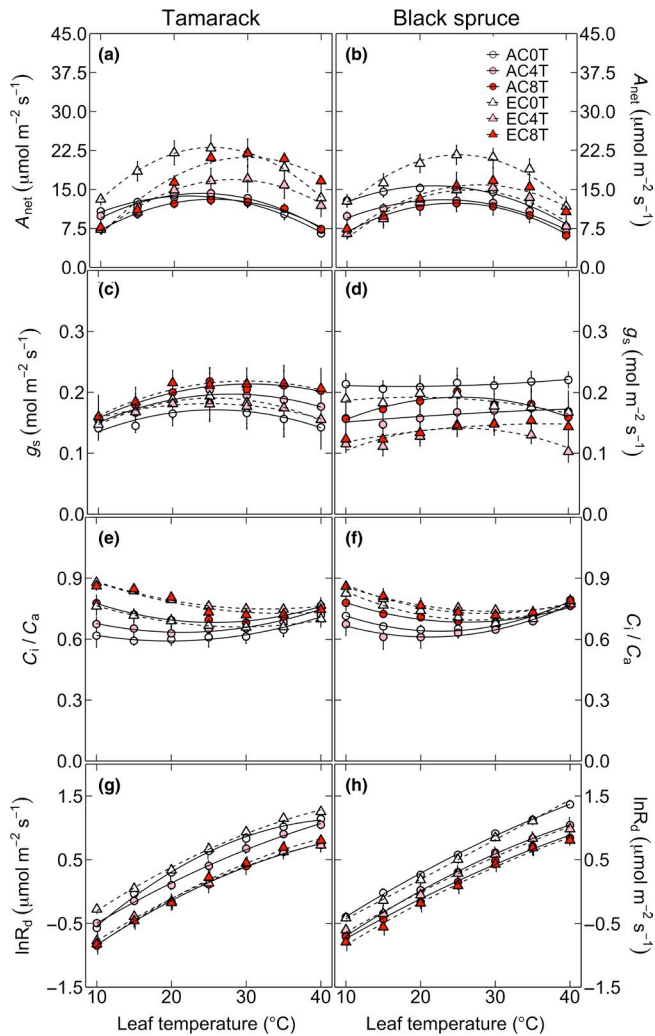


FIGURE 2 Gas exchange responses to leaf temperature in tamarack (a, c, e, g) and black spruce (b, d, f, h) grown under different CO₂ and temperature treatments. (a, b) Net CO₂ assimilation rates (A_{net} , $\mu\text{mol m}^{-2} \text{s}^{-1}$); (c, d) stomatal conductance (g_s , $\text{mol m}^{-2} \text{s}^{-1}$); (e, f) the ratio between intercellular and air CO₂ concentration (C_i/C_a); (g, h) In-transformed dark respiration rates (R_d , $\mu\text{mol m}^{-2} \text{s}^{-1}$). Measurements of A_{net} , g_s , and C_i/C_a were conducted at a CO₂ of 400 and 750 ppm for AC and EC treatments, respectively. Measurements of R_d were taken at 400 ppm only. Lines represent regression lines for growth CO₂ (AC = solid; EC = dashed lines). Symbols represent growth CO₂ (AC = circle; EC = triangle). Colors represent growth temperature (OT = white; 4T = pink; 8T = red). Means \pm SE. $n = 5$. AC, ambient CO₂; EC, elevated CO₂

to individual combinations of CO₂ and temperature as indicated. All analyses were performed in R (version 3.5.2 [20 December 2018]).

3 | RESULTS

3.1 | Experimental treatments

Air temperatures increased from May until midsummer (maximum day temperature of 29°C in the OT treatment), decreasing in late

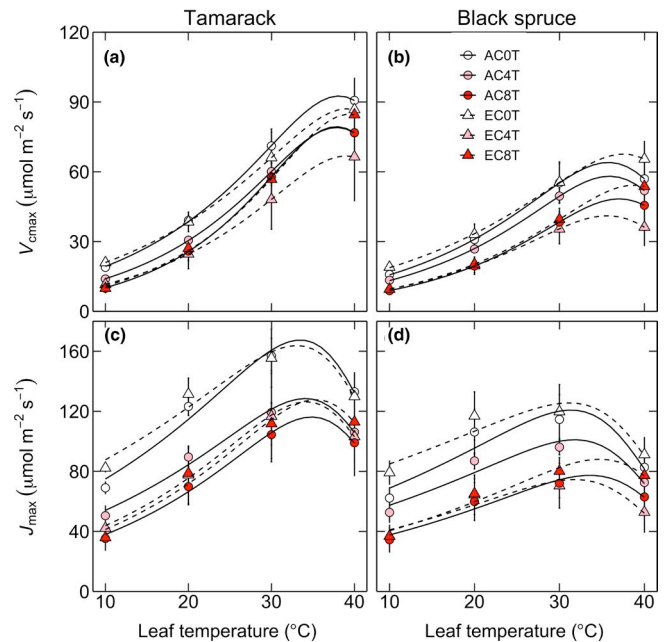


FIGURE 3 Temperature responses of photosynthetic capacity in tamarack (a, c) and black spruce (b, d) grown under different CO₂ and temperature treatments. (a, b) Maximum carboxylation rates of Rubisco (V_{cmax} , $\mu\text{mol m}^{-2} \text{s}^{-1}$) and (c, d) maximum electron transport rates (J_{max} , $\mu\text{mol m}^{-2} \text{s}^{-1}$). Lines represent regression lines for growth CO₂ (AC = solid; EC = dashed lines). Symbols represent growth CO₂ (AC = circle; EC = triangle). Colors represent growth temperature (OT = white; 4T = pink; 8T = red). Means \pm SE. $n = 5$. AC, ambient CO₂; EC, elevated CO₂

summer to a minimum of 19°C (Figure 1a). The warming treatments of +4 and +8 were successfully maintained. Mean CO₂ concentrations were 407 ± 22 ppm and 750 ± 11 ppm in the AC and EC glasshouses, respectively (Figure 1b).

3.2 | Gas exchange measurements

In tamarack, temperature response curves of A_{net} measured at growth CO₂ were altered by both growth temperature and growth CO₂ (Figure 2a; Table 1). Comparing the AC0T and EC8T trees, future growth conditions and measurement CO₂ concentrations greatly stimulated A_{net} above 20°C in tamarack (Figure 2a). Maximum rates of A_{net} (A_{opt}) measured at the growth CO₂ increased in EC tamarack, but were not affected by warming (Tables 2 and 3). The photosynthetic thermal optimum (T_{optA}) of tamarack increased with warming (0.5 and 0.65°C per 1°C warming for AC and EC, respectively), and was ~4°C higher in EC than AC treatments (Tables 2 and 3). In black spruce, the temperature response of A_{net} was also altered by growth conditions (Figure 2b; Table 1): Warming suppressed A_{net} , while elevated CO₂ stimulated A_{net} . While EC8T spruce had higher A_{net} above 35°C than AC0T trees, photosynthetic rates were comparable in the two treatments, even though A_{net} was measured at high CO₂ in the EC8T trees (Figure 2b). This was largely because A_{opt} was 12%–27% higher in EC than AC spruce, but was suppressed by warming (16%–21% in AC and 26%–31% in EC;

TABLE 1 Summary report of the repeated-measures ANOVA in tamarack and black spruce, showing F -values and p -values with leaf temperature (T_{leaf}), CO_2 and temperature treatments as the main effects. Traits analyzed were: A_{net} (net CO_2 assimilation rates); g_s (stomatal conductance); C_i/C_a (ratio of intercellular to air CO_2 concentration); $\ln R_d$ (\ln -transformed dark respiration rates); V_{cmax} (maximum carboxylation rate of Rubisco); J_{max} (maximum rate of electron transport)

	Tamarack		Black spruce	
	F -value	p -value	F -value	p -value
A_{net}				
T_{leaf}	7.7	.006	0.4	.5
CO_2	25.9	<.0001	4.7	.039
Temperature	2.2	.14	5.6	.0096
$T_{\text{leaf}}:\text{CO}_2$	28.5	<.0001	17.4	<.0001
$T_{\text{leaf}}:\text{Temperature}$	9.5	.0001	3.5	.031
$\text{CO}_2:\text{Temperature}$	2.9	.07	0.8	.4
$T_{\text{leaf}}:\text{CO}_2:\text{Temperature}$	1.6	.2	0.02	.9
g_s				
T_{leaf}	13.5	.0003	3.3	.07
CO_2	0.03	.8	3.9	<i>.058</i>
Temperature	1	.3	3.4	.049
$T_{\text{leaf}}:\text{CO}_2$	0.7	.4	0.8	.4
$T_{\text{leaf}}:\text{Temperature}$	4	.019	2.4	<i>.09</i>
$\text{CO}_2:\text{Temperature}$	0.15	.8	0.025	.9
$T_{\text{leaf}}:\text{CO}_2:\text{Temperature}$	0.6	.5	3.7	.026
C_i/C_a				
T_{leaf}	14	.0003	2.1	.2
CO_2	13.7	.0011	18	.0003
Temperature	6.1	.0072	1.4	.2
$T_{\text{leaf}}:\text{CO}_2$	88.9	<.0001	38.8	<.0001
$T_{\text{leaf}}:\text{Temperature}$	9.5	.0001	3.5	.03
$\text{CO}_2:\text{Temperature}$	0.8	.4	1.5	.2
$T_{\text{leaf}}:\text{CO}_2:\text{Temperature}$	0.9	.4	2.9	<i>.056</i>
$\ln R_d$				
T_{leaf}	3,442	<.0001	7,955	<.0001
CO_2	0.23	.6	0.4	.5
Temperature	11	.0004	11.9	.0003
$T_{\text{leaf}}:\text{CO}_2$	0.042	.8	0	.9
$T_{\text{leaf}}:\text{Temperature}$	0.96	.4	3.2	.041
$\text{CO}_2:\text{Temperature}$	2.1	.14	0.046	.9
$T_{\text{leaf}}:\text{CO}_2:\text{Temperature}$	0.8	.4	1.3	.2
V_{cmax}				
T_{leaf}	654	<.0001	525	<.0001
CO_2	0.2	.6	0.16	.69
Temperature	2.5	.1	5.5	.011
$T_{\text{leaf}}:\text{CO}_2$	0.3	.6	0.02	.9
$T_{\text{leaf}}:\text{Temperature}$	1.9	.15	3.4	.039

(Continues)

TABLE 1 (Continued)

	Tamarack		Black spruce	
	F -value	p -value	F -value	p -value
$\text{CO}_2:\text{Temperature}$	0.3	.7	1.8	.18
$T_{\text{leaf}}:\text{CO}_2:\text{Temperature}$	0.8	.4	2.9	<i>.059</i>
J_{max}				
T_{leaf}	131	<.0001	26.8	<.0001
CO_2	0.04	.8	0.017	.9
Temperature	7.2	.0036	7.3	.0032
$T_{\text{leaf}}:\text{CO}_2$	0.0017	.9	0.007	.9
$T_{\text{leaf}}:\text{Temperature}$	0.6	.6	1.8	.2
$\text{CO}_2:\text{Temperature}$	0.2	.8	1.5	.2
$T_{\text{leaf}}:\text{CO}_2:\text{Temperature}$	0.6	.5	0.5	.6

Note: Bold numbers represent p -value less than .05 ($p < .05$), and italicized numbers are p -values between .05 and .1 ($.05 < p < .1$).

Tables 2 and 4). T_{optA} was increased by both growth at warmer temperatures (0.41 and 0.36°C per 1°C warming respectively for AC and EC) and by ~3.6°C by high CO_2 in black spruce (Tables 2 and 4).

In tamarack, there was a trend for stomatal conductance (g_s) to be higher in warm-grown seedlings (0T vs. 8T), with the greatest increases in g_s occurring in 8T seedlings under warm leaf temperatures ($p = .019$; Figure 2c; Table 1). In contrast, in black spruce, g_s was significantly reduced by warming and marginally reduced by CO_2 ($p = .058$; Figure 1d; Table 1). In both species, higher growth and measurement CO_2 increased C_i/C_a . Additionally, the impact of warming and elevated CO_2 on C_i/C_a varied with measurement temperature, with C_i/C_a converging at 40°C in both species (Figure 2e,f; Table 1).

In both species, dark respiration (R_d) decreased with warming, without any effect of CO_2 (Figure 2g,h; Table 1), and R_d measured at 20°C was suppressed in warm-grown plants (Tables 2–4). The Q_{10} was similar across all treatments in tamarack (~1.7), but was reduced by warming in black spruce (Tables 2–4).

3.3 | Photosynthetic capacity

In tamarack, the temperature response curve of V_{cmax} was not significantly altered by the treatments (Figure 3a; Table 1). The leaf temperature at which tamarack V_{cmax} was highest (T_{optV}) was increased by elevated growth CO_2 , but values of V_{cmax} at its thermal optimum (V_{cmaxopt}) were not significantly affected by either CO_2 or temperature (Tables 2 and 3). In contrast, in black spruce, the temperature response curve of V_{cmax} was altered by warming, and V_{cmax} was reduced by warming (Figure 3b; Table 1). In addition, the V_{cmaxopt} was reduced to 41% by warming, but the T_{optV} remained unchanged by the treatments (Tables 2 and 4). In both species, J_{max} was reduced by warming, without any effect of growth CO_2 (Figure 3c,d; Table 1), while the T_{optJ} in both species acclimated to warming (0.25–0.38°C increase per 1°C of warming). J_{maxopt} was reduced to 32% by warming in black spruce (Tables 2 and 4),

TABLE 2 Photosynthetic and respiratory traits measured in this study in tamarack and black spruce seedlings grown under ambient CO₂ (AC) or elevated CO₂ (EC) at either ambient temperatures (OT), ambient + 4°C (4T), or ambient + 8°C (8T). Net CO₂ assimilation rate at the thermal optimum (A_{opt} , $\mu\text{mol m}^{-2} \text{s}^{-1}$); thermal optimum of net CO₂ assimilation (T_{optA} , °C); dark respiration rates measured at 20°C (R_{d20} , $\mu\text{mol m}^{-2} \text{s}^{-1}$); the change in R_d per 10°C increase in temperature (Q_{10}); maximum carboxylation rates of Rubisco at the thermal optimum ($V_{cmaxopt}$, $\mu\text{mol m}^{-2} \text{s}^{-1}$); maximum electron transport rate at the thermal optimum (J_{maxopt} , $\mu\text{mol m}^{-2} \text{s}^{-1}$); thermal optimum of V_{cmax} (T_{optV} , °C); thermal optimum of J_{max} (T_{optJ} , °C); activation energy of V_{cmax} (E_{aV}); activation energy of J_{max} (E_{aJ}); V_{cmax} (V_{cmax20} , $\mu\text{mol m}^{-2} \text{s}^{-1}$) and J_{max} (J_{max20} , $\mu\text{mol m}^{-2} \text{s}^{-1}$) measured at 20°C and the ratio of J_{max20} to V_{cmax20} (J_{max20}/V_{cmax20}). Means \pm SE, $n = 5$

	AC0T	AC4T	AC8T	EC0T	EC4T	EC8T
Tamarack						
A_{opt}	13.9 \pm 0.8a	14.4 \pm 0.7ab	13.1 \pm 0.9a	23.2 \pm 2.6 c	16.9 \pm 2.5abc	21.2 \pm 1.2bc
T_{optA}	21.2 \pm 1.7a	23.5 \pm 1ab	25.3 \pm 0.3ab	24.9 \pm 1.1ab	27.6 \pm 0.6bc	30 \pm 0.4 c
R_{d20}	1.3 \pm 0.2ab	1.2 \pm 0.2ab	0.6 \pm 0.06a	1.5 \pm 0.09b	0.9 \pm 0.2ab	0.7 \pm 0.1a
Q_{10}	1.8 \pm 0.06a	1.7 \pm 0.015a	1.7 \pm 0.08a	1.7 \pm 0.02a	1.7 \pm 0.09a	1.7 \pm 0.07a
$V_{cmaxopt}$	93.9 \pm 9.5a	79.5 \pm 7.9a	79.7 \pm 8.2a	88.6 \pm 14.9a	67.4 \pm 18.9a	85.1 \pm 10.2a
T_{optV}	38.6 \pm 0.9a	38.1 \pm 0.6a	37.9 \pm 0.4a	39.8 \pm 0.8a	38.9 \pm 0.7a	39.4 \pm 0.5a
J_{maxopt}	168 \pm 14a	128 \pm 7a	116 \pm 16a	164 \pm 21a	126 \pm 33a	128 \pm 17a
T_{optJ}	33 \pm 0.3ab	33 \pm 0.2abc	34.9 \pm 0.4cd	32.9 \pm 0.2a	34.3 \pm 0.1bcd	35.2 \pm 0.3d
E_{aV}	47 \pm 4ab	53 \pm 5abc	66 \pm 3 c	40 \pm 4a	51 \pm 1.4abc	60 \pm 2bc
E_{aJ}	30 \pm 4ab	32 \pm 4ab	40 \pm 3b	23 \pm 3a	37 \pm 1b	38 \pm 2b
V_{cmax20}	39 \pm 2a	30 \pm 2a	26 \pm 4a	39 \pm 4a	25 \pm 6a	27 \pm 3a
J_{max20}	123 \pm 6bc	90 \pm 6abc	70 \pm 12a	131 \pm 10 c	78 \pm 19ab	78 \pm 10ab
J_{max20}/V_{cmax20}	3.2 \pm 0.1bc	2.9 \pm 0.1ab	2.6 \pm 0.06a	3.5 \pm 0.09 c	3.2 \pm 0.02bc	2.9 \pm 0.09ab
Black spruce						
A_{opt}	15.7 \pm 0.9ab	13.0 \pm 0.7a	12.3 \pm 1.7a	21.7 \pm 1.9b	14.9 \pm 2ab	15.9 \pm 2.6ab
T_{optA}	21.4 \pm 0.8a	22.9 \pm 0.8ab	24.7 \pm 0.5bc	25.3 \pm 0.4bc	26.5 \pm 0.5cd	28.1 \pm 0.5d
R_{d20}	1.2 \pm 0.3a	0.8 \pm 0.09a	0.80 \pm 0.06a	1.2 \pm 0.2a	0.7 \pm 0.1a	0.74 \pm 0.06a
Q_{10}	1.82 \pm 0.01a	1.78 \pm 0.03a	1.70 \pm 0.02a	1.80 \pm 0.05a	1.70 \pm 0.05a	1.70 \pm 0.05a
$V_{cmaxopt}$	64.4 \pm 7.9a	58.6 \pm 1.9a	49.3 \pm 7.9a	69.8 \pm 9.2a	42 \pm 8a	55 \pm 6a
T_{optV}	36 \pm 1a	36 \pm 1a	37 \pm 1a	39 \pm 2a	36 \pm 1a	39 \pm 1a
J_{maxopt}	121 \pm 17a	101 \pm 4.9a	78 \pm 10a	126 \pm 19a	75 \pm 16a	89 \pm 9a
T_{optJ}	31.3 \pm 0.4a	31.8 \pm 0.3a	33.7 \pm 0.4ab	31 \pm 0.2a	31.2 \pm 1.2a	34.8 \pm 0.7b
E_{aV}	46 \pm 3a	51 \pm 7a	57 \pm 5a	39 \pm 3a	50 \pm 4a	53 \pm 3a
E_{aJ}	23 \pm 2a	23 \pm 4a	30 \pm 5a	15 \pm 2a	26 \pm 4a	29 \pm 5a
V_{cmax20}	31 \pm 3a	27 \pm 1a	20 \pm 3a	33 \pm 5a	20 \pm 4a	20 \pm 2a
J_{max20}	106 \pm 15ab	86 \pm 5ab	59 \pm 12a	116 \pm 16b	64 \pm 13ab	65 \pm 9ab
J_{max20}/V_{cmax20}	3.4 \pm 0.2a	3.3 \pm 0.2a	2.9 \pm 0.2a	3.50 \pm 0.05a	3.3 \pm 0.2a	3.15 \pm 0.13a

Note: Letters after each value represent group comparisons across the six temperature and CO₂ treatments generated from a Tukey post hoc test ($p < .05$).

whereas in tamarack, there was a trend for lower J_{maxopt} in warm-grown seedlings ($p = .07$; Table 2). In tamarack, the J_{max}/V_{cmax} ratio was reduced by warming across the entire measuring temperature range (10–40°C; Figure S1a; Table S1). In contrast, in black spruce, the J_{max}/V_{cmax} ratio was marginally affected by warming ($p = .056$), largely due to convergence of the J_{max}/V_{cmax} ratio across warming treatments with increasing leaf temperature (Figure S1b; Table S1).

The activation energies for V_{cmax} and J_{max} increased with warming (E_{aV} , 2.4 and 1.5 $\text{kJ mol}^{-1} \text{ } ^\circ\text{C}^{-1}$; E_{aJ} , 1.6 and 1.25 $\text{kJ mol}^{-1} \text{ } ^\circ\text{C}^{-1}$ in tamarack and black spruce, respectively) without any CO₂ effects (Tables 2–4). The optimum temperature and activation energy for

J_{max} were consistently lower (T_{optJ} : 33°C; E_{aJ} : 30 kJ/mol) than those of V_{cmax} (T_{optV} : 38°C; E_{aV} : 52 kJ/mol ; Table 2).

4 | RELATIONSHIPS BETWEEN PHOTOSYNTHETIC AND RESPIRATORY PARAMETERS

In both species, V_{cmax20} and J_{max20} were reduced by warming, without any CO₂ effect (Tables 2–4). The ratio between J_{max20} and V_{cmax20} indicates the balance between these two biochemical processes at a moderate leaf temperature. In tamarack, J_{max20}/V_{cmax20} was affected

	Temperature		CO ₂		Temperature × CO ₂	
	F-value	p-value	F-value	p-value	F-value	p-value
Temperature response of gas exchange						
<i>A</i> _{opt}	1.6	.2	24.9	.0001	2.4	.1
<i>T</i> _{optA}	10.5	.0005	25.9	<.0001	0.14	.9
<i>T</i> _{maxA}	9.8	.00075	28.2	<.0001	5.1	.014
<i>R</i> _{d20}	11.4	.0004	0.015	.9	1.9	.17
<i>Q</i> ₁₀	0.3	.8	0.04	.8	0.6	.6
Temperature response of photosynthetic capacity						
<i>V</i> _{cmaxopt}	1	.4	0.2	.7	0.3	.8
<i>T</i> _{optV}	0.6	.6	4.8	.04	0.1	.9
<i>J</i> _{maxopt}	2.9	.07	0.009	.9	0.09	.9
<i>T</i> _{optJ}	23	<.0001	0.1	.8	1.2	.3
<i>E</i> _{av}	14	<.0001	2.9	.09	0.3	.7
<i>E</i> _{aj}	9	.0012	0.3	.6	1.9	.2
<i>V</i> _{cmax20}	5.9	.008	0.3	.6	0.4	.7
<i>J</i> _{max20}	12	.0002	0.035	.9	0.5	.6
<i>J</i> _{max20} : <i>V</i> _{cmax20}	12.8	.0002	9.3	.006	0.03	.9
Plant biomass traits						
Total biomass	5	.0099	0.02	.9	1.6	.2
Root mass ratio	4.3	.017	0.7	.4	1.4	.2
Stem mass ratio	1.4	.3	1.2	.3	0.015	.9
Leaf mass ratio	5.7	.005	0.3	.6	2.7	.07
LMA	7.4	.0014	0.8	.4	1.5	.2
Biochemical traits						
<i>N</i> _a	12.4	<.0001	0.6	.5	2	.1
Chl _a	1.1	.3	0.12	.7	0.4	.7
Chl _b	0.6	.5	0.1	.7	0.6	.5
Carotenoid	2.3	.1	0.1	.7	0.1	.8

Note: Bold numbers represent *p*-value less than .05 ($p < .05$), and bold and italicized are *p*-values between .05 and .1 ($.05 < p < .1$).

by both warming and growth CO₂, such that the ratio was higher in EC plants, and decreased with warming (Tables 2 and 3). In black spruce, $J_{\text{max}20}/V_{\text{cmax}20}$ was only affected by growth temperature, and declined with warming (Tables 2 and 4).

In both species, T_{optA} was positively correlated with both E_{av} (0.1–0.25°C per 1 kJ/mol) and E_{aj} (0.11–0.24°C per 1 kJ/mol) across the warming treatments, but negatively correlated with $J_{\text{max}20}/V_{\text{cmax}20}$ (Figure 4). In addition, there was an elevated CO₂ effect, such that the intercepts of these relationships were higher in EC compared to AC seedlings (Figure 4).

4.1 | Biomass and allocation

In tamarack, warming significantly affected biomass, without any CO₂ effect. Trees in 4T had the highest biomass compared to the 0T (+81% in AC and +29% in EC) and 8T seedlings. However, tamarack

TABLE 3 Summary report of ANOVA of CO₂ and temperature treatments in tamarack on gas exchange parameters, photosynthetic capacity, plant biomass and biochemical traits. Net CO₂ assimilation rate at the thermal optimum (A_{opt} , $\mu\text{mol m}^{-2} \text{s}^{-1}$); thermal optimum of net CO₂ assimilation (T_{optA} , °C); dark respiration rates measured at 20°C ($R_{\text{d}20}$, $\mu\text{mol m}^{-2} \text{s}^{-1}$); the change in R_{d} per 10°C increase in temperature (Q_{10}); maximum carboxylation rates of Rubisco at the thermal optimum (V_{cmaxopt} , $\mu\text{mol m}^{-2} \text{s}^{-1}$); thermal optimum of V_{cmax} (T_{optV} , °C); maximum electron transport rates at the thermal optimum (J_{maxopt} , $\mu\text{mol m}^{-2} \text{s}^{-1}$); thermal optimum of J_{max} (T_{optJ} , °C); activation energy of V_{cmax} (E_{av}); activation energy of J_{max} (E_{aj}); V_{cmax} ($V_{\text{cmax}20}$, $\mu\text{mol m}^{-2} \text{s}^{-1}$) and J_{max} ($J_{\text{max}20}$, $\mu\text{mol m}^{-2} \text{s}^{-1}$) measured at 20°C; the ratio of $J_{\text{max}20}$ to $V_{\text{cmax}20}$ ($J_{\text{max}20}/V_{\text{cmax}20}$); total biomass allocated to roots (root mass ratio); stem (stem mass ratio); and leaf (leaf mass ratio); leaf mass on mass basis (LMA, g/m²); leaf nitrogen per unit leaf area (N_{a} , g/m²); chlorophyll *a* (Chl_a, g/m²), chlorophyll *b* (Chl_b, g/m²), and carotenoid concentrations (g/m²)

grown under 8T were the smallest: AC8T trees had 44% less biomass than AC0T, and EC8T seedlings were 30% smaller than EC0T trees (Figure 5a; Table 3). In black spruce, there was an interaction of growth CO₂ and temperature on total biomass, such that warming consistently reduced biomass, and this decline was more pronounced in EC-grown plants compared to their AC counterparts (Figure 5b; Table 4). In AC, 4T and 8T seedlings were 33% and 63% smaller than 0T trees. In the EC treatments, 4T and 8T trees were 65% and 82% smaller compared to 0T controls. Nevertheless, EC-grown spruce had higher biomass compared to AC spruce for the same temperature treatment (Figure 5b; Table 4).

In both species, the proportion of mass allocated to roots declined with warming (20% in tamarack and 11% in black spruce, 8T compared to 0T; Figure 5c,d; Tables 3 and 4). In tamarack, the proportion of biomass allocated to stems did not differ across warming and CO₂ treatments (Figure 5e; Table 3). In contrast, the proportion of biomass allocated to stems was lowest in 4T spruce (~25% of either

TABLE 4 Summary report of ANOVA of CO₂ and temperature treatments in black spruce on gas exchange parameters, photosynthetic capacity, plant biomass, and biochemical traits. Net CO₂ assimilation rate at the thermal optimum (A_{opt} , $\mu\text{mol m}^{-2} \text{s}^{-1}$), thermal optimum of net CO₂ assimilation (T_{optA} , °C), dark respiration rates measured at 20°C (R_{d20} , $\mu\text{mol m}^{-2} \text{s}^{-1}$), the change in R_d per 10°C increase in temperature (Q_{10}), maximum carboxylation rates of Rubisco at the thermal optimum ($V_{cmaxopt}$, $\mu\text{mol m}^{-2} \text{s}^{-1}$); thermal optimum of V_{cmax} (T_{optV} , °C); maximum electron transport rates at the thermal optimum (J_{maxopt} , $\mu\text{mol m}^{-2} \text{s}^{-1}$); thermal optimum of J_{max} (T_{optJ} , °C); activation energy of V_{cmax} (E_{av}), activation energy of J_{max} (E_{aj}); V_{cmax} (V_{cmax20} , $\mu\text{mol m}^{-2} \text{s}^{-1}$), and J_{max} (J_{max20} , $\mu\text{mol m}^{-2} \text{s}^{-1}$) measured at 20°C; the ratio of J_{max20} to V_{cmax20} (J_{max20}/V_{cmax20}); total biomass allocated to roots (root mass ratio); stem (stem mass ratio); and leaf (leaf mass ratio); leaf mass on mass basis (LMA, g/m²); leaf nitrogen per unit leaf area (N_a , g/m²); chlorophyll *a* (Chl_a , g/m²), chlorophyll *b* (Chl_b , g/m²), and carotenoid concentrations (g/m²)

	Temperature		CO ₂		Temperature × CO ₂	
	F-value	p-value	F-value	p-value	F-value	p-value
Temperature response of gas exchange						
A_{opt}	4.6	.02	6.7	.016	0.7	.5
T_{optA}	13.3	.00012	53.7	<.0001	0.075	.9
R_{d20}	3.9	.034	0.1	.8	0.07	.9
Q_{10}	4.6	.02	0.2	.6	1.5	.3
Temperature response of photosynthetic capacity						
$V_{cmaxopt}$	3.3	.05	0.1	.7	1.6	.2
T_{optV}	1.8	.2	1.8	.2	1.4	.3
J_{maxopt}	5	.015	0.09	.8	1	.4
T_{optJ}	14.8	<.0001	0.03	.9	1.1	.3
E_{av}	3.9	.03	1.3	.3	0.3	.7
E_{aj}	3.7	.038	0.4	.6	0.9	.4
V_{cmax20}	7.6	.0027	0.3	.6	1.2	.3
J_{max20}	8.2	.002	0.05	.8	1	.4
$J_{max20} \cdot V_{cmax20}$	3.4	.050	0.5	.5	0.1	.9
Plant biomass traits						
Total biomass	52.5	<.0001	52.6	<.0001	22	<.0001
Root mass ratio	11	<.0001	1.4	.2	2.2	.1
Stem mass ratio	3.9	.023	0.009	.9	0.3	.8
Leaf mass ratio	14	<.0001	1.8	.2	1.6	.2
LMA	7.2	.0017	2.3	.13	0.9	.4
Biochemical traits						
N_a	13.9	<.0001	0.8	.4	1.8	.2
Chl_a	9.5	.001	0.5	.5	3.6	.04
Chl_b	5.6	.0108	0.2	.7	1.5	.2
Carotenoids	10.7	.0005	1.5	.2	4	.03

Note: Bold numbers represent *p*-value less than .05 ($p < .05$), and bold and italicized are *p*-values between .05 and .1 ($.05 < p < .1$).

OT and 8T; Figure 5f; Table 4). In both species, the proportion of biomass allocated to needles increased with warming, without any CO₂ effect (Figure 5g,h; Tables 3 and 4). Leaf mass per unit area (LMA) was decreased by warming in both species, without a CO₂ effect (Figure 5h,i; Tables 3 and 4).

4.2 | Biochemical traits

In both species, leaf nitrogen per unit leaf area (N_a) declined with warming (14%–44%; Figure 6a,b; Tables 3 and 4). This decline in N_a was not solely driven by declines in LMA, since nitrogen on a mass basis also decreased with warming (Figure S2; Table S2). In tamarack, Chl_a , Chl_b and carotenoid concentrations per unit leaf area did not vary across the treatments (e.g., Figure 6c; Table 3). In contrast, in black spruce, Chl_a declined with warming (3%–51%), as did Chl_b (7%–53%) and carotenoid (8%–55%) concentrations per unit leaf area (Figure 6b,d,f,h; Table 4). There was a warming × CO₂ interaction for

Chl_a and carotenoid concentrations in spruce, such that the warming-induced decline was larger in EC than AC trees (Figure 6d,h; Table 3).

5 | DISCUSSION

We grew two North American boreal tree species under a range of air temperatures and atmospheric CO₂ conditions predicted for the middle and end of the 21st century for high latitude regions, and assessed how their performance might be impacted. In this study, growth temperature and CO₂ conditions both impacted A_{net} , but these effects differed between the two species. As expected, A_{net} measured at the growth CO₂ was stimulated by the elevated CO₂ treatments. However, the stimulation of A_{net} by elevated CO₂ was less pronounced in spruce compared to tamarack due to a reduction of g_s in EC spruce, a response not seen in tamarack. Warming also had contrasting impacts on A_{net} between the two species. In tamarack, A_{net} was largely unaffected by warming (i.e., compare AC0T, AC4T, AC8T in Figure 2a), but A_{net} was

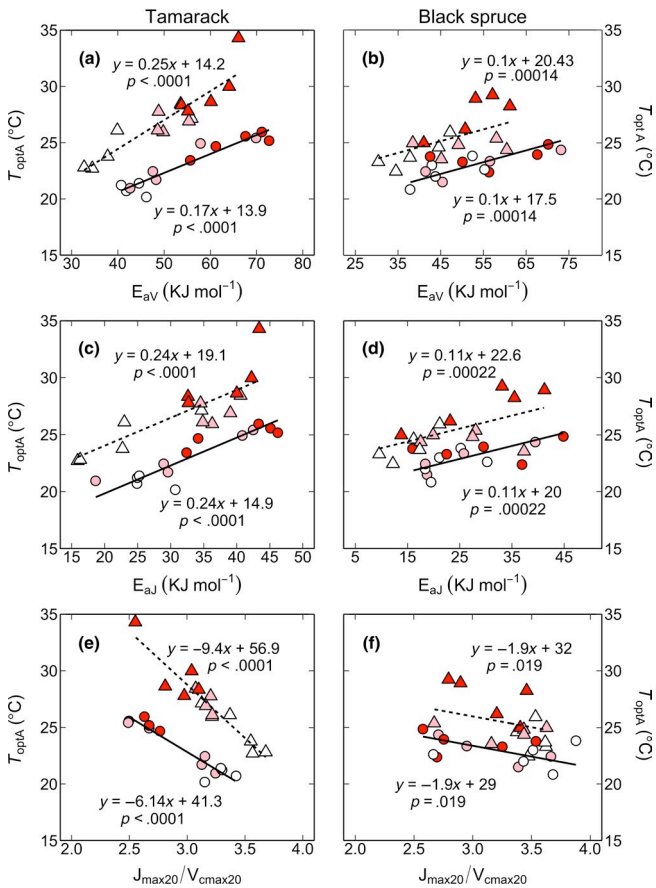


FIGURE 4 The temperature optimum of net photosynthesis measured at growth CO_2 (T_{optA} , °C) as a function of the activation energy of (a, b) the maximum Rubisco carboxylation rate (E_{av} , kJ/mol); (c, d) the maximum electron transport rate (E_{aJ} , kJ/mol); and as function of (e, f) the ratio of the maximum electron transport rate to maximum Rubisco carboxylation rate measured at 20°C (J_{max20}/V_{cmax20}) in tamarack (a, c, e) and black spruce (b, d, f). Lines represent regression lines for growth CO_2 (AC = solid; EC = dashed lines). Symbols represent growth CO_2 (AC = circle; EC = triangle). Colors represent growth temperature (0T = white; 4T = pink; 8T = red). Each data point represents an individual tree. AC, ambient CO_2 ; EC, elevated CO_2

significantly reduced in warm-grown spruce. Furthermore, A_{opt} in tamarack was not affected by warming (see Table 2), while it decreased in 4T and 8T spruce. As with the photosynthetic response to CO_2 , these contrasting responses of A_{net} to warming were partly explained by g_s . In tamarack, g_s tended to be higher in warm-grown plants, while in spruce, g_s decreased with warming. These g_s responses in tamarack, especially at measurement temperatures above 30°C, reduced stomatal limitations to CO_2 diffusion for carboxylation. However, in spruce in the same leaf temperature range, warming induced stomatal closure, which was linked with reductions in A_{net} .

The combined stomatal-photosynthesis model assumes that, under rising growth CO_2 , g_s should decline to maintain a constant C_i/C_a and thus stimulate plant water savings (Gedney et al., 2006; Luo et al., 2008; Medlyn et al., 2011). In contrast to this assumption, C_i/C_a increased in EC-grown seedlings, even in spruce, where g_s declined in high

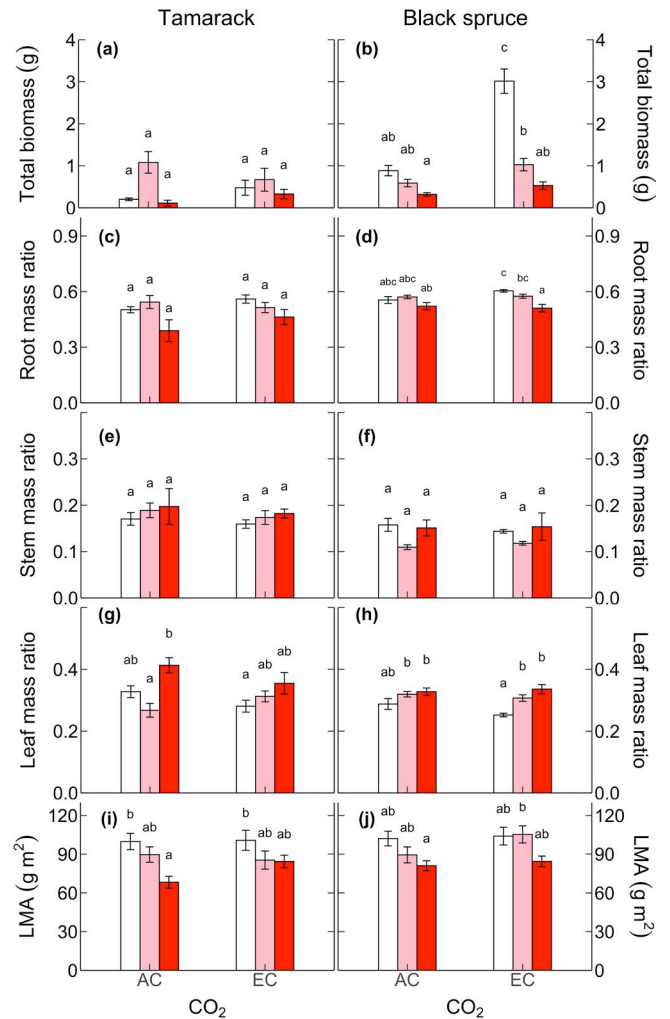


FIGURE 5 Total plant biomass and allocation responses to CO_2 and temperature treatments in tamarack (a, c, e, g, i) and black spruce (b, d, f, h, j). (a, b) Total seedling biomass; (c, d) the proportion of total plant biomass allocated to roots (root mass ratio); (e, f) stem (stem mass ratio); and (g, h) leaves (leaf mass ratio); and (i, j) the leaf mass per unit leaf area (LMA). Colors represent growth temperature (0T = white; 4T = pink; 8T = red); Means \pm SE. Different letters on bars represent differences across the six treatment (Tukey post hoc test, $p < .05$). $n = 4$ –15 for (a)–(h), and $n = 9$ –10 for (i) and (j). AC, ambient CO_2 ; EC, elevated CO_2

CO_2 -grown trees. This may indicate that the EC-induced reduction in g_s was not strong enough to limit an increase in C_i/C_a with elevated CO_2 (as also observed in Kellomaki & Wang, 1996), or it may relate to the suppression of photosynthetic demand for CO_2 in warm-grown spruce seedlings. The responses of g_s in seedlings of both species in our glasshouse experiment add to a growing number of studies from field-grown trees (Hasper et al., 2016; Lamba et al., 2018; Medlyn et al., 2001) that suggest that conifers have a weak stomatal response to elevated CO_2 . Altogether, these findings suggest that a weak g_s response to high CO_2 may be a common feature of conifers regardless of their ontogenetic stage or the type of experimental manipulation (i.e., field vs. glasshouse). Previous studies showed that net photosynthesis in black spruce responds negatively to warming, and the reasons for these declines

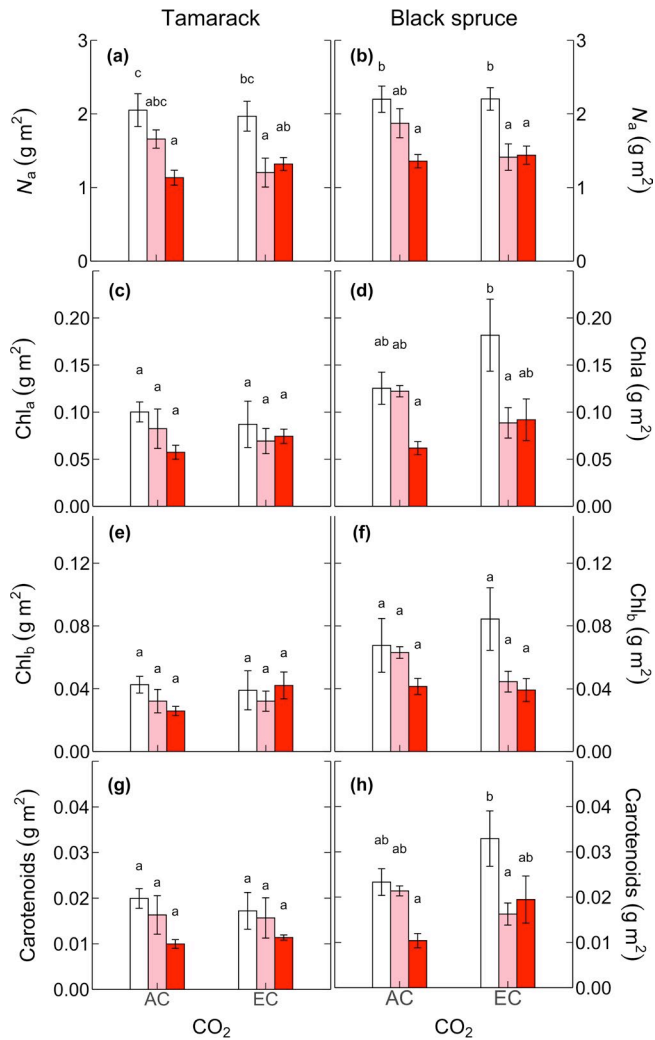


FIGURE 6 Biochemical trait responses to CO₂ and temperature treatments in tamarack (a, c, e, g) and black spruce (b, d, f, h). (a, b) Leaf nitrogen per unit leaf area (N_a , g/m²); (c, d) chlorophyll a (Chl_a , g/m²); (e, f) chlorophyll b (Chl_b , g/m²); and (g, h) total carotenoid concentrations (g/m²) on a leaf area basis. Colors represent growth temperature (0T = white; 4T = pink; 8T = red). Means \pm SE. Different letters on bars represent differences across the six treatment (Tukey post hoc test, $p < .05$). $n = 3$ –5 for (c)–(h), and $n = 9$ –10 for (a) and (b). AC, ambient CO₂; EC, elevated CO₂

include warming-induced reductions in photosynthetic capacity (Way & Sage, 2008b), decreased soil water availability and associated stomatal closure, and increased respiration rates (Girardin et al., 2014; Marchand et al., 2019). Our results highlight that warming-induced stomatal closure can occur in the absence of water limitation. Similarly, warming induced stomatal closure in congeneric, mature Norway spruce growing in its natural habitat (Lamba et al., 2018), suggesting that reductions in g_s may contribute to the observed reduction in carbon gain and growth in black spruce across North America (Marchand et al., 2019), an effect likely exacerbated by soil water limitations (Girardin, Bouriaud, et al., 2016; Girardin et al., 2014; Girardin, Hogg, et al., 2016).

Warming and elevated CO₂ can have compounding effects on g_s since both elevated CO₂ and increased VPD induce stomatal closure

(Dusenge et al., 2019). We observed this effect in spruce, where declines in g_s due to both warming and elevated CO₂ resulted in comparable carbon gain between seedlings grown under current (AC0T) and future climate conditions (EC8T), even when measured at their respective growth CO₂ (Figure 2b). While it is generally assumed that boreal forests are temperature limited (Huang et al., 2019), and might thus benefit from future climate warming and CO₂ fertilization (Stinziano & Way, 2014), these responses have not been observed in black spruce (Girardin, Bouriaud, et al., 2016; Marchand et al., 2019). Our results imply that stomatal responses (among other factors, such as nutrient and water availability) could dictate the long-term trajectory of boreal forest productivity in future climates. Therefore, information on the acclimation of g_s to temperature should be considered (Rogers, Medlyn, et al., 2017) when projecting the impact of climate warming on northern forest carbon gain.

Acclimation of photosynthesis to elevated temperatures usually involves a shift of T_{optA} to higher temperatures (Berry & Bjorkman, 1980; Kroner & Way, 2016; Kumarathunge et al., 2019; Sendall et al., 2015; Way & Sage, 2008a; Way & Yamori, 2014; Yamori et al., 2014; Zhang et al., 2015). In addition, T_{optA} should also increase with elevated CO₂ due to a suppression of photorespiration under high measurement CO₂ (Sage & Kubien, 2007; Wujeska-Klaue et al., 2019). In both species, T_{optA} increased with warming, and was higher in EC-grown and measured plants. In field-grown, North American broad-leaved temperate and boreal tree species subjected to a 2.9°C warming, T_{optA} increased by 1.1°C/°C (Sendall et al., 2015). In our current study, where species were subject to a warming of up to 8°C, T_{optA} only shifted by 0.36–0.65°C/°C. Furthermore, in two dominant European conifers, *P. abies* and *P. sylvestris*, grown under similar treatments as in our study, T_{optA} did not shift with warming (Kurepin et al., 2018). Combined, these results suggest that conifers may have a limited physiological capacity to shift their T_{optA} to keep track with warming predicted for future climates (Way, 2019). However, it should be noted that plants do not necessarily have to adjust their T_{opt} to effectively acclimate to warming (Way & Yamori, 2014; Yamori et al., 2014). Thermal acclimation should improve (or at least maintain) carbon gain under warmer growth temperatures (Way & Yamori, 2014). Therefore, A_{net} measured at the growth temperature provides a better estimate of photosynthetic performance than do changes in the T_{optA} (Dusenge et al., 2019; Way & Yamori, 2014). Comparing seedlings grown in either current (AC0T) or future climate scenario (EC8T), we observed comparable rates of A_{net} experienced by black spruce during a typical growing season (15–30°C), while the small increase in T_{opt} in EC8T spruce resulted in higher photosynthetic performance at temperatures >35°C. In tamarack, plants grown and measured under future climate conditions (EC8T seedlings measured at high leaf temperatures and elevated CO₂) had much higher photosynthetic rates that AC0T tamarack measured under current conditions (cooler leaf temperatures and current CO₂). Since climate change is predicted to be accompanied by increased frequency of extreme heat events (IPCC, 2013), the increases in A_{net} at higher leaf temperatures and T_{opt} may enhance survival of these species as a result of improved carbon gain during extreme heat events compared to non-acclimated plants.

Warming strongly affected photosynthetic physiology, but there were few CO₂ effects in either species. In contrast to our hypothesis, V_{cmax} and J_{max} at 20°C were significantly reduced by warming, while being unaffected by elevated CO₂. These findings contrast with results from mature *P. abies*, which acclimated V_{cmax} to elevated CO₂, but showed no warming effect (Lamba et al., 2018). The responses of photosynthetic capacity observed here were likely due to declines in leaf N with warming (14%–44%), declines that were apparent even when accounting for treatment-induced changes in LMA. In spruce, these reductions in leaf N were also correlated with declines in Chl_a, Chl_b, and carotenoid concentrations. While leaf N usually correlates with photosynthetic capacity (Kattge, Knorr, Raddatz, & Wirth, 2009), and the mechanisms underlying the response of leaf N to elevated CO₂ and subsequent effects on photosynthetic capacity are relatively well characterized (Ainsworth & Long, 2005; Duarte et al., in press), the mechanistic responses of leaf N to warming are not fully understood (Duarte et al., in press). While many studies report little or no effect of warming on basal rates of photosynthetic capacity (Kattge & Knorr, 2007; Kumarathunge et al., 2019; Stefanski et al., 2019; Way & Oren, 2010), when declines in these rates are observed, this is usually correlated with declines in leaf N and Rubisco (Crous et al., 2018; Scafaro et al., 2017; Way & Sage, 2008a, 2008b). Furthermore, the reduced pigment concentrations in warm-grown plants in our study provide further evidence for reductions in RuBP regeneration capacity, particularly in spruce.

The $J_{\text{max}}/V_{\text{cmax}}$ ratio at 20°C declined with warming in both species, but was higher in EC- compared to AC-grown plants in tamarack. Since both $V_{\text{cmax}20}$ and $J_{\text{max}20}$ were reduced by warming, the decline in their ratio is due to a greater decline of $J_{\text{max}20}$ relative to $V_{\text{cmax}20}$. This warming-induced reduction in $J_{\text{max}}/V_{\text{cmax}}$ at a given temperature is common (Crous et al., 2018; Dusenage et al., 2015; Kattge & Knorr, 2007; Kumarathunge et al., 2019; Stefanski et al., 2019; Yamori, Noguchi, & Terashima, 2005), and is proposed to be linked with changes in N partitioning within the photosynthetic machinery (i.e., Rubisco carboxylation vs. RuBP regeneration processes; Hikosaka et al., 2006; Scafaro et al., 2017; Yin et al., 2019). In tamarack, the $J_{\text{max}}/V_{\text{cmax}}$ ratio was lower in warm-grown seedlings across the measuring temperature range (10–40°C), while in black spruce, $J_{\text{max}}/V_{\text{cmax}}$ ratio across warming treatments converged with increasing leaf temperature to achieve largely similar $J_{\text{max}}/V_{\text{cmax}}$ ratio at 30 and 40°C (Figure S1). Although we did not measure N partitioning per se, the ratio of $V_{\text{cmax}20}/N_a$ (Figure S3; Table S2) was not affected by growth temperature in either species, suggesting that there was no reallocation of leaf N to Rubisco carboxylation, and that the activation state of Rubisco was not likely affected by warming. However, warming led to a decrease in $J_{\text{max}20}/N_a$ in tamarack, suggesting that warming negatively affected electron transport, and that photosynthesis was increasingly limited by RuBP regeneration in warm-grown seedlings.

The temperature sensitivity parameters of photosynthetic capacity were impacted by warming, with only weak CO₂ effects. The activation energies of V_{cmax} (E_{av}) and J_{max} (E_{aj}) and the thermal optimum of J_{max} ($T_{\text{opt}J}$) all increased with warming (also seen in other species: Crous et al., 2018; Hikosaka et al., 2006; Kumarathunge et al., 2019; Yamaguchi et al., 2016; Yin et al., 2019), but were not affected by CO₂. The $T_{\text{opt}V}$

was, however, significantly higher in elevated CO₂ treatments. These thermal adjustments in photosynthetic capacity act to improve net CO₂ uptake in a warmer environment, and these adjustments may result from several mechanisms such as the production of a more heat-stable Rubisco activase (Crafts-Brandner, Loo, & Salvucci, 1997; Law & Crafts-Brandner, 2001; Law, Crafts-Brandner, & Salvucci, 2001), increased membrane integrity by increasing the proportion of membrane saturated fatty acids (Murakami et al., 2000; Yamori et al., 2014) and increases in mesophyll conductance (Evans & von Caemmerer, 2013; von Caemmerer & Evans, 2015) with warming. Additionally, the $T_{\text{opt}A}$ was significantly correlated with E_{av} and E_{aj} in both species, (also recently observed in Kumarathunge et al., 2019; Yamaguchi et al., 2019). Importantly, Kumarathunge et al. (2019) recently developed an algorithm that accounts for thermal acclimation of photosynthetic capacity to be incorporated into TBMs, and used data on plants grown under ambient CO₂ conditions. The lack of discernible CO₂ effects on the temperature sensitivity parameters of photosynthetic capacity in our study, the same parameters incorporated into the Kumarathunge et al. (2019) algorithm (E_{av} and E_{aj}), suggest that their temperature response function should apply for both ambient and elevated CO₂-grown plants.

Thermal acclimation of i_d is common (Slot & Kitajima, 2015), and has been seen in a number of boreal tree species (also observed in other boreal forest species: Benomar et al., 2017; Kroner & Way, 2016; Kurepin et al., 2018; Reich et al., 2016; Tjoelker et al., 1999; Wei et al., 2017; Zhang et al., 2015). The reduction in R_d was correlated with decreased leaf N, likely indicating a reduced investment in respiratory proteins (Tjoelker et al., 1999). There is currently no consensus on the effect of CO₂ on R_d (Dusenage et al., 2019; Way et al., 2015). In our study, as well as others (Kroner & Way, 2016; Kurepin et al., 2018), no effect of CO₂ has been seen on R_d . In contrast, some studies found a stimulation of R_d (Lamba et al., 2018; Roberntz & Stockfors, 1998), and this stimulation may be driven by increased carbohydrates in elevated CO₂-exposed trees (Roberntz & Stockfors, 1998; Tjoelker et al., 1998). In addition, enhanced R_d may also be caused by increased energy demand for phloem loading of extra photoassimilates, although this effect remains relatively unexplored (Ainsworth & Lemonnier, 2018). Tjoelker et al. (1999) suggested that the CO₂-induced reduction of leaf N observed in their study partially offsets the stimulation of R_d due to increased carbohydrates in elevated CO₂-grown plants. It is possible that the warming-induced decline in leaf N that we observed was strong enough to completely offset any potential effect of increased carbohydrates produced in EC to stimulate R_d .

The treatments had contrasting effects on growth between tamarack and spruce. In tamarack, there was no effect of elevated CO₂ on biomass, while warming altered growth, with 4T-grown tamarack having the highest biomass. In contrast, elevated CO₂ stimulated spruce biomass, while warming consistently suppressed biomass. Although our current study did not model the long-term carbon budget of the trees with our physiological data, it is likely that the observed contrasting growth responses between species are associated with these physiological responses to the treatments. Our results in tamarack are in line with the assumption that growth in high latitude plants is currently temperature-limited and that moderate warming should stimulate productivity (Huang et al., 2019; Stinziano & Way, 2014).

However, our study also highlights that this warming-induced productivity will be species dependent. These species-specific responses may be linked to plant functional type. For example, evergreen and late-successional species (such as black spruce) often have reduced performance compared to deciduous and early-successional (such as tamarack) when grown in future climatic conditions due to their low plasticity (Dusenge et al., 2019; Kurepin et al., 2018; Way & Oren, 2010; Way & Yamori, 2014). More specifically, our findings add to a growing body of evidence that species in the *Picea* genus will be negatively affected by warming (Kurepin et al., 2018; Way & Sage, 2008b; Zhang et al., 2015), particularly in southern populations (Girardin et al., 2014; Marchand et al., 2019). As our work used seed from southern seed sources, our findings also support the hypothesis that southern populations of boreal species will be strongly affected by future warming (Reich et al., 2015; Sullivan, Pattison, Brownlee, Cahoon, & Hollingsworth, 2017). Since *Larix* and *Picea* species co-occur in boreal forests (Bares & Wali, 1979; Gower & Richards, 1990), decreased performance of spruce species in their southern range implies that *Picea* species may decline as the climate warms, altering the community composition and the structure and function of northern forests.

Biomass allocation was only affected by the warming treatments. The proportion of biomass allocated to roots and needles was significantly decreased and increased by warming, respectively, in line with the review from Way and Oren (2010). Overall, our results suggest that seedlings compensate for reduced photosynthetic metabolism under warming by increasing canopy size to maximize carbon uptake. However, it is not surprising that species reduced their allocation to roots under warming, since they were provided with ample nutrient and water throughout the experiment.

6 | CONCLUSIONS

Overall, our results show that climate change will differentially affect these two boreal species. While photosynthetic capacity declined in both species, mostly driven by reduced leaf N content, these species compensated for the reduced carbon uptake by reducing respiratory CO₂ losses and increasing canopy size. In addition, in tamarack, we observed a slight stimulation of g_s with warming that helped maintain similar A_{net} across the temperature treatments. However, in spruce, g_s was reduced by warming, resulting in further reductions of A_{net} . Altogether, these physiological mechanisms likely underlie the observed growth responses between the species, which resulted in stimulated biomass in 4T tamarack, while biomass was reduced by both warming treatments in black spruce. Additionally, we show that while warming alters the activation energy for V_{cmax} and J_{max} and the thermal optimum of J_{max} , elevated CO₂ had little effect on these parameters, implying that the effect of rising CO₂ can be ignored when modeling thermal acclimation of photosynthetic capacity in vegetation that develops under future climate conditions.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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