EFFECTS OF DROUGHT SEVERITY ON PHYSIOLOGICAL AND MORPHOLOGICAL RESPONSES TO FUTURE CLIMATE IN JACK PINE (*Pinus banksiana*)

By

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ABSTRACT

Climate change is expected to expose boreal trees to higher atmospheric CO_2, warmer temperatures, and more frequent drought. This study examined how drought severity and future climate affected jack pine (*Pinus banksiana*) seedlings. Seedlings were grown for eight weeks under current climate conditions (600 µmol mol^{-1} CO_2 and current temperature) or future climate conditions (1000 µmol mol^{-1} CO_2 and current temperature +4 °C) and 3 soil moisture conditions: control, moderate drought, and severe drought. Foliar gas exchange was measured under 600 µmol mol^{-1} CO_2 for the current climate treatment and both 600 and 1000 µmol mol^{-1} CO_2 for the future climate treatment in the early, middle, and late stages of the drought treatment. Photosynthetic CO_2-response curves were measured to estimate V_{cmax}, J_{max}, and R_d. Seedling height, root collar diameter, and biomass were measured after the completion of all gas exchange measurements. It was found that V_{cmax} and J_{max} were significantly affected interactively by drought and climate treatments, while R_d was not significantly affected by either treatment. The future climate conditions resulted in downregulations in V_{cmax} and J_{max} in the control and severe drought treatment. Under the moderate drought treatment, however, V_{cmax} was higher under the future climate while J_{max} did not show a significant response to climate treatment. The net photosynthetic rate of the seedlings in the future climate treatment measured at 600 µmol mol^{-1} CO_2 was significantly lower than the corresponding measurement in the current climate treatment in the moderate and severe drought, another indication of photosynthetic downregulation. When measured at the corresponding treatment CO_2, the stomatal conductance and transpiration were significantly lower, but intrinsic water-use efficiency was significantly higher, in the seedlings in the future climate treatment, especially under moderate and severe drought. Drought and climate treatments had no significant overall effects on biomass or morphological traits. Under future climate conditions, seedlings tended to allocate more biomass to leaves and showed a trend toward greater height. Moderate drought tended to shift biomass allocation from aboveground to belowground tissues, whereas severe drought showed a stronger trend toward reduced seedling size and biomass accumulation. Overall, these results indicate that future climate conditions may improve water-use efficiency but cause photosynthetic downregulation and shifts in biomass allocation and accumulation in jack pine.

Keywords: climate change, future climate, elevated CO_2, temperature, drought severity, jack pine, photosynthetic downregulation, biomass allocation, biomass accumulation.

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ABBREVIATIONS

A Net photosynthetic rate

A–Ci curve Photosynthetic CO_2 response curve; relationship between net photosynthetic rate and intercellular CO_2 concentration

AGB Aboveground biomass

BGB Belowground biomass

Ci/Ca Ratio of intercellular to ambient CO_2 concentration

CO_2_r Reference CO_2 concentration inside the gas-exchange chamber

E Transpiration rate

ef Effect value; percentage change in the future-climate treatment relative to the current-climate treatment

FC Field capacity

gsw Stomatal conductance to water vapour

iWUE Intrinsic water-use efficiency

J_{max} Maximum rate of electron transport supporting RuBP regeneration.

LMR Leaf mass ratio

PAR Photosynthetically active radiation

R_d Mitochondrial respiration rate in the light

RH Relative humidity

RMR Root mass ratio

RSR Root-to-shoot ratio

SMR Stem mass ratio

TDB Total dry biomass

V_{cmax} Maximum rate of Rubisco carboxylation

VPD Vapour pressure deficit Ψx（P）

Midday Xylem water potential

A600 Photosynthesis of seedlings measured at 600 μmol mol^{-1} CO_2 for both climate conditions

INTRODUCTION

Elevated CO2-induced climate change, particularly increases in global temperature, is placing growing environmental stresses on trees. Since the Industrial Revolution, atmospheric CO_2 concentration has increased from approximately 280 μmol mol^{-1} to more than 410 μmol mol^{-1}, representing an increase of about 45% (Dusenge et al., 2019). By the end of this century, atmospheric CO_2 concentration may reach between 550 and 1000 μmol mol^{-1} (Ciais et al., 2013). As the concentrations of CO_2 and other greenhouse gases continue to rise, the rise in global temperatures will also continue (Dusenge et al., 2019). According to the IPCC (2023), global mean temperature is expected to reach 1.5 °C above the pre-industrial level between 2021 and 2040. At the same time, climate change can lead to changes in regional precipitation patterns and increases in the frequency and severity of droughts (Spinoni et al., 2020; Bose et al., 2020), particularly in high-latitude regions (Hao et al., 2013). As ongoing global change continues, the frequency and severity of soil drought events represent a major challenge to plant physiological processes and broader socio-ecological systems, especially in boreal forest ecosystems (Tripathy et al., 2023).

Boreal forests account for approximately 30% of the global forested areas (Brandt et al., 2013) and play an important role in regulating the global carbon cycle (Bradshaw & Warkentin, 2015). However, the effects of extreme climate events on Canadian boreal forests and the forestry sector are still not well understood (Gauthier et al., 2014). The responses of forest plants to climate change in mid- and high-latitude regions can be either positive or negative (Reich et al., 2022). Warming temperatures may initially increase tree growth and forest productivity in the boreal forest. However, recent studies suggest that climate warming and associated soil moisture deficits may negatively affect boreal conifers in the long run (Marchand et al., 2020). Forest productivity across northern North America is predicted to decline as warming-induced soil drought intensifies (Beck et al., 2011). Because tree physiological responses to soil drought directly govern carbon uptake and survival, understanding these mechanisms is essential for developing proactive forest-management strategies that reduce the negative impacts of climate change on boreal ecosystems (Gauthier et al., 2015).

Elevated atmospheric CO_2 can increase tree growth by increasing photosynthetic rate and water-use efficiency (Habermann et al., 2019; Li et al., 2024). Elevated temperatures can also

increase photosynthesis in some tree species at boreal and temperate latitudes (Saxe et al., 2001). However, higher temperature and elevated CO_2 can interactively affects tree photosynthesis and other physiological processes. Because these two drivers occur simultaneously under climate change, their combined effects cannot be predicted accurately by examining either factor alone (Newaz et al., 2017). It remains unclear whether climate change will strengthen or weaken the ability of trees to acclimate to drought stresses because relatively few studies have examined the combined effects of warming and elevated CO_2 on tree responses to soil-moisture conditions.

Drought stress affects a wide range of physiological and morphological processes in plant. Trees may have the ability to acclimate to drought morphologically and physiologically if the drought treatment or drought spell is long enough (Mencuccini, 2003). Soil drought reduces moisture availability in the root zone, forcing stomatal closure to prevent water loss, which directly decreases photosynthetic carbon assimilation (Adams et al., 2009). As soil water availability declines, xylem water potential becomes more negative and stomatal closure reduces water loss. This response decreases stomatal conductance and transpiration but also restricts CO_2 diffusion into leaves, potentially reducing net photosynthetic rate (Chaves et al., 2003). If stomatal conductance declines proportionally more than carbon assimilation, intrinsic water-use efficiency may increase. With increasing drought severity and/or duration, photosynthetic limitation may extend beyond stomatal regulation to biochemical processes, including Rubisco carboxylation and electron transport capacity. At the whole-plant level, reduced carbon assimilation can limit tree growth and biomass accumulation and alter biomass allocations among leaves, stems, and roots. Drought commonly promotes greater relative biomass allocation to roots, which may improve access to soil water and help plants maintain their water balance (Zhang et al., 2020). Under severe and prolonged soil water deficits, these persistent physiological stresses elevate the risk of hydraulic failure and carbon depletion, substantially increasing drought-related tree mortality (Allen et al., 2010; Ma et al., 2023). However, the strength and direction of these responses depend on drought severity and tree species (Eziz et al., 2017). Tree responses to drought also vary with species and the frequency and duration of droughts (Reich et al., 2022). For instance, while Scots pine is a relatively drought-tolerant species, may not be able to survive increasingly more frequent and prolonged drought events (Bose et al., 2020).

Jack pine (Pinus banksiana Lamb.) is an ecologically dominant pioneer species and the second most commercially valuable conifer in eastern North American boreal forests, playing a pivotal role in post-fire forest regeneration and timber supply (Moulinier et al., 2015). Because of its wide geographic distribution across dry and infertile sites, understanding its physiological responses to future climate scenarios is critical for predicting the carbon sink capacity and resilience of boreal forest ecosystems (Newaz et al., 2017). Jack pine is sensitive to environmental changes (Wang et al., 2025). Previous exposure to drought may also increase the survival of jack pine seedlings when they are exposed to subsequent and more severe drought conditions (Mayne et al., 1994). Its relatively small leaf mass ratio, relatively large root mass ratio, efficient stomatal control of water loss, and resistance to xylem cavitation may partly explain this relative drought tolerance (Blake & Li, 2003). Jack pine may also dehydrate more slowly than some other conifers, which may help protect cellular structures and maintain physiological function during drought (Pallardy et al., 1995). The use of internally stored water may provide an additional short-term buffer against water deficits, although the importance of stem water storage differs among boreal tree species (Thivierge-Lampron et al., 2025). Jack pine that maintains better growth under water stress may show stronger osmotic adjustment, more effective stomatal regulation, and higher net photosynthetic rates (Blake & Yeatman, 1989). However, these adaptations may not completely prevent hydraulic or photosynthetic limitations. As trees become taller, the increasing distance over which water must be transported may increase hydraulic limitation and the risk of xylem cavitation during drought (Olson et al., 2018). Therefore, the relative drought tolerance observed in jack pine seedlings cannot necessarily prevent reductions in physiological function and growth when drought becomes sufficiently severe or is combined with other climatic stresses.

This study aimed to investigate how future climate conditions, represented by the combination of elevated CO_2 and warmer temperature, affect the physiological, and morphological responses of jack pine seedlings to drought stresses of different severity. Tree responses to the combination of elevated CO_2, warming, and drought likely depend on the balance among several processes. Elevated CO_2 may increase the supply of CO_2 photosynthesis and allow seedlings to maintain carbon assimilation while reducing stomatal conductance and transpiration. This response may increase intrinsic water-use efficiency and partially reduce the effects of water limitation. In contrast, warming may increase evaporative demand and intensify

the effect of soil drought. With prolonged exposure, elevated CO_2 and warming may also cause photosynthetic acclimation, resulting in a reduction in biochemical photosynthetic capacity. Such downregulation may be indicated by reductions in the maximum rate of Rubisco carboxylation (V_{cmax}), the maximum rate of electron transport (J_{max}), or net photosynthetic rate when plants grown under different CO_2 concentrations are measured at a common CO_2 concentration. At the whole-plant level, sustained reductions in carbon gain may decrease biomass accumulation, whereas drought may shift biomass allocation toward roots. A better understanding of these connected physiological and morphological responses will improve predictions of how jack pine and other boreal conifers may respond to future climate conditions (Wang & Wang, 2023). In this thesis, I tested the following hypotheses: the photosynthesis and biomass accumulation of jack pine will decline in response to drought, and the degree of the decline will increase with increases in drought severity; Future climate conditions, as represented by the combination of elevated CO2 and warmer air temperature, will increase intrinsic water-use efficiency by decreasing stomatal conductance, but will induce photosynthetic downregulation. Under future climate conditions, different drought severities will cause different degrees of reduction in photosynthesis capacity, stomatal conductance, transpiration, and intrinsic water-use efficiency.

MATERIAL AND METHODS

Plant materials

Pinus banksiana Lamb. (Jack pine) seeds were collected from a jack pine stand west of Thunder Bay within the Lakehead Forest region (PJ Greenmantle 2024, Seed Zone 13). The seeds were stratified by first being soaked in deionized water for 24 h and then placed in a refrigerator at 4 °C for 14 days (Qualtiere, 2008). After the stratification, the seeds were germinated in germination trays with a mixture of peat moss and vermiculite as the growing medium in the Lakehead University greenhouse. The germination was completed in 4 weeks when 85% of the seeds germinated. Seedlings of relatively uniform size were transplanted individually into circular pots (14 cm height × 13 cm diameter) filled with a mixture of peat moss and vermiculite (2:1, v/v). The pots were then transferred to treatment greenhouses.

In the initial two weeks of the treatments, seedlings were irrigated once a week with a solution of conifer starter fertilizer 11-41-8(100.1mg/L N, 373.1mg/L P, 72.8mg/L K). Starting from the third week, when seedlings reached approximately 2–3 cm in height, fertilizer was switched to a conifer growth fertilizer (20-8-20, N–P–K), concentrations were started at 100mg/L N, 40mg/L P, 100mg/L K twice weekly for three weeks and gradually increased to 200mg/L N, 80mg/L P, 200mg/L K until to the end of treatment period (Dang et al., 2025).

Experimental design

The study used a split-plot design to investigate the interactive effects of climate conditions and drought. Whole plots were climate conditions (CO_2 and temperature combinations): current climate condition- 600 µmol mol^{-1} CO_2 and current temperature of the seed source vs. future climate condition-1000 µmol mol^{-1} CO_2 and current +4 °C temperature. The climate treatments were implemented in four greenhouses (two replicates for each treatment). There were three soil-moisture treatments within each climate treatment replicate as split plots: control (80–90 % field capacity (FC)), moderate drought (55–65 % FC), severe drought (25–35 % FC). Eight seedlings of relatively uniform size were assigned to each treatment combination, resulting in a total of 96 seedlings for the study (2 climate condition × 3 moisture × 8 seedlings × 2 replicates). The 8-week drought treatment period commenced 140 days after the start of the climate treatments.

The volumetric soil moisture content was continuously monitored using ML2X Theta Probes (Delta-T Devices, Cambridge, UK) and a Campbell Scientific 1000X datalogger (Campbell Scientific, Edmonton, Canada) connected to a PC via the Campbell Scientific PC400 Datalogger Support Software. When soil moisture content dropped below the lower limit of the target range, pots were irrigated with a calculated volume of nutrient solution to restore soil moisture to the upper limit of the treatment target range.

Greenhouse conditions

The future CO_2 level was maintained using CO_2 generators (GEN-2E; Custom Automated Products Inc., USA). Current temperatures were set based on the 10-year weekly average air temperatures from Thunder Bay, simulating the daily and seasonal temperature patterns. Each day, the temperature was ramped at 8 set points to emulate the diurnal cycle. The temperature in the future climate treatment was the current temperature +4 °C. The temperature and photoperiod settings were adjusted weekly to simulate the seasonal variation.

In the greenhouse, environmental factors including CO_2, temperature, humidity, and photoperiod were automatically controlled and recorded by an Argus Titan Environmental Control System (Argus Control Systems Ltd., Canada). Stirring fans ensured even air distribution. High-pressure sodium lamps (P.L. Systems) provided around 600 μmol m^{-2} s^{-1} PAR at seedling canopy level to supplement natural light when day length was too short, or PAR fell below 500 μmol m^{-2} s^{-1}.

Physiological Measurements

A/Ci response curves

After six weeks of drought treatment, the photosynthetic CO_2 response curves of three seedlings randomly selected from each drought treatment were measured using an open gas-exchange system equipped with a conifer chamber (LI-6800 Portable Photosynthesis System; LI-COR Biosciences, Lincoln, NE, USA). For seedlings grown under the future climate condition, chamber CO_2 concentration (CO_2_r) was set sequentially to 1000, 900, 800, 700, 600, 500, 400, 300, 200, 100, 30, 80, 120, 220, 320, 420, 520, 620, 720, 820, 920, and 1020 μmol mol^{-1}. For seedlings grown under the current climate treatment, chamber CO_2 concentration was set sequentially to 600, 500, 400, 300, 200, 100, 30, 80, 120, 220, 320, 420, 520, 620, 720, 820, 920, and 1020 μmol mol^{-1}. CO_2 response curves were recorded using the LI-6800 auto-log program,

with a flow rate of 400 μmol s^{-1}. The raw ACi curve data were first cleaned by removing obvious outliers to reduce potential bias. The response curves of the cleaned data were then fitted using the FvCB model to estimate V_{cmax}, J_{max}, and R_d. The normality and homogeneity of variance were assessed for V_{cmax}, J_{max}, and R_d using the Shapiro–Wilk test and Levene's test, respectively.

Gas exchange point measurements

Gas exchange measurements were repeated in the following three stages of the 8-week drought treatments: early (week 1), middle (week 4), and late (week 8). Each stage included one week of data collection, with a three-week interval between stages. For each measurement cycle, three seedlings were randomly selected from each treatment combination. Measurements were conducted between 09:00 and 12:00 when gas exchange parameters were found to be relatively stable. For the future climate condition treatment (Future climate condition, FCC), the measurements were taken at 1000 μmol/mol and 600 μmol/mol CO_2_r (A-C1000 and A-C600). For seedlings grown under the current climate condition (Current climate condition, CCC), the measurements were taken at 600 CO_2_r (A-C600). Other measurement conditions were 1000 μmol m^{-2} s^{-1} photosynthetically active radiation (PAR) (above the measured light saturation point at 800 μmol m^{-2} s^{-1}), 25 °C air temperature, and 50% RH (Newaz et al., 2017).

Xylem water potential point measurements

At the end of the 8-week experiment, midday xylem water potential (P) was measured between 13:00 and 15:00 using a Scholander-type pressure chamber (PMS Instruments, Corvallis, OR USA) on all the 92 remaining seedlings.

Morphological Measurements

At the end of the 8-week combined treatment, the height and root collar diameter of all remaining seedlings were measured before the xylem water potential measurements. The roots were carefully washed to remove soil. The aboveground and belowground parts were then placed into labeled paper bags and temporarily stored in a refrigerator. After water potential measurement, all the seedling organs were dried in a drying oven at 75 °C for 48 h. The biomasses of roots, needles, and stems were weighed separately.

Biomass allocation was also assessed using root-to-shoot ratio (RSR = BGB / AGB), leaf mass ratio (LMR = dry leaf biomass / TDB), stem mass ratio (SMR = dry stem biomass / TDB), and root mass ratio (RMR = dry root biomass / TDB).

Leaf area determination

The needles for the gas exchange measurements were scanned using an Epson Expression 10000XL scanner (Epson America Inc., Los Alamitos, CA, USA), and the projected leaf area was then determined from the scanned images using ImageJ software.

Data analysis

The ACi curve data were analyzed using the FvCB model in R according to Duursma (2015), to get estimates of the maximum rate of Rubisco carboxylation (V_{cmax}), the maximum rate of electron transport (J_{max}), and the rate of mitochondrial respiration in the light (R_d). Before statistical analysis, V_{cmax}, J_{max}, and R_d were each tested for normality using the Shapiro–Wilk test and for homogeneity of variance using Levene's test. Since all three variables met the assumptions of normality and homogeneity of variance, two-way ANOVA was performed to test the effects of drought, climate, and their interaction. Prior to the main were significant at $P < 0.05$, Tukey's HSD post-hoc pairwise comparisons were conducted to identify the specific treatment combinations that differed significantly. If no significant heterogeneity was detected among replicate individuals within the same drought treatment, nonlinear trend fitting was performed for each treatment group using the geom_smooth() function in the ggplot2 package in R. The final figures used smoothed trendlines to represent the average response pattern of each treatment group.

Due to the random selection of seedlings at each measurement period, only a subset of individuals was measured across all three periods, resulting in an unbalanced repeated-measures dataset. Climate and drought treatments were treated as fixed effects, while measurement period and greenhouse were included as random effects. Descriptive statistics were first used to examine within-treatment variation across measurement period. No extreme within-group outliers were identified; therefore, all biologically valid observations were retained for subsequent analyses. Two planned comparisons were then conducted. The net photosynthetic rate for two climate treatments was compared to evaluate physiological acclimation to the future climate treatment when gas exchange was measured at a common CO_2 concentration of 600

μmol mol^{-1}. All gas exchange traits measured at corresponding CO_2 concentration to evaluate the actual gas-exchange performance. To accommodate the unbalanced repeated-measures structure and retain all available observations, linear mixed-effects models were used to analyse net photosynthetic rate (A), transpiration rate (E), stomatal conductance (gsw), intrinsic water-use efficiency (iWUE), and the ratio of intercellular to ambient CO_2 concentration (Ci/Ca). Gas-exchange traits measured at the CO_2 concentration corresponding treatment, were visualized using the ggplot2 package in R. For ease of visualization and interpretation, the effects of climate were plotted separately within each drought treatment, and the effects of drought were plotted separately within each climate treatment.

The Levene's test showed that the morphological variables and their log transformations generally did not meet the ANOVA assumptions of normality and homogeneity. Therefore, generalized mixed linear model with a Gamma distribution (GLMM-Gamma) and linear mixed-effects model (LMM), instead of ANOVA were used according to the distributional characteristics of each variable.

The GLMM-Gamma was used to test treatment effects on biomass and morphological traits. For ratio-based variables and midday water potential (P), LMMs were used due to the heterogeneity of variance. When the effect or interaction was significant, post-hoc pairwise comparisons were performed. Principal component analysis (PCA) was performed using the PCA() function in the FactoMineR package in R to investigate the interrelationships among different morphological and physiological variables in different treatments.

RESULTS

Physiological Traits

Drought and climate treatments interactively affected V_{cmax} and J_{max} (Table 1). While V_{cmax} and J_{max} were both generally lower under the future climate conditions in the control and the most severe drought treatment, the trends were the reverse in the intermediate drought treatment, and not all the differences were statistically significant (Figures 1a & 1b). The drought effects on V_{cmax} and J_{max} were different in the two different climate treatments: Under the current climate treatment, V_{cmax} and J_{max} were significantly lower in the intermediate drought than under the other two drought treatments; drought treatments had no significant effects on V_{cmax} and J_{max} in the future climate treatment, although they appeared to be greater in the intermediate drought treatment (Figures 1a & 1b).

None of the treatments had significant effects on R_d (Table 1). However, R_d appeared to have declined with increasing drought severity under the current climate conditions (Figure 1c).

Table 1. Two-way ANOVA results (p values) on the effects of drought, climate condition, and their interactions on V_{cmax}, J_{max} and R_d. The values in bold text are significant at <0.05.

Response variable	Drought	Climate	Drought*Climate
Vcmax	**0.030**	**0.020**	**0.003**
Jmax	**0.015**	**0.002**	**0.001**
Rd	0.825	0.915	0.758

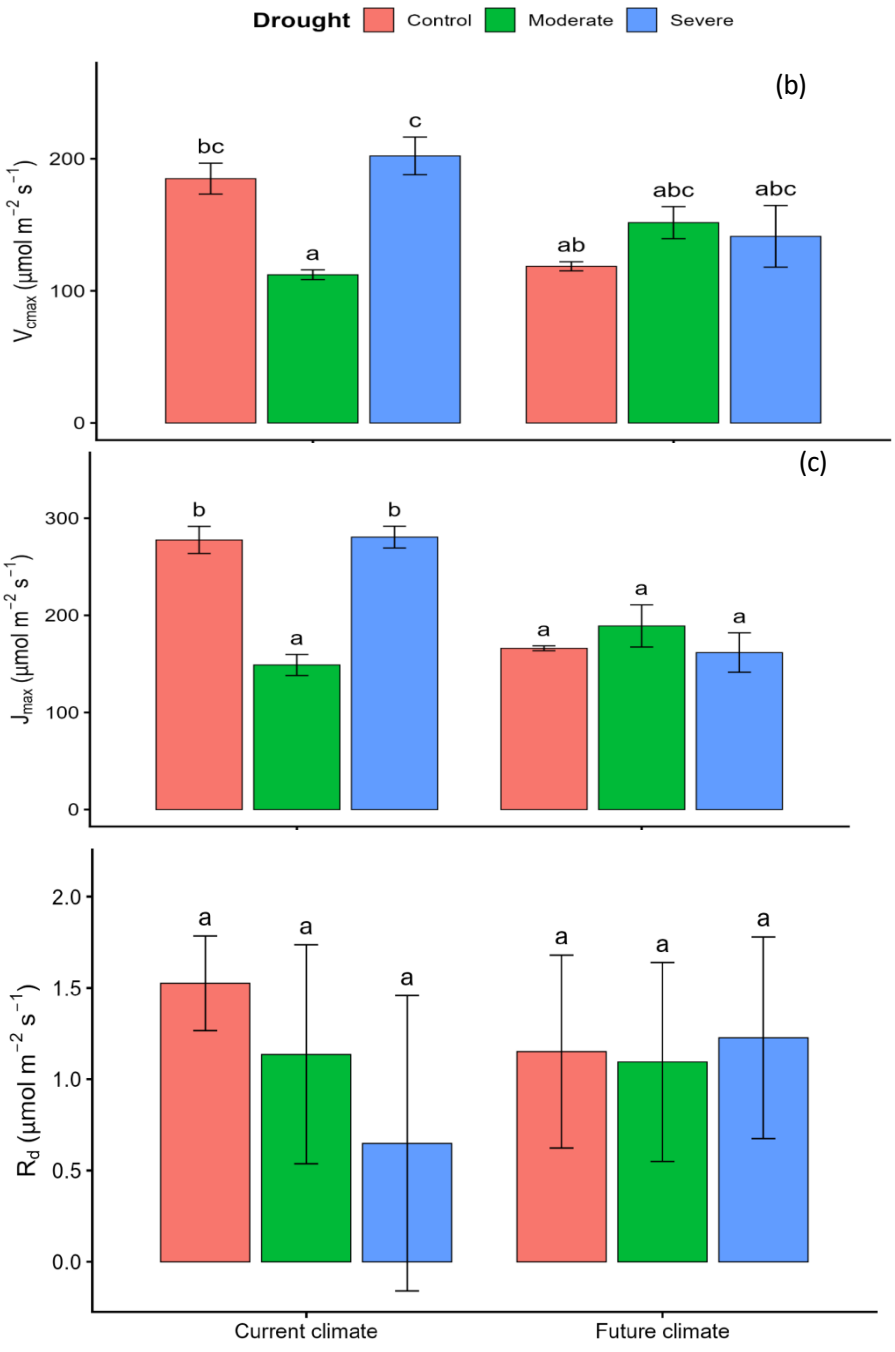


Figure 1. Effects of drought severity and climate conditions on the photosynthetic biochemical photochemical parameters of jack pine (*Pinus banksiana*): (a) maximum Rubisco carboxylation rate (V_{cmax}), (b) maximum electron transport rate (J_{max}), and (c) dark respiration rate (R_d). Bars represent means ± SE. Means sharing at least one letter were not significantly different from each other ($p > 0.05$).

None of the treatments or their interactions significantly affect the rate of photosynthesis measured under the corresponding treatment CO_2 ($P > 0.05$) (Table 2). The rate measured under the same CO_2 for both climate treatments was significantly affected by both treatments and their interaction in the late stage of the drought treatments ($P < 0.01$, Figure 2): it was lower in the future than the current climate treatment (not statistically significant in the control treatment) and the net effects were greater in the moderate than the severe drought treatment (-43.61 % vs. -37.94 %, Table 3, Figure 2a); it was is significantly higher in the moderate drought than the control under the future climate treatment in middle stage (107.70 %)(Table 4).

Table 2. LMM results for photosynthetic rate (A) measured under the corresponding treatment CO_2 concentration during the middle and late stages of drought treatment. Note: A represents net photosynthetic rate measured under treatment CO_2. 'ns' indicates no statistically significant effect ($P > 0.05$).

Trait	Period	Effect	*F*-value	*P*-value	Significance
A	Middle	Climate	1.2270	0.2718	ns
		Drought	0.2420	0.7855	ns
		Climate × Drought	0.8330	0.4389	ns
	Late	Climate	0.2940	0.5897	ns
		Drought	0.1500	0.8612	ns
		Climate × Drought	0.1950	0.8229	ns

Table 3. Effects of climate treatment on gas-exchange traits under different drought treatments and at different stages of the drought treatments. Note: A600 is the photosynthetic rate measured at 600 µmol mol^{-1} CO_2 for both climate treatments, and all other parameters were measured under the corresponding treatment CO_2 (i.e., 600 for the current and 1000 for the future climate conditions): transpiration rate (E), stomatal conductance to water (gsw), intrinsic water-use efficiency (iWUE). "ef" represents the net effect of the future climate. Only statistically significant comparisons are presented. Significance levels are indicated as * ($P < 0.05$), ** ($P < 0.01$), and *** ($P < 0.001$).

Trait	Drought	Period	ef	p.value	Significance
A600	Moderate	Late	-43.61%	0.00275	**
A600	Severe	Late	-37.94%	0.00978	**
E	Moderate	Late	-54.18%	0.001179	**
E	Severe	Late	-40.52%	0.015293	*
gsw	Moderate	Late	-57.60%	5.66E-04	***
gsw	Severe	Late	-43.86%	0.00964	**
iWUE	Moderate	Middle	67.00%	0.012235	*
iWUE	Severe	Middle	111.40%	2.68E-06	***
iWUE	Control	Late	67.87%	0.003861	**
iWUE	Moderate	Late	103.48%	2.68E-05	***
iWUE	Severe	Late	74.76%	0.001613	**

Table 4. Effects of drought treatment on gas-exchange traits under different climate treatments and at different stages of moderate drought treatment. Note: A600 represents the net photosynthetic rate measured at a common CO_2 concentration of 600 µmol mol^{-1} under both climate treatments, whereas intrinsic water-use efficiency (iWUE) was measured at the corresponding treatment CO_2 concentration. "ef" represents the net effect of severe drought. Only statistically significant comparisons are presented. Significance levels are indicated as * ($P < 0.05$), ** ($P < 0.01$), and *** ($P < 0.001$).

Trait	Climate	Period	ef	p.value	Significance
A600	Future climate	Middle	107.70%	0.0093	**
iWUE	Future climate	Middle	40.59%	0.0375	*

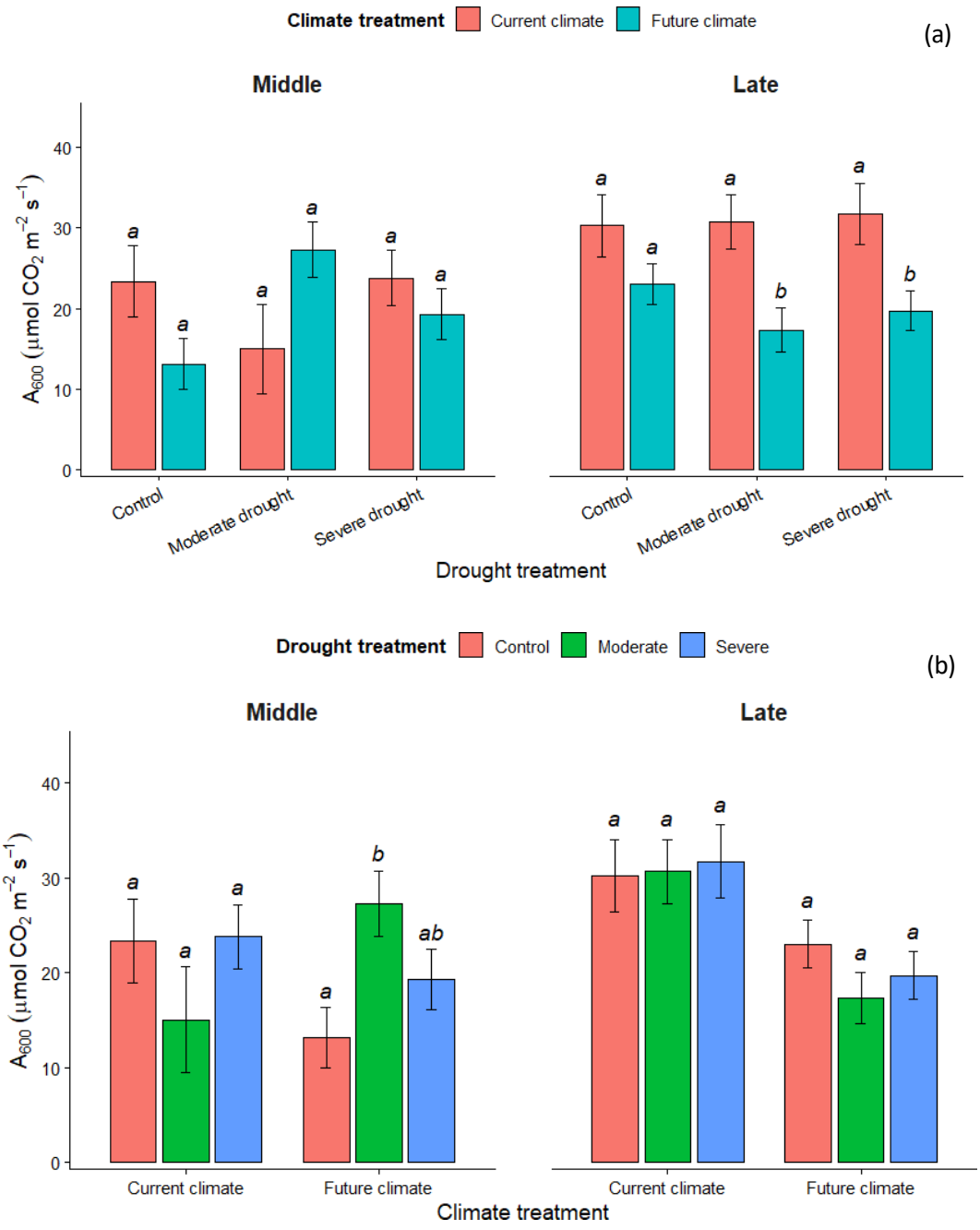
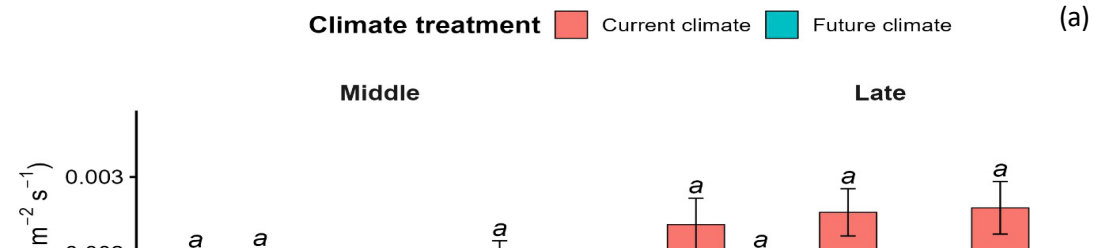


Figure 2. Effects of climate and drought treatments on net photosynthetic rate measured at a common CO_2 concentration of 600 µmol mol^{-1} (A600) during the middle and late stages of drought treatment. (a) Comparisons between current and future climate treatments within each drought treatment. (b) Comparisons among drought treatments within each climate treatment. Bars represent means ± SE. Within each treatment stage and comparison group, means sharing at least one lowercase letter are not significantly different (P>0.05)

Neither climate condition nor any drought treatments significantly affected transpiration rate and stomatal conductance in the middle stage of drought treatments (Figures 3a & 3b). During the late drought treatment stage, however, both were lower in the future than current climate conditions in all the drought treatments, but the differences were not statistically significant in the control treatment (Table 3; Figures 3a & 3b).

In contrast, iWUE was generally greater under the future than under the current climate treatment in the middle and late stages of the drought treatments, but difference was not statistically significant in the control during the middle stage of the drought treatment (Table 3; Figure 3c). Within the future climate treatment, iWUE was significantly higher under severe drought than in the control during the middle stage (Table 4; Figure 4). iWUE tended to increase with increasing drought severity in the future climate conditions in the middle stage of the drought treatments.



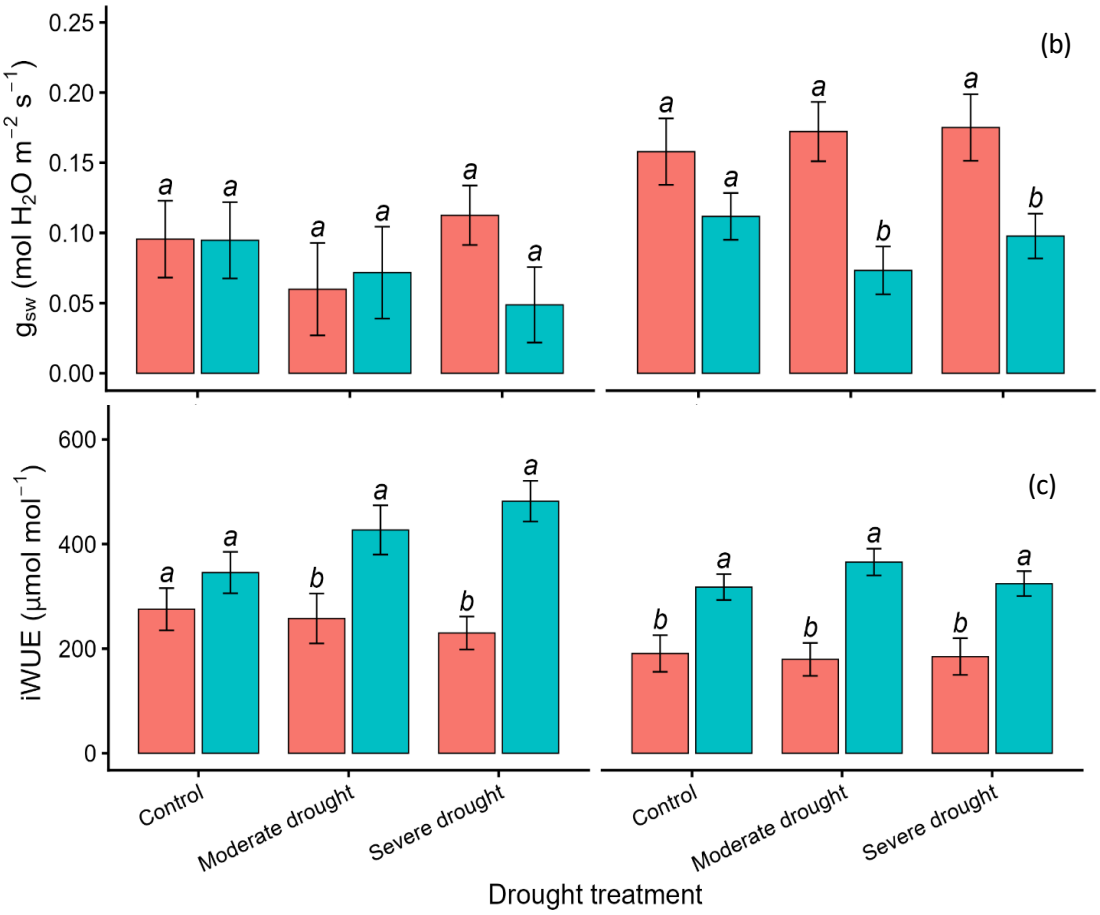


Figure 3. Effects of climate conditions on (a) transpiration rate (E), (b) stomatal conductance (gsw), and (c) intrinsic water-use efficiency (iWUE) in the middle and late stages of the drought treatments. Bars (means ± SE) with the same lower-case letter at the same treatment stage were significantly different from each other ($P<0.05$).

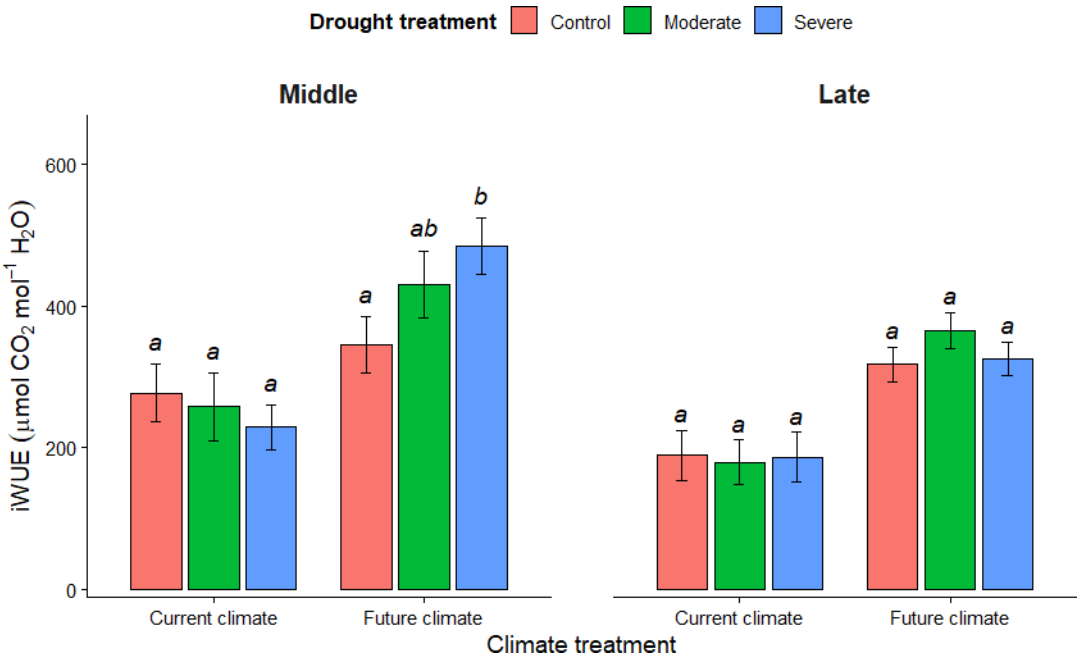


Figure 4. Effects of severe drought treatment on intrinsic water-use efficiency (iWUE) under climate conditions during the middle and late stages of drought treatment. Measurements were conducted at the corresponding treatment CO_2 concentration. Bars represent means ± SE. Different lowercase letters within the same climate treatment and measurement period indicate a significant difference ($P<0.05$).

Morphological Traits

GLMM and LMM analyses did not show any significant effects of drought, climate, or their interactions on any biomass component or biomass ratio ($P > 0.1$). However, PCA revealed coordinated treatment-associated patterns across the measured morphological, biomass, allocation, and midday xylem water-potential traits (Fig. 5). PC1 and PC2 explained 48.7% and 17.4% of the total variance, respectively, accounting for 66.1% cumulatively. PC1 represented overall seedling size and biomass accumulation, whereas PC2 primarily described variation in biomass allocation. The current climate and future climate observations overlapped extensively, indicating no clear multivariate separation between the climate treatments (Fig. 5a). Seedlings in the future climate treatment were distributed more toward negative PC2 scores, in the directions

of LMR, height, and LB, suggesting those seedlings tended to have greater leaf allocation and height relative to root allocation and root collar diameter.

The drought-treatment groups also overlapped but differed in their positions and dispersion (Fig. 5b). Moderate-drought seedlings were distributed more toward positive PC2 scores, in the directions of RMR, RSR, P, BGB, and diameter, suggesting a tendency toward relatively greater belowground allocation. Severe-drought seedlings formed the smallest and most compact ellipse and were concentrated mainly on the negative side of PC1. This distribution indicates generally smaller seedlings, lower biomass accumulation, and less within-treatment variation in the measured traits under severe drought.

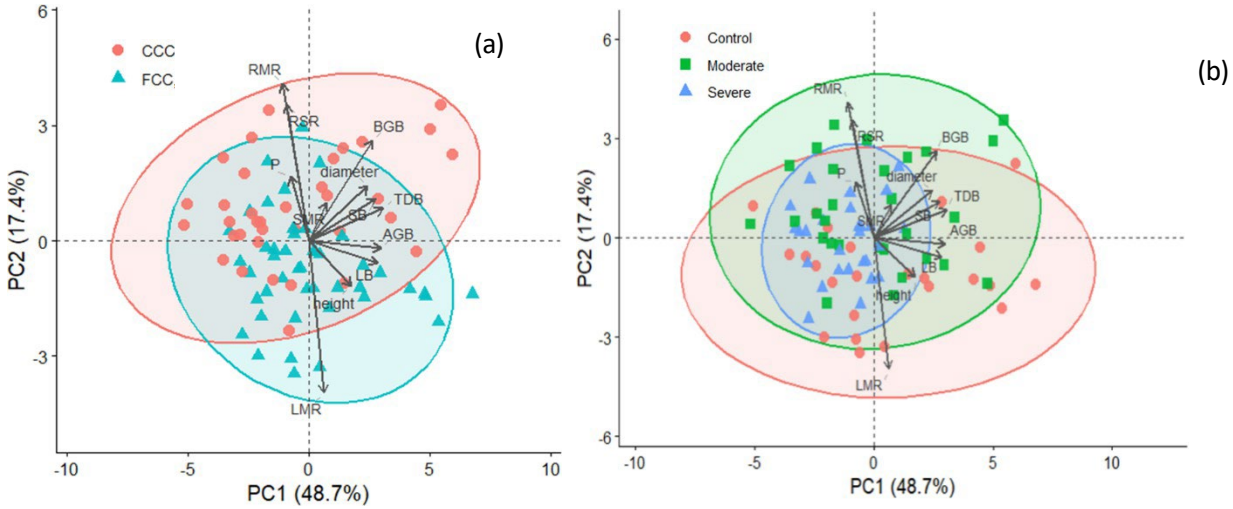


Figure 5. Principal Component Analysis (PCA) on morphological traits under the Current Climate Conditions (CCC) and Future Climate Conditions (FCC) (a) and Control, Moderate, and Severe drought treatments (b). The arrows represent the loadings of morphological, biomass and midday xylem water potential. Points represent individual plant samples, with different colors and shapes denoting corresponding treatment groups. AGB, aboveground biomass; BGB, belowground biomass; TDB, total dry biomass; LMR, leaf mass ratio; SMR, stem mass ratio; RMR, root mass ratio; RSR, root-to-shoot ratio; P, midday xylem water potential; LB, leaf biomass; SB, stem biomass; RB, root biomass.

DISCUSSION

The results of this study suggest that future climate conditions can lead to substantial downregulation of photosynthetic capacity (>40%) in jack pine, as indicated by lower V_{cmax} and J_{max}. This finding is consistent with previous studies showing that prolonged exposure to elevated CO_2 can result in photosynthetic acclimation and reductions in photosynthetic capacity (Ainsworth & Rogers, 2007; Mosseler & Major, 2020). Similar responses have also been reported in boreal tree species. For example, Zhang and Dang (2005) found that elevated CO_2 reduced V_{cmax} and resulted in photosynthetic downregulation in jack pine after several months of treatment. Dang et al. (2021) also reported reductions in V_{cmax}, and under some conditions J_{max}, in black spruce and white spruce exposed to elevated CO_2. These findings suggest that the reductions in V_{cmax} and J_{max} observed in the present study are consistent with photosynthetic acclimation to prolonged CO_2 enrichment reported previously in boreal trees. Shorter term exposure (a few days or weeks) to elevated CO_2, in contrast, can directly and sharply increase net photosynthetic rate by stimulating carbon simulation before photosynthetic acclimation occurs (Ainsworth & Long, 2005). The effects of warming on photosynthetic capacity can vary depending on warming intensity, water availability, species, and exposure duration. Moderate warming may increase V_{cmax} and J_{max} under favorable conditions (Niu et al., 2008), whereas stronger warming can reduce V_{cmax}, J_{max}, and net photosynthetic rate in boreal tree species. Photosynthetic capacity is reduced by warming but unaffected by elevated CO_2 in seedlings of five boreal tree species (Hammer et al., 2025). The results that the future-climate treatment in this study, i.e., a combination of elevated CO_2 and warmer temperature, led to significant declines in both V_{cmax} and J_{max} indicate that elevated CO_2 was the more dominant factor influencing photosynthetic machinery than warmer temperature. This finding further emphasizes the importance of considering multiple factors together if their changes occur together because the combined effects cannot be derived from those of individual factors (Newaz et al., 2017; Olugbadieye et al., 2025). Similar results on other environmental factors have been reported for other boreal tree species (Stinziano and Way, 2014). Furthermore, one factor can modify the tree's response to other factors. For instance, Hammer et al. (2025) have found that elevated CO_2 increased the optimum temperature for photosynthesis by approximately 2 °C in four North American conifer tree species.

This shows that the response of jack pine to drought can vary with drought severity and climate conditions. The moderate drought treatment in this study reduced both V_{cmax} and J_{max} under the current climate but had no significant effect in the future climate treatment. Under the current atmospheric CO_2 concentration, Rubisco is considered one of the main limitations to net photosynthetic rate (A) (Scafaro et al., 2017). Therefore, moderate water deficit may have reduced the biochemical photosynthetic capacity of jack pine. The significant decline in J_{max} also indicates a reduction in maximum electron transport and RuBP regeneration capacity under moderate drought (Zhou et al., 2014). This finding is consistent with Li et al. (2024), who reported that drought significantly reduced photosynthetic performance in *Pinus massoniana.* However, the effects of moderate drought on V_{cmax} and J_{max} differed between the current and future climate treatments, indicating an interactive effect of drought and climate conditions. Drought induces stomatal closure in pine species, which helps limit water loss (Martínez-Vilalta et al., 2004; Tardieu & Simonneau, 1998), while elevated CO_2 may maintain sufficient CO_2 supply even when stomata are partly closed (Costa et al., 2022). Drought may also relieve sink limitation and reduce the need to decrease Rubisco enzyme content, thereby maintaining V_{cmax} and J_{max} at relatively high levels; similar mechanisms have been discussed in previous studies (Zheng et al., 2019; Silveira-Marenco et al., 2023). However, the present results cannot confirm whether this response was directly caused by changes in Rubisco content, enzyme activity, or resource reallocation. Future studies should measure leaf nitrogen concentration, Rubisco content, and other biochemical traits to test these possible mechanisms (Wang et al., 2023). Interestingly, severe drought did not significantly reduce either V_{cmax} or J_{max}, indicating that the biochemical response did not increase linearly with drought severity (Martin-Stpaul et al., 2012). This may suggest that other limitations to photosynthesis, including stomatal limitation, became relatively more important under severe drought (Kenzo et al., 2021; Zhou et al., 2015), although the stomatal conductance measurements in this study did not provide direct evidence for this mechanism. In contrast, under severe drought, both V_{cmax} and J_{max} were significantly lower under future climate than under current climate. This result indicates strong photosynthetic downregulation under future climate when seedlings experienced severe water limitation. Similar drought-related photosynthetic downregulation has also been reported in *Pinus massoniana* (Li et al., 2024). Under some combinations of drought treatment, climate, and measurement stage, when future-climate seedlings were measured at the current atmospheric CO_2 concentration, their

net photosynthetic rate (A) was significantly lower than that of seedlings grown under current climate, confirming a reduction in photosynthetic capacity. In comparison, when future-climate seedlings were measured at their growth CO_2 concentration of 1000 μmol mol^{-1}, their net photosynthetic rate did not differ significantly from that of current-climate seedlings measured at their own growth CO_2 concentration, suggesting that actual photosynthetic performance remained relatively stable. Similar responses have also been reported in black spruce (Bigras and Bertrand, 2006) and tamarack (Murphy and Way, 2021).

The results of this study show that the responses of jack pine to future climate conditions varied with soil moisture conditions and the duration of drought stresses and that the responses of jack pine to droughts varied with climate conditions. We exposed jack pine seedlings to 8 weeks of drought stress with different severity. The seedlings in the future climate treatment started showing lower stomatal conductance and transpiration rates than those in the current climate treatment in the late stage of the moderate and severe drought treatments while the intrinsic water use efficiency showed opposite trends, i.e., higher under the future climate than the current climate conditions. Future climate condition induced stomatal closure is also reported for Aleppo pine (Ainsworth and Rogers, 2007). The iWUE of jack pine also responded to drought treatments differently in the different climate treatments. Under the current climate, iWUE showed no significant response to increases in drought severity. In the future climate treatment, in contrast, iWUE increased with increasing drought severity in the middle stage of the drought treatments but the differences disappeared in the late stage of the treatment. A higher iWUE may help seedlings reduce the effects of water limitation (Dietrich et al., 2016). Elevated CO_2 can reduce stomatal conductance, while warming and elevated CO_2 may have opposing effects on water-use efficiency in C_3 plants (Xu et al., 2013).

The PCA results suggest that jack pine seedlings tended toward greater relative leaf allocation and height under future than current climate conditions. Previous studies have also reported that soil water stress may have relatively limited effects on leaf biomass in some *Pinus* and *Picea* species (Major and Mosseler, 2019). The tendency toward greater leaf allocation may reflect short-term carbohydrate accumulation in leaves rather than increased investment in photosynthetic tissues. Elevated CO_2 can initially increase carbon assimilation faster than newly assimilated carbohydrates can be transported to or utilized by sink organs, resulting in carbohydrate accumulation in source leaves when sink capacity is limited (Wang & Wang,

2021). With prolonged exposure, however, such source–sink imbalance can contribute to photosynthetic acclimation and changes in carbon and nutrient allocation among plant organs. For example, warming increased biomass allocation to needles, while elevated CO_2 also shifted biomass allocation toward needles in *Pinus sylvestris* (Kurepin et al., 2018). This is consistent with the tendency toward greater leaf allocation observed under future climate conditions in the present study. The trend toward greater leaf biomass allocation observed in this study is consistent with the response of *Abies faxoniana* seedlings reported by Yin et al. (2008), whereas *Picea asperata* showed greater allocation to roots under warming. The tendency toward increased height under the combined future-climate treatment is also consistent with responses reported for Minjiang fir, Scots pine, Norway spruce, and white birch (Hou et al., 2011; Wang et al., 2022). Our results showed a trend similar to that reported for white birch by Wang et al. (2022), in which increased leaf biomass under future climate conditions may compensate for the negative effects of photosynthetic downregulation on carbon gain, potentially supporting faster growth and greater biomass accumulation. However, in the present study, these responses were observed only in the PCA results. The lack of statistical significance may partly reflect the relatively small sample size and associated variation among seedlings.

Drought can reduce photosynthesis, biomass accumulation, and plant growth. These negative effects occur broadly from agricultural crops to woody plants and generally become stronger as drought severity increases (Dong et al., 2019). The PCA also suggested different responses between moderate and severe drought. However, future climate conditions did not interactively modify the drought-related shift in biomass allocation. The PCA suggested a tendency toward relatively greater investment belowground under moderate drought. Increased belowground allocation under moderate water limitation has also been reported in other woody plants (e.g., Meier and Leuschner, 2008; Chen et al., 2011). A similar response was varying among species reported for *Pinus tabulaeformis*, in which drought increased root biomass and the root-to-shoot ratio (Chen et al., 2011). In the present study, this shift in allocation was accompanied by little apparent change in root collar diameter. This pattern is also consistent with observations in Norway spruce, where drought promoted preferential carbon allocation to roots while reducing radial growth (Oberhuber et al., 2017). In contrast, seedlings under severe drought tended to have lower overall biomass and smaller seedling size in the PCA. Severe water limitation can strongly constrain photosynthesis and carbohydrate production, and growth

reduction is considered one of the major early responses of plants to drought stress (Sevanto, 2014). Therefore, the smaller seedling size observed under severe drought in the PCA may reflect increasing carbon limitation as drought intensity increased. Overall, these results suggest that moderate drought may primarily induce adjustments in biomass allocation, whereas severe drought may begin to constrain whole-plant growth.

This study provides further evidence to support the importance of the interactive and combined effects of multiple factors on trees to fully understand the potential impact of future climate conditions on trees growing under different site conditions. In particular, the significant interactions between drought and climate conditions for V_{cmax}, J_{max}, and net photosynthetic rate indicate that the physiological response of jack pine to drought cannot be predicted from the effects of drought or future climate alone. In contrast, biomass and morphological traits showed no significant interactive responses, suggesting that physiological traits may be more responsive over the experimental period. However, this study has its limitations that the experiment was carried out using small seedlings only for one growth season and under controlled environmental conditions. Therefore, the responses observed in this study may differ from those of mature trees or seedlings exposed to longer-term and more variable field conditions. There are substantial differences between controlled environmental conditions and field conditions. For example, there are large seasonal and daily variations in all the environmental factors and multiple environmental stresses can occur simultaneously in the field whereas all the environmental conditions are controlled within predefined ranges in controlled environment studies. The results from controlled environment studies should not be extrapolated beyond the environmental conditions of the study without being verified by field experiments (Lu & Yeh, 2024). Combining controlled-environment experiments with longer-term field studies will provide a more comprehensive understanding of how jack pine may respond to future climate change. Future studies could also examine the use of external biological compounds to reduce plant responses to water stress.

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