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Climate change experiment suggests divergent responses of tree seedlings in eastern North America’s Acadian Forest Region over the 21st century

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ABSTRACT

In this study, we conducted a controlled experiment to assess the growth and survival of balsam fir (*Abies balsamea* (L.) Mill.), red spruce (*Picea rubens* Sarg.), and red maple (*Acer rubrum* L.) seedlings in response to warming, drought, and elevated CO₂, as projected under RCP 8.5 for North America's Acadian Forest Region. In response to warming, only red spruce increased in height; however, this effect varied by CO₂ and soil moisture treatments. Under the drought treatment, red spruce biomass was not affected, but mortality increased by two percent. With warming, increases in balsam fir height growth were only detected under certain soil moisture and CO₂ conditions. Balsam fir biomass decreased by 24% under drought, while mortality increased by 5 percent. Warming did not improve red maple height growth, but it remained 7-50 times greater than that of the conifers and no mortality was observed. Overall, CO₂ enrichment increased height growth of droughted seedlings relative to the ambient treatment, demonstrating an amelioration of the negative drought effect. Balsam fir was the least adapted to warming and drought, while red spruce displayed some positive responses. Although growth of red maple seedlings did not increase with warming, they exhibited greater absolute growth and survival, which suggests red maple may outperform both conifers under a warming climate.

Key Words: Plant–climate interactions; Autecology; Seedling; Forest; Climate change; Drought; Growth; Photosynthesis

1 INTRODUCTION

The early life stages of plant species are the most sensitive to environmental stress and may greatly influence plant community assembly and succession under climate change (Johnstone et al., 2010; Kroel-Dulay et al., 2015). By the end of this century, northeastern North America's

36 Acadian Forest Region (AFR) could experience a mean annual temperature increase of 5 to 6°C
37 under the business-as-usual Representative Concentration Pathway 8.5 (RCP; Van Vuuren et al.,
38 2011; McKenney et al. 2013). The AFR lies within North America's boreal-temperate ecotone,
39 an ecological transition zone where many constituent tree species are at either their northern or
40 southern climatic range limits. In particular, cold-adapted species, such as balsam fir (*Abies*
41 *balsamea* (L.) Mill.), are projected to decline in abundance with warming, while warm-adapted,
42 temperate species with more southerly range limits, such as red maple (*Acer rubrum* L.), are
43 expected to thrive under climate change (Boulanger et al., 2018; Bourque & Hassan, 2008;
44 Ashraf et al., 2015; Taylor et al., 2017). However, most forest simulation models used to predict
45 future species' abundances under climate change lack empirically derived physiological limits of
46 seedling growth and survival, undermining the accuracy of their predictions (Bugmann et al.,
47 2001; Price et al., 2001; Urban et al., 2016).

48 In the absence of moisture stress and nutrient limitations, rising atmospheric CO₂ may increase
49 forest growth; however, it is unclear whether this increase in productivity will be sustained over
50 the long-term (Beedlow et al., 2004). Free air CO₂ enrichment (FACE) experiments have shown
51 increased levels of CO₂ may enhance tree growth and stem biomass (Ainsworth & Long, 2005;
52 Norby et al., 2005). Red spruce (*Picea rubens* Sarg.) seedlings grown under 713 CO₂ ppm were
53 40% larger in both size and weight when compared to seedlings under ambient conditions
54 (Samuelson & Seiler, 1994). Similarly, in moist soil conditions, red maple seedlings grown in
55 700 ppm CO₂ produced 70% greater total biomass than seedlings exposed to 350 ppm CO₂
56 (Miao et al., 1992). Without additional mitigation efforts beyond those currently in place,
57 atmospheric concentrations of carbon dioxide (CO₂) are projected to exceed 1000 ppm by the
58 year 2100 under the RCP 8.5 scenario (IPCC, 2014).

While both boreal and temperate tree species in the boreal-temperate ecotone have exhibited increased growth in response to warming experiments (Fisichelli et al., 2014; Carles et al., 2015), rising temperature will also increase plant water use through its effect on vapour pressure deficit (VPD; Will et al., 2013). High VPD has been shown to decrease red spruce net photosynthesis and stomatal conductance by 26 and 43%, respectively (Day, 2000), and increase tree seedling mortality risk during drought (Will et al., 2013). These effects may be counteracted to some degree by elevated CO₂ (Swann et al., 2016; Sperry et al., 2019), which has been found to reduce stomatal conductance and increase the rate of photosynthesis (Long et al., 2004) and water use efficiency (Brodribb et al., 2020). However, it remains unclear how the complex interaction between increasing atmospheric CO₂ and temperature will affect seedlings of tree species in the boreal-temperate ecotone.

When compared with mature trees, young seedlings have minimal rooting depth, which makes them more susceptible to stress when exposed to drought (Donovan & Ehleringer, 1991). During drought, cell turgor pressure decreases, which causes a reduction in cell elongation and plant growth (Proseus et al., 2000). Under severe drought, seedling mortality can result from a decline in the flow of water from roots to leaves (hydraulic failure) or deficient carbohydrate reserves due to a reduction in photosynthesis (carbon starvation) (McDowell et al., 2008). Plants will attempt to avoid moisture stress through stomatal closure (McDowell et al., 2008) or, over the longer term, a reduction in shoot growth, increasing the root:shoot ratio (Brunner et al., 2015).

In this study, we conducted a controlled experiment using a series of specially designed phytotrons (small enclosed greenhouses in which temperature, CO₂ and soil moisture are controlled) to determine the individual and interactive effects of temperature, CO₂, and soil moisture under current and projected climate for the AFR on seedling height, diameter, biomass

and mortality of balsam fir, red spruce and red maple—three ecologically and commercially important tree species in the study area. We hypothesized that (1) warming will increase growth of species due to increased photosynthetic activity; (2) low soil moisture will reduce growth as a result of cell turgor loss and this effect will be more severe with increasing temperature due to high VPD; (3) elevated CO₂ will increase growth and alleviate the drought effect through enhanced photosynthetic activity and improved water use efficiency; and (4) red maple will exhibit greater survival and relative growth than balsam fir and red spruce in response to increasing temperature due to its more southerly range limit (Tirmenstein, 1991).

2 MATERIALS AND METHODS

2.1 Study species

The tree species chosen to compare in our experiment coexist in the AFR, but their geographical ranges vary considerably throughout eastern North America (Fig. 1). Red spruce is predominantly found in Atlantic Canada and northeastern United States and grows in areas with cool, moist summers and cold winters (Sullivan, 1993). Balsam fir and red maple, however, grow on a wider variety of soil types and have much larger distributions. Balsam fir, the most cold-adapted and northerly distributed species in our study, grows in the AFR and Eastern Boreal Ecozone (Uchytel, 1991). Red maple, the most southerly distributed species in our study, is found in the southern portion of the Eastern Boreal Ecozone, through the AFR, and throughout the temperate forests of eastern North America, as far south as Florida (Tirmenstein, 1991). Red maple has a superior capacity for indeterminate growth relative to the conifers (Abrams, 1998), is moderately shade tolerant, and able to establish in a variety of successional stages, including

pioneer and subclimax (Tirmenstein, 1991). Balsam fir and red spruce are considered to be climax species and very shade tolerant (Uchytel, 1991; Sullivan, 1993).

2.2 Experimental design

Six phytotrons were constructed to compare seedling growth and survival of balsam fir, red spruce and red maple under current and projected climate conditions for the city of Fredericton, New Brunswick (45.96° N, 66.64° W), located approximately at the geographical center of the AFR. Current mean annual temperature is 5.6°C and mean January and July temperatures are -9.4 and 19.3°C, respectively. Mean annual precipitation is 1077.7 mm and the average annual frost-free period is 130 days (Environment Canada, 2019). The environmental conditions of the six phytotrons included three temperature treatments (baseline, baseline +3°C, and baseline +6°C) × two CO₂ levels (ambient and elevated), with each phytotron split into two soil moisture treatments (moderate and drought). Each of the 12 unique treatment combinations contained 135 balsam fir and red spruce seedlings, and 15 red maple seedlings. Balsam fir and red spruce seedlings were grown in 45-cell trays (110 cm³/cell), and red maple were grown in six-inch square pots. The disparity between coniferous and deciduous seedling quantities was necessary because of the large difference in first year growth and the limited space within each phytotron. The trial ran from June 4th to Oct 1st, 2019; however, red maple seedlings were removed from the phytotrons on Aug 21st due to height restrictions. The phytotrons were exposed to ambient light during the entire duration of the trial with an average maximum light level of 268.2 μmol m⁻²s⁻¹ (minimum and maximum light levels ranged from 210 to 331 μmol m⁻²s⁻¹).

2.3 Phytotron design

The phytotrons were built in the Atlantic Forestry Centre greenhouse, located in Fredericton, New Brunswick, constructed with pressure treated lumber and covered with clear greenhouse plastic. The phytotrons were 1.0 m wide by 3.0 m long and 1.2 m high. The ventilation system (Appendix A1, Fig. A1.1) for each phytotron consisted of an inline fan that drew air from a galvanized steel container (mixing box) with two inlets (fresh air and recirculated air) and one outlet. The elevated CO₂ phytotrons had an additional inlet for CO₂ installed, which injected pure CO₂ directly into the outlet pipe. Fresh air entered through the opening in the back of the mixing box and mixed with CO₂ from a storage tank (for elevated CO₂ treatments only) and air that was drawn from the phytotron through a corrugated recirculation pipe. The fan pulled the mixed air from the mixing box through the outlet pipe into a 4-inch, perforated manifold that was located along the entire length of one side of the phytotron. Each phytotron had a space heater for warming and a window air conditioner for cooling. The space heater was installed inside the mixing box and the air conditioner was installed on the side of the phytotron.

2.4 Experimental factors

The baseline temperature treatment was determined by averaging the last three years (2016, 2017 and 2018) of weekly temperature data collected from the Fredericton International Airport weather station located in Lincoln, New Brunswick, Canada at 45.87° N and 66.54° W. The +3°C and +6°C treatments represent the projected growing season temperature for Fredericton for the periods of 2041 to 2060 and 2091 to 2100, respectively, under the RCP 8.5 forcing scenario, using climate data from the Coupled Model Intercomparison Project phase 5 (McKenney et al. 2013). The temperature in each phytotron was controlled by a temperature controller (model ITC-310T-B, Inkbird, Shenzhen, P. R. China), which cycled the heater and air conditioner on and off based on a programmed daily temperature schedule consisting of ten

diurnal temperature steps (Appendix A2, Table A2.1). The minimum temperature occurred between 4 and 6 a.m. After 6 a.m., temperature increased every two hours until it reached the maximum temperature at 12 p.m. The maximum temperature was held for 6 hours (12 p.m. to 6 p.m.) and then decreased every two hours until 4 a.m. the next morning. The baseline temperature schedule was changed weekly to match the average weekly minimum and maximum temperatures from the Fredericton International Airport weather station (Table 1). The +3°C and +6°C temperature schedules constantly maintained temperatures that were 3.0°C and 6.0°C above baseline temperature (Table 1). Mean summer temperatures for the baseline, +3°C and +6°C treatments were 19.6, 22.6 and 25.6°C, respectively. Mean summer temperature of each species' southern geographic range boundary was $19.1 \pm 0.9^\circ\text{C}$, $19.6 \pm 1.8^\circ\text{C}$ and $26.7 \pm 1.2^\circ\text{C}$ for balsam fir, red spruce and red maple, respectively. Species' southern boundary temperature data were derived by overlaying tree species distribution maps (Little, 1971) over North American interpolated climate grids obtained from Natural Resources Canada (McKenney et al., 2011) and calculating the average mean summer temperature for 100 randomly located points within the southern 1% of each species range. Unless further mitigation efforts are initiated to reduce greenhouse gas emissions beyond those currently in place, the high (RCP 8.5) emission scenario is becoming increasingly more likely (IPCC, 2014; Foster et al., 2017; UNEP, 2019; Voosen, 2019).

The ambient CO₂ treatment, which represented the current atmospheric CO₂ concentration, had no additional CO₂ added. The elevated treatment held the CO₂ concentration at 900 ppm, mimicking the projected CO₂ concentrations for the end of the century for RCP 8.5 (IPCC, 2014). Carbon dioxide levels in each of the elevated CO₂ phytotrons were regulated by a solenoid valve that was controlled by an Adafruit Feather Huzzah ESP 8266 microcontroller

171 (Adafruit, New York, New York, USA). The microcontroller received data from a CO₂ sensor
172 (model K30, SenseAir, Delsbo, Sweden), located in the recirculation pipe, and injected CO₂ into
173 the phytotrons through the solenoid valve to maintain the target level.

174 Field capacity soil moisture content in the seedling trays/pots was observed to be approximately
175 25% when measured with a digital soil moisture meter (model HSM50, OMEGA, Saint-
176 Eustache, Quebec, Canada). Seedling trays in the moderate and drought soil moisture treatments
177 were allowed to dry down to 15% and 5% soil moisture, respectively, before being hand-watered
178 to field capacity. Three seedlings per species, per soil moisture treatment, in every phytotron
179 were selected for daily soil moisture measurements with the digital soil moisture meter to
180 determine whether watering was required. These methods for measuring soil moisture and
181 watering were carried out for the entire duration of the experiment. The moderate soil moisture
182 treatment was designed to provide adequate soil moisture and prevent negative effects due to
183 overwatering.

184 **2.5 Seed source and seedling preparation**

185 Seed was acquired from the Canadian National Tree Seed Centre
186 (<https://cfs.nrcan.gc.ca/publications?id=36773>). Three parent trees (seedlots) of each of the three
187 tree species were selected from locations within 75 km of Fredericton to minimize variation in
188 the maternal climate. Balsam fir and red maple seeds were stratified according to standard
189 procedures (Bonner & Karrfalt, 2008), and red maple seeds were subsequently germinated in a
190 germinator (model G1000, Conviron, Winnipeg, Manitoba, Canada). Red spruce and stratified
191 balsam fir seeds were sown into 45-cell trays on January 31st and February 1st, respectively, and
192 red maple germinants were planted into 15-cell trays on February 5th (all potting media contained

a mixture of 50% vermiculite and 50% peat moss). Conifer seedlings from cells containing multiple seedlings were transplanted to empty cells within the first two weeks of germination, and all conifer cells were thinned to one seedling at the end of March. Red maple seedlings were grown until May 22nd and then re-potted in 15-cm square pots. During the seedling preparation stage (January 31st to June 3rd), all seedlings were grown in a greenhouse with a 20°C/16°C (day/night) temperature schedule and fertilized with water-soluble 11:41:08 (N:P:K) starter fertilizer (Plant Products Inc., Leamington, Ontario, Canada) at a concentration of 50 ppm N. During the experiment, seedlings were fertilized with water-soluble 20:08:20 (N:P:K) grower fertilizer (Plant Products Inc., Leamington, Ontario, Canada) at a concentration of 100 ppm N until September 9. After this date, a water-soluble finisher fertilizer (08:20:30 (N:P:K); 35 ppm N; Plant Products Inc., Leamington, Ontario, Canada) was used until the end of the experiment.

2.6 Seedling growth and survival

Height and root-collar diameter of all seedlings were measured at the beginning and end of the trial to determine total height and diameter growth. At the start of the trial, average height and diameter of balsam fir, red spruce and red maple seedlings were 34.1 mm (13 to 60 mm) and 0.9 mm (0.1 to 1.9 mm), 112.7 mm (20 to 195 mm) and 1.1 mm (0.5 to 1.6 mm), and 596.4 mm (400 to 920 mm) and 4.9 mm (3.3 to 6.5 mm), respectively. After the trial, seedling mortality was recorded, and full seedling samples for mass measurement ($n = 6$ for red maple; $n = 15$ for red spruce; and $n = 21$ for balsam fir) were randomly selected from each of the 12 treatment combinations and rinsed of soil prior to being oven-dried at 70°C for 48 hours (Taylor et al., 2007) and weighed. Sample sizes varied between species as seedling sizes and processing times were substantially different.

2.7 Measurements of photosynthesis and stomatal conductance

Balsam fir photosynthesis (A_{net}) and stomatal conductance (g_s) were measured for all soil moisture and CO₂ treatments of the baseline and +6°C temperature levels. These data were only collected for balsam fir to assist with interpreting this species' growth and survival results as it is often projected to be one of the most severely impacted by climate change in the AFR (Boulanger et al., 2018; Taylor et al., 2017). Data were collected during the week with the hottest daily temperature schedule (baseline maximum temperature = 28.7°C; +6°C maximum temperature = 34.7°C) between 12:00 and 4:00 p.m., as we expected seedling stress levels to be greatest in the +6°C treatment during this period. Measurements were taken with an LI-6400XT portable photosynthesis system equipped with a 6400-22 opaque conifer chamber and a 6400-18A RGB light source (Li-Cor, Lincoln, Nebraska, USA). The Li-Cor conifer chamber environment was programmed to match the baseline and +6°C temperatures (28.7°C or 34.7°C) and CO₂ levels (400 ppm or 900 ppm) at 60% relative humidity. We chose to compare baseline/ambient CO₂ with +6°C/elevated CO₂ as these treatments represented the current and end of century temperature and atmospheric CO₂ levels. The entire aboveground portions of the balsam fir seedlings were inserted into the 6400-22 opaque conifer chamber and exposed to 1000 $\mu\text{mol m}^{-2}\text{s}^{-1}$ photosynthetic photon flux. We allowed the A_{net} and g_s values to stabilize prior to logging the data. Total leaf surface areas of the seedlings were then measured to interpret the A_{net} and g_s measurements.

2.8 Measurements of xylem water potential

Seedling xylem water potential data were collected for all species and treatments with a model 1505D-EXP pressure chamber (PMS Instrument Company, Albany, Oregon, USA). These data

were used to estimate differences in soil water potential between the soil moisture treatments for each species (Ritchie & Hinckley, 1975; Major & Johnsen, 1996). Three seedlings per species from each of the twelve treatments (totaling 36 seedlings per species) were randomly selected and placed in a dark room for two hours prior to being tested (mimicking predawn xylem water potential). Seedlings from the drought and moderate soil moisture treatments were sampled at 5% soil moisture and the day after watering, respectively. For each sample, a small, freshly cut branch section was placed into the pressure chamber and the pressure was slowly increased. When water was observed on the cut surface, the pressure was recorded.

2.9 Statistical analyses

Analysis of variance (ANOVA) was used to test the interactive effects of temperature, CO₂ and soil moisture treatments on seedling height and diameter growth, total biomass, and root:shoot ratio of each species. The four response variables were tested separately for each species using the following linear model:

$$Y = \beta_0 + \beta_1 T + \beta_2 C + \beta_3 S + \beta_4 T \times C + \beta_5 T \times S + \beta_6 C \times S + \beta_7 T \times C \times S + \varepsilon$$

where Y is one of the four response variables (i.e., seedling height growth, diameter growth, total biomass or root:shoot ratio); T represents temperature treatment; C is CO₂ treatment; S is soil moisture treatment, and ε is random error. Separate models were fit for each species to ease interpretation and to assess the species-specific treatment effects. Mean growth response values of each treatment combination were compared using the “TukeyHSD” post-hoc test in R with the “multcompLetters4” function from the “multcompView” package (Graves et al., 2019). Additionally, the same ANOVA model was used to test the interactive effects of temperature, CO₂ and soil moisture on balsam fir A_{net} and g_s . Each ANOVA was fit with the “aov” function in

259 R (R Core Team, 2020). Non-significant three-way interactions were removed from the final
260 model to increase degrees of freedom and simplify model interpretation. Heteroscedasticity and
261 normality of model residuals were visually assessed by inspecting the model residuals versus
262 fitted values and Normal Q-Q plots, and statistically analyzed with the Shapiro-Wilk test. If
263 heteroscedasticity was found to be significant, the response variable was log-transformed.

264 Logistic regression was used to test the interactive effects of temperature, CO₂ and soil moisture
265 on seedling mortality for balsam fir and red spruce (red maple mortality was not observed in any
266 treatment), and a Chi-square test was used to determine the significance of each treatment factor.
267 Mean xylem water potential values were compared between the two soil moisture treatments for
268 each species using two-sample t-tests.

269 With some treatment combinations being nested within phytotrons (e.g., temperature and CO₂),
270 there is the potential for pseudoreplication to produce spurious effects due to small variations in
271 individual phytotron environments (Hurlbert, 1984). Hurlbert (1984), which addressed the issue
272 of pseudoreplication in ecological studies, has been challenged with regard to the issue of
273 pseudoreplication in growth chamber experiments (Oksanen, 2001; Munzbergova et al., 2017),
274 and experiments using unreplicated growth chamber experimental designs have been
275 successfully published (Bezemer et al., 1998; Cavieres & Arroyo, 2000; Souther et al., 2012;
276 Matias & Jump, 2014; Munzbergova et al., 2017). Nonetheless, to help account for potential
277 increase in Type I error and spurious treatment effects due to pseudoreplication, we chose to set
278 α as 0.01 for all significance tests (as opposed to 0.05) (Banerjee et al., 2009).

279 **3 RESULTS**

280 **3.1 Seedling growth**

281 Increasing temperature significantly influenced three out of four balsam fir growth parameters as
282 either a main or interactive effect (Table 2). These effects included a significant interaction
283 between temperature, CO₂ and soil moisture on height growth; a significant interaction between
284 temperature and CO₂ on diameter growth; and a significant effect of increasing temperature that
285 reduced root:shoot ratio (Table 2). The elevated CO₂ treatments had significant positive effects
286 on all four balsam fir growth variables, with total biomass and root:shoot ratio showing 24.0%
287 and 19.5% increases (Table 2, Fig. 2g, j). In contrast, the drought soil moisture treatment
288 significantly reduced balsam fir diameter growth and total biomass by 9.2% and 23.5%,
289 respectively (Table 2, Fig. 2d, g). When compared with baseline temperature, balsam fir
290 root:shoot ratio was 11.4% and 13.3% smaller in the +3°C and +6°C treatments ($p < 0.01$, Fig. 2j).
291 With warming, increases in balsam fir height growth were only detected under certain soil
292 moisture and CO₂ conditions (Fig. 3a)—seedlings in the +6°C treatment exhibited a small,
293 marginally significant ($p < 0.05$) 14.0% (1.8 mm) increase in average height growth relative to the
294 baseline treatment. When comparing the two CO₂ treatments, the increase in height growth in
295 response to warming is much more evident under the elevated treatment (Fig. 3a). Furthermore,
296 height growth of droughted balsam fir was 22.9% greater under elevated CO₂ relative to the
297 ambient treatment ($p < 0.01$, Fig. 3a). Balsam fir diameter growth under elevated CO₂ was 23.2%
298 greater than the ambient CO₂ treatment under +3°C warming ($p < 0.01$) but was similar under
299 +6°C warming (Fig. 3c).

300 Warming had significant interactive effects on red spruce height and diameter growth, which
301 included a significant interaction between temperature, CO₂ and soil moisture on height growth
302 and a significant interaction between temperature and CO₂ on diameter growth (Table 2). Unlike
303 balsam fir, red spruce root:shoot ratio did not significantly increase with warming (Table 2, Fig.

2k). All red spruce growth parameters increased under elevated CO₂, with total biomass and root:shoot ratio showing 19.9% and 10.4% increases (Table 2, Fig. 2h, k). Red spruce appeared to be much less sensitive to the drought soil moisture treatment than balsam fir with only a 2.3% decrease in diameter growth and no significant reduction in total biomass (Table 2, Fig. 2e, h). Warming increased red spruce average height growth but varied in intensity according to CO₂ and soil moisture conditions (Fig. 2b). Specifically, compared with baseline values, red spruce average height growth was 17.6% and 26.4% greater under +3 and +6°C warming, respectively ($p < 0.01$, Fig. 2b). This was further demonstrated by the clear trend that red spruce height growth increased with warming under every CO₂ and soil moisture treatment combination (Fig. 3b). Additionally, in the drought soil treatment, red spruce height growth was 23.1% greater under elevated CO₂ when compared with ambient ($p < 0.01$, Fig. 3b). Similar to balsam fir, red spruce diameter growth in the elevated CO₂ treatment was 23.2% greater than in the ambient treatment under +3°C warming ($p < 0.01$) but did not differ under +6°C (Fig. 3d). Interestingly, the positive combined effect of warming and elevated CO₂ that was observed at the +3°C level disappeared at the +6°C level, which suggests that this warming-induced increase in growth could be transitory for both conifers.

Red maple height growth and total biomass were influenced by significant interactions between temperature and soil moisture (Table 2). Red maple aboveground growth increased under elevated CO₂, however, unlike the conifers, root:shoot ratio was not affected (Table 2, Fig. 2l). Red maple showed 24.1% greater height growth, 22.4% larger average diameter growth, and 37.7% larger average total biomass under elevated CO₂ compared with ambient (Table 2, Fig. 2c, f, i)—roughly two to three times greater than the responses of the conifers. Moreover, the relative response to elevated CO₂ exhibited by red maple was consistently greater than the two

conifers in both moderate and drought soil moisture treatments for all growth parameters except for root:shoot ratio. Additionally, no interactions between CO₂ and the other treatment factors were observed for any red maple response variable (Table 2). This suggests that the increase in red maple growth in response to elevated CO₂ was not significantly limited or affected by the other treatment factors, which differs from the temperature-limited positive effect of CO₂ that was observed with balsam fir and red spruce diameter growth. Under the drought treatment, red maple exhibited a 20.6% increase in root:shoot ratio (Table 2, Fig. 2l); and, when comparing the baseline/ambient CO₂/drought soil moisture treatment with the +6°C/ambient CO₂/drought soil moisture treatment, root:shoot ratio increased by 49.2% ($p < 0.01$, Fig. 2l). This response to increased moisture stress was not observed in the conifers. Occasionally, droughted red maple growth was significantly greater under elevated CO₂, while seedlings in the moderate soil treatment did not differ between CO₂ treatments (e.g., height growth, diameter growth and total biomass under baseline temperature; Fig. 2c, f, i). Although there was a negative effect of temperature on red maple total biomass overall (Fig. 2i), it varied in intensity between soil moisture treatments—under +6°C, average total biomass of droughted seedlings was 43.6% ($p < 0.01$) smaller than that of seedlings in the moderate soil moisture treatment, while no significant differences were observed between soil moisture treatments at either baseline or +3°C (Fig. 3e). Under the moderate soil moisture treatment, warming did not significantly affect red maple total biomass (Fig. 3e). Similarly, red maple height growth was unaffected by temperature when soil moisture was adequate; however, under drought, we observed 17.3 and 27.4% reductions ($p < 0.01$) in the baseline and +6°C treatments (Fig. 3f). Interestingly, the negative effect of drought on red maple height growth was less severe under elevated CO₂, which elicited a 30.7% increase relative to the ambient CO₂ treatment ($p < 0.01$).

3.2 Impacts of soil moisture on xylem water potential

For all three species, significantly lower xylem water potentials (two-sample t-test, $p < 0.01$) were observed under the drought treatment relative to the moderate treatment (Fig. 4). Under drought, mean xylem water potentials were -1.01, -1.03 and -1.45 MPa for balsam fir, red spruce and red maple, respectively, and -0.42, -0.48 and -0.32 MPa in the moderate soil moisture treatment.

3.3 Balsam fir photosynthesis and stomatal conductance

Balsam fir average A_{net} under both CO_2 treatments decreased by 76.6% in response to moisture stress ($p < 0.01$), and, under elevated CO_2 , A_{net} of both soil moisture treatments increased by 120.6% ($p < 0.01$, Fig. 5). No interaction was observed between soil moisture and CO_2 for balsam fir A_{net} , however, g_s response to moisture stress differed between CO_2 treatments. Under elevated CO_2 , g_s of droughted seedlings improved, while seedlings in the moderate soil moisture treatment experienced a reduction in g_s . A_{net} and g_s values in the drought soil treatments of the ambient CO_2 treatments were nearly zero.

3.4 Seedling mortality

Across temperature and CO_2 treatments, average balsam fir and red spruce mortality increased significantly from 1.6 and 1.0% mortality under moderate soil moisture to 6.8 and 2.7% mortality under the drought treatment, respectively ($p < 0.01$; Figure 6). Balsam fir mortality was significantly affected by an interaction between temperature and CO_2 ($p < 0.01$): under ambient CO_2 , mortality rates for balsam fir were 2.6, 1.5 and 13.3% for the baseline, +3°C and +6°C treatments, respectively, and 0.0, 3.0 and 4.8% under elevated CO_2 . Balsam fir seedlings in the +6°C/ambient CO_2 /drought soil treatment exhibited the greatest mortality at 22.2% (30 of 135 seedlings; Fig. 6). No red maple mortality occurred, so it was excluded from the analysis.

4 DISCUSSION

4.1 Experimental effects of warming, elevated CO₂ and soil moisture on growth

The observed increases in growth in response to warming (red spruce) and elevated CO₂ (all species) support our first and third hypotheses and agree with previous studies (Fisichelli et al., 2014; Carles et al., 2015; Resco de Dios et al., 2016). Red maple, predicted to become more abundant in the AFR under climate change (Ashraf et al., 2015; Boulanger et al., 2018; Taylor et al., 2017), did not show improved growth in response to warming, but maintained greater absolute growth relative to spruce and fir and was only negatively affected by warming when soil moisture was limiting, which supports our second hypothesis. Interestingly, CO₂ did not interact with any other treatment factor for aboveground growth of red maple, which implies that red maple growth will continue to benefit from rising atmospheric CO₂ regardless of the warming that is projected to occur in the AFR. In contrast, we found that there are limits to the increased growth effect of elevated CO₂ for balsam fir and red spruce. This was demonstrated by the increase in diameter growth that these species exhibited under +3°C warming and elevated CO₂, while under +6°C warming and elevated CO₂, diameter growth decreased. Clearly, the increase in fir and spruce diameter growth under warming and elevated CO₂ is temperature-limited. The drought soil moisture treatment, which produced soil water potential estimates similar to those of drought stress field studies (Major & Johnsen, 1996; Olesinski et al., 2011), generally reduced aboveground growth of all species; however, the negative effect of drought was alleviated under elevated CO₂, in accordance with previous studies (Swann et al., 2016; Sperry et al., 2019). This was evident in the height growth of balsam fir, red spruce and red maple seedlings in the drought treatments, which exhibited significant increases under elevated CO₂ relative to the ambient treatments.

395 Some of the observed increases in growth may be more pronounced because of the indeterminate
396 growth pattern expressed by the tested coniferous species during the first growing season (Carles
397 et al., 2015). In the following years, the determinate growth habits typically expressed by red
398 spruce and balsam fir juveniles (Pothier & Margolis, 1991; Samuelson & Seiler, 1993) may limit
399 these species' competitiveness relative to red maple, which has a much greater capacity for
400 indeterminate growth throughout its ontogeny (Abrams, 1998). If the growing season lengthens
401 with climate change as projected (Peñuelas & Filella, 2001), this characteristic will further
402 benefit red maple and likely cause a shift in growth-related competitiveness in favour of red
403 maple.

404 Under ambient CO₂ and drought soil conditions, red maple root:shoot ratio increased in response
405 to warming. This shift in resource allocation to the roots was not observed in balsam fir and red
406 spruce seedlings. An increase in root:shoot ratio limits stress when soil moisture availability is
407 low and can be achieved by restricting shoot growth (Brunner et al., 2015). As the effects of
408 typical drought events become more severe with climate warming (Will et al., 2013; Peñuelas et
409 al., 2017), tree species that lack the ability to limit water stress by altering resource allocation
410 may exhibit lower fitness.

411 Excess soil water resulting from CO₂-induced increases in water use efficiency (Assmann, 1993;
412 Long et al., 2004) could explain why red maple growth in the baseline/moderate soil moisture
413 treatment did not significantly increase in response to elevated CO₂. This is similar to
414 observations of Fisichelli et al. (2014), however, in their case, excessive soil moisture reduced
415 the increased growth effect of warming. This result agrees with past studies that have observed
416 an increase in water use efficiency resulting from rising atmospheric CO₂ (Peñuelas et al., 2011;
417 Soh et al., 2019). Furthermore, the drought soil moisture treatment generally produced smaller

seedlings in the ambient CO₂ phytotrons where improved water use efficiency, as a result of CO₂ enrichment, was absent.

4.2 Effects of climate change on balsam fir photosynthesis and stomatal conductance

Elevated CO₂ has been shown to decrease transpiration through a reduction in g_s (Long et al., 2004; Wertin et al., 2010; Franks et al., 2013); however, the decrease in g_s only negligibly offsets the increase in the rate of photosynthesis (Long et al., 2004). This is in line with the decrease in g_s and the increase in A_{net} that we observed in balsam fir seedlings under elevated CO₂. The low A_{net} and g_s values that were recorded in the ambient CO₂/drought soil moisture treatments may be the result of stomatal closure due to the extremely low soil water potential (Franks et al., 2013). Interestingly, under elevated CO₂, seedlings exposed to similarly low soil water potentials produced larger A_{net} and g_s values, suggesting that the stomata were still open. When subjected to low soil water potentials, plants exposed to elevated CO₂ have been observed to maintain better functionality and higher xylem water potentials than those under ambient conditions (Tyree & Alexander, 1993). Thus, rising levels of atmospheric CO₂ over the 21st century may allow some tree species to continue photosynthesizing during periods of rainfall deficits that, under current atmospheric CO₂ concentrations, would significantly reduce A_{net} . Still, further research is needed to understand the effects of rising atmospheric CO₂ on species with differing stomatal regulation strategies (Duan et al., 2015) and to determine whether the observed increase in photosynthetic capacity will remain heightened or return to baseline levels as a result of photosynthetic adjustment responses (Rey & Jarvis, 1998; Tissue et al., 1999).

4.3 Seedling survival in a changing environment

439 The lack of red maple mortality supports our fourth hypothesis that, under long-term RCP 8.5
440 climatic conditions, red maple seedlings will exhibit better survival than balsam fir and red
441 spruce. With the highest (>20%/year) balsam fir mortality rates occurring in the +6°C/drought
442 soil treatment under ambient CO₂ (Fig. 6), high temperature and reduced soil moisture most
443 likely had the strongest impacts on survival. In comparison, the same treatment under elevated
444 CO₂ produced substantially lower (5.2%/year) balsam fir mortality rates, providing further
445 evidence of the positive effect of improved water use efficiency resulting from CO₂ enrichment,
446 which is clearly shown by the increase in A_{net} and g_s values relative to balsam fir seedlings under
447 the ambient CO₂ treatment (Fig. 5). Seedling mortality is often the result of hydraulic failure
448 when soil moisture is low because their limited root size has less access to soil water than the
449 larger root systems of mature trees (McDowell et al., 2008). The substantial balsam fir mortality
450 that we observed in the +6°C/ambient CO₂/drought soil treatment will require further testing to
451 corroborate these results and may not be meaningful as +6°C warming is not expected to occur
452 in the absence of increased atmospheric CO₂. However, recent modelling studies (Boulanger et
453 al., 2017; Taylor et al., 2017) and field experiments (Reich et al., 2015) have predicted that this
454 species, along with other boreal species in the boreal-temperate ecotone, will be severely
455 impacted under a warming climate, and these findings generally agree with the species'
456 geographic distributions. Balsam fir, having the most northern distribution, was most sensitive to
457 the +6°C treatment, while red maple, the most southerly species, did not show any mortality. Red
458 spruce, which is found in the southern portion of balsam fir's range, exhibited less mortality
459 under the +6°C treatment than balsam fir. In the drought soil treatments, red maple seedlings
460 produced the lowest xylem water potential (Fig. 4), however, no mortality was observed. This
461 agrees with past studies that have observed low mortality rates of red maple during drought

(Elliott & Swank, 1994). Thus, given the potential increase in drought severity under a warming climate (Will et al., 2013; Peñuelas et al., 2017), red maple may be more likely to survive compared with balsam fir and red spruce.

5 CONCLUSIONS

Our experiment showed that some major boreal-temperate tree species in North America's AFR may exhibit divergent physiological responses to the RCP 8.5 radiative forcing scenario. Compared with colder-adapted balsam fir, red spruce exhibited better growth and survival in response to warming and was less sensitive to moisture stress. Red maple, the most southerly distributed species in our study, did not exhibit mortality in any treatment and was the only species that altered resource allocation in response to increasing temperature and hydraulic stress. These results suggest that red spruce may be better adapted than balsam fir to long-term projected warming and increase in drought severity, while red maple seedlings will likely outperform both conifers. As plant community assembly and succession is largely influenced by the sensitivity of seedlings in young forests (Johnstone et al., 2010; Kroel-Dulay et al., 2015), our observations suggest that warm-adapted species, like red maple, may become more abundant in the AFR under a warming climate, which agrees with a growing body of model projections (e.g., Bourque & Hassan, 2008; Ashraf et al., 2015; Taylor et al., 2017). These impacts on forest communities may be most prominent following stand-replacing disturbance, since mature forests have the capacity to buffer fluctuations in underlying environmental conditions (Bouchon & Arseneault, 2004; Johnstone et al., 2010). Tree seedlings in managed forests in the AFR may therefore be more affected by climate change, as typical silvicultural systems utilized in this region involve overstorey removal and planting of cold-adapted spruce species, which may become increasingly less adapted to post-harvest environmental conditions. In natural stands,

485 however, mature overstories will shelter young seedlings and compositional changes will
486 gradually occur as the competitiveness of temperate tree species, relative to cold-adapted species,
487 improves under a warming climate (Fisichelli et al., 2014). To avoid reductions in forest growth
488 caused by a decline in the abundance of cold-adapted tree species in the AFR, adaptive
489 management strategies that either alleviate competition from spruce and fir, or that, alternatively,
490 favour temperate tree species must be considered.

491 Due to equipment and logistical constraints, we were not able to replicate the treatments. This
492 limitation may have led to spurious results; therefore, further investigation will be required to
493 verify our findings. We included Table A3.1 in Appendix A3, which lists all response variable
494 means, standard errors and ranges for all species and treatments to enable species level
495 variability in each treatment to be assessed. This study is limited to first year seedling growth in
496 response to the RCP 8.5 climate change scenario over the course of the 21st century. This
497 experiment should be repeated with two- and three-year-old juveniles to better understand
498 variation between determinate and indeterminate growth patterns expressed by these species and
499 to determine the effects of long-term exposure to elevated CO₂ on photosynthetic activity.

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AUTHORS' CONTRIBUTIONS

WRV, ART, DAM and LD conceived the ideas and designed methodology; WRV and ART collected the data; WRV and ART analyzed the data; WRV led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

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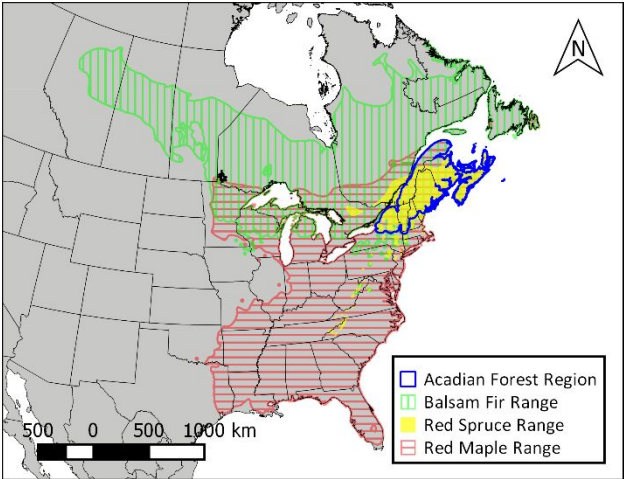
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TABLE 1. Average weekly minimum and maximum temperatures for each temperature treatment.

Date Range	Baseline		Temperature Treatment			
	Max. Temp. (°C)	Min. Temp. (°C)	+3°C		+6°C	
	Max. Temp. (°C)	Min. Temp. (°C)	Max. Temp. (°C)	Min. Temp. (°C)	Max. Temp. (°C)	Min. Temp. (°C)
June 4 to 10	20.0	7.0	23.0	10.0	26.0	13.0
June 11 to 17	20.1	7.6	23.1	10.6	26.1	13.6
June 18 to 24	25.0	9.5	28.0	12.5	31.0	15.5
June 25 to July 1	26.6	11.3	29.6	14.3	32.6	17.3
July 2 to 8	26.4	12.7	29.4	15.7	32.4	18.7
July 9 to 15	26.4	12.2	29.4	15.2	32.4	18.2
July 16 to 22	27.5	13.4	30.5	16.4	33.5	19.4
July 23 to 29	27.4	14.0	30.4	17.0	33.4	20.0
July 30 to August 5	28.7	13.2	31.7	16.2	34.7	19.2
August 6 to 12	28.7	13.2	31.7	16.2	34.7	19.2
August 13 to 19	24.3	11.6	27.3	14.6	30.3	17.6
August 20 to 26	26.0	11.4	29.0	14.4	32.0	17.4
August 27 to September 2	24.2	9.0	27.2	12.0	30.2	15.0
September 3 to 9	24.4	9.3	27.4	12.3	30.4	15.3
September 10 to 16	24.7	9.1	27.7	12.1	30.7	15.1
September 17 to 23	19.1	7.0	22.1	10.0	25.1	13.0
September 24 to 30	19.0	5.2	22.0	8.2	25.0	11.2

TABLE 2. Effects of temperature, CO₂, soil moisture treatments and their interactions on height growth, root-collar diameter growth, total biomass and root:shoot ratio of seedlings. Statistically significant terms ($p < 0.01$) are in bold. Three-way interaction terms that were not significant were removed from the model (NA).

Fixed Effect	Height growth		Diameter growth		Total Biomass		Root:shoot Ratio	
	F-value	P-value	F-value	P-value	F-value	P-value	F-value	P-value
Balsam Fir								
Temperature	3.82	0.022	4.07	0.017	0.86	0.43	12.70	<0.0001
CO ₂	8.66	0.0033	20.11	<0.0001	7.62	0.0062	48.85	<0.0001
Soil moisture	2.30	0.13	14.56	0.00014	11.73	0.00072	1.63	0.20
Temp x CO ₂	2.93	0.054	5.20	0.0056	0.52	0.60	0.42	0.65
Temp x Soil moisture	0.34	0.71	2.00	0.13	2.01	0.14	3.11	0.047
CO ₂ x Soil moisture	2.49	0.11	3.00	0.08	0.37	0.55	0.16	0.69
Temp x CO ₂ x Soil	6.36	0.0017	NA	NA	NA	NA	NA	NA
Red Spruce								
Temperature	58.62	<0.0001	24.65	<0.0001	1.24	0.29	1.76	0.17
CO ₂	53.03	<0.0001	20.07	<0.0001	8.40	0.0042	6.87	0.0095
Soil moisture	1.12	0.29	39.85	<0.0001	1.27	0.26	0.063	0.80
Temp x CO ₂	3.09	0.046	29.82	<0.0001	0.81	0.45	2.26	0.11
Temp x Soil moisture	5.84	0.0030	1.70	0.18	2.74	0.067	0.38	0.68
CO ₂ x Soil moisture	20.54	<0.0001	1.01	0.32	5.71	0.018	0.008	0.93
Temp x CO ₂ x Soil	6.82	0.0011	NA	NA	NA	NA	NA	NA
Red Maple								
Temperature	1.97	0.14	3.14	0.046	5.82	0.0048	1.00	0.38
CO ₂	49.92	<0.0001	74.75	<0.0001	52.11	<0.0001	0.13	0.72
Soil moisture	38.78	<0.0001	2.45	0.12	7.72	0.0072	17.63	<0.0001
Temp x CO ₂	0.41	0.66	4.57	0.012	0.59	0.56	4.25	0.019
Temp x Soil moisture	4.83	0.0091	4.73	0.010	5.01	0.0096	3.84	0.027
CO ₂ x Soil moisture	0.72	0.40	3.38	0.068	0.14	0.71	0.44	0.51
Temp x CO ₂ x Soil	NA	NA	NA	NA	NA	NA	NA	NA



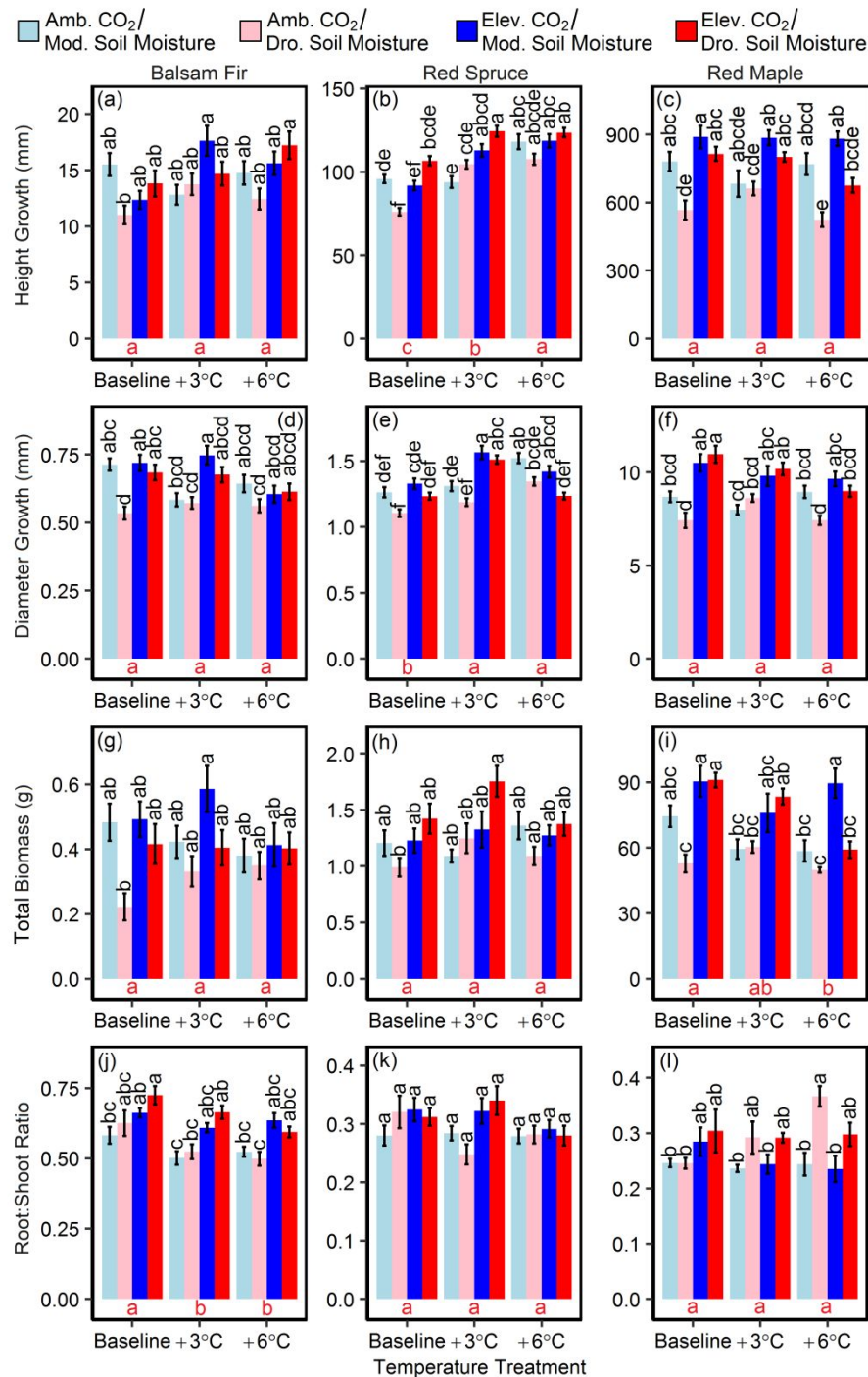
786

787 **FIGURE 1**

788 Map showing the Acadian Forest Region (Two Countries, One Forest, 2014) and the distribution
789 of balsam fir, red spruce and red maple in Canada and the United States (Little, 1971). Map
790 created with QGIS v3.10 software using North America basemap layer provided by Natural
791 Resources Canada.

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FIGURE 2

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Average height growth, root-collar diameter growth, total biomass, and root:shoot ratio of all species in all treatment combinations. Each panel compares a response variable of one species to all combinations of phytotron treatments. Colours represent CO₂ and soil moisture treatment combinations and error bars indicate standard error. Letter displays report the results of a Tukey test with a 99% confidence interval. Groups that share the same letters do not have a significant

801 difference between means. Pairwise comparisons were conducted between temperature
802 treatments (red letters) and between all treatment combinations (black letters).

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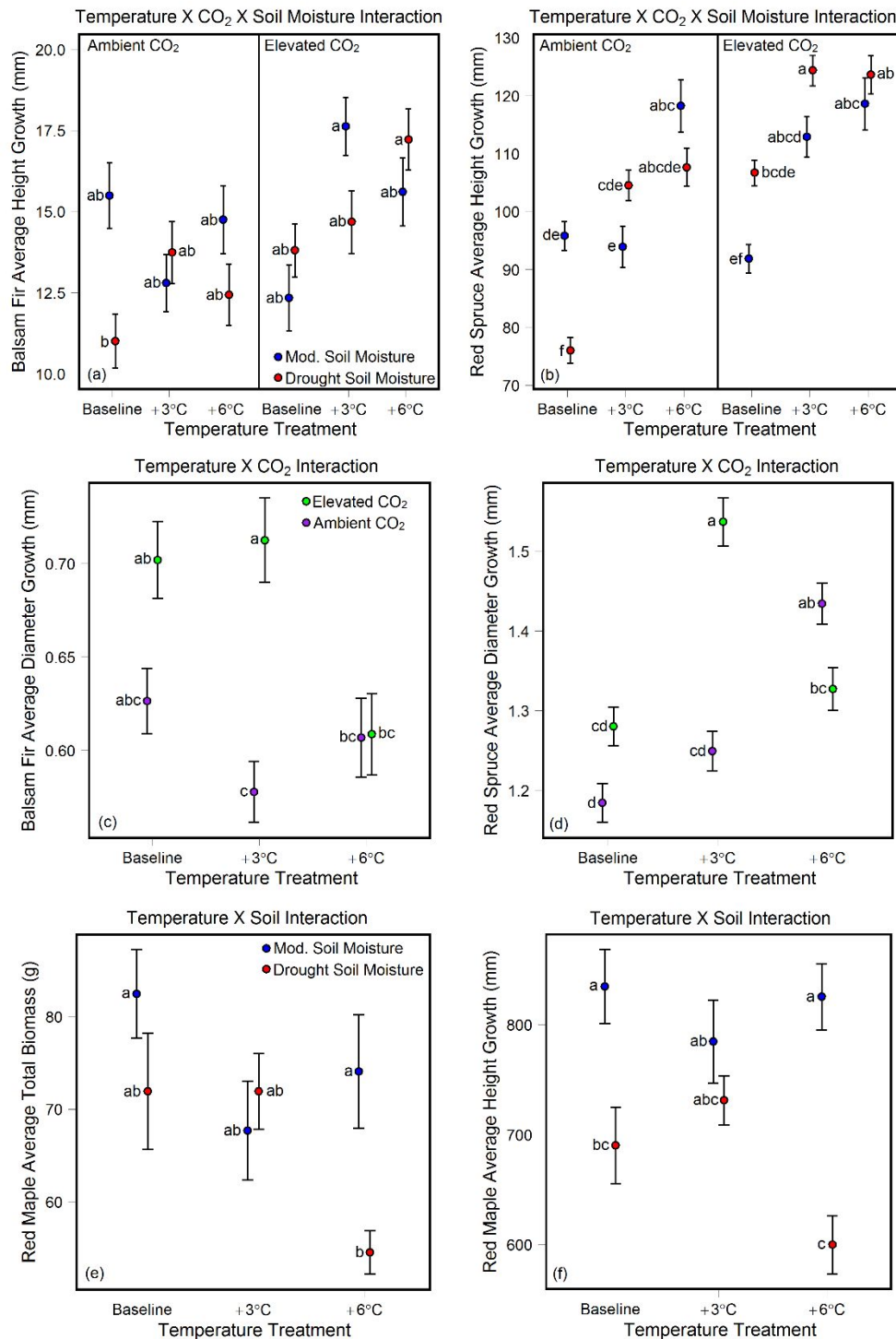


FIGURE 3

Comparison of significant interactions between explanatory variables (temperature, CO₂ and soil moisture). Each panel demonstrates the effects of a significant two-way or three-way interaction on a response variable (average height growth, average diameter growth or average total biomass) for one species. Colours represent CO₂ and soil moisture treatments and error bars

810 indicate standard error. Letter displays report the results of a Tukey test with a 99% confidence
811 interval. Points that share the same letters do not have a significant difference between means.

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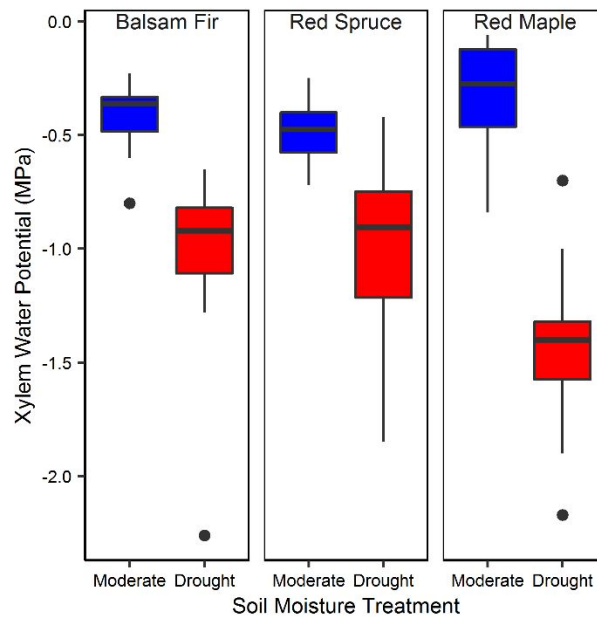
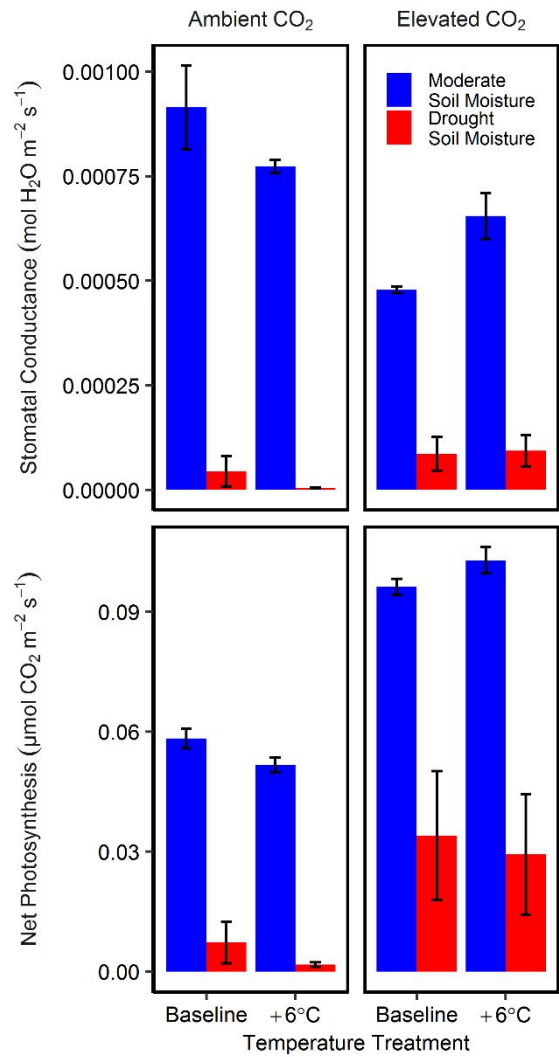


FIGURE 4

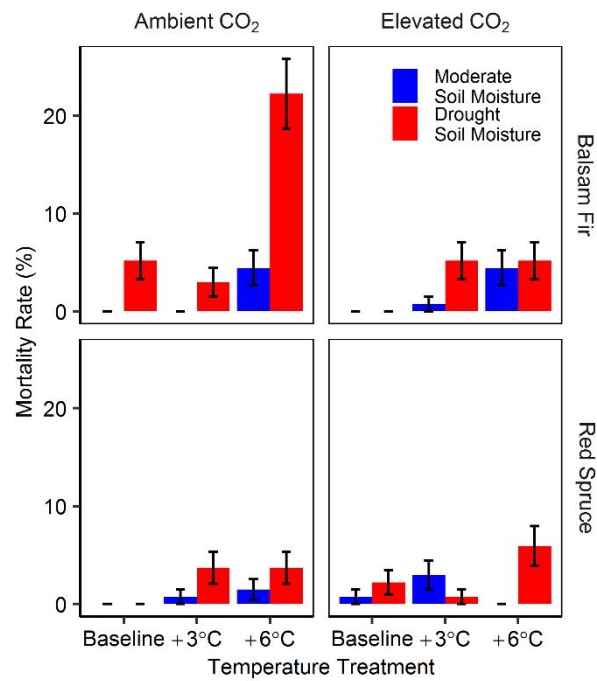
Boxplots showing the xylem water potential for seedlings growing in moderate (blue) and drought (red) soil moisture treatments. The horizontal lines within the boxes are the medians, and the box represents the upper and lower quartiles. Upper and lower whiskers extend to the most extreme observed points above and below the box that fall within 1.5 times the interquartile range, and all points that fall outside of the whiskers are outliers.



821

822 FIGURE 5

823 Mean ± 1 standard error net photosynthesis and stomatal conductance of three balsam fir
824 seedlings from ambient and elevated CO₂ treatments for the baseline and +6°C temperature and
825 drought and moderate soil moisture treatments.



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828 FIGURE 6

829 Mean ± 1 standard error mortality of balsam fir and red spruce seedlings for temperature, CO₂,
 830 and soil moisture treatment combinations. Red maple seedlings did not exhibit mortality.

Appendix 1

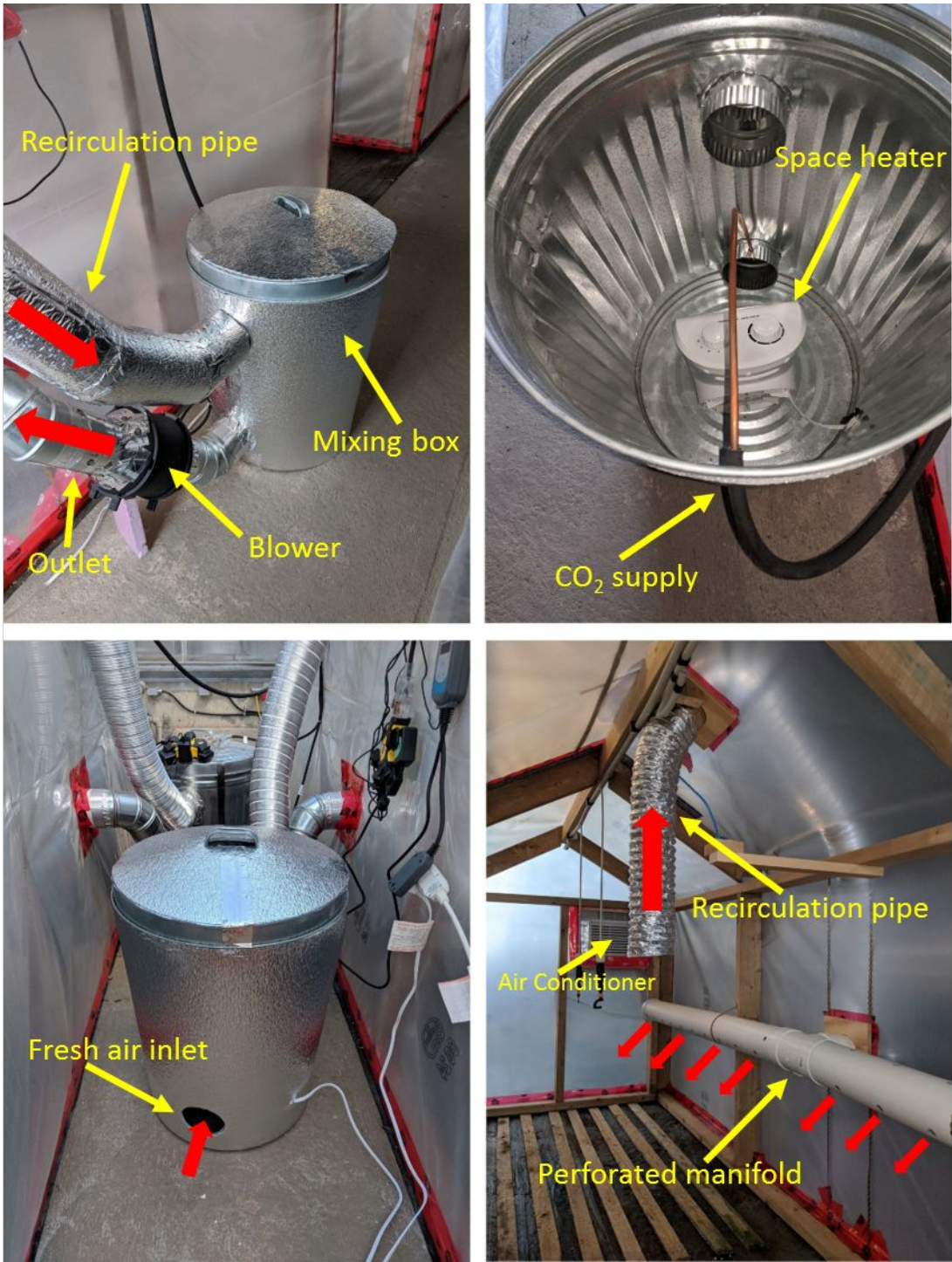


FIGURE A1.1
Phytotron ventilation design. Red arrows indicate the direction of airflow.

Appendix 2.

TABLE A2.1. Phytotron hourly temperature schedule.

Week	Date Range	Max Temp	Min Temp	Scenario	Stage Temperature and Duration									
					12:00 6 hrs	18:00 2 hrs	20:00 2 hrs	22:00 2 hrs	0:00 2 hrs	2:00 2 hrs	4:00 2 hrs	6:00 2 hrs	8:00 2 hrs	10:00 2 hrs
1	June 4 to 10	20.0	7.0	Baseline	20.0	17.8	15.7	13.5	11.3	9.2	7.0	10.3	13.5	16.8
				+3	23.0	20.8	18.7	16.5	14.3	12.2	10.0	13.3	16.5	19.8
				+6	26.0	23.8	21.7	19.5	17.3	15.2	13.0	16.3	19.5	22.8
2	June 11 to 17	20.1	7.6	Baseline	20.1	18.0	15.9	13.9	11.8	9.7	7.6	10.7	13.9	17.0
				+3	23.1	21.0	18.9	16.9	14.8	12.7	10.6	13.7	16.9	20.0
				+6	26.1	24.0	21.9	19.9	17.8	15.7	13.6	16.7	19.9	23.0
3	June 18 to 24	25.0	9.5	Baseline	25.0	22.4	19.8	17.3	14.7	12.1	9.5	13.4	17.3	21.1
				+3	28.0	25.4	22.8	20.3	17.7	15.1	12.5	16.4	20.3	24.1
				+6	31.0	28.4	25.8	23.3	20.7	18.1	15.5	19.4	23.3	27.1
4	June 25 to July 1	26.6	11.3	Baseline	26.6	24.1	21.5	19.0	16.4	13.9	11.3	15.1	19.0	22.8
				+3	29.6	27.1	24.5	22.0	19.4	16.9	14.3	18.1	22.0	25.8
				+6	32.6	30.1	27.5	25.0	22.4	19.9	17.3	21.1	25.0	28.8
5	July 2 to 8	26.4	12.7	Baseline	26.4	24.1	21.8	19.6	17.3	15.0	12.7	16.1	19.6	23.0
				+3	29.4	27.1	24.8	22.6	20.3	18.0	15.7	19.1	22.6	26.0
				+6	32.4	30.1	27.8	25.6	23.3	21.0	18.7	22.1	25.6	29.0
6	July 9 to 15	26.4	12.2	Baseline	26.4	24.0	21.7	19.3	16.9	14.6	12.2	15.8	19.3	22.9
				+3	29.4	27.0	24.7	22.3	19.9	17.6	15.2	18.8	22.3	25.9
				+6	32.4	30.0	27.7	25.3	22.9	20.6	18.2	21.8	25.3	28.9
7	July 16 to 22	27.5	13.4	Baseline	27.5	25.2	22.8	20.5	18.1	15.8	13.4	16.9	20.5	24.0
				+3	30.5	28.2	25.8	23.5	21.1	18.8	16.4	19.9	23.5	27.0
				+6	33.5	31.2	28.8	26.5	24.1	21.8	19.4	22.9	26.5	30.0
8	July 23 to 29	27.4	14.0	Baseline	27.4	25.2	22.9	20.7	18.5	16.2	14.0	17.4	20.7	24.1
				+3	30.4	28.2	25.9	23.7	21.5	19.2	17.0	20.4	23.7	27.1
				+6	33.4	31.2	28.9	26.7	24.5	22.2	20.0	23.4	26.7	30.1
9	July 30 to August 5	28.7	13.2	Baseline	28.7	26.1	23.5	21.0	18.4	15.8	13.2	17.1	21.0	24.8
				+3	31.7	29.1	26.5	24.0	21.4	18.8	16.2	20.1	24.0	27.8
				+6	34.7	32.1	29.5	27.0	24.4	21.8	19.2	23.1	27.0	30.8
10	Aug 6 to 12	28.7	13.2	Baseline	28.7	26.1	23.5	21.0	18.4	15.8	13.2	17.1	21.0	24.8
				+3	31.7	29.1	26.5	24.0	21.4	18.8	16.2	20.1	24.0	27.8
				+6	34.7	32.1	29.5	27.0	24.4	21.8	19.2	23.1	27.0	30.8
11	Aug 13 to 19	24.3	11.6	Baseline	24.3	22.2	20.1	18.0	15.8	13.7	11.6	14.8	18.0	21.1
				+3	27.3	25.2	23.1	21.0	18.8	16.7	14.6	17.8	21.0	24.1
				+6	30.3	28.2	26.1	24.0	21.8	19.7	17.6	20.8	24.0	27.1
12	Aug 20 to 26	26.0	11.4	Baseline	26.0	23.6	21.1	18.7	16.3	13.8	11.4	15.1	18.7	22.4
				+3	29.0	26.6	24.1	21.7	19.3	16.8	14.4	18.1	21.7	25.4
				+6	32.0	29.6	27.1	24.7	22.3	19.8	17.4	21.1	24.7	28.4
13	Aug 27 to Sept 2	24.2	9.0	Baseline	24.2	21.7	19.1	16.6	14.1	11.5	9.0	12.8	16.6	20.4
				+3	27.2	24.7	22.1	19.6	17.1	14.5	12.0	15.8	19.6	23.4
				+6	30.2	27.7	25.1	22.6	20.1	17.5	15.0	18.8	22.6	26.4
14	Sept 3 to 9	24.4	9.3	Baseline	24.4	21.9	19.4	16.9	14.3	11.8	9.3	13.1	16.9	20.6
				+3	27.4	24.9	22.4	19.9	17.3	14.8	12.3	16.1	19.9	23.6
				+6	30.4	27.9	25.4	22.9	20.3	17.8	15.3	19.1	22.9	26.6
15	Sept 10 to 16	24.7	9.1	Baseline	24.7	22.1	19.5	16.9	14.3	11.7	9.1	13.0	16.9	20.8
				+3	27.7	25.1	22.5	19.9	17.3	14.7	12.1	16.0	19.9	23.8
				+6	30.7	28.1	25.5	22.9	20.3	17.7	15.1	19.0	22.9	26.8
16	Sept 17 to 23	19.1	7.0	Baseline	19.1	17.1	15.1	13.1	11.0	9.0	7.0	10.0	13.1	16.1
				+3	22.1	20.1	18.1	16.1	14.0	12.0	10.0	13.0	16.1	19.1
				+6	25.1	23.1	21.1	19.1	17.0	15.0	13.0	16.0	19.1	22.1
17	Sept 24 to 30	19.0	5.2	Baseline	19.0	16.7	14.4	12.1	9.8	7.5	5.2	8.7	12.1	15.6
				+3	22.0	19.7	17.4	15.1	12.8	10.5	8.2	11.7	15.1	18.6
				+6	25.0	22.7	20.4	18.1	15.8	13.5	11.2	14.7	18.1	21.6

Appendix 3.

TABLE A3.1. Response variable means, standard errors and ranges (expressed in table as mean (**standard error**, range)) for all species and treatments.

Species	Temperature Treatment	Soil Moisture Treatment	CO ₂ Treatment	Mean Height Growth (mm)	Mean Diameter Growth (mm)	Mean Total Biomass (g)	Mean Root:Shoot Ratio	Mean Xylem Pressure (MPa)	Net Photosynthesis (µmol CO ₂ m ⁻² s ⁻¹)	Net Stomatal Conductance (mol H ₂ O m ⁻² s ⁻¹)
Balsam Fir	Baseline	Moderate	Elevated	12.3 (0.8 , 45.0)	0.72 (0.030 , 1.3)	0.49 (0.055 , 1.07)	0.65 (0.017 , 0.38)	0.32 (0.021 , 0.07)	0.0340 (0.0161 , 0.00681)	0.0000868 (0.0000407 , 0.0000274)
Balsam Fir	Baseline	Drought	Elevated	13.8 (1.2 , 61.0)	0.68 (0.029 , 1.3)	0.42 (0.061 , 0.90)	0.68 (0.032 , 0.57)	0.96 (0.084 , 0.29)	0.0962 (0.00202 , 0.0543)	0.000478 (0.0000080 , 0.000137)
Balsam Fir	Baseline	Moderate	Ambient	15.5 (1.0 , 65.0)	0.71 (0.023 , 1.4)	0.48 (0.058 , 0.84)	0.54 (0.030 , 0.66)	0.53 (0.140 , 0.46)	0.00725 (0.00523 , 0.00774)	0.0000443 (0.0000362 , 0.000332)
Balsam Fir	Baseline	Drought	Ambient	11.0 (0.8 , 47.0)	0.53 (0.024 , 1.1)	0.22 (0.042 , 0.67)	0.57 (0.045 , 0.86)	0.74 (0.048 , 0.16)	0.0583 (0.00244 , 0.0177)	0.000914 (0.000101 , 0.000115)
Balsam Fir	+3°C	Moderate	Elevated	17.6 (1.3 , 57.0)	0.75 (0.035 , 1.7)	0.59 (0.071 , 1.09)	0.59 (0.016 , 0.28)	0.39 (0.027 , 0.08)		
Balsam Fir	+3°C	Drought	Elevated	14.8 (1.1 , 60.0)	0.68 (0.028 , 1.3)	0.40 (0.055 , 0.74)	0.66 (0.024 , 0.39)	1.48 (0.406 , 1.36)		
Balsam Fir	+3°C	Moderate	Ambient	12.8 (0.9 , 47.0)	0.58 (0.024 , 1.5)	0.42 (0.050 , 0.76)	0.47 (0.024 , 0.40)	0.39 (0.056 , 0.18)		
Balsam Fir	+3°C	Drought	Ambient	13.7 (1.0 , 53.0)	0.57 (0.022 , 1.1)	0.33 (0.047 , 0.73)	0.50 (0.026 , 0.47)	1.02 (0.080 , 0.24)		
Balsam Fir	+6°C	Moderate	Elevated	15.6 (1.1 , 51.0)	0.60 (0.031 , 1.6)	0.41 (0.067 , 0.81)	0.59 (0.026 , 0.46)	0.33 (0.058 , 0.20)	0.0293 (0.0150 , 0.0104)	0.0000931 (0.0000382 , 0.000174)
Balsam Fir	+6°C	Drought	Elevated	16.3 (1.2 , 64.0)	0.60 (0.030 , 1.3)	0.40 (0.050 , 0.83)	0.57 (0.019 , 0.34)	0.85 (0.042 , 0.13)	0.103 (0.00330 , 0.0504)	0.000655 (0.0000550 , 0.000128)
Balsam Fir	+6°C	Moderate	Ambient	14.7 (1.1 , 48.0)	0.64 (0.032 , 1.6)	0.38 (0.051 , 0.98)	0.51 (0.018 , 0.30)	0.57 (0.018 , 0.06)	0.00172 (0.000545 , 0.00646)	0.0000049 (0.0000016 , 0.0000517)
Balsam Fir	+6°C	Drought	Ambient	12.5 (0.9 , 36.0)	0.57 (0.025 , 1.1)	0.35 (0.042 , 0.62)	0.47 (0.025 , 0.45)	1.08 (0.125 , 0.43)	0.0517 (0.00187 , 0.00169)	0.000774 (0.0000151 , 0.0000053)
Red Spruce	Baseline	Moderate	Elevated	91.9 (2.9 , 190.0)	1.33 (0.039 , 1.7)	1.23 (0.11 , 1.69)	0.32 (0.020 , 0.27)	0.50 (0.056 , 0.18)		
Red Spruce	Baseline	Drought	Elevated	106.7 (2.8 , 120.0)	1.23 (0.027 , 1.5)	1.42 (0.13 , 2.02)	0.31 (0.015 , 0.23)	1.22 (0.176 , 0.61)		
Red Spruce	Baseline	Moderate	Ambient	95.8 (2.5 , 154.0)	1.26 (0.039 , 1.9)	1.21 (0.11 , 1.74)	0.28 (0.017 , 0.26)	0.50 (0.037 , 0.12)		
Red Spruce	Baseline	Drought	Ambient	76.0 (2.2 , 116.0)	1.10 (0.028 , 1.6)	0.99 (0.08 , 1.05)	0.32 (0.028 , 0.43)	1.34 (0.258 , 0.84)		
Red Spruce	+3°C	Moderate	Elevated	112.9 (3.8 , 182.0)	1.56 (0.051 , 2.5)	1.32 (0.16 , 3.05)	0.32 (0.022 , 0.53)	0.31 (0.043 , 0.14)		
Red Spruce	+3°C	Drought	Elevated	124.4 (3.4 , 166.0)	1.51 (0.032 , 1.6)	1.75 (0.14 , 1.89)	0.34 (0.025 , 0.33)	0.68 (0.132 , 0.43)		
Red Spruce	+3°C	Moderate	Ambient	93.9 (3.5 , 176.0)	1.31 (0.039 , 1.6)	1.09 (0.06 , 0.71)	0.28 (0.012 , 0.14)	0.57 (0.007 , 0.02)		
Red Spruce	+3°C	Drought	Ambient	104.5 (2.6 , 126.0)	1.19 (0.030 , 1.4)	1.25 (0.13 , 1.65)	0.25 (0.017 , 0.27)	0.69 (0.107 , 0.35)		
Red Spruce	+6°C	Moderate	Elevated	118.6 (4.0 , 186.0)	1.42 (0.044 , 2.1)	1.27 (0.09 , 1.41)	0.29 (0.015 , 0.23)	0.35 (0.068 , 0.22)		
Red Spruce	+6°C	Drought	Elevated	123.6 (2.8 , 139.0)	1.23 (0.027 , 1.5)	1.37 (0.10 , 1.48)	0.28 (0.017 , 0.24)	1.36 (0.328 , 1.11)		
Red Spruce	+6°C	Moderate	Ambient	118.2 (4.5 , 196.0)	1.52 (0.038 , 1.9)	1.36 (0.12 , 1.50)	0.28 (0.013 , 0.16)	0.66 (0.038 , 0.13)		
Red Spruce	+6°C	Drought	Ambient	107.6 (3.3 , 137.0)	1.34 (0.032 , 1.6)	1.09 (0.08 , 1.36)	0.28 (0.015 , 0.21)	0.85 (0.156 , 0.54)		
Red Maple	Baseline	Moderate	Elevated	889.0 (50.2 , 79.0)	10.51 (0.46 , 6.6)	90.43 (7.07 , 43.88)	0.28 (0.025 , 0.17)	0.15 (0.048 , 0.15)		
Red Maple	Baseline	Drought	Elevated	814.3 (31.3 , 37.0)	10.97 (0.46 , 6.0)	91.03 (3.33 , 21.23)	0.30 (0.039 , 0.28)	1.11 (0.211 , 0.70)		
Red Maple	Baseline	Moderate	Ambient	780.7 (42.2 , 72.5)	8.69 (0.29 , 3.4)	74.45 (4.99 , 30.05)	0.25 (0.008 , 0.04)	0.21 (0.052 , 0.18)		
Red Maple	Baseline	Drought	Ambient	566.3 (42.7 , 68.0)	7.42 (0.42 , 5.7)	52.84 (4.08 , 25.41)	0.25 (0.010 , 0.07)	1.42 (0.040 , 0.12)		
Red Maple	+3°C	Moderate	Elevated	886.0 (32.9 , 48.0)	9.81 (0.54 , 7.2)	75.97 (8.86 , 58.00)	0.24 (0.018 , 0.11)	0.09 (0.022 , 0.07)		
Red Maple	+3°C	Drought	Elevated	801.0 (21.0 , 25.0)	10.18 (0.34 , 4.8)	83.46 (3.62 , 24.74)	0.29 (0.009 , 0.06)	1.36 (0.083 , 0.28)		
Red Maple	+3°C	Moderate	Ambient	683.3 (58.3 , 89.5)	7.99 (0.26 , 4.0)	59.41 (4.49 , 30.82)	0.24 (0.007 , 0.05)	0.54 (0.155 , 0.52)		
Red Maple	+3°C	Drought	Ambient	661.7 (30.6 , 49.0)	8.62 (0.21 , 2.7)	60.39 (2.71 , 18.09)	0.29 (0.029 , 0.21)	1.40 (0.265 , 0.90)		
Red Maple	+6°C	Moderate	Elevated	881.7 (31.9 , 46.0)	9.65 (0.39 , 5.7)	89.56 (6.78 , 37.81)	0.24 (0.024 , 0.14)	0.34 (0.061 , 0.21)		

Red Maple	+6°C	Drought	Elevated	675.3 (32.6 , 50.0)	8.99 (0.30 , 5.2)	59.16 (3.74 , 23.38)	0.30 (0.021 , 0.12)	1.57 (0.088 , 0.30)	
Red Maple	+6°C	Moderate	Ambient	769.7 (47.9 , 84.0)	8.95 (0.33 , 4.0)	58.56 (4.89 , 27.71)	0.24 (0.020 , 0.15)	0.61 (0.037 , 0.12)	
Red Maple	+6°C	Drought	Ambient	524.3 (32.1 , 40.0)	7.42 (0.24 , 3.3)	49.88 (1.21 , 7.58)	0.37 (0.018 , 0.10)	1.84 (0.194 , 0.67)	

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