Contrasting acclimation responses to elevated CO$_2$ and warming between an evergreen and a deciduous boreal conifer

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Abstract
Rising atmospheric carbon dioxide (CO$_2$) 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 CO$_2$ 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 CO$_2$ and warming. Black spruce ($Picea mariana$, an evergreen conifer) and tamarack ($Larix laricina$, a deciduous conifer) were grown under ambient (407 ppm) or elevated CO$_2$ (750 ppm) and either ambient temperatures, a 4°C warming, or an 8°C warming. In both species, the thermal optimum of net photosynthesis ($T_{\text{optA}}$) 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, $V_{\text{cmax}}$, and of electron transport, $J_{\text{max}}$) was reduced in warm-grown seedlings, correlating with reductions in leaf N and chlorophyll concentrations. Warming increased the activation energy for $V_{\text{cmax}}$ and $J_{\text{max}}$ ($E_aV$ and $E_aJ$, respectively) and the thermal optimum for $J_{\text{max}}$. In both species, the $T_{\text{optA}}$ was positively correlated with both $E_aV$ and $E_aJ$, but negatively correlated with the ratio of $J_{\text{max}}/V_{\text{cmax}}$. 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 CO$_2$.

KEYWORDS
acclimation, boreal conifers, climate change, evergreen and deciduous, Larix laricina, Picea mariana, $V_{\text{cmax}}$ and $J_{\text{max}}$

1 | INTRODUCTION

Anthropogenic greenhouse gas emissions, such as carbon dioxide (CO$_2$), 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...
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; Kaschek, 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, $A_{\text{net}}$) up to the thermal optimum ($T_{\text{opt}}$), while $A_{\text{net}}$ declines above the $T_{\text{opt}}$ (Berry & Bjorkman, 1980; Way & Yamori, 2014; Yamori, Hikosaka, & Way, 2014). Below the $T_{\text{opt}}$ and at current CO₂ levels, $A_{\text{net}}$ is often limited by the capacity of Rubisco (ribose-1.5-bisphosphate carboxylase/oxygenase) to fix CO₂ (Hikosaka, Ishikawa, Borjigidal, Muller, & Onoda, 2006). Above the $T_{\text{opt}}$ the decline in $A_{\text{net}}$ 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 Camdenner, 2009). At supraoptimal temperatures, $A_{\text{net}}$ 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 $A_{\text{net}}$ at high leaf temperatures (Way, 2019).

When plants are grown in elevated temperatures, they acclimate by increasing the $T_{\text{opt}}$ of $A_{\text{net}}$ ($T_{\text{opt,A}}$), as well as the $T_{\text{max}}$ of maximum Rubisco carboxylation rates ($v_{\text{cmax}}$) and maximum electron transport rates ($U_{\text{max}}$; Kumarathunge et al., 2019; Sendall et al., 2015; Smith & Dukes, 2017; Way & Yamori, 2014). However, shifting these $T_{\text{opt}}$ 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 $A_{\text{net}}$ at growth temperature tends to be reduced in evergreen woody species grown in warmer conditions (Dusenge, 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 $A_{\text{net}}$ 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*, $A_{\text{net}}$ 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 $A_{\text{net}}$ 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, $A_{\text{net}}$ acclimates to elevated CO₂ such that the initial stimulation of $A_{\text{net}}$ 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; Leaky 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 CO$_2$ thus has little impact on boreal tree productivity in field experiments without nutrient addition (Hättenschwiler & Körner, 1997; Sigurdsson, Medhurst, Wallin, Eggertsson, & Linder, 2013; Sigurdsson, Thororgeirsson, & Linder, 2001), while in well-fertilized boreal conifer seedlings, elevated CO$_2$ stimulated $A_{\text{net}}$ equally in Picea mariana, Pinus banksiana, and L. laricina (Tjoelker et al., 1998). Decreased photosynthesis at high CO$_2$ has also been correlated with reduced stomatal conductance associated with elevated CO$_2$-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 CO$_2$ may not vary considerably between evergreen and deciduous conifers growing in similar soils. The effects of elevated CO$_2$ on respiration are more variable than they are on photosynthesis (Amthor, 2000; Amthor, Koch, Willms, & Layzell, 2001), but on longer timescales, respiration can be altered by elevated CO$_2$, 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 CO$_2$ 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., 2015; Tjoelker et al., 1998; Tjoelker, Reich, & Oleksyn, 1999; Uddling & Wallin, 2012; Wallin et al., 2013; Wang, Kellomäki, & Laitinen, 1995; Zha, Kellomäki, & Wang, 2003). The combined effect of CO$_2$ and temperature can either enhance or offset their independent effects on leaf physiology (Long, 1991). High CO$_2$ suppresses photorespiration (which increases with temperature), thus stimulating $A_{\text{net}}$ (Wuéska-Klause, Crous, Ghannoum, & Ellsworth, 2019) and increasing the $T_{\text{opt}}$ (Sage & Kubien, 2007); elevated CO$_2$ thus often enhances plant carbon uptake at high growth temperatures (Uddling & Wallin, 2012; Wallin et al., 2013; Wang et al., 1995). Elevated CO$_2$ and temperature can also have additive effects on carbon uptake (Dusenge et al., 2019). For instance, elevated CO$_2$ 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 $A_{\text{net}}$ and alters the $T_{\text{opt}}$ (Meredo et al., 2018). But strong effects of CO$_2$ 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 CO$_2$ 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 $V_{\text{max}}$ and $J_{\text{max}}$, which are key parameters for estimating CO$_2$ fixation rates. Both $V_{\text{max}}$ and $J_{\text{max}}$ are affected by environmental factors such as growth CO$_2$ (Ainsworth & Long, 2005) and temperature (Kattge & Knorr, 2007; Smith & Dukes, 2017), and values of $V_{\text{max}}$ and $J_{\text{max}}$ vary across plant functional types (Rogers, 2014). In TBMs, $V_{\text{max}}$, $J_{\text{max}}$, and the ratio of $J_{\text{max}}$/$V_{\text{max}}$ are important input parameters (Rogers, Medlyn, et al., 2017; Rogers, Serbin, Ely, Sloan, & Wullschleger, 2017b), as are the thermal sensitivity of $V_{\text{max}}$ and $J_{\text{max}}$ (Meredo et al., 2018). The instantaneous temperature responses of $V_{\text{max}}$ and $J_{\text{max}}$ are often modelled by a peaked Arrhenius function (Medlyn et al., 2002; Kumarathunge et al., 2019; but see Smith & Dukes, 2017):

$$f(T) = k_{\text{opt}} \frac{H_d \exp \left( \frac{E_a}{T - T_{\text{opt}}} \right)}{H_d - E_a \left( 1 - \exp \left( \frac{E_a}{T - T_{\text{opt}}} \right) \right)}.$$  (1)

where $k_{\text{opt}}$ is the process rate (i.e., $V_{\text{max}}$ or $J_{\text{max}}$: μmol m$^{-2}$ s$^{-1}$) at the optimum temperature ($V_{\text{max opt}}$ or $J_{\text{max opt}}$, respectively), $H_d$ (kJ/mol) is the activation 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 $T_{\text{opt}}$ and $T_k$ are the optimum and given temperatures of the process rate. Both $V_{\text{max}}$ and $J_{\text{max}}$ as well as their temperature sensitivity parameters (i.e., $E_a$, $T_{\text{opt}}$, $H_d$, $V_{\text{max opt}}$, and $J_{\text{max opt}}$), 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 CO$_2$ on the temperature sensitivity of photosynthetic capacity, particularly in boreal species (Meredo et al., 2018; Smith & Dukes, 2013). The only two studies on boreal conifers to explore the effects of both warming and elevated CO$_2$ on $V_{\text{max}}$ found different responses: in mature trees of Picea abies, $V_{\text{max}}$ acclimated to CO$_2$ with no effect of warming (Lamba et al., 2018), while in seedlings of Pinus sylvestris, both warming and high CO$_2$ increased $V_{\text{max}}$ (Kellomäki & Wang, 1996). Therefore, more data are needed to improve our understanding of the interactive effects of CO$_2$ and temperature on photosynthetic and respiratory physiology in these important northern trees.

In boreal forests, Lirix and Picea spp. are common, and their distribution extends through North America and across Eurasia.
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 CO$_2$ concentrations (750 ppm). We tested the following hypotheses:

1. $A_{\text{net}}$ will acclimate to warming in tamarack (a deciduous conifer), but in black spruce (an evergreen conifer), $A_{\text{net}}$ will respond negatively to warming. The $A_{\text{net}}$ of both species will increase with elevated CO$_2$ across the warming treatments.
2. Photosynthetic capacity (i.e., $V_{\text{cmax}}$ and $J_{\text{max}}$) will be reduced by elevated CO$_2$, 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 CO$_2$, 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 CO$_2$ 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*: seed lot 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 CO$_2$ and temperature: either ambient CO$_2$ (407 µmol/mol CO$_2$; AC) or elevated CO$_2$ (750 µmol/mol CO$_2$; 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. CO$_2$ concentrations were controlled by blowing pure CO$_2$ into the elevated CO$_2$ glasshouses, using Argus Control Software (Surrey, BC, Canada) TITAN version 1 (Build 00226B.00, Site ID: 1960); CO$_2$ 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...
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 CO$_2$ assimilation rates ($A_{\text{net}}$) were measured at varying intercellular CO$_2$ concentrations ($C_i$) to build A–$C_i$ curves, using a photosynthetic photon flux density of 1,400 µmol photons m$^{-2}$ 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 CO$_2$ of 400 µmol/m$^2$; ambient CO$_2$ concentrations ($C_o$) were then changed sequentially to 400, 200, 150, 100, 50, 400, 750, 1,200, 1,600 and 2,000 µmol/m$^2$.

To assess the temperature response of $A_{\text{net}}$ and dark respiration, separate measurements of $A_{\text{net}}$ were conducted on another five seedlings at 10, 15, 20, 25, 30, 35, and 40°C. At each temperature, light-saturated $A_{\text{net}}$ was first assessed at a cuvette CO$_2$ concentration of 400 ppm, then at 750 ppm. The cuvette CO$_2$ was then returned to 400 ppm, the cuvette irradiance was set to 0 µmol photons m$^{-2}$ s$^{-1}$, and dark respiration ($R_d$) was measured after a 20 min dark-acclimation period. Only one measurement CO$_2$ (400 ppm) was used in the $R_d$ measurements, as there is no direct effect of measurement CO$_2$ 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 $A_{\text{net}}$ temperature response curves (one at 400 ppm CO$_2$, 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 acclimated for ~40 min before starting $A_{\text{net}}$ measurements at the next temperature.

We measured in five consecutive cycles, with each cycle comprising measurements going from the 8T treatment down to the OT treatment to distribute any effects of growth temperature or CO$_2$ on developmental stage of the seedlings over the course of the measurements.

2.3 | Parameterization of photosynthesis models

The C$_3$ photosynthesis model by Farquhar et al. (1980) was used to estimate $V_{\text{cmax}}$ and $J_{\text{max}}$ from the A–$C_i$ curves. Mesophyll conductance was not measured, therefore only apparent $V_{\text{cmax}}$ and $J_{\text{max}}$ based on $C_o$ rather than the CO$_2$ concentration at the site of carboxylation ($C_c$), are reported. $V_{\text{cmax}}$ was calculated from:

$$A_c = \frac{V_{\text{cmax}}(C_i - \Gamma^*)}{C_i + K_c \left(1 + \frac{\Gamma^*}{K_c}\right)} - R_{\text{day}}$$

where $V_{\text{cmax}}$ is the maximum rate of Rubisco carboxylation, $C_i$ and $O$ are the intercellular concentrations of CO$_2$ and O$_2$, respectively, $K_c$ and $K_o$ are the Michaelis–Menten coefficients of Rubisco activity for CO$_2$ and O$_2$, respectively, $\Gamma^*$ is the CO$_2$ compensation point in the absence of mitochondrial respiration, and $R_{\text{day}}$ is mitochondrial respiration in the light. $R_{\text{day}}$ was not measured and the value used was the one generated after fitting the equation.

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

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

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

$$\theta F^2 (aQ + J_{\text{max}}) + aQJ_{\text{max}} = 0$$

where $J_{\text{max}}$ is the potential rate of electron transport, $\theta$ is the curvature of the light response curve, and $a$ is the quantum yield of electron transport. The values of $\theta$ and $a$ 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 $\Gamma^*$ from spinach, a cool-adapted C$_3$ plant (Galmés, Hermida-Carrera, Laanisto, & Niinemets, 2016). The thermal sensitivity of $K_c$ and $K_o$ was modeled from Medlyn et al. (2002):

$$K_c = 274.6 \exp\left[\frac{80,500(T_k - 298)}{(298T_k)^2}\right]$$

$$K_o = 419.8 \exp\left[\frac{14,500(T_k - 298)}{(298T_k)^2}\right]$$
where \( T_o \) denotes the leaf temperature and \( R \) is the universal gas constant (8.314 J mol\(^{-1}\) K\(^{-1}\)).

\[
\Gamma^* = \frac{0.5O}{S_{c/o}},
\]

(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):

\[
S_{c/o} = 2.321 \exp \left[ - \frac{29.000(T_o - 298)}{(298 T_o)} \right],
\]

(8)

which generated a \( \Gamma^* \) at 25°C of 45.2 (\( \mu \)mol/mol), close to the 42.75 (\( \mu \)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 \( \mu \)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 \( \mu \)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 \( A_{sat} \) at growth \( CO_2 \) was fitted using a quadratic regression (Kroner & Way, 2016):

\[
A_{sat} = a T_{leaf}^2 + b T_{leaf} + c,
\]

(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 \( A_{sat} \) was derived from Equation (9). The temperature responses of \( V_{cmax} \) and \( J_{max} \) were fitted using Equation (1) to derive values for \( E_v \), \( E_a \), \( T_{optV} \), \( T_{optJ} \), \( V_{cmaxopt} \) 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):

\[
Q_{10} = \left( \frac{R_2}{R_1} \right)^{10/(T_2 - T_1)}
\]

(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, 653 and 666 nm for total carotenoids (xanthophylls and carotenes), chlorophyll \( a (\text{Chl}_a) \), and chlorophyll \( b (\text{Chl}_b) \) concentrations, respectively, as outlined in Wellburn (1994):

\[
[\text{Chl}_a] = 15.65 \times A_{666} - 7.34 \times A_{653},
\]

(11)

\[
[\text{Chl}_b] = 27.05 \times A_{653} - 11.21 \times A_{666},
\]

(12)

\[
[\text{Carotenoids}] = \frac{1.000 \times A_{470} - 2.86 \times [\text{Chl}_a] - 129.2 \times [\text{Chl}_b]}{221},
\]

(13)

where \( A_{470}, A_{653}, \) and \( A_{666} \) represent absorption at the wavelength of 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 were reported as means ± 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 \( A_{net}, C_i, C_i/C_s, V_{cmax}, J_{max}, R_{dark}, \) and \( J_{max}/V_{cmax} \) ratio to changes in leaf temperature. Post hoc Tukey’s honest significance tests were used to evaluate responses
to individual combinations of CO$_2$ and temperature as indicated. All analyses were performed in R (version 3.5.2 [20 December 2018]).

## RESULTS

### 3.1 Experimental treatments

Air temperatures increased from May until midsummer (maximum day temperature of 29°C in the 0T treatment), decreasing in late summer to a minimum of 19°C (Figure 1a). The warming treatments of +4 and +8 were successfully maintained. Mean CO$_2$ 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 $A_{\text{net}}$ measured at growth CO$_2$ were altered by both growth temperature and growth CO$_2$ (Figure 2a; Table 1). Comparing the AC0T and EC8T trees, future growth conditions and measurement CO$_2$ concentrations greatly stimulated $A_{\text{net}}$ above 20°C in tamarack (Figure 2a). Maximum rates of $A_{\text{net}}$ ($A_{\text{opt}}$) measured at the growth CO$_2$ increased in EC tamarack, but were not affected by warming (Tables 2 and 3). The photosynthetic thermal optimum ($T_{\text{opt}}$) 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 $A_{\text{net}}$ was also altered by growth conditions (Figure 2b; Table 1). Warming suppressed $A_{\text{net}}$ while elevated CO$_2$ stimulated $A_{\text{net}}$. While EC8T spruce had higher $A_{\text{net}}$ above 35°C than AC0T trees, photosynthetic rates were comparable in the two treatments, even though $A_{\text{opt}}$ was measured at high CO$_2$ in the EC8T trees (Figure 2b). This was largely because $A_{\text{opt}}$ 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 (Tleaf), CO₂ and temperature treatments as the main effects. Traits analyzed were: A_{net} (net CO₂ assimilation rates); gₛ (stomatal conductance); C/Co (ratio of intercellular to air CO₂ concentration); lnR_d (ln-transformed dark respiration rates); V_{cmax} (maximum carboxylation rate of Rubisco); J_{max} (maximum rate of electron transport).

| Trait                          | Tamarack F-value | Tamarack p-value | Black spruce F-value | Black spruce p-value |
|-------------------------------|------------------|------------------|----------------------|----------------------|
| A_{net}                       | 7.7              | .006             | 0.4                  | .5                   |
| Tleaf                         | 25.9             | <.0001           | 4.7                  | .039                 |
| CO₂                           | 2.2              | .14              | 5.6                  | .0096                |
| Temperature                   | 28.5             | <.0001           | 17.4                 | <.0001               |
| Tleaf × CO₂                   | 9.5              | .0001            | 3.5                  | .031                 |
| Tleaf × Temperature           | 2.9              | .07              | 0.8                  | .4                   |
| CO₂ × Temperature             | 1.6              | .2               | 0.02                 | .9                   |
| gₛ                            | 13.5             | .0003            | 3.3                  | .07                  |
| Tleaf                         | 0.03             | .8               | 3.9                  | .058                 |
| CO₂                           | 1.3              | 3.4              |                     |                      |
| Temperature                   | 0.7              | .4               | 0.8                  | .4                   |
| Tleaf × CO₂                   | 4                | .019             | 2.4                  | .09                  |
| Tleaf × Temperature           | 0.15             | .8               | 0.025                | .9                   |
| CO₂ × Temperature             | 0.6              | .5               | 3.7                  | .026                 |
| C/Co                          | 1.4              | .0003            | 2.1                  | .2                   |
| Tleaf                         | 13.7             | .0011            | 18                   | .0003                |
| CO₂                           | 6.1              | .0072            | 1.4                  | .2                   |
| Temperature                   | 88.9             | <.0001           | 38.8                 | <.0001               |
| Tleaf × CO₂                   | 9.5              | .0001            | 3.5                  | .03                  |
| Tleaf × Temperature           | 0.8              | .4               | 1.5                  | .2                   |
| CO₂ × Temperature             | 0.9              | .4               | 2.9                  | .056                 |
| In R_d                        | 3.442            | <.0001           | 7.955                | <.0001               |
| Tleaf                         | 0.23             | .6               | 0.4                  | .5                   |
| CO₂                           | 11               | .0004            | 11.9                 | .0003                |
| Temperature                   | 0.042            | .8               | 0                    | .9                   |
| Tleaf × CO₂                   | 0.96             | .4               | 3.2                  | .041                 |
| Tleaf × Temperature           | 2.1              | .14              | 0.046                | .9                   |
| CO₂ × Temperature             | 0.8              | .4               | 1.3                  | .2                   |
| V_{cmax}                      | 654              | <.0001           | 525                  | <.0001               |
| Tleaf                         | 0.2              | .6               | 0.16                 | .69                  |
| CO₂                           | 2.5              | .1               | 5.5                  | .011                 |
| Temperature                   | 0.3              | .6               | 0.02                 | .9                   |
| Tleaf × CO₂                   | 1.9              | .15              | 3.4                  | .039                 |

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

(Continues)

3.3 | Photosynthetic capacity

In tamarack, the temperature response curve of V_{cmax} was not significantly altered by the treatments (Table 1). The leaf temperature at which tamarack V_{cmax} was highest (T_{optv}) was increased by elevated growth CO₂, but values of V_{cmax} at its thermal optimum (V_{cmaxopt}) were not significantly affected by either CO₂ 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 CO₂ (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).
whereas in tamarack, there was a trend for lower $J_{\text{max opt}}$ in warm-grown seedlings ($p = .07$; Table 2). In tamarack, the $J_{\text{max}}/V_{\text{cmax}}$ ratio was reduced by warming across the entire measuring temperature range (10–40°C; Figure S1a; Table S1). In contrast, in black spruce, the $J_{\text{max}}/V_{\text{cmax}}$ ratio was marginally affected by warming ($p = .056$), largely due to convergence of the $J_{\text{max}}/V_{\text{cmax}}$ ratio across warming treatments with increasing leaf temperature (Figure S1b; Table S1).

The activation energies for $V_{\text{cmax}}$ and $J_{\text{max}}$ increased with warming ($E_{\text{av}}$: 2.4 and 1.5 kJ mol$^{-1}$ °C$^{-1}$; $E_{\text{aj}}$: 1.6 and 1.25 kJ mol$^{-1}$ °C$^{-1}$ in tamarack and black spruce, respectively) without any CO$_2$ effects (Tables 2–4). The optimum temperature and activation energy for $J_{\text{max}}$ were consistently lower ($T_{\text{opt J}}$: 33°C; $E_{\text{aj}}$: 30 kJ/mol) than those of $V_{\text{cmax}}$ ($T_{\text{opt V}}$: 38°C; $E_{\text{av}}$: 52 kJ/mol; Table 2).

### Relationships between Photosynthetic and Respiration Parameters

In both species, $V_{\text{cmax20}}$ and $J_{\text{max20}}$ were reduced by warming, without any CO$_2$ effect (Tables 2–4). The ratio between $J_{\text{max20}}$ and $V_{\text{cmax20}}$ indicates the balance between these two biochemical processes at a moderate leaf temperature. In tamarack, $J_{\text{max20}}/V_{\text{cmax20}}$ was affected

### Table 2: Photosynthetic and Respiratory traits measured in this study in tamarack and black spruce seedlings grown under ambient CO$_2$ (AC) or elevated CO$_2$ (EC) at either ambient temperatures (0T), ambient + 4°C (4T), or ambient + 8°C (8T). Net CO$_2$ assimilation rate at the thermal optimum ($A_{\text{opt}}$, µmol m$^{-2}$ s$^{-1}$); thermal optimum of net CO$_2$ assimilation ($T_{\text{opt A}}$, °C); dark respiration rates measured at 20°C ($R_{\text{d20}}$, µmol m$^{-2}$ s$^{-1}$); the change in $R_{\text{d}}$ per 10°C increase in temperature ($Q_{10}$); maximum carboxylation rates of Rubisco at the thermal optimum ($V_{\text{cmax opt}}$, µmol m$^{-2}$ s$^{-1}$); maximum electron transport rate at the thermal optimum ($J_{\text{max opt}}$, µmol m$^{-2}$ s$^{-1}$); thermal optimum of $J_{\text{max opt}}$ ($T_{\text{opt J}, \circ}$); activation energy of $J_{\text{max opt}}$ ($E_{\text{aj}}$); activation energy of $V_{\text{cmax opt}}$ ($E_{\text{av}}$); $V_{\text{cmax20}}$ (µmol m$^{-2}$ s$^{-1}$) and $J_{\text{max20}}$ ($J_{\text{max20}}, µmol m^{-2} s^{-1}$) measured at 20°C and the ratio of $J_{\text{max20}}$ to $V_{\text{cmax20}}$ ($J_{\text{max20}}/V_{\text{cmax20}}$). Means ± SE, n = 5.

|                | AC0T  | AC4T  | AC8T  | EC0T  | EC4T  | EC8T  |
|----------------|-------|-------|-------|-------|-------|-------|
| $A_{\text{opt}}$ | 13.9 ± 0.8a | 14.4 ± 0.7ab | 13.1 ± 0.9a | 23.2 ± 2.6c | 16.9 ± 2.5abc | 21.2 ± 1.2bc |
| $T_{\text{opt A}}$ | 21.2 ± 1.7a | 23.5 ± 1ab | 25.3 ± 0.3ab | 24.9 ± 1.1ab | 27.6 ± 0.6bc | 30.0 ± 0.4c |
| $R_{\text{d20}}$ | 1.3 ± 0.2ab | 1.2 ± 0.2ab | 0.6 ± 0.06a | 1.5 ± 0.09b | 0.9 ± 0.2ab | 0.7 ± 0.1a |
| $Q_{10}$ | 1.8 ± 0.06a | 1.7 ± 0.015a | 1.7 ± 0.08a | 1.7 ± 0.02a | 1.7 ± 0.09a | 1.7 ± 0.07a |
| $V_{\text{cmax opt}}$ | 93.9 ± 9.5a | 79.5 ± 7.9a | 79.7 ± 8.2a | 88.6 ± 14.9a | 67.4 ± 18.9a | 85.1 ± 10.2a |
| $T_{\text{opt V}}$ | 38.6 ± 0.9a | 38.1 ± 0.6a | 37.9 ± 0.4a | 39.8 ± 0.8a | 38.9 ± 0.7a | 39.4 ± 0.5a |
| $J_{\text{max opt}}$ | 168 ± 14a | 128 ± 7a | 116 ± 16a | 164 ± 21a | 126 ± 33a | 128 ± 17a |
| $T_{\text{opt J}}$ | 33 ± 0.3ab | 33 ± 0.2abc | 34.9 ± 0.4cd | 32.9 ± 0.2a | 34.3 ± 0.1bcd | 35.2 ± 0.3d |
| $E_{\text{av}}$ | 47 ± 4ab | 53 ± 5ab | 66 ± 3c | 40 ± 4a | 51 ± 1.4abc | 60 ± 2bc |
| $E_{\text{aj}}$ | 30 ± 4ab | 32 ± 4ab | 34 ± 3b | 30 ± 2a | 37 ± 0.9ab | 40 ± 1.4ab |
| $V_{\text{cmax20}}$ | 39 ± 2a | 30 ± 2a | 26 ± 4a | 39 ± 4a | 25 ± 6a | 27 ± 3a |
| $J_{\text{max20}}$ | 123 ± 6bc | 90 ± 6abc | 70 ± 12a | 131 ± 10c | 78 ± 19ab | 78 ± 10ab |
| $J_{\text{max20}}/V_{\text{cmax20}}$ | 3.2 ± 0.1bc | 2.9 ± 0.1ab | 2.6 ± 0.06a | 3.5 ± 0.09c | 3.2 ± 0.02bc | 2.9 ± 0.09ab |

Note: Letters after each value represent group comparisons across the six temperature and CO$_2$ treatments generated from a Tukey post hoc test ($p < .05$).
Temperature × CO2 declined with warming (Tables 2 and 4). The intercepts of these relationships were higher in EC compared to AC spruce, EC plants, and decreased with warming (Tables 2 and 3). In black spruce, there was an interaction of growth 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 65% and 82% smaller compared to 0T; Figure 5c,d; Tables 3 and 4). In tamarack, the proportion of mass allocated to roots was lowest in 4T spruce (~25% of either 8T or 0T; Figure 5a,c; Tables 3 and 4). In AC, tamarack seedlings were 30% smaller than EC0T trees (Figure 5b; Table 4). In AC, 4T and 8T seedlings were 30% smaller than EC0T trees (Figure 5b; Table 4). In AC, 4T and 8T seedlings were 30% smaller than EC0T trees (Figure 5b; Table 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 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).

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, $J_{max20}/V_{cmax20}$ was only affected by growth temperature, and declined with warming (Tables 2 and 4).

In both species, $T_{optA}$ was positively correlated with both $E_{aw}$ (0.1–0.25°C per 1 kJ/mol) and $E_{aw}$ (0.11–0.24°C per 1 kJ/mol) across the warming treatments, but negatively correlated with $J_{max20}/V_{cmax20}$ (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).

### 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 ($A_{opt}$, µmol m$^{-2}$ s$^{-1}$); thermal optimum of net CO2 assimilation ($T_{optA}$, °C); dark respiration rates measured at 20°C ($R_{20}$, µmol m$^{-2}$ s$^{-1}$); the change in $R_{20}$ per 10°C increase in temperature ($Q_{10}$); maximum carboxylation rates of Rubisco at the thermal optimum ($V_{cmaxopt}$, µmol m$^{-2}$ s$^{-1}$); thermal optimum of $V_{cmax}$ ($T_{optV}$, °C); maximum electron transport rates at the thermal optimum ($J_{maxopt}$, µmol m$^{-2}$ s$^{-1}$); thermal optimum of $J_{max}$ ($T_{optJ}$, °C); activation energy of $V_{cmax}$ ($E_{av}$); activation energy of $J_{max}$ ($E_{aj}$); $V_{cmax}$ ($V_{cmax20}$, µmol m$^{-2}$ s$^{-1}$) and $J_{max}$ ($J_{max20}$, µmol m$^{-2}$ 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/m$^2$); leaf nitrogen per unit leaf area (N, g/m$^2$); chlorophyll a (Chl$_a$, g/m$^2$), chlorophyll b (Chl$_b$, g/m$^2$), and carotenoid concentrations (g/m$^2$)
of CO$_2$ and temperature treatments in black spruce on gas exchange parameters, photosynthetic capacity, plant biomass, and biochemical traits. Net CO$_2$ assimilation rate at the thermal optimum ($A_{\text{opt}}$, µmol m$^{-2}$ s$^{-1}$); thermal optimum of net CO$_2$ assimilation ($T_{\text{optA}}$, °C); dark respiration rates measured at 20°C ($R_{20}$, µmol m$^{-2}$ s$^{-1}$), the change in $R_d$ per 10°C increase in temperature ($Q_{10}$), maximum carboxylation rates of Rubisco at the thermal optimum ($V_{\text{maxopt}}$, µmol m$^{-2}$ s$^{-1}$); thermal optimum of $V_{\text{cmax}}$ ($T_{\text{optV}}$, °C); maximum electron transport rates at the thermal optimum ($J_{\text{maxopt}}$, µmol m$^{-2}$ s$^{-1}$); thermal optimum of $J_{\text{max}}$ ($T_{\text{optJ}}$, °C); activation energy of $V_{\text{cmax}}$ ($Q_{\text{av}}$, µmol m$^{-2}$ s$^{-1}$); activation energy of $J_{\text{max}}$ ($Q_{\text{aj}}$, µmol m$^{-2}$ s$^{-1}$); and $J_{\text{max}}$ ($J_{\text{max20}}$, µmol m$^{-2}$ s$^{-1}$) measured at 20°C; the ratio of $J_{\text{max20}}$ to $V_{\text{cmax20}}$ ($V_{\text{cmax20}}/J_{\text{max20}}$); total biomass allocated to roots (root mass ratio); stem (stem mass ratio); and leaf (leaf mass ratio); leaf mass on mass basis (LMA, g/m$^2$); leaf nitrogen per unit leaf area ($N_a$, g/m$^2$); chlorophyll a ($\text{Chl}_a$, g/m$^2$); chlorophyll b ($\text{Chl}_b$, g/m$^2$); and carotenoid concentrations (g/m$^2$).

### Plant biomass traits
- Total biomass: 52.5 ± 0.0011
- Root mass ratio: 11 ± 0.0001
- Stem mass ratio: 3.9 ± 0.023
- Leaf mass ratio: 14 ± 0.0001
- LMA: 7.2 ± 0.0017

### Biochemical traits
- $N_a$: 13.9 ± 0.0001
- $\text{Chl}_a$: 9.5 ± 0.001
- $\text{Chl}_b$: 5.6 ± 0.0108
- Carotenoids: 10.7 ± 0.0005

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 CO$_2$ effect (Figure 5g,h; Tables 3 and 4). Leaf mass per unit area (LMA) was decreased by warming in both species, without a CO$_2$ 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, $\text{Chl}_a$, $\text{Chl}_b$ and carotenoid concentrations per unit leaf area did not vary across the treatments (e.g., Figure 6c; Table 3). In contrast, in black spruce, $\text{Chl}_a$ declined with warming (3%–51%), as did $\text{Chl}_b$ (7%–53%) and carotenoid (8%–55%) concentrations per unit leaf area (Figure 6b,d,f,h; Table 4). There was a warming × CO$_2$ interaction for $\text{Chl}_a$ 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 CO$_2$ 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 CO$_2$ conditions both impacted $A_{\text{net}}$, but these effects differed between the two species. As expected, $A_{\text{net}}$ measured at the growth CO$_2$ was stimulated by the elevated CO$_2$ treatments. However, the stimulation of $A_{\text{net}}$ by elevated CO$_2$ 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 $A_{\text{net}}$ between the two species. In tamarack, $A_{\text{net}}$ was largely unaffected by warming (i.e., compare AC0T, AC4T, AC8T in Figure 2a), but $A_{\text{net}}$ was...
significantly reduced in warm-grown spruce. Furthermore, $A_{\text{net}}$ 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 $A_{\text{net}}$ 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 $A_{\text{net}}$.

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 $CO_2$-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...
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. Similar results have 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_{opt} 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_{opt} should also increase with elevated CO_2 due to a suppression of photorespiration under high measurement CO_2 (Sage & Kubien, 2007; Wujeska-Klause et al., 2019). In both species, T_{opt} 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_{opt} 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_{opt} 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_{opt} 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_{opt} 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_{opt} (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 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 CO_2 had much higher photosynthetic rates than AC0T tamarack measured under current conditions (cooler leaf temperatures and current CO_2). 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 CO$_2$ effects in either species. In contrast to our hypothesis, $V_{\text{cmax}}$ and $J_{\text{max}}$ at 20°C were significantly reduced by warming, while being unaffected by elevated CO$_2$. These findings contrast with results from mature P. abies, which acclimated $V_{\text{cmax}}$ to elevated CO$_2$, 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 CO$_2$ 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 $V_{\text{cmax20}}$ and $J_{\text{max20}}$ were reduced by warming, the decline in their ratio is due to a greater decline of $J_{\text{max20}}$ relative to $V_{\text{cmax20}}$. This warming-induced reduction in $J_{\text{max}}/V_{\text{cmax}}$ at a given temperature is common (Crous et al., 2018; Dusenge 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 measured 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 $V_{\text{cmax20}}/N_j$ (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 $J_{\text{max20}}/N_j$ 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 CO$_2$ effects. The activation energies of $V_{\text{max}}$ ($E_a$) and $J_{\text{max}}$ ($E_J$) and the thermal optimum of $J_{\text{max}}$ ($T_{\text{optJ}}$) 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 CO$_2$. The $T_{\text{optV}}$ was, however, significantly higher in elevated CO$_2$ treatments. These thermal adjustments in photosynthetic capacity act to improve net CO$_2$ 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 $T_{\text{optV}}$ was significantly correlated with $E_a$ and $E_J$ 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 CO$_2$ conditions. The lack of discernible CO$_2$ effects on the temperature sensitivity parameters of photosynthetic capacity in our study, the same parameters incorporated into the Kumarathunge et al. (2019) algorithm ($E_a$ and $E_J$), suggest that their temperature response function should apply for both ambient and elevated CO$_2$-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 $R_j$ 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 CO$_2$ on $R_j$ (Dusenge et al., 2019; Way et al., 2015). In our study, as well as others (Kroner & Way, 2016; Kurepin et al., 2018), no effect of CO$_2$ has been seen on $R_j$. In contrast, some studies found a stimulation of $R_j$ (Lamba et al., 2018; Roberntz & Stockfors, 1998), and this stimulation may be driven by increased carbohydrates in elevated CO$_2$-exposed trees (Roberntz & Stockfors, 1998; Tjoelker et al., 1998). In addition, enhanced $R_j$ 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 CO$_2$-induced reduction of leaf N observed in their study partially offsets the stimulation of $R_j$ due to increased carbohydrates in elevated CO$_2$-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 $R_j$.

The treatments had contrasting effects on growth between tamarack and spruce. In tamarack, there was no effect of elevated CO$_2$ on biomass, while warming altered growth, with 4T-grown tamarack having the highest biomass. In contrast, elevated CO$_2$ 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 spruce) often have reduced performance compared to deciduous and early-successional species 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 CO$_2$ 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 $V_{c,max}$ and $J_{m,max}$ and the thermal optimum of $J_{m,max}$, elevated CO$_2$ had little effect on these parameters, implying that the effect of rising CO$_2$ 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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